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Endothelins: Molecular Biology, Biochemistry, Pharmacology, Physiology, and Pathophysiology

G. M. RUBANYI* AND M. A. POLOKOFF

Berlex Biosciences, Richmond, California

* Address for Correspondence: Berlex Biosciences, Cardiovascular Department, 15049 San Pablo Avenue, Richmond, CA 94804.

I. Introduction

The discovery of a peptidergic EDCF† (Hickey et al., 1985) and the isolation, sequencing, and cloning of this peptidergic EDCF and naming it endothelin (Yanagisawa et al., 1988b) stimulated worldwide interest and unprecedented research activity. Six years later, it is now evident that the discovery of the ET peptide family initiated a new field of biomedical research which promises to lead to better understanding of the pathomechanism of several diseases and to the development of novel therapeutics.

Within this relatively short period of 6 years, significant progress has been made in ET research. Three different ET genes have been identified in the human genome that have different chromosomal locations and are differentially regulated; the latter processes led to the expression of three different ET isopeptides (ET-1, -2, -3) in animals and humans. The putative enzyme converting the biologically inactive precursor, big ET, to the biologically active mature peptide (ECE) has been purified and characterized. Two different ET receptors (and possibly a third) have been cloned, and the biological (pharmacological) activity of ETs has been tested in numerous animal and human tissues in vivo and in vitro. Signal transduction pathways, mediating both rapid and long-lasting changes in cell function after ligand-receptor interaction, have been identified. The development of sensitive and specific techniques to detect gene expression and mature ET synthesis and production in vitro and in vivo, as well as the discovery of selective ET receptor antagonists, ECE inhibitors, and anti-ET antibodies, have provided the necessary tools to unravel the

† Abbreviations: EDCF, endothelium-derived contracting factor; ACE, angiotensin-converting enzyme; ADP, adenosine diphosphate; ANP, atrial natriuretic peptide; ATP, adenosine triphosphate; AVP, arginine vasopressin; BNP, brain natriuretic peptide; Ca^{2+}, calcium ion; $[Ca^{2+}]_i$, intracellular free calcium ion concentration; cAMP, cyclic adenosine 3′,5′-monophosphate; cDNA, complementary deoxyribonucleic acid; cGMP, cyclic guanosine 3′,5′-monophosphate; CNS, central nervous system; COS, simian virus 40-transformed monkey kidney cell line; CSF, cerebrospinal fluid; DAG, *sn*-1,2-diacylglycerol; ECE, endothelin-converting enzyme; EDRF, endothelium-derived relaxing factor; ET, endothelin; ET-1, -2, -3, endothelin types 1, 2, 3; $ET_{A, B, C}$, endothelin receptor subtypes; G-protein, guanine nucleotide-binding protein; GFR, glomerular filtration rate; GTP, guanosine triphosphate; HUVEC, human umbilical vein endothelial cell; IP_3, 1,4,5-inositol triphosphate; irET, immunoreactive endothelin; K^+, potassium ion; K_f, ultrafiltration coefficient; LDL, low-density lipoprotein; MMP, membrane metalloendopeptidase; mRNA, messenger ribonucleic acid; Na^+, sodium ion; NE, norepinephrine; NEP, neutral endopeptidase; L-NMMA, N^G-monomethyl-L-arginine; NMR, nuclear magnetic resonance; NO, nitric oxide; PAF, platelet-activating factor; PDGF, platelet-derived growth factor; PGI_2, prostacyclin; PI, phosphoinositide; PKC, protein kinase C; PLC, phospholipase C; PMN, polymorphonuclear neutrophil; pO_2, partial pressure of oxygen; RBF, renal blood flow; SAH, subarachnoid hemorrhage; SHR, spontaneously hypertensive rat; SMC, smooth muscle cell; STX, sarafotoxin; TGF, transforming growth factor; TXA_2, thromboxane A_2; VOC, voltage-operated channel; VSMC, vascular smooth muscle cell; WKY, Wistar Kyoto (rat); AP-1, mammalian transcription factor activator protein-1.

physiological and pathophysiological importance of endogenous ETs.

Although an attempt was made to summarize most of the important developments in ET research in the past 6 years, space limitations prevent us from including all of the more than 2500 published original research papers in this review. For more details, the reader is referred to a recently published monograph (Rubanyi, 1992a) and the more than 70 review articles, including general overviews of ET research (Anggard et al., 1990; Chabrier and Braquet, 1990; Doherty, 1992; Haynes and Webb; 1992; Huggins et al., 1993; Lovenberg and Miller, 1990; Luscher, 1991; Marsden and Brenner, 1991; Masaki and Yanagisawa, 1992; Masaki et al., 1992; Miller et al., 1993; Nayler, 1990; Rubanyi and Botelho, 1991; Schini and Vanhoutte, 1991; Webb, 1991; Whittle and Moncada, 1990) and reviews concerning specific areas, including the historical background of EDCFs (Rubanyi, 1988, 1992b; Ryan and Rubanyi, 1988; Vanhoutte and Katusic, 1988; Karwatowska Prokopczuk and Wennmalm, 1990b), endothelial dysfunction (Rubanyi, 1991, 1993; Rubanyi and Vanhoutte, 1990), molecular biology (Yanagisawa and Masaki, 1989; Hiley, 1989; Masaki et al., 1990b, 1991a; Phillips et al., 1992), biosynthesis and ECE (Opgenorth et al., 1992), ET receptors (Sokolovsky, 1991, 1992a,b; Sakurai et al., 1992b; Huggins et al., 1993), signal transduction pathways (Highsmith et al., 1992; Masaki et al., 1990c; Simonson and Dunn, 1990a; Takuwa et al., 1989c), release of vasoactive mediators by ETs, including PGI_2 and NO (Botting and Vane, 1990; Hyslop and De Nucci, 1992), growth regulation (Battistini et al., 1993), cardiovascular effects (Goto et al., 1992; Lerman et al., 1990), effects on coronary arteries (Stewart, 1991), vascular pharmacology (Randall, 1991), heart (Kramer et al., 1992), blood pressure regulation (Masaki et al., 1990a), congestive heart failure (Cody, 1992), kidney (Kon and Badr, 1991; Simonson et al., 1992c; Simonson, 1993), renal diseases and failure (Firth et al., 1988; Kon and Awazu, 1992; Perico and Remuzzi, 1992; Shultz, 1992; Gross et al., 1993a), lung and airways (Lagente et al., 1990; Hay et al., 1993), CNS (Greenberg et al., 1992; Shigeno and Mima, 1990), eye (Chakravarthy and Archer, 1992), and perinatal pharmacology and physiology (Masaki et al., 1991b; Mitchell, 1991).

After a brief historical recollection of the discovery of ETs, the review is divided into several major sections in which are summarized the progress and present state-of-the-art of ET research in the fields of molecular biology, protein chemistry and biochemistry (gene structure and localization, gene expression, biosynthesis, ECE, peptide structure, receptors, structure-activity-relationship), signal transduction mechanisms, pharmacology (reviewing the various in vitro and in vivo biological/pharmacological actions of ETs in different tissues and organs), potential physiological roles of ETs (giving a mostly hypothetical integrated view of how the various biological actions of ETs may fit into complex homeostatic mechanisms in the organism, the potential autocrine-, paracrine-, and endocrine-signaling modes of the peptides, and their presence, metabolism, and elimination in the circulation), and the potential pathophysiological importance of the peptides in various human diseases.

II. Discovery of Endothelins

Key discoveries in the past 15 years revealed that endothelial cells synthesize and release vasorelaxant (e.g., PGI_2 and EDRF; Moncada et al., 1976; Furchgott and Zawadzki, 1980), and vasoconstrictor (EDCF; De Mey and Vanhoutte, 1982; Rubanyi and Vanhoutte, 1986) substances. In 1982, an improperly designed bioassay study led to the discovery of a peptidergic EDCF (Hickey et al., 1985). The plan was to test the biological activity of the culture medium of bovine aortic endothelial cells on isolated pig coronary arteries. It was expected that cultured endothelial cells release EDRF into the culture medium, which will then relax the smooth muscle preparation. Because of the extreme lability of EDRF, no relaxing activity was observed when the culture medium was added to the smooth muscle preparation, because the donor (endothelial cells) and acceptor (smooth muscle) were in two different laboratories. Instead, the culture medium triggered a slowly developing and long-lasting contraction of the vascular smooth muscle, which could not be attributed to any known vasoconstrictor mediators and was shown to be of a peptidergic nature (fig. 1) (Hickey et al., 1985). This pioneering observation was confirmed in subsequent studies (Gillespie et al., 1986; O'Brian et al., 1987). In 1987, Masaki and his colleagues isolated, purified, sequenced, and cloned this peptidergic EDCF, which they named ET (Yanagisawa et al., 1988b).

The 21-amino acid peptide, ET, has no similarity in its sequence to the known peptides of mammalian origin. However, half a year later, the sequence of a rare snake venom, STX, was reported to be very similar to that of ET (Kloog and Sokolovsky, 1989). One of the most remarkable steps in the progress of ET research, following its discovery, was the identification of isotypes of ET. Analysis of human genomic sequences revealed the existence of three distinct genes for ET; these encode three distinct ET peptides (Inoue et al., 1989a,b) and were named ET-1, ET-2, and ET-3.

ETs are widely distributed in the organism of mammals, including humans and nonhuman primates. ET-like immunoreactivity is also found in several species of invertebrates and fish (Kasuya et al., 1991), indicating that ETs found in humans have a long evolutionary history.

III. Structure of Endothelin Peptides

The ET family consists of the three ET isoforms and four highly homologous cardiotoxic peptides isolated from the venom of *Atractaspis engaddensis*, the STXs

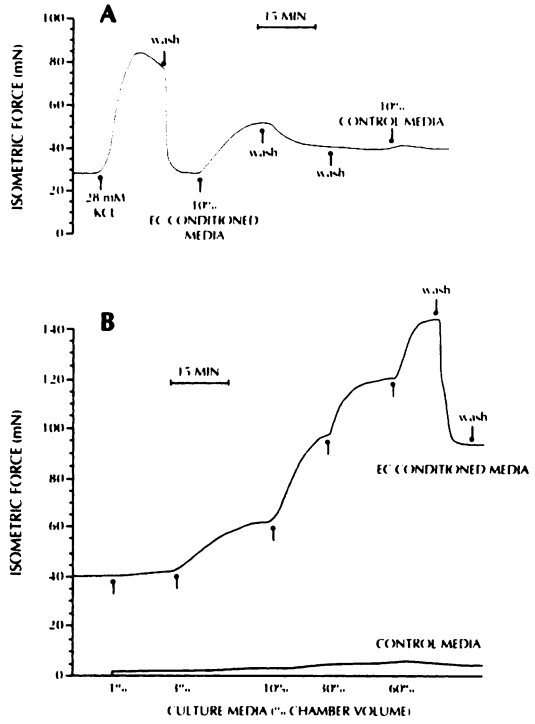

FIG. 1. Discovery of the peptidergic EDCF by bioassay (from Hickey et al., 1985) later identified as the 21-amino acid peptide ET. Conditioned media from cultured bovine aortic endothelial cells evoke concentration-dependent long-lasting contractions of isolated strips of porcine coronary arteries without endothelium. EC, endothelial cell. Reproduced with permission of the American Physiological Society.

(fig. 2). All family members contain 21 amino acid residues and show complete identity at ten positions, including all four cysteine residues (positions 1, 3, 11, and 15), as well as at positions 8 (aspartic acid), 10 (glutamic acid), 16 (histidine), 18 (aspartic acid), 20 (isoleucine), and 21 (tryptophan). Variant amino acids at the other positions tend to be closely related with respect to charge and hydrophobicity, with the exception of the presence of charged residues at position 4 (lysine) in three of the STXs, at position 7 of ET-3 (lysine), and the substitution of a negative for a positive charge at position 9 of STX-c. The COOH-terminal hexapeptide regions of these peptides are largely hydrophobic.

All three ET isoforms are synthesized as larger preproforms which are processed by dibasic amino acid endopeptidase activities to propeptides of 37 to 41 amino acids, the so-called big ETs (fig. 3). The subsequent cleavage of these propeptides to the mature ETs is inefficient both in vitro and in vivo, because big ETs have been identified in plasma (Miyauchi et al., 1989) and in the media of cultured cells (Parker-Botelho et al., 1992). The COOH-terminal portions of the big ETs share considerable sequence homology, although to a somewhat lesser degree than the corresponding mature ETs.

In the ETs, all four cysteine residues participate in disulfide bonding (Cys^1-Cys^{15}; Cys^3-Cys^{11}). It is interesting to note that this disulfide pattern is distinct from that of another naturally occurring toxic peptide, the bee venom protein apamin (Cys^1-Cys^{11}; Cys^3-Cys^{15}) (Pease and Wemmer, 1988). It is now appreciated that the disulfide bonds present in the ETs are vital for high-affinity binding to one class of ET receptors (ET_A) but less important in recognition by another class of ET receptors (ET_B) (see section VI).

Although a preliminary report of the crystallization of ET-1 has appeared (Wolff et al., 1992), to date no detailed X-ray crystal structure has been published for any member of the ET family. In any event, such a static structure may bear little resemblance to the biologically active conformation, because in a small protein interactions between molecules in the crystal lattice are likely to play a dominant role. Several groups have proposed solution structures of ETs based on proton NMR measurements and distance geometry calculations (Andersen et al., 1992; Aumelas et al., 1991; Dalgarno et al., 1992; Donlan et al., 1992; Endo et al., 1989; Mills et al., 1992; Munro et al., 1991; Reily and Dunbar, 1991; Saudek et al., 1989, 1991; Tamaoki et al., 1991a). From these studies, it seems clear that ETs exist in solution as highly compacted structures. Although there is a good deal of consensus about certain structural elements, the physical properties of ET itself have complicated the collection and interpretation of NMR data. ET-1 has been shown to exhibit limited solubility in aqueous solution at neutral pH, to partition into ether/water interfaces (Spinella et al., 1989), and to form micellar aggregates (Calas et al., 1992) with a critical micellar concentration of 22 μM

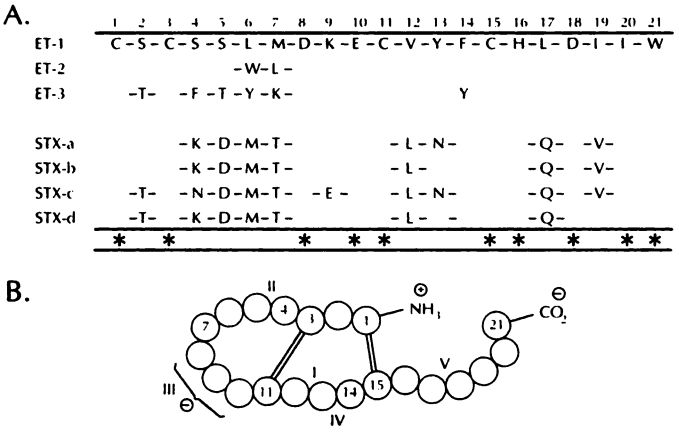

FIG. 2. Structure of the ET/STX family of peptides. A, primary structures of all three human ETs and four STXs are identical at ten of the 21 positions (indicated by asterisks). Residues that differ from the sequence of human ET-1 are shown. B, secondary structure of ET family peptides (key features are indicated in Roman numerals): I, two pairs of disulfide bonds define the compact NH_2-terminal core region consisting of inner and outer loops; II, residues 4 to 7 make up the most highly variable region of these peptides; III, residues 8 to 10 have a net charge of −1 in all family members except STX-c, which has a net charge of −3; IV, residues 12 to 14 are highly nonpolar in all family members; V, COOH terminus (residues 16 to 21) is the most highly conserved region. The terminal tryptophan residue is essential for biological activity.

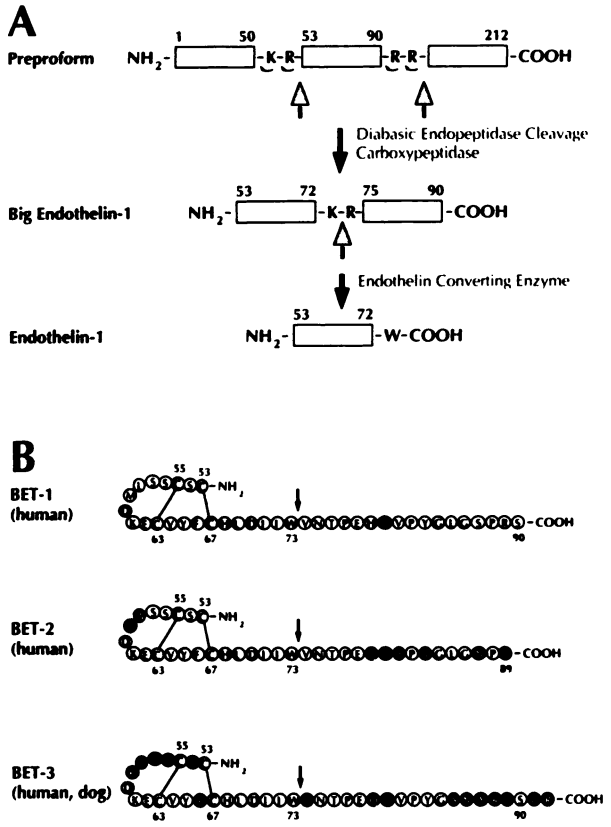

FIG. 3. Posttranslational processing of prepro-ETs. A, 203-amino acid human prepro-ET-1 peptide is cleaved by dibasic amino acid endopeptidase action at two sites (arrows, top), followed by sequential carboxypeptidase activity (small curved arrows) to yield prepro-ET-1 (53 to 91), referred to as pro-ET-1 or big ET-1 (middle). (The COOH-terminal portion of preprop-ET-1 contains the ET-like peptide; see fig. 4.) Big ET-1 is then cleaved by ECE (arrow) at the Trp^{73} to Val^{74} bond to yield the final 21-amino acid product, ET-1 (53 to 73). B, amino acid sequences of the three human big ET-1 isoforms are shown. Arrows indicate the sites of ECE action. ●, amino acid sequence changes compared to the originally identified porcine big ET-1 sequence (Yanagisawa et al., 1988a,b). Illustrations taken from Phillips et al., 1992, and used with permission of Oxford University Press.

(Bennes et al., 1990). Therefore, in most studies, NMR measurements have been made in the presence of organic solvents, or at nonphysiological pH ranges in aqueous/organic solvent mixtures.

Several groups have examined ET-1 structure in dimethyl sulfoxide (Endo et al., 1989; Munro et al., 1991; Saudek et al., 1989, 1991). One common conclusion from these studies was that a helix-like region (although not a regular α-helix) dominates the central portion of the molecule. The data were interpreted as showing a helical region between residues Lys^9 and Cys^{15}, thus encompassing both disulfide bridges (Endo et al., 1989; Saudek et al., 1991), or between Leu^6 and Cys^{11} (Munro et al., 1991). There was no consensus reached about the structure of the COOH-terminal hexapeptide. One model suggested that the tail folded back toward the helical region (Saudek et al., 1991), whereas the other studies concluded that the COOH terminus was flexible and could adopt a number of conformations (Endo et al., 1989; Munro et al., 1991).

Recognizing that dimethyl sulfoxide is a denaturing solvent and that motional averaging is a significant problem in this solvent (Andersen et al., 1992), subsequent studies of ET-1 structure have used aqueous/organic mixtures (Aumelas et al., 1991; Reily and Dunbar, 1991). In these studies, lack of a single prominent conformational isomer was noted. The data appeared to rule out extensive randomization of the structure and to rather support the conclusion that there was rapid interconversion between several tightly defined conformers (Andersen et al., 1992). In agreement with the earlier studies, there was general consensus concerning the presence of a helical core in the region Lys^9-Cys^{15}. This was true both of native ET-1 and of the isosteric norleucine[7] analog of ET-1 (Aumelas et al., 1991). According to one model (Krystek et al., 1991), the two disulfide bonds within the helical region are present on one face of the helix, whereas several residues that contribute to biological activity (e.g., Glu^{10} and Phe^{14}) are present on the opposite face. The structure of the COOH-terminal tail region of the norleucine analog in 50%/50% CD_3CN/water (pH 3.9) was characterized as having considerable mobility, with no evidence of any close interaction with the helical core region (Aumelas et al., 1991). A similar conclusion was reached about the tail region of native ET-1 in 30% CD_3CN (pH 5.4) (Reily and Dunbar, 1991). Others have interpreted their NMR data as indicating that, in mixed water/ethylene glycol, ET-1, and in particular the tail region, may exist in solution in one of several conformations, including states in which the tail resides in close proximity to the helical core (Andersen et al., 1992; Krystek et al., 1991). The conformation of the NH_2-terminal region was not well defined in any of the studies.

Based on far ultraviolet circular dichroism spectra, others have disputed the conclusions from the NMR studies that ET-1 possesses a central helical core (Calas et al., 1992). Instead, a structural model featuring a series of β-turns was proposed.

Two studies have used proton NMR to examine the structure of ET-3 (Bortmann et al., 1991; Mills et al., 1992). It is not surprising that many of the overall structural features of ET-3 appear to be similar to those of ET-1, considering the degree of homology between the ET isoforms. Both studies proposed a well-defined extended conformation at the NH_2 terminus, a less constrained turn region (residues 5 to 7), an irregular helical core region between residues Lys^9 and Cys^{15}, and a COOH-terminal tail that folds back toward the core. As was the case for ET-1, these investigators concluded that ET-3 can exist in one of several interconvertible conformations, which makes the precise localization of critically important residues, such as the COOH-terminal tryptophan, difficult to define.

The structure of big ET-1 was studied under acidic conditions (Donlan et al., 1992). As with ET-1, a family of possible conformations was calculated that exhibit a β-turn at residues 5 to 8 and a helical region between residues 9 and 16. The COOH-terminal portion (residues 17 to 38) was described as a series of different conformers in dynamic equilibrium.

In conclusion, NMR studies have suggested some general features of the tertiary structure of the ETs. What is lacking is a precise picture of the conformation of the peptides when they are bound to the high-affinity receptor sites (in the case of the ETs) or at the active site of the relevant ECE (in the case of big ET). Such data will be of great value in the rational design of ET antagonists and ECE inhibitors.

IV. Endothelin Genes

A. Chromosomal Localization

Screening of genomic libraries at reduced stringency with ET-1 gene fragments has shown that there are three distinct ET-related genes in humans and other mammalian species (Inoue et al., 1989a,b; Itoh et al., 1988, 1989; Kimura et al., 1989a; Marsden et al., 1992; Onda et al., 1991). Southern blot analysis of the mouse genome (Saida et al., 1989) revealed, in addition to mouse ET-1, a second gene whose expression was limited to the intestine. The product of this second gene was named *VIC* (vasoactive intestinal contractile peptide). The vasoactive intestinal contractile peptide gene was later identified as the mouse homolog of the rat ET-2 gene (Bloch et al., 1991). In the human, the ET-1, ET-2, and ET-3 genes have been mapped to chromosome 6 (Arinami et al., 1991; Bloch et al., 1989a,b; Hoehe et al., 1993), chromosome 1 (Arinami et al., 1991; Bloch et al., 1991), and chromosome 20 (Arinami et al., 1991; Bloch et al., 1989a; Rao et al., 1991), respectively. Genetic fine mapping studies of the human ET-1 gene, using a four-allele *Taq*I polymorphism (Berge and Berg, 1992), localized it to the telomeric region of chromosome 6p, close to the gene encoding the α-subunit of clotting factor XIII (Hoehe et al., 1991).

B. Gene Structure

The human ET-1 gene contains five exons, four introns, and 5′- and 3′-flanking regions and spans approximately 6.8 kb of DNA (Inoue et al., 1989a,b) (fig. 4). Each of the five exons encodes a portion of prepro-ET-1: exon 1 encodes the 5′-untranslated region and the first 22 amino acids of the precursor, including the entire signal sequence (Fabbrini et al., 1991); exon 2 encodes the complete sequence of ET-1, the Trp-Val cleavage site at which the ECE processes big ET, and the first four residues of the COOH-terminal portion of big ET-1; exon 3 contains the coding region of the remainder of big ET-1 as well as the "ET-like" peptide of prepro-ET-1; the fifth exon specifies the COOH-terminal portion of pre-

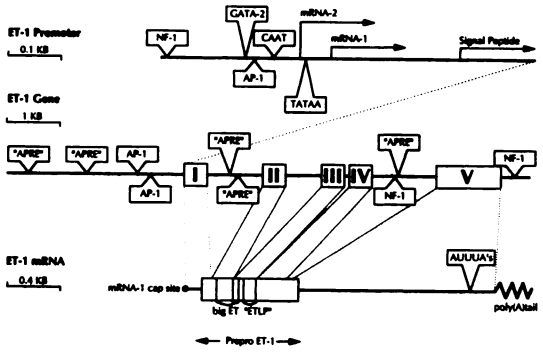

FIG. 4. Structure of the human ET-1 gene. The 6.8-kb coding region (center) comprises five exons (boxes) interrupted by four introns. The primary transcript (ET-1 mRNA, bottom) is 2.3 kb in length and directs translation of the precursor prepro-ET-1 peptide (box). Each of the five exons encodes a portion of prepro-ET-1, including the sequences of pro-ET-1 ("big ET") and the ET-like peptide ("ETLP"). Also shown (top) is the structure of the ET-1 promoter, which contains two functional transcription start sites (Benatti et al., 1993). Also shown are the approximate locations of several important regulating sequences, including consensus TATAA and CAAT boxes, binding sites for nuclear factor-1 ("NF-1"), GATA-2, AP-1/jun, and acute phase reaction regulatory elements ("APRE"). For details, see text.

pro-ET and the 3′-untranslated region of the mRNA. The primary transcription start site in endothelial cells was mapped by S1 nuclease protection to a position 98 base pairs downstream from a CAAT box and 31 base pairs from a TATAA box (Inoue et al., 1989a,b). More recently, a larger transcript was cloned from a human placental cDNA library and was found to contain an additional 80-nucleotide segment 5′ to the previously identified cap site (Benatti et al., 1993). Primer extension and polymerase chain reaction analysis revealed the existence of additional transcription initiation sites.

Several characteristic regulatory elements are found within the ET-1 gene (fig. 4). Motifs of the consensus binding sequence for the transcription factor nuclear factor-1 (Gronostajski, 1987) were identified at the 3′-end of intron 1 (Bloch et al., 1989a,b) and in intron 4 and exon 5 (Inoue et al., 1989a,b). Although the possible function of these sequences has not been defined, nuclear factor-1-binding sites are known to be involved in the induction of the mouse type I collagen gene by TGFβ (Rossi et al., 1988). Interestingly, TGFβ induces ET-1 gene expression in cultured porcine aortic endothelial cells (Kurihara et al., 1989b). Four copies of the hexanucleotide CTGGGA, the acute phase reaction regulatory element, are found within the gene, two in the 5′-flanking region, one within intron 1, and the other within intron 4 (Inoue et al., 1989a,b). One or more of these elements may participate in the known induction of the ET-1 gene following surgery, myocardial infarction, and other stresses (Miyauchi et al., 1989).

Sequences highly homologous to the octanucleotide consensus AP-1/Jun-binding site are present at three positions in the 5′-flanking region and could explain the rapid induction of ET-1 mRNA following treatment of

umbilical vein endothelial cells with phorbol ester (Inoue et al., 1989a,b).

C. Patterns of Gene Expression

Although originally isolated and purified from the culture media of porcine aortic endothelial cells (Yanagisawa et al., 1988b), ET isoforms are produced in a variety of tissues and cell types and there are tissue-specific patterns of isoform expression. Sensitive immunological methods have been developed to detect ET and big ET production in the fentomole range. These methods are not entirely satisfactory for fully characterizing patterns of ET expression, however, because the antibodies used generally show some degree of cross-reactivity between different isoforms. ET gene expression has also been detected by Northern analysis, reverse transcriptase/polymerase chain reaction, in situ hybridization, and RNase protection techniques. Although the data obtained are mainly qualitative in nature, gene probes can be designed with sufficient specificity such that each ET isoform can be studied separately.

The overwhelming majority of studies have focused on ET-1 expression (table 1). Endothelial cells, regardless of their origin, appear to express ET-1 mRNA. This includes umbilical vein (Inoue et al., 1989a,b), mesenteric artery (Dohi et al., 1992), glomerulus (Marsden et al., 1991), corpus cavernosum (Saenz de Tejada et al., 1991), aorta (Tokunaga et al., 1992), and brain microvessel (Yoshimoto et al., 1990). It is now evident that VSMCs as well are capable of ET-1 mRNA expression (Resink et al., 1990a; Tokunaga et al., 1992) and production of mature ET-1 (Resink et al., 1990a). Northern analysis has suggested that ET-2 and ET-3 are not expressed in either vascular endothelium or smooth muscle (Bloch et al., 1989a,b; Firth and Ratcliffe, 1992).

Far from being confined to cells of the vascular wall, ET-1 gene expression also occurs in many other cell types, including breast epithelium (Baley et al., 1990), keratinocytes (Imokawa et al., 1992; Yohn et al., 1993), endometrial stromal and glandular epithelial cells (Economos et al., 1992a,b), macrophages (Ehrenreich et al., 1990; 1993b), bone marrow mast cells (Ehrenreich et al., 1992), astrocytes (Ehrenreich et al., 1993a), mesangial cells (Sakamoto et al., 1990), neurons of the spinal chord and dorsal root ganglia (Giaid et al., 1989, 1991), avascular human amnion (Casey et al., 1991), and cardiomyocytes (Suzuki et al., 1993). Among tumor-derived cells, endometrial carcinoma (Economos et al., 1992a,b), pulmonary squamous cell carcinoma and adenocarcinoma (Giaid et al., 1990), HEp-2 hepatocarcinoma and HeLa cells (Shichiri et al., 1991a,b), and renal adenocarcinoma (Tokito et al., 1991) have all been shown to express ET-1.

Few data concerning expression of mRNA for ET-2 or ET-3 in specific cell types have been reported, although a human renal adenocarcinoma cell line was shown to express ET-2 (Tokito et al., 1991).

At the tissue level, much more is known about the expression of all three ET isoforms. In the adult rat, ET-1 expression was observed, using Northern analysis, in vascular endothelium, lung, brain, uterus, stomach, heart, adrenal gland, and kidney (Sakurai et al., 1991). In a separate study, Northern blots of eye ball, submandibular gland, brain, kidney, jejunum, stomach, and spleen were positive for ET-3 mRNA (Shiba et al., 1992).

A survey of ET gene expression, using a very sensitive RNase protection assay, indicated that mRNAs for ET-1 and ET-3 were detectable in every organ examined (heart, lung, liver, spleen, kidney, placenta, stomach, small and large intestine, testis, skeletal muscle, salivary gland, and brain) (Onda et al., 1990; Firth and Ratcliffe, 1992). Lung was the predominant site of ET-1 expression, being 5-fold higher than large intestine and at least 15-fold higher than any other organ. Major sites of ET-3 expression were small intestine, lung, large intestine, kidney, brain, and stomach. The expression of ET-2 mRNA was much more limited: large and small intestine predominated, with skeletal muscle, heart, and stomach showing much less activity. All other organs tested were negative.

The results of a number of studies of the expression of ET isoforms are summarized in table 1 (for further details see also section VIII).

D. Regulation of Gene Expression

Many of the details of the biosynthetic pathways involved in the production of mature ET isoforms remain to be elucidated. It is clear, however, that regulation of the transcription of ET mRNA plays an important and probably predominant role. The ET-1 promoter region in endothelial cells has been studied extensively to identify *cis*-acting sequences involved in the cell-specific expression of the gene. Bovine aortic endothelial cells were transfected with a series of reporter gene constructs in which the expression of chloramphenicol acetyltransferase was driven by human ET-1 promoter sequences (Lee et al., 1990b). Deletion mutants identified a 143-base pair region upstream of the ET-1-coding region that promoted chloramphenicol acetyltransferase expression. This region was found to contain two sequences, an AP-1 binding motif immediately downstream from a sequence similar to the consensus Eryf-1 (GATA-1) binding motif found in erythroid cells, both of which were required for full chloramphenicol acetyltransferase expression in the transfected cells. Expression of this reporter construct was endothelial cell specific. Site-directed mutagenesis of the AP-1 site at position −109 of the ET-1 gene resulted in a 30-fold reduction in promoter activity, whereas gel shift assays using anti-fos and anti-jun antibodies confirmed the ability of these endothelial cell nuclear factors to bind to and activate

TABLE 1
*ET gene expression in cells and tissues**

Cell/tissue	Method of detection	Expression	Reference
Adrenal gland (human)	Northern, RIA	Positive for ET-1 and ET-3 inadenoma and adjacent normal tissue	Imai et al., 1992a,b
Aorta (human)	In situ hybridization	Strongly positive for ET-1 in endothelial cells; weakly positive for ET-1 smooth muscle cells	Tokunaga et al., 1992
Astrocyte (rat)	RT/PCR, RIA	Positive for ET-1	Ehrenreich et al., 1993a,b
Amnion (human)	Northern, RIA	Positive for ET (isoform specificity of probes not reported); ET expression induced 20-fold by prolactin	Casey et al., 1991
Breast epithelial cell (human)	Northern	Positive for ET-1; negative for ET-2, ET-3	Baley et al., 1990
Carcinoma Cell Lines (human)			
HeLa, HEp-2	Northern	Positive for ET-1	Shichiri et al., 1991a,b
ACHN (renal adenocarcinoma)	Northern	Positive for ET-2	Tokito et al., 1991
Endometrial carcinoma	PCR	Positive for ET-1	Pekonen et al, 1992
HEC 1A endometrial carcinoma	Northern, RIA	Positive for ET-1; upregulated by TGFβ + cycloheximide	Economos et al., 1992a,b
Cardiomyocytes (rat neonatal)	RT/PCR	Positive for ET-1	Suzuki et al., 1993
Endometrial Tissue			
Endometrium (human)	Northern, RIA	Positive for ET-1 in glandular epithelial stromal cells	Economos et al., 1992a,b
Endometrium (human)	RT/PCR	Positive for ET-1, ET-2, ET-3 throughout menstrual cycle	O'Reilly et al., 1992
Endothelial Cells			
Brain microvessel (porcine)	Northern	Positive for ET-1	Yoshimoto et al., 1990
Corpus cavernosum (human)	Northern	Positive for ET-1	Saenz de Tejada et al., 1991
Dental pulp (human)	Immunohistochemistry	Positive for ET-1	Casasco et al., 1991
Glomerulus (bovine)	Northern	Positive for ET-1; upregulated by bradykinin, thrombin	Marsden et al., 1991
Hybrid cell line EA, hy 926 (human)	Northern	Positive for ET-1	Saijonmaa et al., 1990
Mesenteric artery (rat)	Northern, RIA	Positive for ET-1; upregulated by angiotensin II	Dohi et al., 1992
Hypothalamus (human)	In situ hybridization	Positive for ET-1 in neurons and vascular endothelium	Lee et al., 1990a,b,c
Keratinocytes (human)	Northern	Positive for ET-1	Imokawa et al., 1992; Yohn et al., 1993
Kidney (rat)	PCR	Positive for ET-1: glomerulus > inner medullary collecting duct Negative for ET-1: proximal convoluted tubules, medullary thick ascending limbs	Ujiie et al., 1992
Macrophage (human)	Northern, RIA	Positive for ET-1; upregulated by phorbol ester	Ehrenreich et al., 1990
Mast cell (murine)	RT/PCR, RIA	Positive for ET-1; upregulated by phorbol ester or cross-linked IgE	Ehrenreich et al., 1992
Mesangial cells (rat)	Northern	Positive for ET-1	Sakamoto et al., 1990
Neuron (human)	In situ hybridization; immunocytochemistry	Positive for ET-1 in spinal cord and corsal root ganglia Positive for all 3 ET's in neuronal cell bodies	Giaid, 1989; Giaid, 1991
Parathyroid gland (human)	Northern	Positive for ET-1	Eguchi et al., 1992a,b
Pituitary gland (human)	Northern	Positive for ET-1	Takahashi et al., 1992a,b
Placenta (human)	Norther, RIA	ET-1 = bET-1 = ET-3 > ET-2	Benigni et al., 1991a,b
	Northern	Positive for ET-1; enhanced expression at term	Fant et al., 1992
	Northern	Positive for ET-1	Onda et al., 1990
Pulmonary Tumor (human)	In situ hybridization	Positive in squamous cell carcinoma and adenocarcinoma	Giaid et al., 1990

TABLE 1
Continued

Cell/tissue	Method of detection	Expression	Reference
Various tissues (rat)	RNase protection	ET-1: Lung > intestine > other organs ET-2: Intestine > stomach = heart ≫ other organs ET-3: Intestine = lung = kidney > other organs Renal ischemia: ET-1 increased, ET-3 decreased	Firth and Ratcliffe, 1992
	Northern	Positive for ET-1 in vascular endothelial cells, lung, brain, uterus, stomach, heart, adrenal gland, kidney	

* Abbreviations: RIA, radioimmunoassay; RT, reverse transcriptase; PCR, polymerase chain reaction; TGF, transforming growth factor.

transcription at this site (Lee et al., 1991a,b). Two groups independently cloned the cDNA for the protein expressed in HUVECs that binds to the GATA-like region of the human ET-1 promoter (Dorfman et al., 1992; Lee et al., 1991a,b). This protein, GATA-2, which was downregulated by retinoic acid treatment of endothelial cells (Lee et al., 1991a,b), is apparently responsible for the inhibitory effects of retinoic acid on ET-1 expression in HUVECs (Dorfman et al., 1992).

It now appears that ET-1 gene transcription can occur from one or more distal promoter elements, at least in some tissues. As mentioned above, a second species of ET-1 mRNA was recently described, which is transcribed from a promoter region upstream from the transcription start site originally identified in endothelial cell ET-1 mRNA (Benatti et al., 1993). This upstream promoter was found to be efficient in directing transcription of a chloramphenicol acetyltransferase reporter gene construct in COS-1 cells, a kidney epithelial cell line, even in the absence of the downstream GATA-2-binding site which is required for efficient transcription of ET-1 mRNA (mRNA-2; see fig. 4) in endothelial cells (Lee et al., 1991a,b). Thus, ET-1 gene expression seems to be differentially regulated in these two cell types.

An important feature of the ET-1 mRNA, conserved among different species, is the presence of two AUUUA sequences in the 3′-untranslated region. These sequences are known to mediate selective mRNA degradation, and probably account for the short half-life of the message, and the ability of cycloheximide to cause superinduction (Inoue et al., 1989a,b).

Regulation of endothelial cell ET-1 expression has been studied extensively. Increased message levels have been observed after treatment of cultured endothelial cells with growth factors and cytokines such as thrombin (Emori et al., 1992; Kurihara et al., 1989b; Maemura et al., 1992; Marsden et al., 1991), TGFβ (Kurihara et al., 1989b; Yanagisawa et al., 1989; Maemura et al., 1992; Zoja et al., 1991), tumor necrosis factor-α (Lamas et al., 1992; Maemura et al., 1992; Marsden et al., 1992), interleukin-1 (Maemura et al., 1992), and insulin (Hu et al., 1993; Oliver et al., 1991). Although interferon-γ alone had no effect on ET-1 mRNA expression, it potentiated the effects of tumor necrosis factor-α when the two were added simultaneously to endothelial cell cultures (Lamas et al., 1992). Vasoactive substances, such as angiotensin II (Dohi et al., 1992; Imai et al., 1992b), AVP (Imai et al., 1992b), and bradykinin (Marsden et al., 1991), also increased ET-1 mRNA expression in endothelial cells, as did exposure of cells to oxidized or acetylated low-density lipoprotein (Boulanger et al., 1992) or to physiologically low oxygen tension (Kourembanas et al., 1991).

In vitro experiments have indicated that expression of ET mRNA and release of ET from cultured endothelial cells are regulated by fluid shear stress. In primary human umbilical vein endothelial cells, exposure to high shear rates (25 dynes/cm^2) sharply decreased ET-1 mRNA levels relative to control cultures (Sharefkin et al. 1991). Similarly, bovine aortic endothelial cells exhibited a dose-dependent decrease in ET-1 mRNA expression with increasing shear stress (Malek and Izumo, 1992). On the other hand, it was reported that low shear stress (5 dynes/cm^2) increased ET mRNA expression and ET release transiently in cultured porcine aortic endothelial cells, whereas higher shear rates had no effect (Yoshizumi, et al. 1989). These contrasting results may reflect differences based on the origins of the endothelial cells used or in the specific culture conditions.

Regulation of ET mRNA expression has been studied in other cell types as well. Rat VSMCs in culture increased their expression of ET-1 in response to TGFβ, platelet-derived growth factor-AA, angiotensin II, AVP, and ET-1 itself (Hahn et al., 1990). Expression of ET-1 has also been detected in cells of the hemopoietic system. In cultured human macrophages, expression was stimulated by lipopolysaccharide and phorbol esters (Ehrenreich et al., 1990), whereas in murine bone marrow mast cells cross-linked immunoglobulin E, as well as phorbol ester, was stimulatory (Ehrenreich et al., 1992). In human breast epithelial cells in culture, prolactin caused a 20-fold induction in ET-1 mRNA (Baley et al., 1990). TGFβ and interleukin-1 stimulated ET-1 expression in endometrial stromal cells, and in cultured human avas-

cular amnionic cells, epidermal growth factor, interleukin-1, and tumor necrosis factor-α were all found to increase expression of this gene (Casey et al., 1991). Tumor necrosis factor was also found to exert a positive effect in cultured rat mesangial cells (Kohan, 1991), and TGFβ was similarly effective in the canine kidney cell line MDCK (Horie et al., 1991).

V. Endothelin Biosynthesis and Endothelin-converting Enzyme

From the cDNA sequence one can assume that the primary translation product of the human ET-1 gene is a 212-amino acid prepropeptide (Inoue et al., 1989a,b). Processing occurs in three stages (fig. 3): (*a*) dibasic amino acid endopeptidase(s) cleaves the precursor at Arg^{52}-Cys^{53} and at Arg^{92}-Ala^{93}; (*b*) carboxypeptidase(s) sequentially trims the Arg^{92} and Lys^{91} residues from the COOH terminus to produce pro-ET-1, more commonly known as big ET-1; (*c*) specific cleavage between Trp^{73}-Val^{74} by ECE yields ET-1.

The proposal that an enzyme activity must exist that specifically converts pro-ET-1 (big ET-1) to the mature 21-amino acid peptide was contained in the original report of the cloning of porcine ET-1 (Yanagisawa et al., 1988b). The physiological importance of the conversion of big ET to ET was demonstrated by the observation that ET-1 was 140-fold more potent as a vasoconstrictor compared to the precursor peptide (Kimura et al., 1989b), whereas the prepopeptide is devoid of any vasoconstrictor action (Cade et al., 1990). Now more than 6 years later, the nature of ECE remains controversial.

Interestingly, there is now evidence that the biosynthesis of at least one member of the STX family, STX-c, is radically different from that of the ETs (Takasaki et al., 1992). A partial genomic clone covering the coding region and the immediate flanking sequences of STX-c was isolated from *A. engaddensis*. Sequence analysis showed that the characteristic dibasic amino acid pair immediately upstream of the first residue in all three ET isoforms was not conserved in the STX. Furthermore, the nucleotide sequence immediately following the COOH-terminal Trp residue of STX-c was quite different from those of the ET peptides as well. Based on the partial sequence of this gene, the characteristic recognition sequence for ECE, Trp-Val(Ile), is not present in STX-c. Thus, it is possible that the genes for the ETs and the STXs are not evolutionarily related.

A. Vascular Responses to Exogenous Big Endothelin in Vivo

When administered to animals intravenously, big ET-1 is converted to ET-1 in vivo but not in the plasma in vitro (Hemsen et al., 1991b). The great majority of the available data point to the importance of phosphoramidon-sensitive mechanisms in regulating the in vivo conversion of exogenously administered big ET-1 and the resulting vascular responses. An early study (Fukuroda et al., 1990) demonstrated that both blood pressure elevation and airway contractile responses in rats or guinea pigs to intravenous big ET-1, but to not to ET-1, were blocked by phosphoramidon [N-(α-(-rhamnopyranosyloxyhydroxy-phosphinyl)-L-Leu-L-Trp]. Similar observations have been made by others in anesthetized rats. One group (McMahon et al., 1991a,b) showed that the membrane MMP I (neutral endopeptidase; EC 3.4.24.11) inhibitor, thiorphan, was also effective, albeit less potent than phosphoramidon, whereas other known protease inhibitors, such as kelatorphan and captopril (metalloendopeptidases), leupeptin (serine proteases), and *trans*-epoxysuccinyl-L-leucylamido-(4-guanidino) butane (cysteine proteases), were ineffective. The inhibition of big ET-1-induced vascular responses by phosphoramidon appeared to be competitive in nature (Pollock et al., 1993). This compound also blocked responses to big ET-3 (Pollock et al., 1993). A phosphoramidon analog lacking the rhamnose sugar was also found to be effective in vivo (Pollock et al., 1992). In another study (Bird et al., 1992), both phosphoramidon and the cathepsin E inhibitor SQ-32056 blocked the pressor responses in conscious rats challenged with a bolus intravenous injection of big ET-1. However, unlike phosphoramidon, SQ-32056 also inhibited ET-1-induced pressor responses, suggesting that this compound did not act via inhibition of ECE. When injected into the brains of rats (Hashim and Tadepalli, 1991) or dogs (Shinyama et al., 1991), big ET-1 caused increases in systemic blood pressure that were effectively blocked by phosphoramidon, suggesting that phosphoramidon-sensitive ECE activity exists in the CNS as well.

In addition to causing pressor responses, exogenous ET-1 and, less potently, big ET-1 have been shown to increase vascular permeability in the rat trachea, lung, pancreas, liver, spleen, kidney, heart, and diaphragm (Lehoux et al., 1992). Pretreatment of animals with phosphoramidon blocked the big-ET-1-induced permeability changes without affecting the responses to ET-1.

B. Characterization of Endothelin-converting Enzyme

1. Endothelial cell endothelin-converting enzyme. ECE activity has been studied most extensively in endothelial cells. The total homogenate of cultured bovine aortic endothelial cells converted big ET-1 to material with the immunological and chromatographic properties of ET-1 (Ohnaka et al., 1990). The pH dependence of the conversion showed two distinct pH optima (3.0 and 7.0), which apparently represented different proteases based on sensitivity to inhibitors and thermolability. The acidic protease activity was completely inhibited by pepstatin A but was insensitive to metal ion chelators ethyleneglycol-bis(β-aminoethylether)-N,N′-tetraacetic acid and ethylenediamine tetraacetic acid. The neutral protease exhibited the opposite pattern of inhibition by these agents. Neither activity was affected by various serine protease inhibitors, sulfhydryl reagents, or the ACE inhibitor

captopril. Thus, endothelial cells appeared to contain aspartyl and metalloprotease activities capable of converting big ET-1 to ET-1 in vitro.

The acidic, pepstatin-sensitive ECE, present in the cytosolic fraction of endothelial cells (Sawamura et al., 1990a) as well as in bovine adrenal chromaffin granules (Sawamura et al., 1990b), was shown to be similar to cathepsin D based on chromatographic behavior and inhibition by anti-cathepsin D antisera. Subsequent work with purified enzymes suggested that another aspartyl protease, cathepsin E, was a more specific ECE in vitro, because it cleaved specifically between Trp^{21} and Val^{22} of big ET-1 but, unlike cathepsin D, did not significantly degrade ET-1 further under the conditions used (Lees et al., 1990).

The characterization of ECE as an aspartyl protease was unsatisfying for several reasons, however. As discussed above, phosphoramidon inhibited the pressor response to exogenous big ET-1 and lowered blood pressure in SHR. Aspartyl protease inhibitors lack such activity. In cultured endothelial cells, phosphoramidon (Sawamura et al., 1991; Ikegawa et al., 1990), but not pepstatin (Ikegawa et al., 1990), significantly increased the ratio of endogenously synthesized and secreted big ET-1/ET-1. Phosphoramidon was similarly effective in suppressing the conversion of exogenously added big ET-1 by endothelial cell cultures (Ikegawa et al., 1991). Thus, attention has focused on the characterization of a neutral metalloprotease as a possible candidate for the "physiological ECE" in endothelial cells.

At pH 7.0, ECE activity of crude membranes from cultured porcine aortic endothelial cells was sensitive to phosphoramidon (Matsumura et al., 1990; Galvani et al., 1991), whereas the cytosolic fraction contained a neutral pH optimum activity that was insensitive to this agent (Matsumura et al., 1990). The membrane activity was insensitive to the MMP I inhibitor thiorphan (Matsumura et al., 1991b,e) and the ACE inhibitor captopril (Ahn et al., 1992). Limited substrate structure-activity relationship data have been published concerning this membrane ECE from endothelial cells. In one study, big ET-1 and big ET-3 were converted at comparable rates (Matsumura et al., 1992a). However, in another report (Okada et al., 1990), the rate of big ET-2 hydrolysis was only 5% of that seen using big ET-1 as substrate, and big ET-3 was not hydrolyzed at all. These observations are difficult to reconcile even considering the different assay conditions used. In the latter study, several human big ET-1 fragments were also examined as substrates for the membrane ECE. Big ET-1 (1 to 37), lacking the COOH-terminal serine residue, showed activity comparable to the full-length peptide. The NH_2-terminally truncated analog, big ET-1 (16 to 37), was actually cleaved at 3-fold the rate of full-length big ET-1, whereas the COOH-terminal hexapeptide truncated form, big ET-1 (1 to 31), was essentially inactive as a substrate. Thus, the COOH terminus of big ET-1 may influence the conformation of the cleavage site or may interact with a site on the enzyme to promote hydrolysis at the Trp^{21}-Val^{22} bond.

2. Smooth muscle cell endothelin-converting enzyme. ECE activity has also been characterized in cultured VSMCs (Hioki et al., 1991; Matsumura et al., 1991e; Ikegawa et al., 1991). In intact cells incubated with exogenous big ET-1, phosphoramidon (100 μM) strongly suppressed the conversion of the precursor to ET-1 without affecting basal release of ET-1 (Matsumura et al., 1991b,d,e; Ikegawa et al., 1991). Cytosolic fractions prepared from cultured VSMCs contained both pepstatin A-sensitive (acidic pH optimum) ECE activities and phosphoramidon-sensitive (neutral pH optimum) ECE activity (Hioki et al., 1991). These data suggest that unprocessed big ET-1, secreted from endothelial cells or SMCs, may be converted to the active, vasoconstricting peptide at the surface of the SMCs, where it would be available for binding to cell surface ET receptors.

3. Purification of endothelin-converting enzyme. Progress in the purification of a neutral membrane MMP with ECE activity has been slow, despite the intense interest and effort of many academic and industrial research groups worldwide. The reasons for this lack of rapid success in the molecular characterization of ECE probably include (*a*) the relatively low level of expression of activity in most cells and tissues studied; (*b*) the technical difficulties involved in measuring the amount of true product formation against the background of other nonspecific cleavages of the substrate in crude extracts; (*c*) the ability of several different classes of proteases to catalyze the in vitro conversion of big ET to ET; and (*d*) uncertainties about the precise physiological sites of big ET conversion, which led various groups to focus on different tissues and subcellular fractions as the starting material for purification efforts.

In endothelial cells, ECE activity has been solubilized from the membrane fraction using 3-[(cholamidopropyl)dimethylammonio]-1-propanesulfonic acid (Matsumura et al., 1992a), Lubrol PX (Ohnaka et al., 1992), or Triton X-100 (Ahn et al., 1992). The nonionic detergents are preferable to 3-[(cholamidopropyl)dimethylammonio]-1-propanesulfonic acid because the latter detergent is inhibitory to the activity in vitro (Ahn et al., 1992; Ohnaka et al., 1992). After solubilization, the crude activity exhibited an apparent molecular weight, on gel filtration chromatography in the presence of micellar detergent, of 300 to 350 kDa (Ahn et al., 1992; Matsumura et al., 1992a). The enzyme may be glycosylated because the Lubrol-solubilized activity was retained on a *Ricinus communis* agglutinin lectin affinity column (Ohnaka et al., 1992). Elution of the activity from a *R. communis* agglutinin affinity column by the competing sugar D-galactose resulted in a 25-fold purification. Another 20-fold purification was achieved by peanut agglu-

tinin chromatography, because most of the protein retained by the *R. communis* agglutinin column also bound to this column, whereas ECE did not. A final 2-fold purification by high-pressure liquid chromatography anion exchange yielded an ECE preparation enriched 2100-fold over the crude membrane fraction. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the purified material revealed two major silver-stained bands of 100 and 120 kDa. At this stage of purity, the activity was inhibited by phosphoramidon (IC_{50} = 0.8 μM) but not by thiorphan.

Another significant advance in our understanding of ECE was the purification to apparent homogeneity of a phosphoramidon-sensitive activity from rat lung microsomes (Takahashi et al., 1993b). The Triton-solubilized enzyme was sequentially purified over wheat germ agglutinin affinity, zinc chelating, and blue dye agarose columns to achieve an overall enrichment of 6200-fold with an overall yield of 42%. The final preparation exhibited a single major silver-stained band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, under reducing conditions, of 130 kDa. The elution position of the 130-kDa band was correlated with that of ECE activity during chromatography of the purified material on anion exchange and gel filtration columns.

The lung enzyme exhibited properties similar to those of partially purified endothelial cell ECE. Both enzymes were inhibited by phosphoramidon, ethylenediamine tetraacetic acid, and *o*-phenanthroline but not by thiorphan or captopril. Both cleaved big ET-1 in a specific fashion at Trp^{21}-Val^{22}. Both exhibited fairly sharp pH optima between 6.6 and 6.8, and neither preparation degraded ET-1 itself.

C. Membrane Metalloendopeptidase I (EC 3.4.24.11)

This enzyme, more commonly known as NEP or enkephalinase, plays an important role in vivo in the catabolism of a number of biologically active peptides, including atrial natriuretic peptide, enkephalins, bradykinin, and substance P (Erdos and Skidgel, 1989). MMP I is expressed on the external surface of the plasma membrane of a variety of tissues and cell types and is particularly abundant in the kidney (Turner, 1987). This zinc metalloendopeptidase, identical with common acute lymphocytic leukemia antigen, a cell surface antigen expressed on B-lymphocytes (Letarte et al., 1988), has been shown to efficiently cleave mature ET-1, rendering the peptide biologically inactive (Sokolovsky et al., 1990; Vijaraghavan et al., 1990; Fagny et al., 1991). MMP I may be an important ET-1-degrading enzyme in vivo, because SQ-29,072, a potent inhibitor of this enzyme, increased urinary excretion and plasma levels of ET-1 in rats (Abassi et al., 1992). The enzyme is potently inhibited by phosphoramidon, thiorphan, and kelatorphan.

Because others had shown that phosphoramidon and to a lesser extent thiorphan could inhibit the pressor effects of exogenous big ET-1 (McMahon et al., 1991a,b), it seemed possible that MMP I might also have ECE activity. When 3 μM human big ET-1 was incubated with purified rabbit kidney MMP I, a time-dependent accumulation of ET-1 was observed (Patterson et al., unpublished observations, 1991). However, the concentration of ET-1 quickly reached a steady state at approximately 30 nM, whereas degradation products of ET-1 continued to accumulate, probably reflecting a greater catalytic efficiency of MMP I in the ET-1-degradative reaction versus the ECE reaction. Whether MMP I plays any role in vivo in the conversion of big ET to ET remains to be determined. However, it is interesting to note that a potent inhibitor, SQ-28,603, inhibited the hemodynamic responses to exogenous big ET-1, but not to ET-1, in the rat (Gardiner et al., 1992). This effect might have been caused by inhibition of MMP I itself or by an MMP I-like ECE activity.

VI. Endothelin Receptors

A. Pharmacological Studies Suggesting the Presence of Multiple Receptor Subtypes

Shortly after the cloning and characterization of the three ET isoforms, it became obvious that saturable, high-affinity-binding sites for these peptides exist on cell membranes. It was presumed that, like other bioactive peptides, ETs produced their physiological effects through binding to these putative receptor sites. Many studies further suggested that the many diverse physiological and pharmacological effects of ETs could not be mediated by a single receptor subtype. Prior to the discovery of specific ET receptor antagonists, receptor subtype classifications were made on the basis of rank order potencies of either binding or function.

Early studies of the effects of administration of exogenous ET to anesthetized animals revealed a biphasic systemic blood pressure response: a rapid transient decrease, followed by a slowly developing, sustained increase (Spokes et al., 1989). The structure-activity relationships for these two phases were distinct. For the hypotensive phase, ET-1 = ET-2 = ET-3, whereas for the hypertensive phase, ET-1 > ET-2 > ET-3. In vitro correlates of these in vivo effects of different ET isoforms were found by studying isolated rings of rat thoracic aorta with the endothelium either intact or removed (Takayanagi et al., 1991). In the intact rings, ETs invoked slowly developing constriction, ET-1 having greater potency than other ET-related peptides. In rings precontracted with phenylephrine, however, ET-1 was equipotent as other ET-related peptides in causing relaxation. ET-1-selective binding activity was found on membranes prepared from the medial (smooth muscle) layer, whereas membranes from the scraped endothelial layer showed equivalent binding activity for all ET-related peptides tested. Data such as these led to the general concept that vasoconstricting, ET-1-prefering receptors

were located on vascular smooth muscle, whereas vasodilating, ET isotype-nonselective receptors resided in the endothelial cell.

Studies of other smooth muscle preparations showed that this concept was too simplistic, however. In the guinea pig pulmonary artery, for example, ET-3 was a much less potent contractile agent than ET-1 or ET-2, whereas in the trachea the three isoforms were equipotent in promoting contraction (Cardell et al., 1992). These results suggested that different receptors mediated the contractile effects of ET in these tissues. In another study, several different preparations of smooth muscle were stimulated with ETs, STX-b or the COOH-terminal hexapeptide of human ET-1 (Maggi et al., 1989a,b,c,d). In some smooth muscle preparations (e.g., rat thoracic aorta, guinea pig ileum, human urinary bladder), the hexapeptide showed little contractile activity, and the ET receptors were termed ET_A, whereas in other tissues (e.g., guinea pig bronchus, rat vas deferens, rabbit pulmonary artery), the hexapeptide was shown to be a full agonist and the receptors were termed ET_B. Although subsequent studies have shown that the COOH-terminal hexapeptide exhibits very low receptor-binding potency relative to mature ET peptides (Doherty, 1992), the original receptor nomenclature has become universally accepted. (For further details, see section VIII.)

The development of novel ET receptor agonists and antagonists (table 2) provided tools for the identification of multiple ET receptor subtypes in various tissues and has enhanced our understanding of the coupling of receptor activation to physiological responses. STXs containing Glu^9 (STX-c and [Glu^9]STX-b), for example, are potent, highly selective agonists of ET_B receptors (Takayanagi et al., 1991; Williams et al., 1991b). In the isolated rat thoracic aorta, [Glu^9]STX-b evoked endothelium-dependent relaxation of rings precontracted with phenylephrine but, unlike ET-1, did not itself contract endothelium-denuded preparations. In the same study, binding of ^{125}I-[Glu^9]STX-b was limited to endothelium, whereas binding of labeled ET-1 was observed in both endothelium and smooth muscle. Data such as these provided strong evidence that activation of ET_B receptors on endothelial cells causes release of EDRF, whereas activation of ET_A receptors on smooth muscle triggers contraction (see also figs. 8 to 10).

In addition to the clear evidence that there are different ET receptor subtypes differing in their ligand selectivity, recent studies suggest that receptors with similar ligand selectivities, but different ligand affinities, may coexist within the same tissue (Sokolovsky et al., 1992). At present it is not known whether separate genes encode receptors with either high (K_d = nM) or "super high" (K_d = pM) affinities or whether posttranslational modifications of ET receptors can control ligand affinity.

The existence of a third subtype of ET receptor, with a proposed rank order of affinity of ET-3 > ET-1 (i.e., selective for ET-3), has remained controversial. Evidence in favor of a third receptor is derived largely from pharmacological data concerning the relative potency of different ET isoforms in provoking a biological response (Kloog et al., 1989). ET-3 was shown to be the most potent isoform tested in the inhibition of prolactin secretion from pituitary lactotrophs (Samson et al., 1991a,b,c). In cultured bovine carotid artery endothelial cells, ET-1 failed to compete with the binding of 0.25 pM ^{125}I-ET-3 to cell surfaces or to evoke increases in intracellular calcium, whereas ET-3 was effective in both cases (Emori et al., 1990). The conclusions from the latter study were disputed by other investigators who claimed that, in ageing endothelial cell cultures, ET_B receptors were converted to ET-3-preferring receptors (Takayanagi et al., 1991). ET-3 also appeared to be more potent than ET-1 in the activation of PLC in astrocytes (Frelin et al., 1991)

B. Molecular Cloning and Characterization of Endothelin Receptors

Initial progress in the isolation and purification of ET receptor proteins was relatively slow (see below). However, application of direct expression-cloning techniques yielded, by the end of 1990, cDNA for A-type and B-type receptors. Even today, much of what we know about these receptors as molecular entities derives from the deduced amino acid sequences and the molecular probes that these cDNAs provided. Recently, a third receptor conforming generally to the definition of a C subtype (ET-3 selective) has been cloned in *Xenopus laevis*. To date no evidence has been presented that a mammalian homolog of this receptor exists, however.

1. Cloning of endothelin type A and B receptor complementary deoxyribonucleic acids. The original report of the successful cloning of ET_A receptor cDNA (Arai et al., 1990) appeared simultaneously with that of the cloning of ET_B receptor cDNA (Sakurai et al., 1990). Because no protein sequence information was available, expression-cloning techniques were used to isolate these cDNAs. For the ET_A study, a bovine lung cDNA library was expressed in *Xenopus* oocytes and screened with a voltage clamp method, whereas in the ET_B study, a rat lung cDNA library was screened in COS-7 cells, which do not normally express measurable levels of specific, high-affinity binding of ET. The bovine lung ET_A cDNA contained an open reading frame encoding a 427-amino acid protein (Arai et al., 1990), and in the rat lung study (Sakurai et al., 1990), a 415-amino acid protein sequence was predicted. The bovine receptor cDNA, transfected into COS-7 cells, encoded a high-affinity ET-binding protein whose isoform selectivity was ET-1 ≥ ET-2 > ET-3 and, to conform with the previous pharmacological characterization, was called ET_A. The rat lung cDNA was shown to encode a 415-amino acid protein whose binding specificity was ET-1 = ET-2 = ET-3 and thus

TABLE 2
Structure and activities of ET receptor agonists and antagonists

Compound	Type	Structure	IC_{50} ET_A	IC_{50} ET_B	Reference
ET_A-selective					
BQ-123	Antagonist	cyclo (-D-Trp-D-Asp-Pro-D-Val-Leu)	22 nM	18 μM	Ishikawa et al., 1988
FR-139317	Antagonist	2(*R*)-[2(*R*)-[2(*S*)-[[1-(hexahydro-1H-azepinyl)]carbonyl]amino-4-methylpentanoyl]amino-3-[1-methyl-1H-indolyl)]proprionyl]amino-3-(2-pyridyl)propionic acid	1 nM	7 μM	Aramori et al., 1993
50-235	Antagonist	27-0-caffeoyl myricerone	78 nM	>1 μM	Fujimoto et al., 1992
ET_B-selective					
ET-3	Agonist	C-T-C-F-T-Y-K-D-K-E-C-V-Y-Y C-H-L-D-I-I-W	4.5 nM	70 pM	Saeki et al., 1991
STX-c	Agonist	C-T-C-N-D-M-T-A-E-E-C-L-N-F-C-H-Q-D-V-I-W	4.5 μM	20 pM	Williams et al., 1991a,b
4Ala-ET-1 (10–21)	Agonist	E-A-V-Y-F-A-H-L-D-I-I-W	9.9 μM	12 nM	Saeki et al., 1991
BQ-3020	Agonist	Ac-L-M-D-K-E-A-V-Y-F-A-H-L-D-I-I-W	940 nM	200 pM	Ihara et al., 1992a,b
IRL-1620	Agonist	Suc-D-E-E-A-V-Y-F-A-H-L-D-I-I-W	16 pM	200 pM	Ihara et al., 1992a,b
IRL-1038	Antagonist	C-V-Y-F-C-H-D-L-I-I-W	9 nM	700 nM	Urade et al., 1992
Nonselective					
ET-1	Agonist	C-S-C-S-S-L-M-D-K-E-C-V-Y-F-C-H-L-D-I-I-W	160 pM	110 pM	Saeki et al., 1991
Thr^{18}γ-methyl-[Leu^{19}]-ET-1	Antagonist	C-S-C-S-S-L-M-D-K-E-C-V-Y-F-C-H-L-T-γ-methyl-Leu-I-W	0.7 nM	0.25 nM	Shimamoto et al., 1993
PD-142893	Antagonist	Ac-D-diphenylalanine-L-D-I-I-W	15 nM	150 nM	Cody et al., 1992a,b
Ro 46-2005	Antagonist	4-t-butyl-N-[6-(2-hydroxy-ethyoxy)-5-(3-methoxy-phenoxy)-4 pyrimidinyl-benzenesulfonamide	220 nM	1 μM	Clozel et al., 1993a,b

fulfilled the pharmacological criteria of ET_B. Subsequently, rat A10 VSMC ET_A cDNA was identified by expression cloning using an elegant photoemulsion technique (Lin et al., 1991), and porcine cerebellar ET_B cDNA was cloned by screening pools of transfected COS-7 cells for high-affinity ET-1 binding (Elshourbagy et al., 1992).

Homologs of these cloned receptors have been isolated from other species and tissues by screening cDNA libraries with oligonucleotide probes designed around sequences identified from the expression-cloned ET receptors (for review, see Sokolovsky, 1992a,b). Based on the nucleotide sequences of these cloned ET receptor cDNAs, considerable homologies exist between ET_A and ET_B in a given species (approximately 50% identity at the amino acid level; Arai et al., 1993) and between individual subtypes across mammalian species (approximately 85% for human vs. bovine ET_B, for example; Haendler et al., 1992). The mature receptor proteins predicted from these cDNAs all have molecular weights in the range of 45,000 to 50,000 Da. Thus, there is no evidence that ET receptor transcripts exist that encode proteins with molecular weights in the 30,000 to 35,000 Da range, as some membrane cross-linking studies might have suggested. Instead, these smaller ET receptor forms appear to arise from discrete proteolytic cleavage of the mature ET receptor within the NH_2-terminal extracellular domain (Elshourbagy et al., 1992; Saito et al., 1991).

2. Cloning of endothelin type C receptor complementary deoxyribonucleic acid. To date, no ET-3-selective receptor subtype has been cloned by screening cDNA libraries prepared from mammalian cells with probes derived from either ET_A or ET_B sequences. Furthermore, genomic Southern analysis of human DNA probed with gene fragments derived from ET_A or ET_B failed to provide evidence that other closely related genes exist (Haendler et al., 1992; Sakamoto et al., 1991).

Recently (Karne et al., 1993), a cDNA was cloned from dermal melanophores of the frog *X. laevis* that encoded a receptor displaying some of the pharmacological attributes of ET_C. In the melanophore, ET-3 was known to stimulate pigment dispersion >400-fold more potently

than either ET-1 or ET-2, thus making this cell a good candidate for ET_C expression. The key step in the cloning strategy was the use of polymerase chain reaction to amplify a fragment from total melanophore mRNA using degenerate oligonucleotide primers derived from the most highly conserved region of several members of the G-protein-coupled receptor superfamily (transmembrane regions III to IV). The cloned cDNA encoded a protein predicted to have the seven-transmembrane domain structure typical of members of this superfamily, including ET_A and ET_B.

In radioligand-binding studies, neither the wild-type receptor in dermal melanophores nor the cloned ET_C receptor expressed in HeLa cells exhibited the dramatic ET-3 selectivity seen in the biological response of pigment dispersion. In fact, ET-1 was only 4-fold less potent than ET-3 in competing for ^{125}I-ET-3 binding to the ET_C receptor. This finding suggests that very subtle conformational parameters may control the ability of the ligand-receptor complex to activate the signal transduction apparatus. Whether the cloning of this amphibian ET_C-like receptor leads to the identification of mammalian homologs remains to be determined.

3. Structural predictions from cloned endothelin receptor complementary deoxyribonucleic acids. Hydropathicity analyses of the deduced amino acid sequences of ET_A, ET_B, and the recently identified amphibian ET_C receptors revealed a series of seven 20 to 27 residue stretches containing considerable hydrophobic character (Arai et al., 1990; Sakurai et al., 1990; Karne et al., 1993). Sequence homology studies readily identified these proteins as members of the rhodopsin superfamily of transmembrane, G-protein-coupled receptors (Birnbaumer et al., 1990). By analogy, the ET receptors A, B, and C are predicted to have an extracellular NH_2-terminal domain, a seven-transmembrane-spanning domain, and an intracellular COOH-terminal domain (fig. 5).

The NH_2-terminal domains of these receptors are 75 to 100 residues long, considerably larger than the adrenergic receptors, for example, as is typical of the peptide receptors of this class (Nagayama et al., 1991). All three ET receptor subtype cDNAs contain putative NH_2-terminal hydrophobic signal sequences. This property is not shared with the adrenergic receptors and seems to be confined to receptors with long NH_2-terminal extracellular domains (Saudou et al., 1990). Consensus sequences for N-linked glycosylation, Asn-X-Ser/Thr, (Hubbard and Ivatt, 1981) are present in all cloned ET receptor cDNAs. In fact, indirect evidence exists that ET_A present on the surface of Swiss 3T3 cells is a glycoprotein (Devesly et al., 1991). However, to date no reports of the carbohydrate content of a purified ET receptor have appeared.

The transmembrane domains of the ET receptors consist of seven amphipathic membrane-spanning helices joined together by three intracellular and three extracellular loops (fig. 5). The sequences of the transmembrane helices, in particular, are highly conserved between members of the superfamily. In particular, the Asp-Arg-Tyr motif in the second intracellular loop at the end of the third transmembrane helix of all three ET receptors is apparently involved in G-protein coupling (Franke et al., 1990).

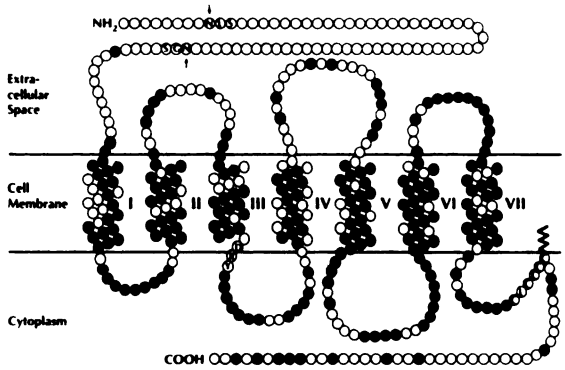

FIG. 5. Primary structure of the human ET_A receptor. The receptor is proposed to consist of an extracellular NH_2-terminal region, seven transmembrane helices (Roman numerals) separated by three extracellular and three cytoplasmic loops, and a cytoplasmic COOH-terminal region. ●, residues that are conserved in the human ET_B receptor sequence. Also shown are two potential N-linked glycosylation sites in the NH_2-terminal region (arrows), the conserved sequence Asp-Arg-Tyr (D-R-Y) at the end of transmembrane helix III, which may be important in G-protein coupling, and the sequence Cys-Leu-Cys-Cys-Cys-Cys (C-L-C-C-C-C) in the COOH-terminal region believed to direct the myristylation of a cysteine residue, which may be important in stabilization of the extracellular ligand-binding site. See text for details.

The COOH-terminal intracellular domain of the ET receptor subtypes shows considerable sequence diversity. One common motif is the sequence Cys-Leu-Cys-Cys-X-Cys that occurs shortly after the end of the seventh transmembrane helix. By analogy to the human β_2-adrenoceptor (O'Dowd et al., 1989), this sequence may be a site for membrane anchoring via palmitoylation. There are several serine residues near the COOH terminus that might serve as phosphorylation sites, although no data have been published that directly support or refute the role of phosphorylation of the receptor.

4. Sequence homology among endothelin receptors. Among the cloned members of a single ET receptor subtype, there is a high degree of sequence identity at the amino acid level (approximately 90%). However, there is considerably less identity between receptor subtypes. As reported by Karne et al. (1993), the optimal alignment of the sequences of rat lung ET_A (Arai et al., 1990), bovine lung ET_B (Sakurai et al., 1990), and *X. laevis* ET_C reveals that each receptor is only about 50% identical with the others. The greatest diversity occurs in the NH_2-terminal extracellular domain, in the extracellular loops, and in the COOH-terminal cytoplasmic domain.

5. Identification of ligand-binding sites on endothelin

receptors. The role of the NH_2-terminal extracellular domain of the ET receptors in ligand binding is unclear. Binding of ET-1 to the ET_A receptor in Swiss 3T3 cells was inhibited by pretreatment of the cells with the lectin wheat germ agglutinin (Devesly et al., 1991). However, the finding that binding to the receptor was not inhibited by treatment of the cells with endoglycosidases suggested that the inhibition by wheat germ agglutinin was strictly steric in nature and not indicative of a role for the (putative) carbohydrate residues in ligand binding. In a study on the rat cerebellar ET_B receptor, a different conclusion was reached (Bousso-Mittler et al., 1991). These workers reported that sialidase treatment of the membranes led to decreased ET-1 binding. What is clear is that glycosylation of human ET_B is not absolutely essential for binding, because *Escherichia coli*, expressing the transfected receptor, demonstrated high-affinity ET cell surface binding (Haendler et al., 1993).

Akiyama (1992) purified the full-length human placental ET_B receptor, as well as a proteolytic fragment lacking essentially the entire NH_2-terminal extracellular domain. This truncated ET_B receptor was equivalent to the full-length receptor in its ability to bind ET-1. Similar results were reported for the purified bovine lung ET_B receptor (Kozuka et al., 1991). However, deletion of 51 amino acids at the NH_2 terminus of the human placental ET_A receptor expressed in COS-7 cells resulted in the complete loss of binding activity, although a mutant receptor lacking only the first 25 NH_2-terminal residues (and expressing one of the two potential N-linked glycosylation sites) retained normal binding activity (Hashido et al., 1992). Finally, chimeric human ET_A and ET_B receptors in which the NH_2-terminal extracellular domains were swapped showed no dramatic alterations in ligand-binding activity (Sakamoto et al., 1993).

A human placental ET_A receptor mutant in which the entire COOH-terminal intracellular domain was deleted was expressed in COS-7 cells. Immunostaining, using specific monoclonal antibodies, demonstrated that the mutant receptor was present in the cell membrane. However, no specific ET-1 binding to this truncated ET_A receptor could be detected. Therefore, the COOH-terminal portion of ET_A may play a significant role in anchoring the receptor properly within the lipid bilayer to maintain the extracellular ligand-binding site.

Chimeric ET receptors represent the state-of-the-art in discovering the function of different domain structures in the binding of agonists and antagonists. The close structural similarities between the ET_A and ET_B receptor subtypes is evidenced by the fact that such chimeras continue to bind ET-1 and couple to signal transduction pathways with activities equivalent to the wild-type receptors. The structural basis for the selective affinity of BQ-123 (table 2) for the ET_A receptor was investigated in a series of receptor chimeras between ET_A and ET_B expressed in Chinese hamster ovary cells (Adachi et al., 1992). The results suggested that the first extracellular loop contains an important binding determinant for high-affinity binding of BQ-123. An essentially similar conclusion was reached in a more recent study. Using receptor chimeras to explore the structural basis of the ability of NH_2-terminally truncated, linear analogs of ET-1 (BQ-2030 and IRL-1620; see table 2), Sakamoto et al. (1993) found that they function as potent, ET_B-selective agonists. The results pointed to the permissive role of transmembrane domains 4 to 6 of ET_B (with adjacent intracellular and extracellular loops) in high-affinity binding of ET_B-selective agonists. In fact, the chimera, containing this region of ET_B substituted for the corresponding region of ET_A, exhibited high affinity for both BQ-123 and the ET_B agonists (Saeki et al., 1991).

Further studies using genetically engineered mutants can be expected to enhance our understanding of the precise structural features that produce the unique ligand selectivities of the ET receptors.

6. Endothelin receptor gene structure. The human ET_A (Hosoda et al., 1992) and ET_B (Arai et al., 1993) receptor genomic DNAs have been cloned and characterized. Southern analysis revealed a single copy of each gene in the human genome. As is typical for members of the G-protein-coupled receptor superfamily, the genes for ET_A and ET_B are large, spanning 40 and 24 kb of DNA, respectively. Human ET_A gene, present on chromosome 4, contains eight exons and seven introns, and ET_B gene, present on chromosome 13, comprises 7 exons and 6 introns. The major overall structural difference between these two genes is the presence of an extra intron in the 5′-noncoding region of ET_A. Otherwise, the exon-intron splice sites are conserved between the two receptors, suggesting their evolution from a common ancestor gene. Introns 2 to 7 of ET_A (1 to 6 of ET_B) occur within the coding region immediately preceding or following one of the transmembrane helix domains, suggesting that the corresponding exons may encode functional units. The 5′-flanking regions of both genes lack consensus TATAA or CAAT boxes but contain putative Sp1-binding sites, GATA-binding motifs, and sequences that in other genes interact with members of the basic helix-loop-helix transcription factor family, such as MyoD, a muscle gene-regulating protein. The ET receptor gene 5′-flanking regions also contain acute phase regulatory element-binding motifs, thought to play a role in gene induction under acute stress in vivo. Despite these gross similarities in the ET_A and ET_B gene 5′-flanking regions, the tissue-specific patterns of expression of these two genes are very different.

7. Tissue-specific expression of endothelin receptors. With the cloning of ET receptors, and the identification of receptor subtype-specific ligands, powerful tools became available with which to characterize tissue-specific patterns of expression.

A variety of different techniques and approaches have been used to localize ET receptors. Electron microscopy coupled with autoradiography was used to visualize ^{125}I-ET-1-binding sites within the kidney and lung of rats in which labeled peptide was intravenously injected (Furuya et al., 1992). In the kidney, silver grains were localized primarily to the fenestrated endothelial cells of glomeruli and to peritubular endothelial cells, whereas few grains were observed on the arteries and large arterioles. This is a gross method, however, and does not give information about the receptor subtype of these binding sites. More precise information has been gained through the use of reverse transcriptase/polymerase chain reaction along microdissected segments of the rat nephron (Terada et al., 1992). The results suggested that ET_B receptors predominate in the collecting duct system, and ET_A was prominent in the vasa recta bundle and in arcuate arteries. Both receptor subtypes were significantly detected in the glomeruli.

8. Regulation of endothelin receptor expression. ET receptors, like many other peptide cell surface receptors, are subject to ligand-induced downregulation. As early as 1988, studies of VSMCs demonstrated that ET-1 pretreatment caused a substantial decrease in ^{125}I-ET-1-binding sites and at the same time attenuated the ability of ET-1 to increase intracellular calcium (Hirata et al., 1988). Similar observations have also been made in Swiss 3T3 cells (Devesly et al., 1991), hepatocytes (Gandhi et al., 1992a), liver Kupffer cells (Gandhi et al., 1992b), cardiocytes (Hirata et al., 1989a), osteosarcoma cells (Sakurai et al., 1992b), astrocytes (Ehrenreich et al., 1993a), and mesangial cells (Thomas et al., 1991). Homologous downregulation of ET-1-binding sites in smooth muscle occurred within 30 minutes of ET-1 exposure and persisted for up to 18 hours (Roubert et al., 1990; Miasiro and Paiva, 1990b). In Swiss 3T3 cells, known to express ET_A receptors exclusively (Devesly et al., 1990), this receptor underwent continuous turnover in the absence of ligand, whereas exposure of cells to ligand increased the rate of receptor turnover (Devesly et al., unpublished observation). In ROS osteosarcoma cells, mRNA for the ET_B receptor was transiently decreased between 2 and 4 hours by incubation of cells with either ET-1 or ET-3. The level of ET_B mRNA then returned to control values by 24 hours, even in the continued presence of ligand. Because the ET_B mRNA exhibited a half-life of at least several hours in these cells, it was suggested that ligand-induced downregulation of the message might be due to increased mRNA degradation (Sakurai et al., 1992b). The sensitivity of cell surface ET receptors to ligand-induced downregulation may have therapeutic implications. For example, in mesangial cells (ET_A) or endothelial cells (ET_B), treatment with the ECE inhibitor phosphoramidon decreased ET release but increased receptor-mediated binding of exogenous ligand and potentiated the ability of ET-1 to release arachidonic acid in mesangial cells (Clozel et al., 1993b).

Other factors have been shown to downregulate cell surface ET receptors. In human VSMCs, activation of PKC by phorbol esters led to a rapid and sustained 50% decrease in ET-1-binding sites as well as ET-induced production of inositol phospholipid second messengers (Resink et al., 1990c). In calf adrenal zona glomerulosa cells, phorbol ester-mediated ET receptor downregulation was inhibited by PKC inhibitors, whereas ET-induced receptor downregulation was unaffected. These results suggested that autologous downregulation of ET receptors was not a PKC-dependent process (Cozza et al., 1990).

9. Isolation of endothelin receptor proteins. Prior to the expression cloning of ET_A and ET_B receptors, a considerable body of work characterizing ET-binding sites on membranes had been reported (for review, see Sokolovsky, 1992a,b). Affinity-labeling studies using bifunctional cross-linking reagents identified major bands with molecular weights ranging from about 30 to >70 kDa. Often, multiple minor bands were also observed. An affinity-labeled protein of M_r 40 kDa was isolated from human placenta but not extensively characterized (Wada et al., 1990). At approximately the same time, a report appeared concerning the successful solubilization, using 3-[(cholamidopropyl)dimethylammonio]-1-propanesulfonic acid and digitonin, of human placental ET receptor activity in a ligand-binding-competent form (Nakajo et al., 1990). The apparent molecular weight of the receptor complex in micellar detergent was estimated to be 340,000 Da, suggesting that the receptor protein was present in an aggregated state. Later, when conditions for the solubilization of bovine lung ET receptor in a nonaggregated, ligand-binding-competent state were devised, affinity labeling of the solubilized material yielded two bands of 34 and 52 kDa (Hagiwara et al., 1991). Protease inhibitor experiments suggested that the smaller band was a limit degradation product of the larger species. Incubation of the solubilized proteins with biotinylated ET-1, followed by avidin affinity chromatography, yielded a purified preparation of the 34-kDa species. Microsequencing of two tryptic peptides gave fragmentary structural data about what was later shown to be bovine lung ET_B. It is likely that such an approach would have soon yielded an ET receptor cDNA had not the expression-cloning efforts described above been successful. Later, the same group succeeded in isolating the 52-kDa form of the bovine lung ET_B receptor and showed that the smaller form was generated by a specific metalloprotease-catalyzed cleavage of the intact receptor in the NH_2-terminal extracellular domain (Kozuka et al., 1991). Interestingly, this biotin affinity purification scheme has not been successfully used to purify the ET_A receptor protein. Apparently, biotinylated ET-1 can bind to ET_A with high affinity, but the ligand-receptor com-

plex is too unstable to survive the avidin affinity chromatography step (Takasuka et al., 1992).

10. Structure-activity relationship of endothelin receptor agonists and antagonists. Because all known actions of the ETs and STXs are mediated by binding to high-affinity receptors, the structure-activity relationships among the members of this family are primarily defined by two parameters: (*a*) relative affinity in binding to and discriminating between the various ET receptor subtypes and (*b*) the ability of the ligand-receptor complexes to couple to the appropriate signal transduction mechanism(s). A large number of studies have addressed these issues both in vitro and in vivo (for review, see Sokolovsky, 1992a,b; Erhardt, 1992).

All of the known naturally occurring members of the ET family of peptides share a high degree of homology, both at the level of amino acid sequence and (probably) structurally (fig. 2). In particular, the disulfide-bonding patterns are invariant among these peptides, and the COOH-terminal hexapeptide sequences are nearly identical. Both of these features are functionally relevant. It is known that either reduction of the disulfide bonds or scrambling of the bonds leads to peptides that are less active in promoting vasoconstriction via activation of ET_A receptors (Randall et al., 1989; Kitazumi et al., 1990). In addition, deletions from or additions to either the NH_2- or COOH terminus of ET-1 vastly reduce or abolish the ability of these peptides to interact with ET_A. Single amino acid substitutions in ET-1 have established that Asp^8, Glu^{10}, and Phe^{14} are critically important residues for vasoconstrictor activity.

Different structural constraints govern interactions with ET_B receptors (table 2). Linear ET analogs, such as [$Ala^{1,3,11,15}$]ET-1, are potent in the mediation of systemic vasodilation (via ET_B), although they lack the sustained vasoconstricting properties (via ET_A) of the native peptide (Bigaud and Pelton, 1992). Even NH_2-terminal truncations of these linear ET analogs [e.g., N-acetyl-4Ala ET-1 (10 to 21)] remain potent agonists of the ET_B receptor but lack ET_A-binding activity (Erhardt, 1992). Thus, the generalization can be made that ET_A binding and activation require both the highly ordered helical core region and the linear COOH-terminal domain present in the native ETs, whereas binding and activation of ET_B needs only the latter structural feature.

Further refinement of this general model has begun through the design and chemical synthesis of analogs of the known ET receptor-specific antagonists. The most intensively studied antagonist is the ET_A-selective cyclic pentapeptide BQ-123. This potent antagonist was designed based on the structure of a naturally occurring component of a bacterial fermentation broth (Ihara et al., 1991a, 1992a,b). A series of amino acid replacements of BQ-123 were then synthesized and tested, and several analogs were identified that had moderately increased selectivity toward ET_A (Ishikawa et al., 1988). Several NMR studies on the solution conformation of BQ-123 have been reported (Atkinson and Pelton, 1992). Although the cyclical nature and small size of the peptide have allowed a good picture of the antagonist to emerge, it is not clear exactly which epitope or epitopes of the ET-1 are modeled by BQ-123.

The development of linear hexapeptide antagonists, modeled after the COOH terminus of ET-1, which retain submicromolar binding potency to both ET_A and ET_B receptors (Cody et al., 1992b), has provided another promising avenue for exploration of the structure-activity relationships of these receptors.

Most recently, random screening of a large chemical library resulted in the identification of a novel class of pyrimidinyl sulfonamides with weak ET receptor antagonist activity. Optimization of this template yielded a moderately potent, orally active, ET_A/ET_B receptor antagonist, Ro 46–2005 (Clozel et al., 1993a) (table 2). This promising lead compound has already shown efficacy in animal models of postischemic renal vasoconstriction and cerebral ischemia following subarachnoid hemorrhage (Clozel et al., 1993a).

VII. Signal Transduction Mechanisms

Among the many biological actions of ETs observed so far, the vasoconstrictor property of ET-1 was the first to be recognized and most widely studied. Similarly, the signal transduction mechanisms triggered by binding of ET-1 to ET_A receptors in vascular smooth muscle are the most extensively analyzed and best understood so far.

Signal transduction pathways stimulated by ETs in other biological systems are only briefly discussed here. They will be mentioned in more detail in the sections describing the actions of ETs in a particular biological system (see section VIII).

A. Signal Transduction Pathways Mediating Short-Term Changes in Cell Function

ET-1-induced contractions of isolated blood vessels are more slowly developing, are maintained for a longer time, and are more resistant to agonist removal than are contractions evoked by most other vasoconstrictor agents. It is generally accepted that, in most vascular smooth muscle preparations, ET-1 interacts with a specific cell surface receptor, the ET_A receptor subtype. In some vascular beds, ET_B receptor subtype is also involved in smooth muscle contraction (for details see section VIII.A.3). Several receptor signal transduction mechanisms were suggested to be involved in ET-1-induced vascular contraction, and include (*a*) increase in cytosolic free calcium concentration ($[Ca^{2+}]_i$) by facilitation of Ca^{2+} influx and mobilization of intracellular Ca^{2+}; (*b*) G-protein-mediated activation of PLC, leading to phosphatidyl inositol hydrolysis and rapid formation of IP_3 and sustained DAG accumulation; (*c*) activation of PKC; (*d*) activation of phospholipase A_2 and D and arachidonic

acid metabolism; and (*e*) changes in intracellular pH (alkalinization) via stimulation of Na^+-H^+ exchange (fig. 6).

1. Increase of cytosolic calcium concentration. Similar to several other vasoconstrictor agonists, ET-1-induced vascular contraction is accompanied by an increase in $[Ca^{2+}]_i$ which, in most preparations, consists of two components: (*a*) a rapid initial transient phase, which is not dependent on the presence of extracellular Ca^{2+} and is the result of mobilization of Ca^{2+} from intracellular stores (presumably the sarcoplasmic reticulum), and (*b*) a sustained phase, which is dependent on external Ca^{2+} and is the result of transmembrane Ca^{2+} influx.

a. STIMULATION OF INFLUX OF EXTRACELLULAR CALCIUM. The first demonstration of the involvement of extracellular calcium in the vascular smooth muscle contraction produced by the peptidergic EDCF was carried out by Hickey et al. (1985) who found that EDCF-induced coronary artery contraction was attenuated by both removal of Ca^{2+} from the medium and by the addition of the L-type Ca^{2+} channel antagonist verapamil. Because of the structural similarities between the newly discovered ET-1 molecule and α-scorpion toxin and the attenuation of ET-1-induced vascular contraction by dihydropyridine Ca^{2+} channel blockers, Yanagisawa et al. (1988b) postulated that the peptide is an endogenous agonist of the voltage-operated L-type Ca^{2+} channel.

In the past 6 years numerous studies addressed this question utilizing various techniques, including testing the effect of L-type Ca^{2+} channel blockers on ET-1-induced vasoconstriction in vivo and in vitro, ^{45}Ca-uptake measurements in isolated blood vessels and SMCs in culture, measuring changes in $[Ca^{2+}]_i$ by the fura-2 technique in cultured cells and blood vessels, detecting Ca^{2+} conductance through single channels by patch-clamp techniques, studying the Ca^{2+} sensitivity of myofilaments, and analyzing the binding of ET-1 to L-type Ca^{2+} channels and the binding of Ca^{2+} antagonists to ET receptors.

Antagonists of L-type Ca^{2+} channels of different structural classes (e.g., verapamil, diltiazem, and the dihydropyridines nifedipine, nicardipine, nitrendipine, isradipine, etc.) were found to markedly attenuate ET-1-induced vasoconstriction in pig coronary arteries in vivo (Egashira et al., 1990), isolated bovine cerebral arteries (Encabo et al., 1992), isolated rat aorta (Sakata et al., 1989; Godfraind et al., 1989), rat mesenteric resistance arterioles (Luscher et al., 1990; Godfraind et al., 1989), isolated pig coronary arteries (Kasuya et al., 1989a,b; Goto et al., 1989), isolated human pial arteries (Hardebo et al., 1989), isolated guinea pig portal vein (Inoue et al., 1990), rat cremaster arterioles in vivo (Lougee et al., 1990), human foram circulation (Luscher et al., 1990), and perfused rat kidney vasculature (Takenaka et al., 1992). However, several studies found no or only marginal effect of the same Ca^{2+} antagonists on ET-1-induced vasoconstriction in rat mesenteric arteries (Wallnofer et al., 1989), rat aorta (Blackburn and Highsmith, 1990; Chabrier et al., 1989; Turner, 1987), rabbit pulmonary vein (Steffan and Russell, 1990), human internal mammary artery (Luscher, 1992), various rabbit and dog blood vessels (D'Orleans-Juste et al., 1989b), human uterine arteries (Bodelsson et al., 1992), and pig coronary artery (Blackburn and Highsmith, 1990).

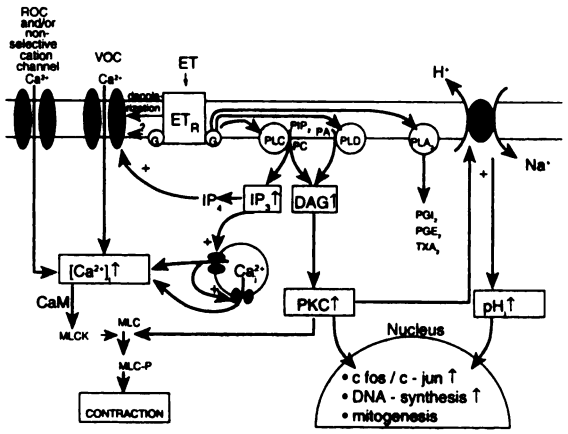

FIG. 6. Signal transduction mechanisms involved in ET-1-induced short-term (e.g., contraction of vascular smooth muscle) and long-term (e.g., mitogenesis) modulation of cell function. For details, see section VII. Abbreviations (see also footnote *): CaM, calmodulin; ET_R, ET receptor; ?, link between ET_R and VOC opening is uncertain; G, G-protein; IP_4, 1,3,4,5-inositol tetrakisphosphate; MLCK, myosin light chain kinase; PA, phosphatidic acid; PC, phosphatidylcholine; PGE_2, prostaglandin E_2; PIP_2, phosphatidylinositol diphosphate; PLA_2, phospholipase A_2; PLD, phospholipase D; ROC, receptor-operated calcium channel.

Similar contradictory observations were made when the effect of L-type Ca^{2+} channel blockers was tested on ET-1-induced $^{45}Ca^{2+}$ uptake or increase in $[Ca^{2+}]_i$ in cultured SMCs. The Ca^{2+} antagonists produced no, or very limited, effect on $^{45}Ca^{2+}$ uptake or the sustained phase of $[Ca^{2+}]_i$ elevation in rat aortic SMCs (Mitsuhashi et al., 1989; Chabrier et al., 1989), in A10 SMCs (Muldoon et al., 1989; Simpson and Ashley, 1989; Takuwa et al., 1991), in rat renal VSMCs (Suzuki et al., 1991c), and in porcine coronary artery SMCs (Wagner Mann et al., 1991; Wagner Mann and Sturek, 1991). Significant inhibition of $^{45}Ca^{2+}$ uptake or the sustained phase of increase in $[Ca^{2+}]_i$ by Ca^{2+} channel blockers was detected in A10 SMCs (Xuan et al., 1989, 1991), rat aortic SMCs in primary culture (Kai et al., 1989), A7r5 SMCs (Huang et al., 1989), rat SMCs (Iijima et al., 1991), and cultured human umbilical artery SMCs (Gardner et al., 1992).

Comparing the effect of ET-1 with the L-type Ca^{2+} channel activator dihydropyridine compound, Bay K 8644, showed similarities (Encabo et al., 1992; Godfraind et al., 1989) but also striking dissimilarities (D'Orleans-Juste et al., 1989a; Hom et al., 1992).

The fact that these results are dependent on the dose of ET-1, the Ca^{2+} antagonist, and the source of the blood

vessel and VSMC used may explain some of the heterogeneous and apparently contradictory observations. Indeed, Sakata and Karaki (1992) reported that, although verapamil strongly inhibited ET-1-induced contractions in the nonpregnant rat uterus, the Ca^{2+} antagonist had little effect in the pregnant uterus.

Several investigators tested the original proposal that ET is an endogenous direct activator of L-type Ca^{2+} channels (Yanagisawa et al., 1988a,b; Goto et al., 1989). All studies, without exception, showed that ET-1 does not compete or displace the binding of several L-type Ca^{2+} channel ligands (Kasuya et al., 1989a,b; Gu et al., 1989b; Hamilton et al., 1989) and that the Ca^{2+} channel antagonists do not interfere with ET-1 binding to blood vessels or isolated SMCs (Clozel et al., 1989; Gardner et al., 1992; Hirata et al., 1988). Therefore, it is certain that ET-1 is not a ligand of L-type Ca^{2+} channels and the observed activation of Ca^{2+} entry via VOC in some preparations must be the consequence of indirect gating of VOC by ET-1.

Studies of single-channel Ca^{2+} currents using the patch-clamp technique confirmed this conclusion. ET-1 does not activate isolated L-type Ca^{2+} channel activity (Naitoh et al., 1990) but increases the activity of an L-channel in cells clamped in the whole cell-attached mode (Inoue et al., 1990; Silberberg et al., 1989; Goto et al., 1989). For example, Silberberg et al. (1989) found that in freshly isolated SMCs from pig coronary artery, studied in the whole cell-attached mode, ET-1 increased Ca^{2+} channel activity without any effect on channel open time or conductance, suggesting that ET-1 acts on these channels via a second-messenger system.

Several indirect mechanisms were proposed for ET-1-induced Ca^{2+} influx via VOCs. The first possibility is that activation of VOC is secondary to ET-1-induced membrane depolarization. Indeed, ET-1 induced sustained depolarization in a variety of smooth muscle preparations including VSMCs (Korbmacher et al., 1989; Lee et al., 1990a; Nakao et al., 1990; Van Renterghem et al., 1988; Wallnofer et al., 1989; Iijima et al., 1991; Takenaka et al., 1992). However, in some preparations the membrane depolarization is very modest and significantly less than that caused by NE or AVP (Kasuya et al., 1989a,b; Wallnofer et al., 1989). The ionic basis of ET-1-induced membrane depolarization is poorly understood. The most convincing indirect mechanism to date is the involvement of activation of Cl^- channels (Iijima et al., 1991; Takenaka et al., 1992; Klockner and Isenbery, 1991). The Cl^- channel antagonist, indanyloxyacetic acid (IAA-94), inhibited the ET-1-induced sustained phase of $[Ca^{2+}]_i$ increase and membrane depolarization in cultured VSMCs (Iijima et al., 1991; Takenaka et al., 1992) and effectively blocked ET-1-induced glomerular afferent arteriolar vasoconstriction in isolated perfused rat kidney (Takenaka et al., 1992). According to this group, ET-1 stimulates intracellular Ca^{2+} mobilization (via IP_3), which activates Ca^{2+}-dependent Cl^--permeable channels, resulting in an efflux of Cl^- and membrane depolarization which, in turn, activates VOCs and causes Ca^{2+} influx.

In addition to activation of Cl^- channels, several other mechanisms have been proposed to explain the ionic basis of ET-1-induced membrane depolarization, including an increase in the intracellular free sodium ion concentration (Nakao et al., 1990; Okada et al., 1991) either via inhibition of Na^+/K^+-ATPase (Meyer Lehnert et al., 1989) or by stimulation of the Na^+/H^+ antiporter (Danthuluri and Brock, 1990).

The findings of Muldoon et al. (1991) that in rat-1 fibroblasts high $[Ca^{2+}]_i$ can inhibit ET-1-induced Ca^{2+} influx may provide some explanation for the observed heterogeneity, because the ET-1-induced initial increase in $[Ca^{2+}]_i$ may be very different at various doses of ET-1 and also in various preparations. Another explanation for the contradictory findings in cultured SMCs relative to ET-1-induced Ca^{2+} influx was provided by Suzuki et al. (1991c), who demonstrated that, in growth-arrested A7r5 SMCs, ET-1 causes only mobilization of intracellular Ca^{2+}, whereas in growth-stimulated A7r5 cells, ET-1 stimulated Ca^{2+} influx without intracellular Ca^{2+} mobilization. ET-1 can also cause membrane hyperpolarization, presumably by enhancing efflux of K^+. In pig coronary artery SMCs, ET-1 stimulated Ca^{2+}-activated K^+ channel activity (Hu et al., 1991).

In addition to membrane depolarization, several second messengers were proposed to be involved in ET-1-induced stimulation of Ca^{2+} uptake into VSMCs. For example, in rat and rabbit VSMCs, PAF antagonists (CV-6209 and WEB-2086) and inhibitors of arachidonic acid metabolism (e.g., indomethacin) significantly inhibited an ET-1-induced increase in $[Ca^{2+}]_i$ (Takayasu et al., 1989), suggesting that PAF or TXA_2 (or another arachidonic acid metabolite) mediates the Ca^{2+} uptake. It was also postulated that a G-protein (maybe G_i) couples the ET receptor to the VOC.

Considerable evidence exists that ET-1 may stimulate Ca^{2+} influx into VSMC via non-dihydropyridine-sensitive Ca^{2+} channels. Several studies demonstrated that at low doses (10^{-9} M) ET-1-induced vascular contraction is predominantly dependent on extracellular Ca^{2+} (i.e., calcium removed from the medium abolishes the response) but L-type Ca^{2+} channel antagonists are not or are only partially effective (Stasch and Kazda, 1989; Wallnofer et al., 1989; Nakamura et al., 1992; Muldoon et al., 1991). Studies demonstrating that Ni^{2+}, which has no effect on L-type Ca^{2+} channels (Blackburn and Highsmith, 1990), La^{3+} (nonspecific cation channel blockers) (Steffan and Russel, 1990), but not L-type Ca^{2+} channel antagonists blocked ET-1-induced contraction or $[Ca^{2+}]_i$ increase, indicating that Ca^{2+} channels other than L-type VOCs (e.g., other VOCs, voltage-insensitive receptor-operated channels or a nonselective cation channel) may be in-

volved in ET-1-induced increased Ca^{2+} influx. Indeed, directly measuring Ca^{2+} conductances in patch-clamped cells, Inoue et al. (1990) found that ET-1 stimulated a nifedipine-insensitive Ca^{2+} channel activity.

Although two studies suggested that ET-1 might activate the Na^+/Ca^{2+} exchange system (Borges et al., 1989a; Criscione et al., 1990), the potential contribution of this ion exchanger in ET-1-induced Ca^{2+} uptake remains speculative.

b. MOBILIZATION OF INTRACELLULAR Ca^{2+}. Numerous studies with cultured SMCs (Bialecki et al., 1989; Danthuluri and Brock, 1990; Gardner et al., 1992; Hirata et al., 1988; Iijima et al., 1991; Kai et al., 1989; Little et al., 1992; Meyer Lehnert et al., 1989; Mitsuhashi et al., 1989; Muldoon et al., 1989; Nakamura et al., 1992; Okishio et al., 1992; Simpson and Ashley, 1989; Suzuki et al., 1991c; Takayasu et al., 1989; Takuwa et al., 1991) and with isolated blood vessels (Pang et al., 1989; Ozaki et al., 1989; Sakata et al., 1989) demonstrated that one of the early cellular events after ET-1 stimulation is a rapid transient increase in $[Ca^{2+}]_i$. This initial transient phase, in contrast to the subsequent sustained elevation of $[Ca^{2+}]_i$, is not sensitive to removal of extracellular Ca^{2+}(Danthuluri and Brock, 1990; Kai et al., 1989; Marsden et al., 1989; Muldoon et al., 1989; Nakamura et al., 1992; Ozaki et al., 1989; Takuwa et al., 1991) or L-type Ca^{2+} channel blockers (Iijima et al., 1991, Kai et al., 1989; Mitsuhashi et al., 1989; Sakata et al., 1989; Simpson and Ashley, 1989; Suzuki et al., 1991c; Takuwa et al., 1991; Wagner Mann et al., 1991) but can be reduced or completely prevented by previous treatment of tissues and smooth cells with caffeine in the absence of extracellular Ca^{2+} (Kai et al., 1989; Wagner Mann et al., 1991) or by ryanodine, a selective inhibitor of Ca^{2+} release from the sarcoplasmic reticulum (Wagner Mann et al., 1991).

Because generation of IP_3 (the intracellular signal molecule that stimulates the release of Ca^{2+} from the sarcoplasmic reticulum and calciosome) is also a very early event in SMCs stimulated by ET-1 (within approximately 10 seconds), it is proposed that ET-1 mobilizes intracellular Ca^{2+} from a store (probably sarcoplasmic reticulum), via IP_3, which overlaps with a caffeine-sensitive store. However, in rat aortic VSMCs in culture, both ET-1 and ET-3 mobilize intracellular Ca^{2+}, but only ET-1 stimulates an increase in IP_3 (Little et al., 1992), suggesting that molecules other than IP_3 (e.g., DAG; see below) may also mediate the ET-induced Ca^{2+} release from intracellular stores. Huang et al. (1993) reported recently that in A7r5 cells a dihydropyridine Ca^{2+} antagonist inhibited both the transient and sustained phases of ET-1-induced increase in $[Ca^{2+}]_i$. Similar results were obtained in human umbilical artery SMC in culture, using the Ca^{2+} channel antagonists verapamil and nicardipine (Gardner et al., 1992), suggesting that influx of extracellular Ca^{2+} may trigger Ca^{2+} release from intracellular stores (Ca^{2+}-induced Ca^{2+} release).

c. SENSITIZATION OF MYOFILAMENTS TO CALCIUM. The ability of ET-1 to cause relatively small but well-maintained vascular contractions in the absence of extracellular Ca^{2+} (Ozaki et al., 1989; Kodama et al., 1989; Ohlstein et al., 1989; Sakata et al., 1989; Wallnofer et al., 1989; Cardell et al., 1990; Huang et al., 1990) and in the absence of a measurable increase in $[Ca^{2+}]_i$ (Ozaki et al., 1989; Huang et al., 1990; Sakata et al., 1989) suggests that, in addition to increased influx of extracellular Ca^{2+} and mobilization of intracellular Ca^{2+}, sensitization of the contractile machinery to Ca^{2+} may also contribute to vasoconstriction under certain conditions. Indeed, in rabbit mesenteric artery strips permeated by *Staphylococcus* L-toxin ET-1 increased myofilament Ca^{2+} sensitivity via a G-protein-dependent pathway probably involving PKC (Nishimura et al., 1992). The probable involvement of PKC activation in ET-1-induced sensitization of contractile proteins to Ca^{2+} was suggested by Sunako et al. (1989) as well.

PKC may phosphorylate contractile proteins. Indeed, ET-1 caused phosphorylation of myosin light chain and caldesmon in pig coronary artery SMCs (Abe et al., 1991) and phosphorylation of myosin heavy chain and caldesmon in pig carotid artery SMC (Adam et al., 1990).

2. Stimulation of phospholipase C and phosphatidylinositol hydrolysis. A wide variety of vasoactive agonists induce contraction of blood vessels by activating PLC and facilitated PI hydrolysis. PLC stimulation catalyzes phosphatidylinositol 4,5-biphosphate breakdown which results in the formation and accumulation of IP_3 and DAG. Numerous reports indicate that ET-1 stimulates PLC-mediated rapid transient IP_3 formation and more sustained DAG generation in vascular smooth muscle.

Soon after the discovery of ET, studies of isolated canine (Pang et al., 1989) and porcine coronary arteries (Kasuya et al., 1989a,b) demonstrated that rapidly after stimulation with ET-1 the tissue level of IP_3 increases in parallel with enhanced $^{45}Ca^{2+}$ uptake and before the onset of vascular contraction. ET-1 stimulates PI hydrolysis in dose-dependent manner, with an EC_{50} similar to that of stimulating $^{45}Ca^{2+}$ uptake and contraction in isolated canine coronary arteries (fig. 7) (Pang et al., 1989). Similar rapid activation of PI hydrolysis has been observed in VSMCs in culture (Van Renterghem et al., 1988; Resink et al., 1988; Araki et al., 1989; Hirata et al., 1988; Huang et al., 1989; Muldoon et al., 1989; Sugiura et al., 1989), in isolated rat (Rapoport et al., 1990) and rabbit aorta (Ohlstein et al., 1989), fibroblasts (Muldoon et al., 1989; Takuwa et al. 1989a,c), glomerular mesangial cells (Simonson et al., 1989; Badr et al., 1989b), and rat atrial myocardial cells (Vigne et al., 1989).

Depending on the activity of kinases and phosphatases in a given target cell, in addition to IP_3, a variety of other inositol phosphates may be found. For example, Simonson et al. (1989) described the formation of inositol tetrakisphosphate in ET-1 stimulated glomerular mes-

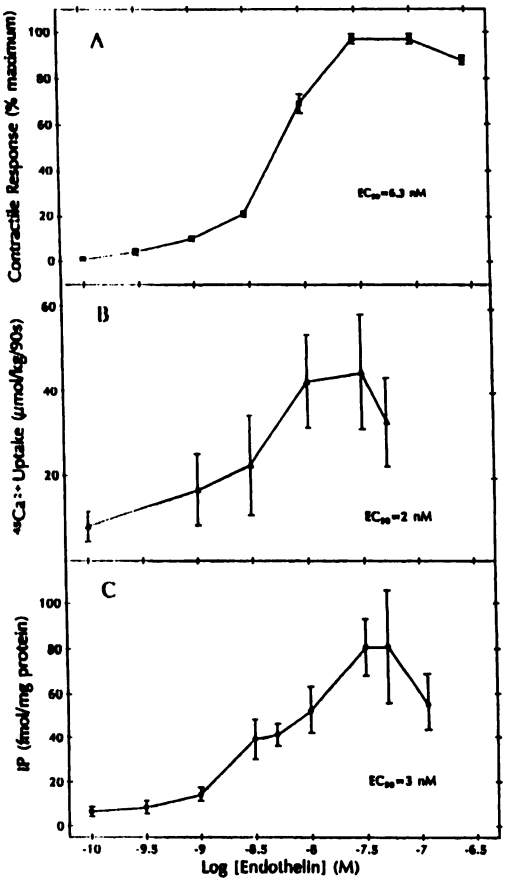

FIG. 7. Concentration-dependent effects of ET-1 on isometric force (A), rate of $^{45}Ca^{2+}$ uptake (B), and [^{3}H]inositol monophosphate (IP) accumulation (C) in canine coronary artery rings (from Pang et al., 1989). Each parameter was tested in three separate rings isolated from the same animal. Data are means ± SEM on seven to 14 rings for seven animals. Reproduced with permission of Raven Press.

angial cells via IP_3-kinase activity, which is a Ca^{2+}-sensitive enzyme.

Consistent with earlier findings with other agonists, ET-1-induced stimulation of PLC and PI hydrolysis in smooth muscle results in a dose-dependent, biphasic increase in DAG that is sustained for 20 minutes or longer (Griendling et al., 1989; Lee et al., 1989a; Muldoon et al., 1989; Sunako et al., 1989, 1990). In addition to PI hydrolysis, the source of DAG may also be hydrolysis of phosphatidylcholine by PLC or phosphatidic acid degraded by phospholipase D. Indeed, ET-1 stimulation resulted in phosphatidylcholine hydrolysis and also activation of phospholipase D in vascular smooth muscle (Resink et al., 1990b). ET-1 stimulates phospholipase D activity (measured as phosphatidylcholine hydrolysis) in rabbit iris sphincter smooth muscle (Zhang et al., 1990), in rat-6 fibroblasts (Pai et al., 1991), in C6 glioma cells and rat-1 and Swiss 3T3 fibroblasts (Ambar and Sokolovsky, 1993), and in rat renal medullary interstitial cells (Friedlander et al., 1993). In rat-6 fibroblasts, which overexpress PKC β1, ET-1 stimulated [^{3}H]thymidine incorporation (Pai et al., 1991). The β-adrenoceptor antagonist propranolol, which is also an inhibitor of phosphatic acid phosphohydrolase, decreased ET-1-induced DAG formation in rat-6 fibroblasts (Pai et al., 1991), suggesting that phosphatidic acid is an important source of DAG formation in this cell type.

The two second messengers of the PI hydrolysis cascade (IP_3 and DAG), formed rapidly after ET-1 stimulation, play important roles in intracellular signaling. IP_3 is responsible for stimulating the release of Ca^{2+} from the sarcoplasmic reticulum and calcisome (Bialecki et al., 1989; Hirata et al., 1989c; Kai et al., 1989; Simpson and Ashley, 1989). Inositol tetrakisphosphate, when formed, acts in synergy with IP_3 to stimulate an increase in $[Ca^{2+}]_i$ in ET-1-stimulated cells, not only by mobilization of Ca^{2+} from intracellular stores but also maybe by enhancing the influx of extracellular Ca^{2+}, the endogenous activator of PKC. Neutral DAG, on the other hand, could mediate ET-1-induced activation of PKC.

3. Activation of protein kinase C. PKC, a Ca^{2+}- and phospholipid-dependent enzyme, can be activated by DAG and tumor-promoting phorbol esters (Nishizuka, 1989). Several studies indicate that activation of this enzyme is involved in ET-1-induced vascular contraction. The slowly developing and long-lasting contractions caused by ET-1 are very similar to those evoked by phorbol esters. Inhibitors of PKC, such as staurosporin, inhibit ET-1-induced contractions. In rat aortic VSMCs, ET-1 and phorbol-12-myristate-13-acetate (but not ionomycin) phosphorylated the same acidic 76-kDa protein, which is a PKC-specific substrate (Griendling et al., 1989). ET-1 also facilitated translocation of PKC from the cytosol to the membrane in cultured bovine aortic VSMCs (Lee et al., 1989a).

Several studies using activators (phorbol esters) and inhibitors of PKC provided some evidence that activation of PKC may play a role in ET-1-induced vascular contraction. However, the evidence to date is at best circumstantial, because (*a*) the PKC inhibitors used (e.g., staurosporin, phloretin, H-7) lack specificity, especially at the high doses at which they were used in most studies, and (*b*) PKC exists in several (at least seven) isoforms (Nishizuka, 1989) with different cellular localization and biological activity (Farago and Nishizuka, 1990).

The PKC inhibitor H-7 effectively blocked ET-1-induced contractions in rabbit pulmonary vein (Steffan and Russell, 1990). Staurosporine, a more selective PKC inhibitor than H-7, significantly attenuated ET-1-induced coronary vasoconstriction in anesthetized pigs (Egashira et al., 1990).

PKC activation-mediated intracellular alkalinization (see below), DNA synthesis, gene transcription, and mitogenesis may play a significant role in ET-1-induced nuclear signaling mechanisms and long-term regulation of cellular function (see below).

4. Activation of phospholipase A_2 and arachidonic acid metabolism. The first (indirect) evidence that ET-1 can stimulate arachidonic acid metabolism via activation of

phospholipase A_2 was provided by De Nucci et al. (1988) who demonstrated that ET-1 caused release of PGI_2 and TXA_2 from isolated perfused rat and guinea pig lung preparations. (By now it is clear that at least PGI_2 comes from endothelial cells after activation of ET_B receptor subtype by ETs; see section IX.A.2.a.) More direct evidence for phospholipase A_2 activation by ET-1 came from studies of cultured SMCs (Reynolds et al., 1989; Resink et al., 1989), iris sphincter smooth muscle (Abdel Latif et al., 1991), and mesangial cells (Simonson and Dunn, 1990a,b). It is not known yet whether ET-1 activates phospholipase A_2 via a G-protein or indirectly by increasing $[Ca^{2+}]_i$ (Azelrod et al., 1988). In vascular smooth muscle ET-1 activates PLC and phospholipase A_2 in parallel but via independent mechanisms (Reynolds et al., 1989).

In addition to the release of PGI_2 from vascular endothelium, ETs were reported to stimulate arachidonic acid metabolism and the consequent release of several of its metabolites in various tissues, which mediate some of the biological actions of the peptides. Stimulation of renal prostaglandin synthesis/release was implicated in some of the effects of ET-1 and ET-3 on the kidney (Chou et al., 1990; Stier et al., 1992; Trybulec et al., 1991; Watanabe et al., 1991; Telemaque et al., 1992). ET-1 stimulates the release of prostaglandin $F_{2\alpha}$ in human endometrium (Cameron et al., 1991), which may contribute to the oxytocic activity of the peptide.

ETs were reported to stimulate the release of prostaglandins in the intestine, mediating ion secretion (Brown and Smith, 1991), in frog adrenal gland where they mediate ET-induced steroidogenesis (Delarue et al., 1990), in rat Kupfer cells (Gandhi et al., 1992b), in the rabbit eye (Granstam et al., 1991), in gestational tissues (Mitchell et al., 1990), in canine airways (Ninomiya et al., 1992b), and in pulmonary resistance vessels (Wang and Coceani, 1992).

Release of TXA_2 and activation of TXA_2 receptors were reported to be involved in ET-1-induced bronchoconstriction (Schumacher et al., 1990) and in ET-3-induced inhibition of ganglionic transmission at preganglionic sites in canine sympathetic ganglia (Kushiku et al., 1991).

5. Intracellular alkalinization: stimulation of Na^+-H^+ exchange. A wide variety of agonists that are linked to PLC activation and to Ca^{2+} mobilization increase intracellular pH via PKC-mediated activation of Na^+-H^+ exchange by increasing the intracellular H^+ affinity for the antiporter (Grinstein and Rothstein, 1986). ET-1 stimulates cytosolic alkalinization in VSMCs, and this effect appears to be a consequence of PKC stimulation. ET-1 stimulates Na^+-H^+ exchange and leads to intracellular alkalinization in VSMCs (Koh et al., 1990; Lonchampt et al., 1991; Rosati et al., 1990) and in human skin fibroblasts (Gardner et al., 1989). The stimulation of Na^+-H^+ exchange and increase in intracellular pH could be prevented by inhibition of PKC (Lonchampt et al., 1991). The time course of intracellular alkalinization is similar to that of the ET-1-stimulated DAG accumulation. When cellular PKC activity was reduced by phorbol 12,13-dibutyrate or by staurosporine, the ET-1-induced alkalinization response was completely blocked (Brock and Danthuluri, 1992). ET-1-induced alkalinization in cultured A-10 VSMCs is also inhibited by 5-N-ethylisopropyl-amiloride, a specific inhibitor of the Na^+-H^+ antiporter, indicating that the cytosolic alkalinization is a consequence of increased Na^+-H^+ exchange (Brock and Danthuluri, 1992).

6. G-proteins. Even before the cloning and discovery of ET receptors (which belong to the G-protein-coupled seven-transmembrane domain rhodopsin receptor superfamily; see section VI), several studies indicated that ET-1 induced stimulation of PLC via receptor-ligand coupling to a G-protein. In cultured VSMCs (Reynolds et al., 1989) and in rat mesangial cells (Simonson and Dunn, 1990a,b), pertussis toxin significantly inhibited ET-1-induced PI hydrolysis. Pertussis toxin also attenuated the ET-1-induced positive inotropic effect in rat ventricular myocytes (Kelly et al., 1990). In rat glomerular mesangial cells, GTP γs potentiated ET-1-induced PI hydrolysis (Simonson and Dunn, 1990a,b). All of these observations suggested that ET-1-induced activation of PLC and consequent PI hydrolysis is mediated by a pertussis toxin-sensitive G-protein (G_i).

However, pertussis toxin had no effect on ET-1-induced PI hydrolysis in rat-1 fibroblasts (Muldoon et al., 1989), in A-10 SMCs (Muldoon et al., 1989), or in cultured VSMCs (Takuwa et al., 1990a). Similarly, pertussis toxin did not suppress ET-1-induced contraction in rat mesenteric arteries and ET-1-induced PI hydrolysis in rat atrial tissue (Vigne et al., 1990a,b). These studies suggest that multiple G-proteins may be involved in ET-1-induced PLC activation in different target cells. The lack of effect of pertussis toxin on ET-1-induced vasoconstriction can also mean that PI hydrolysis plays only a marginal role in mediating the cellular response (contraction) in this tissue.

In pig coronary artery SMCs, pertussis toxin ADP ribosylated a 41-kDa membrane protein and selectively attenuated ET-1-induced contraction and $^{45}Ca^{2+}$ uptake, but not PI hydrolysis (Kasuya et al., 1992). This study suggested that the various signaling pathways activated in parallel by ET-1 in SMCs are coupled to different G-proteins in the same cells. In saponin permeated rabbit rectosigmoidal SMCs, antisera to $G_{o\alpha}$ and G_s had no effect on ET-1-induced biphasic contraction (Bitar et al., 1992). In contrast, antiserum to $G_{i3\alpha}$ prevented the early transient phase of contraction, whereas antiserum to $G_{i1-2\alpha}$ selectively inhibited the sustained phase of contractions (Bitar et al., 1992), suggesting that different G_i protein subtypes are involved in the early transient (mediated by IP_3 generation and intracellular Ca^{2+} mobili-

zation) and later sustained contractions (mediated by influx of extracellular Ca^{2+}, DAG generation, and/or PKC activation).

In cultured rat VSMCs expressing the ET_A receptor, ET-1 dose-dependently stimulated cAMP formation (Eguchi et al., 1992b). In bovine aortic endothelial cells expressing the ET_B receptor subtype, ET-3 dose-dependently inhibited forskolin-induced cAMP formation, which could be prevented by pertussis toxin, which ADP ribosylated the 41-kDa G_i-protein in these cells (Eguchi et al., 1992a).

These data suggest that, in addition to coupling to PLC via G_q-protein, ET_A and ET_B receptor subtypes are coupled to adenylate cyclase, probably via G_s in SMCs (ET_A receptor) and via G_i in endothelial cells (ET_B receptor).

B. Nuclear Signal Transduction Mechanisms Mediating Long-Term Effects of Endothelin on Cell Function

The findings that ET-1 is a weak mitogen for cultured vascular smooth cells (Muldoon et al., 1989; Komuro et al., 1988), fibroblasts (Muldoon et al., 1989; Takuwa et al., 1989c; Kraus et al., 1990), adrenal zona glomerulosa cells (Mazocchi et al., 1992), and glomerular mesangial cells (Simonson et al., 1989; Badr et al., 1989a,b) suggested that, in addition to short-term signal transduction pathways mediating rapid ET-1-induced biological actions such as smooth muscle contraction (see above), ET-1 may regulate gene expression to evoke long-lasting cellular responses as well.

Indeed, ET-1 stimulated the expression of the immediate early response gene c-*fos* in VSMCs (Komuro et al., 1988), 3T3 fibroblasts (Takuwa et al., 1989a), and mesangial cells (Simonson et al., 1989). ET-1 also stimulated the transcription of c-*myc* gene in VSMCs (Komuro et al., 1988) and *VL30* gene transcription in fibroblasts (Muldoon et al., 1989).

Although the signaling pathway(s) mediating the transcription of genes after ET-1 binds to its specific receptor on the plasma membrane are not known, elevation of $[Ca^{2+}]_i$, activation of PKC, tyrosine, and threonine phosphorylation of mitogen-activated protein kinases, and stimulation of Na^+-H^+ exchange, and consequent cytosolic alkalinization, were all proposed to play some role (Simonson and Dunn, 1990a) (Fig 6).

ET-1-induced stimulation of protein synthesis in rat aortic SMCs was partially blocked by inhibitors of PKC, suggesting that activation of PKC mediates, at least in part, the effect of ET-1 on gene transcription in SMCs (Chua et al., 1992).

In rat-1 fibroblasts, ET-1 rapidly increased mRNA levels of five members of the *fos/jun* gene family (c-*fos*, *fos*-B, *fos*-1, c-*jun*, and *jun*-B) (Pribnow et al., 1992). Because the intracellular Ca^{2+} chelator, 1,2-bis(2-aminophenoxy)ethane-N-N-N′-N′-tetraacetic acid, blocked this response, increased $[Ca^{2+}]_i$ must be directly involved in the induction of these *fos/jun* family genes by ET-1 in these cells (Pribnow et al., 1992). The role of increased $[Ca^{2+}]_i$ was further suggested by the finding that the Ca^{2+} channel blocker nifedipine significantly attenuated the ET-1-induced stimulation of DNA synthesis in A7r5 and 1YBA SMC lines (Nakaki et al., 1989).

The ET-1-induced immediate early gene c-*fos* expression was prevented by inhibitors of PKC in VSMCs (Bobik et al., 1990). Stimulation of Na^+-H^+ exchange in rat SMCs could be inhibited by PKC blockers (Lonchampt et al., 1991).

Mitogen-activated protein kinase, which is activated by various growth factors via phosphorylation of tyrosine and threonine residues, plays an important role in the signaling pathways between the growth factor receptors and the nucleus. ET-1 has been reported to activate (phosphorylate) mitogen-activated protein kinase in cultured ventricular cardiomyocytes (Bogoyevitch et al., 1993a), in cultured VSMCs (Koide et al., 1992a,b), and in rat mesangial cells (Wang et al., 1992).

Irrespective of the exact mechanism of action, however, it is possible that via this signaling mechanism ETs can participate in long-term adaptive changes in various tissues, including vascular remodeling, cardiac hypertrophy, and bone remodeling, and also maybe in the pathogenesis of proliferative disorders, such as atherosclerosis, renal diseases and bronchial asthma (see section X).

ET-1 stimulated DNA synthesis ([^{3}H]thymidine incorporation), protein synthesis, and/or proliferation in pulmonary artery SMCs (Janakidevi et al., 1992), in rat-6 fibroblasts (Pai et al., 1991), in A7r5 and 1YB4 SMC lines (Nakaki et al., 1989), and in cultured VSMCs (Chua et al., 1992). In contrast, ET-1 failed to stimulate proliferation on its own in rat aortic SMCs (Koide et al., 1992a,b) and pulmonary artery SMCs (Janakidevi et al., 1992) and produced only very weak mitogenic activity in rat SMCs (Lonchampt et al., 1991, Bobik et al., 1990).

In VSMCs, ET-1 stimulated c-*fos* and c-*myc* expression, [^{3}H]thymidine incorporation, and mitogenesis (Komuro et al., 1988; Bobik et al., 1990), which was much weaker than that produced by fetal calf serum or epidermal growth factor (Bobik et al. 1990).

ET-1-induced DNA synthesis in A7r5 and 1YB4 SMC lines was significantly potentiated by subthreshold doses of PDGF (Nakaki et al., 1989) and in rat SMCs by insulin (Lonchampt et al., 1991).

ET-1 stimulated DNA synthesis and proliferation in pulmonary artery SMCs only in the presence of calf serum (Janakidevi et al., 1992). Antisera to PDGF and epidermal growth factor prevented ET-1-induced proliferation in the presence of calf serum, indicating that the presence of these (or maybe of other) growth factors is required for ET-1-induced SMC growth/proliferation.

Analyzing the potential signaling pathways mediating ET-1-induced gene expression and proliferation in VSMCs, Koide et al. (1992a,b) found that the peptide

tyrosine and threonine phosphorylated at least five proteins, two of which were identical with mitogen-activated protein kinases 1 and 2 (i.e., recognized by anti-mitogen-activated protein kinase antibody). Although these studies showed that ET-1 and PDGF tyrosine phosphorylated the same proteins, ET-1 (in contrast to PDGF) failed to stimulate DNA synthesis and cell proliferation, suggesting that tyrosine phosphorylation of these proteins is not sufficient to stimulate proliferation in these VSMCs (Koide et al., 1992a,b).

In rat VSMCs, ETs had no mitogenic effect alone, but all isopeptides potentiated the mitogenic action of PDGF and calf serum (Weissberg et al., 1990). ET-1 and ET-2 were equipotent, whereas ET-3 had significantly lower potency, suggesting that ET_A receptors mediate the effect (Weissberg et al., 1990).

These studies of cultured rat VSMCs suggest that ET-1 is not a growth factor but may be a potent competence and/or progression factor, stimulating transition from G_0 to G_1 in the cell cycle, leading to increased DNA synthesis in certain conditions and to mitogenesis in combination with other growth factors (e.g., epidermal growth factor or PDGF). However, the lack of a direct mitogenic effect to ET may be due to the different conditions of cell growth in these studies (e.g., difference in passage number, phenotypic changes during passage, explant vs. enzymatically processed cells, species differences, etc.) (Serradeil Le Gal et al., 1991a).

VIII. Biosynthesis, Binding, and Pharmacological Action of Endothelin in Various Biological Systems

A summary of the site of biosynthesis, binding, the ET receptor subtypes, and the pharmacological actions of ETs in various organs and tissues is given in table 3.

A. Cardiovascular System

1. Hemodynamic actions. Intravenous infusion of ET-1 causes rapid and transient vasodilation, followed by a profound and long-lasting increase in blood pressure (Yanagisawa et al., 1988a,b; Liu et al., 1990; De Nucci et al., 1988; Hom et al., 1990). The vasodilator effect was proposed to be due to activation of the vascular endothelium, leading to formation of PGI_2 (De Nucci et al., 1988; Thiemermann et al., 1989) and endothelium-derived NO (Botting and Vane, 1990) or release of ANP (Winquist et al., 1989a,b). However, indomethacin (Hom et al., 1990) or ANP antagonists (Fozard and Part, 1990) had no effect on the transient vasodilation, but they potentiated the sustained pressor response. In some studies, L-N^G-nitro-L-arginine methyl ester and L-NMMA had no effect (Lerman et al., 1992b), but other studies showed that L-N^G-nitro-L-arginine methyl ester can inhibit ET-1-induced vasodilation (Gardiner et al., 1990; Fozard and Part, 1992). Thus, the nature of the initial depressor response is still uncertain and remains to be determined.

The pressor response is due to direct activation of vascular smooth muscle contraction mediated predominantly by the ET_A receptor subtype. However, in some vascular beds activation of ET_B receptors also causes vasoconstriction both in vitro and in situ (for further details, see section VIII.A.3). In anesthetized rats an intravenous bolus of ETs and STXs caused a pressor response with the following order of potency: STX 6b > ET-1 ≫ STX 6c > ET-3. BQ-123 (1 mg/kg) attenuated the response (Ihara et al., 1992a,b; Douglas et al., 1992; Cristol et al., 1993; McMurdo et al., 1993). The same peptides were equieffective in causing renal vasoconstriction in the same animals and BQ-123 was ineffective, suggesting ET_B receptor involvement in the kidney, whereas ET_A receptors were implicated in the systemic pressor response (Cristol et al., 1993; Pollock and Opgenorth, 1993).

Intravenous injection of ET-1 (up to 300 pmol) to anesthetized rats caused an initial transient depressor response, followed by a sustained increase in systemic arterial blood pressure (ED_{50} = 180 pmol) (MacLean et al., 1989). The pressor response was due to increased total peripheral resistance with no change in heart rate or cardiac output. The increase in total peripheral resistance was caused by vasoconstriction in the spleen, stomach, large intestine, small intestine, kidney, and the pancreas/mesentery beds. ET-1 increased blood flow through the heart, lung, liver, fat, and skin because of a redistribution of cardiac output to these vascular beds.

ET not only exerts direct vasoconstrictor effects but also is able to potentiate, at threshold and subthreshold concentrations, contractile responses to other vasoconstrictor substances such as NE and serotonin (Tabuchi et al., 1989; Yang et al., 1990c). In addition, the presence of small amounts of other vasoconstrictor substances (e.g., NE, serotonin, ouabain-like factor) amplified the vasoconstrictor response to ET-1 (Yanagisawa and Masaki, 1989; Dohi et al., 1992; Simonson et al., 1989; Watanabe et al., 1990; Yamasaki et al., 1989). Thus, even small (subthreshold) amounts of locally produced ET may act as a regulator of vascular tone and reactivity in the circulation.

Further details of the hemodynamic actions of ETs are summarized in recent reviews (Lerman et al., 1990; Goto et al., 1992; Randall, 1991; Masaki et al., 1990a).

2. Heart. a. BIOSYNTHESIS AND BINDING. ET is produced by cultured endocardial cells (Lewis and Shah, 1993). The secreted peptide may act in a paracrine fashion on neighboring myocytes, the conduction system, or coronary vessels. Neonatal rat cardiac myocytes express prepro-ET-1 mRNA and synthesize and secrete mature ET-1 (Suzuki et al., 1993), suggesting a potential autocrine mechanism of ET-1 in the heart.

ET-specific, high-affinity-binding sites were identified in cardiac myocytes (Hirata et al., 1989a), in cardiac membranes (Gu et al., 1989a, 1990), and at the atrioventricular node (Yamasaki et al., 1989). In human heart, in

TABLE 3

*Biosynthesis, receptors, and pharmacological actions of ETs in various organs**

Organ	Tissue/cell	Biosynthesis†	Binding‡	Receptors§	Actions‖
Blood pressure				ET_A, ET_B	Transient depressor effect Sustained pressor effect
Heart	Endocardium Myocardium	+	+	ET_A, ET_B	?
	Ventricle	+	+	ET_A, ET_B	Positive inotropy Positive chronotropy Growth (hypertrophy) Prolongation of action potential duration
	Atrium	+	+	ET_A, ET_B	Positive chronotropy Release of ANP Shortening of action potential duration
	Conduction system	−	+	ET_A, ET_B	Arrhythmia
	Coronary vessels	+	+	ET_A, ET_B	Vasoconstriction
Large vessels	Endothelium	+	+	ET_B	Release of PGI_2 and EDRF (NO)
				ET_C (cultured human)	Stimulation of mitogenesis
				ET_A (brain microvessel)	Stimulation of ACE
	Smooth muscle	−	+	ET_A, ET_B	Contraction (veins > arteries) Potentiation of contraction by other agonists Endothelium-dependent relaxation
		+ (culture)	+	ET_A	Stimulation of mitogenesis
	Perivascular nerves	−	+	ET_A	Prejunctional inhibition of NE release Postjunctional potentiation of NE action
Microvessels (isolated)	Endothelium	+	+	ET_A, ET_B	Release of PGI_2 and EDRF (NO) Mitogenesis
	Smooth muscle	−	+	ET_A	Contraction
	Pericytes (retina)	−	+	ET_A	Contraction Mitogenesis
Microcirculation (in situ)		+	+	ET_A ET_B (pancreas) ET_C (liver)	Vasoconstriction (arterioles > venules) Increased permeability
Spleen	Blood vessels	+ (endothelium)	+	ET_A	Vasoconstriction Prostanoid release
	Capsule	−	+	ET_B	Contraction
Lymphatic vessels		?	+	?	Contraction
Blood cells	Platelets	−	−	−	Inhibition of aggregation (indirect via endothelium-derived PGI_2/NO)
	PMNs	−	+	?	Stimulation of aggregation Stimulation of NO production Stimulation of PAF release Potentiation of F-Met-Leu-Phe-induced $\cdot O_2^-$ release
	Monocytes	+	+	?	Chemotaxis (±)
Kidney	Blood vessels	+ (endothelium)	+	ET_A, ET_B	Vasoconstriction
	Glomerulus	+	+	ET_A, ET_B	Vasoconstriction (efferent > afferent) Decrease in GFR Decrease in K_f

TABLE 3
Continued

Organ	Tissue/cell	Biosynthesis†	Binding‡	Receptors§	Actions‖
	Mesangial cells	−	+	ET_A, ET_B	Contraction Mitogenesis
	Juxtaglomerular cell	−	+	?	Decrease in renin release
	Tubules/collecting duct	+ (epithelium)	+	?	Decrease in Na^+ reabsorption
Lung	Blood vessels	+ (endothelium)	+	ET_A, ET_B	Vasoconstriction (artery > vein) Increased vascular permeability
	Airway epithelium (trachea)	+	+	ET_A	Stimulation of Cl^- secretion Stimulation of mucous secretion Stimulation of arachidonate release Stimulation of mitogenesis Stimulation of ciliary beat
	Airway smooth muscle (trachea and bronchus)	−	+	ET_A, ET_B	Contraction Epithelium removal facilitates contraction
Gastrointestinal tract	Mucosa (stomach)	+ (epithelium)	+	ET_A ET_B	Vasoconstriction Ulcerogenicity
	Smooth muscle (ileum)	+ (myenteric nerves)	+	ET_A, ET_B	Contraction/relaxation
	Mucosa (colon)	−	+	ET_A	Stimulation of ion secretion
Liver	Portal blood vessels	+(endothelium)	+	ET_B	Vasoconstriction Contraction of sinusoidal stellate cells
	Hepatocytes	−	+	ET_A, ET_B	Glycogenolysis Contraction of bile canaliculi Cholestasis
	Fat-storing cells/Kupfer cells	−	+	?	ET internalization
Urinary tract	Bladder	+ (epithelium)	+	?	Contraction
	Urethra	−	+	?	Contraction Inhibition of supraspinal micturition reflex
Female reproductive organs	Uterine blood vessels	+ (endothelium)	+	ET_A, ET_B	Vasoconstriction of spiral arteries
	Endometrium	+	+	ET_A, ET_B	Stimulation of PI hydrolysis and $PGF_{2\alpha}$ secretion Progesterone-dependent ET degradation
	Myometrium	−	+	ET_A, ET_B	Stimulation of rhythmic and tonic contractions
	Breast	+ (epithelium)	+ (stroma)	?	Mitogenesis
	Placenta/amnion	+	−	−	Vasoconstriction in placental blood vessels
	Ovaries	?	+	?	Stimulation of steroid synthesis
Male reproductive organs	Vas deferens	?	+	ET_A	Contraction
	Testis	+ (Sertoli cells)	+ (Leydig cells)	?	Stimulation of steroid synthesis

TABLE 3
Continued

Organ	Tissue/cell	Biosynthesis†	Binding‡	Receptors§	Actions‖
Eye	Retinal blood vessels	+ (endothelium)	+ (endothelium, smooth muscle, pericytes)	ET_A, ET_B	Vasoconstriction Endothelial mitogenesis Pericyte contraction/mitogenesis
	Iris (pupil)	+	+	ET_B	Contraction of sphincter Modulation of pupil size and light reflex
	Ciliary body	?	+	?	Inhibition of aqueous humor production Decrease in intraocular pressure
Bone	Osteoclasts	−	+	?	Inhibition (direct)/stimulation (indirect) of osteoclastic bone resorption
	Osteoblasts	−	+	?	Stimulation of osteoblast proliferation
	Bone marrow (endothelial and mast cells)	+	+	?	?
Skin	Keratinocytes	+	?	?	?
	Melanocytes	−	+	?	Stimulation of growth and tyrosinase activity
Adrenal gland	Cortex (glomerulosa cells)	−	+	ET_A, ET_B	Stimulation of aldosterone secretion synergy with angiotensin II
	Medulla	−	+	? ET_C (PC12 cells)	Stimulation of catecholamine secretion
Hypothalamus		+	+	ET_A, ET_B	Stimulation of luteinizing hormone-releasing hormone release Inhibition of prolactin release Stimulation of AVP secretion Stimulation of oxytocin secretion
Pituitary		+	+	ET_A, ET_B ET_C (rat anterior pituitary cells)	Stimulation of adrenocorticotropic hormone release Stimulation of gonadotropin (luteinizing hormone/follicle-stimulating hormone release)
Thyroid gland		+ (follicular cells)	+ (epithelial cells)	?	Inhibition of thyreoglobulin secretion Stimulation of epithelial cell growth
Parathyroid gland		?	+	?	Modulation of parathormone secretion
Pancreas		+	+	ET_A, ET_B	Vasoconstriction Internalization by acinar cells
Central nervous system	Neurons Astrocytes Glia cells	Hypothalamus > striatum > hypophysis > spinal cord (human)	Cerebellum > cortex > brain stem = basal ganglia = hypothalamus = supraoptic nucleus = subformical organ = spinal cord (human/rat)	ET_A, ET_B, ET_C	Increase/decrease of blood pressure Respiratory depression Elevation of plasma catecholamine, AVP, adrenocorticotropic hormone and glucose level Enhanced sympathetic efferent activity (kidney) Inhibition of water intake Stimulation of pyramidal neurons

TABLE 3
Continued

Organ	Tissue/cell	Biosynthesis†	Binding‡	Receptors§	Actions‖
					Decrease in regional cerebral blood flow Stimulation of glucose metabolism in the pituitary Stimulation of substance P release
Peripheral nervous system	Motor and sensory nerves	Dorsal and ventral horn ganglia in spinal cord	?	?	Decrease of motor and sensory nerve conductance Decrease of motor and sensory nerve blood flow Activation of spinal cord neurons (via release of substance P)
	Parasympathetic nerves	Myenteric and submucosal plexuses of the colon	Myenteric and submucosal plexuses of the colon	?	Stimulation of acetylcholine release Potentiation of acetylcholine action (intestine)
	Sympathetic nerves	?	?	?	Inhibition of NE release Potentiation of NE action Suppression/augmentation of baroreflexes

* For details and references, see section VIII.

† Expression of prepro-ET mRNA and/or production of mature ET was reported either in tissues in situ or in cells in culture (for more details, see also Table 1).

‡ Binding of ^{125}I-ET was demonstrated.

§ Receptor subtypes were characterized predominantly by pharmacological rank order of potency of ET isopeptides and sarafotoxins or by the use of selective receptor antagonists. In some instances, expression of ET receptor genes or cloning of the ET receptors was demonstrated (see text for details).

‖ Actions were observed by exogenously administered synthetic ET peptides.

situ hybridization showed ET_A and ET_B receptor mRNA localized to atrial and ventricular myocardium, the atrioventricular and endocardial conducting system, and endocardial cells (Molenaar et al., 1993); these observations were verified by binding studies.

b. ACTIONS. Soon after the discovery that ETs evoke sustained vasoconstriction, evidence that ETs have a direct effect on cardiac tissue was demonstrated (Hu et al., 1988b; Ishikawa et al., 1988). The direct cardiac actions of ETs include positive inotropic and chronotropic effects as well as a prolongation of the action potential duration. ETs also affect heart function indirectly via profound coronary vasoconstriction. Studies with cultured myocytes suggest that ETs may be involved in cardiac hypertrophy as well.

i. Positive inotropic effect. The positive inotropic effect of ET has been documented in isolated perfused hearts (Baydoun et al., 1989; Firth et al., 1990), in isolated human, guinea pig, and rat atria (Davenport et al., 1989), in ferret (Shah et al., 1989) and rabbit papillary muscles (Watanabe et al., 1989a,b), and in isolated adult rat and rabbit ventricular cells (Kelly et al., 1990). The action of ET-1 on heart muscle resembles that observed in vascular smooth muscle, with a delayed onset and a prolonged duration of action (Ishikawa et al., 1988; Eglen et al., 1989; Watanabe et al., 1989a,b; Borges et al., 1989a; Vigne et al., 1989; Shah et al., 1989).

ii. Positive chronotropic effect. Intravenous injection of ET-1 causes bradycardia that is secondary to the accompanying reduction on coronary flow (Karwatowska Prokopczuk and Wennmalm, 1990a). In isolated, spontaneously beating atrial preparations, however, ET-1 causes a dose-dependent increase in heart rate (Ishikawa et al., 1988).

iii. Action potential duration. The positive inotropic response is accompanied by prolongation of the cardiac action potential (Watanabe et al., 1989a,b). In rabbit papillary muscle, for example, 10 nM ET-1 lengthens the action potential duration by 16% (Watanabe et al., 1989a,b). In isolated canine myocardium, ET-1 prolonged action potential duration (followed by early afterdepolarization) in His bundle and ventricular myocardium but shortened action potential duration in atria (Yorikane and Koike, 1990). These pharmacological actions of ET-1 may contribute to the proarrhythmogenic property of the peptide observed frequently after systemic or intracoronary administration (Hom et al., 1992).

iv. Coronary circulation. ETs are potent coronary vasoconstrictors. In anesthetized dogs, a bolus intracoronary injection of 30 pmol/kg ET-1 reduces coronary blood flow by approximately 90% and may even cause total coronary artery occlusion (Kurihara et al., 1989a,c). This effect has been observed in several animal species (Clozel and Clozel, 1989; Chester et al., 1989; Kasuya et al., 1989b; Hom et al., 1992). Coronary veins are more sensitive than are coronary arteries and the effect is endothelium independent (Cocks et al., 1989a,b). Large subepicardial coronary arteries appear to be more sensitive than are endocardial vessels (Clozel and Clozel, 1989).

The coronary vasoconstrictor effect of ET-1 can have serious consequences on heart function. These include a decrease in segmental shortening, an increase in end-diastolic pressure, electrocardiographic signs of myocardial ischemia, a net release of lactate, and, at higher doses, ventricular fibrillation and death (Ezra et al., 1989; Hom et al., 1992; Clozel and Clozel, 1989; Karwatowska Prokopczuk and Wennmalm, 1990a). Several pharmacological interventions, including the administration of nitroglycerin and calcium antagonists, attenuate the constrictor response in large, as well as in small, coronary vessels.

v. Cardiac output. In contrast to the positive inotropic and chronotropic effects seen in isolated heart studies, a decrease in cardiac output is seen with infusion of ET in the intact animal (Lerman et al., 1991c, 1992b). The mechanism of this action may be multifactorial. Administration of ET systemically results in a significant elevation in coronary vascular resistance (see above) and a decline in myocardial perfusion. Decreased cardiac output may also occur in response to increased afterload, although venoconstriction leads to increased preload, which should augment cardiac output.

vi. Signal transduction in cardiac myocytes. In isolated adult rat ventricular myocytes, ET-1 at concentrations of less than 10^{-9} M caused a maximal inotropic response without a detectable increase in $[Ca^{2+}]_i$ (Kelly et al., 1990), suggesting that ET-1 sensitizes the myofilaments to calcium. At higher concentrations (10^{-8} to 10^{-7} M), ET-1 triggers mobilization of intracellular Ca^{2+} and stimulates transmembrane calcium current in isolated rabbit and rat cardiac myocytes (Kelly et al., 1990). Stimulation of PLC resulting in PI hydrolysis has been documented in neonatal ventricular myocytes (Galron et al., 1990b).

Exposure of freshly isolated adult rat ventricular myocytes to pertussis toxin completely abolished the positive inotropic effect of the peptide (Kelly et al., 1990). In contrast, pertussis toxin had no effect on the ET-induced increase in IP_3 in isolated adult rat atrial myocytes (Vigne et al., 1990a).

In cultured cardiac myocytes, ET-1 stimulated myelin basic protein kinase, p42 and p44 mitogen-activated protein kinases (Bogoyevitch et al., 1993a), and the PKC$_\epsilon$ isotype (Bogoyevitch et al., 1993b).

ET-1 (10^{-10} M) increased the contractile amplitude in isolated adult rat myocytes (Kramer et al., 1991). Amiloride, an inhibitor of sarcolemmal Na^+-H^+ exchange, prevented the intracellular alkalinization response to ET-1 and attenuated the inotropic effect. Nonspecific inhibitors of PKC, such as H-7 and sphingosine, diminished or abolished the increase in intracellular pH. However, neither amiloride nor inhibitors of PKC could completely prevent the positive inotropic response (Kramer et al., 1991).

Intracellular alkalinization and the expression of certain protooncogenes, such as c-*fos* and c-*jun*, may also cause hypertrophic growth in cardiac myocytes. Shubeita et al. (1990) demonstrated that ET can cause hypertrophy in cultures of serum-starved neonatal rat ventricular myocytes which was accompanied by activation of immediate early gene expression (e.g., c-*fos*). In ventricular myocytes isolated from adult rat hearts, ET-1 stimulated protein synthesis and PI turnover, via ET_A receptors (Sugden et al., 1993).

3. Large arteries and veins. ET-1 is the most potent endogenous substance known to induce contraction of isolated blood vessels. Ever since the pioneering observations with the peptidergic EDCF on porcine and bovine coronary arteries (Hickey et al., 1985) and the bioassay of the isolated and purified peptide on porcine coronary arteries (Yanagisawa et al., 1988b), numerous studies in the past 6 years confirmed it in arterial preparations of various anatomical origin isolated from a variety of animal species and humans (for references, see reviews by Randall, 1991; Rubanyi and Parker-Botelho, 1991; Rubanyi, 1992a). ET contracts arteries isolated from fish (catfish mesenteric artery), amphibiae (frog systemic arch), and reptiles (turtle left systemic arch) (Poder et al., 1991), as well as the toad aorta (Doi and Fujimoto, 1993). The majority of studies also confirmed the original observation that ET-1 induces slowly developing and long-lasting contractions. The cloning of two different ET receptor subtypes (Arai et al., 1990; Sakurai et al., 1990), classification of them as ET_A and ET_B receptors (Masaki et al., 1994), and the discovery of selective antagonists and ligands for the two receptor subtypes (Williams et al., 1991a,b; Ihara et al., 1991a, 1992a,b; Hiley et al., 1992; Webb et al., 1992; Ohlstein et al., 1992; Atkinson and Pelton 1992; Miyata et al., 1992a,b,c; Bazil et al., 1992) (for further details, see section VI) allowed characterization of the receptor subtype(s) mediating vascular smooth muscle contraction of large artery preparations. In general, these contractions are initiated by the binding of ET isopeptides to ET_A receptors on vascular smooth muscle, as evidenced by (*a*) the presence of ET_A receptors on these blood vessels (Arai et al., 1990), (*b*) the order of potency of the various ET isoforms (ET-1 = ET-2 > ET-3) (Masaki et al., 1994), and (*c*) the

ability of the selective ET_A receptor antagonists (e.g., BQ-123, FR-139317) to prevent or reverse these contractions (Ohlstein et al., 1992; Webb et al., 1992; Cardell et al., 1993). However, recent studies demonstrated that, in addition to ET_A, activation of the ET_B receptor subtype can also mediate vasoconstriction in some vascular preparations (Webb and Lappe, 1993; Harrison et al., 1992; Moreland et al., 1992; Shetty et al., 1993; Pollock and Opgenorth, 1993; Kleha et al., 1994; Noguchi et al., 1993). For example, Kleha et al. (1994) showed that the selective ET_B receptor ligand, STX 6c, contracts isolated rabbit aortic rings, which are desensitized rapidly (fig. 8) and are insensitive to the ET_A receptor antagonist BQ-123 but can be antagonized with the mixed ET_A + ET_B receptor antagonist PD 142,893 (fig. 9, bottom). In the presence of the ET_A receptor antagonist BQ-123, ET-1 triggers partial contraction of this preparation which can be abolished by PD 142,893 (fig. 9, top). In contrast, STX 6c had no contractile effect on isolated rings of rat aorta, and BQ-123 completely blocked the contractions evoked by ET-1 (Kauser and Rubanyi, 1994), suggesting that ET_B receptors are not present in this smooth muscle preparation. In vivo studies confirmed these in vitro observations, showing ET_B receptor-mediated vasoconstriction in coronary arteries of isolated rat hearts (Balwierczak, 1993), in resistance coronary arteries of the dog (Rigel and Lappe, 1993), in the pulmonary circulation of the rabbit (Clozel et al., 1992), in rat renal vasculature (Pollock and Opgenorth, 1993; Cristol et al., 1993), and in the systemic circulation of WKY and SHR (Webb and Lappe, 1993) and guinea pigs (Noguchi et al., 1993).

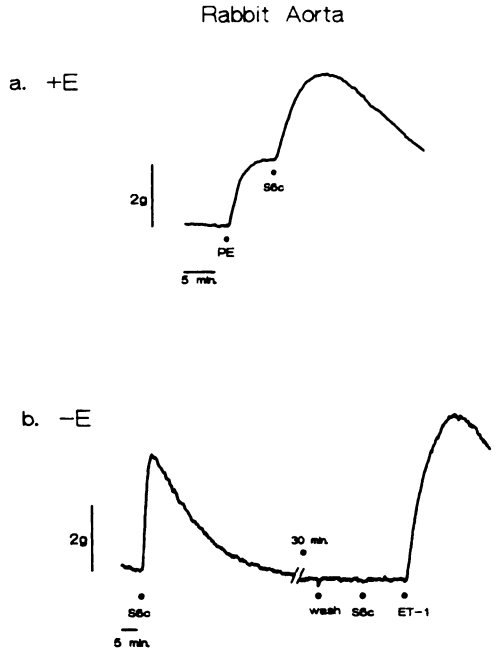

FIG. 8. Effect of the selective ET_B receptor agonist STX 6c (S6c; 30 nM) on isolated rings of rabbit aorta with (a) and without (b) endothelium. In both preparations STX 6c causes transient contractions. Desensitization of ET_B receptors occurs very rapidly (transient contraction) and is maintained for a long time (i.e., no repeated response to STX 6c even after washout) when the aortic ring still contracts in response to ET-1 (b). PE, phenylephrine (100 nM).

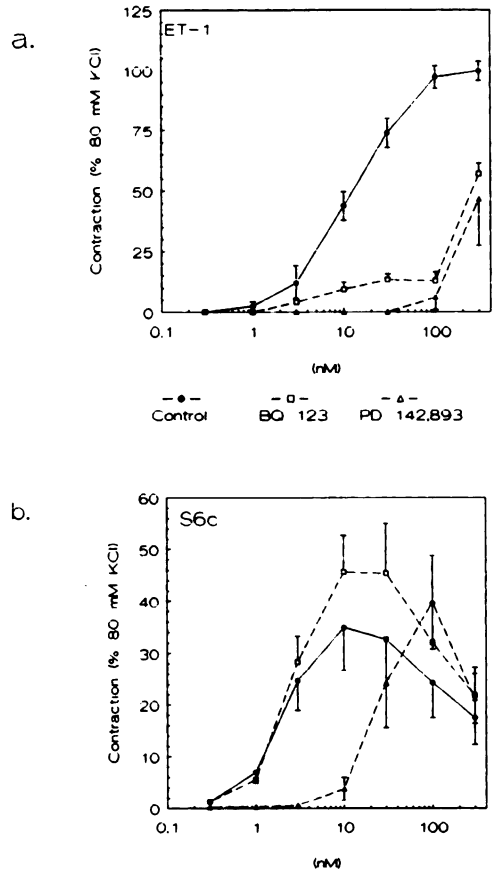

FIG. 9. Dose-response curves to ET-1 (ET_A and ET_B receptor agonist; a) and STX 6c (S6c; selective ET_B receptor ligand; b) in the absence (control) and presence of BQ-123 (10 μM; selective ET_A receptor antagonist) or PD 142,893 (30 μM; mixed ET_A and ET_B receptor antagonist) in isolated rabbit aortic rings without endothelium. BQ-123 partially (small but significant contraction could be still observed) and PD 142,893 competitively antagonized ET-1-induced contractions (a). BQ-123 had no effects, whereas PD 142,893 competitively antagonized STX 6c-induced responses (b). These data indicate that in this preparation both ET_A and ET_B receptors exist on SMCs and activation of both cause vascular contraction.

Although the majority of studies were performed on isolated large arteries, several studies compared the effect of ETs on large arteries and veins. In general, these studies found that ET-1 is 3- to 10-fold more potent in veins than in arteries. Cocks et al. (1989a) were the first to demonstrate that dog veins from the coronary, mesenteric, femoral, renal, and internal mammary vascular beds are 5- to 10 times more sensitive to ET-1 than the corresponding arteries. The same authors found that it is true for isolated human forearm vein and internal mammary vein when compared to internal mammary artery (Cocks et al., 1989b). These observation were later confirmed by comparing the responses to ETs in isolated human saphenous vein and internal mammary artery (Costello et al., 1990), in human internal mammary vein and artery (Yang et al., 1989, 1990b), in human uterine

veins and arteries (Fried and Samuelson, 1991), in human saphenous vein and gastroepiploic artery (McNamara et al., 1992), in fetal lamb pulmonary vein and artery (Wang and Coceani, 1992), in canine arteries and veins (Miller et al., 1989b), and in catfish posterior caudal vein and mesenteric artery (Poder et al., 1991). These in vitro observations were also confirmed in vivo. Infusion of ET-1 (5 pmol/min) into human forearm caused significantly more pronounced constriction of dorsal hand veins than arteries (Haynes et al., 1991). Ca^{2+} antagonists were effective in preventing ET-1-induced vasoconstriction only in arteries but not in hand veins (Haynes et al., 1991), suggesting differences in signal transduction pathways. Potential differences in ET-1 biosynthetic pathways between arteries and veins were also demonstrated in rabbit blood vessels (Auguet et al., 1992); pro-ET-1 (big ET-1) induced vasoconstriction in both the saphenous artery and vein, but phosphoramidon blocked the effect only in the saphenous artery. Whereas ET-1 was 3-fold more potent as a vasoconstrictor in the saphenous vein, big ET-1 was 2-fold more potent in the rabbit saphenous artery (Auguet et al., 1992).

In contrast to these large blood vessels in the systemic circulation, arteries are more sensitive than veins in the pulmonary circulation and in some microcirculatory beds. In the pulmonary circulation of the guinea pig, pulmonary arteries were more sensitive to ET-1 than were pulmonary veins (Cardell et al., 1990). These studies suggested that the increased sensitivity may be due to the difference in blood composition (e.g., lower pO_2 in systemic veins and pulmonary artery). Indeed, hypoxia causes increased sensitivity to ET-1 in arteries (MacLean et al., 1989; Douglas et al., 1991) and the heart (Lin et al., 1989, 1990a).

In the microcirculation of the hamster cheek pouch, rat cremaster muscle, and rat heart, arterioles are more sensitive to ET-1 than are postcapillary venules (Brain, 1989; Joshua, 1990; Homma et al., 1992).

4. Isolated microvessels and microcirculation. Elevation of systemic arterial blood pressure after an intravenous injection of ET-1 (see section VIII.A.1) suggested that, in addition to large arteries and veins, ET is also a potent vasoconstrictor of resistance arterioles in the microcirculation. Direct proof for this hypothesis was provided by studies of isolated microvessels in vitro and microcirculation in situ. Some studies with (cultured) cells isolated from microvessels (endothelial cells or pericytes) provided further evidence for the effect of ETs in the microcirculation.

Using the wire myograph, Deng and Schiffrin (1991) found that ET-1 is a potent vasoconstrictor in isolated rat mesenteric microvessels with a potency higher than ET-3, suggesting that ET_A receptors mediate the vasoconstriction. Topical application of ET-1 caused vasoconstriction in the blood vessels of rat mesenteric microcirculation in situ (De Carvalho et al., 1990; Fortes et al., 1991). Topical application of ET-1 caused long-lasting contractions in both arterioles and veins in the rat cremaster muscle in situ (Joshua, 1990; Lougee et al., 1990).

Similar observations were made in the microcirculation of hamster cheek pouch (Boric et al., 1990; Brain, 1989; Schultz and Muller, 1990), in pial arterioles of piglets (Armstead et al., 1989), rat cortical microvessels (Willette et al., 1990, 1993), rabbit and human skin (Brain et al., 1989), in rat cutaneous microvessels (Lawrence and Brain, 1992), glomerular afferent and efferent arterioles of the rat kidney (Kon et al., 1989; Edwards et al., 1990; Fretschner et al., 1991), in perfused rat heart (Homma et al., 1991), in the in situ canine heart (Hori et al., 1991; Hom et al., 1992), in rat liver (Kurihara et al., 1992), in rat gastric mucosa (Morales et al., 1992), in dog gastric microcirculation (Wood et al., 1992), in rabbit skeletal muscle and ear microvascular network (Randall et al., 1989), in perfused rat lung (Rodman et al., 1992), in ferret lung (Raj et al., 1992), in dog pancreas (Takaori et al., 1992), in rat tumors implanted subcutaneously (Tanda et al., 1991), and in rat peripheral nerve microcirculation (Zochodne et al., 1992).

In the pial arterioles of anesthetized piglets, topical application of 0.1 ng/ml ET-1 caused vasodilation, whereas higher concentrations (1 to 100 ng/ml) evoked vasoconstriction along with a release of prostanoids (PGI_2 and TXA_2). Inhibitors of cyclooxygenase (indomethacin, aspirin) blocked vasodilation and reduced constriction, suggesting that prostanoids [both vasodilator (PGI_2) and vasoconstrictor (TXA_2)] contribute to the effects of ET-1 in porcine pial arterioles.

In the hamster cheek pouch, topical application of 3 pmol ET-1 contracted arterioles selectively (Brain, 1989), whereas higher concentrations (0.1 to 10 nM) constricted both arterioles and venules (Boric et al., 1990). Iloprost, a stable analog of PGI_2, reversed ET-1-induced arteriolar constriction in this preparation (Schultz and Muller, 1990).

In rat cremaster muscle, topical ET-1 is a more potent constrictor of arterioles than venules (Joshua, 1990) and causes complete closure of second-, third-, and fourth-order arterioles (Lougee et al., 1990). Cremaster arterioles in the SHR are more sensitive to ET-1 than those in WKY normotensive rats (Lougee et al., 1990). Ca^{2+} antagonists reduce or inhibit ET-1-induced vasoconstriction in this preparation (Lougee et al., 1990; Joshua, 1990).

The vessels in the mesenteric microcirculation of desoxycorticosterone acetate salt or Goldblatt renal hypertensive rats are more sensitive to topical ET-1 than are those from normotensive rats (De Carvalho et al., 1990). Interestingly, no difference was found in ET-1 sensitivity between aortic rings isolated from the same normotensive and hypertensive rats.

In rat renal arterioles, ET-induced vasoconstriction is mediated by ET_A receptors (ET-1 > ET-3), and Ca^{2+}

antagonists reduced the effect in afferent arterioles but not in efferent arterioles (Edwards et al., 1990). In the split hydronephrotic rat kidney, nitrendipine had no effect on ET-1-induced afferent or efferent arteriolar constrictions (Fretschner et al., 1991).

In isolated perfused rat heart, ET-1 (1 to 40 pmol) constricted resistance arterioles with a higher potency than it did larger arteries (Homma et al., 1991) or postcapillary vessels (Homma et al., 1992). Topical epicardial application of ET-1 (1 to 1000 pmol) causes microvessel constriction and local ischemia (ST segment elevation) in dog hearts in situ (Hori et al., 1991).

Although in most microvascular beds ET-1 is more potent than ET-3 in eliciting vasoconstriction [an effect mediated by ET_A receptors; Edwards et al., 1990 (renal); Lawrence and Brain, 1992 (skin); Deng and Schiffrin, 1991 (mesentery)], the opposite order of potency was observed in rat liver microcirculation (ET_C receptor?) (Kurihara et al., 1992), and equal potency was found for the three isopeptides in dog pancreas microcirculation (ET_B receptor) (Takaori et al., 1992).

In rat liver microcirculation, ET-3-induced, but not ET-1-induced, vasoconstriction was suppressed by inhibitors of cyclooxygenase (indomethacin) and TXA_2 synthase (OKY-046) (Kurihara et al., 1992), suggesting that different signal transduction mechanisms are activated by the two isopeptides in this preparation, and/or they act on different cell types.

In perfused rat (Rodman et al., 1992) and ferret lung (Raj et al., 1992), ET-1 predominantly constricts postcapillary vessels, and in the rat lung, ET-1 causes edema by this mechanism and not by increasing capillary endothelial cell permeability (Rodman et al., 1992).

In rat gastric mucosa, ET-3 causes damage to capillaries and venules, with a potency exceeding that of histamine or leukotriene B_4 (Morales et al., 1992).

In the rabbit ear, ET-1 causes vasoconstriction in first-, second-, third-, and fourth-order arterioles and venules without contracting the central ear artery or large veins (Randall et al., 1989).

In subcutaneously implanted hepatoma tumors in rats, systemic injection of ET-1 increases blood flow to a lesser extent than does angiotensin II (Tanda et al., 1991), presumably because ET-1 (but not angiotensin II) causes vasoconstriction of blood vessels in these tumors (the increase in blood flow is the consequence of an elevated perfusion pressure due to increased arterial blood pressure).

In rat brain cortical microvessels (Willette et al., 1990) and in the rat sciatic nerve microcirculation (Zochodne et al., 1992), topical application of ET-1 causes a reduction in blood flow, vasospasm, and ischemia.

An analysis and demonstration of ET-1 synthesis/release, binding, and action on endothelial cells and pericytes isolated from microvessels and capillaries complemented the above findings in isolated microvessels and microcirculation in situ [Vigne et al., 1990b, 1991; Frelin et al., 1991; Ishibashi et al., 1992 (brain microvessel and capillary EC); Takahashi et al., 1989 (bovine retinal capillary EC); Lee et al., 1989b; Chakravarthy et al., 1992 (bovine retinal microvascular pericytes); Frelin et al., 1991 (astrocytes)].

Bovine retinal pericytes bind ^{125}I-ET-1 with high affinity (Chakravarthy et al., 1992). In the same cells, ET-1 (100 pM) caused contraction, mitogenesis, and increased inositol phosphate turnover (Chakravarthy et al., 1992). Endothelial cells isolated from bovine retinal capillaries synthesize and release ET-1 (Takahashi et al., 1989), which acts in a paracrine fashion on adjacent pericytes.

Astrocytes also respond to ETs with PLC activation (Frelin et al., 1991). However, the order of potency of ET isoforms (i.e., ET-3 > ET-1) suggested that the receptor is neither ET_A nor ET_B but probably a subtype of them or a third receptor isoform (ET_C) (Frelin et al., 1991).

5. Vascular endothelium. a. STIMULATION OF THE SYNTHESIS/RELEASE OF ENDOTHELIUM-DERIVED RELAXING FACTOR (NITRIC OXIDE): ENDOTHELIUM-DEPENDENT VASORELAXATION. ET-1 and ET-3 were reported to stimulate the release of EDRF (NO) from bovine native endothelial cells (Warner et al., 1992), from isolated perfused rat and rabbit blood vessels (Warner et al., 1989a,b), and from several other vascular tissues (Namiki et al., 1992a; Botting and Vane, 1990).

In precontracted isolated rabbit mesenteric artery, but not rabbit aorta (Kleha et al., 1994) and in isolated rat aorta (Kauser and Rubanyi, 1994), the selective ET_B receptor ligand, STX-6c, causes relaxation that can be prevented by removal of the endothelium (fig. 10). In-

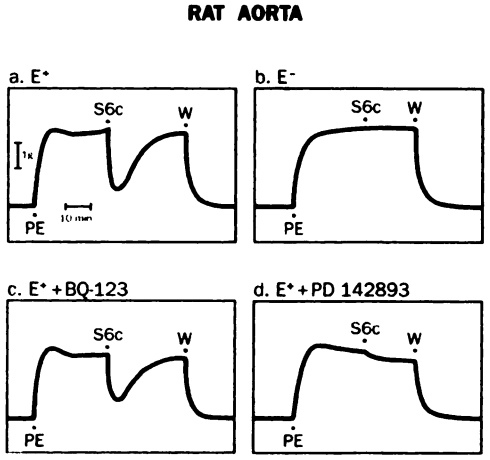

FIG. 10. Endothelium-dependent relaxation by the selective ET_B receptor ligand STX 6c (S6c, 30 nM) in isolated rat aortic rings. In a ring with endothelium (E+, a), STX 6c causes transient relaxation of phenylephrine-induced (PE, 10^{-7} M) contraction (cf. with transient contraction in rabbit aorta, fig. 8; a), which could be prevented by the removal of endothelium (E−; b) or by pretreatment of the ring with the mixed ET_A + ET_B receptor antagonist PD 142893 (30 μM; d) but not by the selective ET_A receptor antagonist BQ-123 (10 μM; c). W, wash.

domethacin had no effect, but N^G-nitro-L-arginine prevented STX 6c-induced endothelium-dependent relaxations, indicating that the relaxations were mediated by EDRF (NO) release from the endothelium (Kauser and Rubanyi, 1994). The relaxation response was insensitive to the ET_A receptor antagonist BQ-123 but could be blocked by the mixed ET_A + ET_B receptor antagonist PD 142,893 (fig. 10), indicating that endothelial ET_B receptors mediate the response. Similar endothelium-dependent and ET_B receptor-mediated (i.e., ET-1 = ET-3) vascular relaxation was observed in rat aorta (Karaki et al., 1993; Moritoki et al., 1993), rabbit mesenteric artery (Shetty et al., 1993), and cultured bovine endothelial cells (Hirata et al., 1993).

Stimulation of NO synthesis in a neuronal cell line was suggested to be involved in ET-1-induced relaxation of guinea pig trachealis muscle (Uchida et al., 1991b) and increase of cGMP (Reiser et al., 1990).

b. STIMULATION OF THE RELEASE OF PROSTACYCLIN: ENDOTHELIUM-DEPENDENT INHIBITION OF PLATELET ACTIVATION. ETs (ET-1 and ET-3) stimulate the release of PGI_2 from bovine and human vascular endothelial cells (Emori et al., 1991a; Filep et al., 1991a,b; Kato et al., 1992; Kibira et al., 1991), from isolated rat mesenteric arteries (Rakugi et al., 1989), from perfused rabbit heart (Karwatowska Prokopczuk and Wennmalm, 1990a), and from perfused rat lung (D'Orleans-Juste et al., 1992). Big ET-1 was also reported to stimulate the release of PGI_2 in vitro and in vivo (D'Orleans-Juste et al., 1991a) via phosphoramidon-sensitive conversion to ET-1. ETs can also stimulate PGI_2 release in vivo in rats (De Nucci et al., 1988; Botting and Vane, 1990), beagle dogs (Filep et al., 1991b,; Herman et al., 1989), and rabbits (Thiemermann et al., 1989).

PGI_2 release was suggested to be responsible for the hypotensive (at least in part) and anti-platelet aggregatory effects of big ET-1, ET-1, and ET-3 in vivo (Filep et al., 1991a,b; Thiemermann et al., 1989) (see also section VIII.A.7).

c. RECEPTORS AND SIGNAL TRANSDUCTION IN VASCULAR ENDOTHELIAL CELLS. In contrast to findings in large vessel endothelium (where only ET_B receptors have been identified so far), several studies showed that in addition to ET_B receptors on brain microvessels (Vigne et al., 1991), capillary endothelial cells in culture contain ET_A receptors as well (ET-1 > ET-3) (Vigne et al., 1991; Ishibashi et al., 1992). Stimulation of ET_A receptors by ET-1 in brain capillary endothelial cells activate PLC via a pertussis toxin-insensitive G-protein and inhibit adenylate cyclase via a pertussis toxin-sensitive pathway (Ishibashi et al., 1992). Another study of brain capillary endothelial cells showed that activation of ET_A receptors by ET-1 leads to mitogenesis (ET-1 being a more potent mitogen than basic fibroblast growth factor; Vigne et al., 1990b), via activation of PLC, Ca^{2+} influx, and probably PKC (Vigne et al., 1991). Stimulation of ET_B receptors in the same cells facilitates Na^+/H^+ exchange via a PKC-independent pathway (Vigne et al., 1991). Studies by Frelin et al. (1991) confirmed that stimulation of ET_B receptors in brain microvascular endothelial cells leads to facilitation of Na^+/H^+ exchange, but they found that it is linked to PKC activation.

6. Spleen. a. BIOSYNTHESIS. RNA blot hybridization with restriction fragments derived from cDNAs and ribonuclease protection assays revealed that ET-1, ET-2, and ET-3 genes are all expressed in the human (Bloch et al., 1989a,b) and rat spleen (Firth and Ratcliffe, 1992). Analyzing the presence of irET by radioimmunoassay and high-pressure liquid chromatography in pig tissues, Hemsen and Lundberg (1991) found high irET-1 in the spleen. Asphyxia for 2 minutes had no effect on plasma irET-1 levels but stimulated overflow of irET-1 from the rabbit spleen (Pernow and Lundberg, 1989; Pernow et al., 1990). Transient release of irET was observed from the spleen after endotoxin administration and after 2 minutes of asphyxia in pigs (Hemsen, 1991).

b. ACTION. In the isolated blood perfused spleen of the dog (Withrington et al., 1992), ET-1 and ET-3 caused a dose-dependent increase in vascular resistance, splenic capsular contraction, and reduction in spleen volume. ET-1 was more potent than ET-3 in evoking vasoconstriction (ET_A receptor), but the two isopeptides were equipotent in causing contraction of the splenic capsular smooth muscle (ET_B receptor). ET_A receptors are present in porcine spleen (Hemsen, 1991; Hemsen et al., 1991a). ET-2 and, to a lesser degree, ET-3 cause vasoconstriction in the porcine spleen in vivo (Hemsen et al., 1991a). Big ET-1 (700 pmol/kg) injection into pigs caused a 30% increase in splenic vascular resistance that was accompanied by a 5-fold increase of plasma irET-1 levels (Modin et al., 1991). ET-1 stimulates the release of PGI_2, prostaglandin E_2 and TXA_2 from perfused rabbit spleen (Rae et al., 1989).

7. Platelets. a. IN VITRO PLATELET FUNCTION. The surveyed studies, with few exceptions, demonstrated that ET has no direct effect on aggregation of animal or human platelets in vitro (Borges et al., 1989b; Edlund and Wennmalm, 1990; Filep et al., 1991a,b; Patel et al., 1989). In aqueorin-loaded gel-filtered human platelets, ET-1 (up to 10^{-6} M) had no effect on aggregation or platelet ionized calcium levels (Joseph et al., 1991). It showed no synergistic effect when platelets were stimulated with collagen, thrombin, or PAF. ET-1 had no effect on human platelet-rich plasma aggregation, TXA_2 generation from platelets, and $[Ca^{2+}]_i$, $[cAMP]_i$, or $[cGMP]_i$ in platelets (Kato et al., 1992). ET-1 (0.01 to 1 nM) had no effect on human platelets in vitro (Ohlstein et al., 1990a). However, in rabbit and canine platelets, ET-1 potentiated aggregation by low concentrations of ADP.

In one study, preincubation of human platelets with ET-1, ET-2, and ET-3 inhibited serotonin-induced plate-

let aggregation via a mechanism involving PKC (Pietraszek et al., 1992) and probably via interaction with serotonin receptors.

b. IN VIVO PLATELET FUNCTION. In contrast to the lack of direct effect on platelets in vitro, several studies showed that after injection of ETs to animals ex vivo or in vivo platelet aggregation was suppressed. In anesthetized beagle dogs, ET-1 (0.03 to 0.3 nmol/kg) injection reduced blood pressure, increased plasma 6-keto-prostaglandin $F_{1\alpha}$ levels and inhibited platelet aggregation in vivo, as monitored by a filter-loop technique (Filep et al., 1991b). Pretreatment of the dogs with indomethacin prevented the antiaggregatory effect of ET-1, whereas PGI_2 injection mimicked the effect.

ET-1 infusion to anesthetized rabbits stimulated PGI_2 release and inhibited ex vivo platelet aggregation (Thiemermann et al., 1989) and also in vivo platelet aggregation, as assessed by ^{111}In-labeled platelets (Thiemermann et al., 1990).

The conditioned culture medium of ET-1-treated HUVECs significantly suppressed platelet aggregation, TXA_2 generation, and $[Ca^{2+}]_i$ elevation and increased platelet cAMP and cGMP levels. These effects could be prevented by treatment of HUVECs with aspirin and L-NMMA, suggesting that PGI_2 and EDRF (NO) released from HUVECs by ET-1 are responsible for the antiaggregatory and platelet inhibitory actions (Kato et al., 1992).

Addition of washed human platelets to cultured bovine pulmonary artery endothelial cells, or to HUVECs, stimulated both ET-1 secretion (Ohlstein et al., 1991, 1992) and expression of prepro-ET-1 mRNA. Because platelet-free buffer from agonist-induced platelet aggregation had similar effects, substance(s) released from activated platelets must mediate the response (Ohlstein et al., 1991).

8. Polymorphonuclear neutrophils. a. BIG ENDOTHELIN CONVERSION AND ENDOTHELIN DEGRADATION BY POLYMORPHONUCLEAR NEUTROPHILS. Activated, but not resting, human PMNs cause marked degradation of ^{125}I-labeled ET-1 (Fagny et al., 1992). Phosphoramidon had no effect the degradation (but soybean trypsin inhibitor prevented it), suggesting the involvement of lysosomal cathepsin G (released from activated PMNs) in ET-1 hydrolysis. Indeed, the purified enzyme led to similar degradation products with a major cleavage site at His^{16}-Leu^{17} (Fagny et al., 1992). Coincubation of formyl-Met-Leu-Phe-activated human PMNs with cultured bovine aortic endothelial cells resulted in a fast (approximately 15 min), and PMN number-dependent, degradation of ET-1 in the medium (Patrignani et al., 1991). Resting PMNs were ineffective. The supernatant of activated PMNs was active as well, a result that could be prevented by protease inhibitors, suggesting that activated PMNs release an ET-1 degrading/inactivating protease(s) (Patrignani et al., 1991). This mechanism could play a role

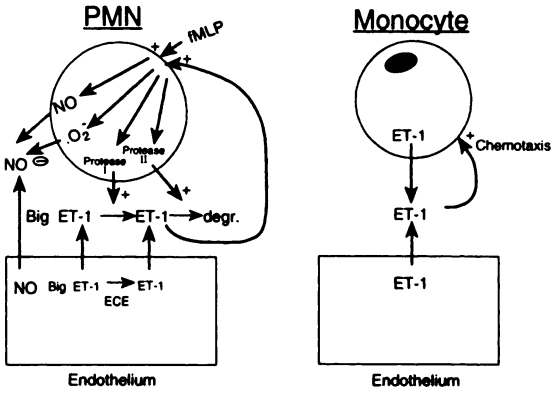

FIG. 11. Interaction between ET-1 and PMNs and monocytes. Activated PMNs release proteases that convert big ET-1 to ET-1 and also degrade ET-1. ET-1 potentiates PMN activation by F-Met-Leu-Phe (fMLP). PMNs produce NO, which inhibits PMN activation and ET-1 production, and also superoxide anion radical ($\cdot O_2^-$), which inactivates NO. Attachment of activated PMNs to endothelial cells stimulates ET-1 synthesis and secretion (not shown). Monocytes secrete ET-1, which stimulates chemotaxis. For further details, see text.

in ET-1 degradation in acute inflammatory reactions when activated PMNs adhere to vascular endothelial cells.

Human PMNs contain serine proteases in their cytosolic fraction that convert big ET-1 to ET-1 (Kaw et al., 1992a,b). These studies showed that human leukocyte elastase forms an intermediate of big ET-1 which is subsequently cleaved by a soluble protease(s) to form mature ET-1 (Kaw et al., 1992a,b). Membrane preparations of PMNs degrade and inactivate ET-1, similar to that which occurs with elastase (Kaw et al., 1992a,b) (fig. 11).

Big ET-1 is converted to ET-1 by peripheral human PMNs (Sessa et al., 1991a,b). This conversion was more pronounced in resting PMNs, suggesting a partial degradation of converting enzyme activity in activated cells (Sessa et al., 1991a,b). Phosphoramidon blocked the conversion, whereas phenylmethylsulfonyl fluoride, pepstatin, and *trans*-epoxysuccinyl-L-leucylamido-(4-guanidino) butane had no effect. Phenylmethylsulfonyl fluoride prevented the degradation of ET-1 by PMNs (Sessa et al., 1991a,b), suggesting that different neutral proteases are responsible for converting big ET-1 to ET-1 and degrading mature ET-1 (fig. 11).

b. EFFECT OF ENDOTHELIN ON POLYMORPHONUCLEAR NEUTROPHIL FUNCTION. In early studies, Borges et al. (1989b) found no effect of ET-1 on PMNs. However, later investigations provided evidence that ET-1 may modulate (stimulate) PMN function (fig. 11). ET-1 dose-dependently stimulated aggregation of human PMNs, which could be prevented by antagonists of PAF receptors (Gomez Garre et al., 1992). ET-1 also caused PAF release from these PMNs which was accompanied by an elevation of cytosolic $[Ca^{2+}]$, and the response was dependent on extra- and intracellular Ca^{2+} levels (Gomez Garre et al., 1992).

ET-1 had no effect on superoxide anion generation in resting PMNs, but the peptide (10^{-10} to 10^{-8} M) significantly potentiated superoxide generation stimulated by formyl-Met-Leu-Phe (Ishida et al., 1990). ET-1 did not cause an elevation of $[Ca^{2+}]_i$ in PMNs, and the potentiating effect was not dependent on extracellular or intracellular Ca^{2+} levels. ET-1 induced a dose-dependent increase in cytosolic Ca^{2+} concentration in isolated human and rat PMNs that was caused by both an influx of extracellular Ca^{2+} and a mobilization of intracellular Ca^{2+}. L-Arginine blocked the ET-1-induced $[Ca^{2+}]_i$ increase. L-NMMA reversed the inhibition by L-arginine, suggesting that ET-1 stimulates NO synthase, and the NO generated exerts a negative feedback influence on PMN activation by ET-1 (fig. 11).

c. EFFECT OF POLYMORPHONUCLEAR NEUTROPHILS ON ENDOTHELIN PRODUCTION. Activated human PMNs caused a 6.3-fold increase in irET-1 release from cultured porcine endothelial cells (Morita et al., 1993) only if they were allowed direct contact. Cell-free PMN-conditioned medium, or coculture in an Intercell chamber (no direct contact of the two cell types), had no stimulatory effect but, instead, decreased irET-1 levels (Morita et al., 1993). These observations suggest that after direct contact activated PMNs stimulate endothelial cell ET-1 gene expression.

9. Monocytes. The effect of ETs on monocyte chemotaxis is controversial. Achmad and Rao (1992) found that ET-1 increased monocyte chemotaxis in a dose-dependent manner (fig. 11). Ca^{2+} channel blockers (nifedipine, diltiazem, and verapamil), aspirin, and indomethacin significantly suppressed monocyte migration, suggesting that enhanced Ca^{2+} influx produced by ET-1 and the stimulation of arachidonic acid metabolism may contribute to the accelerated monocyte migration. ET-1-induced monocyte chemotaxis was attenuated by the radical scavenger and lipid-lowering drug probucol (Achmad and Rao, 1992).

In contrast, Bath et al. (1990) found no affect of ET on monocyte superoxide anion production or monocyte adhesion to porcine aortic endothelial cell monolayers. ET also did not act as a chemoattractant for these monocytes (Bath et al., 1990).

Immunoreactive ET-1 and ET-3 were found in a human promonocytic cell line (U937 cells), in freshly isolated human monocytes, and in cultured human monocytes (Ehrenreich et al., 1990). These cells secrete the peptides into the culture medium, as measured by high-pressure liquid chromatography and radioimmunoassay. The secretion of ET-1, but not ET-3, could be stimulated 6- to 10-fold by lipopolysaccharide and phorbol esters. No irET was found in extracts of human neutrophils and lymphocytes (Ehrenreich et al., 1990).

Human immunodeficiency virus-1 envelope glycoprotein 120 stimulates ET-1 secretion from macrophages (Ehrenreich et al., 1993b). ET-1 gene expression was detected in circulating monocytes and cerebral macrophages of patients with human immunodeficiency virus infection and human immunodeficiency virus encephalopathy, respectively, suggesting that ET-1 may be involved in the AIDS dementia complex.

10. Vascular permeability. Intravenous injection of ET-1 (0.1 to 2.0 nmol/kg) increased the hematocrit in a dose-dependent manner in rats (Filep et al., 1991c) and increased vascular permeability (extravasation of Evans blue) in the trachea, bronchi, stomach, duodenum, kidney, and spleen. Because PAF antagonists attenuated these responses, ET-1-induced stimulation of PAF secretion was suggested to mediate, at least in part, the increased vascular permeability.

In isolated blood perfused rat lungs, ET-1 increased vascular permeability (Helset et al., 1993). In lungs perfused with leukocytes in Krebs-Ringer albumin solution, or with plasma alone, ET-1 caused no change in permeability, suggesting that the effect is dependent on the presence of leukocytes and plasma components and is not the result of a direct effect on the vasculature.

In anesthetized and nephrectomized rats, ANP antibody prevented an ET-1 (25 ng/kg/min) infusion-induced increase in hematocrit, hemoconcentration, and reduction of plasma volume via protein extravasation (Valentin et al., 1991). These findings suggest that ET-1-induced ANP secretion mediates the capillary permeability increase produced by the peptide.

B. Kidney

ET is secreted at several sites in the kidney, where it acts in a paracrine or autocrine fashion on ET receptors on target cells. Because of its biological actions (including vasoactive properties), ET probably contributes to the control of RBF, renal plasma flow, GFR, and sodium and water transport at different nephron sites.

1. Biosynthesis and binding. Prepro-ET-1 mRNA expression was shown in cultured bovine glomerular endothelial cells by Northern blot analysis (Lopez-Farre et al., 1991b); this expression was enhanced by agonists, such as bradykinin, thrombin, ATP, and PAF. Glomerular endothelial cells were shown to release ET-1 that could be enhanced by bradykinin (Lopez-Farre et al., 1991b). A recent study showed that not only endothelial but also glomerular epithelial cells in culture produce ET-1 (Cybulsky et al., 1993). Autoradiographic studies using ^{125}I-labeled ET-1 revealed a high-affinity binding to glomeruli, vasa recta bundles, and the inner medulla (Kohzuki et al., 1989a; Kon et al., 1990; Kloog and Sokolovsky, 1989). High-affinity-binding sites are also present on cultured glomerular mesangial cells (Badr et al., 1989a,b) and cultured renal epithelial cells (Neuser et al., 1990b). Several renal epithelial cell lines (MDCK, LLC-PK_1) have been shown to release ET-1 (Kon and Badr, 1991; Oishi et al., 1991). ET-1 mRNA expression and biosynthesis was shown along rat nephron segments

(Ujiie et al., 1992). The evidence for glomerular, endothelial, and renal epithelial cell ET-1 biosynthesis and release, and the widespread renal ET-1-binding sites, raise the possibility of a local role of ET-1 in the modulation of renal hemodynamics and glomerular and tubular functions.

2. Renal hemodynamics. Systemic infusion of ET-1 increases renal vascular resistance and markedly decreases RBF (Badr et al., 1989b; Cao and Banks, 1990; Chou et al., 1990; Goetz et al., 1988, 1989; Hirata et al., 1989b; King et al., 1989; Miller et al., 1989a). Similar to the systemic effect of ET-1 on mean arterial pressure, renal vasoconstriction is often preceded by a transient renal vasodilation. Indomethacin or aspirin, which block prostaglandin synthesis, potentiate the increase in renal vascular resistance, which implies that vasodilatory prostaglandins attenuate ET-induced renal vasoconstriction (Chou et al., 1990).

ET-1 reduces both cortical and medullary blood flow, although the cortical vasculature is more sensitive to ET-1 (Tsuchiya et al., 1989). ET-1-induced vasoconstriction results from constriction of the glomerular afferent and efferent arterioles and the arcuate and interlobular arteries (Badr et al., 1989b; Edwards et al., 1990; Hirata et al., 1989b; Kon et al., 1989; Loutzenhiser et al., 1990). ET-1, ET-3, and the STXs 6b and 6c can equipotently induce renal vasoconstriction in anesthetized rats (Cristol et al., 1993), suggesting that the ET_B receptor subtype mediates the response. Indeed, ET-1-induced renal vasoconstriction could not be attenuated by the ET_A receptor antagonist BQ-123 in rats (Pollock et al., 1993).

In addition to systemic infusions, which mimic an endocrine rather than the most probable paracrine/autocrine signaling mode of ETs in vivo, the direct intrarenal actions of ETs were investigated in the isolated perfused kidney or on tissue/cell preparations isolated from the kidney. These experiments suggested that ET-1 is indeed a potent renal vasoconstrictor. For example, ET-1 is the most potent agent known to increase renal vascular resistance (approximately 30 times more potent than angiotensin II and 50 times more potent than NE) when added to isolated perfused rat and rabbit kidneys (Cairns et al., 1989; Ferrario et al., 1989; Perico et al., 1990).

3. Glomerular function. a. GLOMERULAR FILTRATION RATE. Intravenous administration of ET-1 in rats and dogs produces a dose-dependent, sustained decrease in GFR (Badr et al., 1989b; Cao and Banks, 1990; Choui et al., 1990; Goetz et al., 1988; Hirata et al., 1989b; King et al., 1989; Madeddu et al., 1989; Miller et al., 1989a). Experiments with isolated perfused kidneys also demonstrate that ET-1 reduces GFR (Cairns et al., 1988; Ferrario et al., 1989; Perico et al., 1991). Decline of the net filtration pressure across the glomerular capillary (Edwards et al., 1990) and the K_f (Badr et al., 1989a,b) were postulated to contribute to the decrease in GFR. However, low doses of ET-1 cause a greater increase in efferent than afferent arteriolar contraction (King et al., 1989). This would favor filtration, but a decline in K_f and a modest decrease in glomerular capillary flow rate cause GFR to remain relatively constant at these low doses of ET-1. Micropuncture studies of individual glomeruli demonstrate that ET-1 directly reduces K_f (Badr et al., 1989a,b; King et al., 1989; Kon et al., 1989). It was postulated that mesangial contraction reduces filtering surface area and K_f (Badr et al., 1989b; Simonson and Dunn, 1990a,b).

b. GLOMERULAR MESANGIAL CELLS. ET-1 contracts and activates multiple cellular signaling pathways in mesangial cells (Simonson et al., 1989; Simonson and Dunn, 1990a,b). Several laboratories have demonstrated an increase in mesangial $[Ca^{2+}]_i$ in response to ET-1, suggesting that increased $[Ca^{2+}]_i$ mediates the contractile response (Badr et al., 1989a; Lin et al., 1991; Pernow et al., 1989).

In addition to its potent contractile actions, ET-1 is also a mitogen for mesangial cells in culture (Badr et al., 1989a; Lopez-Farre et al., 1991b; Simonson et al., 1989; Jaffer et al., 1990). ET-1 induces the expression of several genes in quiescent mesangial cells, including the immediate early response *fos* and *jun* family genes (Simonson et al., 1992a), collagenase (Simonson et al., 1992a,b), and platelet-derived growth factor A and B chains (Jaffer et al., 1990).

Mesangial cells express both ET_A and ET_B receptor subtypes (Badr et al., 1989a; Baldi and Dunn, 1991), but the receptor subtype responsible for the contractile and mitogenic actions of ET on mesangial cells is not well characterized (Simonson et al., 1989; Simonson and Dunn, 1990a,b).

4. Tubular function. a. SODIUM EXCRETION. Systemic infusion of ET-1 decreases natriuresis (sodium excretion) because of the reduction in filtered load (Miller et al., 1989a; Goetz et al., 1988; Hirata et al., 1989b). Infusion of ET-1 also increases circulating aldosterone, which may contribute to the antinatriuretic effect of circulating ET-1 (see section VIII.L). However, both the decrease in filtered load and the increase in circulating aldosterone occur when ET-1 is tested after systemic infusions (i.e., endocrine-signaling mode). In contrast, studies of the isolated perfused kidney have consistently shown an increase in sodium excretion (Ferrario et al., 1989; Perico et al., 1990). Similarly, several studies document a natriuretic effect of ET-1 in vivo despite a decline in RBF and GFR (Garcia et al., 1990; King et al., 1989; Perico et al., 1991).

A possible explanation for these apparently contradictory findings is that ET-1, acting in an intrarenal, paracrine, or autocrine mode, would be natriuretic, whereas ET-1, acting in an endocrine mode, would be antinatriuretic (table 4).

b. WATER EXCRETION. Systemic infusion of pressor

TABLE 4
*Local (autocrine/paracrine) and systemic (endocrine) actions of endothelin-1**

	Local†	Systemic‡
Blood vessels		
Smooth muscle tone	↑	↑
Smooth muscle proliferation	↑	?
Endothelial PGI_2/EDRF (NO) release	↑	↑
Heart		
Contractility	↑	↓§
Heart rate	↑	↓§
Action potential duration	↑	
Arrhythmia‡		
ANP release	↑	↑
Coronary artery vasoconstriction	↑	↑
Cardiac output		↓/↑§
Kidney		
RBF/renal plasma flow	↓	↓¶
GFR	↓	↓¶
Na^+ excretion	↑	↓/**
Diuresis	↑	↓/**
Renin release	↓	↑††
Mesangial cell proliferation	↑	?
Adrenal gland		
Aldosterone secretion (cortex)	↑	↑
Catecholamine secretion (medulla)	↑	↑
Reflex (neural) control		
CNS		
Sympathoadrenal activity	↑‡‡	↑‖
Vasopressin (antidiuretic hormone) secretion	↑‡‡	↑‖
Peripheral reflexes		
Baroreceptor sensitivity	↓	−/↑
Neuromuscular junction		
Prejunctional	↓	−/↓
Postjunctional	↑	↑

* For details see text in sections VIII and IX.

† Local (direct) actions observed in isolated organs, tissues, or cultured cells with exogenously administered ET-1.

‡ Biological changes observed after intravenous systemic application of ET-1 into intact animals. These effects are due to both direct and indirect mechanisms.

§ The (opposite) effects observed after systemic ET-1 application are due to severe coronary vasoconstriction-induced myocardial ischemia.

‖ Subpressor doses of ET-1 increase cardiac output due to venoconstriction.

¶ Decrease of RBF/RPF and GFR was observed after injection of both pressor and subpressor doses of ET-1.

** The apparent antidiuretic and antinatriuretic actions were observed after systemic application of pressor doses of ET-1. In contrast, at subpressor doses (i.e., no effect on RBF or GFR) systemic application of ET-1 causes natriuresis and diuresis.

†† The increased plasma renin activity observed after systemic ET-1 application is due to indirect effects via activation of the sympathoadrenal system and via renal vasoconstriction (stimulation of juxtaglomerular osmoreceptors).

‡‡ Effect observed after intracerebroventricular (CNS) application of ET-1 in intact animals.

‖ Intravenous (systemic) application of ET-1.

doses of ET-1 decreased diuresis presumably indirectly due to a reduction in RBF and GFR. However, in some studies, systemic infusion of ET-1 increases diuresis despite a decrease in RBF and GFR (Badr et al., 1989b; Goetz et al., 1988). These studies suggested that ET-1 inhibits water reabsorption in the renal tubular network. Indeed, ET-1 inhibits AVP-mediated cAMP accumulation in the cortical collecting duct and in the outer and inner medullary collecting duct (Tomita et al., 1990) and inhibits AVP-stimulated water permeability in microperfused inner medullary collecting duct segments (Oishi et al., 1991).

Cultured rat inner medullary collecting duct cells express prepro-ET-1 mRNA and produce ET-1, both of which are significantly inhibited by increasing media osmolality (Kohan and Padilla, 1993). In cultured endothelial cells, increased osmolality had no effect on ET-1 production. Urinary ET-1 excretion and inner medullary collecting duct prepro-ET-1 mRNA expression were significantly reduced in volume-depleted (high medullary osmolality) versus volume expanded (low medullary osmolality) rats (Kohan and Padilla, 1993). Because ET-1 is an autocrine inhibitor of inner medullary collecting duct water and sodium reabsorption, these data suggest that ET-1 may be an important link between volume status and inner medullary collecting duct water and sodium reabsorption.

C. Lung

1. Biosynthesis. Cultured guinea pig tracheal epithelial cells produce ET-1, which is regulated by several growth factors and cytokines (Endo et al., 1992); TGFβ, tumor necrosis factor α, interleukins 1, 2, 6, and 8, and insulin-like growth factor-1 increased ET-1 synthesis. Some of them stimulated epithelial cell mitogenesis as well (interleukins 2 and 6 and insulin-like growth factor-1). In contrast, the growth factors epidermal growth factor, PDGF, and granulocyte-monocyte colony-stimulating factor promoted proliferation without a stimulation of ET-1 synthesis. Thrombin stimulates ET-1 secretion from rabbit tracheal epithelial cells (Rennick et al., 1993). Endotoxin stimulates the production of ET-1 by cultured guinea pig tracheal epithelial cells (Ninomiya et al., 1991). ET-1 is secreted by human bronchial epithelial cells and binds to human bronchial SMCs (Mattoli et al., 1990).

2. Airway smooth muscle. ET-1 is a potent constrictor of smooth muscle in trachea and bronchus isolated from mouse, rat, guinea pig, rabbit, and humans (Ninomiya et al., 1992a; Uchida et al., 1988; Maggi et al., 1989a,d; Advenier et al., 1990; Henry et al., 1990; Macquin-Mavier et al., 1989; Grunstein et al., 1991a,b; Hay et al., 1993; Sarria et al., 1990; McKay et al., 1991; Mattoli et al., 1991b; O'Donnell et al., 1990; Battistini et al., 1990b, c; White et al., 1991, 1992). Similar results were obtained in vivo (Macquin-Mavier et al., 1989; White et al., 1991, 1992). Because of the three isopeptides, ET-1 proved to be the most potent constrictor of airway smooth muscle (Advenier et al., 1990), the response is probably mediated by the ET_A receptor subtype.

Removal of the epithelium potentiated the contractile

effect of ET-1 (Maggi et al., 1989d; Hay et al., 1993), suggesting that, similarly to the endothelium in blood vessels, airway epithelium suppresses the direct smooth muscle activation produced by ET-1. In rabbit trachea half-maximally contracted with methacholine, low concentrations ($\leq 10^{-9}$ M) of ET-1 caused a relaxation that could be attenuated by indomethacin and removal of epithelium (Grunstein et al., 1991a,b), suggesting that smooth muscle relaxant prostanoids (e.g., PGI_2, prostaglandin E_2) may mediate, at least in part, the relaxation.

Removal of the epithelium increased the responsiveness of isolated guinea pig trachea (Hay et al., 1993) and guinea pig bronchus to ETs (Maggi et al., 1989d). In trachea with epithelium, only phosphoramidon (but not indomethacin, captopril, bacitracin, or leupeptin) mimicked the potentiation caused by epithelium removal, suggesting that the increased responsiveness is caused by removal of an epithelium-derived phosphoramidon-sensitive peptidase (Hay et al., 1993). Indeed, epithelium removal augmented ET-1- and ET-2-induced, but not ET-3-induced, guinea pig tracheal contraction, an observation that could be mimicked by phosphoramidon (Tschirhart et al., 1991), suggesting that enkephalinase (EC 3.4.24.11) or another phosphoramidon-sensitive peptidase in tracheal epithelium was able to degrade ET-1 and ET-2 but not ET-3.

Intravenous administration of ET-1 (10^{-8} mol/kg) to guinea pigs caused tracheal smooth muscle relaxation, followed by sustained contraction in vivo (White et al., 1991). Both removal of the epithelium and indomethacin attenuated the contraction but had no effect on the initial relaxation. In contrast, topical application of ET-1 to the epithelial surface of guinea pig trachea caused only a contraction, which could be potentiated by epithelium removal (White et al., 1992).

The signal transduction pathways mediating airway smooth muscle contraction are less studied than those present in vascular smooth muscle. Apparently, several pathways are activated, including a stimulation of the influx of extracellular Ca^{2+} (Advenier et al., 1990; Grunstein et al., 1991a,b), elevation of IP_3 (Grunstein et al., 1991b), activation of PKC (Grunstein et al., 1991b), increases in cyclooxygenase metabolites of arachidonic acid (Macquin-Mavier et al., 1989; McKay et al., 1991; O'Donnell et al., 1990), and increases in PAF (Battistini et al., 1990c).

ET-1 also causes a bronchoconstriction in vivo (Macquin-Mavier et al., 1989) that can be potentiated by the β-adrenoceptor antagonist propranolol. These findings suggest that systemic ET-1-induced sympathetic activation may counteract the direct bronchoconstrictor activity of the peptide. Indeed, the β-adrenoceptor agonist isoprenaline effectively attenuated the ET-1-induced airway smooth muscle contraction in vitro (Maggi et al., 1989a,d).

3. Airway epithelium. ET stimulates chloride secretion across canine tracheal epithelium (Plews et al., 1991; Tamaoki et al., 1991b; Satoh et al., 1992). The response was inhibited by the Na^+/Cl^- cotransport inhibitor, furosemide. It was evoked with equal potency by ET-1 and ET-2, whereas ET-3 was less active, suggesting that the ET_A receptor subtype on epithelial cells mediates the response. Indomethacin attenuated the response, suggesting that cyclooxygenase metabolite(s) of arachidonic acid may be involved (Plews et al., 1991; Satoh et al., 1992). ET-1 increased [^{3}H]arachidonate release and also increased $[Ca^{2+}]_i$ and cAMP in these epithelial cells (Plews et al., 1991).

ET stimulates mucus glycoprotein secretion from feline tracheal submucosal glands via an elevation of $[Ca^{2+}]_i$ (Shimura et al., 1992).

ET-1 stimulates ciliari beat frequency in cultured canine tracheal epithelial cells by elevating $[Ca^{2+}]_i$ (Tamaoki et al., 1991b), suggesting that the peptide may play a role in modulating airway mucociliary transport function.

ET-1 (0.1 to 100 nM) inhibited methacholine and phenylephrine-stimulated ferret tracheal submucosal gland secretion of mucus and lysosomal enzymes and of active albumin transport across the epithelium (Yurdakos and Webber, 1991; Webber et al., 1992).

D. Gastrointestinal Tract

1. Biosynthesis. Primary cultures of myenteric neuronal cells (but not glia cells, fibroblasts, or SMCs) from neonatal rat intestine express prepro-ET-1 mRNA and secrete mature ET-1. Membrane depolarization or veratridine had no affect on the rate of ET-1 secretion.

Rabbit gastric epithelial (mucosal) cells in culture produce ET-1, which can act in a paracrine manner on blood vessels and other tissues in the mucosa (Ota et al., 1991).

2. Smooth muscle. ET-1 is a potent vasoconstrictor in the isolated perfused rat stomach (Peskar et al., 1992; Wallace et al., 1989a,b). Similar observations were made in the rat stomach in situ after intravenous or intraarterial injections of ET (Wallace et al., 1989a,b; Whittle and Esplugues, 1988; Whittle and Lopez Belmonte, 1991; Masuda et al., 1992; Peskar et al., 1992). ET-1 was 10 times more potent than ET-3 in causing gastric vasoconstriction after intravenous injection (Wallace et al., 1989b), suggesting that ET_A receptors mediate the response.

In the isolated guinea pig ileum, cumulative application of ET-1 had only minor effects on smooth muscle tone, probably because of rapid desensitization (Borges et al., 1989b). However, when ET-1 was applied in a noncumulative fashion, it caused a biphasic response: an initial transient relaxation, followed by a sustained contraction (Lin and Lee, 1990). Competitive binding assays and affinity-labeling studies indicated a single class of binding sites for ET in the guinea pig ileum (Galron et

al., 1991; Wollberg et al., 1991). However, the biphasic smooth muscle response (Lin and Lee, 1992) and cross-tachyphylaxis observed between ET isopeptides (Miasiro and Paiva, 1992) demonstrated the existence of at least two different receptor types in this tissue. Indeed, the initial relaxation was suggested to be mediated by the ET_B receptor subtype (BQ-123 had no effect, and ET-1 and ET-3 were equipotent) (Lin and Lee, 1992; Hiley et al., 1992), whereas the sustained contraction was mediated by ET_A receptors (BQ-123 effectively antagonized it, and ET-1 was more potent than ET-3) (Lin and Lee, 1992; Hiley et al., 1992). In contrast, in the longitudinal muscle of guinea pig ileum, the three isopeptides were equipotent in evoking contractions (Yoshinaga et al., 1992), suggesting that the ET_B receptor mediates the contraction. Urade et al. (1992) reported that the selective ET_B receptor antagonist, IRL 1038, antagonized ET-3-induced contractile responses in the guinea pig ileum. It appears, therefore, that both ET_A and ET_B receptors are present, and both may mediate contractions in the guinea pig ileum.

Similar to ET-1 and ET-3, the COOH-terminal hexapeptide ET-1 (16 to 21) induced relaxation followed by contraction in the guinea pig ileum (Miasiro and Paiva, 1990a; Miasiro et al., 1993). In contrast to ET-1 and ET-3, however, the COOH-terminal peptide caused no desensitization (tachyphylaxis). These findings suggest that, whereas the COOH-terminal domain is responsible for the biological effect, the NH_2-terminal domain may be responsible for the strong binding and tachyphylaxis of ET-1 and ET-3.

The signal transduction pathways and cellular mechanism(s) of ET-1-induced relaxation and contraction of intestinal smooth muscle are not clarified yet.

3. Intestinal mucosa. In isolated rat colon, ET-1 stimulates an ion transport that involves ET_A receptors and may be mediated, in part, by PAF and prostanoids (Kiyohara et al., 1993; Brown and Smith, 1991).

In contrast to an ET-1-induced direct action on intestinal smooth muscle (i.e., tetrodotoxin and cholinergic or adrenergic antagonists had no effect) (Lin and Lee, 1990), stimulation of colonic mucosal secretion by ET-1 could be attenuated by tetrodotoxin and atropine (Kiyohara et al., 1993). These latter data suggested that ET-1-induced colonic mucosal secretion is mediated, at least in part, by submucosal cholinergic nerves.

E. Liver

Sinusoidal endothelial cells produce ET-1, which can be stimulated by TGF_β (Rieder et al., 1991). After intravenous injection of ^{125}I-ET-1, significant binding of ET-1 was detected in the liver (Furuya et al., 1992). Electron microscopic autoradiography revealed that the fat-storing cells had the highest binding, followed by Kupfer cells and liver cells. Fat-storing cells internalized into their cytoplasm most of the bound ET-1 (Shiba et al., 1989; Sirvio et al., 1990).

ET-1 binds to hepatocytes with high affinity and is rapidly internalized (Gandhi et al., 1992a). ET-1 stimulates PI hydrolysis in these cells with an ED_{50} of 1 pM via a pertussis toxin-sensitive G-protein.

ET-1 causes portal vessel constriction and glycogenolysis in the perfused rat liver (Gandhi et al., 1990; Serradeil Le Gal et al., 1991b; Tran Thi et al., 1993; Yang et al., 1990a). Whereas the glycogenolytic effect was rapidly desensitized after repeated ET-1 challenge, the vasoconstrictor effect was not, suggesting the involvement of two different receptors. ET-1 had no effect on liver cell cAMP but increased $[Ca^{2+}]_i$. The glycogenolysis effect was abolished by Ca^{2+}-free solution but not by Ca^{2+} antagonists. Glycogen phosphorylase enzyme was stimulated in hepatocytes by ET-1 with an EC_{50} of 0.03 pM (Serradeil Le Gal et al., 1991b).

In addition to stimulation of glycogenolysis and vasoconstriction (Tran Thi et al., 1993), several biological (pharmacological) actions of ET-1 were observed in various tissues in the liver, including contraction of bile canaliculi in isolated rat hepatocytes (Kamimura et al., 1993), contraction of sinusoidal stellate cells associated with an increase in $[Ca^{2+}]_i$ and IP_3 formation (Kawada et al., 1993), cholestasis due to an increase in portal pressure (Isales et al., 1993), and mediation of ethanol-induced vasoconstriction (i.e., anti-ET-1 antibody caused approximately 80% inhibition of ethanol-induced vasoconstriction in rat liver) (Oshita et al., 1993). In perfused rat liver, the ET-1 induced glycogenolysis, and elevation in portal pressure can be attenuated by the stable PGI_2 analog iloprost and by the NO donor Sin-1 (Tran Thi et al., 1993).

F. Urinary Tract

ET-binding sites have been localized in the lower urinary tract (bladder and urethra) of the rabbit (Garcia Pascual et al., 1990), pig (Persson et al., 1992), and human (Maggi et al., 1989a,c, 1990; Konda et al., 1992). ET-1 is synthesized by the epithelium and SMCs of the urinary bladder, and ET receptor subtypes were identified on bladder smooth muscle (Traish et al., 1992), suggesting autocrine and paracrine regulation of bladder function by the peptide. Indeed, ET-1 is a potent constrictor of human (Maggi et al., 1989a,c), porcine (Persson et al., 1992), rat (Secrest and Cohen, 1989; Lecci et al., 1991), and rabbit (Garcia Pascual et al., 1990) urinary bladder. The contractions induced by ET-1 are accompanied by an elevation of intracellular $[Ca^{2+}]$ and stimulation of PI hydrolysis in isolated pig urinary bladder detrusor muscle (Persson et al., 1992) and in rabbit detrusor and urethral smooth muscle (Garcia Pascual et al., 1993).

Intracerebroventricular administration of ET-1 or ET-3 (100 pmol) to rats inhibited the supraspinal micturition

reflex (Lecci et al., 1991). Guanethidine or vagotomy had no effect. Intravenous injection of ET-1 (350 pmol) also inhibited supraspinal micturition reflex with a delay of several minutes, which could be attenuated by guanethidine.

G. Female Reproductive System

1. Uterus. a. BIOSYNTHESIS BY THE ENDOMETRIUM. IrET-1 was detected in glandular epithelium and vascular endothelium of human endometrium and myometrium (Cameron et al., 1992). Specific immunostaining for ET-1 was observed in the endometrium, but not the myometrium, of rabbit uterus (Maggi et al., 1991). IrET-1 is produced by rabbit endometrial, but not myometrial cells, in culture (Orlando et al., 1990). ET-1 secretion from primary cultures of rabbit endometrial cells is stimulated by the neurohypophyseal hormones oxytocin and vasopressin (Orlando et al. 1990). Human endometrial adenocarcinoma cells express ET-1 (Pekonen et al., 1992).

Human endometrium contains specific binding sites for ET-1 and ET-3 (Davenport et al., 1991), and ET-1 stimulates PI hydrolysis and prostaglandin $F_{2\alpha}$ synthesis (Cameron et al., 1991) from explants of proliferative human endometrium in culture (Ahmed et al., 1992).

b. BINDING AND ACTION IN MYOMETRIUM. Both ET_A (ET-1 > ET-3) and ET_B (ET-1 = ET-3) receptor subtypes are present in rabbit myometrium (Maggi et al., 1991). Rat myometrium has ^{125}I-ET-1-binding sites (Borges et al., 1989b) and contains an ET receptor different from that of uterine vascular tissue (low affinity vs. high affinity, respectively). ET-1-binding sites are present in human nonpregnant and pregnant uteri and uterine blood vessels (Svane et al., 1993). The existence of ET_A receptors in human myometrium was demonstrated by expressing them in *X. laevis* oocytes (Kimura et al., 1992).

In premenopausal, nonpregnant or pregnant women, myometrial ET-binding sites are more abundant than in the myometrium of postmenopausal women (Schiff et al., 1993), suggesting that myometrial ET receptors are under the control of ovarian steroid hormones.

ET-1, ET-2, and ET-3 cause contraction of rat (Borges et al., 1989b; Kozuka et al., 1989; Sakata et al., 1989), rabbit (Suzuki, 1990), guinea pig (Eglen et al., 1989), sheep (Yang and Clark, 1992), and human uterus (Svane et al., 1993). ET-1 causes two types of contractions in rat uterus: phasic (rhythmic) contractions, which can be inhibited by Ca^{2+} channel antagonists, and tonic contractions, which are extracellular Ca^{2+} concentration dependent but insensitive to Ca^{2+} channel antagonists (Kozuka et al., 1989). ET-1 contracts both nonpregnant and midpregnant rabbit uterus, being more potent in nonpregnant uterus (Suzuki, 1990). The phasic, but not the tonic, contractions could be inhibited by Ca^{2+} channel antagonists.

In cultured human myometrial cells ET-1 dose-dependently increased $[Ca^{2+}]_i$ (Maher et al., 1991). Ca^{2+} channel antagonists reduced the effect by approximately 50%, whereas removal of extracellular Ca^{2+} abolished it. ET-1 induces PI hydrolysis in nonpregnant (Wollberg et al., 1992a,b) and pregnant rat uteri (Okawa et al., 1991). ET-3 increases $[Ca^{2+}]_i$ and causes myosin light chain phosphorylation in human myometrium (Word et al., 1990).

2. Placenta and amnion. Human placenta expresses prepro-ET-1 mRNA and produces equal amounts of big ET-1, ET-1, and ET-3 and very low amounts of ET-2 (Benigni et al., 1991a). ET-1 is synthesized in the human amnion and can penetrate fetal membranes and the endometrium toward the myometrium (Eis et al., 1992).

3. Estrus and menstrual cycle. Prepro-ET-1, -ET-2, and -ET-3 mRNA are present in human endometrium in all phases (proliferative, secretory, and menstrual) of the menstrual cycle (O'Reilly et al., 1992). The ratio of ET_A and ET_B receptor mRNA, however, changes during the cycle. In the proliferative phase, only ET_A mRNA could be identified, whereas in the later phases, an increase in ET_B receptor mRNA occurred. Low-level immunostaining for ET was found in the stromal human endometrium throughout the menstrual cycle (Salamonsen et al., 1992). Strong staining was present in luminal epithelium during the secretory phase and in glandular epithelium in the late secretory phase.

The effect of ETs was studied on rat uterus at different stages of the estrus cycle (Wollberg et al., 1992b). The rank order of sensitivity of the uterus to the contractile effect of ETs was proestrus > estrus > metestrus.

The exact mechanism of menstruation is not fully understood. Specifically, it is not clear what mediates the progesterone withdrawal-related vasoconstriction of the spiral arteries. It is believed that uterine prostaglandins, increased during menstruation, may be responsible for this vasoconstriction. Prostaglandins, however, may not be the only factor initiating menstruation because inhibitors of prostaglandin biosynthesis do not prevent menstruation.

A steroid-dependent degradation system of ET-1 (NEP) has been identified in the human endometrium. It has been found that NEP is a progesterone-dependent protein in human endometrium. It correlates with plasma progesterone levels in a highly significant manner (Casey et al., 1992).

It has been suggested that the decreasing endometrial enkephalinase at the time of luteolysis, together with the increase in the synthesis of ET-1 by stromal cells, may result in the increase in the local concentration of ET-1 and, in turn, in menstruation (Casey et al., 1992). Thus, the ET-1/enkephalinase system in the endometrium may be considered an important mechanism of menstruation in humans.

4. Sexual steroid hormones. Ovarian steroid hormones

changed the localization of ET-1 in rabbit endometrium: in immature animals, ET-1 was present in epithelial cells, whereas after estrogen treatment, the peptide was localized primarily in stromal cells of the endometrium.

17β-Estradiol treatment (but not estradiol + progesterone treatment) significantly increased ET receptors in rabbit myometrium (Maggi et al., 1991). However, estradiol failed to affect ET receptor number in primary cultures of rabbit myometrium (Maggi et al., 1991), suggesting that the effect is not direct, but other mediators are also involved.

Uterus from ovariectomized rats showed weak responsiveness to ETs. 17β-Estradiol treatment increased sensitivity significantly (Wollberg et al., 1992a).

Studying sexual dimorphism in circulating plasma irET levels, Polderman et al. (1993) found that the irET level is higher in men than in women and higher in nonpregnant than in pregnant women. In 12 male-to-female transsexuals treated with 17β-estradiol and cyproterone acetate, the irET level decreased significantly, whereas in 13 female-to-male transsexuals treated with testosterone, irET levels increased significantly (Polderman et al., 1993). These findings indicate that male and female sexual steroid hormones have opposite effects on irET levels.

H. Male Reproductive System

ET-1 gene is expressed and mature ET-1 is synthesized and secreted in rat testicular Sertoli cells (Ergul et al., 1993), irET was detected in the seminal fluid (Casey et al., 1992), and specific ET-1 binding sites were detected on rat testicular Leydig cells (Ergul et al., 1993). In Leydig cells isolated from rat testis, ET-1 and, to a lesser extent, ET-3 stimulate basal and human chorionic gonadotropin-induced testosterone production (Conte et al., 1993). Indomethacin had no effect, but nifedipine abolished ET-induced testosterone secretion. In a murine Leydig tumor cell line (MA-10), ET-1 stimulated an approximately 6-fold increase of progesterone production and enhanced the expression of c-*jun* and c-*myc* protooncogenes. However, in contrast to epidermal growth factor, ET-1 caused no mitogenesis in these cells.

These findings suggest that ET-1 may play a role in modulating steroid production in the testis via a paracrine mechanism.

I. Eye

1. Biosynthesis. High levels of prepro-ET mRNA have been found in the rat (MacCumber et al., 1989) and rabbit (MacCumber et al., 1991) eye. In the rat, the levels of ET mRNA in the iris are among the highest of all tissues analyzed (MacCumber et al., 1989). IrET-1 and irET-3 are present in the rat (MacCumber et al., 1989) and rabbit eye (MacCumber et al., 1991). IrET-3 levels are much higher than irET-1 levels, and irET-3 is most concentrated in the iris and ciliary body in the rat and rabbit eye (MacCumber et al., 1989, 1991). The source of ETs is uncertain, but vascular endothelial cells and neurons are the most likely sites of production.

Dense ET-binding sites are present in the iris, choroid, and retina in the rat eye (MacCumber et al., 1989). Mature ET-1 is produced by cultured human retinal microvascular endothelial cells (Takahashi et al., 1989), and these endothelial cells and corresponding pericytes contain high-affinity-binding sites for ET-1 (Takahashi et al., 1989).

2. Action. The reported biological (pharmacological) effects of ETs in tissues of the eye include stimulation of contraction and mitogenesis of human retinal pericytes in vitro (Chakravarthy et al., 1992; Granstam et al., 1992). Other effects include rapid and reversible contraction of isolated retinal branches of bovine short posterior ciliary arteries (Nyborg et al., 1991), constriction of iris and preretinal blood vessels in the rabbit eye (MacCumber et al., 1991), constriction of the pupil and attenuation of the light response, and a marked reduction of intraocular pressure in the rabbit eye (MacCumber et al., 1991). The decrease in intraocular pressure is probably due to reduced production of aqueous humor by the ciliary body rather than by an increased outflow (MacCumber et al., 1991).

The effect of ET-1 and ET-3 on pupil size in the rabbit eye depends on whether they are applied in vivo or in vitro. In vitro both ET-1 and ET-3 cause pupillary constriction. In vivo, however, ET-1 causes dilation, and ET-3 inhibits the light response. Two different receptors were identified in the eye: an ET_A receptor, presumably on vascular tissue, and an ET_B receptor, on the pupillary sphincter (MacCumber et al., 1991).

In bovine retinal pericytes, ET-1 causes contraction with a potency much higher than any other agonist tested (Ramachandran et al., 1993).

In a coculture system, HUVEC cells stimulate bovine retinal pericyte proliferation, which is mediated by ET-1 produced by the endothelial cells (Yamagishi et al., 1993). Retinal endothelial cells and pericytes influence each others' function (metabolic activity, growth/proliferation), and coculture of these cells activates latent TGFβ. Because active TGFβ stimulates expression of the ET-1 gene in endothelial cells and the secreted ET-1 acts on pericytes, it is tempting to speculate that this biofeedback mechanism plays some role in the maintenance of normal retinal microvascular function. Dysfunction of this system in diabetes or hypertension may play a pathogenic role in microangiopathies in the eye (see section X).

J. Bone

Bone marrow-derived mast cells synthesize and secrete ET-1 and have specific ET receptors (Ehrenreich et al., 1992), suggesting that ET-1 may be involved in mediating long-term changes in the microenvironment of mast cells, including bone remodeling.

There is an abundance of ET-producing endothelial cells with close proximity to bone-resorbing osteoclasts in the bone marrow, and ET could be localized in osteoclasts themselves (Sasaki and Hong, 1991). Osteoclastic bone resorption (studied by settling disaggregated osteoclasts from neonatal rat long bones on devitalized cortical bone substrate) was significantly suppressed by ET-1 in a dose-dependent manner (EC_{50} 2.5 nM), without inhibiting acid phosphatase secretion (Alam et al., 1992). ET-1 (EC_{50} 8 nM) significantly suppressed, in a reversible fashion, mobility of isolated osteoclasts without changing $[Ca^{2+}]_i$ in these cells (Alam et al., 1992).

In osteoblast-like MC3T3-E1 cells, ET-1 stimulated [^{3}H]thymidine incorporation, synergistically stimulated PDGF-induced [^{3}H]thymidine incorporation (EC_{50} 2.5 nM), and tyrosine phosphorylated several proteins (Schvartz et al., 1992).

In UMR 106 osteoblastic cells, ET-1 increased $[Ca^{2+}]_i$ and PI hydrolysis but had no effect on cAMP levels (Tatrai et al., 1992b). Ca^{2+} channel blockers had no effect, but removal of extracellular Ca^{2+} attenuated the Ca^{2+} transient. In cultured osteoblastic cells from rat calvariae ET-1 activates PLC and mobilizes intracellular Ca^{2+} (Takuwa et al., 1989b, 1990b).

In contrast to the direct suppression of osteoclast activity and stimulation of osteoblast proliferation, ET-1 stimulated bone resorption in neonatal mouse calvaria and fetal rat limb bone cultures, effects that could be inhibited by indomethacin (Tatrai et al., 1992a). ET-1 stimulated collagen and noncollagen protein synthesis only in the presence of indomethacin, suggesting that the catabolic actions of ET-1 are mediated by endogenous prostaglandins, compounds that mask the direct anabolic effect of the peptide on bone metabolism. ET stimulated PI hydrolysis, an effect that may be involved in the anabolic actions of ET-1. Based on these observations, a potential role for ETs in bone remodeling was proposed (Tatrai et al., 1992a,b).

K. Skin

Human keratinocytes in culture express prepro-ET-1 mRNA and secrete mature ET-1 (Yohn et al., 1993). In the human epidermal-melanin unit, melanocytes produce melanin and transfer it to neighboring keratinocytes, which in turn produce factors that regulate melanocyte function. ET-1 stimulates growth and tyrosinase activity in human skin melanocytes.

ET-1 may act in an anti-inflammatory manner following its stimulated local production by inflammatory cytokines and as a consequence of its potent vasoconstrictor activity. Indeed, ET-1 inhibited edema formation and neutrophil accumulation induced by chemotactic agents in rabbit skin (Brain et al., 1989). In human skin, ET-1 evoked vasoconstriction, which was surrounded by a flare, due to increased blood flow presumably as a consequence of an axon reflex (Brain et al., 1989). However,

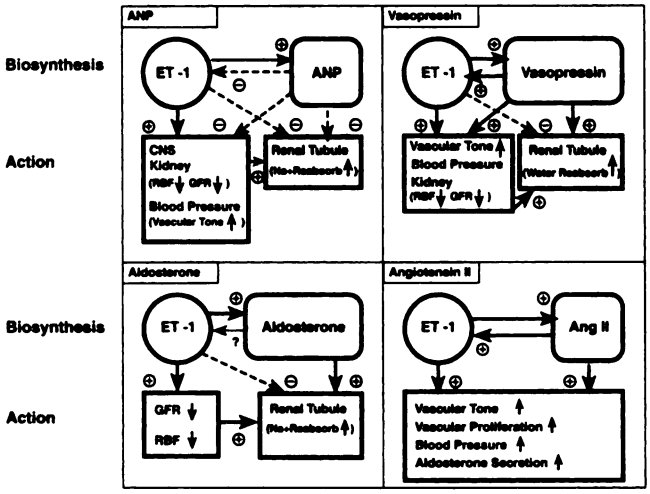

FIG. 12. Interaction at the level of biosynthesis/release and biological activity between ET-1 and various hormones. For details, see section VIII.L. ?, Effect unknown; Ang II, angiotensin II.

intradermal injection of ET-1 together with formyl-Met-Leu-Phe did not suppress neutrophil accumulation in rats (Chander et al., 1989).

L. Endocrine Systems

ETs interact with several endocrine systems and hormones, including the renin-angiotensin system, aldosterone, AVP, and atrial natriuretic peptide. These interactions exist at the level of both biosynthesis and biological actions (fig. 12).

1. Renin-angiotensin system. ET modulates renal and extrarenal renin secretion in vivo and in vitro, stimulates angiotensin II production, and synergizes with many of the biological actions of angiotensin II, which stimulates ET-1 production in endothelial cells (fig. 12).

a. RENIN. Both in vitro and in vivo studies suggest that ET can modulate the secretion of renin from the juxtaglomerular cells of the renal cortex. However, these studies lead to apparently opposite results: in vitro ET-1 invariably suppressed renin secretion, whereas injection of ET-1 into animals either did not affect or increased plasma renin activity.

ET-1 suppresses renin secretion from isolated perfused rat kidney (Munter and Hackenthal, 1989), rat kidney cortical slices, dispersed rat juxtaglomerular cells (Matsumura et al., 1989c; Moe et al., 1991), and isolated rat glomeruli (Rakugi et al., 1988; Takagi et al., 1988, 1989). In isolated perfused rat kidney, ET-3 also suppressed renin release (Yamada and Yoshida, 1991). ET-1 suppressed not only basal, but also isoproterenol-induced, release of renin from isolated juxtaglomerular cells (Moe et al., 1991; Takagi et al., 1988).

The direct inhibitory action of renin release by ET-1 in these in vitro preparations is mediated by an elevated intracellular calcium level, because (*a*) a decrease in the cytosolic Ca^{2+} concentration in juxtaglomerular cells [by removal of extracellular Ca^{2+} or by treatment with Ca^{2+} chelators (e.g., BAPTA) (Moe et al., 1991; Rakugi et al.,

1988) or Ca^{2+} antagonists (TMB-8; Takagi et al., 1989)] stimulates renin release and (*b*) removal of extracellular Ca^{2+} prevented the inhibitory effect of ET-1 in juxtaglomerular cells (Takagi et al., 1988, 1989) in renal cortical slices (Matsumura et al., 1989c) and in isolated perfused rat kidney (Munter and Hackenthal, 1989).

In contrast to findings in vitro, systemic injection of pressor doses (>0.2 nM/kg) of ET-1 to anesthetized (Miller et al., 1989a; Otsuka et al., 1989) or conscious (Goetz et al., 1988; Nakamoto et al., 1989) dogs caused significant elevation of plasma renin activity. Subpressor doses of ET-1 caused no changes in plasma renin activity in conscious rats (Madeddu et al., 1989) or even significantly reduced it in anesthetized dogs (Otsuka et al., 1989) and conscious normal and cirrhotic rats (Claria et al., 1991a,b). Similar observations were made in healthy human volunteers, in whom infusion of 1 to 5 ng/kg/min ET-1, which elevated plasma irET-1 to levels similar to that found in several pathological conditions, increased diastolic blood pressure, reduced heart rate and renal plasma flow, and increased renal vascular resistance but had no effect on plasma renin activity (Gasic et al., 1992; Vierhapper et al., 1990). Similarly to the in vitro findings, infusion of ET-1 (1–4 ng/kg/min) directly into the renal artery decreased renin release significantly in anesthetized dogs (Chou et al., 1990) and in anesthetized rats (Matsumura et al., 1989a). In conscious rats, ET-1 antiserum decreased plasma renin activity and increased urinary sodium excretion (Yamada and Yoshida, 1991). Subcutaneous infusion of ET-1 for a week (0.2 mg/kg/h) decreased plasma renin activity in rats (Mazzocchi et al., 1990b).

In extrarenal tissues (e.g., human decidua) ET-1, ET-2, and ET-3 stimulated (rather than inhibited, as in the kidney) prorenin and renin release (Chao et al., 1993). The cause of opposite effects of ETs on renal and extrarenal renin production is not understood.

b. ANGIOTENSIN-CONVERTING ENZYME AND ANGIOTENSIN II. Although ACE inhibitors do not affect the conversion of pro-ET-1 (big ET-1) to ET-1 (Komuro et al., 1988), several studies suggested an interaction between the two systems. Infusion of ET-1 (5 pmol/kg/min) for 7 days into rats caused a sustained increase in mean arterial blood pressure without changes in plasma angiotensin II level. Coinfusion of captopril (1 mg/kg/h) prevented ET-1-induced hypertension, suggesting either that elevation of blood pressure is caused by ET-1-induced stimulation of the renin-angiotensin system or that existing levels of angiotensin II "permit" or facilitate ET-1-induced vasoconstriction (Takeshita et al., 1991).

Similar observations were made by Yasujima et al. (1991) who found that elevation of mean arterial blood pressure by a 24-hour infusion of ET-1 (60 ng/kg/day) to rats could be prevented by captopril and enalapril. Because the effect of these ACE inhibitors could be reversed by infusion of subpressor doses of angiotensin II, these authors postulated that the ACE inhibitors act by reducing the sensitivity of blood vessels to ET-1 (i.e., subpressor levels of angiotensin II increase the responsiveness). This hypothesis was supported by the finding that treatment of SHR with enalapril reduced the ET-1 sensitivity of perfused hearts isolated from these animals (Takeshita et al., 1991).

In the isolated perfused rat heart, captopril reversed the ET-1-induced coronary vasoconstriction (Neubauer et al., 1990). Neubauer et al. suggested an alternative mechanism for the ACE inhibitors: vasodilation by inhibiting the breakdown of bradykinin, which in turn stimulates the synthesis/release of the potent endogenous vasodilator EDRF (NO).

In cultured HUVECs, captopril and enalapril inhibit calf serum-induced ET release (Yoshida and Nakamura, 1992). Angiotensin II or ACE had no effect, but bradykinin and sodium nitroprusside inhibited ET production. These findings suggest that by inhibiting bradykinin breakdown, ACE inhibitors may increase EDRF (NO) production, which inhibits ET-biosynthesis (Boulanger and Luscher, 1990).

Another potentially important interaction between ET and the renin-angiotensin system was identified by Kawaguchi et al. (1990, 1991). In cultured pulmonary arterial endothelial cells, ET-1 dose-dependently increased the conversion of angiotensin I to angiotensin II by an enalapril-sensitive mechanism. ET-1 (10^{-8} M) stimulated ACE activity 2.5-fold in these cells.

As described above, ET-1 can augment the production of angiotensin II, by elevating plasma renin activity and/or by enhancing ACE activity. Angiotensin II is one of the most potent stimulators of ET-1 synthesis/release (Scott-Burden et al., 1991). Angiotensin II and ET-1 act synergistically to induce vasoconstriction, VSMC proliferation, and aldosterone secretion from the adrenal cortex (Cozza et al., 1992).

2. Aldosterone. Specific ET-binding sites exist in the aldosterone-producing zona glomerulosa of the adrenal gland, and ETs stimulate aldosterone secretion both in vitro and in vivo. There are no data available concerning the effect of aldosterone on ET-1 production. Aldosterone and ET-1 influence renal tubular Na^+ reabsorption in opposite directions (i.e., stimulation by aldosterone and inhibition by ET-1) (fig. 12).

Tissue autoradiography studies revealed significant ^{125}I-ET-1 binding in the medulla and zona glomerulosa (but not in zona fasciculata or reticularis) of the rat adrenal gland (Kohzuki et al., 1989b; Koseki et al., 1989). Similar specific ET-1 binding was demonstrated in cultured bovine adrenal glomerulosa cells (Cozza et al., 1989, 1992; Gomez-Sanchez et al., 1990) and in porcine, human, and rat adrenal gland (Nakamoto et al., 1991).

In bovine adrenal glomerulosa cells ET-1 stimulates aldosterone (but not cortisol) secretion with an EC_{50} of 300 pM (Cozza et al., 1992). Angiotensin II is a more

potent agonist (EC_{50} 40 pM). ET-1 potentiates angiotensin II-induced aldosterone secretion (Cozza et al., 1992). ET-1 was more potent than ET-3, suggesting that the ET_A receptor subtype mediates this effect in the bovine adrenal gland (Cozza et al., 1989; Gomez-Sanchez et al., 1990).

However, in isolated rat glomerulosa cells, ET-1, ET-2, and ET-3 were equipotent in stimulating aldosterone secretion, suggesting that ET_B receptors mediate the response in the rat adrenal tissue (Hinson et al., 1991a,b,c). In frog adrenocortical slices, ET-1 stimulates both aldosterone and corticosterone secretion, which could be inhibited by inhibitors of prostaglandin synthesis and Ca^{2+} entry blockers (Delarue et al., 1990). In rat dispersed zona glomerulosa cells, ET-1 stimulates aldosterone and corticosterone secretion and potentiates adrenocorticotropic hormone-induced aldosterone secretion (Mazzocchi et al., 1990a). In rabbit dispersed adrenal capsular cells, ET-1 stimulated aldosterone secretion with an EC_{50} of 10^{-14} M (Morishita et al., 1989). In dispersed rat adrenal glomerulosa cells, ET-1 did not affect basal release but potentiated adrenocorticotropic hormone- and 8-Br-cAMP-induced aldosterone secretion (Rosolowsky et al., 1990). In rat adrenal glomerulosa cells, ET-1 stimulates aldosterone secretion via IP_3 formation and elevation of $[Ca^{2+}]_i$ (Woodcock et al., 1990). Angiotensin II was more potent than ET-1 or vasopressin.

In slices of normal human adrenal cortex, ET-1 stimulates aldosterone but not cortisol secretion (Zeng et al., 1992). However, ET-1 had no effect on adrenocortical adenomatous tissue from patients with primary hyperaldosteronism (Zeng et al., 1992).

In the perfused rat adrenal gland, adrenocorticotropic hormone stimulates the release of ET-1 (Hinson et al., 1991c). In rat and human dispersed zona glomerulosa cells, ET-1 and ET-3 stimulate aldosterone secretion (Hinson et al., 1991a). In good agreement with these in vitro observations, systemic injection of ET-1 in vivo elevated plasma aldosterone levels in anesthetized dogs (Tsuchiya et al., 1990b,c; Miller et al., 1989a), in conscious dogs (Goetz et al., 1988), and in conscious rats (Mazzocchi et al., 1990b).

Subcutaneous infusion of ET-1 (0.2 mg/kg/h) to conscious rats for a week increased mean arterial blood pressure and circulating levels of aldosterone and caused hypertrophy of the zona glomerulosa (but not the zona fasciculata) in the adrenal gland (Mazzocchi et al., 1990b). Zona glomerulosa cells isolated from these animals secreted significantly more aldosterone and corticosterone under basal conditions than did cells isolated from untreated rats (Mazzocchi et al., 1990b).

3. Arginine vasopressin. Several interactions between ETs and AVP have been described, including stimulation of ET-1 production by AVP, stimulation of AVP secretion by ETs, and synergism, as well as antagonism, in their biological activities (fig. 12).

a. ARGININE VASOPRESSIN STIMULATES ENDOTHELIN SYNTHESIS/RELEASE. Infusion of AVP to conscious dogs increased plasma irET-1 levels (Emmeluth and Bie, 1992). AVP stimulates ET production in human mesangial cells (Bakris et al., 1991), in rat mesangial cells (Sakamoto et al., 1992), in cultured bovine aortic endothelial cells (Emori et al., 1991b), and in primary cultures of rabbit endometrial cells (Oishi et al., 1991). ET and AVP are colocalized in freshly harvested rabbit aortic endothelial cells (Milner et al., 1990; Loesch et al., 1991). In human mesangial cells, AVP-induced mitogenic action is apparently mediated by ET-1, because ET-1 antibodies suppressed the mitogenic action of AVP (Bakris et al., 1991). In cultured bovine endothelial cells, AVP-induced ET-1 production is mediated by V_1 vasopressinergic receptors (Emori et al., 1991b). AVP induces ET-1 gene expression in cultured bovine carotid endothelial cells (Imai et al., 1992b).

b. ENDOTHELINS STIMULATE ARGININE VASOPRESSIN SECRETION IN THE NEUROHYPOPHYSIS AND ELEVATE PLASMA LEVELS OF ARGININE VASOPRESSIN. Infusion of ET-1 (30 ng/kg/min) to conscious dogs increased circulating plasma levels of AVP (Goetz et al., 1988). ET-1 (20 ng/kg/min) infusion for 1 hour decreased RBF and blocked the antidiuretic effect of AVP in conscious dogs (Miller et al., 1989a; Goetz et al., 1989). Intravenous infusion of 40 fmol/kg/min ET-1 for 40 minutes decreased mean arterial blood pressure, baroreceptor sensitivity, pressor response to NE and angiotensin II, and circulating plasma levels of AVP (Nakamoto et al., 1991). Infusion of higher doses of ET-1 (400 fmol/kg/min) increased blood pressure and circulating levels of AVP, suggesting that changes in blood pressure modulate AVP secretion (Nakamoto et al., 1991).

Studies with centrally (i.e., into the third ventricle) injected ETs confirmed these observations but suggested that ETs can stimulate AVP secretion directly and that the secreted AVP contributes to elevation of blood pressure. Intracerebroventricular injection of ET-3 (11 and 23 pmol) caused a 2- and 5- to 7-fold elevation of plasma AVP, respectively, and reduced stimulated water drinking (Gasic et al., 1992). Intracerebroventricular application of ET-1 increased mean arterial blood pressure, an effect that was mediated, at least in part, by vasopressin (i.e., vasopressinergic receptor blockade decreased the pressor response) (Yamamoto et al., 1991, 1992b; Kawano et al., 1989). These findings suggested that ET-1-induced AVP secretion from the CNS, and the consequent elevation in circulating plasma levels of AVP, contributes to the pressor response to centrally applied ET-1. Indeed, systemic injection of ET-1 (50 to 100 pmol) enhanced the activity of neurons in the subfornical organ, which lacks the blood-brain barrier, and stimulated the secretion/release of AVP (Wall and Ferguson, 1992).

Microinjection of ET-1 (5 pmol) into the subfornical organ increased the activity of vasopressinergic neurons in the neurohypophysis (Wall et al., 1992). Thus, both systemically or centrally injected ET-1 can increase AVP secretion in the hypothalamo-neurohypophysial system.

ET-1 and ET-3 potentiate membrane depolarization-induced AVP release from isolated nerve endings of the posterior pituitary via ET_A receptor activation (Ritz et al., 1992). The same nerve endings contain irET, the level of which is regulated by homeostatic mechanisms involved in water balance (Ritz et al., 1992). ET-1 (10^{-9} to 10^{-8} M) stimulates AVP release from perfused rat hypothalamus, an effect that can be blocked by the Ca^{2+} antagonist nicardipine (Shichiri et al., 1989). IrET-3 is present in the median eminence and in the anterior and neurointermediate lobes of the hypothalamus at concentrations much higher than those in the aorta (Samson et al., 1991a,b,c). ET-1 and ET-3 stimulate release of AVP from rat supraoptic nucleus in vitro (brain slices) with equal potency, suggesting that the ET_B receptor subtype may mediate the response (Yamamoto et al., 1992a).

c. INTERACTION BETWEEN THE BIOLOGICAL ACTIONS OF ENDOTHELINS AND ARGININE VASOPRESSIN. Several reports indicate potential interactions between the biological actions of ETs and AVP. In general, ET and AVP act synergistically as vasoconstrictors, but ET appears to inhibit AVP-induced water reabsorption in renal collecting tubules. ET-1 potentiates the vasoconstrictor action of AVP in the perfused rabbit ear artery (Wong Dusting et al., 1991) and the pressor effect of AVP in pregnant rats (Molnar and Hertelendy, 1990).

In isolated mouse kidney cortical collecting tubules, outer medullary collecting tubules, and inner medullary collecting tubules, ET-1 (10^{-10} to 10^{-8} M) inhibited AVP-induced cAMP accumulation. ET-1 had no effect on AVP-induced cAMP accumulation in cortical and medullary ascending limbs of the Henle loop or on parathormone-, calcitonin-, glucagon-, and isoproterenol-induced changes in cAMP in outer medullary collecting tubules (Tomita et al., 1990). Tomita et al. postulated that the observed effects of ET-1 may have the physiological function of maintaining urine volume via an antagonism of the AVP-induced reduction of urine volume. ET-1 counteracts the decrease in renal plasma flow and GFR. Similarly, in freshly prepared rat renal papillary tubules, ETs inhibited AVP-induced cAMP accumulation. ET-1 and ET-3 were equieffective, suggesting that the ET_B receptor subtype mediates the effects (Woodcock and Land, 1992). Infusion of ET-1 into rats caused diuresis, despite a decrease in the renal plasma flow and GFR (Oishi et al., 1991). In isolated perfused rat inner medullary collecting tubules segments, ET-1 (10^{-10} to 10^{-8} M) reversibly inhibited AVP-induced osmotic water permeability without affecting AVP-stimulated urea permeability or dibutyril-cAMP-induced osmotic water permeability. These studies suggest that ET acts at these cells at sites prior to cAMP production in the AVP-induced signal transduction pathway (Oishi et al., 1991).

4. Atrial and brain natriuretic peptide. Interactions between the ETs and ANP/BNP include stimulation of ANP secretion by ET-1, inhibition of ET production by ANP and BNP, and functional antagonism between ETs and ANP in several biological systems (fig. 12).

a. EFFECT OF ENDOTHELINS ON ATRIAL NATRIURETIC PEPTIDE/BRAIN NATRIURETIC PEPTIDE SECRETION. ET-1 stimulates the release of ANP from isolated rat atrial myocytes (Fozard and Part, 1990), neonatal rat cardiomyocytes and fetal rat hypothalamic cell cultures (Gardner et al., 1991; Uusimaa et al., 1992), rat atria (Hu et al., 1988a), cultured fetal rat diencephalic neurons (Levin et al., 1991), perfused rat heart (Mantymaa et al., 1990; Pitkanen et al., 1990), superfused rat atria (Schiebinger and Gomez Sanchez, 1990), isolated rat atria (Stasch et al., 1989), and cultured rat atrial myocytes (Fukuda et al., 1988). ET-1 also stimulates the release of BNP from rat atrial and ventricular cardiomyocytes (Horio et al., 1992).

ET-1-induced ANP release from rat atrial myocytes is significantly higher in cells isolated from the SHR than from normotensive WKY rats (Fozard and Part, 1990). Similarly, ET-1 stimulates basal and stretch-induced ANP release from perfused hearts, an effect that is more pronounced in hearts from SHR than from WKY rats (Mantymaa et al., 1990). ANP secretion stimulation by ET-1 in cardiac myocytes involves an elevation of cytosolic Ca^{2+} (Schiebinger and Gomez Sanchez, 1990) and an activation of PKC (Mantymaa et al., 1990; Pitkanen et al., 1991).

In good agreement with the in vitro studies, intravenous injection of ET-1 elevates circulating plasma ANP levels in the conscious newborn calf (Amadieu et al., 1991), in conscious normal and cirrhotic rats (Stasch et al., 1989; Claria et al., 1991b), in conscious dogs (Nakamoto et al., 1991; Tsuchiya et al., 1990a,b,c), in anesthetized rats (Ohman et al., 1990), and in anesthetized, bilaterally nephrectomized rats (Valentin et al., 1991). In anesthetized rats, ET-1 injection elevates plasma immunoreactive BNP (Valentin et al., 1991).

In several of these studies, only pressor doses of ET-1 elevated plasma ANP or BNP levels, an observation that correlated well with increases in mean arterial pressure (Horio et al., 1992; Tsuchiya et al., 1990a,b,c). Indeed, subpressor doses of ET-1 had no effect on the plasma ANP level in conscious dogs (40 pmol/kg) and in the conscious newborn calf (Tsuchiya et al., 1990a,b,c), suggesting that, in contrast to in vitro studies (in which ET-1 acted directly on cardiac myocytes), in vivo the stimulus for ANP secretion is right atrial stretch due to elevated blood pressure and right atrial pressure. However, in conscious rats ET-1 (0.3 to 3.0 nmol/kg) increased plasma ANP with and without an elevation of right atrial pressure, suggesting that ET-1 can act di-

rectly on atrial myocytes in vivo as well (Ohman et al., 1990). Indeed, in conscious dogs a subpressor dose of ET-1 (40 fmol/kg/min) reduced blood pressure and increased plasma ANP (Nakamoto et al., 1991). However, in conscious and unrestrained rats, intravenous infusion of 1 pmol/kg/min ET-1, which doubled circulating plasma irET-1 levels, decreased plasma ANP (Shirakami et al., 1993). In isolated perfused rat hearts, low concentrations (10^{-11} M) of ET-1 suppressed ANP secretion by an indomethacin-sensitive mechanism (Shirakami et al., 1993).

b. EFFECT OF ATRIAL NATRIURETIC PEPTIDE ON ENDOTHELIN PRODUCTION. The majority of studies showed that ANP effectively reduced ET-1 production in cultured cells. ANP was found to inhibit angiotensin II or thrombin-induced ET-1 secretion from cultured bovine aortic endothelial cells (Hu et al., 1992), cultured rat mesangial cells (Kohno et al., 1992a), cultured HUVECs (Kohno et al., 1991b), isolated porcine aorta (Kohno et al., 1992d), and cultured human endothelial cells (Saijonmaa et al., 1990). In contrast, in cloned rat parathyroid cells and primary cultures of human parathyroid cells (both have ANP/BNP receptors), ANP and BNP stimulate ET-1 synthesis/release in parallel with an elevation of cellular cGMP level (De Feo et al., 1991).

The ANP-induced elevation of cellular cGMP content (Kohno et al., 1991b; 1992b) should play a role in the inhibition of ET-1 production because the nitrovasodilator sodium nitroprusside (Saijonmaa et al., 1990; Boulanger and Luscher, 1991), authentic NO and endothelium-derived NO (Boulanger and Luscher, 1991), and 8-Br-cGMP (Kohno et al., 1992b; Saijonmaa et al., 1990) had similar effects. Only one study reported that ANP inhibited ET-1 production in endothelial cells via a mechanism different from elevation of cGMP (Hu et al., 1992) This study demonstrated that the ANP analog, C-ANP (4 to 23) (which acts only on C-receptors and does not activate guanylate cyclase) had the same effect as ANP (i.e., reduced ET-1 gene expression and mature peptide production by approximately 50%). The guanylate cyclase antagonist LY 83583 did not affect the response. Hu et al. speculated that C-receptor activation by ANP or its analogs leads to a decrease in cellular cAMP level which then mediates the suppression of ET-1 gene expression and protein production (Hu et al., 1992). In addition to ANP, the analogs BNP-45 (Kohno et al., 1992c), CNP-22, BNP-26 (Kohno et al., 1992b,c), and BNP-32 (Kohno et al., 1991b) also suppressed ET-1 production via elevation of cGMP.

c. INTERACTION BETWEEN THE BIOLOGICAL ACTIONS OF ENDOTHELINS AND ATRIAL NATRIURETIC PEPTIDE/BRAIN NATRIURETIC PEPTIDE. ANP is a potent vasodilator and, via stimulation of natriuresis, reduces plasma volume and osmolarity. Theoretically, these actions of the peptide can counteract the vasoconstrictor and renal effects (reduction of renal plasma flow, GFR, and consequently natriuresis and diuresis) of ET-1. Indeed, several studies showed that ANP (and BNP) effectively antagonizes the biological actions of ET-1 both in vitro and in vivo.

In cultured VSMCs, ANP antagonized the mitogenic activity of ET-1 and ET-3 (Neuser et al., 1990a). Blood pressure elevation after an intracerebroventricular injection of ET-1 was effectively attenuated by coinjection of BNP (Makino et al., 1990). Similarly, coinjection of BNP reduces the elevation of plasma AVP level following centrally applied ET-1 (Makino et al., 1992). Intravenous infusion of ET-1 caused a pressor response in conscious rats that was reduced by coinjection of ANP (Ohman et al., 1990). ANP also inhibited ET-1-induced renal vasoconstriction (Suzuki et al., 1991a). Earlier studies suggested that ANP release may mediate the initial transient depressor response to a bolus injection of ET-1 in rats (Winquist et al., 1989b). However, Fozard and Part (1990) demonstrated that ANP plays no role in the vasodilator response to ET-1 in the SHR. ANP may attenuate the pressor response to ET-1, because anti-ANP antibodies facilitated it in anesthetized rats (Valentin et al., 1991).

There are a few studies showing that ET-1 can also effectively antagonize the biological actions of ANP. In cultured rat diencephalic glia cells, ET-1 and ET-3 stimulate mitogenesis and inhibit ANP-induced cGMP elevation (Levin et al., 1992). Because ET-1 and ET-3 were equipotent, the ET_B receptor subtype was postulated to mediate these effects.

ET-1 also effectively antagonized the cardiovascular (decrease in blood pressure), renal (natriuresis and diuresis), and endocrine (AVP, renin, aldosterone) effects of ANP in vivo (Ota et al., 1992).

5. Thyroid gland. In human thyroid epithelial cells (isolated from patients with Grave's disease), ET-1 (10^{-9} M) stimulated [^{3}H]thymidine incorporation and mitogenesis (Eguchi et al., 1992a).

Primary cultures of human thyroid follicular cells produce irET (Tseng et al., 1993). TGFβ increased irET secretion, which could be significantly augmented by thyroxine-stimulating hormone. Thyroxine-stimulating hormone alone had no effect on irET production. Immunostaining for ET could be localized in these cells in the perinuclear region; staining was augmented by thyroxine-stimulating hormone but not by TGFβ (Tseng et al., 1993). The thyroid follicular cells also exhibited a specific high-affinity ET-1-binding site; these sites could be increased by TGFβ but not by thyroxine-stimulating hormone (Tseng et al., 1993). These observations suggest that ET may play an autocrine role in thyroid gland cells.

6. Pancreas. Both prepro-ET-1 and ET-3 mRNA expression and ET receptors were found in the pancreas, where the ETs cause vasoconstriction. In dispersed rat pancreatic acinar cells, specific and high-affinity ^{125}I-ET

binding was observed and identified as ET_A and ET_B receptor subtypes. ETs, however, caused no change in amylase secretion or intracellular Ca^{2+}, IP_3, or cAMP levels. After binding to acinar cells, both ET-1 and ET-3 were rapidly internalized, and the internalization could be attenuated by the secretagogue cholecystokinin-8 (Hildebrand et al., 1993). These data suggest that pancreatic acinar cells may be involved in elimination (clearance) of circulating or locally produced ETs.

M. Central Nervous System

Experimental evidence, such as the following, suggests that ETs are localized in the CNS, modulate several CNS functions, and, therefore, may be regarded as neuropeptides: (*a*) ET gene expression and mature ET production can be detected in the brain, (*b*) specific ET-binding sites are identified in various regions of the human and animal brain, (*c*) ETs activate several cell types in the brain, and (*d*) intracerebroventricular injection of ETs cause significant changes in cardiovascular, respiratory, and neuroendocrine system function and in fluid and electrolyte balance.

1. Endothelin gene expression and production in the brain. RNA blot hybridization provided evidence of ET gene expression in a variety of regions of the human brain (Lee et al., 1990c) and spinal cord (Giaid et al., 1989). Among the regions studied, the hypothalamus and striatum have the highest density of cells containing prepro-ET mRNA (Lee et al., 1990c). In the spinal cord, laminae IV and VI of the dorsal horn and lamina IX of the ventral horn showed the greatest activity (Giaid et al., 1989). The presence of irET-1 and ET-3 was detected in porcine brain (Shinmi et al., 1989a,b). ET-3 is produced in the magnocellular neurons of the hypothalamo-neurohypophyseal system (Samson et al., 1991a,b,c).

Using highly specific and sensitive radioimmunoassays, Ando et al. (1991) found irET-1 and irET-3 in human CSF. The concentration of irET-3 was 150% higher than that of irET-1.

Circulating plasma ET-1 levels increase rapidly with upright posture and decrease with volume expansion in humans. The site of release was postulated to be the neurohypophysis or anteroventral hypothalamus. This sudden change in circulating irET level is in sharp contrast to the slow and delayed production of ET-1 in the circulation by the endothelium, suggesting a different mechanism of peptide release from the CNS.

2. Endothelin-binding sites in the brain. Specific ET-binding sites labeled with ^{125}I-ET-1 have been demonstrated in human brain (Jones et al., 1989; Kurihara et al., 1990). The highest levels were found in the stratum molecular of the dentate gyrus and in the granular layer of the cerebellum, with intermediate levels in the caudate, molecular layer of the cerebellum, and layer VI of temporal cortex; low levels occurred in more superficial cortical layers and in the spinal cord (Jones et al., 1989). Binding sites for ^{125}I-ET-1 and ^{125}I-ET-3 have similar distributions in rat brain (Nambi et al., 1990). High-affinity binding of ^{125}I-ET-1 was observed in cerebellar granule cells and astrocytes (MacCumber et al., 1990) and in primary cultures of brain capillary endothelial cells (Vigne et al., 1990b).

Autoradiograms of ^{125}I-ET-1 binding in the rat brain demonstrated that ET receptors are predominantly localized in the brain stem, basal ganglia, and cerebellum (Koseki et al., 1989). In addition, specific ET-1-binding sites were identified in nuclei that are involved in the CNS regulation of the cardiovascular system: nuclei of the anteroventral hypothalamus, the supraoptic nucleus, and the subfornical organ. ET-1-binding sites were also detected on rat pituitary cell membranes (Calvo et al., 1990).

Quantitative receptor autoradiographic methods revealed high concentrations of specific ^{125}I-ET-1-binding sites in the choroid plexus, subfornical organ, lacunosum molecular layer of the hippocampus, and granular layer of the cerebellum of the rat brain (Niwa et al., 1991a,b). ^{125}I-ET-1 and ^{125}I-STX 6b bind to membranes of rat cerebral cortex, hypothalamus, medulla, and spinal cord on a single, high-affinity site. There was no difference found in binding between WKY rats and SHR (Gulati and Rebello, 1992).

3. Biological actions of endothelins in the brain and neural tissues. Siren and Feurerstein (1989) first demonstrated that intracerebroventricular injection of ET-1 (30 pmol/kg) in rats produced profound pressor and vasoconstrictor responses. Similar observations were made after a microinjection of ET-1 into the area postrema (Ferguson and Smith, 1990), the fourth cerebral ventricle of anesthetized rats (Hashim and Tadepalli, 1992), the cerebral ventricles of conscious rats (Kawano et al., 1989; Makino et al., 1990; Yamamoto et al., 1991) and conscious rabbits (Matsumura et al., 1991a), and the cisterna magna of mongrel cats (Morimoto et al., 1991).

Microinjections of ET-1 into the area postrema region of anesthetized rats resulted in complex changes in blood pressure. Low doses of ET-1 (0.2 to 1.0 pmol) evoked increases, a higher dose (5.0 pmol) evoked decreases, and an intermediate dose (2.0 pmol) evoked biphasic (increase followed by decrease) responses in blood pressure (Ferguson and Smith, 1990). Because other vasoconstrictor substances (injected into the area postrema region) had no effect on blood pressure, the effect of ET-1 on area postrema appears to be specific for the peptide.

Micropneumophoresis of ET-1 (100 to 300 fmol) into the nucleus tractus solitarius produced depressor and bradycardic responses in anesthetized rats (Hashim and Tadepalli, 1992). In anesthetized, but spontaneously breathing, rats, injection of ET-1 (3 to 10 pmol) into the fourth ventricle evoked respiratory depression and a transient pressor response (Hashim and Tadepalli, 1992). Injection of ET-1 (10 to 1000 pmol) into the

cisterna magna caused a significant decrease in regional cerebral blood flow and an increase in arterial pressure in mongrel cats (Morimoto et al., 1991).

In parallel with increased mean arterial blood pressure, intracerebroventricular infusion of ET-1 in rats and rabbits increased plasma catecholamine, AVP, glucose, and adrenocorticotropic hormone levels and enhanced renal sympathic nerve activity (Makino et al., 1990; Kawano et al., 1989; Yamamoto et al., 1991; Matsumura et al., 1991a). V_1-vasopressinergic receptor antagonists (e.g., TMe-AVP), α-adrenergic receptor antagonists (e.g., prazosin), and ganglionic blockade (e.g., pentolinium, hexamethonium) attenuated or completely abolished the pressor response (Kawano et al., 1989; Yamamoto et al., 1991; Matsumura et al., 1991a), suggesting that centrally administered ET-1 activates the sympathoadrenal and AVP systems, which mediate the central pressor response.

Coadministration of BNP (0.2 to 1.0 nmol) attenuated intracerebroventricularly applied ET-1-induced pressor responses and the increase in plasma AVP levels occurring in conscious, freely moving rats (Makino et al., 1992).

Systemic administration of ET-1 increased the activity of subfornical organ neurons which have projections to the paraventricular nucleus of the hypothalamus. Systemic ET-1 (50 to 100 pmol) injection activated vasopressin- and oxytocin-secreting neurons in the paraventricular nucleus and supraoptic nucleus (Wall and Ferguson, 1992). Because ET-1 does not cross the blood-brain barrier (i.e., cannot act directly in paraventricular nucleus and supraoptic nucleus) (Mima et al., 1989; Kadel et al., 1990), it should act on the subfornical organ which lacks this barrier. Indeed, in animals in which the subfornical organ has been lesioned, systemic ET-1 had little or no effect on vasopressin- and oxytocin-releasing neurons (Wall and Ferguson, 1992).

Injection of ET-3 (100 pmol) into the cisterna magna of anesthetized rats caused an initial increase in blood pressure, heart rate, and renal sympathetic nerve activity, which was followed by a depression of all three parameters (Gasic et al., 1992). Simultaneously, the arterial baroreceptor reflex was significantly suppressed. These cardiovascular effects of centrally applied ET-3 were abolished by hexamethonium but were not influenced by vasopressin or angiotensin II antagonists.

When administered into the third cerebral ventricle, ET-3 dose-dependently inhibited water intake in conscious rats exposed to exogenous and endogenous stimuli for drinking (Gasic et al., 1992). Selective ET-3 antibodies (injected into the third ventricle) potentiate angiotensin II-induced water drinking, suggesting that endogenous ET-3 may play a role in the central control of fluid and electrolyte homeostasis.

In conscious rats, intravenous injection of ET-1 (14 nmol/min) stimulates glucose metabolism exclusively in the pituitary intermediate and anterior lobes, whereas intracerebroventricular application (9 pmol) causes a hypermetabolic response in several neuroendocrine structures in the forebrain involved in the regulation of fluid homeostasis, body temperature, and cardiovascular system (Gross et al., 1993b).

ET-1 (Stojilkovic et al., 1990) and ET-3 (Samson et al., 1991c; Kanyicska et al., 1991) stimulate gonadotropin (luteinizing hormone or follicle-stimulating hormone) release in the anterior pituitary, similarly to that produced by hypothalamic gonadotropin-releasing hormone. Substance P was released from perfused rat hypothalamus and anterior pituitary after stimulation with ET-1 (Calvo et al., 1990). ET-3 inhibited prolactin release from cultured pituitary cells (Samson et al., 1991a,b,c). ET-3 (probably the most abundant ET isoform in the CNS) stimulated luteinizing hormone-releasing hormone release from the arcuate nucleus-median eminence in vivo and from an luteinizing hormone-releasing hormone-secreting GT1 neuronal cell line (Moretto et al., 1993).

ET-1 and the neurohypophyseal hormones AVP and oxytocin are colocalized in the neural lobe of rat pituitary (Nakamura et al., 1993).

4. Signal transduction in neural tissues. Less is known about ET-activated signal transduction in neural than in vascular tissue. Both ET-1 and ET-3 increase free intracellular Ca^{2+} levels in cultured astrocytes and C6 glioma cells (Marsault et al., 1990; Marin et al., 1991). ETs stimulate the production of IP_3 in cerebellar and cerebral cortical slices (MacCumber et al., 1990; Ambar et al., 1989; Crawford et al., 1990), cultured cerebellar granule cells and astrocytes (Lin et al., 1990b,c), and C6 glioma cells (Reiser et al., 1990), suggesting that release of Ca^{2+} from inositol phosphate-sensitive intracellular stores contributes to the elevation of $[Ca^{2+}]_i$. ET-1 does not stimulate Ca^{2+} influx or alter $[Ca^{2+}]_i$ levels in rat brain synaptosomes (Hamilton et al., 1989), suggesting that neuronal ET receptors involved in Ca^{2+} signaling may be localized preferentially to postsynaptic sites. Both ET-1 and ET-3 promote astrocyte mitogenesis (MacCumber et al., 1990; Supattapone et al., 1989).

Studies with NG108–15 neuroblastoma cells showed that ET-1 produced a biphasic increase in $[Ca^{2+}]_i$, which consisted of a transient peak elevation, followed by a sustained plateau (Chan and Greenber, 1991). Similar to VSMCs (see section VII), both phases are dependent on extracellular Ca^{2+}, but whereas plateau responses are abolished by the L-type Ca^{2+} channel antagonist nimodipine and enhanced by the dihydropyridine agonist Bay K 8644, the phasic response is insensitive to these agents. Neither component is affected by ω-conotoxin or other drugs that block dihydropyridine-insensitive voltage-operated Ca^{2+} channels.

N. Peripheral Nervous System

The presence of high-affinity-binding sites on ganglia and nerve terminals of peripheral motor, sensory, and

autonomic nerves and modulation of peripheral nerve function by exogenously applied ETs suggests that the peptides may play a role in peripheral nervous system function.

1. Motor and sensory nerves. The presence of ET mRNA in dorsal and ventral horn ganglia in the spinal cord suggested that ETs may play some role in peripheral motor and sensory nerve functions and/or spinal cord motor and sensory reflexes (Giaid et al., 1989). ET-1 reduced sciatic and saphenous motor and sensory nerve conductance in rats probably via reduction of endoneurial nerve blood flow (Zochodne et al., 1992). In the spinal cord, ET-1 activated neurons which were inhibited by spandite, a substance P antagonist (Yoshizawa et al., 1989).

2. Autonomic nervous system. a. SYMPATHETIC NERVES. Most experimental evidence indicates that ETs (especially ET-1) inhibit neurotransmitter (NE) release from sympathetic nerve terminals but potentiate the biological actions of NE on postjunctional sites (vascular smooth muscle) (Wong Dusting et al., 1989, 1990). In isolated perfused rat mesenteric arteries, subthreshold (for direct vascular contraction) doses of ET-1 (10^{-11} to 10^{-10} M) significantly enhanced exogenous NE-induced vasoconstriction but inhibited periarterial (transmural) nerve stimulation-induced vasoconstriction (Nakamura et al., 1989). These studies suggested that in subpressor doses ET-1 modulates adrenergic neuroeffector mechanisms: facilitates it at postjunctional sites and inhibits it prejunctionally. Indeed, ET-1 inhibited transmural electrical stimulation-induced [^{3}H]NE release in the same preparation (Nakamura et al., 1989). Inhibition of [^{3}H]NE overflow was significantly less pronounced in mesenteric arteries isolated from SHR than from WKY rats (Tabuchi et al., 1990).

ET-1, ET-2, and, to a lesser degree, ET-3 (0.3 to 30 nM) significantly potentiated electrical field stimulation-induced contractions in mouse vas deferens, without exerting any effect on basal tension (Rae and Calixto, 1990). Similarly, perivascular nerve stimulation-induced [^{3}H]NE release was suppressed, but exogenous NE-induced vasoconstriction was potentiated, by ET-1 in isolated guinea pig pulmonary artery (Wiklund et al., 1989a,b). Infusion of ETs (0.3 to 3.0 ng/kg/min) into the renal artery of anesthetized dogs suppressed NE efflux but did not affect the decrease in RBF induced by low frequency (0.7 to 1.2 Hz) renal nerve stimulation (Takagi et al., 1991a).

In the rat and guinea pig vas deferens, ET-1 enhanced nerve stimulation and exogenous ATP-induced contraction but had no affect on exogenous NE-induced contractions (Wiklund et al., 1990). [^{3}H]NE overflow evoked by nerve stimulation was suppressed by ET-1, but not by ET-3, in rat vas deferens (Wiklund et al., 1991).

In the venous system, ET-1 facilitated nerve stimulation-induced venoconstriction (rat mesenteric vascular bed) (Warner et al., 1990), and the stimulation of adrenergic nerves augmented the effect of ET-1 on venous tone (Waite and Pang, 1992).

In dog cardiac sympathetic ganglia, ET-3 inhibited ganglionic transmission at preganglionic sites via stimulation of TXA_2 production (Kushiku et al., 1991).

Studies in healthy human volunteers showed that an intraarterial infusion of low concentrations of ET-1 (1 pmol/min) had no effect on sympathetic nerve activation (evoked by lower body negative pressure) or exogenous NE-induced vasoconstriction in the forearm (Haynes et al., 1991; Harrison et al., 1992; Cockcroft et al., 1991). However, intravenous injection of subpressor doses of ET-1 caused significant augmentation of renal sympathetic nerves in dogs (Lerman et al., 1991c).

b. PARASYMPATHETIC NERVES. In contrast to sympathetic neuroeffector mechanisms, very little is known about the effect of ETs on parasympathetic neurons or neuroeffector mechanisms. Using immunohistochemistry, Inagaki et al. (1991) localized ir-ET-1 in both the parasympathetic myenteric and submucosal nerve plexuses of the human colon. In vitro autoradiography revealed specific ^{125}I-ET-1-binding sites in the same neural plexuses, indicating a possible role of ET in the modulation of parasympathetic innervation and mobility and secretion in the human intestine (Inagaki et al., 1991). Indeed, ET-1 stimulated acetylcholine release from parasympathetic neurons (Wiklund et al., 1989c) and potentiated acetylcholine-induced contraction of intestinal smooth muscle. Prejunctional inhibition by ET-3 was demonstrated on cholinergic (guinea pig ileum) neurotransmission (Wiklund et al., 1991).

3. Baroreflex. Local exposure of isolated and endothelium-denuded carotid sinus of anesthetized dogs to ET-1 (10^{-7} M) produced vasoconstriction and suppression of baroreceptor activity (Chapleau et al., 1992). At a lower concentration (10^{-8} M), ET-1 caused only vasoconstriction, suggesting that at higher doses ET-1 acts directly on baroreceptor nerve fibers. Such an action could limit the buffering capacity of the baroreflex and promote hypertension.

Baroreflex sensitivity was attenuated by intravenous infusion of 40 fmol/kg/min ET-1 in conscious dogs but was not affected by 400 fmol/kg/min ET-1 (Nakamoto et al., 1991). Intravenous bolus injection of ET-1 (0.3 to 3.0 nmol/kg) had no significant effect on baroreceptor activity in anesthetized rats (Ohman et al., 1990). In conscious rats, intravenous ET-1 (0.67 nmol/kg) did not alter baroreceptor reflex control of sympathetic efferent nerve activity or heart rate (Knuepfer et al., 1989).

In contrast to topical (desensitization) and systemic intravenous application (desensitization or no effect), injection of ET-1, ET-2, or ET-3 (25 pmol/kg) into the cisterna magna in conscious normotensive rats caused a significant increase of baroreceptor sensitivity by affecting the vagal, but not the sympathetic, component of the

baroreflex (i.e., methylatropine, but not atenolol, prevented the facilitation) (Itoh and Van Den Buuse, 1991).

Activation of the baroreflex stimulates ET release into the plasma (Kaufmann et al., 1991). The results of studies in normal subjects and in patients with primary autonomic failure (no baroreflex) showed that activation of a baroreflex stimulates the release of ET into plasma (probably from the neurohypophysis), and it was suggested that impaired ET release may contribute to orthostatic hypotension of patients with primary autonomic failure (Kaufmann et al., 1991).

O. Presence of Endothelins in Body Fluids

Significant quantities of irET-1 are detected in circulating plasma in several species, including humans (Parker-Botelho et al., 1992; Morel et al., 1989; Pernow et al., 1989; Saito et al., 1989; Suzuki et al., 1990a,b; Yoshimi et al., 1989). The circulating plasma concentrations of ET-1 are in the low picomolar range in healthy humans, which can be elevated 2- to 30-fold in various pathological conditions (for review, see Masaki et al., 1992).

In addition to the circulating peptide, irET has been detected in human urine (Berbinschi and Ketelslegers, 1989). It was found that concentrations of ET were on average 6-fold higher in urine samples than in plasma. Similarly, ET is present in normal human CSF at levels that are significantly greater (approximately 7-fold) than in plasma (Hoffman et al., 1989; Nomura et al., 1989). ET has also been quantified in bronchial lavage fluid where the levels were elevated during the bronchospastic phase of an asthma attack and returned to basal levels after recovery (Nomura et al., 1989).

P. Plasma Half-Life, Elimination, and Metabolism of Endothelins in the Circulation

Although ETs are stable in blood and plasma, the plasma half-life of intravenously injected ETs is short. After intravenous bolus injection of ^{125}I-ET-1 the plasma half-life of intact ET-1 in the rat was 40 seconds and of total radioactivity 68 seconds in anesthetized rats (Sirvio et al., 1990). This rapid decay was virtually unchanged even when pressor amounts (1 to 2 nmol/kg) of cold ET-1 were coadministered with the radiolabeled tracer. In the pig, the plasma half-life of ET-2 and ET-3 was 1 to 2 minutes, whereas that of big ET-1 was 9 minutes (Hemsen et al., 1991b).

In the anesthetized rat approximately 60% of intravenously injected ET-1 or ET-3 is removed from the circulation within the first minute (Anggard et al., 1989). The quick disappearance of ETs from the circulation is due to first-pass elimination by the lung and kidney (Striba et al., 1989). The role of the lungs in the removal of circulating ET was demonstrated by De Nucci et al. (1988) who found that more than 50% of infused ET-1 was removed by the lungs after one passage. Although the lung removes approximately 90% of circulating ETs, excretion via the kidney, and to a smaller extent via the liver, also contributes to the elimination of the peptides from the circulation. For example, ^{125}I-ET-1 was taken up predominantly by the lungs (82%) and, to a lesser extent, by the kidneys (10%), heart (3.6%), liver (2.7%), and spleen (1.5%) in rats (Sirvio et al., 1990). Thus, the characteristically long-lasting pressor responses evoked by the ETs in whole animals are not due to their persistence in the plasma but, rather, to the slow dissociation from the receptors.

The neutral metalloendopeptidase (EC 3.4.24.11; NEP or MMP I) cleaves ET-1 and ET-3; this enzyme has been identified in rat kidneys (Vijaraghavan et al., 1990). Initial cleavage of ET-1 occurs between Ser^5 and Leu^6 within one of the intramolecular loops, whereas the action against ET-2 and ET-3 is initiated between Asp^{18} and Ile^{19} in the COOH terminus. Inhibition of NEP by SQ-29,072 significantly elevated plasma levels and urinary excretion of endogenous and exogenous ET-1 in anesthetized rat (Abassi et al., 1992), suggesting that ET-1 degradation by NEP contributes to the elimination and inactivation of the peptide from the circulation in vivo.

IX. Potential Physiological Significance of Endothelins

The discovery that ETs, which are close structural and functional relatives of components (STXs) of a deadly snake venom, are expressed in several human tissues and circulate in human blood did not inspire many in the scientific community to envision a physiological role for the peptides. Even after 6 years of extensive research, it is widely assumed that ETs must contribute to many pathological events (see section X) rather than to play a role in physiological homeostatic mechanisms. Indeed, at this time, it requires some stretch of imagination to propose such a role for ETs, mostly because of lack of sufficient data to support it. The mechanisms listed below are hypothetical, and only future studies with potent and selective tools (e.g., receptor antagonists) will determine whether these hypothesis are true or not. In addition to the few examples discussed here, potential physiological roles of ETs were postulated in other organs as well (e.g., contribution to menstruation), which are briefly mentioned in previous sections.

A. Integrated Role of Endothelins in Cardiovascular Homeostasis

1. Maintenance of basal vascular tone. In some vascular beds, after all known neurohumoral control mechanisms have been inhibited, the vascular smooth muscle is not completely relaxed, indicating the presence of basal vascular tone. The mechanism of maintenance of basal vascular tone is still unknown. One postulate is an intrinsic mechanism in the SMCs sensitive to changes in transmural pressure. Another possibility is a still unknown circulating substance. A third is that the endo-

thelial cells produce factors that may contribute to basal vascular tone.

ET is produced by cultured endothelial cells at a slow basal rate, and although its expression can be stimulated by various agonists, facilitation of ET release is detectable only several hours after stimulation. There is no evidence that ET is stored in endothelial cells; therefore, its release may be directly connected to its de novo synthesis. Because of its high vasoconstrictor potency and long-lasting action, the continuous release of small amounts of ET could contribute to the maintenance of vascular tone (Rubanyi, 1989). An important feature of ET-induced vascular contraction is that it can be inhibited by most known vasodilator agents and by the potent endogenous vasodilator EDRF (NO). If basal tone is maintained by ET, it may be balanced by the tonic release of EDRF (NO) under physiological conditions, and it should be effectively antagonized by local vasodilator mechanisms in case of increased metabolic requirements. An imbalance between the production of ET and EDRF (NO) could lead to pathologically elevated vascular tone (see section X).

2. Modulation of endothelin biosynthesis and action in the vascular wall. Both biosynthesis by the endothelium and bioactivity of ET-1 on vascular smooth muscle and adrenergic nerve terminals are modulated by several factors in the intact blood vessel wall (fig. 13).

a. MODULATION BY THE ENDOTHELIUM. ET-induced contraction of isolated blood vessels in vitro is effectively antagonized by the endothelium-derived vasorelaxant substances, PGI_2 and EDRF (NO) (Schini et al., 1991; Ito et al., 1991; Myatt et al., 1992; Ohde et al., 1992; Berti et al., 1990). De Nucci et al. (1988), Otsuka et al. (1990), and Hom et al. (1990), using inhibitors of cyclooxygenase (e.g., indomethacin, aspirin), and Lerman et al. (1992b), Madeddu et al. (1991), and Whittle et al. (1989), using inhibitors NO synthase (e.g., L-NMMA), showed that both PGI_2 and EDRF (NO) could suppress the pressor activity of ETs in vivo.

PGI_2 and its stable analogs (e.g., carbacyclin) effectively antagonize ET-1-induced DNA synthesis in cultured VSMCs (Nakaki et al., 1991). Defibrotide prevented ET-1-induced vasoconstriction in human saphenous veins by releasing PGI_2 (Berti et al., 1990).

In addition to reducing bioactivity, nitric oxide also inhibits endothelin production in vascular endothelium. Boulanger and Luscher (1990) first demonstrated that, in isolated porcine aortic preparations, thrombin- and A23187-induced, stimulated release of ET-1 could be potentiated by L-NMMA and methylene blue and suppressed by superoxide dismutase and 8-bromo-cGMP. Because thrombin and the Ca^{2+} ionophore both stimulate the release of EDRF (NO) as well, the data suggested that NO could inhibit ET-1 synthesis/release from the endothelium of porcine aorta. However, basal release of ET-1 was not affected by any of the inhibitors used. In contrast, L-NMMA had no effect on thrombin-induced ET-1 release from cultured endothelial cells (Boulanger and Luscher, 1991), suggesting that this interaction between NO (cGMP) and ET-1 synthesis does not exist in cultured cells. It may require the presence of other cell types (e.g., smooth muscle) found in the blood vessel wall (see below) or may be an inherent property of native endothelial cells that are lost in culture. Indeed, cGMP-dependent protein kinase activation rapidly declines in cultured endothelial cells after the first few passages, providing a potential explanation for why NO-induced, elevated cGMP is unable to modulate ET-1 production in cultured endothelial cells after several passages. However, Cocks et al. (1991) found that oxyhemoglobin (which inactivates NO) increased the production of ET-1 in cultured endothelial cells. In brain microvessel endothelial cells, 8-Br-cAMP suppressed ET-1 production (Durieu-Trautmann et al., 1993), suggesting that both cyclic nucleotides may modulate ET-1 production. Exposure of cultured HUVECs to 6 to 25 dyn/cm^2 for 6 hours or more inhibited ET-1 release, an

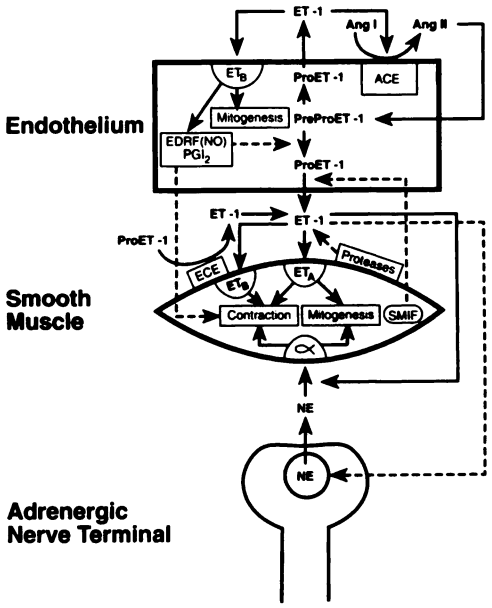

FIG. 13. Factors modulating the biosynthesis and bioactivity of ET-1 in the blood vessel wall. ET-1, synthesized and secreted by endothelial cells, acts on ET_A and ET_B receptors on underlying vascular SMCs and initiates contraction and mitogenesis in a paracrine fashion. ET-1 also interferes with noradrenergic neurotransmission by inhibiting NE release from adrenergic nerve endings but potentiating NE action on smooth muscle. ET-1 also acts on ET_B receptors on endothelial cells in an autocrine-signaling mode stimulating EDRF (NO) and PGI_2 synthesis and mitogenesis. ET-1 activates ACE, and angiotensin II (Ang II) stimulates ET-1 biosynthesis and acts synergistically with ET-1 on vascular smooth muscle contraction and mitogenesis (not shown). EDRF (NO) inhibits ET-1 biosynthesis in endothelial cells and together with PGI_2 inhibits smooth muscle contraction. SMCs produce a diffusible factor(s) (smooth muscle-derived inhibitory factor, SMIF) which inhibits conversion of big ET-1 to ET-1 in endothelial cells (see also fig. 14). SMCs also convert big ET-1 to mature ET-1 (ECE) and contain proteases that degrade ET-1. For further details, see section IX. Solid arrow, stimulation; dashed arrow, inhibition; α, α-adrenergic receptor.

action that could be prevented or reversed by N^G-nitro-L-arginine (Kuchan and Frangos, 1993). 8-Bromo-cGMP and ANP mimicked, whereas isobutyl methylxanthine potentiated, the inhibitory effect of shear stress, suggesting that shear stress-induced inhibition of endothelial ET-1 production is NO and cGMP mediated.

b. MODULATION BY SMOOTH MUSCLE CELLS. In coculture systems, the presence of vascular (Stewart et al., 1990) or bronchial (Cade et al., 1991) SMCs significantly inhibited the accumulation of ET-1 and big ET-1 in the culture medium of endothelial cells. At least two mechanisms may be responsible for the observed phenomenon: (*a*) SMCs release a diffusible factor(s) that suppresses the production of ET-1 by endothelial cells and/or (*b*) proteases associated with the SMC degrade ET-1 and big ET-1.

Exposure of bovine pulmonary artery endothelial cells in culture to conditioned medium of human bronchial SMCs significantly suppressed the amount of ET-1 released from bovine pulmonary endothelial cell (Cade et al., 1991). A decrease in the amount of ET-1 can be explained by two different mechanisms: the diffusible factor(s) either inhibits the production of ET-1 by endothelial cells and/or proteolytically degrades it. The second possibility could be ruled out because the conditioned medium of human bronchial SMCs had no significant effect on the concentration of ET-1 given to the medium in the absence of bovine pulmonary endothelial cells. Thus, the diffusible and stable (at least for 24 hours under the given conditions) factor(s) (smooth muscle inhibitory factor, or smooth muscle-derived inhibitory factor) must inhibit the production of ET-1 by endothelial cells. The observation that, in contrast to ET-1, the amount of big ET-1 was not significantly influenced by the conditioned medium of human bronchial SMCs (fig. 14) suggested that the diffusible factor(s) interfered with the final step of ET-1 biosynthesis (i.e., conversion of big ET-1 to ET-1 by ECE) (Cade et al., 1991). Neither the precise mechanism of action nor the nature of the diffusible factor(s) is known at the present time.

In contrast to the conditioned medium alone, the presence of human bronchial SMCs themselves prevent the accumulation of ET-1 and big ET-1 in the conditioned medium of bovine pulmonary endothelial cell (Cade et al., 1991). Because human bronchial SMCs had a similar effect on ET-1 and big ET-1 in the absence of endothelial cells, the phenomenon cannot be due to inhibition of ET-1 production.

The factor(s) associated with the SMCs that prevents ET-1 or big ET-1 from accumulating in the culture media appears to be either physically attached to the human bronchial SMC or present in higher concentrations only when the SMCs are present. This is based on the fact that no ET-1 or big ET-1 accumulated in cell cultures, but the media from human bronchial SMC did not exert the same effect as the cells themselves.

These mechanisms, if present in blood vessels in vivo, may provide effective local protection against the potent vasoconstrictor peptide.

3. Indirect control of vascular tone and plasma volume via interaction with neuroendocrine mechanisms. In addition to direct contraction of vascular smooth muscle, ETs may play a role in the control of vascular tone and resistance by a variety of indirect mechanisms as well, including (*a*) modulation of central control mechanisms (via enhancing sympathetic nerve activity or vasopressin release and baroreceptor sensitivity) by ET produced in the CNS or circulating ET acting on areas of the CNS lacking the blood-brain barrier (e.g., subfornical organ); (*b*) ET released in the vicinity of baroreceptors (carotid sinus, aortic arch, etc.) may suppress their activity; (*c*) modulation of adrenergic neurotransmission in chromaffin cells of the adrenal medulla and in the blood vessel wall can lead to an increased/decreased liberation of NE, respectively, and augmentation of postsynaptic effects of the neurotransmitter; and (*d*) local modulation of the synthesis/release of other endothelium-derived vasoactive substances (EDRF, PGI_2) and of angiotensin II.

The known effects of ET on renal function suggest a synergistic action with aldosterone (i.e., antinatriuresis) but via a different mechanism. Exogenous administration of ET causes a dose-dependent decrease in GFR. Corresponding reductions in urine flow and sodium excretion occur, leading to Na^+ retention, increased plasma osmolality, and volume. These latter changes can influence cardiovascular function (e.g., blood pressure) independently of changes in vascular tone.

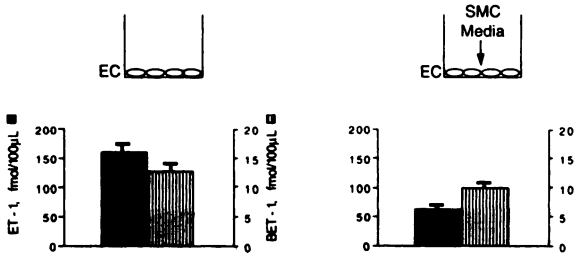

FIG. 14. Effect of culture media from human bronchial SMCs on ET-1 and big ET-1 (BET-1) accumulation (24 hours) in bovine aortic endothelial cell (EC) culture medium. Addition of SMC media to cultured endothelial cells (right) significantly reduces ET-1, but not big ET-1, accumulation, suggesting that an SMC-derived diffusible factor(s) inhibits conversion of big ET-1 to ET-1 in endothelial cells. For further details, see text.

B. Regulation of Water Balance

Water loss stimulates compensatory mechanisms, which include increased sympathetic activity, via baroreceptor inhibition, and secretion of vasopressin or antidiuretic hormone, via stimulation of osmoreceptors in the CNS. Inhibition of baroreceptors can also stimulate the release of antidiuretic hormone. Both mechanisms will reduce water excretion in the kidney with resultant normalization of plasma volume. Local production of ET

(either in peripheral tissues or the CNS) may contribute to these regulatory (homeostatic) pathways in several ways: (*a*) inhibition of baroreceptor function, (*b*) constriction of glomerular afferent arterioles, (*c*) reduction of GFR in the kidney, and (*d*) effects on vasopressin (antidiuretic hormone) secretion (via a central mechanism or through baroreceptor resetting) (for review, see Rubanyi and Shepherd, 1992). An increase in circulating ET was reported in severe hypotension (e.g., cardiogenic, endotoxin, and hemorrhagic shock) and release of ET from the neurohypophysis during water deprivation (Yoshizawa et al., 1990). These data suggest that ET may be involved in a compensatory homeostatic mechanism controlling water balance. The demonstration that centrally applied (or produced) ET-3 and ET-1 can inhibit water intake further supports this hypothesis.

C. Contribution to Local (Hemostasis) and Systemic Homeostatic Mechanisms in Hemorrhage

The currently available data may suggest that the ET system serves an important role in protecting the organism from the lethal consequences of hemorrhage, both locally (hemostasis) and systemically (fig. 15). Thrombin and TGFβ are potent stimulators of ET biosynthesis and release. One may speculate that, in the event of blood vessel injury and consequent blood coagulation, the locally generated TGFβ- and thrombin-stimulated release of ET and the long-lasting vasoconstriction triggered by the peptide contribute to local hemostasis, a role thus far attributed primarily to serotonin released from activated platelets (fig. 15). This mechanism may be especially effective in the case of endothelial dysfunction (i.e., loss of EDRF production), because thrombin is also a potent stimulant of EDRF release.

At the systemic level, in the case of significant hemorrhage, there is marked activation of the sympathoadrenal and the renin-angiotensin-aldosterone systems. As noted already, angiotensin II is also a stimulator of ET secretion, and the two vasoactive peptides act in synergy in many biological systems, including vasoconstriction and elevation of total peripheral vascular resistance. Furthermore, ET might function systemically during hemorrhage to enhance fluid retention via its indirect antinatriuretic effects (reduction of glomerular filter load in the kidney and stimulation of aldosterone secretion) and to preserve perfusion pressure. Additionally, ET-induced venoconstriction would increase preload, thereby possibly further augmenting cardiac output in this setting.

It seems then that during hemorrhage ETs (especially ET-1) might serve as important local mediators at the site of bleeding to reduce the amount of blood loss, as well as acting systemically to restore the balance between vascular bed capacity and plasma volume via enhanced fluid retention in an attempt to increase circulating plasma volume and to maintain perfusion pressure through arterial vasoconstriction and increased cardiac output (fig. 15).

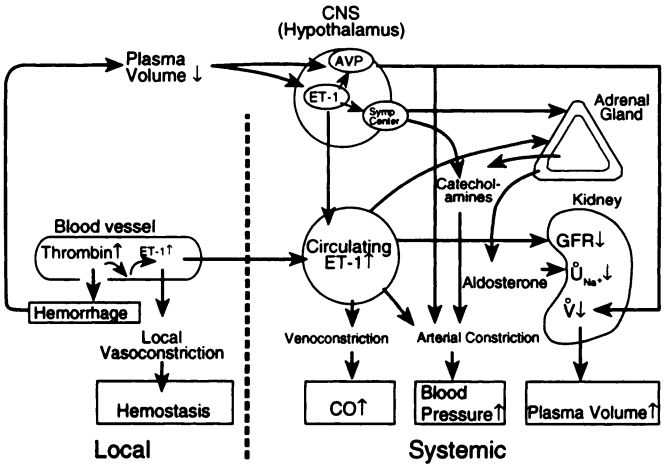

FIG. 15. Local (left) and systemic (right) effects of ET-1 that may play a role in physiological homeostatic mechanisms in the cardiovascular system after blood loss. Stimulation of ET-1 secretion by thrombin (and other locally produced factors, e.g., TGFβ) during vascular injury and rupture of blood vessels (hemorrhage) cause long-lasting vasoconstriction that contributes to local hemostasis. Decrease in plasma volume following hemorrhage (and in other pathological conditions, such as septicemia, congestive heart failure, etc.) initiates complex neuroendocrine compensatory mechanisms. Circulating and CNS-derived ET-1 was postulated to play a role in this homeostatic mechanism by (*a*) stimulating AVP secretion and activation sympathoadrenal centers in the CNS, (*b*) stimulation of aldosterone and catecholamine secretion from the adrenal gland, and (*c*) reducing RBF and GFR and, as a consequence, Na^+ (UNa^+) and water excretion (V) in the kidney. In synergy with renal actions of aldosterone (Na^+ excretion) and AVP (water excretion), ET-1 may contribute to restoration of plasma volume. In synergy with the sympathoadrenal system and angiotensin II (not shown), ET-1 many contribute to the restoration of systemic arterial blood pressure. Being a potent venoconstrictor and positive inotropic and chronotropic agent in the heart (not shown), ET-1 may increase cardiac output (CO). The following biological actions of locally produced ET-1 may cause effects that counteract these systemic actions: (*a*) direct natriuretic and diuretic action in renal tubules and augmented vascular permeability in the lung may lead to reduction of plasma volume and (*b*) coronary vasoconstriction and increased total peripheral resistance may reduce cardiac output. For further details, see text.

D. Paracrine-, Autocrine-, and Endocrine-signaling Modes of Endothelins

1. Paracrine-signaling mode. Data supporting a paracrine action of ETs are numerous, and therefore, this type of signaling mode of the peptides is the most widely recognized and accepted to date. In general, the local source of ETs is the endothelium (e.g., blood vessels, heart), epithelium (e.g., airways, renal tubules/collecting ducts, intestine, uterus, urinary tract), neurons (CNS, intestine), or specialized cells (e.g., Sertoli cells in testis, mast cells in bone marrow, keratinocytes in skin) (for details, see section VIII).

Furthermore, because ET-1 appears to be released from the posterior pituitary during water deprivation and has been shown to promote vasopressin release, it has been suggested that ET released from nerve termi-

nals in the pituitary may act locally to modulate release of neurosecretory hormones such as vasopressin and oxytocin. Recently, a paracrine function for ET was also proposed in human mammary tissue. Cultured breast epithelial cells were found to exhibit low constitutive expression of mRNA for ET-1, and release of immunoreactive peptide was enhanced in response to prolactin administration. Breast stromal cells located proximally to the epithelial cells did not express mRNA for ET but were found to contain specific cell surface receptors for ET-1; ET-1 mRNA is synthesized in close proximity to ET-1-binding sites in several other organs tested, including the lung, kidney, intestine, eye, bone, skin, testis, and uterus.

2. Autocrine-signaling mode. Reports of the existence of functional receptors on ET-producing cells, including endothelial, smooth muscle, epithelial, and cancer cells, raised the possibility that ETs may act in an autocrine fashion as well.

Indeed, studies of brain and retinal microvessel and capillary endothelial and neighboring cells (pericytes, astrocytes) suggested that, in addition to paracrine mechanisms (pericytes, astrocytes), ET-1 produced by the endothelium may act in an autocrine fashion to modulate endothelial cell function as well (Vigne et al., 1990b, 1991; Frelin et al., 1991; Ishibashi et al., 1992; Chakravarthy et al., 1992; Takahashi et al., 1989; Yokokowa et al., 1991a).

A potential sustained positive feedback loop of autocrine stimulation of VSMC growth/proliferation by ET was proposed by Hahn et al. (1990). These authors showed that, although quiescent rat VSMCs did not constitutively express prepro-ET-1 mRNA, it could be induced by various growth factors (e.g., TGFβ, PDGF-A), vasoactive hormones (angiotensin II and AVP), and ET-1 itself. In addition, ET-1 stimulated the expression of transcripts for PDGF-A chain, TGFβ, and thrombospondin in quiescent VSMCs. In a recent study Alberts et al. (1994) showed that transfection of SMCs with the ET-1 gene leads to release of mature human ET-1 into the culture medium which, in turn, stimulates SMC proliferation in an autocrine manner.

Further sites of potential autocrine-signaling mode for ETs include the cardiac myocytes, thyroid and parathyroid gland cells, renal inner medullary collecting duct epithelial cells, and urinary bladder SMCs.

3. Endocrine-signaling mode. There are some data that support a potential endocrine function of ETs. Exogenously administered ETs have a wide range of actions on cardiovascular, renal, and several endocrine systems. However, in the majority of these studies pharmacological doses of ETs were administered that increased plasma irET to levels exceeding those observed in physiological or pathological conditions (Haynes and Webb, 1991).

Three studies (one in dogs, two in healthy human volunteers) tested the effects produced by a 2- to 3-fold elevation of circulating plasma irET-1 level. In six healthy, sodium-loaded human volunteers, injection of human ET-1 (1, 2.5, and 5 ng/kg/min) increased ir-ET-1 plasma levels from 1.2 ± 0.3 to 3.2 ± 1.9, 9.9 ± 7.6, and 56.5 ± 50.3 pmol/liter, respectively. Mean arterial blood pressure and serum K^+ level increased and serum Na^+ level decreased, but no change occurred in plasma aldosterone, renin, or ANP levels (Vierhapper et al., 1990). The lowest dose, at which a 3-fold elevation in plasma irET-1 was obtained, resulted in no significant change in blood pressure. Higher doses caused a significant reduction in RBF. Interpretation of this human study is limited by the lack of monitoring of key parameters, including cardiac output and systemic vascular resistance, as these might have been changed by ET-1 despite the lack of effect on mean arterial blood pressure.

Indeed, in another study with healthy human volunteers, infusion of 1 to 5 ng/kg/min ET-1 elevated plasma irET-1 to levels similar to that found in several pathological conditions and increased diastolic blood pressure, reduced heart rate and renal plasma flow, and increased renal vascular resistance (Gasic et al., 1992).

ET-1, infused into normal dogs at a dose that simulated pathophysiological levels of plasma irET-1 levels had no significant effect on systemic blood pressure but caused a significant increase in systemic vascular resistance, a reduction in RBF, and a decrease in cardiac output (Lerman et al., 1991c). These studies showed that moderate elevation of circulating ET-1 levels can cause significant biological responses, suggesting an endocrine-signaling mode for the peptide, especially in pathological conditions in which plasma irET levels are high and/or the responsiveness of target organs to ETs increases (see section X). A summary of direct local (paracrine, autocrine) and indirect systemic (endocrine) actions of ETs in several organ systems is presented in table 4.

X. Pathophysiology

Fulfillment of the following criteria would argue for a role of ETs in the pathophysiology of human diseases: (*a*) the local production by vascular (or other) tissues and/or the circulating plasma levels of the peptide are elevated because of increased production, decreased degradation, and/or delayed elimination; (*b*) the vasoconstrictor, proliferative, or other pathologically relevant cellular responses of ETs are augmented either because of increased responsiveness of target cells to ET and/or because of reduced counterbalance mechanisms (e.g., reduced production of vasodilators or diminished degradation/inactivation of the peptide); and (*c*) anti-ET antibodies, selective ET receptor antagonists, or inhibitors of its production show beneficial effects on the respective pathology in animal models of the disease and, ultimately, in humans.

In most of the diseases discussed below (for an overview, see table 5) the first and, in part, the second criteria

TABLE 5
*Diseases for which a pathophysiological role for ETs was postulated**

Cardiovascular diseases
Hypertension†
Malignant forms/stages
Complications (stroke, acute myocardial infarction, renal failure)
Hemangioendothelioma‡
Heart
Coronary vasospasm
Unstable angina
Myocardial ischemia†
Acute myocardial infarction
Reperfusion injury
Congestive heart failure
Brain
Vasospasm following SAH†
Stroke
Blood vessels
Restenosis following percutaneous transluminal coronary angioplasty
Atherosclerosis
Raynaud's disease
Diabetic microangiopathies (retinopathy, neuropathy,† nephropathy)
Shock
Cardiogenic
Hemorrhagic
Septic
Kidney diseases
Acute renal failure due to:
Ischemia/reperfusion†
Cyclosporin A†
X-ray contrast agents
Chronic renal failure (glomerulosclerosis)
Glomerulonephritis
Hepatorenal syndrome (cirrhosis of the liver)
Lung diseases
Bronchial asthma
Pulmonary hypertension
Hypoxic pulmonary vasoconstriction
Gastrointestinal diseases
Gastric ulcer
Inflammatory bowel disease
Crohn's disease
Ulcerative colitis
Disorders of the Female Reproductive System
Premature labor†
Dysmenorrhea
Preeclampsia
Carcinogenesis

* Predominantly based on circumstantial evidence, including increased local ET gene expression and production, elevated circulating plasma irET levels, augmented binding and/or action of ET-1. (For details, see text in section X.)

† Selective ET receptor antagonists (e.g., BQ-123, Ro 46-2005), ET biosynthesis inhibitor (e.g., phosphoramidon), or anti-ET antibody showed beneficial effects in animal models.

‡ Tumor removal decreased blood pressure and plasma irET-1 level.

have been fulfilled. So far, only in a few cases were ET receptor antagonists or ET production inhibitors used. Thus, the demonstration of true pathological significance of endogenous ETs in these (and maybe also in other) disease states must await future animal and, eventually, clinical studies with the available tools and also with the more selective and potent receptor antagonists and ECE inhibitors to be developed in the future.

A. Vasospasm

Being the most potent endogenous vasoconstrictor known, ET-1 was initially postulated to be involved in the pathogenesis of a local (vasospasm) or generalized (hypertension) increase in vascular tone. The long-lasting vasoconstriction induced by ET-1 make it an ideal candidate for initiation and/or maintenance of vasospasm. The pathogenic role of overproduction of and/or increased reactivity to ET-1 was considered in coronary, cerebral, and peripheral (e.g., Raynaud's disease) vasospastic episodes.

1. Coronary vasospasm. The following findings support a potential role of ET-1 in coronary vasospasm: (*a*) irET-1 plasma levels are elevated locally (coronary circulation) during vasospastic episodes (angina) in patients (Matsuyama et al., 1991; Toyo-oka et al., 1991; Toyo-oka and Sugimoto, 1991); (*b*) ET-1 causes long-lasting (spastic) contractions in human (Chester et al., 1992; Yang et al., 1990b,c) and canine coronary arteries (Kurihara et al., 1989a,c; Saito et al., 1992) which is augmented in coronary arteries with endothelial denudation or dysfunction due to ischemia or reperfusion (Clozel and Sprecher, 1991; Neubauer et al., 1991; Saito et al., 1992), and (*c*) subthreshold concentrations of ET-1 potentiate the coronary vasoconstrictor action of other substances (e.g., serotonin) in isolated human coronary arteries (Yang et al., 1990b,c; Chester et al., 1992).

In patients with coronary artery disease, stimulation of vasospasm by acetylcholine or ergonovine causes significant elevation of irET-1 in coronary sinus plasma without changes in the irET-1 plasma level of a peripheral artery (Toyo-oka et al., 1991) or the aortic arch (Matsuyama et al., 1991). The latter study also found that the irET-1 level increased only in patients whose coronary sinus lactate levels were increased as well (sign of myocardial ischemia). Elevated plasma irET-1 levels were found in patients with provoked (acetylcholine, ergonovine) coronary vasospasm, with signs of myocardial ischemia (Miyauchi et al., 1991a).

2. Cerebral vasospasm following subarachnoid hemorrhage. SAH is followed by cerebral vasospasm in the majority of patients. Several pathogenic factors (e.g., hemoglobin) were suggested to be responsible for the link between SAH and vasospasm. The following evidence (accumulated over the past years) suggest that ET may be one of the potential factors mediating SAH-induced cerebral vasospasm: (*a*) plasma and CSF irET-1 levels are significantly elevated in patients with SAH (Levesque et al., 1990; Mosaoka et al., 1989; Shigeno and Mima, 1990; Suzuki et al., 1990a,b, 1992a; Fujimoto et al., 1992; Kraus et al., 1991; Lam et al., 1991) and in the canine two-stage model of SAH (Yamaura et al., 1992; Shigeno and Mima, 1990; Matsumura et al., 1991c); (*b*)

inhibition of ET-1 biosynthesis (e.g., by phosphoramidon; Matsumura et al., 1991c), monoclonal antibodies against ET-1 (Yamaura et al., 1992), and administration of the ET_A receptor antagonist BQ-123 (Clozel and Watanabe, 1993) significantly reduced/prevented vasospasm following SAH in dogs; (*c*) injection of exogenous ET-1 mimics cerebral vasospasm after SAH (Asano et al., 1990; Ide et al., 1989; Kobayashi et al., 1991; Hunrich et al., 1991); (*d*) ET-1 is a potent vasoconstrictor in isolated basilar arteries (most common site of vasospasm) of several species, including human, and the reactivity of cerebral blood vessels to ET-1 increases significantly after SAH (Nakagomi et al., 1989; Alafaci et al., 1990, 1991; Papadopoulos et al., 1990; Ide et al., 1989; Asano et al., 1990; Willette and Sauermilch, 1990); and (*e*) hemoglobin stimulates the synthesis/release of ET-1 from cultured endothelial cells (Ohlstein and Storer, 1992).

In patients with SAH plasma irET-1 is elevated for 2 weeks after the onset of disease; the plasma level was higher in patients with cerebral vasospasm (Suzuki et al., 1992a). In contrast, no change was observed in the irET-1 level in CSF in the first 3 days following hemorrhage. In patients with vasospasm, CSF irET-1 level increased significantly on days 5 to 7 after hemorrhage, coinciding with the onset of cerebral vasospasm. In SAH patients in whom vasospasm did not develop, no change in the CSF irET-1 level was observed (Suzuki et al., 1992a).

Kraus et al. (1991) demonstrated significant elevation of irET-3 level in CSF only in patients with SAH and vasospasm but not in healthy volunteers or in patients with head injury or epilepsy. Similarly, the CSF irET-3 level elevation was demonstrated in patients with SAH but not in patients with cerebral infarction, subdural hematoma, or brain tumor (Suzuki et al., 1990a,b).

In the two-stage dog model of SAH, irET-1 in plasma of basilar artery was elevated only on day 2, but not on day 7, following SAH (Ikuya et al., 1992). Interestingly, topical application of anti-ET-1 monoclonal antibody effectively prevented basilar artery vasospasm when applied on day 2 but not on day 7. These data suggested that ET-1 may play the role of initiation of vasospasm in the early stages of the disease in this animal model.

Intraarterial injection of ET-1 (1 nmol/kg) and big ET-1 (10 nmol/kg) cause SAH in anesthetized rabbits (in the distal part of the basilar artery vascular bed) (Huneidi et al., 1991). Although angiotensin II infusion caused a greater increase in systemic blood pressure, it did not initiate SAH in this model (Huneidi et al., 1991). Intracisternal application of BQ-123 (10 nmol) completely prevented cerebral vasospasm following SAH in the rat, but intravenous injection (3 mg/kg) had no effect (Clozel and Watanabe, 1993). The mixed ET_A + ET_B receptor antagonist Ro 46–2005 (3 mg/kg, intravenously) significantly attenuated a decrease in cerebral blood flow 30, 60, and 120 minutes following SAH in rats (Clozel et al., 1993a).

However, there are an equal number of studies that do not support the concept that ET-1 plays an important role in the pathogenesis of cerebral vasospasm after SAH. These studies found that, in patients and dogs with SAH, irET plasma and CSF levels did not correlate with the development of cerebral vasospasm (Shigeno and Mima, 1990). Phosphoramidon did not prevent cerebral vasospasm in dogs (Shigeno and Mima, 1990). Intracerebroventricular administration of BQ-123 (10^{-4} M) or phosphoramidon (2×10^{-4} M) did not prevent SAH-induced cerebral vasospasm in canine basilar arteries exposed to autologous blood (Cosentino et al., 1993).

3. Raynaud's disease. Raynaud's disease is a progressive vascular disorder that is provoked by exposure to cold and is characterized by frequent and sudden decreases in blood flow to the fingertips. It is postulated that excessive and long-lasting contraction (spasm) of arteries in the fingers leads to these symptoms. Endothelial cell dysfunction (i.e., reduced synthesis/release of such vasodilator substances as PGI_2 and NO) and/or augmented synthesis/release of endothelium-derived vasoconstrictor factors (e.g., ET-1) were suggested to play an important role in the pathogenesis of this disease (Cimminiello et al., 1991). Indeed, the irET-1 level was found to be elevated in patients with Raynaud's disease (Biondi et al., 1991). Zamora et al. (1990) reported a 3-fold elevation of plasma irET-1 in these patients compared to healthy volunteers (5.3 vs. 1.7 pg/ml, respectively). Cooling of the hands caused a moderate elevation of plasma irET-1 in healthy subjects (from 1.7 to 2.7 pg/ml), whereas a significant increase was detected in patients with Raynaud's disease (from 5.3 to 10.3 pg/ml), a finding that correlated well with cooling-induced vasospasm (Zamora et al., 1990). Elevated ET levels were found in the cutaneous vasculature of patients with Raynaud's disease (Dowd et al., 1990). Plasma irET-1 levels were also significantly elevated in patients with systemic sclerosis and diffuse scleroderma (Yamane et al., 1991, 1992).

B. Hypertension

At the time of the discovery of the ETs, the first clinical syndrome in which a pathological role for the peptide was postulated was hypertension (Hickey et al., 1985; Yanagisawa et al., 1988a,b). Irrespective of the etiology of the disease (e.g., essential, renal), hypertension is characterized by a general increase in peripheral vascular resistance, which is due, in part, to an elevated vascular tone and also to structural alterations of the blood vessel wall. The sometimes fatal consequences of hypertension (e.g., acute myocardial infarction and stroke), on the other hand, are caused by tissue ischemia and vascular disorders in specific regions of the body. Because ET is the most potent vasoconstrictor substance

known both in vitro and in vivo, and several of its biological actions may cause elevated peripheral vascular resistance or local vascular disorders, it was postulated that the peptide is, indeed, involved in the pathogenesis of hypertension and its sequelae. However, to date the evidence for ET's involvement in mild to moderate hypertension is controversial. In contrast, its role in fulminant forms or stages of the disease and its renal, myocardial, and cerebral complications is supported by convincing evidence.

1. Vascular production and circulating levels of endothelin in hypertension. Increased basal and stimulated release of ET from the vasculature was reported in animal models of hypertension. The mesenteric artery of the SHR releases more peptide under basal conditions than do the same arteries from WKY rats (Miyamori et al., 1991). In prehypertensive, salt-sensitive Dahl rats, ouabain stimulates more ET production than in normotensive controls (Goligorsky et al., 1991). In desoxycorticosterone acetate hypertensive rats, the plasma clearance of ET-1 is prolonged (Yokokawa et al., 1992).

The circulating plasma levels of irET-1 are not significantly altered in rats with various forms of experimental hypertension (Suzuki et al., 1991b; Vemulapalli et al., 1991), unless malignant forms of hypertension were studied (Kohno et al., 1991a).

In some studies of patients with essential hypertension, moderately elevated plasma irET levels were found (Kohno et al., 1990b; Saito et al., 1991; Shichiri et al., 1990; Lerman et al., 1991a), whereas in other studies no elevation could be observed (Haak et al., 1992; Davenport et al., 1991; Predel et al., 1990; Schiffrin and Thibault, 1991). These inconclusive data could mean that circulating irET levels are not elevated in mild to moderate human essential hypertension but could also be due to problems with the techniques of detection (e.g., cross-reactivity between ET isoforms, big ET and their degradation products) and/or due to the fact that vascular endothelial cells may release the peptide preferentially toward the underlying vascular wall and circulating levels do not represent accurately the local production of the peptides that act predominantly in a paracrine/autocrine fashion at their site of release rather than in an endocrine-signaling mode.

In contrast, consistently elevated circulating irET was demonstrated in patients with malignant hypertension (Yokokawa et al., 1991b,c, 1992) and in severe hypertension with end-organ complications, such as renal failure (Widimsky et al., 1991), advanced arteriosclerosis (Luscher et al., 1990), or preeclampsia (Taylor et al., 1990; Florijn et al., 1991).

2. Altered responsiveness to endothelin in hypertension. The observed changes in pressor responsiveness in animals with hypertension or in vasoconstrictor reactivity of isolated blood vessels are inconclusive. In the SHR, the pressor responses to ET are reported to be moderately elevated (Miyauchi et al., 1989), reduced (Winquist et al., 1989a), or not altered (Hirata et al., 1989b). In isolated aorta of SHR the sensitivity to ETs (compared to aortas isolated from WKY rats) was reported to be enhanced (Clozel, 1989; Vemulapalli et al., 1991), reduced (Ashida et al., 1991; Dohi and Luscher, 1991), or unchanged (Criscione et al., 1990). Reduced responsiveness was demonstrated in the aorta and mesenteric arteries of desoxycorticosterone acetate salt hypertensive (Nguyen et al., 1992; Deng and Schiffrin, 1992) and renovascular hypertensive (two-kidney, one clip) rats (Dohi et al., 1991). Reduced responsiveness does not necessarily argue against a role of ETs in the development of hypertension. It may reflect previous exposure of these blood vessels to elevated levels of ET-1, leading to receptor downregulation, tachyphylaxis, or sustained receptor occupancy. Indeed, in contrast to adult animals, resistance arteries of young prehypertensive rats are very sensitive to the vasoconstrictor effect of ET-1 (Dohi and Luscher, 1991). In contrast to the inconclusive results with the direct responsiveness, the potentiating effect of subpressor or subthreshold concentrations of ET on vasoconstriction induced by other agonists is increased in hypertension (Tabuchi et al., 1989; Dohi and Luscher, 1991; Yang et al., 1990c; Dohi et al., 1992).

Renal arteries isolated from SHR show increased reactivity to ET (Tomobe et al., 1988). Based on its importance in blood pressure regulation, alteration of ET responsiveness in the renal vascular bed may play an important role in the long-term regulation (elevation) of systemic blood pressure.

In addition to the direct smooth muscle responsiveness, ET-induced vasoconstriction can be increased by impaired endothelial generation of counterbalancing vasodilator substances [e.g., PGI_2 and EDRF (NO)]. Indeed, in several animal models of hypertension (e.g., SHR, desoxycorticosterone acetate) endothelium-dependent relaxation is impaired (for review, see Luscher, 1991). Endothelium-dependent relaxation of resistance arterioles from hypertensive animals to ET-1 is significantly reduced (Dohi and Luscher, 1991), suggesting that endothelial protection against ET-1-induced vasoconstriction is depressed in hypertension.

It is unknown, whether other regulatory mechanisms (e.g., ET-1 inactivation by SMCs or PMNs) are also altered in hypertension.

3. Effect of endothelin biosynthesis inhibitors and endothelin receptor antagonists on blood pressure in hypertension. Phosphoramidon, a nonselective, neutral metalloendopeptidase inhibitor and an effective inhibitor of ET-1 production (Sawamura et al., 1990a,b, 1991; Matsumura et al., 1990) was reported to decrease blood pressure in SHR (McMahon et al., 1991a), suggesting that ET-1 production contributes to increased peripheral vascular resistance in SHR. In contrast, anti-ET anti-

body had no effect on blood pressure in SHR (Takagi et al., 1991b).

The selective ET_A receptor antagonist BQ-123 inhibited the pressor effect of an intravenous injection of ET-1 in rats (Bazil et al., 1992). BQ-123 significantly decreased blood pressure in stroke-prone, but not in normal, SHR (Nishikibe et al., 1993), suggesting that ETs may contribute to the elevation of blood pressure and probably also to its complications (atherosclerosis, myocardial and cerebral ischemia, renal failure, etc.) especially in malignant forms of hypertension. However, even in stroke-prone SHR, BQ-123 caused only partial reduction in blood pressure. This finding may be due to the fact that ETs play only a minor role or that blockade of ET_A receptors is insufficient to totally prevent the effect of ETs. A recent study with the orally active mixed ET_A + ET_B receptor antagonist, Ro 46–2005, was highly effective in decreasing blood pressure in rats (Clozel et al., 1993a), demonstrating that ETs may play an important role in this disease and that both ET_A and ET_B receptor subtypes may be involved.

4. Hypertension associated with endothelin-secreting hemangioendothelioma. The pathophysiological role for ET-1 in hypertension was supported by the clinical findings of Yokokawa and colleagues (1991b,c, 1992). Two cases of hemangioendothelioma, a rare malignant tumor associated with hypertension and elevated plasma ET-1 levels, were reported. Blood pressure and plasma irET-1 were decreased in both cases after surgical excision of the tumor, and in one patient recurrence was associated with redevelopment of hypertension and elevated circulating ET levels. In one case, tumor content of ET-1 and ET-1 mRNA was shown to be higher than in normal skin. Plasma ET levels in these patients were 15- to 20-fold higher than in normal subjects which, considering the rapid clearance of ET-1 from the circulation, suggests profound secretion of ET-1 by the tumor.

C. Pregnancy-associated Hypertension (Preeclampsia)

Progressive hypertension developing during pregnancy (preeclampsia, toxemia) is associated with a generalized endothelial cell activation (dysfunction) in the maternal circulation, which results (among others) in overproduction of ET-1, reduced production of EDRF (NO) and PGI_2, and the acquisition of prothrombotic properties of endothelial cells. These changes were postulated to contribute to the symptoms of high blood pressure, inadequate tissue perfusion/oxygenation, and the syndrome of disseminated intravascular coagulation.

Several clinical studies demonstrated significant elevation of plasma irET-1 in preeclampsia (Clark et al., 1992; Ihara et al., 1991c; Mastrogiannis et al., 1991, 1992; Nova et al., 1991; Schiff et al., 1992; Taylor et al., 1990; Florijn et al., 1991; Otani et al., 1991). However, in some studies no change of circulating irET-1 level was found in the maternal plasma during preeclampsia (Benigni et al., 1992; Lumme et al., 1992). That hypertension alone is not the cause of increased production of ET-1 was demonstrated by Ihara et al. (1991c) who found no change in irET-1 in pregnant women with preexisting hypertension.

Several studies showed good correlation between symptoms of preeclampsia and circulating plasma irET-1 levels. Magnesium infusion decreased blood pressure and plasma irET-1 level in patients with preeclampsia but had no affect on these parameters in preterm or term (normal) pregnant women (Mastrogiannis et al., 1992). Elevated plasma irET-1 levels returned to control values within 48 hours following cesarean section, showing good correlation with the time course of mean arterial blood pressure reduction (Taylor et al., 1990). In patients with preeclampsia and disseminated intravascular coagulation, the plasma irET-1 level was significantly higher than in patients with hypertension alone (Ihara et al., 1991c). Plasma irET-1 levels in normal pregnant women abusing cocaine were elevated similarly to those found in women with preeclampsia (Samuels et al., 1993).

It is still unknown, however, whether the elevated plasma irET-1 level is only the marker of endothelial cell activation/dysfunction or rather the local overproduction of ET-1 contributes to the pathogenesis of the disease.

D. Pulmonary Hypertension and Hypoxic Vasoconstriction

Measuring irET-1 concentrations in the arterial and venous blood, Levy et al. (1990; Stewaer et al., 1991b) found that the arterial to venous ratio was significantly less than 1 in normal subjects, close to 1 in patients with secondary pulmonary hypertension of various etiologies, and significantly greater than 1 in patients with primary pulmonary hypertension. It appears that the normal lung clears ET from the circulation and that this clearance function is decreased in secondary pulmonary hypertension which can contribute to elevated circulatory ET-1 levels in this condition (Levy et al., 1990; Stewart et al., 1991b). In addition to decreased clearance, increased production of ET-1 may occur in the lung of patients with primary pulmonary hypertension. Increased expression of ET-1 mRNA in an animal model of primary pulmonary hypertension (Stelzner et al., 1990) and in patients with pulmonary hypertension (Giaid et al., 1993) seems to support this hypothesis. In patients with secondary pulmonary hypertension due to valvular heart disease, circulating plasma irET-1 level is significantly elevated; these levels are reduced to normal after surgical correction of valvular disease (Chang et al., 1993).

During pulmonary alveolar hypoxia, irET-1 in rat lung is increased (Shirakami et al., 1991), and with prolonged hypoxia, constrictor sensitivity to ET-1 increases and vasodilation is lost (Eddahibi et al., 1991). These findings

raise the possibility that ET-1 may be involved in sustaining chronic hypoxic pulmonary hypertension.

Normobaric hypoxia (10% O_2) increased circulating ET-1 levels and stimulated ET-1 gene expression in the lung and right atrium but not in the organs supplied by the systemic circulation of the rat (Elton et al., 1992).

E. Ischemia

The potent and long-lasting vasoconstriction produced by ETs raised the possibility that the peptides may contribute to a reduction of blood flow (ischemia) to several organs, including the heart, brain, lung, and kidney. Increased synthesis/release of ET-1 from the endothelium during hypoxia and ischemia, combined with findings of (*a*) elevated tissue and circulatory plasma levels of ET-1, (*b*) enhanced reactivity of blood vessels during ischemia/reperfusion, (*c*) augmented binding of ET-1 to ischemic tissues, (*d*) sporadic reports of prevention of ischemic tissue damage by ET antibodies, and (*e*) ET biosynthesis inhibitors (e.g., phosphoramidon) or ET_A receptor antagonists (e.g., BQ-123), further supports the potential role of ETs in the pathomechanism of ischemic tissue injury.

1. Myocardial ischemia, reperfusion injury, and acute myocardial infarction. Animal studies provided compelling evidence for the potential involvement of ET in myocardial damage during ischemia. Exogenously administered ET-1 is a potent vasoconstrictor of large and small coronary arteries leading to signs of myocardial ischemia in dogs (Hori et al., 1991; Kurihara et al., 1989a,c; Hom et al., 1992; Muramatsu et al., 1991; Salvati et al., 1991) and pigs (Adachi et al., 1991a; Ezra et al., 1989). Coronary artery occlusion (ischemia) followed by reperfusion causes significant facilitation of ET-1-induced coronary artery vasoconstriction in the dog heart in vivo and in vitro (Clozel and Sprecher, 1991; Saito et al., 1992) and in the isolated perfused rat heart (Brunner et al., 1992; Watts et al., 1992; Neubauer et al., 1991). Exogenous ET-1 has direct proarrhythmic effects on the myocardium in dogs in vivo and in vitro (Salvati et al., 1991; Hom et al., 1992). ^{125}I-ET-1 binding to cardiac membranes is increased following ischemia and reperfusion in rat hearts (Liu et al., 1990; Nayler et al., 1992). Ischemia/reperfusion stimulates ET-1 synthesis/release from isolated rat heart (Brunner et al., 1992), from dog heart in vivo (Tsuji et al., 1991), and from rat heart in vivo (Watanabe et al., 1991). The ET-1 biosynthesis (ECE) inhibitor phosphoramidon causes significant reduction of myocardial infarction size in anesthetized rats 24 hours after ischemia (Grover et al., 1992). The monoclonal antibody against ET-1 (Aw ET N 40; which antagonized the effect of ET-1 and ET-2, but not that of ET-3, in vitro and in vivo) significantly reduced myocardial infarction size in anesthetized rats (Watanabe et al., 1991).

These findings in animal experiments are supported by findings of a significant elevation of circulating plasma irET-1 levels in patients with acute myocardial infarction (Lam et al., 1991; Matsuyama et al., 1991; Miyauchi et al., 1989, 1991a, 1992a; Naruse et al., 1991a,b; Stewart et al., 1991a,c; Takahara et al., 1991; Yasuda et al., 1990).

a. ENDOTHELIN CAUSES MYOCARDIAL ISCHEMIA. In anesthetized open-chest pigs and dogs, an intravenous injection of both ET-1 and the Ca^{2+} channel opener Bay K 8644 causes coronary vasoconstriction and elevation of mean arterial blood pressure (Adachi et al., 1991a; Hom et al., 1992). However, although Bay K 8644 increased, ET-1 decreased the maximal rate of force development (Hom et al., 1992). Intracoronary injection of ET-1 caused myocardial ischemia, and Bay K 8644 did not. Ca^{2+} antagonists prevented the effect of Bay K 8644 but not the coronary vasoconstrictor effect of ET-1; thus, ET-1 must act via a different mechanism (Adachi et al., 1991a).

In isolated rat heart ET-1 causes coronary vasoconstriction and reduction of the maximal rate of force development, which could be prevented by BQ-123, suggesting that these effects are mediated by an ET_A receptor (Grover et al., 1992). ET-1 did not reduce the maximal rate of force development, if coronary flow was maintained constant, indicating that its negative effect on myocardial contractility is secondary to a reduction in blood supply (Grover et al., 1992). Topical application of low concentrations (pmol) of ET-1 on the epicardium of dog hearts causes local ischemia (Hori et al., 1991). In anesthetized dogs, intracoronary injection of 60 to 100 pmol ET-1 causes a reduction of coronary blood flow and the maximal rate of force development, which are followed by ST segment elevation, atrioventricular conductance disturbances, and left ventricular fibrillation (Muramatsu et al., 1991).

b. ISCHEMIA/REPERFUSION POTENTIATES CORONARY VASOCONSTRICTION INDUCED BY ENDOTHELIN. Low perfusion pressure potentiates coronary vasoconstriction induced by ET-1, but not that of angiotensin II, in the dog heart in situ (Clozel and Sprecher, 1991). In the isolated perfused rat heart, 30 minutes of coronary artery occlusion (ischemia), followed by reperfusion, potentiated ET-1 (0.04 to 400 pmol) induced coronary vasoconstriction, probably via increased number/affinity of ET receptors and absence of EDRF (NO) and vasodilator prostaglandin (PGI_2) release from the endothelium (Neubauer et al., 1991). In contrast, hypoxia/reoxygenation suppressed ET-1-induced coronary vasoconstriction in the same preparation (Neubauer et al., 1991). In dog hearts in situ, ischemia-reperfusion augmented ET-1-induced coronary vasoconstriction and reduced endothelium-dependent relaxation (EDRF production) (Saito et al., 1992). Interestingly, in isolated coronary arteries, the postischemic potentiation could be observed only if activated PMNs were added to the preparation (Saito et al., 1992). In perfused rat heart ischemia/reperfusion augmented an ET-2-induced increase in coronary vascular resistance.

In perfused rat hearts, ischemia (30 minutes) and reperfusion potentiated ET-1-induced coronary vasoconstriction (Watts et al., 1992). The potentiation was not accompanied by significant changes in ^{125I}-ET-1 binding, suggesting that the potentiation is probably due to reduced synthesis/release of EDRF (NO) (Watts et al., 1992).

Ischemia/reperfusion-induced increased ET-1 production and myocardial infarction size were both significantly attenuated by adenosine in anesthetized dogs (Velasco et al., 1993).

c. CHANGE IN ^{125}I-ENDOTHELIN BINDING IN ISCHEMIC MYOCARDIUM. Conflicting results were reported concerning changes in myocardial ET-binding sites following ischemia. ^{125}I-ET-1 binding to cardiac membranes increased following 20 to 90 minutes of ischemia, and further increased after reperfusion, in isolated rat hearts (Liu et al., 1990; Nayler et al., 1992). In contrast, no change in ET-1 binding was found in the ischemic rat heart (Neubauer et al., 1991; Watts et al., 1992), except for a receptor downregulation in the left atria (Neubauer et al., 1991).

d. ISCHEMIA-REPERFUSION STIMULATES SYNTHESIS/RELEASE OF ENDOTHELIN IN THE MYOCARDIUM. In the isolated perfused rat heart, irET-1 release was reduced by approximately 90% during ischemia, but it was increased by approximately 400% during reperfusion (Brunner et al., 1992). In anesthetized rats, ischemia followed by 60 minutes of reperfusion caused a significant elevation in plasma (approximately 4-fold) and myocardial (approximately 7-fold) irET-1 (Watanabe et al., 1990, 1991). The irET-1 level in the plasma of the coronary central vein in the heart of anesthetized dogs in situ did not change during 45 minutes of coronary occlusion but increased 2-fold after 60 minutes of reperfusion (Tsuji et al., 1991).

e. PLASMA IMMUNOREACTIVE ENDOTHELIN LEVEL IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION, ANGINA, AND PERCUTANEOUS TRANSLUMINAL CORONARY ANGIOPLASTY. In patients with acute myocardial infarction, the plasma irET-1 level was reported to be approximately 7-fold higher (3.43 ± 1.03 pmol) than in healthy volunteers (0.54 ± 0.05 pmol) (Lam et al., 1991). Especially high plasma irET-1 levels are found in patients with hemodynamic complications (Tomoda, 1993). In patients with acute myocardial infarction plasma and urine irET-1 and immunoreactive big ET-1 levels are elevated, and a novel high molecular weight irET form appears, suggesting that biosynthesis and/or degradation of ET is changed as well (Naruse et al., 1991a). Naruse et al. found that there is no change in circulating thrombomodulin level (endothelial cell activation/injury marker) when irET-1 levels increase in patients with acute myocardial infarction (Naruse et al., 1991b). Plasma irET-1 level elevation was found to be an early sign of acute myocardial infarction (Stewart et al., 1991a). No change in irET-1 plasma level (coronary sinus) occurs in patients with stable angina (i.e., myocardial ischemia but no infarction) (Stewart et al., 1991c). Yasuda et al. (1990) found elevated plasma irET-1 levels in the acute phase of acute myocardial infarction; the plasma level correlated well with the severity (extent) of the infarction. No change in plasma irET-1 level was found in patients with stable angina pectoris (Yasuda et al., 1990). In patients following percutaneous transluminal coronary angioplasty, coronary sinus irET-1 is significantly increased, whereas no change can be observed in the plasma from the femoral artery (Tahara et al., 1991).

2. *Cerebral ischemia: stroke.* Although there are less experimental data available than for myocardial ischemia, the potential role of ETs in the pathomechanism of cerebral ischemia is supported by both animal studies and clinical observations in patients. The following experimental data support the potential involvement of ETs in cerebral ischemia: (*a*) ETs are potent vasoconstrictors of large and small cerebral arteries both in vivo and vitro, and the long-lasting and extensive vasoconstriction produced by exogenously administered peptides causes tissue damage that is similar to that observed following ischemia in rat, cat, and dog brain (Mima et al., 1989; Agnati et al., 1991; Fuxe et al., 1992; Kurosawa et al., 1991; Macrae et al., 1991a,b; Robinson et al., 1991); (*b*) bilateral carotid artery occlusion (cerebral ischemia) followed by reperfusion is associated with elevated plasma irET-1 in gerbils (Willette et al., 1993); and (*c*) in anesthetized rabbits intracerebroventricular injection of ET-1 causes significant reduction of CSF production via sustained vasoconstriction in the choroid plexus (Schalk et al., 1992).

Transient forebrain ischemia reduced (by approximately 15%) ET receptor density and increased (approximately 2-fold) irET-1 in gerbil brain (Willette et al., 1993). Transient forebrain ischemia significantly augmented ET-1- and ET-3-like immunoreactivity in the hippocampus of stroke-prone SHR (Yamashita et al., 1993).

In anesthetized rats, intravenous or intraarterial injection of ET-1 (100 to 300 pmol) causes cerebral vasoconstriction and electroencephalographic signs of cerebral ischemia (Willette et al., 1990). Intracerebroventricular injection of ET-1 causes focal ischemia in the cortex of rat brain (Agnati et al., 1991). Intracisternal injection of ET-1 causes vasoconstriction leading to ischemic cerebral lesions developing 24 to 48 hours after the injection (Fuxe et al., 1992). Occlusion of one common carotid artery in gerbils for 30 minutes followed by reperfusion causes an elevation of plasma irET-1 that persists for at least 12 hours (Giuffrida et al., 1992). Intracisternal injection of ET-1 (0.01 to 0.03 nmol) to conscious rats causes reductions in blood flow and signs of ischemic tissue damage in the brainstem (Macrae et al., 1991a). Topical application of 0.25 nmol ET-1 to the middle

cerebral artery in the rat in vivo causes a reduction in blood flow and ischemic cell damage similar to that observed after occlusion of the same arteries (Robinson et al., 1991).

These findings in animal experiments were supported by clinical studies demonstrating significant elevation of plasma irET-1 in patients with cerebral ischemia/infarct (stroke). In 16 patients with nonhemorrhagic cerebral infarction, the plasma irET-1 level was elevated 4-fold compared to healthy volunteers, whereas the plasma irET-3 level showed no change (Ziv et al., 1992). Ziv et al. postulated that excessive production of ET-1 may cause vasoconstriction in the collateral circulation, thereby enlarging the area of tissue damage (infarct size).

In addition to promoting ischemia through vasoconstriction, ETs might contribute to the pathogenesis of stroke by direct effects on neurons or glia. Damage (death) to neurons after stroke is thought to result largely from increases in $[Ca^{2+}]_i$ (Choi and Rothman, 1990). Indeed, ET-1 and ET-3 increase $[Ca^{2+}]_i$ in cultured glia and neuroblastoma cells (Yue et al., 1990; Marsault et al., 1990). ETs might also contribute to ischemic neuronal injury indirectly by stimulating the release of excitatory amino acids (Lin et al., 1990c), which play a key role in neuronal damage in stroke (Choi and Rothman, 1990).

Intrahippocampal injection of ibotenic acid increases ET-like immunoreactivity in pyramidal neurons and astrocytes (Cintra et al., 1989). Intrathecal (Hokfelt et al., 1989) and intrastriatal (Fuxe et al., 1989) injection of ET-1 produces lesions characterized by neuronal loss, which resemble those observed in ischemia.

3. Acute renal ischemia and nephrotoxic substances. Acute renal failure following an ischemic episode or caused by toxic substances (e.g., cyclosporin A, X-ray contrast agents, endotoxin) is characterized by a reversible, but severe, depression (sometimes complete cessation) of renal functions, frequently requiring hemodialysis in these patients. The syndrome is usually accompanied by significant vasoconstriction and reduction of renal plasma flow, GFR, and tubular functions, all of which are reminiscent of the renal actions of exogenously administered ETs. The renal vasculature is one of the most sensitive vascular beds to the vasoconstrictor action of ET-1. Indeed, systemic injection of subpressor doses of ET-1 causes a reduction of RBF in conscious dogs (Lerman et al., 1991c) and human volunteers (Gasic et al., 1992).

Experimental evidence suggests the involvement of ETs (especially ET-1) in the development of acute renal failure following ischemia and in response to nephrotoxic substances. Injection of exogenous ET-1 causes potent and long-lasting renal vasoconstriction and mimics the syndrome of acute renal failure (Kon et al., 1989; Shibouta et al., 1990; Firth et al., 1988; McMurray et al., 1992). ^{125}I-ET-1-binding affinity and/or receptor number on renal vasculature was found to be increased following ischemia/reperfusion in rats (Clozel et al., 1991; Nambi et al., 1993; Kon et al., 1992), although Wilkes et al. (1991) found no change in ET-1-binding affinity or receptor number in postischemic rat kidney. Ischemia (45 minutes) and reperfusion (6 hours) significantly augmented ET-1 mRNA but decreased ET-3 mRNA in the kidney (Firth and Ratcliffe, 1992). These changes were long lasting, persisting for days. These findings support a role for ET-1 in postischemic renal vascular phenomena and also demonstrate that the two ET genes are differentially regulated. Ischemia/reperfusion-induced and renal toxic substance-induced (cyclosporin A, X-ray contrast media, endotoxin, amphotericin-B) acute renal failure is associated with elevation of plasma and urine levels of irET-1 in rats [Kon et al., 1992 (ischemia); Lopez-Farre et al., 1991a (ischemia); Shibouta et al., 1990 (ischemia); Heyman et al., 1992 (amphotericin-B); Margulies et al., 1991 (X-ray contrast medium); Morel et al., 1991 (endotoxin); Perico et al., 1990 (cyclosporin A)]. A nonionic, low osmolar contrast agent (ioversol) caused much smaller stimulation of ET-1 production in the rat kidney in vivo and in cultured endothelial cells than an ionic and high osmolar agents (e.g., iothalamate) (Heyman et al., 1993).

Anti-ET-1 monoclonal antibody prevented renal vasoconstriction following ischemia-, cyclosporin A-, and endotoxin-induced acute renal failure in the rat (Kon et al., 1992), postischemic renal failure and stimulated glomerular PAF production in the rat (Lopez-Farre et al., 1991b), ischemia-induced tubular necrosis and Ca^{2+} accumulation in necrotic tissue (Shibouta et al., 1990), and cyclosporin A-induced renal vasoconstriction in isolated perfused rat kidney and in rat kidney in situ (Perico et al., 1990). However, ET-1 antibody was ineffective in reversing or preventing postischemic renal failure in one study (Takabatake et al., 1991). The selective ET_A receptor antagonist BQ-123 (0.5 mg/kg/min for 2.5 hours) protected the rat kidney in situ against ischemia-induced acute renal failure and tubular cell injury (Mino et al., 1992). Ro 46–2005 (3 mg/kg, intravenously), a mixed ET_A + ET_B receptor antagonist, significantly reduced renal vasoconstriction following reperfusion (Clozel et al., 1993a).,

Cyclosporin A contracted, in a dose-dependent manner, isolated glomerular afferent and efferent arterioles (Lanese and Conger, 1993). In afferent arterioles, BQ-123 completely blocked the vasoconstriction, but in efferent arterioles, the ET_A receptor antagonist had no effect on cyclosporin A-induced vasoconstriction. Pentoxifylline attenuated cyclosporin A-induced ET-1 secretion and vasoconstriction in autoperfused dog kidney (Carrier et al., 1993).

Cyclosporin A stimulates ET-1 secretion from cultured VSMCs (Takeda et al., 1993), suggesting a potential

autocrine regulatory mechanism for cyclosporin-induced vasoconstriction.

In good agreement with these findings, plasma irET-1 concentrations are significantly elevated in patients with acute renal failure (Tomita et al., 1989).

F. Congestive Heart Failure

Congestive heart failure is characterized by low cardiac output, sodium and water retention, and peripheral vasoconstriction. Some of the well-documented biological actions (vasoconstriction, reduction of cardiac output, sodium retention, modulation of the renin-angiotensin-aldosterone system) of ET suggest that it may be involved in the development and/or progression of the congestive heart failure syndrome.

Plasma irET level is elevated 2- to 3-fold above normal in an experimental animal model of heart failure induced by rapid ventricular pacing (Cavero et al., 1990; Margulies et al., 1990). Elevated circulating plasma irET-1 levels were also reported in humans with heart failure (Lerman et al., 1992a; Stewart et al., 1992). Although the source of the elevated plasma ET level in congestive heart failure is unclear, at least two mechanisms may be responsible. The first possibility is increased production of ET-1. Studies in vitro showed increased ET-1 secretion by the endothelium in the presence of angiotensin II or AVP (Resink et al., 1990a; Emori et al., 1989, 1991b). With both angiotensin II and AVP being elevated in congestive heart failure, these may serve as stimuli for ET production.

In addition to increased ET production, decreased clearance of ET from the circulation may also occur in congestive heart failure. Indeed, Cavero et al. (1990) found that, after infusion of the same concentration of ET-1, a 3-fold or greater increase in plasma irET-1 occurred in patients when compared to healthy controls. This finding indicated decreased clearance of ET-1, at least in this animal model of heart failure.

The selective ET_A receptor antagonist FR-139317 reduced systemic blood pressure and peripheral resistance in a dog model of chronic heart failure (Clavell et al., 1994), suggesting that elevated endogenous ET contributes to the increase of systemic vascular resistance in severe congestive heart failure.

Because congestive heart failure is a low cardiac output state similar to that seen after hemorrhage, one may speculate that ET-1 may be elevated in heart failure because of the decrease in intravascular volume. Similar to the sympathoadrenal and renin-angiotensin-aldosterone systems, the vasoconstricting effects of ET-1 may be beneficial early in heart failure, augmenting preload via venoconstriction, increasing systemic vascular resistance to maintain perfusion pressure, and increasing plasma volume by fluid and water retention. With time, however, the chronic effects of ET-1 likely become excessive and contribute to a continuing deterioration of cardiac function.

G. Shock Syndrome

The shock syndrome is characterized by decreasing systemic blood pressure because the capacity of the vasculature exceeds the volume of circulating blood due to either excessive vasodilation (septic shock), failure of the heart to pump blood (cardiogenic shock), or loss of blood (hemorrhagic shock) or plasma (burn). In the initial stages neurohumoral mechanisms (e.g., sympathoadrenal system, AVP) are activated to maintain blood pressure via peripheral vasoconstriction. Stimulated synthesis/release of ETs also was postulated to contribute to this compensatory mechanism (see also section IX.C; fig. 15). Indeed, significantly elevated circulating plasma irET-1 levels were reported in patients with acute cardiac failure (cardiogenic shock) (Cernacek and Stewart, 1989) with central hypovolemia following upright tilting or hemorrhage (Maten et al., 1992) and in patients with septic shock syndrome (Weitzberg et al., 1991).

There is an approximately 5-fold increase of plasma irET level in septic shock patients during the early stage of this disorder, whereas in patients with anuria the increase is approximately 10-fold (from 2.4 to 11.3 and 24 pmol/liter, respectively) (Weitzberg et al., 1991). Similar to findings in patients, septic shock caused by injection of *Escherichia coli* bacterium or lipopolysaccharide leads to a significant elevation of plasma irET-1 level in sheep (Clozel et al., 1989; 1991), dogs (Nakamura et al., 1991), pigs (Pernow et al., 1990), and rats (Vemulapalli et al., 1991).

Studies with cultured endothelial cells demonstrated that endotoxin is a potent stimulant of ET-1 production. Lipopolysaccharide stimulates the release of ET-1 from bovine pulmonary artery endothelial cells in culture (Nakamura et al., 1991) and from human endothelial cells and macrophages (Voerman et al., 1992). Tumor necrosis factor, the cytokine mediating some of the tissue damage caused by lipopolysaccharide, stimulates ET-1 mRNA expression and release of ET-1 from bovine aortic, renal arterial, and glomerular capillary endothelial cells in rats (Marsden and Brenner, 1992).

Endotoxin injection into pigs caused an approximately 7-fold elevation of plasma irET-1, which was caused predominantly by release from the spleen, because it could be prevented by splenectomy (Pernow et al., 1990; Lundberg et al., 1991). The release of ET-1 from the spleen correlated well with renal artery vasoconstriction. It is postulated that local (i.e., in renal vasculature) and systemic (i.e., spleen) production of ET-1 plays an important role in renal vasoconstriction and renal failure in endotoxic shock.

H. Hypercholesterolemia and Atherosclerosis

The potential involvement of ETs in the progression of atheromatous vascular disease is supported by the

following findings: (*a*) plasma irET-1 level is increased in patients with atherosclerosis and in animal models of hypercholesterolemia; (*b*) expression of the ET-1 gene is induced, the synthesis and release of ET-1 peptide increased, and the binding of exogenous ET-1 enhanced in the atheromatous vascular lesion; (*c*) oxidized LDL and several cytokines (tumor necrosis factor α, interleukin-1β, TGFβ1), all involved in the vascular injury process, stimulate ET-1 production by the endothelium and macrophages; (*d*) ET-1 is a (weak) mitogen or comitogen in vascular smooth muscle cells; and (*e*) ET-1-induced vasoconstriction is potentiated in atherosclerosis.

1. Increased circulating immunoreactive endothelin levels. Plasma irET-1 level increased significantly in rats fed a high cholesterol diet (Horio et al., 1991a; Miyauchi et al., 1992b). The lipid-lowering drug clinofibrate prevented plasma irET-1 elevation (Horio et al., 1991a). Elevation of plasma irET-1 occurred in cholesterol-fed rats before any signs of atherosclerosis developed (Horio et al., 1991a). Similar to the results of these animal studies, elevated plasma irET-1 levels were found in patients with hypercholesterolemia (Bath and Martin, 1991) and in patients with advanced symptomatic (angina) coronary artery atherosclerosis (Lerman et al., 1991b). In the latter patients, the plasma irET-1 level correlated well with the number of atherosclerotic lesions (Lerman et al., 1991b).

2. Stimulated production of endothelin. Oxidized LDL (30 to 300 mg/ml) and acetylated LDL, but not native LDL, stimulated the expression of prepro-ET mRNA and the release of mature ET-1 in cultured human and porcine endothelial cells and in isolated porcine aorta (Boulanger et al., 1992). The LDL scavenger receptor antagonist, dextran sulfate, inhibited the stimulation and downregulation of PKC by pretreatment with phorbol esters (Boulanger et al., 1992), suggesting that the effect is mediated by the LDL scavenger receptor and activation of PKC in endothelial cells. In human macrophages, modified, but not native, LDL stimulated ET-1 secretion (oxidized LDL > acetylated LDL > native LDL) (Martin Nizard, 1991).

In cultured porcine aortic endothelial cells, cytokines (interleukin-1β, tumor necrosis factor α), the growth factor TGF$_\beta$ and thrombin stimulated the synthesis/release of ET-1 (Maemura et al., 1992). The ET-1 gene in these endothelial cells has tissue-specific "response elements" for these factors (Maemura et al., 1992). Because all of these factors may be involved in the initiation/progression of atheromatous vascular lesions, it was postulated that local ET-1 release may also contribute to the disease process. Immunohistochemistry revealed the presence of irET-1 in smooth muscle and endothelial cells at the lesion sites (Lerman et al., 1991b).

In comparison to normal human aorta, prepro-ET-1 mRNA expression is elevated, whereas ET_A and ET_B receptor mRNA expressions decreased in human atherosclerotic lesions (Winkles et al., 1993).

3. Augmented vasoconstriction. ET-1 is a potent vasoconstrictor of normal and atherosclerotic human coronary arteries (Chester et al., 1992). ET-1 potentiates the vasoconstrictor effect of serotonin only in diseased human coronary arteries (Chester et al., 1992). Intraarterial injection of ET-1 to the hindlimb of cynomolgus monkeys caused vasoconstriction of the large arteries only in animals fed an atherogenic diet (Lopez et al., 1990). Some of the other biological actions of ET-1 (e.g., smooth muscle mitogenesis, monocyte chemotaxis; see section VIII) further support the postulated role of the peptide in atherosclerosis.

I. Bronchial Asthma

ET production by bronchial epithelial cells, potent bronchoconstriction by ETs in vitro and in vivo, and stimulation of mucous secretion (see section VIII) suggested that ETs may be involved in lung diseases, including bronchial asthma. Indeed, significant elevation of lung and bronchial lavage fluid irET content in animal models and in patients during asthma attack suggests a potential pathogenetic role of ETs in this disease.

Aerosolized ET-1 increases airway resistance in guinea pigs in vivo (Kanazawa et al., 1992). Very low concentrations (10^{-12} M) of ET-1 potentiated histamine-induced bronchoconstriction in this preparation. Because bronchi isolated from asthmatic and nonasthmatic patients showed similar reactivity to ET-1 in vitro (Goldie et al., 1990), increased production, rather than augmented responsiveness, of bronchial smooth muscle may contribute to an asthma attack. Indeed, increased ET-1 production in the lung was found in animal models and patients. Repeated intratracheal instillation of Sephadex beads induces long-lasting inflammation and a 3.5-fold increase of ET-1 content in rat lungs (Andersson et al., 1992). A novel glycocorticoid drug, budesonide, abolished the inflammation and reduced lung ET-1 levels approximately 70%.

IrET-1 level was significantly elevated in bronchoalveolar lavage fluid of patients during asthma attacks compared to that of normal volunteers (Mattoli et al., 1991a). No change in circulating plasma irET-1 was observed. After 15 days of treatment with glycocorticoids and β-adrenergic agonists, the patients became asymptomatic and the irET-1 content of bronchial lavage fluid decreased approximately 3-fold. In endobronchial biopsy specimens, ET-1 could be detected immunohistochemically in 11 of 17 patients with bronchial asthma but in only one of 11 controls (Springall et al., 1991).

J. Gastric Ulcer

Reduced gastric mucosal blood flow (local vasoconstriction) was postulated to be involved in the pathogenesis of gastric ulcer. Experiments carried out on rat stomach in vivo and in vitro suggest that mucosal vaso-

constriction produced by local production or elevated circulating levels of ET-1 may contribute to the development of mucosal lesions and ultimately to gastric ulcer. ET-1 potentiates the mucosal damage (ulcerogenic activity) of intragastric administration of ethanol, HCl, and capsaicin (Peskar et al., 1992; Wallace et al., 1989a, b; Whittle and Lopez Belmonte, 1991).

Intravenous infusion of 50 pmol/kg/min ET-1 for 10 minutes to rats caused no mucosal damage on its own but significantly potentiated intragastric ethanol-induced mucosal injury (Peskar et al., 1992). Although the stable prostacyclin analog iloprost had no effect on vasoconstriction induced by ET-1 in the isolated perfused rat stomach, it prevented the ulcerogenic effect of intravenously administered ET-1 in the rat stomach in situ (Peskar et al., 1992). Inhibition of prostanoid synthesis by pretreatment with indomethacin facilitated the ulcerogenic action of intravenously or intraarterially infused ET-1 (4 to 20 pmol/kg/min) in the rat stomach in situ (Wallace et al., 1989a, b; Whittle and Lopez Belmonte, 1991). Similarly, depletion of sensory neuropeptides by capsaicin or prevention of their release by morphine potentiated the ulcerogenic effect of ET-1 in the in situ rat stomach (Whittle and Lopez Belmonte, 1991). Intravenous or intraarterial injection of ET-1 increased the gastric mucosal sensitivity to intragastric ethanol and HCl (Wallace et al., 1989a,b) and caused hemorrhagic and necrotic mucosal lesions (Whittle and Esplugues, 1988) in the rat stomach in situ.

The most direct evidence for the potential involvement of endogenous ET-1 in the pathogenesis of gastric mucosal damage was provided by studies of isolated perfused rat stomach (Masuda et al., 1992). These experiments showed that ethanol-induced mucosal vasoconstriction and damage were associated with the release of ET-1. Both the ethanol-induced vasoconstriction and mucosal damage could be prevented by anti-ET-1 antibody (Masuda et al., 1992).

K. Inflammatory Bowel Disease

Evidence exists that both immune hypersensitivity and ischemia-reperfusion injury may play a role in the pathogenesis of inflammatory bowel disease. At present, no unifying hypothesis linking these two potential causes exists. However, Murch et al. (1992) found that tissue biopsies or resections from patients with either Crohn's disease or ulcerative colitis exhibited remarkably increased local production of ET compared to control samples, as assessed by immunohistochemical techniques. In both disorders, the percentage of positively staining cells in the lamina propria increased by 10- to 20-fold compared to controls. Perivascular ET immunoreactivity was prominent in the submucosa of Crohn's disease tissue samples, with many positively stained macrophages. Although this was not observed in ulcerative colitis, the irET levels in the supernatants of samples from both diseases were about 12-fold higher than in control supernatants. The finding of focal ischemia and vascular obliteration noted in Crohn's disease, which was never fully reconciled with a strictly T-cell-mediated process, further supports a pathogenic role of ETs in this disease. The source of ET immunoreactivity in ulcerative colitis could be activated monocytes or polymorphonuclear leukocytes stimulated by mucosal ingress of bacterial lipopolysaccharide.

L. Diabetes and Its Complications

Several lines of evidence suggest that stimulated production of ET-1 may contribute to diabetic vasculopathies: (*a*) circulating plasma irET levels are elevated in some patients with diabetes and in STZ-diabetic rats, (*b*) high levels of glucose and insulin stimulate ET-1 production in endothelial cells, and (*c*) increased local production and the relevant biological action of ET-1 make it a candidate in the development of diabetic complications such as retinopathy, neuropathy, and nephropathy.

1. Circulating plasma endothelin level in diabetes. Several authors demonstrated an elevation in circulating plasma irET-1 levels in diabetic patients (Collier et al., 1992; Haak et al., 1992; Kawamura et al., 1992; Lam et al., 1991; Takahashi et al., 1990a), whereas others found no change in urinary or plasma irET-1 levels compared to those of healthy volunteers (Totsune et al., 1991; Predel et al., 1990; Kanno et al., 1991). Significant correlation was found between plasma irET-1 level and diabetic complications: irET-1 was higher in patients with microalbuminia (nephropathy) in type I and type II diabetic patients (Collier et al., 1992). IrET-1 increased with advanced age (duration of disease), with the level of glycosylated hemoglobin (Hb_{A1}), and with the presence of hypertension (Haak et al., 1992) in type II diabetic patients only if proliferative retinopathy already existed (Kawamura et al., 1992). In contrast, no correlations were found between irET-1 plasma levels and age, renal function, retinopathy, or hypertension in another study of diabetic patients (Takahashi et al., 1990a). One study demonstrated a reduced conversion of big ET-1 to ET-1 in patients with diabetes mellitus (Tsunoda et al., 1991). Both significant elevation (Takeda et al., 1991) and no change (Takahashi et al., 1991c) of plasma irET-1 levels were reported in STZ-diabetic rats.

2. High glucose and insulin concentrations stimulate endothelin production. Elevated glucose (from 5.5 to 11.1 or 22.2 mM) in the culture medium stimulated ET-1 secretion from bovine aortic endothelial cells (Yamauchi et al., 1990). Aldose reductase inhibitors had no effect, suggesting that the stimulation is not mediated by sorbitol (polyol) accumulation. In contrast, elevated glucose (27.5 mM) reduced ET-1 release from cultured porcine aortic endothelial cells under basal and stimulated (calf serum) conditions (Hattori et al., 1991a).

Insulin and insulin-like growth factor-1 stimulated ET-1 production in these cells (Hattori at al., 1991a). Similarly, in cultured bovine aortic endothelial cells, insulin increased prepro-ET-1 mRNA expression (Oliver et al., 1991; Hu et al., 1993). These experiments revealed that insulin does not act via activation of PKC but probably via an insulin-responsive element on the 5′-region of the ET-1 gene in these cells (Oliver et al., 1991). Basal ET-1 production in mesenteric arteries isolated from STZ-diabetic rats is significantly higher than in arteries from normal rats of the same strain (Takeda et al., 1991).

3. Cardiovascular responsiveness to endothelin in diabetes. In general, reduced responsiveness to ET-1 was reported in blood vessels of diabetic animals (with the exception of the increased responsiveness of the renal circulation). Reduced responsiveness to ET-1 was reported in aorta isolated from STZ-diabetic rats (Fulton et al., 1991; Hodgson and King, 1992) and in glomeruli from STZ-diabetic rats (Awazu et al., 1991a). Although the precise mechanism is not known, downregulation of PKC due to sorbitol accumulation (Awazu et al., 1991a) or the downregulation of ET receptors found in STZ-diabetic rat heart muscle cell membranes (Nayler et al., 1989c) may explain it.

4. Diabetic retinopathy. ET-1 is produced by retinal microvessel endothelial cells and contract adjacent pericytes (Takahashi et al., 1989; De la Rubia et al., 1992). Increased production of ET-1 in retinal capillary endothelial cells during diabetes can have "toxic" effects on pericytes, probably contributing to their loss and the consequent retinopathy in diabetic patients.

5. Diabetic neuropathy. ET-1 causes a significant reduction in blood flow to peripheral sensory nerves (Zochodne et al., 1992) in rats, similar to that observed after STZ-induced diabetes (Cameron et al., 1992). The ischemia/hypoxia-induced neuropathy was similar in diabetic and ET-1-treated animals, suggesting the involvement of enhanced local production of ET-1 (or elevated circulatory ET-1 levels) in the pathogenesis of diabetic neuropathy.

6. Diabetic nephropathy. The high sensitivity of renal vasculature to the vasoconstrictor effects of ET-1 in normal animals is significantly augmented in STZ-diabetic rats in vivo (Kiff et al., 1991) and in perfused kidneys isolated from STZ-diabetic rats (Tammesild et al., 1992). However, in glomeruli isolated from STZ-diabetic rats, ET-1 vasoreactivity was found to be suppressed (Awazu et al., l991b).

M. Kidney Diseases

1. Renal insufficiency and chronic renal failure. Circulating plasma levels of irET were reported to be significantly elevated in patients with advanced renal insufficiency and in patients with chronic renal failure receiving dialysis (Koyama et al., 1989; Warrens et al., 1990; Deray et al., 1992). Fractionation of plasma samples revealed that the elevation in irET is not due to increases in irET-1 levels but, rather, to marked increases in immunoreactive big ET-1 degradation products (larger than ET-1) (Saito et al., 1991). These findings suggest that the primary deficiency is big ET-1 metabolism rather than systemic overproduction or slowed clearance of ET-1 in these diseases. Indeed, using radioimmunoassay, highly specific for ET-1, Shichiri et al. (1990) found no difference in irET-1 plasma levels between healthy volunteers and normotensive patients with renal insufficiency or failure. However, significant elevation of plasma irET-1 was observed in patients with renal insufficiency and hypertension (Shichiri et al., 1990).

Urinary excretion of irET-1 increased, rather than decreased, in patients with renal insufficiency (Ohta et al., 1991), suggesting that renal production of ET-1 increases. Similarly, increased production of ET-1 by renal tissues and elevated irET-1 urinary excretion was demonstrated in renal mass ablation-induced renal insufficiency animal models (Benigni et al., 1991b; Brooks et al., 1991).

Based on the known biological effects of ET-1 on glomerular tissue (see section VIII), it was proposed that increased renal production of ET-1 may play an important role in functional and structural changes of the glomeruli in response to injury caused by loss of functional renal tissue or inflammation (Benigni et al., 1992; Force and Bonventre, 1992; Simonson et al., 1992c).

2. Hepatorenal syndrome. Circulating venous plasma levels of irET-1 are significantly (severalfold) elevated in patients with liver cirrhosis (Moore et al., 1992) which declines after liver transplantation (Kraus et al., 1992). The plasma levels observed may be sufficient to induce renal vasoconstriction (the most sensitive vascular bed to ET-1-induced vasoconstriction), as suggested by observations in conscious animals (Lerman et al., 1991c) and in healthy volunteers (Gasic et al., 1992) following intravenous injection of small amounts of ET-1. It was, therefore, postulated that renal vasoconstriction and insufficiency in cirrhotic patients (hepatorenal syndrome) may be caused (at least in part) by elevated circulating ET-1 levels. Indeed, a good correlation was found between circulating irET-1 levels and renal (dys)function in cirrhotic patients (Gross et al., 1993a; Uchihara et al., 1992). Furthermore, the highest plasma irET-1 level among patients with liver cirrhosis was found in those with hepatorenal syndrome (Moore et al., 1992).

3. Other kidney diseases. At least circumstantial evidence exists for the potential pathophysiological role of ET in the following renal abnormalities: obstructive nephropathy, i.e., severe vasoconstriction following release of obstruction (Kelleher et al., 1992); lupus nephritis (Ida et al., 1990); systemic vasculitis (Kanno et al., 1990); and vascular renal transplant rejection (Watschinger et al., 1991). Treatment of anemia due to

chronic renal disease with recombinant erythropoietin causes hypertension in some patients which was postulated to be caused by erythropoietin-induced stimulation of ET production (Carlini et al., 1993; Takahashi et al., 1993; Takayama et al., 1991).

N. Carcinogenesis

Several cultured cell lines or tissues originating from various tumors express prepro-ET mRNA, secrete mature ET peptides, contain ET receptors, and respond to exogenously administered ETs.

Human parathyroid hormone adenoma tissue contains ET_A and ET_B receptors and expresses prepro-ET-1 mRNA, and ET-1 inhibits parathyroid hormone secretion under basal conditions. ET-2 expression was demonstrated in a renal adenocarcinoma cell line (Ohkubo et al., 1990b; Onda et al., 1991). ET-1 expression was observed in human endometrial adenocarcinoma cells (Pekonen et al., 1992). Several human cancer cell lines produce and respond to ET-1 (Shichiri et al., 1991a,b). Human renal adenocarcinoma cells express ET-2 and phosphoramidon reduces conversion of the 38-amino acid residue big (pro) ET-2 to ET-2 (Yorimitsu et al., 1992).

Elevated tissue expression and circulating plasma levels of ET were reported in several human tumors, including adrenocorticotropic hormone-secreting bronchial carcinoid (Murakami et al., 1993) and hepatocellular carcinoma (Ishibashi et al., 1993). Circulating plasma irET-1 and immunoreactive big ET-1 levels are significantly elevated (from normal 9.4 to 30.3 pg/mL) in patients with hepatocellular carcinoma (Parkes et al., 1991; Ishibashi et al., 1993). The increase was more pronounced than that observed in patients with liver cirrhosis (22.1 pg/mL) and correlated well with the size of the liver tumor. Because circulating thrombomodulin levels were not elevated (a marker of generalized or local endothelial cell activation/injury), the authors speculated that the source of ET-1 may be the tumor cells themselves (Parkes et al., 1991).

Tissue irET-1 level was significantly elevated in about half of all human pheochromocytomas analyzed, and the circulating plasma irET-1 level increased significantly in these patients (Sone et al., 1991). In four patients with the rare mammary phyllodes tumor, irET-1 was 18- to 27-fold higher in the tumor tissue than in mammary fibroadenomas (Yamashita et al., 1992). ET-1 was localized histochemically only in epithelial, but not in the stromal, cells of these tumors. High expression levels of ET-1 were detected in human breast cancer (Yamashita et al., 1991b, 1992). The pathology associated with the rare ET-1-secreting skin tumor hemangioendothelioma (Yokokawa et al., 1991b,c, 1992) has been discussed in section X.B.4.

Although not proven yet, some of these data suggest that ETs may contribute to carcinogenesis in certain tumors via autocrine/paracrine mechanisms.

O. Summary

The data available to date suggest, but do not prove, that ETs may play an important pathophysiological role in human diseases. All of the main criteria (i.e., increased production of and/or reactivity to ETs and efficacy of an ET receptor antagonist, antibody, or ECE inhibitor) have not yet been met in any of the disease states listed. The criteria fulfilled so far for the cardiovascular indications reviewed in this paper are summarized in table 6. It is expected that, with the use of the available and newly discovered tools (receptor antagonists and ECE inhibitors), this table will be filled soon. When this is achieved for these and other diseases not listed in table 6, the true significance of ETs in human diseases can and will be determined.

XI. Conclusions and Perspectives

The discovery in 1985 and isolation in 1988 of a peptidergic EDCF produced by cultured endothelial cells generated worldwide interest and exponentially growing research activity. The plethora of publications has revealed important information about genes, gene expression, and potential biochemical processing of the ET family of peptides. In addition, data about specific membrane receptors, signal transduction pathways, and the wide variety of biological actions of ETs are abundant.

These studies have also demonstrated that ET(s) can be formed in many cells of the body, as well as the endothelium, and thus have the potential for many of the effects suggested by in vitro and in vivo studies. ET appears to function predominantly as a local, rather than a circulating, hormone. These autocrine and paracrine actions suggest that ET has the potential to regulate many organ functions, either directly or indirectly, by interaction with neuroendocrine regulatory systems. Based on data generated by in vivo binding and in situ hybridization studies, by administration of exogenous synthetic peptides, and by measuring changes in plasma levels of irET, some hypotheses have been proposed concerning the potential significance of ETs in health and disease.

Despite these speculations, however, the physiological and pathophysiological significance of ETs remains to be established. Several key questions need to be analyzed and answered. These include a better understanding of the distribution and regulation of function of ET receptor subtypes, the localization and isolation of ECE, and the physiological and pathological significance of endogenously produced ETs. For example, although receptor antagonists are important tools in analyzing the potential role of ETs in the pathophysiology of diseases, it is possible that ET_A and ET_B receptors are differently regulated in different forms of diseases, at different stages of the disease, and in different organs. Addi-

TABLE 6

*Criteria met to support pathophysiological role of ET in cardiovascular diseases**

Disease	Criteria					
	ET levels↑		Reactivity to ET↑		ET antagonist effective	
	Animal	Human	Animal	Human	Animal	Human
Cerebral vasospasm following subarachnoid hemorrhage	+	+	+	+	+	?
Hypertension (fulminant/complications)	+	+	+	+	+	?
Acute renal failure	+	+	+	+	+	?
Congestive heart failure	+	+	+	?	+	?
Coronary vasospasm	+	+	+	+	?	?
Myocardial ischemia (acute myocardial infarction)	+	+	+	?	+/−	?
Atherosclerosis (hypercholesterolemia)	+	+	+	+	?	?
Essential hypertension	+/−	+/−	+/−	?	+/−	?
Shock	+	+	?	?	?	?
Pulmonary hypertension	+	+	?	?	?	?

* Detailed review of these diseases can be found in section X. +, criteria tested and met; −, criteria tested but not met; ?, criteria not tested yet.

tionally, lack of significant acute effects of a receptor antagonist does not necessarily rule out a role for ETs, because the tight binding to its receptors and long-lasting effects may require administration of a more potent antagonist than the presently available ones for a much longer time. Additionally, ETs may be involved in the development of disease symptoms only at an early stage of the disease, and may have no importance at later (more progressive) stages.

From the perspective of therapeutics, based on inhibition of ET biosynthesis, the questions of how many different types of enzymes can convert big ET to ET and whether there are isoform- or tissue-specific ECEs are particularly important. To answer these questions, appropriate tools (specific antibodies, gene probes) will be needed. Recent developments, however, raise the possibility that these important questions will be answered in the near future. Cloning of the ET receptors, characterization of ET-converting enzyme, and the availability of specific receptor antagonists, monoclonal antibodies, and converting enzyme inhibitors provided the long-awaited necessary tools to study further the physiological and pathological significance of this unique peptide family.

The discovery of potent ET receptor antagonists and the recent demonstration of their effectiveness in several pathological animal models provided convincing evidence that endogenous ETs may indeed be of importance in the pathogenesis of several diseases. Therefore, it is expected that with the use of such tools many of the still hypothetical mechanisms summarized in this review will be tested in the future. Molecular biology has already contributed significantly to the rapid progress of ET research (e.g., discovery of ET isopeptides, cloning of the receptor subtypes), and it is expected that this trend will continue. For example, first attempts were made to develop transgenic animals with ET gene-knockout (Kurihara et al., 1993). Establishment of such models will also significantly contribute to better understanding of the true physiological and pathological significance of the ET peptide family. Thus, research concerning ETs can now enter into a new exciting phase, and we can expect further breakthroughs in the coming years.

REFERENCES

ABASSI, Z., GOLOMB, E., AND KEISER, H. R.: Neutral endopeptidase inhibition increases the urinary excretion and plasma levels of endothelin. Metabolism **41:** 683–685, 1992.

ABDEL LATIF, A. A., ZHANG, Y., AND YOUSUFZAI, S. Y.: Endothelin 1 stimulates the release of arachidonic acid and prostaglandins in rabbit iris sphincter smooth muscle: activation of phospholipase A2. Curr. Eye Res. **10:** 259–265, 1991.

ABE, Y., KASUYA, Y., KUDO, M., YAMASHITA, K., GOTO, K., MASAKI, T., AND TAKUWA, Y.: Endothelin 1 induced phosphorylation of the 20 kDa myosin light chain and caldesmon in porcine coronary artery smooth muscle. Jpn. J. Pharmacol. **57:** 431–435, 1991.

ACHMAD, T. H., AND RAO, G. S.: Chemotaxis of human blood monocytes toward endothelin 1 and the influence of calcium channel blockers. Biochem. Biophys. Res. Commun. **169:** 994–1000, 1992.

ADACHI, H., SHOJI, T., AND GOTO, K.: Comparison of the effects of endothelin and Bay k 8644 on cardiohemodynamics in anesthetized pigs. Eur. J. Pharmacol. **193:** 57–65, 1991a.

ADACHI M., YANG Y. Y., TRZECIAK A., FURUICHI Y., AND MIYAMOTO C.: Identification of a domain of ET_A receptor required for ligand binding. FEBS Lett. **311:** 179–183, 1992.

ADAM, L. P., MILIO, L., BRENGLE, B., AND HATHAWAY, D. R.: Myosin light chain and caldesmon phosphorylation in arterial muscle stimulated with endothelin 1. J. Mol. Cell. Cardiol. **22:** 1017–1023, 1990.

ADVENIER, C., SARRIA, B., NALINE, E., PUYBASSET, L., AND LAGENTE, V.: Contractile activity of three endothelins (ET 1, ET 2 and ET 3) on the human isolated bronchus. Br. J. Pharmacol. **100:** 168–172, 1990.

AGNATI, L. F., ZOLI, M., KUROSAWA, M., BENFENATI, F., BIAGINI, G., ZINI, I., HALLSTROM, A., UNGERSTEDT, U., TOFFANO, G., AND FUXE, K.: A new model of focal brain ischemia based on the intracerebral injection of endothelin 1. Ital. J. Neurol. Sci. **12** (Suppl. 11): 49–53, 1991.

AHMED, A., CAMERON, I. T., FERRIANI, R. A., AND SMITH, S. K.: Activation of phospholipase A2 and phospholipase C by endothelin 1 in human endometrium. J. Endocrinol. **135:** 383–390, 1992.

AHN, K., BENINGO, K., OLDS, G., AND HUPE, D.: The endothelin converting enzyme from human umbilical vein is a membrane bound metalloprotease similar to that from bovine aortic endothelial cells. Proc. Natl. Acad. Sci. USA **89:** 8606–8610, 1992.

AKIYAMA, N., HIRAOKA, O., FUJII, Y., TERASHIMA, H., SATOH, M., WADA, K., AND FURUICHI, Y.: Biotin derivatives of endothelin: utilization for affinity purification of endothelin receptor. Protein Expression Purification **3:** 427–433, 1992.

ALAFACI, C., JANSEN, I., ARBAB, M. A., SHIOKAWA, Y., SVENDGAARD, N. A., AND EDVINSSON, L.: Enhanced vasoconstrictor effect of endothelin in cerebral arteries from rats with subarachnoid haemorrhage. Acta Physiol. Scand. **138:** 317–319, 1990.

ALAFACI, C., SALPIETRO, F. M., IACOPINO, D. G., EDVINSSON, L., AND TOMA-

SELLO, F.: Endothelin: an endothelium derived vasoactive peptide and its possible role in the pathogenesis of cerebral vasospasm. Ital. J. Neurol. Sci. **12** (Suppl. 11): 55–58, 1991.

ALAM, A. S., GALLAGHER, A., SHANKAR, V., GHATEI, M. A., DATTA, H. K., HUANG, C. L., MOONGA, B. S., CHAMBERS, T. J., BLOOM, S. R., AND ZAIDI, M.: Endothelin inhibits osteoclastic bone resorption by a direct effect on cell motility: implications for the vascular control of bone resorption. Endocrinology **130**: 3617–3624, 1992.

ALBERTS, G. F., PEIFLEY, K. A., JOHNS, A., KLEHA, J. F., AND WINKLES, J. A.: Constitutive ET-1 overexpression promotes smooth muscle cell proliferation via an external autocrine loop. J. Biol. Chem. **269**: 10112–10118, 1994.

AMADIEU, FARMAKIS M., DAVICCO, M. J., GIRY, J., AND BARLET, J. P.: Effects of angiotensin II, arginine vasopressin, endothelin and catecholamines on plasma atrial natriuretic peptide concentrations in the conscious newborn calf. J. Dev. Physiol. (Oxf.) **16**: 51–56, 1991.

AMBAR, I., KLOOG, Y., SCHVARTZ, I., HAXUM, E., AND SOKOLOVSKY, M.: Competitive interaction between endothelin and sarafotoxin: binding and phosphoinositides hydrolysis in rat atria and brain. Biochem. Biophys. Res. Commun. **158**: 195–201, 1989.

AMBAR, I., AND SOKOLOVSKY, M.: Endothelin receptors stimulate both phospholipase C and phospholipase D activities in different cell lines. Eur. J. Pharmacol. **245**: 31–41, 1993.

ANDERSEN, N. H., CHEN, C. P., MARSCHNER, T. M., KRYSTEK, S. R., JR., AND BASSOLINO, D. A.: Conformational isomerism of endothelin in acidic aqueous media: a quantitative NOESY analysis. Biochemistry **31**: 1280–1295, 1992.

ANDERSSON, S. E., ZACKRISSON, C., HEMSEN, A., AND LUNDBERG, J. M.: Regulation of lung endothelin content by the glucocorticosteroid budesonide. Biochem. Biophys. Res. Commun. **188**: 1116–1121, 1992.

ANDO, K., HIRATA, Y., TOGASHI, K., KAWAKAMI, M., AND MARUMO, F.: Endothelin 1 and endothelin 3 like immunoreactivity in human cerebrospinal fluid. J. Cardiovasc. Pharmacol. **7** (Suppl.): S434–S436, 1991.

ANGGARD, E., GALTON, S., RAE, G., THOMAS, R., MCLOUGHLIN, L., DE NUCCI, G., AND VANE, J. R.: The fate of radioiodinated endothelin 1 and endothelin 3 in the rat. J. Cardiovasc. Pharmacol. **13** (Suppl. 5): S46–S49, 1989.

ANGGARD, E. E., BOTTING, R. M., AND VANE, J. R.: Endothelins. Blood Vessels **27**: 269–281, 1990.

ARAI, H., HORI, S., ARAMORI, I., OHKUBO, H., AND NAKANISHI, S.: Cloning and expression of a cDNA encoding an endothelin receptor. Nature (Lond.) **348**: 730–732, 1990.

ARAI, H., NAKAO, K., TAKAYA, K., HOSODA, K., OGAWA, Y., NAKANISHI, S., AND IMURA, H.: The human endothelin B receptor gene. Structural organization and chromosomal assignment. J. Biol. Chem., **268**: 3463–3470, 1993.

ARAKI, S., KAWAHARA, Y. KARIYA, K., SUNAKO, M., FUKUZAKI, H., AND TAKAI, Y.: Stimulation of phospholipase C mediated hydrolysis of phosphoinositides by endothelin in cultured rabbit aortic smooth muscle cells. Biochem. Biophys. Res. Commun. **159**: 1072–1079, 1989.

ARAMORI, C., NIREI, H., SHOUBO, M., SOGABE, K., NAKAMURA, K., KOJO, H., NOTSU, Y., ONO, T., AND NAKANISHI, S.: Subtype selectivity of a novel endothelin antagonist, FR139317, for the two endothelin receptors in transfected Chinese hamster ovary cells. Mol. Pharmacol. **43**: 127–131, 1993.

ARINAMI, T., ISHIKAWA, M., INOUE, A., YANAGISAWA, M., MASAKI, T., YOSHIDA, M. C., AND HAMAGUCHI, H.: Chromosomal assignments of the human endothelin family genes: the endothelin 1 gene (EDN1) to 6p23 p24, the endothelin 2 gene (EDN2) to 1p34, and the endothelin 3 gene (EDN3) to 20q13.2 q13.3. Am. J. Hum. Genet. **48**: 990–996, 1991.

ARMSTEAD, W. M., MIRRO, R., LEFFLER, C. W., AND BUSIJA, D. W.: Influence of endothelin on piglet cerebral microcirculation. Am. J. Physiol. **257**: H707–H710, 1989.

ASANO, T., IKEGAKI, I., SATOH, S., SUZUKI, Y., SHIBUYA, M., SUGITA, K., AND HIDAKA, H.: Endothelin: a potential modulator of cerebral vasospasm. Eur. J. Pharmacol., **190**: 365–372, 1990.

ASHIDA, T., KAWANO, Y., YOSHIMI, H., AKABANE, S., KURAMOCHI, M., AND OMAE, T.: Effects of ouabain and verapamil on endothelin 1 induced contraction of mesenteric artery in young spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S174–S176, 1991.

ATKINSON, R. A., AND PELTON, J. T.: Conformational study of cylco [D Trp D Asp Pro D Val Leu], and endothelin A receptor selective antagonist. FEBS Lett. **296**: 1–6, 1992.

AUGUET, M., DELAFLOTTE, S., CHABRIER, P. E., AND BRAQUET, P.: The vasoconstrictor action of big endothelin 1 is phosphoramidon sensitive in rabbit saphenous artery, but not in saphenous vein. Eur. J. Pharmacol., **224**: 101–102, 1992.

AUMELAS, A., CHICHE, L., MAHE, E., LE NGUYEN, D., SIZUN, P., BERTHAULT, P., AND PERLY, B.: Determination of the structure of [Nle7] endothelin by 1H NMR. Int. J. Pept. Protein Res. **37**: 315–324, 1991.

AWAZU, M., PARKER, R. E., HARVIE, B. R., ICHIKAWA, I., AND KON, V.: Down regulation of endothelin 1 receptors by protein kinase C in streptozotocin diabetic rats. J. Cardiovasc. Pharmacol., **17** (Suppl. 7): S500–S502, 1991.

AWAZU, M., SUGIURA, M., INAGAMI, T., ICHIKAWA, I., AND KON, V.: Cyclosporine promotes glomerular endothelin binding in vivo. J. Am. Soc. Nephrol. **1**: 1253–1258, 1991.

AZELROD, J., BURCH, R. M., AND JELSEMA, C. L.: Receptor-mediated activation of phospholipase A_2 via GTP-binding proteins arachidonic acid and its metabolites as second messengers. Trends Neurol. Sci. **11**: 117–123, 1988.

BADR, K. F., MUNGER, K. A., SUGIURA, M., SNAJDAR, R. M., SCHWARTZBERG, M., AND INAGAMI, T.: High and low affinity binding sites for endothelin on cultured rat glomerular mesangial cells. Biochem. Biophys. Res. Commun. **161**: 776–781, 1989.

BADR, K. F., MURRAY, J. J., BREYER, M. D., TAKAHASHI, K., INAGAMI, T., AND HARRIS, R. C.: Mesangial cell, glomerular and renal vascular responses to endothelin in the rat kidney. Elucidation of signal transduction pathways. J. Clin. Invest. **83**: 336–342, 1989.

BAKRIS, G. L., FAIRBANKS, R., AND TRAISH, A. M.: Arginine vasopressin stimulates human mesangial cell production of endothelin. J. Clin. Invest. **87**: 1158–1164, 1991.

BALDI, E., AND DUNN, M. J.: Endothelin binding and receptor down regulation in rat glomerular mesangial cells. J. Pharmacol. Exp. Ther. **256**: 581–586, 1991.

BALEY, P. A., RESINK, T. J., EPPENBERGER, U., AND HAHN, A. W.: Endothelin messenger RNA and receptors are differentially expressed in cultured human breast epithelial and stromal cells. J. Clin. Invest. **85**: 1320–1323, 1990.

BALWIERCZAK, J. L.: Two subtypes of the endothelin-1 (ET-1) receptor (ETA and ETB) mediate the coronary vasoconstrictor effect of ET-1. *In* Proceeding of the 3rd International Conference on Endothelin, p. 4, 1993. Houston, TX. February 15–17, 1993.

BATH, P. M., AND MARTIN, J. F.: Serum platelet derived growth factor and endothelin concentrations in human hypercholesterolaemia. J. Intern. Med. **230**: 313–7, 1991.

BATH, P. M., MAYSTON, S. A., AND MARTIN, J. F.: Endothelin and PDGF do not stimulate peripheral blood monocyte chemotaxis, adhesion to endothelium, and superoxide production. Exp. Cell. Res. **187**: 339–342, 1990.

BATTISTINI, B., CHAILLER, P., D'ORLEANS-JUSTE, P., BRIERE, N., AND SIROIS, P.: Growth regulatory properties of endothelins. Peptides (Elmsford) **14**: 385–399, 1993.

BATTISTINI, B., FILEP, J. G., HERMAN, F., AND SIROIS, P.: Endothelin 1 does not affect human platelet aggregation in whole blood and platelet rich plasma. Thromb. Res. **60**: 105–108, 1990.

BATTISTINI, B., FILEP, J., AND SIROIS, P.: Potent thromboxane mediated in vitro bronchoconstrictor effect of endothelin in the guinea pig. Eur. J. Pharmacol. **178**: 141–142, 1990.

BATTISTINI, B., SIROIS, P., BRAQUET, P., AND FILEP, J. G.: Endothelin induced constriction of guinea pig airways: role of platelet activating factor. Eur. J. Pharmacol. **186**: 307–310, 1990.

BAYDOUN, A. R., PEERS, S. H., CIRINO, G., AND WOODWARD, B.: Effects of endothelin 1 on the rat isolated heart. J. Cardiovasc. Pharmacol. **13** (Suppl. 5): S193–S196, 1989.

BAZIL, M. K., LAPPE, R. W., AND WEBB, R. L.: Pharmacologic characterization of an endothelin A (ETA) receptor antagonist in conscious rats. J. Cardiovasc. Pharmacol. **20**: 940–948, 1992.

BENATTI, L., BONECCHI, L., COZZI, L., AND SARMIENTOS, P.: Two preproendothelin 1 mRNAs transcribed by alternative promoters. J. Clin. Invest. **91**: 1149–1156, 1993.

BENIGNI, A., GASPARI, F., ORISIO, S., BELLIZZI, L., AMUSO, G., FRUSCA, T., AND REMUZZI, G.: Human placenta expresses endothelin gene and corresponding protein is excreted in urine in increasing amounts during normal pregnancy. Am. J. Obstet. Gynecol. **164**: 844–848, 1991.

BENIGNI, A., ORISIO, S., GASPARI, F., FRUSCA, T., AMUSO, G., AND REMUZZI, G.: Evidence against a pathogenetic role for endothelin in preeclampsia. Br. J. Obstet. Gynaecol. **99**: 798–802, 1992.

BENIGNI, A., PERICO, N., LADNY, J. R., IMBERTI, O., BELLIZZI, L., AND REMUZZI, G.: Increased urinary excretion of endothelin 1 and its precursor, Big endothelin 1, in rats chronically treated with cyclosporine. Transplantation (Baltimore) **52**: 175–177, 1991.

BENNES, R., CALAS, B., CHABRIER, P. E., DEMAILLE, J., AND HEITZ, F.: Evidence for aggregation of endothelin 1 in water. FEBS Lett. **276**: 21–24, 1990.

BERBINSCHI, A., AND KETELSLEGERS, J. M.: Endothelin in urine. Lancet **2**: 46, 1989.

BERGE, K. E., AND BERG, K.: No effect of a Taq1 polymorphism in DNA at the endothelin I (EDN1) locus on normal blood pressure level or variability. Clin. Genet. **41**: 90–95, 1992.

BERTI, F., ROSSONI, G., BIASI, G., BUSCHI, A., MANDELLI, V., AND TONDO, C.: Defibrotide, by enhancing prostacyclin generation, prevents endothelin 1 induced contraction in human saphenous veins. Prostaglandins **40**: 337–350, 1990.

BIALECKI, R. A., IZZO, N. J., JR., AND COLUCCI, W. S.: Endothelin 1 increases intracellular calcium mobilization but not calcium uptake in rabbit vascular smooth muscle cells. Biochem. Biophys. Res. Commun. **164**: 474–479, 1989.

BIGAUD, M., AND PELTON, J. T.: Discrimination between ETA and ETB receptor mediated effects of endothelin 1 and [Ala1,3,11, 15]endothelin 1 by BQ 123 in the anaesthetised rat. Br. J. Pharmacol. **107**: 912–918, 1992.

BIONDI, M. L., MARASINI, B., BASSANI, C., AND AGOSTONI, A.: Increased plasma endothelin levels in patients with Raynaud's phenomenon. N. Engl. J. Med. **324**: 1139–1140, 1991.

BIRD, J. E., WALDRON, T. L., LITTLE, D. K., ASAAD, M. M., DORSO, C. R., DIDONATO, G., AND NORMAN, J. A.: The effects of novel cathepsin E inhibitors on the big endothelin pressor response in conscious rats. Biochem. Biophys. Res. Commun. **182**: 224–231, 1992.

BIRNBAUMER, L., ABRAMOWITZ, J., AND BROWN, A. M.: Receptor-effector coupling by G-proteins. Biochim. Biophys. Acta **1031**: 163–224, 1990.

BITAR, K. N., STEIN, S., AND OMANN, G. M.: Specific G proteins mediate endothelin induced contraction. Life Sci. **50:** 2119–2124, 1992.

BLACKBURN, K., AND HIGHSMITH, R. F.: Nickel inhibits endothelin induced contractions of vascular smooth muscle. Am. J. Physiol. **258:** C1025–C1030, 1990.

BLOCH, K. D., EDDY, R. L., SHOWS, T. B., AND QUERTERMOUS, T.: cDNA cloning and chromosomal assignment of the gene encoding endothelin 3. J. Biol. Chem. **264:** 18156–18161, 1989.

BLOCH, K. D., FRIEDRICH, S. P., LEE, M. E., EDDY, R. L., SHOWS, T. B., AND QUERTERMOUS, T.: Structural organization and chromosomal assignment of the gene encoding endothelin. J. Biol. Chem. **264:** 10851–10857, 1989.

BLOCH, K. D., HONG, C. C., EDDY, R. L., SHOWS, T. B., AND QUERTERMOUS, T.: cDNA cloning and chromosomal assignment of the endothelin 2 gene: vasoactive intestinal contractor peptide is rat endothelin 2. Genomics **10:** 236–242, 1991.

BOBIK, A., GROOMS, A., MILLAR, J. A., MITCHELL, A., AND GRINPUKEL, S.: Growth factor activity of endothelin on vascular smooth muscle. Am. J. Physiol. **258:** C408–C415, 1990.

BODELSSON, G., SJOBERG, N. O., AND STJERNQUIST, M.: Contractile effect of endothelin in the human uterine artery and autoradiographic localization of its binding sites. Am. J. Obstet. Gynecol. **167:** 745–750, 1992.

BOGOYEVITCH, M. A., GLENNON, P. E., AND SUGDEN, P. H.: Endothelin 1, phorbol esters and phenylephrine stimulate MAP kinase activities in ventricular cardiomyocytes. FEBS Lett. **317:** 271–275, 1993.

BOGOYEVITCH, M. A., PARKER, P. J., AND SUGDEN, P. H.: Characterization of protein kinase C isotype expression in adult rat heart. Protein kinase C epsilon is a major isotype present, and it is activated by phorbol esters, epinephrine, and endothelin. Circ. Res. **72:** 757–767, 1993.

BORGES, R., CARTER, D. V., VON GRAFENSTEIN, H., HALLIDAY, J., AND KNIGHT, D. E.: Ionic requirements of the endothelin response in aorta and portal vein. Circ. Res. **65:** 265–271, 1989.

BORGES, R., VON GRAFENSTEIN, H., AND KNIGHT, D. E.: Tissue selectivity of endothelin. Eur. J. Pharmacol. **165:** 223–230, 1989.

BORIC, M. P., DONOSO, V., FOURNIER, A., ST. PIERRE, S., AND HUIDOBRO TORO, J. P.: Endothelin reduces microvascular blood flow by acting on arterioles and venules of the hamster cheek pouch. Eur. J. Pharmacol. **190:** 123–133, 1990.

BORTMANN, P., HOFLACK, J., PELTON, J. T., AND SAUDEK, V.: Solution conformation of endothelin-3 by ^{1}H. NMR and distance geometry calculations. Neurochem. Int. **18:** 491–496, 1991.

BOTTING, R. M., AND VANE, J. R.: Endothelins: potent releasers of prostacyclin and EDRF. Pol. J. Pharmacol. Pharm. **42:** 203–218, 1990.

BOULANGER, C., AND LUSCHER, T. F.: Release of endothelin from the porcine aorta. Inhibition by endothelium derived nitric oxide. J. Clin. Invest. **85:** 587–590, 1990.

BOULANGER, C. M., AND LUSCHER, T. F.: Differential effect of cyclic GMP on the release of endothelin 1 from cultured endothelial cells and intact porcine aorta. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S264–S266, 1991.

BOULANGER, C. M., TANNER, F. C., BEA, M. L., HAHN, A. W., WERNER, A., AND LUSCHER, T. F.: Oxidized low density lipoproteins induce mRNA expression and release of endothelin from human and porcine endothelium. Circ. Res. **70:** 1191–1197, 1992.

BOUSSO-MITTLER, D., GALRON, R., AND SOKOLOVSKY, M.: Endothelin/sarafotoxin receptor heterogeneity: evidence for different glycosylation in receptors from different tissues. Biochem. Biophys. Res. Commun. **178:** 921–926, 1991.

BRAIN, S. D.: The direct observation of arteriolar constriction induced by endothelin in vivo. Eur. J. Pharmacol. **160:** 401–403, 1989.

BRAIN, S. D., CROSSMAN, D. C., BUCKLEY, T. L., AND WILLIAMS, T. J.: Endothelin 1: demonstration of potent effects on the microcirculation of humans and other species. J. Cardiovasc. Pharmacol. **13** (Suppl. 5): S147–S149 (discussion, S150), 1989.

BROCK, T. A., AND DANTHULURI, N. R.: Cellular actions of endothelin in vascular smooth muscle. *In* Endothelin, edited by G. M. Rubanyi, Oxford University Press, New York, pp. 103–124, 1992.

BROOKS, D. P., CONTINO, L. C., STORER, B., AND OHLSTEIN, E. H.: Increased endothelin excretion in rats with renal failure induced by partial nephrectomy. Br. J. Pharmacol. **104:** 987–989, 1991.

BROWN, M. A., AND SMITH, P. L.: Endothelin: a potent stimulator of intestinal ion secretion in vitro. Regul. Pept. **36:** 1–19, 1991.

BRUNNER, F., DU TOIT, E. F., AND OPIE, L. H.: Endothelin release during ischaemia and reperfusion of isolated perfused rat hearts. J. Mol. Cell. Cardiol. **24:** 1291–1305, 1992.

CADE, C., ILOZU, C. V., RUBANYI, G. M., AND BOTELHO, L. H.: Smooth muscle cells contain a factor responsible for decreasing big endothelin and endothelin-1 produced by cultured endothelial cells. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S71–S75, 1991.

CADE, C., LUMMA, W. C., JR., MOHAN, R., RUBANYI, G. M., PARKER-BOTELHO, L. H.: Lack of biological activity of preproendothelin [110 130] in several endothelin assays. Life Sci. **47:** 2097–2103, 1990.

CAIRNS, H. S., ROGERSON, M., FAIRBANKS, L. D., WESTWICK, J., AND NEILD, G. H.: Endothelin and cyclosporin nephrotoxicity (letter). Lancet **2:** 1496–1497, 1988.

CAIRNS, H. S., ROGERSON, M. E., FAIRBANKS, L. D., NEILD, G. H., AND WESTWICK, J.: Endothelin induces an increase in renal vascular resistance and a fall in glomerular filtration rate in the rabbit isolated perfused kidney. Br. J. Pharmacol. **98:** 155–160, 1989.

CALAS, B., HARRICANE, M. C., GUIMARD, L., HEITZ, F., MENDRE, C., CHABRIER, P. E., AND BENNES, R.: Endothelin 1: conformation and aggregation. Pept. Res. **5:** 97–101, 1992.

CALVO, J. J., GONZALEZ, R., DE CARVALHO, L. F., TAKAHASHI, K., KANSE, S. M., HART, G. R., GHATEI M. A., AND BLOOM, S. R.: Release of substance P from rat hypothalamus and pituitary by endothelin. Endocrinology **126:** 2288–2295, 1990.

CAMERON, I. T., DAVENPORT, A. P., BROWN, M. J., AND SMITH, S. K.: Endothelin 1 stimulates prostaglandin F2 alpha release from human endometrium. Prostaglandins Leukotrienes Essent Fatty Acids **42:** 155–157, 1991.

CAMERON, I. T., DAVENPORT, A. P., VAN PAPENDORP, C., BARKER, P. J., HUSKISSON, N. S., GILMOUR, R. S., BROWN, M. J., AND SMITH, S. K.: Endothelin like immunoreactivity in human endometrium. J. Reprod. Fertil. **95:** 623–628, 1992.

CAO, L. Q. AND BANKS, R. O.: Cardiovascular and renal actions of endothelin: effects of calcium channel blockers. Am. J. Physiol. **258:** 254–258, 1990.

CARDELL, L. O., UDDMAN, R., AND EDVINSSON, L.: Analysis of endothelin 1 induced contractions of guinea pig trachea, pulmonary veins and different types of pulmonary arteries. Acta Physiol. Scand. **139:** 103–111, 1990.

CARDELL, L. O., UDDMAN, R., AND EDVINSSON, L.: Evidence for multiple endothelin receptors in the guinea pig pulmonary artery and trachea. Br. J. Pharmacol. **105:** 376–380, 1992.

CARDELL, L. O., UDDMAN, R., AND EDVINSSON, L.: A novel ETA receptor antagonist, FR 139317, inhibits endothelin induced contractions of guinea pig pulmonary arteries, but not trachea. Br. J. Pharmacol. **108:** 448–452, 1993.

CARLINI, R., OBIALO, C. I., AND ROTHSTEIN, M.: Intravenous erythropoietin (rHuEPO) administration increases plasma endothelin and blood pressure in hemodialysis patients. Am. J. Hypertens. **6:** 103–107, 1993.

CARRIER, M., PERRAULT, L. P., TRONC, F., STEWART, D. J., AND PELLETIER, C. L.: Pentoxifylline decreases cyclosporine induced renal endothelin release and vasoconstriction. Ann. Thorac. Surg. **55:** 490–492, 1993.

CASASCO, A., CALLIGARO, A., CASASCO, M., SPRINGALL, D. R., TENTI, P., MARCHETTI, C., POGGI, P., AND POLAK, J. M.: Immunohistochemical localization of endothelin-like immunoreactivity in human tooth germ and mature dental pulp. Anat. Embryol. Berl. **183:** 515–520, 1991.

CASEY, M. L., BYRD, W., AND MACDONALD, P. C.: Massive amounts of immunoreactive endothelin in human seminal fluid. J. Clin. Endocrinol. Metab. **74:** 223–225, 1992.

CASEY, M. L., WORD, R. A., AND MACDONALD, P. C.: Endothelin 1 gene expression and regulation of endothelin mRNA and protein biosynthesis in avascular human amnion. Potential source of amniotic fluid endothelin. J. Biol. Chem. **266:** 5762–5768, 1991.

CAVERO, P. G., MILLER, W. L., HEUBLEIN, D. M., MARGULIES, K. B., AND BURNETT, J. C., JR.: Endothelin in experimental congestive heart failure in the anesthetized dog. Am. J. Physiol. **259:** F312–F317, 1990.

CERNACEK, P., AND STEWART, D. J.: Immunoreactive endothelin in human plasma: marked elevations in patients in cardiogenic shock. Biochem. Biophys. Res. Commun. **161:** 562–567, 1989.

CHABRIER, P. E., AUGUET, M., ROUBERT, P., LONCHAMPT, M. O., GILLARD, V., GUILLON, J. M., DELAFLOTTE, S., AND BRAQUET, P.: Vascular mechanism of action of endothelin 1: effect of Ca^{2+} antagonists. J. Cardiovasc. Pharmacol. **13** (Suppl. 5): 32–35, 1989.

CHABRIER, P. E., AND BRAQUET, P.: Endothelin. Horm. Res. **34:** 169–174, 1990.

CHAKRAVARTHY, U., AND ARCHER, D. B.: Endothelin: a new vasoactive ocular peptide. Br. J. Ophthalmol. **76:** 107–108, 1992.

CHAKRAVARTHY, U., GARDINER, T. A., ANDERSON, P., ARCHER, D. B., AND TRIMBLE, E. R.: The effect of endothelin 1 on the retinal microvascular pericyte. Microvasc. Res. **43:** 241–254, 1992.

CHAN, J., AND GREENBER, D. A.: Endothelin and calcium signaling in NG 108–15 neuroblastoma [times] glioma cells. J. Pharmacol. Exp. Ther. **258:** 524–530, 1991.

CHANDER, C. L., MOORE, A. R., DESA, F. M., HOWAT, D. W., AND WILLOUGHBY, D. A.: Anti inflammatory effects of endothelin 1. J. Cardiovasc. Pharmacol. **13** (Suppl. 5): S218–S219, 1989.

CHANG, H., WU, G. J., WANG, S. M., AND HUNG, C. R.: Plasma endothelin levels and surgically correctable pulmonary hypertension. Ann. Thorac. Surg. **55:** 450–458, 1993.

CHAO, H. S., POISNER, A., POISNER, R., AND HANDWERGER, S.: Endothelins stimulate the synthesis and release of prorenin from human decidual cells. J. Clin. Endocrinol. Metab. **76:** 615–619, 1993.

CHAPLEAU, M. W., HAJDUCZOK, G., AND ABBOUD, F. M.: Suppression of baroreceptor discharge by endothelin at high carotid sinus pressure. Am. J. Physiol. **263:** R103–R108, 1992.

CHESTER, A. H., DASHWOOD, M. R., CLARKE, J. G., LARKIN, S. W., DAVIES, G. J., TADJKARIMI, S., MASERI, A., AND YACOUB, M. H.: Influence of endothelin on human coronary arteries and localization of its binding sites. Am. J. Cardiol. **63:** 1395–1398, 1989.

CHESTER, A. H., O'NEIL, G. S., ALLEN, S. P., LUU, T. N., TADJKARIMI, S., AND YACOUB, M. H.: Effect of endothelin on normal and diseased human coronary arteries. Eur. J. Clin Invest. **22:** 210–213, 1992.

CHOI, D. W., AND ROTHMAN, S. M.: The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. Annu. Rev. Neurosci. **13:** 171–182, 1990.

CHOU, S. Y., DAHHAN, A., AND PORUSH, J. G.: Renal actions of endothelin: interaction with prostacyclin. Am. J. Physiol. **259:** F645–F652, 1990.

CHUA, B. H., KREBS, C. J., CHUA, C. C., AND DIGLIO, C. A.: Endothelin stimulates

protein synthesis in smooth muscle cells. Am. J. Physiol. **262:** E412–E416, 1992.

CIMMINIELLO, C., MILANI, M., UBERTI, T., ARPAIA, G., PEROLINI, S., AND BONFARDECI, G.: Endothelin, vasoconstriction, and endothelial damage in Raynaud's phenomenon (letter; comment). Lancet **337:** 114–115, 1991.

CINTRA, A., FUXE, K., ANGGARD, E., TINNER, B., STAINES, W., AND AGNATI, L. F.: Increased endothelin like immunoreactivity in ibotenic acid lesioned hippocampal formation of the rat brain. Acta Physiol. Scand. **137:** 557–558, 1989.

CLARIA, J., JIMENEZ, W., ARROYO, V., CASTRO, A., ASBERT, M., ROS, J., RIVERA, F., AND RODES, J.: Doses of endothelin have natriuretic effects in conscious rats with cirrhosis and ascites. Kidney Int. **40:** 182–187, 1991.

CLARIA, J., JIMENEZ, W., LA, VILLA G., ASBERT, M., CASTRO, A., LLIBRE, J. L., ARROYO, V., AND RIVERA, F.: Effects of endothelin on renal haemodynamics and segmental sodium handling in conscious rats. Acta Physiol. Scand. **141:** 305–308, 1991.

CLARK, B. A., HALVORSON, L., SACHS, B., AND EPSTEIN, F. H.: Plasma endothelin levels in preeclampsia: elevation and correlation with uric acid levels and renal impairment. Am. J. Obstet. Gynecol. **166:** 962–968, 1992.

CLAVELL, A. L., WRIGHT, R. S., THOMAS, M. R., BRANDT, R. R., OPGENORTH, T. J., AND BURNETT, J. C.: Elevated endogenous endothelin mediates systemic vasoconstriction in experimental chronic congestive heart failure. J. Am. Coll. Cardiol. (Aabstracts of the 43rd Annual Scientific Session of ACC) 172A, 1994.

CLOZEL, J. P., AND CLOZEL, M.: Effects of endothelin on the coronary vascular bed in open chest dogs. Circ. Res. **65:** 1193–1200, 1989.

CLOZEL, J. P., AND SPRECHER, U.: Influence of low perfusion pressure on effect of endothelin on coronary vascular bed. Am. J. Physiol. **260:** H893–H901, 1991.

CLOZEL, M.: Endothelin sensitivity and receptor binding in the aorta of spontaneously hypertensive rats. J. Hypertens. **7:** 913–917, 1989.

CLOZEL, M., BREU, V., BURRI, K., CASSAL, J. M., FISCHLI, W., GRAY, G. A., HIRTH, G., LOFFLER, B. M., MULLER, M., NEIDHART, W., AND RAMUZ, H.: Pathophysiological role of endothelin revealed by the first orally active endothelin receptor antagonist. Nature (Lond.) **365:** 759–761, 1993.

CLOZEL, M., FISCHLI, W., AND GUILLY, C.: Specific binding of endothelin on human vascular smooth muscle cells in culture. J. Clin. Invest. **83:** 1758–1761, 1989.

CLOZEL, M., GRAY, G. A., BREU, V., LOFFLER, B. M., AND OSTERWALDER, R.: The endothelin ETB receptor mediates both vasodilation and vasoconstriction in vivo. Biochem. Biophys. Res. Commun. **186:** 867–873, 1992.

CLOZEL, M., LOFFLER, B. M., BREU, V., HILFIGER, L., MAIRE, J. P., AND BUTSCHA, B.: Downregulation of endothelin receptors by autocrine production of endothelin 1. Am. J. Physiol. **265:** C188–C192, 1993.

CLOZEL, M., LOFFLER, B. M., AND GLOOR, H.: Relative preservation of the responsiveness to endothelin 1 during reperfusion following renal ischemia in the rat. J. Cardiovasc. Pharmacol. **17** (Suppl. 7)**:** S313–S315, 1991.

CLOZEL, M., AND WATANABE, H.: BQ 123, a peptidic endothelin ETA receptor antagonist, prevents the early cerebral vasospasm following subarachnoid hemorrhage after intracisternal but not intravenous injection. Life Sci. **52:** 825–834, 1993.

COCKCROFT, J. R., CLARKE J. G., AND WEBB, D. J.: The effect of intra arterial endothelin on resting blood flow and sympathetically mediated vasoconstriction in the forearm of man. Br. J. Clin. Pharmacol. **31:** 521–524, 1991.

COCKS, T. M., BROUGHTON, A., DIB, M., SUDHIR, K., AND ANGUS, J. A.: Endothelin is blood vessel selective: studies on a variety of human and dog vessels in vitro and on regional blood flow in the conscious rabbit. Clin. Exp. Pharmacol. Physiol. **16:** 243–246, 1989.

COCKS, T. M., FAULKNER, N. L., SUDHIR, K., AND ANGUS, J.: Reactivity of endothelin 1 on human and canine large veins compared with large arteries in vitro. Eur. J. Pharmacol. **171:** 17–24, 1989.

COCKS, T. M., MALTA, E., KING, S. J., WOODS, R. L., AND ANGUS, J. A.: Oxyhaemoglobin increases the production of endothelin 1 by endothelial cells in culture. Eur. J. Pharmacol. **196:** 177–182, 1991.

CODY, R. J.: The potential role of endothelin as a vasoconstrictor substance in congestive heart failure. Eur. Heart J. **13:** 1573–1578, 1992.

CODY, R. J., HAAS, G. J., BINKLEY, P. F., CAPERS, Q., AND KELLEY, R.: Plasma endothelin correlates with the extent of pulmonary hypertension in patients with chronic congestive heart failure. Circulation **85:** 504–509, 1992.

CODY, W. L., DOHERTY, A. M., HE, J. X., DEPUE, P. L., RAPUNDALO, S. T., HINGORANI, G. A., MAJOR, T. C., PANEK, R. L., DUDLEY, D. T., HALEEN, S. J., ET AL.: Design of a functional hexapeptide antagonist of endothelin. J. Med. Chem. **35:** 3301–3303, 1992.

COLLIER, A., LEACH, J. P., MCLELLAN, A., JARDINE, A., MORTON, J. J., AND SMALL, M.: Plasma endothelinlike immunoreactivity levels in IDDM patients with microalbuminuria. Diabetes Care **15:** 1038–1040, 1992.

CONTE, D., QUESTINO, P., FILLO, S., NORDIO, M., ISIDORI, A., AND ROMANELLI, F.: Endothelin stimulates testosterone secretion by rat Leydig cells. J. Endocrinol. **136:** R1–R4, 1993.

COSENTINO F., MCMAHON E. G., CARTER J. S., AND KATUSIC Z. S.: Effect of ETA-receptor antagonist BQ-123 and phosphoramidon on cerebral vasospasm J. Cardiovasc. Pharmacol. **22** (Suppl. 8)**:** S332-S335, 1993.

COSTELLO, K. B., STEWART, D. J., AND BAFFOUR, R.: Endothelin is a potent constrictor of human vessels used in coronary revascularization surgery. Eur. J. Pharmacol. **186:** 311–314, 1990.

COZZA, E. N., CHIOU, S., AND GOMEZ SANCHEZ, C. E.: Endothelin 1 potentiation of angiotensin I. I. stimulation of aldosterone production. Am. J. Physiol. **262:** R85–R89, 1992.

COZZA, E. N., AND GOMEZ SANCHEZ, C. E.: Effects of endothelin 1 on its receptor concentration and thymidine incorporation in calf adrenal zona glomerulosa cells: a comparative study with phorbol esters. Endocrinology **127:** 549–554, 1990.

COZZA, E. N., GOMEZ SANCHEZ C. E., FOECKING, M. F., AND CHIOU, S.: Endothelin binding to cultured calf adrenal zona glomerulosa cells and stimulation of aldosterone secretion. J. Clin. Invest. **84:** 1032–1035, 1989.

CRAWFORD, M. L., HILEY, C. R., AND YOUNG, J. M.: Characteristics of endothelin 1 and endothelin 3 stimulation of phosphoinositide breakdown differ between regions of guinea pig and rat brain. Naunyn Schmiedebergs Arch. Pharmacol. **341:** 268–271, 1990.

CRISCIONE, L., NELLIS, P., RINIKER, B., THOMANN, H., AND BURDET, R.: Reactivity and sensitivity of mesenteric vascular beds and aortic rings of spontaneously hypertensive rats to endothelin: effects of calcium entry blockers. Br. J. Pharmacol. **100:** 31–36, 1990.

CRISTOL, J. P., WARNER, T. D., THIEMERMANN, C., AND VANE, J. R.: Mediation via different receptors of the vasoconstrictor effects of endothelins and sarafotoxins in the systemic circulation and renal vasculature of the anaesthetized rat. Br. J. Pharmacol. **108:** 776–779, 1993.

CYBULSKY, A. V., STEWART, D. J., AND CYBULSKY, M. I.: Glomerular epithelial cells produce endothelin 1. J. Am. Soc. Nephrol. **3:** 1398–1404, 1993.

D'ORLEANS-JUSTE, P., DE NUCCI, G., AND VANE, J. R.: Endothelin 1 contracts isolated vessels independently of dihydropyridine sensitive Ca^{2+} channel activation. Eur. J. Pharmacol. **165:** 289–295, 1989.

D'ORLEANS-JUSTE, P., FINET, M., DE NUCCI, G., AND VANE, J. R.: Pharmacology of endothelin 1 in isolated vessels: effect of nicardipine, methylene blue, hemoglobin, and gossypol. J. Cardiovasc. Pharmacol. **13** (Suppl. 5)**:** S19–S22 (discussion p. S45), 1989.

D'ORLEANS-JUSTE, P., LIDBURY, P. S., TELEMAQUE, S., WARNER, T. D., AND VANE, J. R.: Human big endothelin releases prostacyclin in vivo and in vitro through a phosphoramidon sensitive conversion to endothelin 1. J. Cardiovasc. Pharmacol. **17** (Suppl. 7)**:** S251–S255, 1991.

D'ORLEANS-JUSTE, P., TELEMAQUE, S., AND CLAING, A.: Different pharmacological profiles of big endothelin 3 and big endothelin 1 in vivo and in vitro. Br. J. Pharmacol. **104:** 440–444, 1991.

D'ORLEANS-JUSTE, P., TELEMAQUE, S., CLAING, A., IHARA, M., YANO, M.: Human big endothelin 1 and endothelin 1 release prostacyclin via the activation of ET1 receptors in the rat perfused lung. Br. J. Pharmacol. **105:** 773–775, 1992.

DALGARNO, D. C., SLATER, L., CHACKALAMANNIL, S., AND SENIOR, M. M.: Solution conformation of endothelin and point mutants by nuclear magnetic resonance spectroscopy. Int. J. Pept. Protein Res. **40:** 515–523, 1992.

DANTHULURI, N. R., AND BROCK, T. A.: Endothelin receptor coupling mechanisms in vascular smooth muscle: a role for protein kinase C. J. Pharmacol. Exp. Ther. **254:** 393–399, 1990.

DAVENPORT, A. P., CAMERON, I. T., SMITH, S. K., AND BROWN, M. J.: Binding sites for iodinated endothelin 1, endothelin 2 and endothelin 3 demonstrated on human uterine glandular epithelial cells by quantitative high resolution autoradiography. J. Endocrinol. **129:** 149–154, 1991.

DAVENPORT, A. P., NUNEZ, D. J., AND BROWN, M. J.: Binding sites for ^{125}I labelled endothelin 1 in the kidneys: differential distribution in rat, pig and man demonstrated by using quantitative autoradiography. Clin. Sci. **77:** 129–131, 1989.

DE CARVALHO, M. H., NIGRO, D., SCIVOLETTO, R., BARBEIRO, H. V., DE OLIVEIRA, M. A., DE NUCCI, G., AND FORTES, Z. B.: Comparison of the effect of endothelin on microvessels and macrovessels in Goldblatt II and deoxycorticosterone acetate salt hypertensive rats. Hypertension **15** (Suppl.)**:** I68–I71, 1990.

DE FEO, M. L., BARTOLINI, O., ORLANDO, C., MAGGI, M., SERIO, M., PINES, M., HURWITZ, S., FUJII, Y., SAKAGUCHI, K., AURBACH, G. D., ET AL.: Natriuretic peptide receptors regulate endothelin synthesis and release from parathyroid cells. Proc. Natl. Acad. Sci. USA **88:** 6496–6500, 1991.

DE LA RUBIA, G., OLIVER, F. J., INOGUCHI, T., AND KING, G. L.: Induction of resistance to endothelin 1's biochemical actions by elevated glucose levels in retinal pericytes. Diabetes **41:** 1533–1539, 1992.

DE MEY, J. G., AND VANHOUTTE P. M.: Heterogeneous behavior of the canine arterial and venous wall: importance of endothelium. Circ. Res. **51:** 439–447, 1982.

DE NUCCI, G., THOMAS, R., D'ORLEANS, JUSTE P., ANTUNES, E., WALDER, C., WARNER, T. D., AND VANE, J. R.: Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium derived relaxing factor. Proc. Natl. Acad. Sci. USA **85:** 9797–9800, 1988.

DELARUE, C., DELTON, I., FIORINI, F., HOMO DELARCHE, F., FASOLO, A., BRAQUET, P., AND VAUDRY H.: Endothelin stimulates steroid secretion by frog adrenal gland in vitro: evidence for the involvement of prostaglandins and extracellular calcium in the mechanism of actin of endothelin. Endocrinology **127:** 2001–2008, 1990.

DENG, L. Y., AND SCHIFFRIN, E. L.: Calium dependence of effects of endothelin on rat mesenteric microvessels. Can. J. Physiol. Pharmacol. **69:** 798–804, 1991.

DENG, L. Y., AND SCHIFFRIN, E. L.: Effects of endothelin on resistance arteries of DOCA salt hypertensive rats. Am. J. Physiol. **262:** H1782–H1787, 1992.

DERAY, G., CARAYON, A., MAISTRE, G., BENHMIDA, M., MASSON, F., BARTHE-

LEMY, C., PETITCLERC, T., AND JACOBS, C.: Endothelin in chronic renal failure. Nephrol. Dial. Transplant. **7:** 300–305, 1992.

DEVESLY, P., CADE, C., POLOKOFF, M. A., AND BOTELHO, L. H.: Evidence of glycosylated sites on the endothelin 1 receptor in Swiss 3T3 cells. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S134–S136, 1991.

DEVESLY, P., PHILLIPS, P. E., JOHNS, A., RUBANYI, G. M., AND PARKER-BOTELHO, L. H.: Receptor kinetics differ for endothelin 1 and endothelin 2 binding to Swiss 3T3 fibroblasts. Biochem. Biophys. Res. Commun. **172:** 126–134, 1990.

DEVESLY P., PHILLIPS P. E., POLOKOFF M. A., AND PARKER BOTELHO, L. H.: Turnover and down-regulation of functional ET_A receptors on the surface of Swiss 3T3 cells. J. Cell. Physiol., submitted for publication.

DOHERTY, A. M.: Endothelin: a new challenge. J. Med. Chem. **35:** 1493–1508, 1992.

DOHI, Y., CRISCIONE, L., AND LUSCHER, T. F.: Renovascular hypertension impairs formation of endothelium derived relaxing factors and sensitivity to endothelin 1 in resistance arteries. Br. J. Pharmacol. **104:** 349–354, 1991.

DOHI, Y., HAHN, A. W., BOULANGER, C. M., BUHLER, F. R., AND LUSCHER, T. F.: Endothelin stimulated by angiotensin II augments contractility of spontaneously hypertensive rat resistance arteries. Hypertension **19:** 131–137, 1992.

DOHI, Y., AND LUSCHER, T. F.: Endothelin in hypertensive resistance arteries. Intraluminal and extraluminal dysfunction. Hypertension **18:** 543–549, 1991.

DOI, Y., AND FUJIMOTO, S.: Vasocontractions of the in vitro toad aortas induced by endothelin 1 and sarafotoxin S6b. Anat. Rec. **235:** 253–260, 1993.

DONLAN, M. L., BROWN, F. K., AND JEFFS, P. W.: Solution conformation of human big endothelin 1. J. Biomol. NMR **2:** 407–420, 1992.

DORFMAN, D. M., WILSON, D. B., BRUNS, G. A., AND ORKIN, S. H.: Human transcription factor GATA 2. Evidence for regulation of preproendothelin 1 gene expression in endothelial cells. J. Biol. Chem. **267:** 1279–1285, 1992.

DOWD, P. M., BUNKER, C. B., BULL, H. A., FOREMAN, J. C., TERENGHI, G., SPRINGALL, D. R., AND POLAK, J. M.: Raynaud's phenomenon, calcitonin gene related peptide, endothelin, and cutaneous vasculature (*letter*). Lancet **336:** 1014, 1990.

DOUGLAS, S. A., ELLIOTT, J. D., AND OHLSTEIN, E. G.: Regional vasodilation to endothelin-1 is mediated by a non ETA receptor subtype in the anaesthetized rat: effect of BQ 123 on systemic haemodynamic responses. Eur. J. Pharmacol. **221:** 315–324, 1992.

DOUGLAS, S. A., JAMES, S., AND HILEY, C. R.: Endothelial modulation and changes in endothelin pressor activity during hypoxia in rat isolated perfused superior mesenteric arterial bed. Br. J. Pharmacol. **103:** 1441–1448, 1991.

DURIEU-TRAUTMANN, O., FEDERICI, C., CREMINON, C., FOIGNANT CHAVEROT, N., ROUX, F., CLAIRE, M., STROSBERG, A. D., AND COURAUD, P. O.: Nitric oxide and endothelin secretion by brain microvessel endothelial cells: regulation by cyclic nucleotides. J. Cell. Physiol. **155:** 104–111, 1993.

ECONOMOS, K., MACDONALD, P. C., AND CASEY, M. L.: Endothelin 1 gene expression and biosynthesis in human endometrial HEC 1A cancer cells. Cancer Res. **52:** 554–557, 1992.

ECONOMOS, K., MACDONALD, P. C., AND CASEY, M. L.: Endothelin 1 gene expression and protein biosynthesis in human endometrium: potential modulator of endometrial blood flow. J. Clin. Endocrinol. Metab. **74:** 14–19, 1992b.

EDDAHIBI, S., RAFFESTIN, B., BRAQUET, P., CHABRIER, P. E., AND ADNOT, S.: Pulmonary vascular reactivity to endothelin 1 in normal and chronically pulmonary hypertensive rats. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S358–S361, 1991.

EDLUND, A., AND WENNMALM, A.: Endothelin does not affect aggregation of human platelets. Clin. Physiol. **10:** 585–590, 1990.

EDWARDS, R. M., TRIZNA, W., AND OHLSTEIN, E. H.: Renal microvascular effects of endothelin. Am. J. Physiol. **259:** F217–F221, 1990.

EGASHIRA, K., PIPERS, F. S., RUSH, J. E., AND MORGAN, J. P.: Effects of calcium channel blockers on coronary vasoconstriction induced by endothelin 1 in closed chest pigs. J. Am. Coll. Cardiol. **16:** 1296–1303, 1990.

EGLEN, R. M., MICHEL, A. D., SHARIF, N. A., SWANK, S. R., AND WHITING, R. L.: The pharmacological properties of the peptide, endothelin. Br. J. Pharmacol. **97:** 1297–1307, 1989.

EGUCHI, S., HIRATA, Y., IMAI, T., KANNO, K., AKIBA, T., SAKAMOTO, A., YANAGISAWA, M., MASAKI, T., AND MARUMO, F.: Endothelin receptors in human parathyroid gland. Biochem. Biophys. Res. Commun. **184:** 1448–1455, 1992.

EGUCHI, S., HIRATA, Y., IHARA, M., YANO, M., AND MARUMO, F.: A novel ETA antagonist (BQ 123) inhibits endothelin 1 induced phosphoinositide breakdown and DNA synthesis in rat vascular smooth muscle cells. FEBS Lett. **302:** 243–246, 1992.

EHRENREICH, H., ANDERSON, R. W., FOX, C. H., RIECKMANN, P., HOFFMAN, G. S., TRAVIS, W. D., COLIGAN, J. E., KEHRL, J. H., AND FAUCI, A. S.: Endothelins, peptides with potent vasoactive properties, are produced by human macrophages. J. Exp. Med. **172:** 1741–1748, 1990.

EHRENREICH, H., BURD, P. R., ROTTEM, M., HULTNER, L., HYLTON, J. B., GARFIELD, M., COLIGAN, J. E., METCALFE, D. D., AND FAUCI, A. S.: Endothelins belong to the assortment of mast cell derived and mast cell bound cytokines. New Biol. **4:** 147–156, 1992.

EHRENREICH, H., COSTA, T., CLOUSE, K. A., PLUTA, R. M., OGINO, Y., COLIGAN, J. E., AND BURD, P. R.: Thrombin is a regulator of astrocytic endothelin 1. Brain Res. **600:** 201–207, 1993.

EHRENREICH, H., RIECKMANN, P., SINOWATZ, F., WEIH, K. A., ARTHUR, L. O., GOEBEL, F. D., BURD, P. R., COLIGAN, J. E., AND CLOUSE, K. A.: Potent stimulation of monocytic endothelin 1 production by HIV 1 glycoprotein 120. J. Immunol. **150:** 4601–4609, 1993.

EIS, A. W., MITCHELL, M. D., AND MYATT, L.: Endothelin transfer and endothelin effects on water transfer in human fetal membranes. Obstet. Gynecol. **79:** 411–415, 1992.

ELSHOURBAGY, N. A., LEE, J. A., KORMAN, D. R., NUTHALAGANTI, P., SYLVESTER, D. R., DILELLA, A. G., SUTIPHONG, J. A., AND KUMAR, C. S.: Molecular cloning and characterization of the major endothelin receptor subtype in porcine cerebellum. Mol. Pharmacol. **41:** 465–473, 1992.

ELTON, T. S., OPARIL, S., TAYLOR, G. R., HICKS, P. H., YANG, R. H., JIN, H., AND CHEN, Y. F.: Normobaric hypoxia stimulates endothelin 1 gene expression in the rat. Am. J. Physiol. **263:** R1260–R1264, 1992.

EMMELUTH, C., AND BIE, P.: Effects, release and disposal of endothelin 1 in conscious dogs. Acta Physiol. Scand. **146:** 197–204, 1992.

EMORI, T., HIRATA, Y., IMAI, T., OHTA, K., KANNO, K., EGUCHI, S., AND MARUMO, F.: Cellular mechanism of thrombin on endothelin 1 biosynthesis and release in bovine endothelial cell. Biochem. Pharmacol. **44:** 2409–2411, 1992.

EMORI, T., HIRATA, Y., AND MARUMO, F.: Endothelin 3 stimulates prostacyclin production in cultured bovine endothelial cells. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S140–S142, 1991.

EMORI, T., HIRATA, Y., AND MARUMO, F.: Specific receptors for endothelin 3 in cultured bovine endothelial cells and its cellular mechanism of action. FEBS Lett. **263:** 261–264, 1990.

EMORI, T., HIRATA, Y., OHTA, K., KANNO, K., EGUCHI, S., IMAI, T., SHICHIRI, M., AND MARUMO, F.: Cellular mechanism of endothelin 1 release by angiotensin and vasopressin. Hypertension **18:** 165–170, 1991.

EMORI, T., HIRATA, Y., OHTA, K., SHICHIRI, M., AND MARUMO, F.: Secretory mechanism of immunoreactive endothelinin cultured bovine endothelial cells. Biochem. Biophys. Res. Commun. **160:** 93–100, 1989.

ENCABO, A., FERRER, M., MARIN, J., VILLAMOR, J., AND BALFAGON, G.: Vasoconstrictive responses elicited by endothelin in bovine cerebral arteries. Gen. Pharmacol. **23:** 263–267, 1992.

ENDO, S., INOOKA, H., ISHIBASHI, Y., KITADA, C., MIZUTA, E., AND FUJINO, M.: Solution conformation of endothelin determined by nuclear magnetic resonance and distance geometry. FEBS Lett. **25:** 149–154, 1989.

ENDO, T., UCHIDA, Y., MATSUMOTO, H., SUZUKI, N., NOMURA, A., HIRATA, F., AND HASEGAWA, S.: Regulation of endothelin 1 synthesis in cultured guinea pig airway epithelial cells by various cytokines. Biochem. Biophys. Res. Commun. **186:** 1594–1599, 1992.

ERDOS E. G., AND SKIDGEL R. A.: Neutral endopeptidase 24.11 (enkephalinase) and related regulators of peptide hormones. FASEB J. **3:** 145–151, 1989.

ERGUL, A., GLASSBERG, M. K., MAJERCIK, M. H., AND PUETT, D.: Endothelin 1 promotes steroidogenesis and stimulates protooncogene expression in transformed murine Leydig cells. Endocrinology **132:** 598–603, 1993.

ERHARDT P. W.: Endothelin structure and structure—activity relationships. *In* Endothelin, edited by G. M. Rubanyi, Oxford University Press, New York, pp. 41–57, 1992.

EZRA, D., GOLDSTEIN, R. E., CZAJA, J. F., AND FEUERSTEIN, G. Z.: Lethal ischemia due to intracoronary endothelin in pigs. Am. J. Physiol. **257:** H339–H343, 1989.

FABBRINI, M. S., VALSASINA, B., NITTI, G., BENATTI, L., AND VITALE, A.: The signal peptide of human preproendothelin 1. FEBS Lett. **286:** 91–94, 1991.

FAGNY, C., MICHEL, A., LEONARD, I., BERKENBOOM, G., FONTAINE, J., AND DESCHODT LANCKMAN, M.: In vitro degradation of endothelin 1 by endopeptidase 24.11 (enkephalinase). Peptides (Elmsford) **12:** 773–778, 1991.

FAGNY, C., MICHEL, A., NORTIER, J., AND DESCHODT LANCKMAN, M.: Enzymatic degradation of endothelin 1 by activated human polymorphonuclear neutrophils. Regul. Pept. **42:** 27–37, 1992.

FARAGO, A., AND NISHIZUKA, Y.: Protein kinase C in transmembrane signaling. FEBS Lett. **268:** 350–354, 1990.

FERGUSON, A. V., AND SMITH, P.: Cardiovascular responses induced by endothelin microinjection into area postrema. Regul. Pept. **27:** 75–85, 1990.

FERRARIO, R. G., FOULKES, R., SALVATI, P., AND PATRONO, C.: Hemodynamic and tubular effects of endothelin and thromboxane in the isolated perfused rat kidney. Eur. J. Pharmacol. **171:** 127–134, 1989.

FILEP, J. G., BATTISTINI, B., COTE, Y. P., BEAUDOIN, A. R., AND SIROIS, P.: Endothelin 1 induces prostacyclin release from bovine aortic endothelial cells. Biochem. Biophys. Res. Commun. **177:** 171–176, 1991.

FILEP, J. G., HERMAN, F., BATTISTINI, B., CHABRIER, P. E., BRAQUET, P., AND SIROIS, P.: Antiaggregatory and hypotensive effects of endothelin 1 in beagle dogs: role for prostacyclin. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S216–S218, 1991.

FILEP, J. G., SIROIS, M. G., ROUSSEAU, A., FOURNIER, A., AND SIROIS, P.: Effects of endothelin 1 on vascular permeability in the conscious rat: interactions with platelet activating factor. Br. J. Pharmacol. **104:** 797–804, 1991.

FIRTH, J. D., AND RATCLIFFE, P. J.: Organ distribution of the three rat endothelin messenger RNAs and the effects of ischemia on renal gene expression. J. Clin. Invest. **90:** 1023–1031, 1992.

FIRTH, J. D., RATCLIFFE, P. J., RAINE, A. E., AND LEDINGHAM, J. G.: Endothelin: an important factor in acute renal failure? Lancet **2:** 1179–1182, 1988.

FIRTH, J. D., ROBERTS, A. F., AND RAINE, A. E.: Effect of endothelin on the function of the isolated perfused working rat heart. Clin. Sci. **79:** 221–226, 1990.

FLORIJN, K. W., DERKX, F. H., VISSER, W., HOFMAN, J. A., ROSMALEN, F. M.,

WALLENBURG, H. C., AND SCHALEKAMP, M. A.: Plasma immunoreactive endothelin 1 in pregnant women with and without pre eclampsia. J. Cardiovasc. Pharmacol. 7 (Suppl. 7): S446–S448, 1991.

FORTES, Z. B., NIGRO, D., SCIVOLETTO, R., AND CARVALHO, M. H.: Influence of sex on the reactivity to endothelin 1 and noradrenaline in spontaneously hypertensive rats. Clin. Exp. Hypertens. [A] 13: 807–816, 1991.

FOZARD, J. R., AND PART, M. L.: No major role for atrial natriuretic peptide in the vasodilator response to endothelin 1 in the spontaneously hypertensive rat. Eur. J. Pharmacol. 180: 153–159, 1990.

FOZARD, J. R., AND PART, M. L.: The role of nitric oxide in the regional vasodilator effects of endothelin 1 in the rat. Br. J. Pharmacol. 105: 744–750, 1992.

FRANKE, R. R., KONIG, B., SAKMAR, T. P., KHORANA, H. G., AND HOFMANN, K. P.: Rhodopsin mutants that bind but fail to activate transducin. Science (Wash. DC) 250: 123–125, 1990.

FRELIN, C., LADOUX, A., MARSAULT, R., AND VIGNE, P.: Functional properties of high and low affinity receptor subtypes for endothelin 3. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S131–S133, 1991.

FRETSCHNER, M., ENDLICH, K., GULBINS, E., LANG, R. E., SCHLOTTMANN, K., AND STEINHAUSEN, M.: Effects of endothelin on the renal microcirculation of the split hydronephrotic rat kidney. Renal Physiol. Biochem. 14: 112–127, 1991.

FRIED, G., AND SAMUELSON, U.: Endothelin and neuropeptide Y are vasoconstrictors in human uterine blood vessels. Am. J. Obstet. Gynecol. 164: 1330–1336, 1991.

FRIEDLAENDER, M. M., JAIN, D., AHMED, Z., HART, D., BARNETT, R. L., AND NORD, E. P.: Endothelin activation of phospholipase D: dual modulation by protein kinase C and Ca^{2+}. Am. J. Physiol. 264: F845–F853, 1993.

FUJIMOTO, M., MIHARA, S., NAKAJIMA, S., UEDA, M., NAKAMURA, M., AND SAKURAI, K.: A novel non peptide endothelin antagonist isolated from bayberry, *Myrica cerifera*. FEBS Lett. 305: 41–44, 1992.

FUKUDA, Y., HIRATA, Y., YOSHIMI, H., KOJIMA, T., KOBAYASHI, Y., YANAGISAWA, M., AND MASAKI, T.: Endothelin is a potent secretagogue for atrial natriuretic peptide in cultured rat atrial myocytes. Biochem. Biophys. Res. Commun. 155: 167–172, 1988.

FUKURODA, T., NOGUCHI, K., TSUCHIDA, S., NISHIKIBE, M., IKEMOTO, F., OKADA, K., AND YANO, M.: Inhibition of biological actions of big endothelin 1 by phosphoramidon. Biochem. Biophys. Res. Commun. 172: 390–395, 1990.

FULTON, D. J., HODGSON, W. C., SIKORSKI, B. W., AND KING, R. G.: Attenuated responses to endothelin 1, KCl and $CaCl_2$, but not noradrenaline, of aortae from rats with streptozotocin induced diabetes mellitus. Br. J. Pharmacol. 104: 928–932, 1991.

FURCHGOTT, R. F., AND ZAWADZKI, J. V.: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholin. Nature (Lond.) 288: 373–376, 1980.

FURUYA, S., NARUSE, S., NAKAYAMA, T., AND NOKIHARA, K.: Binding of ^{125}I endothelin 1 to fat storing cells in rat liver revealed by electron microscopic radioautography. Anat. Embryol. Berl. 185: 97–100, 1992.

FUXE, K., CINTRA, A., ANDBJER, B., ANGGARD, E., GOLDSTEIN, M., AND AGNATI, L. F.: Centrally administered endothelin 1 produced lesions in the brain of the male rat. Acta Physiol. Scand. 137: 155–156, 1989.

FUXE, K., KUROSAWA, N., CINTRA, A., HALLSTROM, A., GOINY, M., ROSEN, L., AGNATI, L. F., AND UNGERSTEDT, U.: Involvement of local ischemia in endothelin 1 induced lesions of the neostriatum of the anaesthetized rat. Exp. Brain Res. 88: 131–139, 1992.

GALRON, R., BDOLAH, A., KLOOG, Y., AND SOKOLOVSKY, M.: Endothelin/sarafotoxin receptor induced phosphoinositide turnover: effects of pertussis and cholera toxins and of phorbol ester. Biochem. Biophys. Res. Commun. 71: 949–954, 1990.

GALRON, R., BDOLAH, A., KOCHVA, E., WOLLBERG, Z., KLOOG, Y., AND SOKOLOVSKY, M.: Kinetic and cross linking studies indicate different receptors for endothelins and sarafotoxins in the ileum and cerebellum. FEBS Lett. 283: 11–14, 1991.

GALRON, R., KLOOG, Y., BDOLAH, A., AND SOKOLOVSKY, M.: Different pathways of endothelin/sarafotoxin stimulated phosphoinositide hydrolysis in myocytes. Eur. J. Pharmacol. 188: 85–88, 1990.

GALVANI, A. P., ZAMAI, M., NITTI, G. P., CURATOLO, L., CIAVOLELLA, A., AND CAIOLFA, V. R.: Big endothelin converting enzyme activities in subcellular fractions of bovine aortic endothelial cells. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S47–S51, 1991.

GANDHI, C. R., BEHAL, R. H., HARVEY, S. A., NOUCHI, T. A., AND OLSON, M. S.: Hepatic effects of endothelin. Receptor characterization and endothelin induced signal transduction in hepatocytes. Biochem. J. 287: 897–904, 1992.

GANDHI, C. R., STEPHENSON, K., AND OLSON, M. S.: Endothelin, a potent peptide agonist in the liver. J. Biol. Chem. 265: 17432–17435, 1990.

GANDHI, C. R., STEPHENSON, K., AND OLSON, M. S.: A comparative study of endothelin and platelet activating factor mediated signal transduction and prostaglandin synthesis in rat Kupffer cells. Biochem. J. 281: 485–492, 1992.

GARCIA PASCUAL, A., LARSSON, B., AND ANDERSSON, K. E.: Contractile effects of endothelin 1 and localization of endothelin binding sites in rabbit lower urinary tract smooth muscle. Acta Physiol. Scand. 140: 545–555, 1990.

GARCIA PASCUAL, A., PERSSON, K., HOLMQUIST, F., AND ANDERSSON, K. E.: Endothelin 1 induced phosphoinositide hydrolysis and contraction in isolated rabbit detrusor and urethral smooth muscle. Gen. Pharmacol. 24: 131–138, 1993.

GARCIA, R., LACHANCE, D., AND THIBAULT, G.: Positive inotropic action, natriuresis and atrial natriuretic factor release induced by endothelin in the conscious rat. J. Hypertens. 8: 725–731, 1990.

GARDINER, S. M., COMPTON, A. M., KEMP, P. A., AND BENNETT, T.: Regional and cardiac haemodynamic responses to glyceryl trinitrate, acetycholine, bradykinin and endothelin-1 in conscious rats: effects of NG-nitro-L-arginine methyl ester. Br. J. Pharmacol. 101: 632–639, 1990.

GARDINER, S. M., KEMP, P. A., AND BENNETT, T.: Effects of the neutral endopeptidase inhibitor SQ 28,603, on regional haemodynamic responses to atrial natriuretic peptide or proendothelin-1 [1–38] in conscious rats. Br. J. Pharmacol. 106: 180–186, 1992.

GARDNER, D. G., NEWMAN, E. D., NAKAMURA, K. K., AND NGUYEN, K. P.: Endothelin increases the synthesis and secretion of atrial natriuretic peptide in neonatal rat cardiocytes. Am. J. Physiol. 261: E177–E182, 1991.

GARDNER, J. P., MAHER, E., AND AVIV, A.: Calcium mobilization and Na^+/H^+ antiport activation by endothelin in human skin fibroblasts. FEBS Lett. 256: 38–42, 1989.

GARDNER, J. P., TOKUDOME, G., TOMONARI, H., MAHER, E., HOLLANDER, D., AND AVIV, A.: Endothelin induced calcium responses in human vascular smooth muscle cells. Am. J. Physiol. 262: C148–C155, 1992.

GASIC, S., WAGNER, O. F., VIERHAPPER, H., NOWOTNY, P., AND WALDHAUSL, W.: Regional hemodynamic effects and clearance of endothelin 1 in humans: renal and peripheral tissues may contribute to the overall disposal of the peptide. J. Cardiovasc. Pharmacol. 19: 176–180, 1992.

GIAID, A., GIBSON, S. J., HERRERO, M. T., GENTLEMAN, S., LEGON, S., YANAGISAWA, M., MASAKI, T., IBRAHIM, N. B., ROBERTS, G. W., ROSSI, M. L., ET AL.: Topographical localisation of endothelin mRNA and peptide immunoreactivity in neurones of the human brain. Histochemistry 95: 303–314, 1991.

GIAID, A., GIBSON, S. J., IBRAHIM, B. N., LEGON, S., BLOOM, S. R., YANAGISAWA, M., MASAKI, T., VARNDELL, I. M., AND POLAK, J. M.: Endothelin 1, an endothelium derived peptide, is expressed in neurons of the human spinal cord and dorsal root ganglia. Proc. Natl. Acad. Sci. USA 86: 7634–7638, 1989.

GIAID, A., HAMID, Q. A., SPRINGALL, D. R., YANAGISAWA, M., SHINMI, O., SAWAMURA, T., MASAKI, T., KIMURA, S., CORRIN, B., AND POLAK, J. M.: Detection of endothelin immunoreactivity and mRNA in pulmonary tumours. J. Pathol. 162: 15–22, 1990.

GIAID, A., YANAGISAWA, M., LANGLEBEN, D., MICHEL, R. P., LEVY, R., SHENNIB, H., KIMURA, S., MASAKI, T., DUGUID, W. P., AND STEWART, D. J.: Expression of endothelin 1 in the lungs of patients with pulmonary hypertension. N. Engl. J. Med. 328: 1732–1739, 1993.

GILLESPIE, M. N., OWASOYO, J. D., MCMURTRY, I. F., AND O'BRIEN, R. F.: Sustained coronary vasoconstriction provoked by a peptidergic substance released from endothelial cells in culture. J. Pharmacol. Exp. Ther. 236: 339–343, 1986.

GIUFFRIDA, R., BELLOMO, M., POLIZZI, G., AND MALATINO, L. S.: Ischemia-induced changes in the immunoreactivity for endothelin and other vasoactive peptides in the brain of the Mongolian gerbil. J. Cardiovasc. Pharmacol. 20 (Suppl. 12): 41–44, 1992.

GODFRAIND, T., MENNIG, D., MOREL, N., AND WIBO, M.: Effect of endothelin 1 on calcium channel gating by agonists in vascular smooth muscle. J. Cardiovasc. Pharmacol. 13 (Suppl. 5): 112–117, 1989.

GOETZ, K., WANG, B. C., LEADLEY, R., JR; ZHU, J. L., MADWED, J., AND BIE, P.: Endothelin and sarafotoxin produce dissimilar effects on renal blood flow, but both block the antidiuretic effects of vasopressin. Proc. Soc. Exp. Biol. Med. 191: 425–427, 1989.

GOETZ, K. L., WANG, B. C., MADWED, J. B., ZHU, J. L., AND LEADLEY, R. J., JR.: Cardiovascular, renal, and endocrine responses to intravenous endothelin in conscious dogs. Am. J. Physiol. 255: R1064–R1068, 1988.

GOLDIE, R. G., HENRY, P. J., PATERSON, J. W., PREUSS, J. M., AND RIGBY, P. J.: Contractile effects and receptor distributions for endothelin 1 (ET 1) in human and animal airways. Agents Actions 31 (Suppl.): 229–232, 1990.

GOLIGORSKY, M. S., ILJIMA, K., MORGAN, M., YANAGISAWA, M., MASAKI, T., LIN, L., NASJLETTI, A., KASKEL, F., FRAZER, M., AND BADR, K. F.: Role of endothelin in the development of Dahl hypertension. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S484–S491, 1991.

GOMEZ GARRE, D., GUERRA, M., GONZALEZ, E., LOPEZ FARRE, A., RIESCO, A., CARAMELO, C., ESCANERO, J., AND EGIDO, J.: Aggregation of human polymorphonuclear leukocytes by endothelin: role of platelet activating factor. Eur. J. Pharmacol. 224: 167–172, 1992.

GOMEZ-SANCHEZ, C. E., COZZA, E. N., FOECKING, M. F., CHIOU, S., AND FERRIS, M. W.: Endothelin receptor subtypes and stimulation of aldosterone secretion. Hypertension 15: 744–747, 1990.

GOTO, K., KASUYA, Y., MATSUKI, N., TAKUWA, Y., KURIHARA, H., ISHIKAWA, T., KIMURA, S., YANAGISAWA, M., AND MASAKI, T.: Endothelin activates the dihydropyridine sensitive, voltage dependent Ca^{2+} channel in vascular smooth muscle. Proc. Natl. Acad. Sci. USA 86: 3915–3918, 1989.

GOTO, K., YANAGISAWA, M., KIMURA, S., AND MASAKI, T.: Cardiovascular effects of endothelin. Jpn. Circ. J. 56: 162–169, 1992.

GRANSTAM, E., WANG, L., AND BILL, A.: Effects of endothelins (ET 1, ET 2 and ET 3) in the rabbit eye: role of prostaglandins. Eur. J. Pharmacol. 194: 217–223, 1991.

GRANSTAM, E., WANG, L., AND BILL, A.: Ocular effects of endothelin 1 in the cat. Curr. Eye Res. 11: 325–332, 1992.

GREENBERG, D. A., CHAN, J., AND SAMPSON, H. A.: Endothelins and the nervous system. Neurology 42: 25–31, 1992.

GRIENDLING, K. K., TSUDA, T., AND ALEXANDER, R. W.: Endothelin stimulates diacylglycerol accumulation and activates protein kinase C in cultured vascular smooth muscle cells. J. Biol. Chem. **264:** 8237–8240, 1989.

GRINSTEIN, S., AND ROTHSTEIN, A.: Mechanism of regulation of the Na^+/H^+ exchanger. J. Membr. Biol. **90:** 1–12, 1986.

GRONOSTAJSKI, R. M.: Site specific DNA binding of nuclear factor I: effect of spacer region. Nucleic Acids Res. **15:** 5545–5559, 1987.

GROSS, P., RENN, C., WALDHERR, R., SEIFERT, M., VON BAEHR, R., AND HOCHER, B.: Potential role of endothelin in the physiological and pathological regulation of kidney function. Endothelium **1:** 71–84, 1993.

GROSS, P. M., WAINMAN, D. S., CHEW, B. H., ESPINOSA, F. J., AND WEAVER, D. F.: Calcium mediated metabolic stimulation of neuroendocrine structures by intraventricular endothelin 1 in conscious rats. Brain Res. **606:** 135–142, 1993.

GROVER, G. J., SLEPH, P. G., FOX, M., AND TRIPPODO, N. C.: Role of endothelin 1 and big endothelin 1 in modulating coronary vascular tone, contractile function and severity of ischemia in rat hearts. J. Pharmacol. Exp. Ther. **263:** 1074–1082, 1992.

GRUNSTEIN, M. M., CHUANG, S. T., SCHRAMM, C. M., AND PAWLOWSKI, N. A.: Role of endothelin 1 in regulating rabbit airway contractility. Am. J. Physiol. **260:** L75–L82, 1991.

GRUNSTEIN, M. M., ROSENBERG, S. M., SCHRAMM, C. M., AND PAWLOWSKI, N. A.: Mechanisms of action of endothelin 1 in maturing rabbit airway smooth muscle. Am. J. Physiol. **260:** L434–L443, 1991.

GU, X. H., CASLEY, D. J., CINCOTTA, M., AND NAYLER, W. G.: ^{125}I endothelin 1 binding to brain and cardiac membranes from normotensive and spontaneously hypertensive rats. Eur. J. Pharmacol. **177:** 205–209, 1990.

GU, X. H., CASLEY, D., AND NAYLER, W.: Specific high affinity binding sites for ^{125}I labelled porcine endothelin in rat cardiac membranes. Eur. J. Pharmacol., 167: 281–290, 1989.

GU, X. H., LIU, J. J., DILLON, J. S., AND NAYLER, W. G.: The failure of endothelin to displace bound, radioactively labelled, calcium antagonists (PN 200/110, D888 and diltiazem). Br. J. Pharmacol. **96:** 262–264, 1989.

GULATI, A., AND REBELLO, S.: Characteristics of endothelin receptors in the central nervous system of spontaneously hypertensive rats. Neuropharmacology **31:** 243–250, 1992.

HAAK, T., JUNGMANN, E., FELBER, A., HILLMANN, U., AND USADEL, K. H.: Increased plasma levels of endothelin in diabetic patients with hypertension. Am. J. Hypertens. **5:** 161–166, 1992.

HAENDLER, B., HECHLER, U., BECKER, A., AND SCHLEUNING, W. D.: Expression of human endothelin receptor ETB by *Escherichia coli* transformants. Biochem. Biophys. Res. Commun. **191:** 633–638, 1993.

HAENDLER, B., HECHLER, U., AND SCHLEUNING, W. D.: Molecular cloning of human endothelin (ET) receptors ETA and ETB. J. Cardiovasc. Pharmacol. **20** (Suppl. 12): S1–S4, 1992.

HAGIWARA, H., KOZUKA, M., SAKAGUCHI, H., EGUCHI, S., ITO, T., AND HIROSE, S.: Separation and purification of 34 and 52 kDa species of bovine lung endothelin receptors and identification of the 34 kDa species as a degradation product. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S117–S118, 1991.

HAHN, A. W., RESINK, T. J., SCOTT-BURDEN, T., POWELL, J., DOHI, Y., AND BUHLER, F. R.: Stimulation of endothelin mRNA and secretion in rat vascular smooth muscle cells: a novel autocrine function. Cell Regul. **1:** 649–659, 1990.

HAMILTON, M. G., FREW, R., AND LUNDY, P. M.: Effect of endothelin on Ca^{2+} influx, intracellular free Ca^{2+} levels and ligand binding to N and L type Ca^{2+} channels in rat brain. Biochem. Biophys. Res. Commun. **162:** 1332–1338, 1989.

HARDEBO, J. E., KAHRSTROM, J., OWMAN, C., AND SALFORD, L. G.: Endothelin is a potent constrictor of human intracranial arteries and veins. Blood Vessels **26:** 249–253, 1989.

HARRISON, V. J., RANDRIANTSOA, A., AND SCHOEFFTER, P.: Heterogeneity of endothelin sarafotoxin receptors mediating contraction of pig coronary artery. Br. J. Pharmacol. **105:** 511–513, 1992.

HASHIDO, K., GAMOU, T., ADACHI, M., TABUCHI, H., WATANABE, T., FURUICHI, Y., AND MIYAMOTO, C.: Truncation of N-terminal extracellular or C-terminal intracellular domains of the human ET_A receptor abrogated the binding activity to ET-1. Biochim. Biophys. Acta **187:** 1241–1248, 1992.

HASHIM, M. A., AND TADEPALLI, A. S.: Functional evidence for the presence of a phosphoramidon sensitive enzyme in rat brain that converts big endothelin 1 to endothelin 1. Life Sci. **49:** PL207–PL211, 1991.

HASHIM, M. A., AND TADEPALLI, A. S.: Hemodynamic responses evoked by endothelin from central cardiovascular neural substrates. Am. J. Physiol. **262:** H1–H9, 1992.

HATTORI, Y., KASAI, K., NAKAMURA, T., EMOTO, T., AND SHIMODA, S.: Effect of glucose and insulin on immunoreactive endothelin 1 release from cultured porcine aortic endothelial cells. Metabolism **40:** 165–169, 1991.

HATTORI, Y., NAKAYA, H., ENDOU, M., AND KANNO, M.: Pharmacological analysis of the positive inotropic effect of endothelin 1 in guinea pig left atria. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S194–S196, 1991b.

HAY, D. W., HENRY, P. J., AND GOLDIE, R. G.: Endothelin and the respiratory system. Trends Pharmacol. Sci. **14:** 29–32, 1993.

HAYNES, B., AND WEBB, D. J.: Administration of endothelin 1 in humans. Circulation **83:** 1121, 1991.

HAYNES, W. G., CLARKE, J. G., COCKCROFT, J. R., AND WEBB, D. J.: Pharmacology of endothelin 1 in vivo in humans. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S284–S286, 1991.

HAYNES, W. G., AND WEBB, D. J.: Endothelin: a long acting local constrictor hormone. Br. J. Hosp. Med. **47:** 340–349, 1992.

HELSET, E., KJAEVE, J., AND HAUGE, A.: Endothelin 1 induced increases in microvascular permeability in isolated, perfused rat lungs requires leukocytes and plasma. Circ. Shock **39:** 15–20, 1993.

HEMSEN, A.: Biochemical and functional characterization of endothelin peptides with special reference to vascular effects. Acta Physiol. Scand. Suppl. **602:** 1–61, 1991.

HEMSEN, A., LARSSON, O., AND LUNDBERG, J. M.: Characteristics of endothelin A and B binding sites and their vascular effects in pig peripheral tissues. Eur. J. Pharmacol. **208:** 313–322, 1991.

HEMSEN, A., AND LUNDBERG, J. M.: Presence of endothelin 1 and endothelin 3 in peripheral tissues and central nervous system of the pig. Regul. Pept. **36:** 71–83, 1991.

HEMSEN, A., PERNOW, J., AND LUNDBERG, J. M.: Regional extraction of endothelins and conversion of big endothelin to endothelin 1 in the pig. Acta Physiol. Scand. **141:** 325–334, 1991.

HENRY, P. J., RIGBY, P. J., SELF, G. J., PREUSS, J. M., AND GOLDIE, R. G.: Relationship between endothelin 1 binding site densities and constrictor activities in human animal airway smooth muscle. Br. J. Pharmacol. **100:** 786–792, 1990.

HERMAN, F., MAGYAR, K., CHABRIER, P. E., BRAQUET, P., AND FILEP, J.: Prostacyclin mediates antiaggregatory and hypotensive actions of endothelin in anesthetized beagle dogs. Br. J. Pharmacol. **98:** 38–40, 1989.

HEYMAN, S. N., CLARK, B. A., CANTLEY, L., SPOKES, K., ROSEN, S., BREZIS, M., AND EPSTEIN, F. H.: Effects of ioversol versus iothalamateon endothelin release and radiocontrast nephropathy. Invest. Radiol. **28:** 313–318, 1993.

HEYMAN, S. N., CLARK, B. A., KAISER, N., EPSTEIN, F. H., SPOLKES, K., ROSEN, S., AND BREZIS, M.: In vivo and in vitro studies on the effect of amphotericin B on endothelin release. J. Antimicrob. Chemother. **29:** 69–77, 1992.

HICKEY, K. A., RUBANYI, G. M., PAUL, R. J., AND HIGHSMITH, R. F.: Characterization of a coronary vasoconstrictor produced by cultured endothelial cells. Am. J. Physiol. **248:** C550–C556, 1985.

HIGHSMITH, R. F., BLACKBURN, K., AND SCHMIDT, D. J.: Endothelin and calcium dynamics in vascular smooth muscle. Annu. Rev. Physiol. **54:** 257–277, 1992.

HILDEBRAND, P., MROZINSKI, J. E., JR., MANTEY, S. A., PATTO, R. J., AND JENSEN, R. T.: Pancreatic acini possess endothelin receptors whose internalization is regulated by PLC activating agents. Am. J. Physiol. **264:** G984–G993, 1993.

HILEY, C. R.: Functional studies on endothelin catch up with molecular biology. Trends Pharmacol. Sci. **10:** 47–49, 1989.

HILEY, C. R., COWLEY, D. J., PELTON, J., AND HARGREAVES, A. C.: BQ 123, cyclo (D Trp D Asp Pro D Val Leu), is a non competitive antagonist of the actions of endothelin 1 in SK N MC human neuroblastoma cells. Biochem. Biophys. Res. Commun. **184:** 504–510, 1992.

HINSON, J. P., KAPAS, S., TEJA, R., AND VINSON, G. P.: Effect of the endothelins on aldosterone secretion by rat zona glomerulosa cells in vitro. J. Steroid Biochem. Mol. Biol. **40:** 437–439, 1991.

HINSON, J. P., VINSON, G. P., KAPAS, S., AND TEJA, R.: The relationship between adrenal vascular events and steroid secretion: the role of mast cells and endothelin. J. Steroid Biochem. Mol. Biol. **40:** 381–389, 1991.

HINSON, J. P., VINSON, G. P., KAPAS, S., AND TEJA, R.: The role of endothelin in the control of adrenocortical function: stimulation of endothelin release by ACTH and the effects of endothelin 1 and endothelin 3 on steroidogenesis in rat and human adrenocortical cells. J. Endocrinol. **128:** 275–280, 1991.

HIOKI, Y., OKADA, K., ITO, H., MATSUYAMA, K., AND YANO, M.: Endothelin converting enzyme of bovine carotid artery smooth muscles. Biochem. Biophys. Res. Commun. **174:** 446–451, 1991.

HIRATA, Y., EMORI, T., EGUCHI, S., KANNO, K., IMAI, T., OHTA, K., AND MARUMO, F.: Endothelin receptor subtype B mediates synthesis of nitric oxide by cultured bovine endothelial cells. J. Clin. Invest. **91:** 1367–1373, 1993.

HIRATA, Y., FUKUDA, Y., YOSHIMI, H., EMORI, T., SHICHIRI, M., AND MARUMO, F.: Specific receptor for endothelin in cultured rat cardiocytes. Biochem. Biophys. Res. Commun. **160:** 1438–1444, 1989.

HIRATA, Y., MATSUOKA, H., KIMURA, K., FUKUI, K., HAYAKAWA, H., SUZUKI, E., SUGIMOTO, T., SUGIMOTO, T., YANAGISAWA, M., AND MASAKI, T.: Renal vasoconstriction by the endothelial cell derived peptide endothelin in spontaneously hypertensive rats. Circ. Res. **65:** 1370–1379, 1989.

HIRATA, Y., TAKAGI, Y., FUKUDA, Y., AND MARUMO, F.: Endothelin is a potent mitogen for rat vascular smooth muscle cells. Atherosclerosis **78:** 225–228, 1989.

HIRATA, Y., YOSHIMI, H., TAKAICHI, S., YANAGISAWA, M., AND MASAKI, T.: Binding and receptor down regulation of a novel vasoconstrictor endothelin in cultured rat vascular smooth muscle cells. FEBS Lett. **239:** 13–17, 1988.

HODGSON, W. C., AND KING, R. G.: Effects of glucose, insulin or aldose reductase inhibition on responses to endothelin 1 of aortic rings from streptozotocin induced diabetic rats. Br. J. Pharmacol. **106:** 644–649, 1992.

HOEHE, M. R., EHRENREICH, H., CAENAZZO, L., AND BERRETTINI, W. H.: *TaqI* identifies a four allele DNA polymorphism of the human endothelin 1 gene (EDN1). Nucleic Acids Res. **19:** 3161, 1991.

HOEHE, M. R., EHRENREICH, H., OTTERUD, B., CAENAZZO, L., PLAETKE, R., ZANDER, H., AND LEPPERT, M.: The human endothelin 1 gene (EDN1) encoding a peptide with potent vasoactive properties maps distal to HLA on chromosome arm 6p in close linkage to D6S89. Cytogenet. Cell Genet. **62:** 131–135, 1993.

HOFFMAN, A., KEISER, H. R., GROSSMAN, E., GOLDSTEIN, D. S., GOLD, P. W., AND KLING, M.: Endothelin concentration in cerebrospinal fluid in depressive patients. Lancet 2: 1519, 1989.
HOKFELT, T., POST, C., FREEDMAN, J., LUNDBERG, J. M., AND TERENIUS, L.: Endothelin induced spinal lesion afer intrathecal administration. Acta Physiol. Scand. 137: 555–556, 1989.
HOM, G. J., SCHUTT, W. A., AND RUBANYI, G. M.: Potential mechanisms of endothelin-induced transient decreases in arterial pressure in SHR. *In* Endothelium-derived Contracting Factors, edited by G. M. Rubanyi and P. M. Vanhoute, Karger, Basel, Switzerland, pp. 98–103, 1990.
HOM, G. J., TOUHEY, B., AND RUBANYI, G. M.: Effects of intracoronary administration of endothelin in anesthetized dogs: comparison with Bay k 8644 and U 46619. J. Cardiovasc. Pharmacol. 19: 194–200, 1992.
HOMMA, S., MIYAUCHI, T., GOTO, K., SUGISHITA, Y., SATO, M., AND OHSHIMA, N.: Effects of endothelin 1 on coronary microcirculation in isolated beating hearts of rats. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S276–S278, 1991.
HOMMA, S., MIYAUCHI, T., SUGISHITA, Y., GOTO, K., SATO, M., AND OHSHIMA, N.: Vasoconstrictor effects of endothelin 1 on myocardium microcirculation studied by the Langendorff perfusion method: differential sensitivities among microvessels. Microvasc. Res. 43: 205–217, 1992.
HORI, S., KYOTANI, S., INOUE, S., FUKUDA, K., OHNISHI, Y., KUSUHARA, M., AIKAWA, N., YAMAGUCHI, K., NAKAMURA, Y., AND HANDA, S.: Subepicardial microischemia formation induced by epicardial application of endothelin 1. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S300–S301, 1991.
HORIE, M., UCHIDA, S., YANAGISAWA, M., MATSUSHITA, Y., KUROKAWA, K., AND OGATA, E.: Mechanisms of endothelin 1 mRNA and peptides induction by TGF beta and TPA in MDCK cells. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S222–S225, 1991.
HORIO, T., KOHNO, M., MURAKAWA, K., YASUNARI, K., YOKOKAWA, K., UEDA, M., AND TAKEDA, T.: Increased plasma immunoreactive endothelin 1 concentration in hypercholesterolemic rats. Atherosclerosis 89: 239–246, 1991.
HORIO, T., KOHNO, M., AND TAKEDA, T.: Effects of arginine vasopressin, angiotensin II and endothelin 1 on the release of brain natriuretic peptide in vivo and in vitro. Clin. Exp. Pharmacol. Physiol. 19: 575–582, 1992.
HORIO, T., KOHNO, M., YOKOKAWA, K., MURAKAWA, K., YASUNARI, K., FUJIWARA, H., KURIHARA, N., AND TAKEDA, T.: Effect of hypoxia on plasma immunoreactive endothelin 1 concentration in anesthetized rats. Metabolism 40: 999–1001, 1991.
HOSODA, K., NAKAO, K., TAMURA, N., ARAI, H., OGAWA, Y., SUGA, S., NAKANISHI, S., AND IMURA, H.: Organization, structure, chromosomal assignment, and expression of the gene encoding the human endothelin A receptor. J. Biol. Chem. 267: 18797–18804, 1992.
HU, J. R., BERNINGER, U. G., AND LANG, R. E.: Endothelin stimulates atrial natriuretic peptide (ANP) release from rat atria. Eur. J. Pharmacol. 158: 177–178, 1988.
HU, J. R., VON HARSDORF, R., AND LANG, R. E.: Endothelin has potent inotropic effects in rat atria. Eur. J. Pharmacol. 158: 275–278, 1988.
HU, R. M., LEVIN, E. R., PEDRAM, A., AND FRANK, H. J.: Atrial natriuretic peptide inhibits the production and secretion of endothelin from cultured endothelial cells. Mediation through the C receptor. J. Biol. Chem. 267: 17384–17389, 1992.
HU, R. M., LEVIN, E. R., PEDRAM, A., AND FRANK, H. J.: Insulin stimulates production and secretion of endothelin from bovine endothelial cells. Diabetes 42: 351–358, 1993.
HU, S. L., KIM, H. S., AND JENG, A. Y.: Dual action of endothelin 1 on the Ca^{2+} activated K^+ channel in smooth muscle cells of porcine coronary artery. Eur. J. Pharmacol. 194: 31–36, 1991.
HUANG, S., SIMONSON, M. S., AND DUNN, M. J.: Mandidipine inhibits endothelin 1 induced [Ca^{2+}]$_i$ signaling but potentiates endothelin's effect on c fos and c jun induction in vascular smooth muscle and glomerular mesangial cells. Am. Heart J. 125: 589–597, 1993.
HUANG, X. N., HISAYAMA, T., AND TAKAYANAGI, I.: Endothelin 1 induced contraction of rat aorta: contributions made by Ca^{2+} influx and activation of contractile apparatus associated with no change in cytoplasmic Ca^{2+} level. Naunyn Schmiedebergs Arch. Pharmacol. 341: 80–87, 1990.
HUANG, Y. T., HAMILTON, C. A., AND REID, J. L.: Endothelin stimulates phosphatidylinositol hydrolysis in rat vascular smooth muscles. J. Hypertens. 7: 703–705, 1989.
HUBBARD, S. C., AND IVATT, R. J.: Synthesis and processing of asparagine-linked oligosaccharides. Annu. Rev. Biochem. 50: 555–583, 1981.
HUGGINS, J. P., PELTON, J. T., AND MILLER, R. C.: The structure and specificity of endothelin receptors: their importance in physiology and medicine. Pharmacol. Ther. 59: 55–123, 1993.
HUNEIDI, A. H., THIEMERMANN, C., LIDBURY, P. S., D'ORLEANS-JUSTE, P., ANGGARD, E. E., AFSHAR, F., AND VANE, J. R.: Endothelin 1 and big endothelin cause subarachnoid hemorrhage in the anesthetized rabbit. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S492–S495, 1991.
HYSLOP, S., AND DE NUCCI, G.: Vasoactive mediators released by endothelins. Pharmacol. Res. 26: 223–242, 1992.
IDE, K., YAMAKAWA, K., NAKAGOMI, T., SASAKI, T., SAITO, I., KURIHARA, H., YOSIZUMI, M., YAZAKI, Y., AND TAKAKURA, K.: The role of endothelin in the pathogenesis of vasospasm following subarachnoid haemorrhage. Neurol. Res. 11: 101–104, 1989.
IHARA, M., FUKURODA, T., SAEKI, T., NISHIKIBE, M., KOJIRI, K., SUDA, H., AND YANO, M.: An endothelin receptor (ETA) antagonist isolated from *Streptomyces misakiensis*. Biochem. Biophys. Res. Commun. 178: 132–137, 1991a.
IHARA, M., ISHIKAWA, K., FUKURODA, T., SAEKI, T., FUNABASHI, K., FUKAMI, T., SUDA, H., AND YANO, M.: In vitro biological profile of a highly potent novel endothelin (ET) antagonist BQ 123 selective for the ETA receptor. J. Cardiovasc. Pharmacol. 20 (Suppl. 12): S11–S14, 1992a.
IHARA, M., NOGUCHI, K., SAEKI, T., FUKURODA, T., TSUCHIDA, S., KIMURA, S., FUKAMI, T., ISHIKAWA, K., NISHIKIBE, M., AND YANO, M.: Biological profiles of highly potent novel endothelin antagonists selective for the ETA receptor. Life Sci. 50: 247–255, 1992b.
IHARA, M., SAEKI, T., FUNABASHI, K., NAKAMICHI, K., YANO, M., FUKURODA, T., MIYAJI, M., NISHIKIBE, M., AND IKEMOTO, F.: Two endothelin receptor subtypes in porcine arteries. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S119–S121, 1991b.
IHARA, Y., SAGAWA, N., HASEGAWA, M., OKAGAKI, A., LI, X. M., INAMORI, K., ITOH, H., MORI, T., SAITO, Y., SHIRAKAMI, G., ET AL.: Concentrations of endothelin 1 in maternal and umbilical cord blood at various stages of pregnancy. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S443–S445, 1991c.
IIJIMA, K., LIN, L., NASJLETTI, A., AND GOLIGORSKY, M. S.: Intracellular signaling pathway of endothelin 1. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S146–S149, 1991.
IKEGAWA, R., MATSUMURA, Y., TSUKAHARA, Y., TAKAOKA, M., AND MORIMOTO, S.: Phosphoramidon, a metalloproteinase inhibitor, suppresses the secretion of endothelin 1 from cultured endothelial cells by inhibiting a big endothelin 1 converting enzyme. Biochem. Biophys. Res. Commun. 171: 669–675, 1990.
IKEGAWA, R., MATSUMURA, Y., TSUKAHARA, Y., TAKAOKA, M., AND MORIMOTO, S.: Phosphoramidon inhibits the generation of endothelin 1 from exogenously applied big endothelin 1 in cultured vascular endothelial cells and smooth muscle cells. FEBS Lett. 293: 45–48, 1991.
IMAI, T., HIRATA, Y., EGUCHI, S., KANNO, K., OHTA, K., EMORI, T., SAKAMOTO, A., YANAGISAWA M., MASAKI, T., AND MARUMO, F.: Concomitant expression of receptor subtype and isopeptide of endothelin by human adrenal gland. Biochem. Biophys. Res. Commun. 182: 1115–1121, 1992a.
IMAI, T., HIRATA, Y., EMORI, T., YANAGISAWA, M., MASAKI, T., AND MARUMO, F.: Induction of endothelin 1 gene by angiotensin and vasopressin in endothelial cells. Hypertension 19: 753–757, 1992b.
IMOKAWA, G., YADA, Y., AND MIYAGISHI, M.: Endothelins secreted from human keratinocytes are intrinsic mitogens for human melanocytes. J. Biol. Chem. 267: 24675–24680, 1992.
INAGAKI, H., BISHOP, A. E., YURA, J., AND POLAK, J. M.: Localization of endothelin 1 and its binding sites to the nervous system of the human colon. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S455–S457, 1991.
INOUE, Y., OIKE, M., NAKAO, K., KITAMURA, K., AND KURIYAMA, H.: Endothelin augments unitary calcium channel currents on the smooth muscle cell membrane of guinea pig portal vein. J. Physiol. (Lond.) 423: 171–191, 1990.
INOUE, A., YANAGISAWA, M., KIMURA, S., KASUYA, Y., MIYAUCHI, T., GOTO, K., AND MASAKI, T.: The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc. Natl. Acad. Sci. USA 86: 2863–2867, 1989a.
INOUE, A., YANAGISAWA, M., TAKUWA, Y., MITSUI, Y., KOBAYASHI, M., AND MASAKI, T.: The human preproendothelin 1 gene. Complete nucleotide sequence and regulation of expression. J. Biol. Chem. 264: 14954–14959, 1989b.
ISALES, C. M., NATHANSON, M. H., AND BRUCK, R.: Endothelin 1 induces cholestasis which is mediated by an increase in portal pressure. Biochem. Biophys. Res. Commun. 191: 1244–1251, 1993.
ISHIBASHI, M., FUJITA, M., NAGAI, K., KAKO, M., FURUE, H., HAKU, E., OSAMURA, Y., AND YAMAJI, T.: Production and secretion of endothelin by hepatocellular carcinoma. J. Clin. Endocrinol. Metab. 76: 378–383, 1993.
ISHIBASHI, M., HAIZUKA, H., TSUKAMURA, T., FURUE, H. YAMAJI, T., LADOUX, A., AND FRELIN, C.: Endothelin 1 and blood pressure: endothelins inhibit adenylate cyclase in brain capillary endothelial cells. Am. J. Hypertens. 5: 772–774, 1992.
ISHIDA, K., TAKESHIGE, K., AND MINAKAMI, S.: Endothelin 1 enhances superoxide generation of human neutrophils stimulated by the chemotactic peptide N. formyl methionyl leucyl phenylalanine. Biochem. Biophys. Res. Commun. 173: 496–500, 1990.
ISHIKAWA, T., YANAGISAWA, M., KIMURA, S., GOTO, K., AND MASAKI, T.: Positive chronotropic effects of endothelin, a novel endothelium derived vasoconstrictor peptide. Pflugers Arch. 413: 108–110, 1988.
ITO, S., JUNCOS, L. A., NUSHIRO, N., JOHNSON, C. S., AND CARRETERO, O. A.: Endothelium derived relaxing factor modulates endothelin action in afferent arterioles. Hypertension 17: 1052–1056, 1991.
ITOH, S., AND VAN DEN BUUSE, M.: Sensitization of baroreceptor reflex by central endothelin in conscious rats. Am. J. Physiol. 260: H1106–H1112, 1991.
ITOH, Y., KIMURA, C., ONDA, H., AND FUJINO, M.: Canine endothelin 2: cDNA sequence for the mature peptide. Nucleic Acids Res. 17: 5389, 1989.
ITOH, Y., YANAGISAWA, M., OHKUBO, S., KIMURA, C., KOSAKA, T., INOUE, A., ISHIDA, N., MITSUI, Y., ONDA, H., FUJINO, M., ET AL.: Cloning and sequence analysis of cDNA encoding the precursor of a human endothelium derived vasoconstrictor peptide, endothelin: identity of human and porcine endothelin. FEBS Lett. 231: 440–444, 1988.
JAFFER, F. E., KNAUSS, T. C., POPTIC, E., AND ABBOUD, H. E.: Endothelin stimulates PDGF secretion in cultured human mesangial cells. Kidney Int. 38: 1193–1198, 1990.
JANAKIDEVI, K., FISHER, M. A., DEL VECCHIO, P. J., TIRUPPATHI, C., FIGGE, J.,

AND MALIK, A. B.: Endothelin 1 stimulates DNA synthesis and proliferation of pulmonary artery smooth muscle cells. Am. J. Physiol. **263:** C1295–C1301, 1992.

JONES, C. R., HILEY, C. R., PELTON, J. T., AND MILLER, R. C.: Autoradiographic localization of endothelin binding sites in kidney. Eur. J. Pharmacol. **163:** 379–382, 1989.

JOSEPH, R., SCICLI, A. G., HAN, E., GRUNFELD, S., CARRETERO, O. A., AND WELCH, K. M.: Endothelin 1 and human platelet activity. Thromb. Res. **61:** 529–536, 1991.

JOSHUA, I. G.: Endothelin induced vasoconstriction of small resistance vessels in the microcirculation of the rat cremaster muscle. Microvasc. Res. **40:** 191–198, 1990.

KADEL, K. A., HEISTAD, D. D., AND FARACI, F. M.: Effects of endothelin on blood vessels of the brain and choroid plexus. Brain Res. **518:** 78–82, 1990.

KAI, H., KANAIDE, H., AND NAKAMURA, M.: Endothelin sensitive intracellular Ca^{2+} store overlaps with caffeine sensitive one in rat aortic smooth muscle cells in primary cultures. Biochem. Biophys. Res. Commun. **158:** 235–243, 1989.

KAMIMURA, Y., SAWADA, N., AOKI, M., AND MORI, M.: Endothelin 1 induces contraction of bile canaliculi in isolated rat hepatocytes. Biochem. Biophys. Res. Commun. **191:** 817–822, 1993.

KANAZAWA, H., KURIHARA, N., HIRATA, K., FUJIWARA, H., MATSUSHITA, H., AND TAKEDA, T.: Low concentration endothelin 1 enhanced histamine mediated bronchial contractions of guinea pigs in vivo. Biochem. Biophys. Res. Commun. **187:** 717–721, 1992.

KANNO, K., HIRATA, Y., NUMANO, F., EMORI, T., OHTA, SHICHIRI, M., AND MARUMO, F.: Endothelin 1 and vasculitis (letter). JAMA **264:** 2868, 1990.

KANNO, K., HIRATA, Y., SHICHIRI, M., AND MARUMO, F.: Plasma endothlin 1 levels in patients with diabetes mellitus with or without vascular complication. J. Cardiovasc. Pharmacol. (Suppl. 7): S475–S476, 1991.

KANYICSKA, B., BURRIS, T. P., AND FREEMAN, M. E.: Endothelin 3 inhibits prolactin and stimulates LH, FSH and TSH secretion from pituitary cell culture. Biochem. Biophys. Res. Commun. **174:** 338–343, 1991.

KARAKI, H., SUDJARWO, S. A., HORI, M., SAKATA, K., URADE, Y., TAKAI, M., AND OKADA, T.: ETB receptor antagonist, Irl 1038, selectively inhibits the endothelin induced endothelium dependent vascular relaxation. Eur. J. Pharmacol. **231:** 371–374, 1993.

KARNE, S., JAYAWICKREME, C. K., AND LERNER, M. R.: Cloning and characterization of an endothelin-3 specific receptor (ETc receptor) from *Xenopus laevis* dermal melanophores. J. Biol. Chem. **268:** 19126–19133, 1993.

KARWATOWSKA PROKOPCZUK, E., AND WENNMALM, A.: Effects of endothelin on coronary flow, mechanical performance, oxygen uptake, and formation of purines and on outflow of prostacyclin in the isolated rabbit heart. Circ. Res. **66:** 46–54, 1990a.

KARWATOWSKA PROKOPCZUK, E., AND WENNMALM, A.: Endothelium derived constricting factor(s): the last novelty endothelin. Clin. Physiol. **10:** 113–121, 1990b.

KASUYA, Y., DOBAYASHI, H., AND UEMURA, H.: Endothelin like immunoreactivity in the nervous system of invertebrates and fish. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S463–S466, 1991.

KASUYA, Y., ISHIKAWA, T., YANAGISAWA, M., KIMURA, S., GOTO, K., AND MASAKI, T.: Mechanism of contraction to endothelin in isolated porcine coronary artery. Am. J. Physiol. **257:** H1828–H1835, 1989a.

KASUYA, Y., TAKUWA, Y., YANAGISAWA, M., KIMURA, S., GOTO, K., AND MASAKI, T.: Endothelin 1 induces vasoconstriction through two functionally distinct pathways in porcine coronary artery: contribution of phosphoinositide turnover. Biochem. Biophys. Res. Commun. **161:** 1049–1055, 1989b.

KASUYA, Y., TAKUWA, Y., YANAGISAWA, M., MASAKI, T., AND GOTO, K.: A pertussis toxin sensitive mechanism of endothelin action in porcine coronary artery smooth muscle. Br. J. Pharmacol. **107:** 456–462, 1992.

KATO, K., SAWADA, S., TOYODA, T., KOBAYASHI, K., SHIRAI, K., YAMAMOTO, K., TAMAGAKI, T., YAMAGAMI, M., YONEDA, M., TAKADA, O., ET AL.: Influence of endothelin on human platelet aggregation and prostacyclin generation from human vascular endothelial cells in culture. Jpn. Circ. J. **56:** 422–431, 1992.

KAUFMANN, H., ORIBE, E., AND OLIVER, J. A.: Plasma endothelin during upright tilt: relevance for orthostatic hypotension? Lancet **338:** 1542–1545, 1991.

KAUSER, K., AND RUBANYI, G. M.: ET_B receptor mediates endothelium-dependent relaxation to sarafotoxin C and ET-1 in rat aorta. Endothelium, in press, 1994.

KAW, S., HECKER, M., SOUTHAN, G. J., WARNER, T. D., AND VANE, J. R.: Characterization of serine protease derived metabolites of big endothelin in the cytosolic fraction from human polymorphonuclear leukocytes. J. Cardiovasc. Pharmacol. **20** (Suppl. 12): S22–S24, 1992a.

KAW, S., HECKER, M., AND VANE, J. R.: The two step conversion of big endothelin 1 to endothelin 1 and degradation of endothelin 1 by subcellular fractions from human polymorphonuclear leukocytes. Proc. Natl. Acad. Sci. USA **89:** 6886–6890, 1992b.

KAWADA, N., TRAN THI, T. A., KLEIN, H., AND DECKER, K.: The contraction of hepatic stellate (Ito) cells stimulated with vasoactive substances. Possible involvement of endothelin 1 and nitric oxide in the regulation of the sinusoidal tonus. Eur. J. Biochem. **213:** 815–823, 1993.

KAWAGUCHI, H., SAWA, H., AND YASUDA, H.: Endothelin stimulates angiotensin I to angiotensin II conversion in cultured pulmonary artery endothelial cells. J. Mol. Cell. Cardiol. **22:** 839–842, 1990.

KAWAGUCHI, H., SAWA, H., AND YASUDA, H.: Effect of endothelin on angiotensin converting enzyme activity in cultured pulmonary artery endothelial cells. J. Hypertens. **9:** 171–174, 1991.

KAWAMURA, M., OHGAWARA, H., NARUSE, M., SUZUKI, N., IWASAKI, N., NARUSE, K., HORI, S., DEMURA, H., AND OMORI, Y.: Increased plasma endothelin in NIDDM patients with retinopathy. Diabetes Care **15:** 1396–1397, 1992.

KAWANO, Y., YOSHIDA, K., YOSHIMI, H., KURAMOCHI, M., AND OMAE, T.: The cardiovascular effect of intracerebroventricular endothelin in rats. J Hypertens **7** (Suppl.): S22–S23, 1989.

KELLEHER, J. P., SHAH, V., GODLEY, M. L., WAKEFIELD, A. J., GORDON, I., RANSLEY, P. G., SNELL, M. E., AND RISDON, R. A.: Urinary endothelin (ET1) in complete ureteric obstruction in the miniature pig. Urol. Res. **20:** 63–65, 1992.

KELLY, R. A., EID, H., KRAMER, B. K., O'NEILL, M., LIANG, B. T., REERS, M., AND SMITH, T. W.: Endothelin enhances the contractile responsiveness of adult rat ventricular myocytes to calcium by a pertussis toxin sensitive pathway. J. Clin. Invest. **86:** 1164–1171, 1990.

KIBIRA, S., DUDEK, R., NARAYAN, K. S., AND BING, R. J.: Release of prostacyclin, endothelium derived relaxing factor and endothelin by freshly harvested cells attached to microcarrier beads. Mol. Cell. Biochem. **108:** 75–84, 1991.

KIFF, R. J., GARDINER, S. M., COMPTON, A. M., AND BENNETT, T.: The effects of endothelin 1 and NG nitro L arginine methyl ester on regional haemodynamics in conscious rats with streptozotocin induced diabetes mellitus. Br. J. Pharmacol. **103:** 1321–1326, 1991.

KIMURA, C., ITOH, Y., OHKUBO, S., OGI, K., ONDA, H., AND FUJINO, M.: Cloning and sequencing of a canine gene segment encoding mature endothelin. Nucleic Acids Res. **17:** 3290, 1989a.

KIMURA, S., KASUYA, Y., SAWAMURA, T., SHINIMI, O., SUGITA, Y., YANAGISAWA, M., GOTO, K., AND MASAKI, T.: Conversion of big endothelin 1 to 21 residue endothelin 1 is essential for expression of full vasoconstrictor activity: structure activity relationships of big endothelin 1. J. Cardiovasc. Pharmacol. **13** (Suppl. 5): S5–7, S18, 1989b.

KIMURA, T., AZUMA, C., SAJI, F., TOKUGAWA, Y., TAKEMURA, M., MIKI, M., ONO, M., MORI, K., AND TANIZAWA, O.: Functional expression of human myometrial endothelin receptors in *Xenopus laevis* oocytes. Acta Endocrinol. (Copenh.) **126:** 64–66, 1992.

KING, A. J., BRENNER, B. M., AND ANDERSON, S.: Endothelin: a potent renal and systemic vasoconstrictor peptide. Am. J. Physiol. **256:** F1051–F1058, 1989.

KITAZUMI, K., SHIBA, T., NISHIKI, K., FURUKAWA, Y., TAKASAKI, C., AND TASAKA, K.: Structure activity relationship in vasoconstrictor effects of sarafotoxins and endothelin 1. FEBS Lett. **260:** 269–272, 1990.

KIYOHARA, T., OKUNO, M., NAKANISHI, T., SHINOMURA, Y., AND MATSUZAWA, Y.: Effect of endothelin 1 on ion transport in isolated rat colon. Gastroenterology **104:** 1328–1336, 1993.

KLEHA, J., RUBANYI, G. M., AND JOHNS, A.: ET_A and ET_B receptors mediate contraction in rabbit aorta. Endothelium, in press, 1994.

KLOCKNER, U., AND ISENBERY, G.: Endothelin depolarizes myocytes from porcine coronary and human mesenteric arteries through a Ca activated chloride current. Pflugers Arch. **418:** 168–175, 1991.

KLOOG, Y., BOUSSO-MITTLER, D., BDOLAH, A., AND SOKOLOVSKY, M.: Three apparent receptor subtypes for the endothelin/sarafotoxin family. FEBS Lett. **253:** 199–202, 1989.

KLOOG, Y., AND SOKOLOVSKY, M.: Similarities in mode and sites of action of sarafotoxins and endothelins. Trends Pharmacol. Sci. **10:** 212–214, 1989.

KNUEPFER, M. M., HAN, S. P., TRAPANI, A. J., FOK, K. F., AND WESTFALL, T. C.: Regional hemodynamic and baroreflex effects of endothelin in rats. Am. J. Physiol. **257:** H918–H926, 1989.

KOBAYASHI, M. KONDO, M., AND MITSUI, Y.: Establishment of human endothelial cell lines in a serum free culture and its application for expression of transfected prepro endothelin gene. Hum. Cell. **4:** 296–305, 1991.

KODAMA, M., KANAIDE, H., ABE, S., HIRANO, K., KAI, H., AND NAKAMURA, M.: Endothelin induced Ca independent contraction of the porcine coronary artery. Biochem. Biophys. Res. Commun. **160:** 1302–1308, 1989.

KOH, E., MORIMOTO, S., KIM, S., NABATA, T., MIYASHITA, Y., AND OGIHARA, T.: Endothelin stimulates Na^+/H^+ exchange in vascular smooth muscle cells. Biochem. Int. **20:** 375–380, 1990.

KOHAN, D. E.: Endothelin synthesis by rabbit renal tubule cells. Am. J. Physiol. **261:** F221–F226, 1991.

KOHAN, D. E., AND PADILLA, E.: Osmolar regulation of endothelin 1 production by rat inner medullary collecting duct. J. Clin. Invest. **91:** 1235–1240, 1993.

KOHNO, M., HORIO, T., IKEDA, M., YOKOKAWA, K., FUKUI, T., YASUNARI, K., KURIHARA, N., AND TAKEDA, T.: Angiotensin II stimulates endothelin 1 secretion in cultured rat mesangial cells. Kidney Int. **42:** 860–866, 1992a.

KOHNO, M., HORIO, T., YOKOKAWA, K., KURIHARA, N., AND TAKEDA, T.: C type natriuretic peptide inhibits thrombin and angiotensin II stimulated endothelin release via cyclic guanosine 3′,5′ monophosphate. Hypertension **19:** 320–325, 1992b.

KOHNO, M., MURAKAWA, K., HORIO, T., YOKOKAWA, K., YASUNARI, K., FUKUI, T., AND TAKEDA, T.: Plasma immunoreactive endothelin 1 in experimental malignant hypertension. Hypertension **18:** 93–100, 1991a.

KOHNO, M., YASUNARI, K., MURAKAWA, K., YOKOKAWA, K., HORIO, T., FUKUI, T., AND TAKEDA, T.: Plasma immunoreactive endothelin in essential hypertension. Am. J. Med. **88:** 614–618, 1990.

KOHNO, M., YASUNARI, K., YOKOKAWA, K., MURAKAWA, K., HORIO, T., AND TAKEDA, T.: Inhibition by atrial and brain natriuretic peptides of endothelin

1 secretion after stimulation with angiotensin II and thrombin of cultured human endothelial cells. J. Clin. Invest. **87:** 1999–2004, 1991b.

KOHNO, M., YOKOKAWA, K., HORIO, T., YASUNARI, K., MURAKAWA, K., AND TAKEDA, T.: Atrial and brain natriuretic peptides inhibit the endothelin 1 secretory response to angiotensin II in porcine aorta. Circ. Res. **70:** 241–247, 1992c.

KOHNO, M., YOKOKAWA, K., HORIO, T., YASUNARI, K., MURAKAWA, K., IKEDA, M., AND TAKEDA, T.: Release mechanism of endothelin 1 and big endothelin 1 after stimulation with thrombin in cultured porcine endothelial cells. J. Vasc. Res. **29:** 56–63, 1992d.

KOHZUKI, M., JOHNSTON, C. I., CHAI, S. Y., CASLEY, D. J., AND MENDELSOHN, F. A.: Localization of endothelin receptors in rat kidney. Eur. J. Pharmacol. **160:** 193–194, 1989a.

KOHZUKI, M., JOHNSTON, C. I., CHAI, S. Y., CASLEY, D. J., ROGERSON, F., AND MENDELSOHN, F. A.: Endothelin receptors in rat adrenal gland visualized by quantitative autoradiography. Clin. Exp. Pharmacol. Physiol. **16:** 239–242, 1989b.

KOIDE, M., KAWAHARA, Y., TSUDA, T., ISHIDA, Y., SHII, K., AND YOKOYAMA, M.: Endothelin 1 stimulates tyrosine phosphorylation and the activities of two mitogen activated protein kinases in cultured vascular smooth muscle cells. J. Hypertens. **10:** 1173–1182, 1992a.

KOIDE, M., KAWAHARA, Y., TSUDA, T., ISHIDA, Y., SHII, K., AND YOKOYAMA, M.: Stimulation of protein tyrosine phosphorylation by endothelin 1 in cultured vascular smooth muscle cells. Atherosclerosis **92:** 1–7, 1992b.

KOMURO, I., KURIHARA, H., SUGIYAMA, T., YOSHIZUMI, M., TAKAKU, F., AND YAZAKI, Y.: Endothelin stimulates c fos and c myc expression and proliferation of vascular smooth muscle cells. FEBS Lett. **238:** 249–252, 1988.

KON, V., AND AWAZU, M.: Endothelin and cyclosporine nephrotoxicity. Renal Failure **14:** 345–350, 1992.

KON, V., AND BADR, K. F.: Biological actions and pathophysiologic significance of endothelin in the kidney. Kidney Int. **40:** 1–12, 1991.

KON, V., SUGIURA, M., INAGAMI, T., HARVIE, B. R., ICHIKAWA, I., AND HOOVER, R. L.: Role of endothelin in cyclosporine induced glomerular dysfunction. Kidney Int. **37:** 1487–1491, 1990.

KON, V., YOSHIOKA, T., FOGO, A., AND ICHIKAWA, I.: Glomerular actions of endothelin in vivo. J. Clin. Invest. **83:** 1762–1767, 1989.

KONDA, S., FUSHIMI, E., MOITA, T., AND TASHIMA, Y.: Direct measurement of endothelin receptor in human bladder base and dome using ^{125}I endothelin. Tohoku J. Exp. Med. **167:** 159–161, 1992.

KORBMACHER, C., HELBIG, H., HALLER, H., ERICKSON, LAMY K. A., AND WIEDERHOLT, M.: Endothelin depolarizes membrane voltage and increases intracellular calcium concentration in human ciliary muscle cells. Biochem. Biophys. Res. Commun. **164:** 1031–1039, 1989.

KOSEKI, C., IMAI, M., HIRATA, Y., YANAGISAWA, M., AND MASAKI, T.: Autoradiographic localization of [^{125}I] endothelin 1 binding sites in rat brain. Neurosci. Res. **6:** 581–585, 1989.

KOUREMBANAS, S., MARSDEN, P. A., MCQUILLAN, L. P., AND FALLER, D. V.: Hypoxia induces endothelin gene expression and secretion in cultured human endothelium. J. Clin. Invest. **88:** 1054–1057, 1991.

KOYAMA, H., TABATA, T., NISHZAWA, Y., INOUE, T., MORII, H., AND YAMAJI, T.: Plasma endothelin levels in patients with uraemia. Lancet **1:** 991–992, 1989.

KOZUKA, M., ITO, T., HIROSE, S., LODHI, K. M., AND HAGIWARA, H.: Purification and characterization of bovine lung endothelin receptor. J. Biol. Chem. **266:** 16892–16896, 1991.

KOZUKA, M., ITO, T., HIROSE, S., TAKAHASHI, K., AND HAGIWARA, H.: Endothelin induces two types of contractions of rat uterus: phasic contractions by way of voltage dependent calcium channels and developing contractions through a second type of calcium channels. Biochem. Biophys. Res. Commun. **159:** 317–323, 1989.

KRAMER, B. K., NISHIDA, M., KELLY, R. A., AND SMITH, T. W.: Endothelins. Myocardial actions of a new class of cytokines. Circulation **85:** 350–356, 1992.

KRAMER, B. K., SMITH, T. W., AND KELLY, R. A.: Endothelin and increased contractility in adult rat ventricular myocytes. Role of intracellular alkalosis induced by activation of the protein kinase C dependent Na^+ H^+ exchanger. Circ. Res. **68:** 269–279, 1991.

KRAUS, G. E., BUCHOLZ, R. D., YOON, K. W., KNUEPFER, M. M., SMITH, K. R., JR., MULDOON, L. L., PRIBNOW, D., RODLAND, K. D., AND MAGUN, B. E.: Endothelin 1 stimulates DNA synthesis and anchorage independent growth of rat 1 fibroblasts through a protein kinase C dependent mechanism. Cell Reg. **1:** 379–390, 1990.

KRAUS, G. E., BUCHOLZ, R. D., YOON, K. W., KNUEPFER, M. M., SMITH, K. R., JR., MULDOON, L. L., PRIBNOW, D., RODLAND, K. D., AND MAGUN, B. E.: Cerebrospinal fluid endothelin 1 and endothelin 3 levels in normal and neurosurgical patients: a clinical study and literature review. Surg. Neurol. **35:** 20–29, 1991.

KRAUS, T., MEHRABI, A., KLAR, E., MATHIAS, D., ARNOLD, J., OTTO, G., AND HERFARTH, C.: Peri and postoperative plasma kinetics of big endothelin and endothelin 1/2 after liver transplantation. Transplant. Proc. **24:** 2569–2571, 1992.

KRYSTEK, S. R., JR., BASSOLINO, D. A., BRUCCOLERI, R. E., HUNT, J. T., PORUBCAN, M. A., WANDLER C. F., AND ANDERSEN, N. H.: Solution conformation of a cyclic pentapeptide endothelin antagonist. Comparison of structure obtained from constrained dynamics and conformational search. FEBS Lett. **299:** 255–261, 1992.

KRYSTEK, S. R., JR., BASSOLINO, D. A., NOVOTNY, J., CHEN, C., MARSCHNER, T. M., AND ANDERSEN, N. H.: Conformation of endothelin in aqueous ethylene glycol determined by 1H NMR and molecular dynamics simulations. FEBS Lett. **281:** 212–218, 1991.

KUCHAN, M. J., AND FRANGOS, J. A.: Shear stress regulates endothelin 1 release via protein kinase C and cGMP in cultured endothelial cells. Am. J. Physiol. **264:** H150–H156, 1993.

KURIHARA, M., OCHI, A., KAWAGUCHI, T., NIWA, M., KATAOKA, Y., AND MORI, K.: Localization and characterization of endothelin receptors in human gliomas: a growth factor? Neurosurgery **27:** 275–281, 1990.

KURIHARA, H., YAMAOKI, K., NAGAI, R., YOSHIZUMI, M., TAKAKU, F., SATOH, H., INUI, J., AND YAZAKI, Y.: Endothelin: a potent vasoconstrictor associated with coronary vasospasm. Life Sci. **44:** 1937–1943, 1989a.

KURIHARA, H., KURIHARA, Y., KUWAKI, T., MAEMURA, K., CAO, W., SUZUKI, H., KODAMA, T., YASU-YOSHI, O., KUMADA, M., AND YAZAKI, Y.: Targeted disruption of mouse endothelin-1 gene (II): Elevated blood pressure in heterozygotes. Circulation **88** (Suppl.): I-332, 1993.

KURIHARA, H., YOSHIZUMI, M., SUGIYAMA, T., TAKAKU, F., YANAGISAWA, M., MASAKI, T., HAMAOKI, M., KATO, H., AND YAZAKI, Y.: Transforming growth factor beta stimulates the expression of endothelin mRNA by vascular endothelial cells. Biochem. Biophys. Res. Commun. **159:** 1435–1440, 1989b.

KURIHARA, H., YOSHIZUMI, M., SUGIYAMA, T., YAMAOKI, K., NAGAI, R., TAKAKU, F., SATOH, H., INUI, J., YANAGISAWA, M., MASAKI, T., ET AL.: The possible role of endothelin 1 in the pathogenesis of coronary vasospasm. J. Cardiovasc. Pharmacol. **13** (Suppl. 5): S132–S137, S142, 1989c.

KURIHARA, T., AKIMOTO, M., KUROKAWA, K., ISHIGURO, H., NIIMI, A., MAEDA, A., SIGEMOTO, M. YAMASHITA, K., YOKOYAMA, I., HIRAYAMA, Y., ET AL.: Relationship between endothelin and thromboxane A2 in rat liver microcirculation. Life Sci. **51:** 281–285, 1992.

KUROSAWA, M., FUXE, K., HALLSTROM, A., GOINY, M., CINTRA, A., AND UNGERSTEDY, U.: Responses of blood flow, extracellular lactate, and dopamine in the striatum to intrastriatal injection of endothelin 1 in anesthetized rats. J. Cardiovasc. Parmacol. **17** (Suppl. 7): S340–S342, 1991.

KUSHIKU, K., OHJIMI, H., YAMADA, H., TOKUNAGA, R., AND FURUKAWA, T.: Endothelin 3 inhibits ganglionic transmission at preganglionic sites through activation of endogenous thromboxane A2 production in dog cardiac sympathetic ganglia. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S197–S199, 1991.

LAGENTE, V., TOUVAY, C., MENCIA HUERTA, J. M., CHABRIER, P. E., AND BRAQUET, P.: Bronchopulmonary effects of endothelin. Clin. Exp. Allergy **20:** 343–348, 1990.

LAM, H. C., TAKAHASHI, K., GHATEI, M. A., WARRENS, A. N., REES, A. J., AND BLOOM, S. R.: Immunoreactive endothelin in human plasma, urine, milk, and saliva. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S390–S393, 1991.

LAMAS, S., MICHEL, T., COLLINS, T., BRENNER, B. M., AND MARSDEN, P. A.: Effects of interferon gamma on nitric oxide synthase activity and endothelin 1 production by vascular endothelial cells. J. Clin. Invest. **90:** 879–887, 1992.

LANESE, D. M., AND CONGER, J. D.: Effects of endothelin receptor antagonist on cyclosporine induced vasoconstriction in isolated rat renal arterioles. J. Clin. Invest. **91:** 2144–2149, 1993.

LAWRENCE, E., AND BRAIN, S. D.: Responses to endothelins in the rat cutaneous microvasculature: a modulatory role of locally produced nitric oxide. Br. J. Pharmacol. **106:** 733–738, 1992.

LECCI, A., GIULIANI, S., SANTICIOLI, P., ROVERO, P., MAGGI, C. A., AND GIACHETTI, A.: Intracerebroventricular administration of endothelins: effects on the supraspinal micturition reflex and blood pressure in the anaesthetized rat. Eur. J. Pharmacol. **199:** 201–207, 1991.

LEE, H. K., LEIKAUF, G. D., AND SPERELAKIS, N.: Electromechanical effects of endothelin on ferret bronchial and tracheal smooth muscle. J. Appl. Physiol. **68:** 417–420, 1990a.

LEE, M. E., BLOCH, K. D., CLIFFORD, J. A., AND QUERTERMOUS, T.: Functional analysis of the endothelin 1 gene promoter. Evidence for an endothelial cell specific *cis* acting sequence. J. Biol. Chem. **265:** 10446–10450, 1990b.

LEE, M. E., DE LA MONTE, S. M., NG, S. C., BLOCH, K. D., AND QUERTERMOUS, T.: Expression of the potent vasoconstrictor endothelin in the human central nervous system. J. Clin. Invest. **86:** 141–147, 1990c.

LEE, M. E., DHADLY, M. S., TEMIZER, D. H., CLIFFORD, J. A., YOSHIZUMI, M., AND QUERTERMOUS, T.: Regulation of endothelin 1 gene expression by Fos and Jun. J. Biol. Chem. **266:** 19034–19039, 1991a.

LEE, M. E., TEMIZER, D. H., CLIFFORD, J. A., AND QUERTERMOUS, T.: Cloning of the GATA binding protein that regulates endothelin 1 gene expression in endothelial cells. J. Biol. Chem. **266:** 16188–161892, 1991b.

LEE, T. S., CHAO, T., HU, K. Q., AND KING, G. L.: Endothelin stimulates a sustained 1,2 diacylglycerol increase and protein kinase C activation in bovine aortic smooth muscle cells. Biochem. Biophys. Res. Commun. **162:** 381–386, 1989a.

LEE, T. S., HU, K. Q., CHAO, T., AND KING, G. L.: Characterization of endothelin receptors and effects of endothelin on diacylglycerol and protein kinase C in retinal capillary pericytes. Diabetes **38:** 1643–1646, 1989b.

LEES, W. E., KALINKA, S., MEECH, J., CAPPER, S. J., COOK, N. D., AND KAY, J.: Generation of human endothelin by cathepsin E. FEBS Lett. **273:** 99–102, 1990.

LEHOUX, S., PLANTE, G. E., SIROIS, M. G., SIROIS, P., AND D'ORLEANS-JUSTE, P.: Phosphoramidon blocks big endothelin 1 but not endothelin 1 enhancement of vascular permeability in the rat. Br. J. Pharmacol. **107:** 996–1000, 1992.

LERMAN, A., CLICK, R. L., NARR, B. J., WIESNER, R. H., KROM, R. A., TEXTOR,

S. C., AND BURNETT, J. C., JR.: Elevation of plasma endothelin associated with systemic hypertension in humans following orthotopic liver transplantation. Transplantation (Baltimore) **51:** 646–650, 1991a.

LERMAN, A., EDWARDS, B. S., HALLETT, J. W., HEUBLEIN, D. M., SANDBERG, S. M., AND BURNETT, J. C., JR.: Circulating and tissue endothelin immunoreactivity in advanced atherosclerosis. N. Engl. J. Med. **325:** 997–1001, 1991b.

LERMAN, A., HILDEBRAND, F. L., JR., AARHUS, L. L., AND BURNETT, J. C., JR.: Endothelin has biological actions at pathophysiological concentrations. Circulation **83:** 1808–1814, 1991c.

LERMAN, A., HILDEBRAND, F. L., JR., MARGULIES, K. B., O'MURCHU, B., PERRELLA, M. A., HEUBLEIN, D. M., SCHWAB, T. R., AND BURNETT, J. C., JR.: Endothelin, a new cardiovascular regulatory peptide. Mayo Clin. Proc. **65:** 1441–1455, 1990.

LERMAN, A., KUBO, S. H., TSCHUMPERLIN, L. K., AND BURNETT, J. C., JR.: Plasma endothelin concentrations in humans with end stage heart failure and after heart transplantation [see comments]. J. Am. Coll. Cardiol. **20:** 849–853, 1992a.

LERMAN, A., SANDOK, E. K., HILDEBRAND, F. L., JR., AND BURNETT, J. C., JR.: Inhibition of endothelium derived relaxing factor enhances endothelin mediated vasoconstriction. Circulation **85:** 1894–1898, 1992b.

LETARTE, M., VERA, S., TRAN, R., ADDIS, J. B. L., ONIZUKA, R. J., QUACKENBUSH, E. J., JONGENEEL, C. V., AND MCINNES, R. R.: Common acute lymphocytic leukemia antigen is identical to neutral endopeptidase. J. Exp. Med. **168:** 1247–1253, 1988.

LEVESQUE, H., SEVRAIN, L., FREGER, P., TADIE, M., COURTOIS, H., AND CREISSARD, P.: Raised plasma endothelin in aneurysmal subarachnoid haemorrhage. Lancet **335:** 290, 1990.

LEVIN, E. R., FRANK, H. J., AND PEDRAM, A.: Endothelin receptors on cultured fetal rat diencephalic glia. J. Neurochem. **58:** 659–666, 1992.

LEVIN, E. R., ISACKSON, P. J., AND HU, R. M.: Endothelin increases atrial natriuretic peptide production in cultured rat diencephalic neurons. Endocrinology **128:** 2925–2930, 1991.

LEVY, R. D., LANGLEBEN, D., CERNACEK, P., AND STEWART, D. J.: Increased plasma levels of the endothelium-derived vasoconstrictor endothelin in pulmonary hypertension marker or mediator of disease? (abstract). Am. Rev. Resp. Dis. **141:** 889, 1990.

LEWIS M. J., AND SHAH A. M.: Endothelial modulation of myocardial contraction. Endothelium, in press, 1994.

LIN, H. Y., KAJI, E. H., WINKEL, G. K., IVES, H. E., AND LODISH, H. F.: Cloning and functional expression of a vascular smooth muscle endothelin1 receptor. Proc. Natl. Acad. Sci. USA **88:** 3185–3189, 1991.

LIN, S. Y., CAI, H., GONG, Q. Y., YANG, Z. C., ZHOU, J. H., ZHANG, D., AND ZHANG, Z. K.: Role of endothelin in the pathogenesis of hypertension in spontaneously hypertensive and 2 kidneys 1 clip rats. Chin. Med. J. Engl. **103:** 748–753, 1990a.

LIN, W. W., AND LEE, C. Y.: Biphasic effects of endothelin in the guinea pig ileum. Eur. J. Pharmacol. **176:** 57–62, 1990.

LIN, W. W., AND LEE, C. Y.: Intestinal relaxation by endothelin isopeptides: involvement of Ca^{2+} activated K^+ channels. Eur. J. Pharmacol. **219:** 355–360, 1992.

LIN, W. W., LEE, C. Y., AND CHUANG, D. M.: Cross desensitization of endothelin and sarafotoxin induced phosphoinositide turnover in neurons. Eur. J. Pharmacol. **166:** 581–582, 1989.

LIN, W. W., LEE, C. Y., AND CHUANG, D. M.: Comparative studies of phosphoinositide hydrolysis induced by endothelin related peptides in cultured cerebellar astrocytes, C6 glioma and cerebellar granule cells. Biochem. Biophys. Res. Commun. **168:** 512–519, 1990b.

LIN, W. W., LEE, C. Y., AND CHUANG, D. M.: Endothelin 1 stimulates the release of preloaded [^{3}H]D aspartate from cultured cerebellar granule cells. Biochem. Biophys. Res. Commun. **167:** 593–599, 1990c.

LITTLE, P. J., NEYLON, C. B., TKACHUK, V. A., AND BOBIK, A.: Endothelin 1 and endothelin 3 stimulate calcium mobilization by different mechanisms in vascular smooth muscle. Biochem. Biophys. Res. Commun. **183:** 694–700, 1992.

LIU, J., CHEN, R., CASLEY, D. J., AND NAYLER, W. G.: Ischemia and reperfusion increase ^{125}I labeled endothelin 1 binding in rat cardiac membranes. Am. J. Physiol. **258:** 829–835, 1990.

LOESCH, A., BODIN, P. AND BURNSTOCK, G.: Colocalization of endothelin, vasopressin and serotonin in cultured endothelial cells of rabbit aorta. Peptides **12:** 1095–1103, 1991.

LONCHAMPT, M. O., PINELIS, S., GOULIN, J., CHABRIER, P. E., AND BRAQUET, P.: Proliferation and Na^+/H^+ exchange activation by endothelin in vascular smooth muscle cells. Am. J. Hypertens. **4:** 776–779, 1991.

LOPEZ, J. A., ARMSTRONG, M. L., PIEGORS, D. J., AND HEISTAD, D. D.: Vascular responses to endothelin 1 in atherosclerotic primates. Arteriosclerosis **10:** 1113–1118, 1990.

LOPEZ-FARRE, A., GOMEZ GARRE, D., BERNABEU, F., AND LOPEZ NOVOA, J. M.: A role for endothelin in the maintenance of post ischemic renal failure inthe rat. J. Physiol. (Lond.) **444:** 513–522, 1991a.

LOPEZ-FARRE, A., GOMEZ GARRE, D., BERNABEU, F., MONTANES, I., MILLAS, I., AND LOPEZ NOVO, J. M.: Renal effects and mesangial cell contraction induced by endothelin are mediated by PAF. Kidney Int. **39:** 624–630, 1991b.

LOUGEE, L., HINOJOSA LABORDE, C., HARDER, D. R., AND LOMBARD, J. H.: Effect of nifedipine on endothelin induced contractions of skeletal muscle arterioles of spontaneously hypertensive rats. Microcirc. Endothelium Lymphatics **6:** 355–368, 1990.

LOUTZENHISER, R., EPSTEIN, M., HAYASHI, K., AND HORTON, C.: Direct visualization of effects of endothelin in the renal microvasculature. Am. J. Physiol. **258:** F61–F68, 1990.

LOVENBERG, W., AND MILLER, R. C.: Endothelin: a review of its effects and possible mechanisms of action. Neurochem. Res. **15:** 407–417, 1990.

LUMME, R., LAATIKAINEN, T., VUOLTEENAHO, O., AND LEPPALUOTO, J.: Plasma endothelin, atrial natriuretic peptide (ANP) and uterine and umbilical artery flow velocity waveforms in hypertensive pregnancies. Br. J. Obstet. Gynecol. **99:** 761–764, 1992.

LUNDBERG, J. M., AHLBORG, G., HEMSEN, A., NISELL, H., LUNELL, N. O., PERNOW, J., RUDEHILL, A., AND WEITZBERG, E.: Evidence for release of endothelin 1 in pigs and humans. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S350–S353, 1991.

LUSCHER, T. F.: Endothelin. J. Cardiovasc. Pharmacol. **18** (Suppl. 10): S15–S22, 1991.

LUSCHER, T. F.: Endothelin: systemic arterial and pulmonary effects of a new peptide with potent biologic properties. Am. Rev. Respir. Dis. **146:** S56–S60, 1992.

LUSCHER, T. F., YANG, Z., TSCHUDI, M., VON SEGESSER, L., STULZ, P., BOULANGER, C., SIEBENMANN, R., TURNIA, M., AND BUHLER, F. R.: Interaction between endothelin 1 and endothelium derived relaxing factor in human arteries and veins. Circ. Res. **66:** 1088–1094, 1990.

MACCUMBER, M. W., JAMPEL, H. D., AND SNYDER, S. H.: Ocular effects of the endothelins. Abundant peptides in the eye. Arch. Ophthalmol. **109:** 705–709, 1991.

MACCUMBER, M. W., ROSS, C. A., GLASER, B. M., AND SNYDER, S. H.: Endothelin: visualization of mRNAs by in situ hybridization provides evidence for local action. Proc. Natl. Acad. Sci. USA **86:** 7285–7289, 1989.

MACCUMBER, M. W., ROSS, C. A., AND SNYDER, S. H.: Endothelin in brain: receptors, mitogenesis, and biosynthesis in glial cells. Proc. Natl. Acad. Sci. USA **87:** 2359–2363, 1990.

MACLEAN, M. R., RANDALL, M. D., AND HILEY, C. R.: Effects of moderate hypoxia, hypercapnia and acidosis on haemodynamic changes induced by endothelin 1 in the pithed rat. Br. J. Pharmacol. **98:** 1055–1065, 1989.

MACQUIN-MAVIER, I., LEVAME, M., ISTIN, N., AND HARF, A.: Mechanisms of endothelin mediated bronchoconstriction in the guinea pig. J. Pharmacol. Exp. Ther. **250:** 740–745, 1989.

MACRAE, I., ROBINSON, M., MCAULEY, M., REID, J., AND MCCULLOCH, J.: Effects of intracisternal endothelin 1 injection on blood flow to the lower brain stem. Eur. J. Pharmacol. **203:** 85–91, 1991a.

MACRAE, I. M., MCAULEY, M. A., ROBINSON, M. J., REID, J. L., AND MCCULLOCH, J.: Endothelin 1 induced hypertension: a consequence of medullary ischemia? J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S496–S499, 1991b.

MADEDDU, P., ANANIA, V., PARPAGLIA, P. P., TROFFA, C., PAZZOLA, A., SORO, A., MANUNTA, P., TONOLO, G., DEMONTIS, M. P., VARONI, M., ET AL.: Endothelin 1 induced renal vasoconstriction is blunted by enalaprilat and enhanced by EDRF antagonist in awake normotensive rats. Clin. Invest. Med. **14:** 600–606, 1991.

MADEDDU, P., TROFFA, C., GLORIOSO, N., PAZZOLA, A., SORO, A., MANUNTA, P., TONOLO, G., DEMONTIS, M. P., VARONI, M. V., AND ANANIA, V.: Effect of endothelin on regional hemodynamics and renal function in awake normotensive rats. J. Cardiovasc. Pharmacol. **14:** 818–825, 1989.

MAEMURA, K., KURIHARA, H., MORITA, T., HASHI, Y., AND YAZAKI, Y.: Production of endothelin 1 in vascular endothelial cells is regulated by factors associated with vascular injury. Gerontology **38** (Suppl. 1): 29–35, 1992.

MAGGI, C. A., GIULIANI, S., PATACCHINI, R., BARBANTI, G., TURINI, D., AND MELI, A.: Contractile responses of the human urinary bladder, renal pelvis and renal artery to endothelins and sarafotoxin S6b. Gen. Pharmacol. **21:** 247–249, 1990.

MAGGI, C. A., GIULIANI, S., PATACCHINI, R., ROVERO, P., GIACHETTI, A., MELI, A.: The activity of peptides of the endothelin family in various mammalian smooth muscle preparations. Eur. J. Pharmacol. **174:** 23–31, 1989a.

MAGGI, C. A., GIULIANI, S., PATACCHINI, R., SANTICIOLI, P., ROVERO, P., GIACHETTI, A., AND MELI, A.: The C terminal hexapeptide, endothelin (16 21), discriminates between different endothelin receptors. Eur. J. Pharmacol. **166:** 121–122, 1989b.

MAGGI, C. A., GIULIANI, S., PATACCHINI, R., SANTICIOLI, P., TURINI, D., BARBANTI, G., AND MELI A.: Potent contractile activity of endothelin on the human isolated urinary bladder. Br. J. Pharmacol. **96:** 755–757, 1989c.

MAGGI, C. A., PATACCHINI, R., GIULIANI, S., AND MELI, A.: Potent contractile effect of endothelin in isolated guinea pig airways. Eur. J. Pharmacol. **160:** 179–182, 1989d.

MAGGI, M., VANNELLI, G. B., PERI, A., BRANDI, M. L., FANTONI, G., GIANNINI, S., TORRISI, C., GUARDABASSO, V., BARNI, T., TOSCANO, V., ET AL.: Immunolocalization, binding, and biological activity of endothelin in rabbit uterus: effect of ovarian steroids. Am. J. Physiol. **260:** E292–E305, 1991.

MAHER, E., BARDEQUEZ, A., GARDNER, J. P., GOLDSMITH, L., WEISS, G., MASCARINA, M., AND AVIV, A.: Endothelin and oxytocin induced calcium signaling in cultured human myometrial cells. J. Clin. Invest. **87:** 1251–1258, 1991.

MAKINO, S., HASHIMOTO, K., HIRASAWA, R., HATTORI, T., KAGEYAMA, J., AND OTA, Z.: Central interaction between endothelin and brain natriuretic peptide on pressor and hormonal responses. Brain Res. **534:** 117–121, 1990.

MAKINO, S., HASHIMOTO, K., HIRASAWA, R., HATTORI, T., AND OTA, Z.: Central interaction between endothelin and brain natriuretic peptide on vasopressin secretion. J Hypertens. 10: 25–28, 1992.
MALEK, A., AND IZUMO, S: Physiological fluid shear stress causes downregulation of endothelin 1 mRNA in bovine aortic endothelium. Am. J. Physiol. 263: C389–C396, 1992.
MANTYMAA, P., LEPPALUOTO, J., AND RUSKOAHO, H.: Endothelin stimulates basal and stretch induced atrial natriuretic peptide secretion from the perfused rat heart. Endocrinology 126: 587–595, 1990.
MARGULIES, K. B., HILDEBRAND, F. L., HEUBLEIN, D. M., AND BURNETT, J. C., JR.: Radiocontrast increases plasma and urinary endothelin. J. Am. Soc. Nephrol. 2: 1041–1045, 1991.
MARGULIES, K. B., HILDEBRAND, F. L., JR., LERMAN, A., PERRELLA, M. A., AND BURNETT, J. C., JR.: Increased endothelin in experimental heart failure. Circulation 82: 2226–2230, 1990.
MARIN, P., DELUMEAU, J. C., DURIEU-TRAUTMANN, O., LE NGUYEN, D., PREMONT, J., STROSBERG, A. D., AND COURAUD, P. O.: Are several G proteins involved in the different effects of endothelin 1 in mouse striatal astrocytes? J. Neurochem. 56: 1270–1275, 1991.
MARSAULT, R., VIGNE, P., BREITTMAYER, J. P., AND FRELIN, C.: Astrocytes are target cells for endothelins and sarafotoxin. J. Neurochem. 54: 2142–2144, 1990.
MARSDEN, P. A., AND BRENNER, B. M.: Nitric oxide and endothelins: novel autocrine/paracrine regulators of the circulation. Semin. Nephrol. 11: 169–185, 1991.
MARSDEN, P. A., AND BRENNER, B. M.: Transcriptional regulation of the endothelin 1 gene by TNF alpha. Am. J. Physiol. 262: C854–C861, 1992.
MARSDEN, P. A., DANTHULURI, N. R., BRENNER, B. M., BALLERMANN, B. J., AND BROCK, T. A.: Endothelin action on vascular smooth muscle involved inositol trisphosphate and calcium mobilization. Biochem. Biophys. Res. Commun. 158: 86–93, 1989.
MARSDEN, P. A., DORFMAN, D. M., COLLINS, T., BRENNER, B. M., ORKIN, S. H., AND BALLERMANN, B. J.: Regulated expression of endothelin 1 in glomerular capillary endothelial cells. Am. J. Physiol. 261: F117–F125, 1991.
MARSDEN, P. A., SULTAN, P., CYBULSKY, M., GIMBRONE, M. A., JR., BRENNER, B. M., AND COLLINS, T.: Nucleotide sequence of endothelin 1 cDNA from rabbit endothelial cells. Biochim. Biophys. Acta 1129: 249–250, 1992.
MARTIN NIZARD, F., HOUSSAINI, H. S., LESTAVEL DELATTRE, S., DURIEZ, P., AND FRUCHART, J. C.: Modified low density lipoproteins activate human macrophages to secrete immunoreactive endothelin. FEBS Lett. 293: 127–130, 1991.
MASAKI, T., VANE, J. R., AND VANHOUTTE, P. M.: V. International Union of Pharmacology nomenclature of endothelin receptors. Pharmacol. Rev. 46: 137–142, 1994.
MASAKI, T., KIMURA, S., YANAGISAWA, M., AND GOTO, K.: Molecular and cellular mechanism of endothelin regulation. Implications for vascular function. Circulation 84: 1457–1468, 1991a.
MASAKI, T., USUKI, S., YANAGISAWA, M., AND SUZUKI, N.: Endothelin in perinatal pharmacology. Semin. Perinatol. 15: 27–29, 1991b.
MASAKI, T., AND YANAGISAWA, M.: Endothelins. Essays Biochem. 27: 79–89, 1992.
MASAKI, T., YANAGISAWA, M., AND GOTO, K.: Physiology and pharmacology of endothelins. Med. Res. Rev. 12: 391–421, 1992.
MASAKI, T., YANAGISAWA, M., GOTO, K., AND KIMURA, S.: Role of endothelin in mechanisms of local blood pressure control. J. Hypertens. 8 (Suppl.): S107–S112, 1990a.
MASAKI, T., YANAGISAWA, M., INOUE, A., KIMURA, S., AND GOTO, K.: Study on the endothelin gene and its expression. Jpn. Circ. J. 54: 1221–1225, 1990b.
MASAKI, T., YANAGISAWA, M., TAKUWA, Y., KASUYA, Y., KIMURA, S., AND GOTO, K.: Cellular mechanism of vasoconstriction induced by endothelin. Adv. Second Messenger Phosphoprotein Res. 24: 425–428, 1990c.
MASTROGIANNIS, D. S., KALTER, C. S., O'BRIEN, W. F., CARLAN, S. J., AND REECE, E. A.: Effect of magnesium sulfate on plasma endothelin 1 levels in normal and preeclamptic pregnancies. Am. J. Obstet. Gynecol. 167: 1554–1559, 1992.
MASTROGIANNIS, D. S., O'BRIEN, W. F., KRAMMER, J., AND BENOIT, R.: Potential role of endothelin 1 in normal and hypertensive pregnancies. Am. J. Obstet. Gynecol. 165: 1711–1716, 1991.
MASUDA, E., KAWANO, S., NAGANO, K., TSUJI, S., ISHIGAMI, Y., TSUJII, M., HAYASHI, N., FUSAMOTO, H., AND KAMADA, T.: Effect of intravascular ethanol on modulation of gastric mucosal integrity: possible role of endothelin 1. Am. J. Physiol. 262: G785–G790, 1992.
MATSUMURA, K., ABE, I., TSUCHIHASHI, T., TOMINAGA, M., KOBAYASHI, K., AND FUJISHIMA, M.: Central effect of endothelin on neurohormonal responses in conscious rabbits. Hypertension 17: 1192–1196, 1991a.
MATSUMURA, Y., HISAKI, K., OHYAMA, T., HAYASHI, K., AND MORIMOTO, S.: Effects of endothelin on renal function and renin secretion in anesthetized rats. Eur. J. Pharmacol. 166: 577–580, 1989a.
MATSUMURA, Y., IKEGAWA, R., HISAKI, K., TSUKAHARA, Y., TAKAOKA, M., AND MORIMOTO, S.: Conversion of big endothelin 1 to endothelin 1 by phosphoramidon sensitive metalloproteinase derived from aortic endothelial cells. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S65–S67, 1991b.
MATSUMURA, Y., IKEGAWA, R., SUZUKI, Y., TAKAOKA, M., UCHIDA, T., KIDO, H., SHINYAMA, H., HAYASHI, K., WATANABE, M., AND MORIMOTO, S.: Phosphoramidon prevents cerebral vasospasm following subarachnoid hemorrhage in dogs: the relationship to endothelin 1 levels in the cerebrospinal fluid. Life Sci. 49: 841–848, 1991c.
MATSUMURA, Y., IKEGAWA, R., TSUKAHARA, Y., TAKAOKA, M., AND MORIMOTO, S.: Conversion of big endothelin 1 to endothelin 1 by two types of metalloproteinases derived from porcine aortic endothelial cells. FEBS Lett. 272: 166–170, 1990.
MATSUMURA, Y., IKEGAWA, R., TSUKAHARA, Y., TAKAOKA, M., AND MORIMOTO, S.: N ethylmaleimide differentiates endothelin converting activity by two types of metalloproteinases derived from vascular endothelial cells. Biochem. Biophys. Res. Commun. 178: 531–538, 1991d.
MATSUMURA, Y., IKEGAWA, R., TSUKAHARA, Y., TAKAOKA, M., AND MORIMOTO, S.: Conversion of big endothelin 1 to endothelin 1 by two types of metalloproteinases of cultured porcine vascular smooth muscle cells. Biochem. Biophys. Res. Commun. 178: 899–905, 1991e.
MATSUMURA, Y., NAKASE, K., IKEGAWA, R., HAYASHI, K., OHYAMA, T., MORIMOTO, S.: The endothelium derived vasoconstrictor peptide endothelin inhibits renin release in vitro. Life Sci. 44: 149–157, 1989c.
MATSUMURA, Y., TSUKAHARA, Y., KUNINOBU, K., TAKAOKA, M., AND MORIMOTO, S.: Phosphoramidon sensitive endothelin converting enzyme in vascular endothelial cells converts big endothelin 1 and big endothelin 3 to their mature form. FEBS Lett. 305: 86–90, 1992a.
MATSUYAMA, K., YASUE, H., OKUMURA, K., SAITO, Y., NAKAO, K., SHIRAKAMI, G., AND IMURA, H.: Increased plasma level of endothelin 1 like immunoreactivity during coronary spasm in patients with coronary spastic angina. Am. J. Cardiol. 68: 991–995, 1991.
MATTOLI, S., MEZZETTI, M., RIVA, G., ALLEGRA, L., AND FASOLI, A.: Specific binding of endothelin on human bronchial smooth muscle cells in culture and secretion of endothelin like material from bronchial epithelial cells. Am. J. Respir. Cell Mol. Biol. 3: 145–151, 1990.
MATTOLI, S., SOLOPERTO, M., MARINI, M., AND FASOLI, A.: Levels of endothelin in the bronchoalveolar lavage fluid of patients with symptomatic asthma and reversible airflow obstruction. J. Allergy Clin. Immunol. 88: 376–384, 1991a.
MATTOLI, S., SOLOPERTO, M., MEZZETTI, M., AND FASOLI, A.: Mechanisms of calcium mobilization and phosphoinositide hydrolysis in human bronchial smooth muscle cells by endothelin 1. Am. J. Respir. Cell Mol. Biol. 5: 424–430, 1991b.
MAZZOCCHI, G., MALENDOWICZ, L. K., MENEGHELLI, V., AND NUSSDORFER, G. G.: Endothelin 1 stimulates mitotic activity in the zona glomerulosa of the rat adrenal cortex. Cytobios 69: 91–96, 1992.
MAZZOCCHI, G., MALENDOWICZ, L. K., AND NUSSDORFER, G. G.: Endothelin-1 acutely stimulates the secretory activity of rat zona glomerulosa cells. Peptides (Elmsford) 11: 763–765, 1990a.
MAZZOCCHI, G., REBUFFAT, P., MENEGHELLI, V., MALENDOWICZ, L. K., KASPRZAK, A., AND NUSSDORFER G. G.: Effects of prolonged infusion with endothelin 1 on the function and morphology of rat adrenal cortex. Peptides (Elmsford) 11: 767–772, 1990b.
MCKAY, K. O., BLACK, J. L., AND ARMOUR, C. L.: The mechanism of action of endothelin in human lung. Br. J. Pharmacol. 102: 422–428, 1991.
MCMAHON, E. G., PALOMO, M. A., AND MOORE, W. M.: Phosphoramidon blocks the pressor activity of big endothelin[1–39] and lowers blood pressure in spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S29–S33, 1991a.
MCMAHON, E. G., PALOMO, M. A., MOORE, W. M., MCDONALD, J. F., AND STERN, M. K.: Phosphoramidon blocks the pressor activity of porcine big endothelin 1 (1 39) in vivo and conversion of big endothelin 1 (139) to endothelin 1 (1 21) in vitro. Proc. Natl. Acad. Sci. USA 88: 703–707, 1991b.
MCMURDO, L., CORDER, R., THIEMERMANN, C., AND VANE, J. R.: Incomplete inhibition of the pressor effects of endothelin 1 and related peptides in the anaesthetized rat with BQ 123 provides evidence for more than one vasoconstrictor receptor. Br. J. Pharmacol. 108: 557–561, 1993.
MCMURRAY, J. J., RAY, S. G., ABDULAH, I., DARGIE, H. J., AND MORTON, J. J.: Plasma endothelin in chronic heart failure. Circulation 85: 1374–1379, 1992.
MCNAMARA, D. B., LIGHT, J. T., MINKES, R. K., SAROYAN, R. M., KVAMME, P., ROWE, N., WEBB, W. R., FOX, L., KERSTEIN, M. D., MILLS, N. L., ET AL.: Comparative effects of endothelin (ET1) and U46619 on human saphenous vein and gastroepiploic artery, sources of human autologous grafts. Mol. Cell. Biochem. 117: 81–85, 1992.
MEYER LEHNERT, H., WANNING, C., PREDEL, H. G., BACKER, A., STELKENS, H., AND KRAMER, H. J.: Effects of endothelin on sodium transport mechanisms: potential role in cellular Ca^{2+} mobilization. Biochem. Biophys. Res. Commun. 163: 458–465, 1989.
MIASIRO, N., NAKAIE, C. R., AND PAIVA, A. C.: Endothelin (16 21): biphasic effect and no desensitization on the guinea pig isolated ileum. Br. J. Pharmacol. 109: 68–72, 1993.
MIASIRO, N., AND PAIVA, A. C.: Effects of endothelin 1 on the isolated guinea pig ileum: role of Na^+ ions. Naunyn Schmiedebergs Arch. Pharmacol. 342: 706–712, 1990a.
MIASIRO, N., AND PAIVA, A. C.: Homologous desensitization of the effects of endothelin on rabbit aorta rings and on cultured rat aorta smooth muscle cells. Eur. J. Pharmacol. 179: 151–158, 1990b.
MIASIRO, N., AND PAIVA, A. C.: Effects of endothelin 3 on the isolated guinea pig ileum: role of Na^+ ions and endothelin receptor subtypes. Eur. J. Pharmacol. 214: 133–141, 1992.
MILLER, R. C., PELTON, J. T., AND HUGGINS, J. P.: Endothelins from receptors to medicine. Trends Pharmacol. Sci. 14: 54–60, 1993.

MILLER, W. L., REDFIELD, M. M., AND BURNETT, J. C., JR.:Integrated cardiac, renal, an endocrine actions of endothelin. J. Clin. Invest. **83:** 317–320, 1989a.
MILLER, V. M., KOMORI, K., BURNETT, J. C., JR., AND VANHOUTTE, P. M.: Differential sensitivity to endothelin in canine arteries and veins. Am. J. Physiol. **257:** H1127–H1131, 1989b.
MILLS, R. G., O'DONOGHUE, S. I., SMITH, R., AND KING, G. F.: Solution structure of endothelin 3 determined using NMR spectroscopy. Biochemistry **31:** 5640–5645, 1992.
MILNER, P., BODIN, P., LOESCH, A., AND BURNSTOCK, G.: Rapid release of endothelin and ATP from isolated aortic endothelial cells exposed to increased flow. Biochem. Biophys. Res. Commun. **170:** 649–656, 1990.
MIMA, T., YANAGISAWA, M., SHIGENO, T., SAITO, A., GOTO, K., TAKAKURA, K., AND MASAKI, T.: Endothelin acts in feline and canine cerebral arteries from the adventitial side. Stroke **20:** 1553–1556, 1989.
MINO, N., KOBAYASHI, M., NAKAJIMA, A., AMANO, H., SHIMAMOTO, K., ISHIKAWA, K., WATANABE, K., NISHIKIBE, M., YANO, M., AND IKEMOTO, F.: Protective effect of a selective endothelin receptor antagonist, BQ 123, in ischemic acute renal failure in rats. Eur. J. Pharmacol. **221:** 77–83, 1992.
MITCHELL, M. D.: Endothelins in perinatal biology. Semin. Perinatol. **15:** 79–85, 1991.
MITCHELL, M. D., ROMERO, R. J., LEPERA, R., RITTENHOUSE, L., AND EDWIN, S. S.: Actions of endothelin 1 on prostaglandin production by gestational tissues. Prostaglandins **40:** 627–635, 1990.
MITSUHASHI, T., MORRIS, R. C., JR., AND IVES, H. E.: Endothelin induced increases in vascular smooth muscle Ca^{2+} do not depend on dihydropyridine sensitive Ca^{2+} channels. J. Clin. Invest. **84:** 635–639, 1989.
MIYAMORI, I., TAKEDA, Y., YONEDA, T., AND TAKEDA, R.: Endothelin 1 release from mesenteric arteries of spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. **17** (Suppl. 7)**:** S408–S410, 1991.
MIYATA, S., FUKAMI, N., NEYA, M., TAKASE, S., AND KIYOTO, S.: WS 7338, new endothelin receptor antagonists isolated from *Streptomyces* sp. No. 7338 III. Structures of WS 7338 A, B, C and D and total synthesis of WS 7338 B. J. Antibiot. (Tokyo) **45:** 788–791, 1992a.
MIYATA, S., HASHIMOTO, M., FUJIE, K., NISHIKAWA, M., KIYOTO, S., OKUHARA, M., AND KOHSAKA, M.: WS 7338, new endothelin receptor antagonists isolated from *Streptomyces* sp. No. 7338. II. Biological characterization and pharmacological characterization of WS 7338 B. J. Antibiot. (Tokyo) **45:** 83–87, 1992b.
MIYATA, S. OHHATA, N., MURAI, H., MASUI, Y., EZAKI, M., TAKASE, S., NISHIKAWA, M., KIYOTO S., OKUHARA, M., AND KOHSAKA, M.: WS009 A and B, new endothelin receptor antagonists isolated from *Streptomyces* sp. no. 89009. I. Taxonomy, fermentation, isolation, physico chemical properties and biological activities. J. Antibiot. (Tokyo) **45:** 1029–1040, 1992c.
MIYAUCHI, T., DOI, T., SUZUKI, N., KAKIHANA, M., YAMAGUCHI, I., SUGISHITA, Y., MITSUI, T. HORI, M., MASAKI, T., AND GOTO, K.: Plasma endothelin 1 concentration in the coronary sinus in dogs with artificially induced myocardial infarction. Peptides (Elmsford) **13:** 1013–1015, 1992a.
MIYAUCHI, T., SUGISHITA, Y., MATSUDA, M. SAKAI, H., SUZUKI, N., MASAKI, T., AND GOTO, K.: Increased plasma concentration of endothelin 1 in cholesterol fed rats (letter). Atherosclerosis **93:** 257–259, 1992b.
MIYAUCHI, T., SUGISHITA, Y., YAMAGUCHI, I., AJISAKA, R., TOMIZAWA, T., ONIZUKA, M., MATSUDA, M., KONO, I., YANAGISAWA, M., GOTO, K., ET AL.: Plasma concentrations of endothelin 1 and endothelin 3 are altered differently in various pathophysiological conditions in humans. J. Cardiovasc. Pharmacol. **17** (Suppl. 7)**:** S394–S397, 1991a.
MIYAUCHI, T., YANAGISAWA, M., TOMIZAWA, T., SUGISHITA, Y., SUZUKI, N., FUJINO, M., AJISAKA, R., GOTO, K., AND MASAKI, T.: Increased plasma concentrations of endothelin 1 and big endothelin 1 in acute myocardial infarction. Lancet **2:** 53–54, 1989.
MODIN, A., PERNOW, J., AND LUNDBERG, J. M.: Phosphoramidon inhibits the vasoconstrictor effects evoked by big endothelin 1 but not the elevation of plasma endothelin 1 in vivo. Life Sci. **49:** 1619–1625, 1991.
MOE, O., TEJEDOR, A., CAMPBELL, W. B., ALPERN, R. J., AND HENRICH, W. L.: Effects of endothelin on in vitro renin secretion. Am. J. Physiol. **260:** E521–E525, 1991.
MOLENAAR, P., O'REILLY, G., SHARKEY, A., KUC, R. E., HARDING, D. P., PLUMPTON, C., GRESHAM, G. A., AND DAVENPORT, A. P.: Characterization and localization of endothelin receptor subtypes in the human atrioventricular conducting system and myocardium. Circ. Res. **72:** 526–538, 1993.
MOLNAR, M., AND HERTELENDY, F.: Pressor responsiveness to endothelin is not attenuated in gravid rats. Life Sci. **47:** 1463–1468, 1990.
MONCADA, S., GRYGLEWSKI, R., BUNTING, S., AND VANE, J. R.: An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature (Lond.) **263:** 663–665, 1976.
MOORE, K., WENDON, J., FRAZER, M., KARANI, J., WILLIAMS, R., AND BADR, K.: Plasma endothelin immunoreactivity in liver disease and the hepatorenal syndrome. N. Engl. J. Med. **327:** 1774–1778, 1992.
MORALES, R. E., JOHNSON, B. R., AND SZABO, S.: Endothelin induces vascular and mucosal lesions, enhances the injury by HCl/ethanol, and the antibody exerts gastroprotection. FASEB J. **6:** 2354–2360, 1992.
MOREL, D. R., LACROIX, J. S., HEMSEN, A., STEINIG, D. A., PITTET, J. F., AND LUNDBERG, J. M.: Increased plasma and pulmonary lymph levels of endothelin during endotoxin shock. Eur. J. Pharmacol. **167:** 427–428, 1989.
MOREL, D. R., PITTET, J. F., GUNNING, K., HEMSEN, A., LACROIX, J. S., AND LUNDBERG, J. M.: Time course of plasma and pulmonary lymph endothelin like immunoreactivity during sustained endotoxaemia in chronically instrumented sheep. Clin. Sci. **81:** 357–365, 1991.
MORELAND, R. S., CILEA, J., AND MORELAND, S.: Staurosporine decreases stiffness but not stress n endothelin 1 stimulated arterial muscle. Am. J. Physiol. **262:** C862–C869, 1992.
MORETTO, M., LOPEZ, F. J., AND NEGRO VILAR, A.: Endothelin 3 stimulates luteinizing hormone releasing hormone (LHRH) secretion from Lhrh neurons by a prostaglandin dependent mechanism. Endocrinology **132:** 789–794, 1993.
MORIMOTO, T., HANAMURA, T., SASAKI, T., SAITO, I., AND TAKAKURA, K.: Effect of intracisternal injection of endothelin 1 on regional cerebral blood flow in cats. Neurol. Med. Chir. **31:** 635–640, 1991.
MORISHITA, R., HIGAKI, J., AND OGIHARA, T.: Endothelin stimulates aldosterone biosynthesis by dispersed rabbit adreno capsular cells. Biochem. Biophys. Res. Commun. **160:** 628–632, 1989.
MORITA, T., KURIHARA, H., YOSHIZUMI, M., MAEMURA, K., SUGIYAMA, T., NAGAI, R., AND YAZAKI, Y.: Human polymorphonuclear leukocytes have dual effects on endothelin 1: the induction of endothelin 1 mRNA expression in vascular endothelial cells and modification of the endothelin 1 molecule. Heart Vessels **8:** 1–6, 1993.
MORITOKI, H., MIYANO, H., TAKEUCHI, S., YAMAGUCHI, M., HISAYAMA, T., AND KONDOH, W.: Endothelin 3 induced relaxation of rat thoracic aorta: a role for nitric oxide formation. Br. J. Pharmacol. **108:** 1125–1130, 1993.
MULDOON, L. L., ENSLEN, H., RODLAND, K. D., AND MAGUN, B. E.: Stimulation of Ca^{2+} influx by endothelin 1 is subject to negative feedback by elevated intracellular Ca^{2+}. Am. J. Physiol. **260:** C1273–C1281, 1991.
MULDOON, L. L., RODLAND, K. D., FORSYTHE, M. L., AND MAGUN, B. E.: Stimulation of phosphatidylinositol hydrolysis, diacylglycerol release, and gene expression in response to endothelin, a potent new agonist for fibroblasts and smooth muscle cells. J. Biol. Chem. **264:** 8529–8536, 1989.
MUNRO, S., CRAIK, D., MCCONVILLE, C., HALL, J., SEARLE, M., BICKNELL, W., SCANLON, D., AND CHANDLER, C.: Solution conformation of endothelin, a potent vasoconstricting bicyclic peptide. A combined use of 1H NMR spectroscopy and distance geometry calculations. FEBS Lett. **278:** 9–13, 1991.
MUNTER, K., AND HACKENTHAL, E.: The effects of endothelin on renovascular resistance and renin release. J. Hypertens. **7** (Suppl.)**:** S276–S277, 1989.
MURAKAMI, O., TAKAHASHI, K., SONE, M., TOTSUNE, K., OHNEDA, M., ITOI, K., YOSHINAGA, K., AND MOURI, T.: An ACTH secreting bronchial carcinoid: presence of corticotropin releasing hormone, neuropeptide Y and endothelin 1 in the tumor tissue. Acta Endocrinol. (Copenh.) **128:** 192–196, 1993.
MURAMATSU, K., TOMOIKE, H., OHARA, Y. EGASHRA, S., AND NAKAMURA, M.: Effects of endothelin 1 on epicardial coronary tone, coronary blood flow, ECG ST change and regional wall motion in anesthetized dogs. Heart Vessels **6:** 191–196, 1991.
MURCH, S. H., BRAEGGER, C. P., SESSA, W. C., AND MACDONALD, T. T.: High endothelin 1 immunoreactivity in Crohn's disease and ulcerative colitis. Lancet **339:** 381–385, 1992.
MYATT, L., BREWER, A. S., LANGDON, G., AND BROCKMAN, D. E.: Attenuation of the vasoconstrictor effects of thromboxane and endothelin by nitric oxide in the human fetal placental circulation. Am. J. Obstet. Gynecol. **166:** 224–230, 1992.
NAGAYAMA, Y., WADSWORTH, H. L., CHAZENBALK, G. D., RUSSO, D., SETO, P., AND RAPOPORT, B.: Thyrotropin-leutinizing hormone/chorionic gonadotropin receptor extracellular domain chimeras as probes for thyrotropin receptor function. Proc. Natl. Acad. Sci. USA **88:** 902–905, 1991.
NAITOH, T., TOYO-OKA, T., AND SUGIMOTO, T.: An endogenous Ca^{2+} channel agonist, endothelin 1, does not directly activate partially purified dihydropyridine sensitive Ca^{2+} channel from cardiac muscle in a reconstituted system. Biochem. Biophys. Res. Commun. **171:** 1205–1210, 1990.
NAKAGOMI, T., IDE, K., YAMAKAWA, K., SASAKI, T., KURIHARA, H., SAITO, I., AND TAKAKURA, K.: Pharmacological effect of endothelin, an endothelium derived vasoconstrictive peptide, on canine basilar arteries. Neurol. Med. Chir. **29:** 967–974, 1989.
NAKAJO, S., SUGIURA, M., INAGAMI, T., NITTA, K., NARUSE, M., SANAKA, T., TSUCHIYA, K., NARUSE, K., ZENG, Z. P., DEMURA, H., AND SUGINO N.: Native form of endothelin receptor in human placental membranes. Biochem. Biophys. Res. Commun. **167:** 280–286, 1990.
NAKAKI, T., NAKAYAMA, M., YAMAMOTO, S., AND KATO, R.: Endothelin mediated stimulation of DNA synthesis in vascular smooth muscle cells. Biochem. Biophys. Res. Commun. **158:** 880–883, 1989.
NAKAKI, T., OHTA, M., AND KATO, R.: Inhibition by prostacyclin and carbacyclins of endothelin induced DNA synthesis in cultured vascular smooth muscle cells. Prostaglandins Leukotrienes Essent. Fatty Acids **44:** 237–239, 1991.
NAKAMOTO, H., SUZUKI, H., MURAKAMI, M., KAGEYAMA, Y., NAITOH, M., SAKAMAKI, Y., OHISHI, A., AND SARUTA, T.: Different effects of low and high doses of endothelin on haemodynamics and hormones in the normotensive conscious dog. J. Hypertens. **9:** 337–344, 1991.
NAKAMOTO, H., SUZUKI, H., MURAKAMI, M., KAGEYAMA, Y., OHISHI, A., FUKUDA, K., HORI, S., AND SARUTA, T.: Effects of endothelin on systemic and renal haemodynamics and neuroendocrine hormones in conscious dogs. Clin. Sci. **77:** 567–572, 1989.
NAKAMURA M., ABELL, T. J., RICHARDS, A. M., IKRAM, H., ESPINER, E. A., AND YANDLE, T. G.: Endothelin induced changes in intracellular calcium concentration in rat vascular smooth muscle cells: effects of extracellular calcium and atrial natriuretic factor. Heart Vessels **7:** 37–41, 1992.

NAKAMURA, M., TABUCHI, Y., RAKUGI, H., NAGANO, M., AND OGIHARA, T.: Actions of endothelin on adrenergic neuroeffector junction. J. Hypertens. 7 (Suppl.): S132–S133, 1989.

NAKAMURA, S., NARUSE, M., NARUSE, K., SHIODA, S., NAKAI, Y., AND UEMURA, H.: Colocalization of immunoreactive endothelin 1 and neurohypophysial hormones in the axons of the neural lobe of the rat pituitary. Endocrinology 132: 530–533, 1993.

NAKAMURA, T., KASAI, K., SEKIGUCHI, Y., BANBA, N., TAKAHASHI, K., EMOTO, T., HATTORI, Y., AND SHIMODA, S.: Elevation of plasma endothelin concentrations during endotoxin shock in dogs. Eur. J. Pharmacol. 205: 277–282, 1991.

NAKAO, K., INOUE, Y., OIKE, M., KITAMURA, K., AND KURIYAMA, H.: Mechanisms of endothelin induced augmentation of the electrical and mechanical activity in rat portal vein. Pflugers Arch. 415: 526–532, 1990.

NAMBI, P., PULLEN, M., AND FEUERSTEIN, G.: Identification of endothelin receptors in various regions of rat brain. Neuropeptides 16: 195–199, 1990.

NAMBI, P., PULLEN, M., JUGUS, M., AND GELLAI, M.: Rat kidney endothelin receptors in ischemia induced acute renal failure. J. Pharmacol. Exp. Ther. 264: 345–348, 1993.

NAMIKI, A., HIRATA, Y., FUKAZAWA, M., ISHIKAWA, M., MOROI, M., AIKAWA, J., YABUKI, S., AND MACHII, K.: Endothelin 1 and endothelin 3 induced vasorelaxation via endothelium derived nitric oxide. Jpn. J. Pharmacol. 58 (Suppl. 2): 326P, 1992a.

NAMIKI, A., HIRATA, Y., FUKAZAWA, M., ISHIKAWA, M., MOROI, M., AIKAWA, J., YAMAGUCHI, T., AND MACHII, K.: Granulocyte colony stimulating factor stimulates immunoreactive endothelin 1 release from cultured bovine endothelial cells. Eur. J. Pharmacol. 227: 339–341, 1992b.

NARUSE, K., NARUSE, M., WATANABE, Y., YOSHIHARA, I., OHSUMI, K., HORIUCHI, J., MONZEN, C. KATO, Y., NAKAMURA, N., SUGINO, N., ET AL.: Molecular form of immunoreactive endothelin in plasma and urine of normal subjects and patients with various disease states. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S506–S508, 1991a.

NARUSE, M., KAWANA, M., HIFUMI, S., NARUSE, K., YOSHIHARA, I., OKA, T., KATO, Y., MONZEN, C., KURIMOTO, F., OHSUMI, K., ET AL.: Plasma immunoreactive endothelin, but not thrombomodulin, is increased in patients with essential hypertension and ischemic heart disease. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S471–S474, 1991b.

NAYLER, W. G.: Endothelin: isoforms, binding sites and possible implications in pathology. Trends Pharmacol. Sci. 11: 96–99, 1990.

NAYLER, W. G., LIU, J. J., PANAGIOTOPOULOS, S., AND CASLEY, D. J.: Streptozotocin induced diabetes reduces the density of [^{125}I] endothelin binding sites in rat cardiac membranes. Br. J. Pharmacol. 97: 993–995, 1989c.

NAYLER, W. G., OU, R. C., GU, X. H., AND CASLEY, D. J.: Effect of amlodipine pretreatment on ischaemia reperfusion induced increase in cardiac endothelin 1 binding site density. J. Cardiovasc. Pharmacol. 20: 416–420, 1992.

NEUBAUER, S., ERTL, G., HAAS, U., PULZER, F., AND KOCHSIEK, K.: Effects of endothelin 1 in isolated perfused rat heart. J. Cardiovasc. Pharmacol. 16: 1–8, 1990.

NEUBAUER, S., ZIMMERMANN, S., HIRSCH, A., PULZER, F., TIAN, R., BAUER, W., BAUER, B., AND ERTL G.: Effects of endothelin 1 in the isolated heart in ischemia/reperfusion and hypoxia/reoxygenation injury. J. Mol. Cell. Cardiol. 23: 1397–1409, 1991.

NEUSER, D., KNORR, A., STASCH, J. P., AND KAZDA, S.: Mitogenic activity of endothelin 1 and 3 on vascular smooth muscle cells is inhibited by atrial natriuretic peptides. Artery 17: 311–324, 1990a.

NEUSER, D., ZAISS, S., AND STASCH, J. P.: Endothelin receptors in cultured renal epithelial cells. Eur. J. Pharmacol. 176: 241–243, 1990b.

NGUYEN, P. V., PARENT, A., DENG, L. Y., FLUCKIGER, J. P., THIBAULT, G., AND SCHIFFRIN, E. L.: Endothelin vascular receptors and responses in deoxycorticosterone acetate salt hypertensive rats. Hypertension 19 (Suppl.): II98–II104, 1992.

NINOMIYA, H., UCHIDA, Y., ISHII, Y., NOMURA, A., KAMEYAMA, M., SAOTOME, M., ENDO, T., AND HASEGAWA, S.: Endotoxin stimulates endothelin release from cultured epithelial cells of guinea pig trachea. Eur. J. Pharmacol. 203: 299–302, 1991.

NINOMIYA, H., UCHIDA, Y., SAOTOME, M., NOMURA, A., OHSE, H., MATSUMOTO, H., HIRATA, F., AND HASEGAWA, S.: Endothelins constrict guinea pig tracheas by multiple mechanisms. J. Pharmacol. Exp. Ther. 262: 570–576, 1992a.

NINOMIYA, H., YU, X. Y., HASEGAWA, S., AND SPANNHAKE, E. W.: Endothelin 1 induces stimulation of prostaglandin synthesis in cells obtained from canine airways by bronchoalveolar lavage. Prostaglandins 43: 401–411, 1992b.

NISHIKIBE, M., TSUCHIDA, S., OKADA, M., FUKUODA, T., SHIMAMOTO, K., YANO, M., ISHIKAA, K., AND IKEMOTO, F.: Antihypertensive effect of a newly synthesized endothelin antagonist, BQ 123, in a genetic hypertensive model. Life Sci. 52: 717–724, 1993.

NISHIMURA, J., MORELAND, S., AHN, H. Y., KAWASE, T., MORELAND, R. S., AND VAN BREEMEN, C.: Endothelin increases myofilament Ca^{2+} sensitivity in alpha toxin permeabilized rabbit mesenteric artery. Circ. Res. 71: 951–959 1992.

NISHIZUKA, Y.: Studies and perpspectives of the protein kinase C family for cellular regulation. Cancer (Phila.) 63: 1892–1903, 1989.

NIWA, M., KAWAGUCHI, T., FUJIMOTO, M., KATAOKA, Y., AND TANIYAMA, K.: Receptors for endothelin in the central nervous system. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S137–S139, 1991a.

NIWA, M., KAWAGUCHI, T., YAMASHITA, K., MAEDA, T., KURIHARA, M., KATAOKA, Y., AND OZAKI, M.: Specific ^{125}I endothelin 1 binding sites in the central nervous system. Clin. Exp. Hypertens. [A] 13: 799–806, 1991b.

NOGUCHI, K., NOGUCHI, Y., HIROSE, H., NISHIKIBE, M., IHARA, M., ISHIKAWA, K., AND YANO, M.: Role of endothelin ETB receptors in bronchoconstrictor and vasoconstrictor responses in guinea pigs. Eur. J. Pharmacol. 233: 47–51, 1993.

NOMURA, A., UCHIDA, Y., KAMEYANA, M., SAOTOME, M., OKI, K., AND HASEGAWA, S.: Endothelin and bronchial asthma (letter). Lancet 2: 747–748, 1989.

NOVA, A., SIBAI, B. M., BARTON, J. R., MERCER, B. M., AND MITCHELL, M. D.: Maternal plasma level of endothelin is increased in preeclampsia. Am. J. Obstet. Gynecol. 165: 724–727, 1991.

NYBORG, N. C., PRIETO, D., BENEDITO, S., AND NIELSEN, P. J.: Endothelin 1 induced contraction of bovine retinal small arteries is reversible and abolished by nitrendipine. Invest. Ophthalmol. Vis. Sci. 32: 27–31, 1991.

O'BRIAN, R. F., ROBBINS, R. J., AND MCMURTRY, I. F.: Endothelial cells in culture produce a vasoconstrictor substance. J. Cell. Physiol 132: 263–270, 1987.

O'DONNELL, S. R., WANSTALL, J. C., AND ZENG, X. P.: Pinacidil antagonism of endothelin induced contractions of smooth muscle in the lungs: differences between tracheal and pulmonary artery preparations. J. Pharmacol. Exp. Ther. 252: 1318–1323, 1990.

O'DOWD, B. F., HNATOWICH, M., CARON, M. G., LEFKOWITZ, R. J., AND BOUVIER, M.: Palmitoylation of the human beta$_2$-adrenergic receptor. J. Biol. Chem. 264: 7564–7569, 1989.

OHDE, H., MORIMOTO, S., OHNISHI, K., YAMAOKA, E. FUJUO, K., AND YASUDA, O., OGIHARA, T.: Bradykinin suppresses endothelin induced contraction of coronary, renal and femoral arteries through its B2 receptor on the endothelium. Agents Actions 38 (Suppl.): 14–22, 1992.

OHKUBO, S., OGI, K., HOSOYA, M., MATSUMOTO, H., SUZUKI, N., KIMURA, C., ONDO, H., AND FUJINO, M.: Specific expression of human endothelin 2 (ET 2) gene in a renal adenocarcinoma cell line. Molecular cloning of cDNA encoding the precursor of ET 2 and its characterization. FEBS Lett. 274: 136–140, 1990b.

OHLSTEIN, E. H., ARLETH, A., BRYAN, H., ELLIOTT, J. D., AND SUNG, C. P.: The selective endothelin ETA receptor antagonist BQ123 antagonizes endothelin 1 mediated mitogenesis. Eur. J. Pharmacol. 225: 347–350, 1992.

OHLSTEIN, E. H., HOROHONICH, S., AND HAY, D. W.: Cellular mechanisms of endothelin in rabbit aorta. J. Pharmacol. Exp. Ther. 250: 548–555, 1989.

OHLSTEIN, E. H., AND STORER, B. L.: Oxyhemoglobin stimulation of endothelin production in cultured endothelial cells. J. Neurosurg. 77: 274–278, 1992.

OHLSTEIN, E. H., STORER, B., NAMBI, P., GIVEN, M., AND LIPPTON, H.: Endothelin and platelet function. Thromb. Res. 57: 967–974, 1990a.

OHLSTEIN, E. H., STORER, B. L., BUTCHER, J. A., DEBOUCK, C., AND FEUERSTEIN, G.: Platelets stimulate expression of endothelin mRNA and endothelin biosynthesis in cultured endothelial cells. Circ. Res. 69: 832–841, 1991.

OHMAN, K. P., HOFFMAN, A., AND KEISER, H. R.: Endothelin induced vasoconstriction and release of atrial natriuretic peptides in the rat. Acta Physiol. Scand. 138: 549–556, 1990.

OHNAKA, K., NISHIKAWA, M., TAKAYANAGI, R., HAJI, M., AND NAWATA, H.: Partial purification of phosphoramidon sensitive endothelin converting enzyme in porcine aortic endothelial cells: high affinity for *Ricinus communis* agglutinin. Biochem. Biophys. Res. Commun. 185: 611–616, 1992.

OHNAKA, K., TAKAYANAGI, R., YAMAUCHI, T., OKAZAKI, H., OHASHI, M., UMEDA, F., AND NAWATA, H.: Identification and characterization of endothelin converting activity in cultured bovine endotheial cells. Biochem. Biophys. Res. Commun. 168: 1128–1136, 1990.

OHTA, K., HIRATA, Y., SHICHIRI, M., KANNO K., EMORI, T., TOMITA, K., AND MARUMO, F.: Urinary excretion of endothelin 1 in normal subjects and patients with renal disease. Kidney Int. 39: 307–311, 1991.

OISHI, R., NONOGUCHI, H., TOMITA, K., AND MARUMO, F.: Endothelin 1 inhibits AVP stimulated osmotic water permeability in rat inner medullary collecting duct. Am. J. Physiol. 261: F951–F956, 1991.

OKADA, K., ISHIKAWA, S., AND SAITO, T.: Interaction between endothelin induced Na^+ and Ca^{2+} kinetics in cultured rat vascular smooth muscle cells. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S124–S126, 1991.

OKADA, K., MIYAZAKI, Y., TAKADA, J., MATSUYAMA, K., YAMAKI, T., AND YANO, M.: Conversion of big endothelin 1 by membrane bound metalloendopeptidase in cultured bovine endothelial cells. Biochem. Biophys. Res. Commun. 171: 1192–1198, 1990.

OKAWA, T., SUZUKI, Y., SUZUKI, H., ENDO, C., HOSHI, K., SATO, A., AND NAKANISHI, H.: Endothelin 1 induces inositol phosphate production in rat pregnant myometrium. Acta Obstet. Gynaecol. Jpn. (Jpn. Ed.) 43: 1695–1696, 1991.

OKISHIO, M. OHKAWA, S., ICHIMORI, Y., AND KONDO, K.: Interaction between endothelium derived relaxing factors, nitrosothiols, and endothelin 1 on Ca^{2+} mobilization in rat vascular smooth muscle cells. Biochem. Biophys. Res. Commun. 183: 849–855, 1992.

OLIVER, F. J., DE LA RUBIA G., FEENER, E. P., LEE, M. E., LOEKEN, M. R., SHIBA, T., QUERTERMOUS T., AND KING, G. L.: Stimulation of endothelin 1 gene expression by insulin in endothelial cells. J. Biol. Chem. 266: 23251–23256, 1991.

ONDA, H., OHKUBO, S., KOSAKA, T., YASUHARA, T., OGI, K., HOSOYA, M.,

MATSUMOTO, H., SUZUKI, N., KITADA, C., ISHIBASHI, Y., ET AL.: Expression of endothelin 2 (ET 2) gene in a human renal adenocarcinoma cell line: purification and cDNA cloning of ET 2. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S39–S43, 1991.

ONDA, H., OHKUBO, S., OGI, K., KOSAKA, T., KIMURA, C., MATSUMOTO, H., SUZUKI, N., AND FUJINO, M.: One of the endothelin gene family, endothelin 3 gene, is expressed in the placenta. FEBS Lett. **261:** 327–330, 1990.

OPGENORTH, T. J., WU WONG, J. R., AND SHIOSAKI, K.: Endothelin converting enzymes. FASEB J. **6:** 2653–2659, 1992.

O'REILLY, G., CHARNOCK JONES, D. S., DAVENPORT, A. P., CAMERON, I. T., AND SMITH, S. K.: Presence of messenger ribonucleic acid for endothelin 1, endothelin 2, and endothelin 3 in human endometrium and a change in the ratio of ETA and ETB receptor subtype across the menstrual cycle. J. Clin. Endocrinol. Metab. **75:** 1545–1549, 1992.

ORLANDO, C., BRANDI, M. L., PERI, A., GIANNINI, S., FANTONI, G., CALABRESI, E., SERIO, M., AND MAGGI, M.: Neurohypophyseal hormone regulation of endothelin secretion from rabbit endometrial cells in primary culture. Endocrinology **126:** 1780–1782, 1990.

OSHITA, M., TAKEI, Y., KAWANO, S., YOSHIHARA, H., HIJIOKA, T., FUKUI, H., GOTO, M., MASUDA E., NISHIMURA, Y., FUSAMOTO, H., ET AL.: Roles of endothelin 1 and nitric oxide in the mechanism for ethanol induced vasoconstriction in rat liver. J. Clin. Invest. **91:** 1337–1342, 1993.

OTA, K., KIMURA, T., SHOJI, M., INOUE, M., SATO, K., OHTA, M., YAMAMOTO, T., TSUNODA, K., ABE, K., AND YOSHINAGA, K.: Interaction of ANP with endothelin on cardiovascular, renal, and endocrine function. Am. J. Physiol. **262:** E135–E141, 1992.

OTA, S., HIRATA, Y., SUGIMOTO, T., KOHMOTO, O., HATA, Y., YOSHIURA, K., NAKADA, R., TERANO A., AND SUGIMOTO, T.: Endothelin 1 secretion from cultured rabbit gastric epithelial cells. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S406–S407, 1991.

OTANI, S., USUKI, S., SAITOH, T., YANAGISAWA, M., IWASAKI, H., TANAKA, J., SUZUKI, N., FUJINO, M., GOTO, K., AND MASAKI, T.: Comparison of endothelin 1 concentrations in normal and complicated pregnancies. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S308–S312, 1991.

OTSUKA, A., MIKAMI, H., KATAHIRA, K., TSUNETOSHI, T., MINAMITANI, K., AND OGIHARA, T.: Changes in plasma renin activity and aldosterone concentration in response to endothelin injection in dogs. Acta Endocrinol. (Copenh.) **121:** 361–364, 1989.

OTSUKA, A., MIKAMI, H., TSUNETOSHI, T., KATAHIRA, K., KOHARA, K., MORIGUCHI, A., NAKAMURA, F., AND OGIHARA, T.: Effect of aspirin on the pressor activity of endothelin. Biochem. Int. **21:** 1153–1160, 1990.

OZAKI, H., SATO, K., SAKATA, K., AND KARAKI, H.: Endothelin dissociates muscle tension from cytosolic Ca^{2+} in vascular smooth muscle of rat carotid artery. Jpn. J. Pharmacol. **50:** 521–524, 1989.

PAI, J. K., DOBEK, E. A., AND BISHOP, W. R.: Endothelin 1 activates phospholipase D. and thymidine incorporation in fibroblasts overexpressing protein kinase C beta 1. Cell Regul. **2:** 897–903, 1991.

PANG, D. C., JOHNS, A., PATTERSON, K., BOTELHO, L. H., AND RUBANYI, G. M.: Endothelin 1 stimulates phosphatidylinositol hydrolysis and calcium uptake in isolated canine coronary arteries. J. Cardiovasc. Pharmacol. **13** (Suppl. 5): S75–S79, 1989.

PAPADOPOULOS, S. M., GILBERT, L. L., WEBB, R. C., AND D'AMATO, C. J.: Characterization of contractile responses to endothelin in human cerebral arteries: implication for cerebral vasospasm. Neurosurgery. **26:** 810–815, 1990.

PARKER-BOTELHO, L. H., CADE, C., PHILLIPS, P. E., AND RUBANYI, G. M.: Tissue specificity of endothelin synthesis and binding. *In* Endothelin, edited by G. M. Rubanyi, Oxford University Press, New York, pp. 72–102, 1992.

PARKES, D. G., REID, A. F., COGHLAN, J. P., SCOGGINS, B. A., WHITWORTH, J. A., ET AL.: Effects of calcium channel blockade on the hemodynamic responses to endothelin infusion in conscious sheep. Am. J. Hypertens. **4:** 719–722, 1991.

PATEL, A., FARBANKS, L., GORDGE, M. P., AND NEILD, G. H.: Failure of endothelin to influence in vitro platelet responses. Thromb. Res. **56:** 769–770, 1989.

PATRIGNANI, P., DEL MASCHIO A., BAZZONI, G., DAFFONCHIO, L., HERNANDEZ, A., MODICA, R., MONTESANTI, L., VOLPI, D., PATRONO, C., AND DEJANA, E.: Inactivation of endothelin by polymorphonuclear leukocyte derived lytic enzymes. Blood **78:** 2715–2720, 1991.

PATTERSON, K., POLOKOFF, M. A., AND PARKER-BOTELHO, L. H.: Unpublished observations, 1991.

PEASE, J. H. H., AND WEMMER, D. E.: Solution structure of apamin determined by nuclear magnetic resonance and distance geometry. Biochemistry **27:** 8491–8498, 1988.

PEKONEN, F., SAIJONMAA, O., NYMAN, T., AND FYHRQUIST, F.: Human endometrial adenocarcinoma cells express endothelin 1. Mol. Cell. Endocrinol. **84:** 203–207, 1992.

PERICO, N., CORNEJO, R. P., BENIGNI, A., MALACHINI, B., LADNY, J. R., AND REMUZZI, G.: Endothelin induces diuresis and natriuresis in the rat by acting on proximal tubular cells through a mechanism mediated by lipoxygenase products. J. Am. Soc. Nephrol. **2:** 57–69, 1991.

PERICO, N., DADAN, J., AND REMUZZI, G.: Endothelin mediates the renal vasoconstriction induced by cyclosporine in the rat. J. Am. Soc. Nephrol. **1:** 76–83, 1990.

PERICO, N., AND REMUZZI, G.: Vascular endothelin and renal disease progression. Contrib. Nephrol. **100:** 236–253, 1992.

PERNOW, J., HEMSEN, A., HALLEN, A., AND LUNDBERG, J. M.: Release of endothelin like immunoreactivity in relation to neuropeptide Y and catecholamines during endotoxin shock and asphyxia in the pig. Acta Physiol. Scand. **140:** 311–322, 1990.

PERNOW, J., HEMSEN, A., AND LUNDBERG, J. M.: Increased plasma levels of endothelin like immunoreactivity during endotoxin administration in the pig. Acta Physiol. Scand. **137:** 317–318, 1989.

PERNOW, J., AND LUNDBERG, J. M.: Endothelin like immunoreactivity is released from the pig spleen during asphyxia. Acta Physiol. Scand. **137:** 553–554, 1989.

PERSSON, K., GARCIA, PASCUAL A., HOLMQUIST, F., AND ANDERSSON, K. E.: Endothelin 1 induced contractions of isolated pig detrusor and vesical arterial smooth muscle: calcium dependence and phosphoinositide hydrolysis. Gen. Pharmacol. **23:** 445–453, 1992.

PESKAR, B. M., NOWAK, P., AND LAMBRECHT, N.: Effect of prostaglandins and capsaicin on gastric vascular flow and mucosal injury in endothelin 1 treated rats. Agents Actions **37** (Suppl.): 85–91, 1992.

PHILLIPS, P. E., CADE, C., PARKER-BOTELHO, L. H., AND RUBANYI, G. M.: Molecular biology of endothelins. *In* Endothelin, edited by G. M. Rubanyi, Oxford University Press, New York, pp. 31–40, 1992.

PIETRASZEK, M. H., TAKADA, Y., AND TAKADA, A.: Endothelins inhibit serotonin induced platelet aggregation via a mechanism involving protein kinase C. Eur. J. Pharmacol. **219:** 289–293, 1992.

PITKANEN, M., MANTYMAA, P., AND RUSKOAHO, H.: Staurosporine, a protein kinase C inhibitor, inhibits atrial natriuretic peptide secretion induced by sarafotoxin, endothelin and phorbol ester. Eur. J. Pharmacol. **195:** 307–315, 1991.

PLEWS, P. I., ABDEL, MALEK Z. A., DOUPNIK, C. A., AND LEIKAUF, G. D.: Endothelin stimulates chloride secretion across canine tracheal epithelium. Am. J. Physiol. **261:** L188–L194, 1991.

PODER, T. C., SILBERBERG, S. D., AND RAMPE, D.: Contraction of reptile, amphibian, and fish blood vessels by endothelin 1. Can J. Physiol Pharmacol. **69:** 215–217, 1991.

POLDERMAN, K. H., STEHOUWER, C. D., VAN, KAMP G. J., DEKKER, G. A., VERHEUGT, F. W., AND GOOREN, L. J.: Influence of sex hormones on plasma endothelin levels. Ann. Intern. Med. **118:** 429–432, 1993.

POLLOCK, D. M., DIVISH, B. J., MILICIC, I., NOVOSAD, E. I., BURRES, N. S., AND OPGENORTH, T. J.: In vivo characterization of endothelin converting enzyme in the rat. Eur. J. Pharmacol. **231:** 459–464, 1993.

POLLOCK, D. M., AND OPGENORTH, T. J.: Evidence for endothelin induced renal vasoconstriction independent of ETA receptor activation. Am. J. Physiol. **264:** R222–R226, 1993.

POLLOCK, D. M., SHIOSAKI, K., SULLIVAN, G. M., AND OPGENORTH, T. J.: Rhamnose moiety of phosphoramidon is not required for in vivo inhibition of endothelin converting enzyme. Biochem. Biophys. Res. Commun. **186:** 1146–1150, 1992.

PREDEL, H. G., MEYER, LEHNERT, H., BACKER, A., STELKENS, H., AND KRAMER, H. J.: Plasma concentration of endothelin in patients with abnormal vascular reactivity. Effects of ergometric exercise and acute saline loading. Life Sci. **47:** 1837–1842, 1990.

PRIBNOW, D., MULDOON, L. L., FAJARDO, M., THEODOR, L., CHEN, L. Y., AND MAGUN, B. E.: Endothelin induces transcription of fos/jun family genes: a prominent role for calcium ion. Mol. Endocrinol. **6:** 1003–1012, 1992.

RAE, G. A., AND CALIXTO, J. B.: Effects of endothelins on nerve mediated contractions of the mouse vas deferens. Life Sci. **47:** PL83–PL89, 1990.

RAE, G. A., TRYBULEC, M., DE NUCCI, G., AND VANE, J. R.: Endothelin 1 releases eicosanoids from rabbit isolated perfused kidney and spleen. J. Cardiovasc. Pharmacol., 13 (Suppl. 5): S89-S-92 (discussion, S102), 1989.

RAJ, J. U., TOGA, H., IBE, B. O., AND ANDERSON, J.: Effects of endothelin, platelet activating factor and thromboxane A2 in ferret lungs. Respir. Physiol. **88:** 129–140, 1992.

RAKUGI, H., NAKAMARU, M., SAITO, H., HIGAKI, J., AND OGIHARA, T.: Endothelin inhibits renin release from isolated rat glomeruli. Biochem. Biophys. Res. Commun. **155:** 1244–1247, 1988.

RAKUGI, H., NAKAMARU, M., TABUCHI, Y., NAGANO, M., MIKAMI, H., AND OGIHARA, T.: Endothelin stimulates the release of prostacyclin from rat mesenteric arteries. Biochem. Biophys. Res. Commun. **160:** 924–928, 1989.

RAMACHANDRAN, E., FRANK, R. N., AND KENNEDY, A.: Effects of endothelin on cultured bovine retinal microvascular pericytes. Invest. Ophthalmol. Vis. Sci. **34:** 586–595, 1993.

RANDALL, M. D.: Vascular activities of the endothelins. Pharmacol. Ther. **50:** 73–93, 1991.

RANDALL, M. D., DOUGLAS, S. A., AND HILEY, C. R.: Vascular activities of endothelin 1 and some alanyl substituted analogues in resistance beds of the rat. Br. J. Pharmacol. **98:** 685–699, 1989.

RAO, V. V., LOFFLER, C., AND HANSMANN, I.: The gene for the novel vasoactive peptide endothelin 3 (EDN3) is localized to human chromosome 20q13.2 qter. Genomics **10:** 840–841, 1991.

RAPOPORT, R. M., STAUDERMAN, K. A., AND HIGHSMITH, R. F.: Effects of EDCF and endothelin on phosphatidylinositol hydrolysis and contraction in rat aorta. Am. J. Physiol. **258:** C122–C131, 1990.

REILY, M. D., AND DUNBAR, J. B., JR.: The conformation of endothelin 1 in aqueous solution: NMR derived constraints combined with distance geometry and molecular dynamics calculations. Biochem. Biophys. Res. Commun. **178:** 570–577, 1991.

REISER, G.: Endothelin and a Ca^{2+} ionophore raise cyclic GMP levels in a

neuronal cell line via formation of nitric oxide. Br. J. Pharmacol. **101:** 722–726, 1990.

RENNICK, R. E., MILNER, P., AND BURNSTOCK, G.: Thrombin stimulates release of endothelin and vasopressin, but not substance P, from isolated rabbit tracheal epithelial cells. Eur. J. Pharmacol. **230:** 367–370, 1993.

RESINK, T. J., HAHN, A. W., SCOTT-BURDEN T., POWELL, J., WEBER, E., AND BUHLER, F. R.: Inducible endothelin mRNA expression and peptide secretion in cultured human vascular smooth muscle cells. Biochem. Biophys. Res. Commun. **168:** 1303–1310, 1990a.

RESINK, T. J., SCOTT-BURDEN, T., AND BUHLER, F. R.: Endothelin stimulates phospholipase C in cultured vascular smooth muscle cells. Biochem. Biophys. Res. Commun. **157:** 1360–1368, 1988.

RESINK, T. J., SCOTT-BURDEN, T., AND BUHLER, F. R.: Activation of phospholipase A2 by endothelin in cultured vascular smooth muscle cells. Biochem. Biophys. Res. Commun. **158:** 279–286, 1989.

RESINK, T. J., SCOTT-BURDEN T., AND BUHLER, F. R.: Activation of multiple signal transduction pathways by endothelin in cultured human vascular smooth muscle cells. Eur. J. Biochem. **189:** 415–421, 1990b.

RESINK, T. J., SCOTT-BURDEN T., WEBER, E., AND BUHLER, F. R.: Phorbol ester promotes a sustained down regulation of endothelin receptors and cellular responses to endothelin in human vascular smooth muscle cells. Biochem. Biophys. Res. Commun. **166:** 1213–1219, 1990c.

REYNOLDS, E. E., MOK, L. L., AND KUROKAWA, S.: Phorbol ester dissociates endothelin stimulated phosphoinositide hydrolysis and arachidonic acid release in vascular smooth muscle cells. Biochem. Biophys. Res. Commun. **160:** 868–873, 1989.

RIEDER, H., RAMADORI, G., AND MEYER ZUM BUSCHENFELDE, K. H.: Sinusoidal endothelial liver cells in vitro release endothelin augmentation by transforming growth factor beta and Kupffer cell conditioned media. Klin. Wochenschr. **69:** 387–391, 1991.

RIGEL, D. F., AND LAPPE, R. W.: Differential responsiveness of conduit and resistance coronary arteries to endothelin A and B receptor stimulation in anesthetized dogs. *In* Proceedings of the 3rd International Conference on Endothelin, p. 106, 1993. Houston, TX. February 15–17, 1993.

RITZ, M. F., STUENKEL, E. L., DAYANITHI, G., JONES, R., AND NORDMANN, J. J.: Endothelin regulation of neuropeptide release from nerve endings of the posterior pituitary. Proc. Natl. Acad. Sci. USA **89:** 8371–8375, 1992.

ROBINSON, M. J., MACRAE, I. M., TODD, M., REID, J. L., AND MCCULLOCH, J.: Reduction in local cerebral blood flow induced by endothelin 1 applied topically to the middle cerebral artery in the rat. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S354–S357, 1991.

RODMAN, D. M., STELZNER, T. J., ZAMORA, M. R., BONVALLET, S. T., OKA, M., SATO, K., O'BRIEN, R. F., AND MCMURTRY, I. F.: Endothelin 1 increases the pulmonary microvascular pressure and causes pulmonary edema in salt solution but not blood perfused rat lungs. J. Cardiovasc. Pharmacol. **20:** 658–663, 1992.

ROSATI, C., JEANCLOS, E., CAVALIE, S., CHABRIER, P. E., HANNAERT, P., BRAQUET, P., AND GARAY, R.: Stimulatory action of endothelin 1 on membrane Na^+ transport in vascular smooth muscle cells in culture. Am. J. Hypertens. **3:** 711–713, 1990.

ROSOLOWSKY, L. J., AND CAMPBELL, W. B.: Endothelin enhances adrenocorticotropin stimulated aldosterone release from cultured bovine adrenal cells. Endocrinology **126:** 1860–1866, 1990.

ROSSI, P., KARSENTY, G., ROBERTS, A. B., ROCHE, N. S., SPORN, M. B., AND DE CROMBRUGGHE, B.: A nuclear factor 1 binding site mediates the transcriptional activation of type I collagen promoter by transforming growth factor-β. Cell **52:** 405–414, 1988.

ROUBERT, P., GILLARD, V., PLAS, P., CHABRIER, P. E., AND BRAQUET, P.: Down regulation of endothelin binding sites in rat vascular smooth muscle cells. Am. J. Hypertens. **3:** 310–312, 1990.

RUBANYI G. M.: Endothelium-derived vasoconstrictor factors. *In* Endothelial Cell, Vol. III, edited by U. S. Ryan, CRC Press, Boca Raton, FL, pp. 61–75, 1988.

RUBANYI G. M.: Maintenance of "basal" vascular tone may represent a physiological role for endothelin. J. Vasc. Med. Biol. **1:** 315–316, 1989.

RUBANYI, G. M. (ED.): Cardiovascular Significance of Endothelium-derived Vasoactive Factors. Futura Publishing Co., Inc., Mount Kisco, NY, pp. 1–357, 1991.

RUBANYI G. M. (ED.): Endothelin. Oxford University Press, New York, pp. 1–277, 1992a.

RUBANYI G. M.: Endothelium-derived vasoconstrictor factors: an overview. *In* Endothelial Regulation of Vascular Tone, edited by U. S. Ryan and G. M. Rubanyi, Marcel Dekker, Inc., New York, pp. 375–386, 1992b.

RUBANYI G. M.: The role of endothelium in cardiovascular homeostasis and diseases. J. Cardiovasc Pharmacol. **22** (Suppl. 4): S1–S14, 1993.

RUBANYI, G. M., AND PARKER-BOTELHO, L. H.: Endothelins. FASEB J. **5:** 2713–2720, 1991.

RUBANYI, G. M., AND SHEPHERD J. T.: Hypothetical role of endothelin in the control of the cardiovascular system. *In* Endothelin, edited by G. M. Rubanyi, Oxford University Press, Oxford, UK, pp. 258–271, 1992.

RUBANYI G. M., AND VANHOUTTE P. M.: Hypoxia releases a vasoconstrictor substance from the canine vascular endothelium. J. Physiol. (Lond.) **364:** 45–56, 1986.

RUBANYI G. M., AND VANHOUTTE P. M. (EDS.): Endothelium-derived Relaxing Factors. Karger, Basel, Switzerland, pp. 1–237, 1992.

RYAN U. S., AND RUBANYI G. M. (EDS.): Endothelial Regulation of Vascular Tone, Marcel Dekker, Inc., New York, pp. 1–396, 1992.

SAEKI, T., IHARA, M., FUKURODA, T., YAMAGIWA, M., AND YANO, M.: Endothelin-1 analogs with ETB agonistic activity. Biochem. Biophys. Res. Comm. **179:** 286–292, 1991.

SAENZ DE TEJADA, I., CARSON, M. P., DE, LAS MORENAS A., GOLDSTEIN, I., AND TRAISH, A. M.: Endothelin: localization, synthesis, activity, and receptor types in human penile corpus cavernosum. Am. J. Physiol. **261:** H1078–H1085, 1991.

SAIDA, K., MITSUI, Y., AND ISHIDA, N.: A novel peptide, vasoactive intestinal contractor, of a new (endothelin) peptide family. Molecular cloning, expression, and biological activity. J. Biol. Chem. **264:** 14613–14616, 1989.

SALJONMAA, O., RISTIMAKI, A., AND FYHRQUIST, F.: Atrial natriuretic peptide, nitroglycerine, and nitroprusside reduce basal and stimulated endothelin production from cultured endothelial cells. Biochem. Biophys. Res. Commun. **173:** 514–520, 1990.

SAITO, T., FUSHIMI, E., ABE, T., KIMURA, Y., TAKAHASHI, K., KUDO, Y., NAKAGOMI, A., AND MIURA, M.: Augmented contractile response to endothelin and blunted endothelium dependent relaxation in post ischemic reperfused coronary arteries. Jpn. Circ. J. **56:** 657–670, 1992.

SAITO, Y., MIZUNO, T., ITAKURA, M., SUZUKI, Y., ITO, T., HAGIWARA, H., AND HIROSE, S.: Primary structure of bovine endothelin ETB receptor and identification of signal peptidase and metal proteinase cleavage sites. J. Biol. Chem. **266:** 23433–23437, 1991.

SAITO, Y., NAKAO, K., SHIRAKAMI, G., JOUGASAKI, M., YAMADA, T., ITOH, H., MUKOYAMA, M., ARAI, H., HOSODA, K., SUGA, S., ET AL.: Detection and characterization of endothelin 1 like immunoreactivity in rat plasma. Biochem. Biophys. Res. Commun. **163:** 1512–1516, 1989.

SAKAMOTO, A., YANAGISAWA, M., SAKURAI, T., TAKUWA, Y., YANAGISAWA, H., AND MASAKI, T.: Cloning and functional expression of human cDNA for the ETB endothelin receptor. Biochem. Biophys. Res. Commun. **178:** 656–663, 1991.

SAKAMOTO, A., YANAGISAWA, M., SAWAMURA, T., ENOKI, T., OHTANI, T., SAKURAI, T., NAKAO, K., TOYO-OKA, T., AND MASAKI, T.: Distinct subdomains of human endothelin receptors determine their selectivity to endothelin A selective antagonist and endothelin B selective agonists. J. Biol. Chem. **268:** 8547–8553, 1993.

SAKAMOTO, H., SASAKI, S., HIRATA, Y., IMAI, T., ANDO, K., IDA, T., SAKURAI, T., YANAGISAWA, M., MASAKI, T., AND MARUMO, F.: Production of endothelin 1 by rat cultured mesangial cells. Biochem. Biophys. Res. Commun. **169:** 462–468, 1990.

SAKAMOTO, H., SASAKI, S., NAKAMURA, Y., FUSHIMI, K., AND MARUMO, F.: Regulation of endothelin 1 production in cultured rat mesangial cells. Kidney Int. **41:** 350–355, 1992.

SAKATA, K., AND KARAKI, H.: Effects of endothelin on cytosolic Ca^{2+} level and mechanical activity in rat uterine smooth muscle. Eur. J. Pharmacol. **221:** 9–15 1992.

SAKATA, K., OZAKI, H., KWON, S. C., AND KARAKI, H.: Effects of endothelin on the mechanical activity and cytosolic calcium level of various types of smooth muscle. Br. J. Pharmacol. **98:** 483–492, 1989.

SAKURAI, T., YANAGISAWA, M., INOUE, A., RYAN, U. S., KIMURA, S., MITSUI, Y., GOTO, K., AND MASAKI, T.: cDNA cloning, sequence analysis and tissue distribution of rat preproendothelin 1 mRNA. Biochem. Biophys. Res. Commun. **175:** 44–47, 1991.

SAKURAI, T., YANAGISAWA, M., AND MASAKI, T.: Molecular characterization of endothelin receptors. Trends Pharmacol, Sci. **13:** 103–108, 1992b.

SAKURAI, T., YANAGISAWA, M., TAKUWA, Y., MIYAZAKI, H., KIMURA, S., GOTO, K., AND MASAKI, T.: Cloning of a cDNA encoding a non isopeptide selective subtype of the endothelin receptor. Nature (Lond.) **348:** 732–735, 1990.

SALAMONSEN, L. A., BUTT, A. R., MACPHERSON, A. M., ROGERS, P. A., AND FINDLAY, J. K.: Immunolocalisation of the vasoconstrictor endothelin in human endometrium during the menstrual cycle and in umbilical cord at birth. Am. J. Obstet. Gynecol. **167:** 163–167, 1992.

SALVATI, P., CHIERCHIA, S., DHO, L., FERRARIO, R. G., PARENTI, P., VICEDOMINI, G., AND PATRONO C.: Proarrhythmic activity of intracoronary endothelin in dogs: relation to the site of administration and to changes in regional flow. J. Cardiovasc. Pharmacol. **17:** 1007–1014, 1991.

SAMSON, W. K., SKALA, K. D., ALEXANDER, B. D., AND HUANG, F. L.: Hypothalamic endothelin: presence and effects related to fluid and electrolyte homeostasis. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S346–S349, 1991a.

SAMSON, W. K., SKALA, K. D., ALEXANDER, B., AND HUANG, F. L.: Possible neuroendocrine actions of endothelin 3. Endocrinology **128:** 1465–1473, 1991b.

SAMSON, W. K., SKALA, K., HUANG, F. L., GLUNTZ, S., ALEXANDER, B., AND GOMEZ SANCHEZ, C. E.: Central nervous system action of endothelin 3 to inhibit water drinking in the rat. Brain Res. **539:** 347–351, 1991c.

SAMUELS, P., STEINFELD, J. D., BRAITMAN, L. E., RHOA, M. F., CINES, D. B., AND MCCRAE, K. R.: Plasma concentration of endothelin 1 in women with cocaine associated pregnancy complications. Am. J. Obstet. Gynecol. **168:** 528–533, 1993.

SARRIA, B., NALINE, E., MORCILLO, E., CORTIJO, J., ESPLUGUES, J., AND ADVENIER, C.: Calcium dependence of the contraction produced by endothelin (ET1) in isolated guinea pig trachea. Eur. J. Pharmacol. **187:** 445–453, 1990.

SASAKI, T., AND HONG, M. H.: Localization of endothelin-1 in the osteoclast. J Electron Microsc. **42:** 193–196, 1993.

SATOH, M., SHIRMURA, S., ISHIHARA, H., NAGAKI, M., SASAKI, H., AND TAKISH-

IMIA, T.: Endothelin 1 stimulates chloride secretion across canine tracheal epithelium. Respiration **59:** 145–150, 1992.

SAUDEK, V., HOFLACK, J., AND PELTON, J. T.: 1H NMR study of endothelin, sequence specific assignment of the spectrum and a solution structure. FEBS Lett. **257:** 145–148, 1989.

SAUDEK, V., HOFLACK, J., AND PELTON, J. T.: Solution conformation of endothelin 1 by 1H NMR, CD, and molecular modeling. Int. J. Pept. Protein Res. **37:** 174–179, 1991.

SAUDOU, F., AMLAIKY, N., PLASSAT, J. L., BORELLI, E., AND HEN, R.: Cloning and characterization of a *Drosophila* tyramine receptor. EMBO J. **9:** 3611–3617, 1990.

SAWAMURA, T., KASUYA, Y., MATSUSHITA, Y., SUZUKI, N., SHINMI, O., KISHI, N., SUGITA, Y., YANAGISAWA, M., GOTO, K., MASAKI, T., ET AL.: Phosphoramidon inhibits the intracellular conversion of big endothelin 1 to endothelin 1 in cultured endothelial cells. Biochem. Biophys. Res. Commun. **174:** 779–784, 1991.

SAWAMURA, T., KIMURA, S., SHINMI, O., SUGITA, Y., KOBAYASHI, M., MITSUI, Y., YANAGISAWA, M., GOTO, K., AND MASAKI, T.: Characterization of endothelin converting enzyme activities in soluble fraction of bovine cultured endothelial cells. Biochem. Biophys. Res. Commun. **169:** 1138–1144, 1990a.

SAWAMURA, T., KIMURA, S., SHINMI, O., SUGITA, Y., YANAGISAWA, M., GOTO, K., AND MASAKI, T.: Purification and characterization of putative endothelin converting enzyme in bovine adrenal medulla: evidence for a cathepsin D like enzyme. Biochem. Biophys. Res. Commun. **168:** 1230–1236, 1990b.

SCHALK, K. A., FARACI, F. M., AND HEISTAD, D. D.: Effect of endothelin on production of cerebrospinal fluid in rabbits. Stroke **23:** 560–563, 1992.

SCHIEBINGER, R. J., AND GOMEZ SANCHEZ, C. E.: Endothelin: a potent stimulus of atrial natriuretic peptide secretion by superfused rat atria and its dependency on calcium. Endocrinology **127:** 119–125, 1990.

SCHIFF, E., BEN BARUCH, G., GALRON, R., MASHIACH, S., AND SOKOLOVSKY, M.: Endothelin 1 receptors in the human myometrium: evidence for different binding properties in postmenopausal as compared to premenopausal and pregnant women. Clin. Endocrinol. **38:** 321–324, 1993.

SCHIFF, E., BEN BARUCH, G., PELEG, E. ROSENTHAL, T., ALCALAY, M., DEVIR, M., AND MASHIACH, S.: Immunoreactive circulating endothelin 1 in normal and hypertensive pregnancies. Am. J. Obstet. Gynecol. **166:** 624–628, 1992.

SCHIFFRIN, E. L., AND THIBAULT, G.: Plasma endothelin in human essential hypertension. Am. J. Hypertens. **4:** 303–308, 1991.

SCHINI, V. B., KIM, N. D., AND VANHOUTTE, P. M.: The basal and stimulated release of EDRF inhibits the contractions evoked by endothelin 1 and endothelin 3 in aortae of normotensive and spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. **17** (Suppl. 7)**:** S267–S271, 1991.

SCHINI, V. B., AND VANHOUTTE, P. M.: Endothelin 1: a potent vasoactive peptide. Pharmacol Toxicol. **69:** 303–309, 1991.

SCHULTZ, B. G., AND MULLER, B.: Iloprost antagonizes endothelin induced vasoconstriction in macro and microcirculation. Eicosanoids **3:** 135–138, 1990.

SCHUMACHER, W. A., STEINBACHER, T. E., ALLEN, G. T., AND OGLETREE, M. L.: Role of thromboxane receptor activation in the bronchospastic response to endothelin. Prostaglandins **40:** 71–79, 1990.

SCHVARTZ, I., ITTOOP, O., DAVIDAI, G., AND HAZUM, E.: Endothelin rapidly stimulates tyrosine phosphorylation in osteoblast like cells. Peptides (Elmsford) **13:** 159–163, 1992.

SCOTT-BURDEN, T., RESINK, T. J., HAHN, A. W., AND VANHOUTTE, P. M.: Induction of endothelin secretion by angiotensin II: effects on growth and synthetic activity of vascular smooth muscle cells. J. Cardiovasc. Pharmacol. **17** (Suppl. 7)**:** S96–S100, 1991.

SECREST, R. J., AND COHEN, M. L.: Endothelin: differential effects in vascular and nonvascular smooth muscle. Life Sci. **45:** 1365–1372, 1989.

SERRADEIL LE GAL, C., HERBERT, J. M., GARCIA, C., BOUTIN, M., AND MAFFRAND, J. P.: Importance of the phenotypic state of vascular smooth muscle cells on the binding and the mitogenic activity of endothelin. Peptides (Elmsford) **12:** 575–579, 1991a.

SERRADEIL LE GAL, C., JOUNEAUX, C., SANCHEZ BUENO, A., RAUFASTE, D., ROCHE, B., PREAUX, A. M., MAFFRAND, J. P., COBBOLD, P. H., HANOUNE, J., AND LOTERSZTAJN, S.: Endothelin action in rat liver. Receptors, free Ca^{2+} oscillations, and activation of glycogenolysis. J. Clin. Invest. **87:** 133–138, 1991b.

SESSA, W. C., KAW, S., HECKER, M., AND VANE, J. R.: The biosynthesis of endothelin 1 by human polymorphonuclear leukocytes. Biochem. Biophys. Res. Commun. **174:** 613–618, 1991a.

SESSA, W. C., KAW, S., ZEMBOWICZ, A., ANGGARD, E., HECKER, M., AND VANE, J. R.: Human polymorphonuclear leukocytes generate and degrade endothelin 1 by two distinct neutral proteases. J. Cardiovasc. Pharmacol. **17** (Suppl. 7)**:** S34–S38, 1991b.

SHAH, A. M., LEWIS, M. J., AND HENDERSON, A. H.: Inotropic effects of endothelin in ferret ventricular myocardium. Eur. J. Pharmacol. **63:** 365–367 1989.

SHAREFKIN, J. B., DIAMOND, S. L., ESKIN, S.G., MCINTIRE, L. V., AND DIEFFENBACH, C. W.: Fluid flow decreases preproendothelin mRNA levels and suppresses endothelin 1 peptide release in cultured human endothelial cells. J. Vasc. Surg. **14:** 1–9, 1991.

SHETTY, S. S., OKADA, T., WEBB, R. L., DELGRANDE, D., AND LAPPE, R. W.: Functionally distinct endothelin B receptors in vascular endothelium and smooth muscle. Biochem. Biophys. Res. Commun. **191:** 459–464, 1993.

SHIBA, R., SAKURAI, T., YAMADA, G., MORIMOTO, H., SAITO, A., MASAKI, T., AND GOTO, K.: Cloning and expression of rat preproendothelin 3 cDNA. Biochem. Biophys. Res. Commun. **186:** 588–594, 1992.

SHIBA, R., YANAGISAWA, M., MIYAUCHI, T., ISHII, Y., KIMURA, S., UCHIYAMA, Y., MASAKI, T., AND GOTO, K.: Elimination of intravenously injected endothelin 1 from the circulation of the rat. J. Cardiovasc. Pharmacol. **13** (Suppl. 5)**:** S98–S101 (discussion S102), 1989.

SHIBOUTA, Y., SUZUKI, N., SHINO, A., MATSUMOTO, H., TERASHITA, Z., KONDO, K., AND NISHIKAWA, K.: Pathophysiological role of endothelin in acute renal failure. Life Sci. **46:** 1611–1618, 1990.

SHICHIRI, M., HIRATA, Y., KANNO, K., OHTA, K., EMORI, T., AND MARUMO, F.: Effect of endothelin 1 on release of arginine vasopresain from perifused rat hypothalamus. Biochem. Biophys. Res. Commun. **163:** 1332–1337, 1989.

SHICHIRI, M., HIRATA, Y., ANDO, K., KANNO, K., EMORI, T., OHTA, K., AND MARUMO, F.: Postural change and volume expansion affect plasma endothelin levels. JAMA **263:** 661, 1990.

SHICHIRI, M., HIRATA, Y., AND MARUMO, F.: Endothelin 1 as an autocrine/paracrine factor for human tumor cell lines. J. Cardiovasc. Pharmacol. **17** (Suppl. 7)**:** S76–S78, 1991a.

SHICHIRI, M., HIRATA, Y., NAKAJIMA, T., ANDO, K., IMAI, T., YANAGISAWA, M., MASAKI, T., AND MARUMO, F.: Endothelin 1 is an autocrine/paracrine growth factor for human cancer cell lines. J. Clin. Invest. **87:** 1867–1871, 1991b.

SHIGENO, T., AND MIMA, T: A new vasoconstrictor peptide, endothelin: profiles as vasoconstrictor and neuropeptide. Cerebrovasc. Brain Metab. Rev. **2:** 227–239, 1990.

SHIMAMOTO, N., KUBO, K., SUZUKI, N., ABE, M., WAKIMASU, M., AND FUJINO, M.: Pharmacological profile of endothelin A/B receptor antagonist, [Thr18, gamma methyl Leu19] endothelin-1. Abstracts of the 3rd International Conference on Endothelin, p. 115, 1993. Houston, TX. February 15–17, 1993.

SHIMURA, S., ISHIHARA, H., SATOH, M., MASUDA, T., NAGAKI, N., SASAKI, H., AND TAKISHIMA, T.: Endothelin regulation of mucus glycoprotein secretion from feline tracheal submucosal glands. Am. J. Physiol. **262:** L208–L213, 1992.

SHINMI, O., KIMURA, S., SAWAMURA, T., SUGITA, Y., YOSHIZAWA, T., UCHIYAMA, Y., YANAGISAW A. M., GOTO, K., MASAKI, T., AND KANAZAWA, I.: Endothelin 3 is a novel neuropeptide: isolation and sequence determination of endothelin 1 and endothelin 3 in porcine brain. Biochem. Biophys. Res. Commun. **164:** 587–593, 1989a.

SHINMI, O., KIMURA, S., YOSHIZAWA, T., SAWAMURA, T., UCHIYAMA, Y., SUGITA, Y., KANAZAWA, I., YANAGISAWA, M., GOTO, K., AND MASAKI, T.: Presence of endothelin 1 in porcine spinal cord: isolation and sequence determination. Biochem. Biophys. Res. Commun. **162:** 340–346, 1989b.

SHINYAMA, H., UCHIDA, T., KIDO, H., HAYASHI, K., WATANABE, M., MATSUMURA, Y., IKEGAWA, R. TAKAOKA, M., AND MORIMOTO, S.: Phosphoramidon inhibits the conversion of intracisternally administered big endothelin 1 to endothelin 1. Biochem. Biophys. Res. Commun. **178:** 24–30, 1991.

SHIRAKAMI, G., NAKAO, K., SAITO, Y., MAGARIBUCHI, T., JOUGASAKI, M., MUKOYAMA, M., ARAI, H., HOSODA, K., SUGA, S., OGAWA, Y., ET AL.: Acute pulmonary alveolar hypoxia increases lung and plasma endothelin 1 levels in conscious rats. Life Sci. **48:** 969–976, 1991.

SHIRAKAMI, G., NAKAO, K., SAITO, Y., MAGARIBUCHI, T., MUKOYAMA, M., ARAI, H., HOSODA, K., SUGA, S., MORI, K., AND IMURA, H.: Low doses of endothelin 1 inhibit atrial natriuretic peptide secretion. Endocrinology **132:** 1905–1912, 1993.

SHUBEITA, H. E., MCDONOUGH, P. M., HARRIS, A. N., KNOWLTON, K. U., GLEMBOTSKI, C. C., BROWN, J. H. AND CHIEN, K. R.: Endothelin induction of inositol phospholipid hydrolysis, sarcomere assembly, and cardiac gene expression in ventricular myocytes. A paracrine mechanism for myocardial cell hypertrophy. J. Biol. Chem. **265:** 20555–20562, 1990.

SHULTZ, P. J.: An emerging role for endothelin in renal disease (editorial; comment). J. Lab. Clin. Med. **119:** 448–449, 1992.

SILBERBERG, S. D., PODER, T. C., LACERDA, A. E.: Endothelin increases single channel calcium currents in coronary arterial smooth muscle cells. FEBS Lett. **247:** 68–72, 1989.

SIMONSON, M. S.: Endothelins: multifunctional renal peptides. Physiol. Rev. **73:** 375–411, 1993.

SIMONSON, M. S., AND DUNN, M. J.: Cellular signaling by peptides of the endothelin gene family. FASEB J. **4:** 2989–3000, 1990a.

SIMONSON, M. S., AND DUNN, M. J.: Endothelin 1 stimulates contraction of rat glomerular mesangial cells and potentiates beta adrenergic mediated cyclic adenosine monophosphate accumulation. J. Clin. Invest. **85:** 790–797, 1990b.

SIMONSON, M. S., JONES, J. M., AND DUNN, M. J.: Cytosolic and nuclear signaling by endothelin peptides: mesangial response to glomerular injury. Kidney Int. **41:** 542–545, 1992a.

SIMONSON, M. S., JONES, J. M., AND DUNN, M. J.: Differential regulation of *fos* and *jun* gene expression and AP 1 *cis* element activity by endothelin isopeptides. Possible implications for mitogenic signaling by endothelin. J. Biol. Chem. **267:** 8643–8649, 1992b.

SIMONSON, M. S., KESTER, M., BALDI, E., OSANAI, T., THOMAS, C. P., MENE, P., AND DUNN, M. J.: Endothelins: renal and cardiovascular actions. Adv. Nephrol. Necker. Hosp. **21:** 177–194, 1992c.

SIMONSON, M. S., WANN, S., MENE, P., DUBYAK, G. R., KESTER, M., AND DUNN, M. J.: Endothelin 1 activates the phosphoinositide cascade in cultured glomerular mesangial cells. J. Cardiovasc. Pharmacol. **13** (Suppl. 5)**:** S80–S83, 1989.

SIMPSON, A. W., AND ASHLEY, C. C.: Endothelin evoked Ca^{2+} transients and

oscillations in A1 vascular smooth muscle cells. Biochem. Biophys. Res. Commun. **163:** 1223–1229, 1989.

SIREN, A. L., AND FEURERSTEIN, G.: Hemodynamic effects of endothelin after systemic and central nervous system administration in the conscious rat. Neuropeptides **14:** 231–236, 1989.

SIRVIO, M. L., METSARINNE, K., SALJONMAA, O., AND FYHRQUIST, F.: Tissue distribution and half life of ^{125}I endothelin in the rat: importance of pulmonary clearance. Biochem. Biophys. Res. Commun. **167:** 1191–1195, 1990.

SOKOLOVSKY, M.: Endothelins and sarafotoxins: physiological regulation, receptor subtypes and transmembrane signaling. Trends Biochem. Sci. **16:** 261–264, 1991.

SOKOLOVSKY, M.: Endothelins and sarafotoxins: physiological regulation, receptor subtypes and transmembrane signaling. Pharmacol. Ther. **54:** 129–149, 1992a.

SOKOLOVSKY, M.: Structure function relationships of endothelins, sarafotoxins, and their receptor subtypes. J. Neurochem. **59:** 809–821, 1992b.

SOKOLOVSKY, M., AMBAR, I., AND GALRON, R.: A novel subtype of endothelin receptors. J. Biol. Chem. **267:** 20551–20554, 1992.

SOKOLOVSKY, M., GALRON, R., KLOOG, Y., BDOLAH, A., INDIG, F. E., BLUMBERG, S., AND FLEMINGER, G.: Endothelins are more sensitive than sarafotoxins to neutral endopeptidase: possible physiological significance. Proc. Natl. Acad. Sci. USA **87:** 4702–4706, 1990.

SONE, M., TOTSUNE, K., TAKAHASHI, K., OHNEDA, M., ITOI, K., MURAKAMI, O., MIURA, Y., MOURI, T., AND YOSHINAGA, K.: Immunoreactive endothelin in pheochromocytomas. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S427–S429, 1991.

SPINELLA, M. J., KRYSTEK, S. R., JR., PEAPUS, D. H., WALLACE, B. A., BRUNER, C., AND ANDERSEN, T. T.: A proposed structural model of endothelin. Pept. Res. **2:** 286–291, 1989.

SPOKES, R. A., GHATEI, M. A., AND BLOOM, S. R.: Studies with endothelin 3 and endothelin 1 on rat blood pressure and isolated tissues: evidence for multiple endothelin receptor subtypes. J. Cardiovasc. Pharmacol. **13** (Suppl. 5): S191–S192, 1989.

SPRINGALL, D. R., HOWARTH, P. H., COUNIHAN, H., DJUKANOVIC, R., HOLGATE, S. T., AND POLAK, J. M.: Endothelin immunoreactivity of airway epithelium in asthmatic patients. Lancet **337:** 697–701, 1991.

STASCH, J. P., HIRTH, DIETRICH C., KAZDA, S., AND NEUSER, D.: Endothelin stimulates release of atrial natriuretic peptides in vitro and in vivo. Life Sci. **45:** 869–875, 1989.

STASCH, J. P., AND KAZDA, S.: Endothelin 1 induced vascular contractions: interactions with drugs affecting the calcium channel. J. Cardiovasc. Pharmacol. **13** (Suppl. 5): S63–S66, 1989.

STEFFAN, M., AND RUSSELL, J. A.: Signal transduction in endothelin induced contraction of rabbit pulmonary vein. Pulm. Pharmacol. **3:** 1–7, 1990.

STELZNER, T. J., O'BRIEN, R. F., YANAGISAWA, M., SAKURAI, T., SATO, K., WEBB, S., ZAMORA, M., MCMURTRY, I. F. AND FISHER, J. H.: Increased lung endothelin 1 production in rats with idiopathic pulmonary hypertension. Am. J. Physiol. **262:** 614–620, 1992.

STEWART, D. J.: Role of EDRF and endothelin in coronary vasomotor control. Basic Res. Cardiol. **86** (Suppl. 2): 77–87, 1991.

STEWART, D. J., CERNACEK, P., COSTELLO, K. B., AND ROULEAU, J. L.: Elevated endothelin 1 in heart failure and loss of normal response to postural change. Circulation **85:** 510–517, 1992.

STEWART, D. J., KUBAC, G., COSTELLO, K. B., AND CERNACEK, P.: Increased plasma endothelin 1 in the early hours of acute myocardial infarction. J. Am. Coll. Cardiol. **18:** 38–43, 1991a.

STEWART, D. J., LANGLEBEN, D., CERNACEK, P., AND CIANFLONE, K.: Endothelin release is inhibited by coculture of endothelial cells with cells of vascular media. Am. J. Physiol. **259:** 1928–1932, 1990.

STEWART, D. J., LEVY, R. D., CERNACEK, P., AND LANGLEBEN, D.: Increased plasma endothelin 1 in pulmonary hypertension: marker or mediator of disease? Ann. Intern. Med. **114:** 464–469, 1991b.

STEWART, J. T., NISBET, J. A., AND DAVIES, M. J.: Plasma endothelin in coronary venous blood from patients with either stable or unstable angina. Br Heart J. **66:** 7–9, 1991c.

STIER, C. T., JR., QUILLEY, C. P., AND MCGIFF, J. C.: Endothelin 3 effects on renal function and prostanoid release in the rat isolated kidney. J. Pharmacol. Exp. Ther. **262:** 252–256, 1992.

STOJILKOVIC, S. S., MERELLI, F., IIDA, T., KRSMANOVIC, L. Z., AND CATT, K. J.: Endothelin stimulation of cytosolic calcium and gonadotropin secretion in anterior pituitary cells. Science (Wash. DC). **248:** 1663–1666, 1990.

SUGDEN, P. H., FULLER, S. J., MYNETT, J. R., AND HATCHETT, R. J.: 4th; Bogoyevitch, M. A., Sugden, M. C.: Stimulation of adult rat ventricular myocyte protein synthesis and phosphoinositide hydrolysis by the endothelins. Biochim. Biophys. Acta **1175:** 327–332, 1993.

SUGIURA, M., INAGAMI, T., HARE, G. M. AND JOHNS, J. A.: Endothelin action: Inhibition by a protein kinase C inhibitor and involvement of phosphoinositols. Biochem. Biophys. Res. Commun. **158:** 170–176, 1989.

SUGIURA, M., INAGAMI, T., AND KON, V.: Endotoxin stimulates endothelin release in vivo and in vitro as determined by radioimmunoassay. Biochem. Biophys. Res. Commun. **161:** 1220–1227, 1989.

SUNAKO, M., KAWAHARA, Y., HIRATA, K., TSUDA, T., YOKOYAMA, M., FUKUZAKI, H., AND TAKAI, Y.: Mass analysis of 1,2 diacylglycerol in cultured rabbit vascular smooth muscle cells. Comparison of stimulation by angiotensin II and endothelin. Hypertension **15:** 84–88, 1990.

SUNAKO, M., KAWAHARA, Y., KARIYA, K., ARAKI, S., FUKUZAKI, H., AND TAKAI, Y.: Endothelin induced biphasic formation of 1,2 diacylglycerol in cultured rabbit vascular smooth muscle cells mass analysis with a radioenzymatic assay. Biochem. Biophys. Res. Commun. **160:** 744–750, 1989.

SUPATTAPONE, S., SIMPSON, A. W., AND ASHLEY, C. C.: Free calcium rise and mitogenesis in glial cells caused by endothelin. Biochem. Biophys. Res. Commun. **165:** 1115–1122, 1989.

SUZUKI, E., HIRATA, Y., MATSUOKA, H., SUGIMOTO, T., HAYAKAWA, H., SHIN, W. S., TOYOOKA, T., AND SUGIMOTO, T.: Effects of atrial natriuretic peptide on endothelin induced vasoconstriction and intracellular calcium mobilization. J. Hypertens. **9:** 927–934, 1991a.

SUZUKI, H., SATO, S., SUZUKI, Y., OKA, M., TSUCHIYA, T., IINO, I., YAMANAKA, T., ISHIHARA, N., AND SHIMODA, S.: Endothelin immunoreactivity in cerebrospinal fluid of patients with subarachnoid haemorrhage. Ann. Med. **22:** 233–236, 1990a.

SUZUKI, H., SATO, S., SUZUKI, Y., TAKEKOSHI, K., ISHIHARA, N., AND SHIMODA, S.: Increased endothelin concentration in CSF from patients with subarachnoid hemorrhage. Acta Neurol. Scand. **81:** 553–554, 1990b.

SUZUKI, N., MIYAUCHI, T., TOMOBE, Y., MATSUMOTO, H., GOTO, K., MASAKI, T., AND FUJINO, M.: Plasma concentrations of endothelin 1 in spontaneously hypertensive rats and DOC artery. Circ. Res. **69:** 1361–1368, 1991b.

SUZUKI, R., MASAOKA, H., HIRATA, Y., MARUMO, F., ISOTANI, E., AND HIRAKAWA, K.: The role of endothelin 1 in the origin of cerebral vasospasm in patients with aneurysmal subarachnoid hemorrhage. J. Neurosurg. **77:** 96–100, 1992a.

SUZUKI, T., KUMAZAKI, T., AND MITSUI, Y.: Endothelin 1 is produced and secreted by neonatal rat cardiac myocytes in vitro. Biochem. Biophys. Res. Commun. **191:** 823–830, 1993.

SUZUKI, T., TOYO-OKA, T., SHIN, W. S., AND SUGIMOTO, T.: Cell growth dependent expression of endothelin 1 provocable Ca^{2+} channels in cloned vascular smooth muscle cells. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S187–S189, 1991c.

SUZUKI, Y.: Properties of endothelin induced contractions in the rabbit non pregnant and pregnant myometria. Fukushima J. Med. Sci. **36:** 29–40, 1990.

SVANE, D., LARSSON, B., ALM, P., ANDERSSON, K. E., AND FORMAN, A.: Endothelin 1: immunocytochemistry, localization of binding sites, and contractile effects in human uteroplacental smooth muscle. Am. J. Obstet. Gynecol. **168:** 233–241, 1993.

TABUCHI, Y., NAKAMARU, M., RAKUGI, H., NAGANO, M., HIGASHIMORI, K., MIKAMI, H., AND OGIHARA, T.: Effects of endothelin on neuroeffector junction in mesenteric arteries of hypertensive rats. Hypertension **15:** 739–743, 1990.

TABUCHI, Y., NAKAMARU, M., RAKUGI, H., NAGANO, M., MIKAMI, H., AND OGIHARA, T.: Endothelin inhibits presynaptic adrenergic neurotransmission in rat mesenteric artery. Biochem. Biophys. Res. Commun. **161:** 803–808, 1989.

TAHARA, A., KOHNO, M., YANAGI, S., ITAGANE, H., TODA, I., AKIOKA, I., TERAGAKI, M., YASUDA M., TAKEUCHI, K., AND TAKEDA, T.: Circulating immunoreactive endothelin in patients undergoing percutaneous transluminal coronary angioplasty. Metabolism **40:** 1235–1237, 1991.

TAKABATAKE, T., ISE, T., OHTA, K., AND KOBAYASHI, K.: Endothelin effects on renal function and tubuloglomerular feedback. Kidney Int. **32** (Suppl.): S122–S124, 1991.

TAKAGI, H., HISA, H., AND SATOH, S.: Effects of endothelin on adrenergic neurotransmission in the dog kidney. Eur. J. Pharmacol. **203:** 291–294, 1991a.

TAKAGI, M., MATSUOKA, H., ATARASHI, K., AND YAGI, S.: Endothelin: a new inhibitor of renin release. Biochem. Biophys. Res. Commun. **157:** 1164–1168, 1988.

TAKAGI, M., TSUKADA, H., MATSUOKA, H., AND YAGI, S.: Inhibitory effect of endothelin on renin release in vitro. Am. J. Physiol. **257:** E833–E838, 1989.

TAKAGI, Y., FUKASE, M., TAKATA, S., KAWAKAMI, M., MASUI, M., UEDA, M., AND FUJITA, T.: Role of endogenous endothelin in the development of hypertension in rats. Am. J. Hypertens. **4:** 389–391, 1991b.

TAKAHARA, S., MOUTABARRIK, A., ISHIBASHI, M., FUKUNAGA, M., KAMEOKA, H., TAKANO, Y., KOKAGO, Y., JIANG, H., SONODA, T., AND OKUYAMA, A.: FK 506 mechanism of nephrotoxicity: stimulatory effect on endothelin secretion by cultured kidney cells and tubular cell toxicity in vitro. Transplant Proc. 23: 3133–3136, 1991.

TAKAHASHI, K., BROOKS, R. A., KANSE, S. M., GHATEI, M. A., KOHNER, E. M., AND BLOOM, S. R.: Production of endothelin 1 by cultured bovine retinal endothelial cells and presence of endothelin receptors on associated pericytes. Diabetes **38:** 1200–1202, 1989.

TAKAHASHI, K., GHATEI, M. A., LAM, H. C., O'HALLORAN, D. J., AND BLOOM, S. R.: Elevated plasma endothelin in patients with diabetes mellitus. Diabetologia **33:** 306–310, 1990a.

TAKAHASHI, K., SUDA, K., LAM, H. C., GHATEI, M. A., AND BLOOM, S. R.: Endothelin like immunoreactivity in rat models of diabetes mellitus. J. Endocrinol. **130:** 123–127, 1991c.

TAKAHASHI, K., TOTSUNE, K., IMAI, Y., SONE, M., NOZUKI, M., MURAKAMI, O., SEKINO, H., AND MOURI, T.: Plasma concentrations of immunoreactive endothelin in patients with chronic renal failure treated with recombinant human erythropoietin. Clin. Sci. **84:** 47–50, 1993a.

TAKAHASHI, M., MATSUSHITA, Y., IIJIMA, Y., AND TANZAWA, K.: Purification and characterization of endothelin-converting enzyme from rat lung. J. Biol. Chem. **268:** 21394–21398, 1993b.

TAKAI, M., UMEMURA, I., YAMASAKI, K., WATAKABE, T., FUJITANI, Y., ODA, K.,

URADE, Y., INUI, T., YAMAMURA, T., AND OKADA, T.: A potent and specific agonist, Suc-[Glu[9], Ala[11], [15]]- endothelin-1 (8–21), iRL 1620, for the ETB receptor. Biochem. Biophys. Res. Commun. **184:** 953–959, 1992.

TAKAORI, K., INOUE, K., KOGIRE, M., HIGASHIDE, S., TUN, T., AUNG, T., DOI, R., FUJII, N., AND TOBE, T.: Effects of endothelin on microcirculation of the pancreas. Life Sci. **51:** 615–622, 1992.

TAKASAKI, C., ITOH, Y., ONDA, H., AND FUJINO, M.: Cloning and sequence analysis of a snake, Atractaspis engaddensis gene encoding sarafotoxin S6c. Biochem. Biophys. Res. Commun. **189:** 1527–1533, 1992.

TAKASUKA, T., AKIYAMA, N., HORII, I., FURUICHI, Y., AND WATANABE, T.: Different stability of ligand receptor complex formed with two endothelin receptor species, ETA and ETB. J. Biochem. (Tokyo) **111:** 748–753, 1992.

TAKAYAMA, K., NAGAI, T., KINUGASA, E., AKIZAWA, T., AND KOSHIKAWA, S.: Changes in endothelial vasoactive substances under recombinant human erythropoietin therapy in hemodialysis patients. ASAIO-Trans. **37:** M187–188, 1991.

TAKAYANAGI, R., KITAZUMI, K., TAKASAKI, C., OHNAKA, K., AIMOTO, S., TASAKA, K., OHASHI, M., AND NAWATA, H.: Presence of non selective type of endothelin receptor on vascular endothelium and its linkage to vasodilation. FEBS Lett. **282:** 103–106, 1991.

TAKAYASU, M., KONDO, K., AND TERAO, S.: Endothelin induced mobilization of Ca^{2+} and the possible involvement of platelet activating factor and thromboxane A2. Biochem. Biophys. Res. Commun. **160:** 751–757, 1989.

TAKEDA, Y., ITOH, Y., YONEDA, T., MIYAMORI, I., AND TAKEDA, R.: Cyclosporine A induces endothelin 1 release from cultured rat vascular smooth muscle cells. Eur. J. Pharmacol. **233:** 299–301, 1993.

TAKEDA, Y., MIYAMORI, I., YONEDA, T., AND TAKEDA, R.: Production of endothelin 1 from the mesenteric arteries of streptozotocin induced diabetic rats. Life Sci. **48:** 2553–2556, 1991.

TAKENAKA, T., EPSTEIN, M., FORSTER, H., LANDRY, D. W., IIJIMA, K., AND GOLIGORSKY, M. S.: Attenuation of endothelin effects by a chloride channel inhibitor, indanyloxyacetic acid. Am. J. Physiol. **262:** F799–F806, 1992.

TAKESHITA, H., NISHIKIBE, M., YANO, M., AND IKEMOTO, F.: Coronary vascular response to endothelin in isolated perfused hearts of spontaneously hypertensive rats. Clin. Exp. Pharmacol. Physiol. **18:** 661–669, 1991.

TAKUWA, N., TAKUWA, Y., YANAGISAWA, M., YAMASHITA, K., AND MASAKI, T.: A novel vasoactive peptide endothelin stimulates mitogenesis through inositol lipid turnover in Swiss 3T3 fibroblasts. J. Biol. Chem. **264:** 7856–7861, 1989a.

TAKUWA, Y., KASUYA, Y., TAKUWA, N., KUDO, M., YANAGISAWA, M., GOTO, K., MASAKI, T., AND YAMASHITA, K.: Endothelin receptor is coupled to phospholipase C via a pertussis toxin insensitive guanine nucleotide binding regulatory protein in vascular smooth muscle cells. J. Clin. Invest. **85:** 653–658, 1990a.

TAKUWA, Y., MASAKI, T., AND YAMASHITA, K.: The effects of the endothelin family peptides on cultured osteoblastic cells from rat calvariae. Biochem. Biophys. Res. Commun. **170:** 998–1005, 1990b.

TAKUWA, Y., MASAKI, T., AND YAMASHITA, K.: The mechanisms of endothelin action in vascular smooth muscle cells. Contrib Nephrol. **90:** 99–104, 1991.

TAKUWA, Y., OHUE, Y., TAKUWA, N., AND YAMASHITA, K.: Endothelin 1 activates phospholipase C. and mobilizes Ca^{2+} from extra and intracellular pools in osteoblastic cells. Am. J. Physiol. **257:** E797–E803, 1989b.

TAKUWA, Y., YANAGIZAWA, M., TAKUWA, N., AND MASAKI, T.: Endothelin, its diverse biological activities and mechanisms of action. Prog. Growth Factor Res. **1:** 195–206, 1989c.

TAMAOKI, H., KOBAYASHI, Y., NISHIMURA, S., OHKUBO, T., KYOGOKU, Y., NAKAJIMA, K., KUMAGAYE, S., KIMURA, T., AND SAKAKIBARA, S.: Solution conformation of endothelin determined by means of 1H NMR spectroscopy and distance geometry calculations. Protein Eng. **4:** 509–518, 1991a.

TAMAOKI, J., KANEMURA, T., SAKAI, N., ISONO, K., KOBAYASHI, K., AND TAKIZAWA, T.: Endothelin stimulates ciliary beat frequency and chloride secretion in canine cultured tracheal epithelium. Am. J. Respir. Cell Mol. Biol. **4:** 426–431, 1991b.

TAMMESILD, P. J., HODGSON, W. C., AND KING, R. G.: Increased sensitivity to endothelin 1 in isolated Krebs' perfused kidneys of streptozotocin diabetic rats. Clin. Exp. Pharmacol. Physiol. **19:** 261–265, 1992.

TANDA, S., HORI, K., SAITO, S., SHINOZAKI, M., ZHANG, Q. H., AND SUZUKI, M.: Comparison of the effects of intravenously bolus administered endothelin 1 and infused angiotensin II on the subcutaneous tumor blood flow in anesthetized rats. Jpn. J. Cancer Res. **82:** 958–963, 1991.

TATRAI, A., FOSTER, S., LAKATOS, P., SHANKAR, G., AND STERN, P. H.: Endothelin 1 actions on resorption, collagen and noncollagen protein synthesis, and phosphatidylinositol turnover in bone organ cultures. Endocrinology **131:** 603–607, 1992a.

TATRAI, A., LAKATOS, P., THOMPSON, S., AND STERN, P. H.: Effects of endothelin 1 on signal transduction in UMR 106 osteoblastic cells. J. Bone Miner. Res. **7:** 1201–1209, 1992b.

TAYLOR, R. N., VARMA, M., TENG, N. N., AND ROBERTS, J. M.: Women with preeclampsia have higher plasma endothelin levels than women with normal pregnancies. J. Clin. Endocrinol. Metab. **71:** 1675–1677, 1990.

TELEMAQUE, S., LEMAIRE, D., CLAING, A. AND D'ORLEANS, JUSTE P.: Phosphoramidon sensitive effects of big endothelins in the perfused rabbit kidney. Hypertension **20:** 518–523, 1992.

TERADA, Y., TOMITA, K., NONOGUCHI, H., AND MARUMO, F.: Different localization of two types of endothelin receptor mRNA in microdissected rat nephron segments using reverse transcription and polymerase chain reaction assay. J. Clin. Invest. **90:** 107–112, 1992.

THIEMERMANN, C., LIDBURY, P. S., THOMAS, G. R., AND VANE, J. R.: Endothelin 1 releases prostacyclin and inhibits ex vivo platelet aggregation in the anesthetized rabbit. J. Cardiovasc. Pharmacol. **13** (Suppl. 5): S138–S141, 1989.

THIEMERMANN, C., MAY, G. R., PAGE, C. P., AND VANE, J. R.: Endothelin 1 inhibits platelet aggregation in vivo: a study with [111]indium labelled platelets. Br. J. Pharmacol. **99:** 303–308, 1990.

THOMAS, C. P., BALDI, E., SIMONSON, M. S., KESTER, M., AND DUNN, M. J.: Endothelin receptors and coupled GTP binding proteins in glomerular mesangial cells. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S79–S84, 1991.

TOKITO, F., SUZUKI, N., HOSOYA, M., MATSUMOTO, H., OHKUBO, S., AND FUJINO, M.: Epidermal growth factor (EGF) decreased endothelin 2 (ET 2) production in human renal adenocarcinoma cells. FEBS Lett. **295:** 17–21, 1991.

TOKUNAGA, O., FAN, J., WATANABE, T., KOBAYASHI, M., KUMAZAKI, T., AND MITSUI, Y.: Endothelin. Immunohistologic localization in aorta and biosynthesis by cultured human aortic endothelial cells. Lab. Invest. **67:** 210–217, 1992.

TOMODA, H.: Plasma endothelin 1 in acute myocardial infarction with heart failure. Am. Heart J. **125:** 667–672, 1993.

TOMITA, K., NONOGUCHI, H., AND MARUMO, F.: Effects of endothelin on peptide dependent cyclic adenosine monophosphate accumulation along the nephron segments of the rat. J. Clin. Invest. **85:** 2014–2018, 1990.

TOMITA, K., UJIIE, K., NAKANISHI, T., TOMURA, S., MATSUDA, O., ANDO, K., SHICHIRI, M., HIRATA, Y., AND MARUMO, F.: Plasma endothelin levels in patients with acute renal failure. N. Engl. J. Med. **321:** 1127, 1989.

TOMOBE, Y., MIYAUCHI, T., SAITO, A., YANAGISAWA, M., KIMURA, S., GOTO, K., AND MASAKI, T.: Effects of endothelin on the renal artery from spontaneously hypertensive and Wistar Kyoto rats. Eur. J. Pharmacol. **152:** 373–374, 1988.

TOTSUNE, K., SONE, M., TAKAHASHI, K., OHNEDA, M., ITOI, K., MURAKAMI, O., SAITO, T., MOURI T., AND YOSHINAGA, K.: Immunoreactive endothelin in urine of patients with and without diabetes mellitus. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S423–S424, 1991.

TOYO-OKA, T., AIZAWA, T., SUZUKI, N., HIRATA, Y., MIYAUCHI, T., SHIN, W. S., YANAGISAWA, M., MASAKI, T., AND SUGIMOTO, T.: Increased plasma level of endothelin 1 and coronary spasm induction in patients with vasospastic angina pectoris. Circulation **83:** 476–483, 1991.

TOYO-OKA, T., AND SUGIMOTO, T.: Editorial comment: endothelin: key to coronary vasospasm? Circulation **84:** 1451–1452, 1991.

TRAISH, A. M., MORAN, E., DALEY, J. T., DE LAS MORENAS, A., AND SAENZ DE TEJADA, I.: Monoclonal antibodies to human endothelin 1: characterization and utilization in radioimmunoassay and immunocytochemistry. Hybridoma **11:** 147–163, 1992.

TRAN THI, T. A., KAWADA, N., AND DECKER, K.: Regulation of endothelin 1 action on the perfused rat liver. FEBS Lett. **318:** 353–357, 1993.

TRYBULEC, M., DUDEK, R. R., AND GRYGLEWSKI, R. J.: Effects of endothelin 1 and endothelin 3 on the release of prostanoids from isolated perfused rat kidney. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S229–S232, 1991.

TSCHIRHART, E. J., DRIJFHOUT, J. W., PELTON, J. T., MILLER, R. C., AND JONES, C. R.: Endothelins: functional and autoradiographic studies in guinea pig trachea. J. Pharmacol. Exp. Ther. **258:** 381–387, 1991.

TSENG, Y. C., LAHIRI, S., JACKSON, S., BURMAN, K. D., AND WATOFSKY, L.: Endothelin binding to receptors and endothelin production by human thyroid follicular cells: effects of transforming growth factor beta and thyrotropin. J. Clin. Endocrinol. Metab. **76:** 156–161, 1993.

TSUCHIYA, H., OTSUKA, A., MIKAMI, H., KATAHIRA, K., TSUNETOSHI, T., KOHARA, K., HIGAKI, J., NUGENT, C. A., AND OGIHARA, T.: Increased atrial natriuretic factor secretion after endothelin injection in dogs. Methods Find. Exp. Clin. Pharmacol. **12:** 379–383, 1990a.

TSUCHIYA, K., NARUSE, M., SANAKA, T., NARUSE, K., NITTA, K., DEMURA, H., AND SUGINO, N.: Effects of endothelin on renal regional blood flow in dogs. Eur. J. Pharmacol. **166:** 541–543, 1989.

TSUCHIYA, K., NARUSE, M., SANAKA, T., NARUSE, K., KATO, Y., ZENG, Z. P., NITTA, K., SHIZUME, K., DEMURA, H., AND SUGINO, N.: Effects of endothelin on renal hemodynamics and excretory functions in anesthetized dogs. Life Sci. **46:** 59–65, 1990b.

TSUCHIYA, K., NARUSE, M., SANAKA, T., NARUSE, K., ZENG, Z. P., NITTA, K., DEMURA, H., SHIZUM, E. K., AND SUGINO, N.: Renal and hemodynamic effects of endothelin in anesthetized dogs. Am. J. Hypertens. **3:** 792–795, 1990c.

TSUJI, S., SAWAMURA, A., WATANABE, H., TAKIHARA, K., PARK, S. E., AND AZUMA, J.: Plasma endothelin levels during myocardial ischemia and reperfusion. Life Sci. **48:** 1745–1749, 1991.

TSUNODA, K., ABE, K., SATO, T., YOKOSAWA, S., AND YOSHINAGA, K.: Decreased conversion of big endothelin 1 to endothelin 1 in patients with diabetes mellitus. Clin. Exp. Pharmacol. Physiol. **18:** 731–732, 1991.

TURNER A. J.: Endopeptidase-24.11 and neuropeptide metabolism. *In* Neuropeptides and Their Peptidases, edited by A. J. Turner, Ellis-Horwood, Health Science Ser., pp. 183–201, 1987.

UCHIDA, Y., NINOMIYA, H., SAOTOME, M., NOMURA, A., OHTSUKA, M., YANAGISAWA, M., GOTO, K., MASAKI, T., AND HASEGAWA, S.: Endothelin, a novel vasoconstrictor peptide, as potent bronchoconstrictor. Eur. J.. Pharmacol. **154:** 227–228, 1988.

UCHIDA, Y., SAOTOME, M., NOMURA, A., NINOMIYA, H., OHSE, H., HIRATA, F., AND HASEGAWA, S.: Endothelin 1 induced relaxation of guinea pig trachealis muscles. J. Cardiovasc. Pharmacol. **17** (Suppl. 7): S210–S212, 1991b.

UCHIHARA, M., IZUMI, N., SATO, C., AND MARUMO, F.: Clinical significance of elevated plasma endothelin concentration in patients with cirrhosis. Hepatology 16: 95–99, 1992.

UJIIE, K., TERADA, Y., NONOGUCHI, H., SHINOHARA, M., TOMITA, K., AND MARUMO, F.: Messenger RNA expression and synthesis of endothelin 1 along rat nephron segments. J. Clin. Invest. 90: 1043–1048, 1992.

URADE, Y., FUJITANI, Y., ODA, K., WATANABE, T., UMEMURA, I., TAKAI, M., OKADA, T., SAKATA, K., AND KARAKI, H.: An endothelin B receptor-selective antagonist: IRL 1038, [Cys11-Cys15]-endothelin-1(11–21). FEBS Lett. 311: 12–16, 1992.

UUSIMAA, P. A., HASSINEN, I. E., VUOLTEENAHO, O., AND RUSKOAHO, H.: Endothelin induced atrial natriuretic peptide release from cultured neonatal cardiac myocytes: the role of extracellular calcium and protein kinase C. Endocrinology 130: 2455–2464, 1992.

VALENTIN, J. P., GARDNER, D. G., WIEDEMANN, E., AND HUMPHREYS, M. H.: Modulation of endothelin effects on blood pressure and hematocrit by atrial natriuretic peptide. Hypertension 17: 864–869, 1991.

VAN RENTERGHEM, C., VIGNE, P., BARHANIN, J., SCHMID ALLIANA, A., FRELIN, C., AND LAZDUNSKI, M.: Molecular mechanism of action of the vasoconstrictor peptide endothelin. Biochem. Biophys. Res. Commun. 157: 977–985, 1988.

VANHOUTTE, P. M., AND KATUSIC, Z. S.: Endothelium derived contracting factor: endothelin and/or superoxide anion? Trends Pharmacol. Sci. 9: 229–230, 1988.

VELASCO, C. E., JACKSON, E. K., MORROW, J. A., VITOLA, J. V., INAGAMI, T., AND FORMAN, M. B.: Intravenous adenosine suppresses cardiac release of endothelin after myocardial ischaemia and reperfusion. Cardiovasc Res. 27: 121–128, 1993.

VEMULAPALLI, S., CHIU, P. J., RIVELLI, M., FOSTER, C. J., AND SYBERTZ, E. J.: Modulation of circulating endothelin levels in hypertension and endotoxemia in rats. J. Cardiovasc. Pharmacol. 18: 895–903, 1991.

VIERHAPPER, H., WAGNER, O., NOWOTNY, P., AND WALDHAUSL, W.: Effect of endothelin 1 in man. Circulation 81: 1415–1418, 1990.

VIGNE, P., BREITTMAYER, J. P., MARSAULT, R., AND FRELIN, C.: Endothelin mobilizes Ca^{2+} from a caffeine and ryanodine insensitive intracellular pool in rat atrial cells. J. Biol. Chem. 265: 6782–6787, 1990a.

VIGNE, P., LADOUX, A., AND FRELIN, C.: Endothelins activate Na^+/H^+ exchange in brain capillary endothelial cells via a high affinity endothelin 3 receptor that is not coupled to phospholipase C. J. Biol. Chem. 266: 5925–5928, 1991.

VIGNE, P., LAZDUNSKI, M., AND FRELIN, C.: The inotropic effect of endothelin 1 on rat atria involves hydrolysis of phosphatidylinositol. FEBS Lett. 249: 143–146, 1989.

VIGNE, P., MARSAULT, R., BREITTMAYER, J. P., AND FRELIN, C.: Endothelin stimulates phosphatidylinositol hydrolysis and DNA synthesis in brain capillary endothelial cells. Biochem. J. 266: 415–420, 1990b.

VIJARAGHAVAN, J., SCICHI, A. G., CARETERIO, O. A., SLAUGHTER, C., MOOMAW, C., AND HERSCH, L. B.: The hydrolysis of endothelins by neutral endopeptidase 24.1.1. (encephalinase). J. Biol. Chem. 265: 14150–14155, 1990.

VOERMAN, H. J., STEHOUWER, C. D., VAN KAMP, G. J., STRACK VAN SCHIJNDEL, R. J., GROENEVELD, A. B., AND THIJS, L. G.: Plasma endothelin levels are increased during septic shock. Crit. Care Med. 20: 1097–1101, 1992.

WADA, K., TABUCHI, H., OHBA, R., SATOH, M., TACHIBANA, Y., AKIYAMA, N., HIRAOKA, O., ASAKURA, A., MIYAMOTO, C., AND FURUICHI, Y.: Purification of an endothelin receptor from human placenta. Biochem. Biophys. Res. Commun. 167: 251–257, 1990.

WAGNER MANN, C., BOWMAN, L., AND STUREK, M.: Primary action of endothelin on Ca release in bovine coronary artery smooth muscle cells. Am. J. Physiol. 260: C763–C770, 1991.

WAGNER MANN, C., AND STUREK, M.: Endothelin mediates Ca influx and release in porcine coronary smooth muscle cells. Am. J. Physiol. 260: C771–C777, 1991.

WAITE, R. P., AND PANG, C. C.: The sympathetic nervous system facilitates endothelin 1 effects on venous tone. J. Pharmacol. Exp. Ther. 260: 45–50, 1992.

WALL, K. M., AND FERGUSON, A. V.: Endothelin acts at the subfornical organ to influence the activity of putative vasopressin and oxytocin secreting neurons. Brain Res. 586: 111–116, 1992.

WALL, K. M., NASR, M., AND FERGUSON, A. V.: Actions of endothelin at the subfornical organ. Brain Res. 570: 180–187, 1992.

WALLACE, J. L., CIRINO, G., DE NUCCI, G., MCKNIGHT, W., AND MACNAUGHTON, W. K.: Endothelin has potent ulcerogenic and vasoconstrictor actions in the stomach. Am. J. Physiol. 256: G661–G666, 1989a.

WALLACE, J. L., KEENAN, C. M., MACNAUGHTON, W. K., AND MCKNIGHT, G. W.: Comparison of the effects of endothelin 1 and endothelin 3 on the rat stomach. Eur. J. Pharmacol. 167: 41–47, 1989b.

WALLNOFER, A., WEIR, S., RUEGG, U., AND CAUVIN, C.: The mechanism of action of endothelin 1 as compared with other agonists in vascular smooth muscle. J. Cardiovasc. Pharmacol. 13 (Suppl. 5): S23–S31, 1989.

WANG, Y., AND COCEANI, F.: Isolated pulmonary resistance vessels from fetal lambs. Contractile behavior and responses to indomethacin and endothelin 1. Circ. Res. 71: 320–330, 1992.

WANG, Y., SIMONSON, M. S., POUYSSEGUR, J., AND DUNN, M. J.: Endothelin rapidly stimulates mitogen activated protein kinase activity in rat mesangial cells. Biochem. J. 287: 589–594, 1992.

WARNER, T. D., DE NUCCI, G., AND VANE, J. R.: Rat endothelin is a vasodilator in the isolated perfused mesentery of the rat. Eur. J. Pharmacol. 159: 325–326, 1989a.

WARNER, T. D., D'ORLEANS-JUSTE, P., AND VANE, J. R.: Endothelin 1 and U46619 potentiate selectively the venous responses to nerve stimulation within the perfused superior mesenteric vascular bed of the rat. Biochem. Biophys. Res. Commun. 172: 745–750, 1990.

WARNER, T. D., MITCHELL, J. A., DE NUCCI, G., AND VANE, J. R.: Endothelin 1 and endothelin 3 release EDRF from isolated perfused arterial vessels of the rat and rabbit. J. Cardiovasc. Pharmacol. 13 (Suppl. 5): S85–S88, 1989b.

WARNER, T. D., SCHMIDT, H. H., AND MURAD, F.: Interactions of endothelins and EDRF in bovine native endothelial cells: selective effects of endothelin 3. Am. J. Physiol. 262: H1600–H1605, 1992.

WARRENS, A. N., CASSIDY, M. J., TAKAHASHI, K., GHATEI, M. A., AND BLOOM, S. R.: Endothelin in renal failure. Nephrol. Dial. Transplant. 5: 418–422, 1990.

WATANABE, H., MIYAZAKI, H., KONDOH, M., MASUDA, Y., KIMURA, S., YANAGISAWA, M., MASAKI, T., AND MURAKAMI, K.: Two distinct types of endothelin receptors are present on chick cardiac membranes. Biochem. Biophys. Res. Commun. 161: 1252–1259, 1989a.

WATANABE, T., KUSUMOTO, K., KITAYOSHI, T., AND SHIMAMOTO, N.: Positive inotropic and vasoconstrictive effects of endothelin 1 in in vivo and in vitro experiments: characteristics and the role of L type calcium channels. J. Cardiovasc. Pharmacol. 13 (Suppl. 5): S108–S111, 1989b.

WATANABE, T., SUZUKI, N., SHIMAMOTO, N., FUJINO, M., AND IMADA, A.: Contribution of endogenous endothelin to the extension of myocardial infarct size in rats. Circ. Res. 69: 370–377, 1991.

WATANABE, T., SUZUKI, N., SHIMAMOTO, N., FUJINO, M., AND IMADA, A.: Endothelin in myocardial infarction. Nature (Lond.) 344: 114, 1990.

WATSCHINGER, B., VYCHYTIL, A., SCHULLER, M., HARTTER, E., TRAINDL, O., POHANKA, E., ULRICH, W., AND KOVARIK, J.: The pathophysiologic role of endothelin in acute vascular rejection after renal transplantation. Transplantation (Baltimore) 52: 743–746, 1991.

WATTS, J. A., CHAPAT, S., JOHNSON, D. E., AND JANIS, R. A.: Effects of nisoldipine upon vasoconstrictor responses and binding of endothelin 1 in ischemic and reperfused rat hearts. J. Cardiovasc. Pharmacol. 19: 929–936, 1992.

WEBB, D. J.: Endothelin receptors cloned, endothelin converting enzyme characterized and pathophysiological roles for endothelin proposed. Trends Pharmacol. Sci. 12: 43–46, 1991.

WEBB, M. L., DICKINSON, K. E., DELANEY, C. L., LIU, E. C., SERAFINO, R., COHEN, R. B., MONSHIZADEGAN, H., AND MORELAND, S.: The endothelin receptor antagonist, BQ 123, inhibits angiotensin II induced contractions in rabbit aorta. Biochem. Biophys. Res. Commun. 185: 887–892, 1992.

WEBB, R. L., AND LAPPE, R. W.: Hemodynamic effects of an endothelin (ETB) selective agonist, IRL 1620 in conscious Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR). *In* Proceedings of the 3rd International Conference on Endothelin, p. 137, 1993. Houston, Tx. February 15–17, 1993.

WEBBER, S. E., YURDAKOS, E., WOODS, A. J., AND WIDDICOMBE, J. G.: Effects of endothelin 1 on tracheal submucosal gland secretion and epithelial function in the ferret. Chest 101 (Suppl. 3): 63S–67S, 1992.

WEISSBERG, P. L., WITCHELL, C., DAVENPORT, A. P., HESKETH, T. R., AND METCALFE, J. C.: The endothelin peptides ET-1, ET-2, ET-3 and sarafotoxin S6b are comitogenic with platelet derived growth factor for vascular smooth muscle cells. Atherosclerosis 85: 257–262, 1990.

WEITZBERG, E., LUNDBERG, J. M., AND RUDEHILL, A.: Elevated plasma levels of endothelin in patients with sepsis syndrome. Circ. Shock. 33: 222–227, 1991.

WHITE, S. R., HATHAWAY, D. P., UMANS, J. G., AND LEFF, A. R.: Direct effects on airway smooth muscle contractile response caused by endothelin 1 in guinea pig trachealis. Am. Rev. Respir. Dis. 145: 491–493, 1992.

WHITE, S. R., HATHAWAY, D. P., UMANS, J. G., TALLET, J., ABRAHAMS, C., AND LEFF, A. R.: Epithelial modulation of airway smooth muscle response to endothelin 1. Am. Rev. Respir. Dis. 144: 373–378, 1991.

WHITTLE, B. J., AND ESPLUGUES, J. V.: Induction of rat gastric damage by the endothelium derived peptide, endothelin. Br. J. Pharmacol. 95: 1011–1013, 1988.

WHITTLE, B. J., AND LOPEZ BELMONTE, J.: Interactions between the vascular peptide endothelin 1 and sensory neuropeptides in gastric mucosal injury. Br. J. Pharmacol. 102: 950–954, 1991.

WHITTLE, B. J., LOPEZ BELMONTE, J., AND REES, D. D.: Modulation of the vasodepresor actions of acetylcholine, bradykinin, substance P and endothelin in the rat by a specific inhibitor of nitric oxide formation in the rat by a specific inhibitor of nitric oxide formation. Br. J. Pharmacol. 98: 646–652, 1989.

WHITTLE, B. J., AND MONCADA, S.: The endothelin explosion. A pathophysiological reality or a biological curiosity? Circulation 81: 2022–2025, 1990.

WIDIMSKY, J., JR., HORKY, K., AND DVORAKOVA, J.: Plasma endothelin 1,2 levels in mild and severe hypertension. J. Hypertens. 9 (Suppl.): S194–S195, 1991.

WIKLUND, N. P., OHLEN, A., AND CEDERQVIST, B.: Adrenergic neuromodulation by endothelin in guinea pig pulmonary artery. Neurosci. Lett. 101: 269–273, 1989a.

WIKLUND, N. P., OHLEN, A., WIKLUND, C. U., CEDERQVIST, B., HEDQVIST, P., AND GUSTAFSSON, L. E.: Neuromuscular actions of endothelin on smooth, cardiac and skeletal muscle from guinea pig, rat and rabbit. Acta Physiol. Scand. 137: 399–407, 1989b.

WIKLUND, N. P., OHLEN, A., WIKLUND, C. U., HEDQVIST, P., AND GUSTAFSSON, L. E.: Endothelin modulation of neuroeffector transmission in rat and guinea pig vas deferens. Eur. J. Pharmacol. 185: 25–33, 1990.

WIKLUND, N. P., WIKLUND, C. U., CEDERQVIST, B., OHLEN, A., HEDQVIST, P., AND GUSTAFSSON, L. E.: Endothelin modulation of neuroeffector transmission in smooth muscle. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S335–S339, 1991.
WIKLUND, N. P., WIKLUND, C. U., OHLEN, A., AND GUSTAFSSON, L. E.: Cholinergic neuromodulation by endothelin in guinea pig ileum. Neurosci. Lett. 101: 342–346, 1989c.
WILKES, B. M., PEARL, A. R., MENTO, P. F., MAITA, M. E., MACICA, C. M., AND GIRAARDI, E. P.: Glomerular endothelin receptors during initiation and maintenance of ischemic acute renal failure in rats. Am. J. Physiol. 260: F110–F118, 1991.
WILLETTE, R. N., OHLSTEIN, E. H., PULLEN, M., SAUERMELCH, C. F., COHEN, A., AND NAMBI, P.: Transient forebrain ischemia alters acutely endothelin receptor density and immunoreactivity in gerbil brain. Life Sci. 52: 35–40, 1993.
WILLETTE, R. N., AND SAUERMELCH, C. F.: Abluminal effects of endothelin in cerebral microvasculature assessed by laser Doppler flowmetry. Am. J. Physiol. 259: H1688–H1693, 1990.
WILLETTE, R. N., SAUERMELCH, C., EZEKIEL, M., FEUERSTEIN, G., AND OHLSTEIN, E. H.: Effect of endothelin on cortical microvascular perfusion in rats. Stroke 21: 451–458, 1990.
WILLIAMS, D. L., JR., JONES, K. L., COLTON, C. D., AND NUTT, R. F.: Identification of high affinity endothelin 1 receptor subtypes in human tissues. Biochem. Biophys. Res. Commun. 180: 475–480, 1991a.
WILLIAMS, D. L., JR., JONES, K. L., PETTIBONE, D. J., LIS, E. V., AND CLINESCHMIDT, B. V.: Sarafotoxin S6c: an agonist which distinguishes between endothelin receptor subtypes. Biochem. Biophys. Res. Commun. 175: 556–561, 1991b.
WINKLES, J. A., ALBERTS, G. F., BROGI, E., AND LIBBY, P.: Endothelin 1 and endothelin receptor mRNA expression in normal and atherosclerotic human arteries. Biochem. Biophys. Res. Commun. 191: 1081–1088, 1993.
WINQUIST, R. J., BUNTING, P. B., GARSKY, V. M., LUMMA, P. K., AND SCHOFIELD, T. L.: Prominent depressor response to endothelin in spontaneously hypertensive rats. Eur. J. Pharmacol. 163: 199–203, 1989a.
WINQUIST, R. J., SCOTT, A. L., AND VLASUK, G. P.: Enhanced release of atrial natriuretic factor by endothelin in atria from hypertensive rats. Hypertension 14: 111–114, 1989b.
WITHRINGTON, P. G., ANSARI, N., CROXTON, R., DE NUCCI, G., AND VANE, J. R.: The actions of endothelins 1 and 3 on the vascular and capsular smooth muscle of the isolated blood perfused spleen of the dog. Br. J. Pharmacol. 105: 490–494, 1992.
WOLFF, M., DAY, J., GREENWOOD, A., LARSON, S., AND MCPHERSON, A.: Crystallization and preliminary × ray analysis of human endothelin. Acta Crystallogr. Sect. B Struct. Sci. 48: 239–240, 1992.
WOLLBERG, Z., BDOLAH, A., GALRON, R., SOKOLOVSKY, M., AND KOCHVA, E.: Contractile effects and binding properties of endothelins/sarafotoxins in the guinea pig ileum. Eur. J. Pharmacol. 198: 31–36, 1991.
WOLLBERG, Z., BOUSSO MITTLER, D., BDOLAH, A., KLOOG, Y., KOCHVA, E., AND SOKOLOVSKY, M.: Endothelins and sarafotoxins: effects on motility, binding properties and phosphoinositide hydrolysis during the estrous cycle of the rat uterus. J. Basic Clin. Physiol. Pharmacol. 3: 41–57, 1992a.
WOLLBERG, Z., SHINNAR, N., BDOLAH, A., AND KOCHVA, E.: Endothelin and sarafotoxin: influence on steroid regulated motility of rat uterus. Life Sci. 51: PL57—PL60, 1992b.
WONG DUSTING, H. K., REID, J. J., AND RAND, M. J.: Paradoxical effects of endothelin on cardiovascular noradrenergic neurotransmission. Clin. Exp. Pharmacol. Physiol. 16: 229–233, 1989.
WONG DUSTING, H. K., LA, M., AND RAND, M. J.: Mechanisms of the effects of endothelin on responses to noradrenaline and sympathetic nerve stimulation. Clin. Exp. Pharmacol. Physiol. 17: 269–273, 1990.
WONG DUSTING, H. K., LA, M., AND RAND, M. J.: Endothelin 1 enhances vasoconstrictor responses to sympathetic nerve stimulation and noradrenaline in the rabbit ear artery. Clin. Exp. Pharmacol. Physiol. 18: 131–136, 1991.
WOOD, J. G., YAN, Z. Y., AND CHEUNG, L. Y.: Relative potency of endothelin analogues on changes in gastric vascular resistance. Am. J. Physiol. 262: G977–G982, 1992.
WOODCOCK, E. A., AND LAND, S.: Interaction between vasopressin and endothelin in renal papillary tubules: uncoupling following cell isolation and culture. Clin. Exp. Pharmacol. Physiol. 19: 384–387, 1992.
WOODCOCK, E. A., LITTLE, P. J., AND TANNER, J. K.: Inositol phosphate release and steroidogenesis in rat adrenal glomerulosa cells. Comparison of the effects of endothelin, angiotensin II and vasopressin. Biochem. J. 271: 791–796, 1990.
WORD, R. A., KAMM, K. E., STULL, J. T., AND CASEY, M. L.: Endothelin increases cytoplasmic calcium and myosin phosphorylation in human myometrium. Am. J. Obstet. Gynecol. 162: 1103–1108, 1990.
XUAN, Y. T., WATKINS, W. D., AND WHORTON, A. R.: Regulation of endothelin mediated calcium mobilization in vascular smooth muscle cells by isoproterenol. Am. J. Physiol. 260: C492–C502, 1991.
XUAN, Y. T., WHORTON, A. R., AND WATKINS, W. D.: Inhibition by nicardipine of endothelin mediated inositol phosphate formation and Ca^{2+} mobilization in smooth muscle cell. Biochem. Biophys. Res. Commun. 160: 758–764, 1989.
YAMADA, K., AND YOSHIDA, S.: Role of endogenous endothelin in renal function during altered sodium balance. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S290–S292, 1991.
YAMAGISHI, S., HSU, C. C., KOBAYASHI, K., AND YAMAMOTO, H.: Endothelin 1 mediates endothelial cell dependent proliferation of vascular pericytes. Biochem. Biophys. Res. Commun. 191: 840–846, 1993.
YAMAMOTO, S., MORIMOTO, I., YAMASHITA, H., AND ETO, S.: Inhibitory effects of endothelin 3 on vasopressin release from rat supraoptic nucleus in vitro. Neurosci. Lett. 141: 147–150, 1992a.
YAMAMOTO, T., KIMURA, T., OTA, K., SHOJI, M., INOUE, M., SATO, K., OHTA, M., AND YOSHINAGA, K.: Central effects of endothelin 1 on vasopressin and atrial natriuretic peptide release and cardiovascular and renal function in conscious rats. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S316–S318, 1991.
YAMAMOTO, T., KIMURA, T., OTA, K., SHOJI, M., INOUE, M., SATO, K., OHTA, M., AND YOSHINAGA, K.: Central effects of endothelin 1 on vasopressin release, blood pressure, and renal solute excretion. Am. J. Physiol. 262: E856–E862, 1992b.
YAMANE, K., KASHIWAGI, H., SUZUKI, N., MIYAUCHI, T., YANAGISAWA, M., GOTO, K., AND MASAKI, T.: Elevated plasma levels of endothelin 1 in systemic sclerosis. Arthritis Rheum. 34: 243–244, 1991.
YAMANE, K., MIYAUCHI, T., SUZUKI, N., YUHARA, T., AKAMA, T., SUZUKI, H., AND KASHIWAGI, H.: Significance of plasma endothelin 1 levels in patients with systemic sclerosis. J. Rheumatol. 19: 1566–1571, 1992.
YAMASAKI, H., NIWA, M., YAMASHITA, K., KATAOKA, Y., SHIGEMATSU, K., HASHIBA, K., AND OZAKI, M.: Specific ^{125}I endothelin 1 binding sites in the atrioventricular node of the porcine heart. Eur. J.. Pharmacol. 168: 247–250, 1989.
YAMASHITA, J., OGAWA, M., EGAMI, H., MATSUO, S., KIYOHARA, H., INADA, K., YAMASHITA, S., AND FUJITA, S.: Abundant expression of immunoreactive endothelin 1 in mammary phyllodes tumor: possible paracrine role of endothelin 1 in the growth of stromal cells in phyllodes tumor. Cancer Res. 52: 4046–4049, 1992.
YAMASHITA, J., OGAWA, M., INADA, K., YAMASHITA, S., MATSUO, S., AND TAKANO, S.: A large amount of endothelin 1 is present in human breast cancer tissues. Res. Commun. Chem. Pathol. Pharmacol. 74: 363–369, 1991b.
YAMASHITA, K., KATAOKA, Y., NIWA, M., SHIGEMATSU, K., HIMENO, A., KOIZUMI, S., AND TANIYAMA, K.: Increased production of endothelins in the hippocampus of stroke prone spontaneously hypertensive rats following transient forebrain ischemia: histochemical evidence. Cell. Mol. Neurobiol. 13: 15–23, 1993.
YAMAUCHI, T., OHNAKA, K., TAKAYANAGI, R., UMEDA, F., AND NAWATA, H.: Enhanced secretion of endothelin 1 by elevated glucose levels from cultured bovine aortic endothelial cells. FEBS Lett. 267: 16–18, 1990.
YAMAURA, I., TANI, E., MAEDA, Y., MINAMI, N. AND SHINDO, H.: Endothelin 1 of canine basilar artery in vasospasm. J. Neurosurg. 76: 99–105, 1992.
YANAGISAWA, M., INOUE, A., ISHIKAWA, T., KASUYA, Y., KIMURA, S., KUMAGAYE, S., NAKAJIMA, K., WATANABE, T. X., SAKAKIBARA, S., GOTO, K., ET AL.: Primary structure, synthesis, and biological activity of rat endothelin, an endothelium derived vasoconstrictor peptide. Proc. Natl. Acad. Sci. USA 85: 6964–6967, 1988a.
YANAGISAWA M., KURIHARA H., KIMURA S., TOMOBE Y., KOBAYASHI M., MITSUI Y., YAZAKI Y., GOTO K., AND MASAKI, T.: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature (Lond.) 332: 411–415, 1988b.
YANAGISAWA, M., AND MASAKI, T.: Molecular biology and biochemistry of the endothelins. Trends Pharmacol. Sci. 10: 374–378, 1989.
YANAGISAWA, M., MASAKI, T., HAMAOKI, M., KATO, H., AND YAZAKI, Y.: Transforming growth factor beta stimulates the expression of endothelin mRNA by vascular endothelial cells. Biochem. Biophys. Res. Commun. 159: 1435–1440, 1989.
YANG, D., AND CLARK, K. E.: Effect of endothelin 1 on the uterine vasculature of the pregnant and estrogen treated nonpregnant sheep. Am. J. Obstet. Gynecol. 167: 1642–1650, 1992.
YANG, M. C., YU, P. C., TU, M. S., LAY, C. S., HONG, C. Y., CHOU, C. K., CHEN, C. F., AND KUO, J. S.: Effects of endothelin and vasopressin on portal pressure of rats. Life Sci. 46: 1929–1936, 1990a.
YANG, Z., BAUER, E., VON SEGESSER, L., STULZ, P., TURINA, M., AND LUSCHER, T. F.: Different mobilization of calcium in endothelin 1 induced contractions in human arteries and veins: effects of calcium antagonists. J. Cardiovasc. Pharmacol. 16: 654–660, 1990b.
YANG, Z. H., BUHLER, F. R., DIEDERICH, D., AND LUSCHER, T. F.: Different effects of endothelin 1 on cAMP and cGMP mediated vascular relaxation in human arteries and veins: comparison with norepinephrine. J. Cardiovasc. Pharmacol. 13 (Suppl. 5): S129–S131, 1989.
YANG, Z. H., RICHARD, V., VON SEGESSER L., BAUER, E., STULZ, P., TURINA, M., AND LUSCHER, T. F.: Threshold concentrations of endothelin 1 potentiate contractions to norepinephrine and serotonin in human arteries. A new mechanism of vasospasm? Circulation 82: 188–195, 1990c.
YASUDA, M., KOHNO, M., TAHARA, A., ITAGANE, H., TODA, I., AKIOKA, K., TERAGAKI, M., OKU, H. TAKEUCHI, K., AND TAKEDA, T.: Circulating immunoreactive endothelin in ischemic heart disease. Am. Heart J. 119: 801–806, 1990.
YASUJIMA, M., ABE, K., KANAZAWA, M., YOSHIDA, K., KOHZUKI, M., TAKEUCHI, K., TSUNODA, K., KUDO, K., HIWATARI, M., SATO, T., ET AL.: Antihypertensive effect of captopril and enalapril in endothelin infused rats. Tohoku J. Exp. Med. 163: 219–227, 1991.
YOHN, J. J., MORELLI, J. G., WALCHAK, S. J., RUNDELL, K. B., NORRIS, D. A., AND ZAMORA, M. R.: Cultured human keratinocytes synthesize and secrete endothelin 1. J. Invest. Dermatol. 100: 23–26, 1993.

YOKOKAWA, K., KOHNO, M., MURAKAWA, K., YASUNARI, K. AND TAKEDA, T.: Effect of endothelin 1 on cytosolic calcium ions in cultured human endothelial cells. J. Hypertens. 8: 843–849, 1990.

YOKOKAWA, K., KOHNO, M., YASUNARI, K., MURAKAWA, K., AND TAKEDA, T.: Endothelin 3 regulates endothelin 1 production in cultured human endothelial cells. Hypertension 18: 304–315, 1991a.

YOKOKAWA, K., MANDAL, A. K., KOHNO, M., HORIO, T., MURAKAWA, K., YASUNARI, K., AND TAKEDA, T.: Heparin suppresses endothelin 1 action and production in spontaneously hypertensive rats. Am. J. Physiol. 263: R1035–R1041, 1992.

YOKOKAWA, K., TAHARA, H., KOHNO, M., MURAKAWA, K., YASUNARI, K., NAKAGAWA, K., HAMADA, T. OTANI, S., YANAGISAWA, M., AND TAKEDA, T.: Endothelin secreting tumor. J. Cardiovasc. Pharmacol. 17 (Suppl. 7): S398–S401, 1991b.

YOKOKAWA, K., TAHARA, H., KOHNO, M., MURAKAWA, K., YASUNARI, K., NAKAGAWA, K., HAMADA, T. OTANI, S., YANAGISAWA, M., AND TAKEDA, T.: Hypertension associated with endothelin secreting malignant hemangioendothelioma. Ann. Intern. Med. 114: 213–215, 1991c.

YORIKANE, R., AND KOIKE, H.: The arrhythmogenic action of endothelin in rats. Jpn. J. Pharmacol. 53: 259–263, 1990.

YORIMITSU, K., SHINMI, O., NISHIYAMA, M., MOROI, K., SUGITA, Y., SAITO, T., INAGAKI, Y., MASAKI, T., AND KIMURA, S.: Effect of phosphoramidon on big endothelin 2 conversion into endothelin 2 in human renal adenocarcinoma (ACHN) cells. Analysis of endothelin 2 biosynthetic pathway. FEBS Lett. 314: 395–398, 1992.

YOSHIDA, H., AND NAKAMURA, M.: Inhibition by angiotensin converting enzyme inhibitors of endothelin secretion from cultured human endothelial cells. Life Sci. 50: PL195–PL200, 1992.

YOSHIMI, H., HIRATA, Y., FUKUDA, Y., KAWANO, Y., EMORI, T., KURAMOCHI, M., OMAE, T., AND MARUMO, F.: Regional distribution of immunoreactive endothelin in rats. Peptides (Elmsford) 10: 805–808, 1989.

YOSHIMOTO, S., ISHIZAKI, Y., KURIHARA, H., SASAKI, T., YOSHIZUMI, M., YANAGISAWA, M., YAZAKI, Y., MASAKI, T., TAKAKURA, K., AND MUROTA, S.: Cerebral microvessel endothelium is producing endothelin. Brain Res. 508: 283–285, 1990.

YOSHINAGA, M., CHIJIIWA, Y., MISAWA, T., HARADA, N., AND NAWATA, H.: Endothelin B receptor on guinea pig small intestinal smooth muscle cells. Am. J. Physiol. 262: G308–G311, 1992.

YOSHIZAWA, T., KIMURA, S., KANAZAWA, I., YANAGISAWA, M., MASAKI, T.: Endothelin 1 depolarizes a ventral root potential in the newborn rat spinal cord. J. Cardiovasc. Pharmacol. 13 (Suppl. 5): S216–S217, 1989.

YOSHIZAWA, T., SHINMI, O., GIAID, A., YANAGISAWA, M., GIBSON, S. J., KIMURA, S., UCHIYAMA, Y., POLAK, J. M., MASAKI, T., AND KANAZAWA, I.: Endothelin: a novel peptide in the posterior pituitary system. Science (Wash. DC). 247: 462–464, 1990.

YOSHIZUMI, M., KURIHARA, H., SUGIYAMA, T., TAKAKU, F., YANAGISAWA, M., MASAKI, T., AND YAZAKI, Y.: Hemodynamic shear stress stimulates endothelin production by cultured endothelial cells. Biochm. Biophys. Res. Commun. 161: 859–864, 1989.

YUE, T. L., NAMBI, P., WU, H. L. AND FEUERSTEIN, G.: Effect of endothelins on cytosolic free calcium concentration in neuroblastoma NG108 15 and NCB 20 cells. Neuropeptides 17: 7–12, 1990.

YURDAKOS, E., AND WEBER, S. E.: Endothelin 1 inhibits pre stimulated tracheal submucosal gland secretion and epithelial albumin transport. Br. J. Pharmacol. 104: 1050–1056, 1991.

ZAMORA, M. R., O'BRIEN, R. F., RUTHERFORD, R. B., AND WEIL, J. V.: Serum endothelin 1 concentrations and cold provocation in primary Raynaud's phenomenon. Lancet 336: 1144–1147, 1990.

ZENG, Z. P., NARUSE, M., GUAN, B. J., NARUSE, K., SUN, M. L., ZANG, M. F., DEMURA, H., AND SHI, Y. F.: Endothelin stimulates aldosterone secretion in vitro from normal adrenocortical tissue, but not adenoma tissue, in primary aldosteronism. J. Clin. Endocrinol. Metab. 74: 874–878, 1992.

ZHANG, W., SAKAI, N., YAMADA, H., FU, T., AND NAZAWA, Y. L.: Endothelin 1 induces intracellular calcium rise and inositol 1,4,5 trisphosphate formation in cultured rat and human glioma cells. Neurosci. Lett. 112: 199–204, 1990.

ZIV, I., FLEMINGER, G., DJALDETTI, R., ACHIRON, A., MELAMED, E., AND SOKOLOVSKY, M.: Increased plasma endothelin 1 in acute ischemic stroke. Stroke 23: 1014–1016, 1992.

ZOCHODNE, D. W., HO, L. T., AND GROSS, P. M.: Acute endoneurial ischemia induced by epineurial endothelin in the rat sciatic nerve. Am. J. Physiol. 263: H1806–H1810, 1992.

ZOJA, C., ORISIO, S., PERICO, N., BENIGNI, A., MORIGI, M., BENATTI, L., RAMBALDI, A., AND REMUZZI, G.: Constitutive expression of endothelin gene in cultured human mesangial cells and its modulation by transforming growth factor beta, thrombin, and a thromboxane A2 analogue. Lab. Invest. 64: 16–20, 1991.