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V. International Union of Pharmacology Nomenclature of Endothelin Receptors*

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I. Introduction

Following the discovery of ET‡ (Yanagisawa et al., 1988), the existence of three distinct isoforms of ET, designated ET-1, ET-2, and ET-3, was predicted from the finding of three separate genes (Inoue et al., 1989). The expression of the three isotypes has been verified in many tissues and found to occur in various proportions. Only ET-1 is made by endothelial cells. In addition to these endogenous ligands, sarafotoxins, constituents of a rare snake venom, have been included as members of the ET superfamily. ETs act not only on blood vessels but also in nonvascular systems. These diverse responses to ETs appear to be mediated by specific receptors. Results of many pharmacological and ligand-binding experiments have suggested the existence of multiple subtypes of ET receptors. At present, the existence of two receptor subtypes, designated ET_A and ET_B, has been confirmed. Molecular characterization of these two receptors has been summarized (Sakurai et al., 1992). In addition, the existence of the third type, ET_C, was reported recently in *Xenopus laevis* (Karne et al., 1993); whether this represents a species variant of the ET_A or ET_B receptors or is a new subtype that has a distinct mammalian homologue is not yet clear. This report represents the consensus view of the subcommittee of the International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification.

II. Differential Potencies of Endothelins

A dissociation of the potency order of the three ET isopeptides has been observed in a variety of systems. In general, responses can be divided into two groups according to the pharmacological potency of the three isopeptides. In the first group of responses, which includes vasoconstriction in most arteries, bronchoconstriction, and stimulation of aldosterone secretion, ET-1 and ET-2 are more potent than ET-3 (table 1). In the second group, which includes vasodilation, the three isopeptides have a similar potency (table 2). For example, the EC_{50} of the three endogenous agonists in constricting deendothelialized strips of porcine coronary artery are 5.2×10^{-10} M for ET-1, 1.9×10^{-9} M for ET-2, and 3.7×10^{-9} M for ET-3 (Inoue et al., 1989), whereas the conductance of the rat hindquarter vascular bed (in the presence of indomethacin), measured 0.25 min after bolus injection of 4 pmol of ET-1, ET-2, or ET-3, increases 44, 26, and 71%, respectively (Gardiner et al., 1990).

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‡ Abbreviation: ET, endothelin.

TABLE 1
Type I responses to endothelins (potency order: ET-1 > ET-3)

Tissue or organ (species)	Assay*	Reference
Vascular smooth muscle cells	Pressor effect in vivo, contraction, PI generation, binding assay, etc.	Inoue et al., 1989
Bronchial smooth muscle (human)	Contraction	Advenier et al., 1990
Uterine smooth muscle (rat)	Contraction, PI generation, binding assay	Bousso-Mittler et al., 1989
Renal medullary interstitial cells (rat)	Binding assay	Wilkes et al., 1991
Cardiac muscle (neonatal rat)	PI generation, binding assay	Galron et al., 1989
Adrenocortical cells (zona glomerulosa, bovine)	Stimulation of aldosterone secretion, binding assay	Gomez-Sanchez et al., 1990
C6 glioma cells (rat)	PI generation, binding assay	Martin et al., 1990
Fibroblast cells (human, mouse)	Ca^{2+} transient, binding assay	Ohnishi-Suzaki et al., 1990
Osteoblast cells (rat)	DNA synthesis, PI generation, binding assay, etc.	Takuwa et al., 1990

* PI, inositol phosphate.

TABLE 2
Type II responses to endothelins (potency order: ET-1 = ET-3)

Tissue or organ (species)	Assay	Reference
Whole animal (rat)	Initial depressor response in vivo	Inoue et al., 1989
Mesenteric artery (rat)	Transient relaxation, endothelium-derived relaxing factor release	Warner et al., 1989
Stomach (rat)	Ulcerogenicity	Wallace et al., 1989
Whole animal (rabbit)	Inhibition of ex vivo platelet aggregation	Lidbury et al., 1989
Cerebral cortex, cerebellum (rat)	PI generation	Crawford et al., 1990
Astrocytes (rat)	PI generation, Ca^{2+} transient	Marsault et al., 1990
Mesangium cells (rat)	PI generation, binding assay	Watanabe et al., 1989

* PI, inositol phosphate.

TABLE 3
Comparison of recombinant endothelin receptors

Receptor subtype*	Affinity rank order	Origin	Amino acid residues (inclusive signal sequences)	Tissue distribution	References
ET_A	ET-1 > ET-2 >> ET-3	Bovine lung	427	Heart, lung, intestine, brain	Arai et al., 1990
		Rat A10 cell	426	Heart, lung, intestine, brain	Lin et al., 1991
		Human	427	Aorta, heart, lung, intestine, brain	Hosoda et al., 1991
ET_B	ET-1 = ET-2 = ET-3	Bovine lung	441	Brain, lung	Saito et al., 1991
		Rat lung	441	Brain, lung, kidney	Sakurai et al., 1991
		Human jejunum/liver/placenta	442	Brain, lung, kidney, heart	Sakamoto et al., 1991
					Nakamuta et al., 1991
				Endothelial cells	Ogawa et al., 1991
ET_C	ET-1 < ET-3	Dermal melanophores of *X. laevis*	444	*Xenopus* dermal melanophores	Karne et al., 1993

* Both subtypes have a heptahelical tertiary structure and couple to phospholipase C via G-proteins.

III. Radioligand Binding to Endothelin Receptors

In radioligand-binding studies, the order of affinity of ETs for membrane fractions prepared from various tissues such as vascular smooth muscle (Martin et al., 1990), chick heart (Watanabe et al., 1989), rat lung (Masuda et al., 1989), rat mesangial cells (Sugiura et al., 1989), human placenta (Nakajo et al., 1989), and bovine cerebellum (Schwartz et al., 1990) has also demonstrated that there are at least two distinct subtypes of ET receptors.

IV. Molecular Cloning Studies

So far, two classes of cDNA clones encoding ET receptors have been isolated from mammalian tissues (Arai et al., 1990; Sakurai et al., 1990; Lin et al., 1991; Hosoda et al., 1991; Saito et al., 1991; Sakamoto et al., 1991; Nakamuta et al., 1991; Ogawa et al., 1991). The structures of the mature ET receptors have been deduced from the nucleotide sequences of cDNAs. The apparent discrepancy between the reported molecular size of binding sites in membrane preparations and the size of the recombinant receptors may be due to different glycosylation and truncation of the fully processed receptor protein (Kozuka et al., 1991; Hashido et al., 1992). The encoded proteins contain seven stretches of 20 to 27 hydrophobic amino acid residues in all ET receptor subtypes, revealing a seven-turn G-protein-coupled receptor belonging to the rhodopsin-type receptor superfamily. Both recep-

TABLE 4
Representative ET antagonists

Drug class	Structure	References
ET_A antagonist		
BQ-123	Cyclo (-D-Trp-D-Asp-Pro-D-Val-Leu-)	Ihara et al., 1992
FR-139317	2(*R*)-[2(*R*)-[2(*S*)-[[1-hexahydro-1H-azepinyl]carbonyl]amino-4-methylpentanoyl]amino-3-]3-(1-methyl-1H-indolyl)]propionyl] amino-3-(2-pyridyl)propionic acid	Aramori et al., 1993
50-235	27-0-caffeoyl myricerone	Fujimoto et al., 1992
TTA-386	1-(Hexahydro-1H-azepinyl)-carbonyl-Leu-D-Trp-D-Ala-Ala-Tyr-D-Phe	Kitada et al., 1993
ET_B antagonist		
IRL 1038	Cys-Val-Tyr-Phe-Cys-His-Leu-Asp-Ile-Ile-Trp	Urade et al., 1992
Nonselective ET antagonist		
[Thr^{18},$MeLeu^{19}$]-ET-1	Cys-Ser-Cys-Ser-Ser-Leu-Met-Asp-Lys-Glu-Cys-Val-Tyr-Phe-Cys-His-Leu-Thr-MeLeu-Ile-Trp	Wakimasu et al., 1993
PD145065	Ac-(5H-dibenzyl[a,d]cycloheptane-10,11-dihydro-glycine)-Leu-Asp-Ile-Ile-Trp	Doherty et al., 1993
Ro46-2005	4-tert-butyl-*N*-[6-(2-hydroxy-ethoxy)-5(-3-methoxy-phenoxy)-4 pyrimidinyl-benzensulfonamide	Breu et al., 1993

tors have an NH_2-terminal signal sequence, which is rare among such heptahelical receptors, with a relatively long extracellular NH_2-terminal portion preceding the first transmembrane domain. These receptors can be classified into two groups. The order of affinity of ETs for the first receptor type, designated ET_A, is ET-1 $\gg$ ET-3. ET-1 and ET-2 have similar, although not identical, affinities for this subtype of ET receptor. The second receptor type, designated ET_B, shows equal affinity for all three ETs and for sarafotoxins. According to the displacement of radioligand ET-1 bound to ET_A or ET_B receptor expressed in transfected COS7 cells with unlabeled ET-1 or ET-3, the apparent K_i values of ET-1 and ET-3 for ET_A were 3.5×10^{-9} and 1.0×10^{-6} M, and those for ET_B were 9.5×10^{-10} and 2.0×10^{-9} M, respectively (Sakamoto et al., 1993). These results are compatible with the previous pharmacological and ligand-binding experimental results (table 3).

Human or bovine ET_A and ET_B receptors have about 63% amino acid homology. The human ET_B sequence shows a high homology (85%) to the rat or bovine ET_B receptor.

V. Signal Transduction Systems Stimulated by Endothelins

Both types of ET receptors are coupled to phospholipase C via a G-protein (Takuwa et al., 1989; Aramori and Nakanishi, 1992; Sakamoto et al., 1993) after stimulation by ETs. The concentration of intracellular free calcium increases transiently and is then followed by a sustained elevation of the intracellular concentration of the ion. The later sustained level depends on the exterior calcium ion concentration, suggesting that it is due to the opening of a calcium channel(s) in the plasma membrane by ETs. The mechanism(s) of the opening of calcium channel(s) is unclear.

VI. Tissue and Cellular Distribution

Northern blot analysis demonstrated that ET receptor mRNA is widely distributed in various tissues (Arai et al., 1990; Sakurai et al., 1990; Hosoda et al., 1991; Sakamoto et al., 1991; Hori et al., 1992). However, there is an important difference in tissue distribution patterns between the two subtypes. The ET_B receptor is not expressed in the denuded aorta but is found in aortic endothelial cells (Sakurai et al., 1990; Sakamoto et al., 1991; Nakamuta et al., 1991; Fujitani et al., 1993). Hence, ET_B may play some role in the release of endothelium-derived relaxing factor and vasodilator prostanoids from endothelial cells. The ET_A receptor is present on vascular smooth muscle cells and may be responsible for contraction (Hori et al., 1992). However, in some blood vessels, e.g., the rabbit saphenous vein, the rabbit jugular vein, the rat renal vascular bed, and the porcine pulmonary vein, vasoconstriction is mediated by ET_B-like but not ET_A receptors (Sumner et al., 1992; Moreland et al., 1992; Pollack and Opgenorth, 1993; Cristol et al., 1993; Sudjarwo et al., 1993).

VII. Antagonists and Synthetic Agonists for ET_A and ET_B

A linear peptide analogue of ET-1, in which four cysteine residues were replaced by four alanine residues (called 4Ala ET-1), binds to porcine cerebellar membranes. However, the truncated linear peptides 4Ala ET-1 (6 to 21), 4Ala ET-1 (8 to 21), and *N*-acetyl-4Ala ET-1 (10 to 21) selectively bind to ET_B receptors (Saeki et al., 1991). In addition, BQ3020 (AcLMDKEAVYFAHLDIIW) Ihara et al., 1992) and IRL 1620 (suc-DEEAVYFAHLDIIW) (Takai et al., 1992; Watanabe et al., 1992; Karaki et al., 1993a) are selective agonists for the ET_B receptor. The amino acid sequences of the carboxyl-terminal ends of those peptides are the same as in ET-1. These peptides cause endothelium-dependent vasodi-

TABLE 5
Summary of ET receptors

Endogenous ligands	ET-1, ET-2, ET-3	
Nonselective antagonists	PD145065, Ro-46- 2005 [Thr^{18},Meleu^{19}]-ET-1	
Receptor subtype	ET_A	ET_B
Response	Vasoconstriction	Vasodilation (vasoconstriction) Aldosterone release
Selective agonists		Sarafotoxin S6c 4Ala ET-1 BQ-3020 IRL-1620
Selective antagonists	BQ-123 FR-139317 50-235 TTA-381	IRL-1038
Potency order (K_i for ^{125}I-ET-1 binding, M)	ET-1 > ET-3 ET-1 = 3.5×10^{-9} ET-3 = 1.0×10^{-6}	ET-1 = ET-3 ET-1 = 9.5×10^{-10} ET-3 = 2.0×10^{-9}
Antagonist potency (K_i for ^{125}I-ET-1 binding)	BQ-123 = 7.3 nM 50-235 = 86 nM FR-139317 = 0.53 nM TTA-386 = 0.34 nM	IRL-1038 = 6–11 nM BQ-123 = 18 M
Radioligands	^{125}I-ET-1, ^{125}I-ET-2 ^{125}I-Sarafotoxin S6b	^{125}I-ET-1, ^{125}I-ET-2, ^{125}I-ET-3 ^{125}I-Sarafotoxin S6b ^{125}I-BQ3020, ^{125}I-IRL-1620
Gene	Human chromosome 4 (hET-AR)	Human chromosome 13 (hET-BR)
Structural information	427 amino acid human 7TM	442 amino acid human 7TM

lator activity in porcine pulmonary arterial strips (Karaki et al., 1993b).

Specific antagonists to ET_A and/or ET_B receptors are presented in table 4 (Ihara et al., 1991a; Spinella et al., 1991; Urade et al., 1992; Fujimoto et al., 1992; Aramori et al., 1993; Doherty et al., 1993; Kitada et al., 1993; Breu et al., 1993). The nonselective ET antagonist PD145065 is a pentapeptide analogue of the carboxyl-terminal sequence of ET-1 (Doherty et al., 1993). The ET-1 analogue [diaminopropionic acid 1, Asp^{15}]ET-1 was reported to be an ET_A-specific antagonist (Spinella et al., 1991). BE18257B, cyclo(-D-Glu-L-Ala-allo-D-Ile-L-Leu-D-Trp), a product of *Streptomyces misakiensis* (Ihara et al., 1991a) and its derivatives, particularly BQ-123, which are cyclic pentapeptides, discriminate between ET_A and ET_B receptors (Ihara et al., 1991a). The sequence of BQ-123 is similar to the sequence of the carboxyl-terminal side of ET-1. It is potent and highly selective for ET_A receptors [IC_{50} 7.3 nM and 18 M for ET_A and ET_B, respectively, for binding of iodine-labeled ET-1 (Ihara et al., 1991b)]. FR-139317 (Aramori et al., 1993) was also obtained through chemical modifications of BE-18257 B and is selective for ET_A. 50–235 is a nonpeptide ET_A antagonist isolated from bayberry, *Myrica cerifera* (Fujimoto et al., 1992). The structure of 50–235 is also similar to that of BQ-123. So far only one antagonist selective for the ET_B receptor IRL 1038 has been described (Urade et al., 1992; Karaki et al., 1993a). This ET_B antagonist is based on the carboxyl-terminal sequence of ET-1 (11–21) but includes a disulfide bond between Cys^1 and Cys^5 (Urade et al., 1992). These results strongly suggest that binding sites of ET_A and ET_B receptors for the agonists and antagonists are very similar (Adachi et al., 1992; Sakamoto et al., 1993).

VIII. Pharmacological Evidence Suggesting the Existence of Additional Receptor(s)

Based on studies of cultured rat anterior pituitary cells (Martin et al., 1990), rat PC12 pheochromocytoma cells (Samson et al., 1990), and cultured human endothelial cells (Yokokawa et al., 1991), the existence of a receptor specific for ET-3 has been suggested. Northern blots of rat mRNA probed with ET_A cDNA revealed two mRNA species (Hori et al., 1992). However, Southern blot analysis of human DNA revealed the existence of only two ET receptor genes, probably corresponding to the ET_A and ET_B genes (Sakamoto et al., 1991). Indeed, an analysis of human ET genes revealed eight and seven exons for ET_A and ET_B genes, respectively, and suggested only one product from each gene (Hosoda et al., 1992; Arai et al., 1993). Additional mammalian ET receptors, if they exist, must be markedly differently from both ET_A and ET_B receptors.

IX. Conclusion

A survey of currently published papers shows that known ET receptors can be classified basically into three types. Because ET-2 as an agonist is very similar to ET-1, the ET receptors are characterized by the affinity rank order ET-1 > ET-3, ET-1 = ET-3, and ET-1 < ET-3 (table 5). These receptors have been designated ET_A, ET_B, and ET_C, respectively. The molecular cloning of the latter, ET_C receptor, has only been demonstrated so far in nonmammalian cells.

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