0031-6997/96/4802-0253$03.00/0
PHARMACOLOGICAL REVIEWS

Vol. 48, No. 2
Printed in U.S.A.

Calcitonin Gene-Related Peptide in the Cardiovascular System: Characterization of Receptor Populations and Their (Patho)physiological Significance

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I. Introduction

The importance of the autonomic nervous system in the regulation of cardiovascular function is well established. Many investigators have attempted to classify the populations of selective receptors and the intracellular coupling mechanisms associated with the physiological effects of the classical autonomic neurotransmitters, acetylcholine and noradrenaline. Within the last decade, it has become apparent that many more substances exert a regulatory influence upon myocardium, endothelium and vascular smooth muscle, either as neurotransmitters, neuromodulators, hormones, or as paracrine agents released from neighbouring cells. Among a growing list of such substances are cardioactive neuropeptides, which co-exist with the traditional autonomic neurotransmitters in the cardiovascular nervous supply. These include those peptides colocalized with noradrenaline in the sympathetic innervation, such as neuropeptide Y, those colocalized with acetylcholine in the parasympatheic innervation, such as vasoactive intestinal peptide, peptide histidine isoleucine and somatostatin, and those colocalized in the nonadrenergic noncholinergic (NANC) sensory innervation, such as the tachykinins and calcitonin gene-related peptide (CGRP). Although the effects of many of these substances on the cardiovascular system were identified initially in intact animals, much of the information about the biochemical mechanisms that mediate these physiological effects has been obtained from simpler experimental models in vitro, such as isolated perfused hearts, blood vessels and strips of papillary muscles. Characterization of receptors for these substances on, and receptor-effector coupling within, isolated cardiovascular cells is a relatively new scientific pursuit.

The molecular genetics, biology and structure of CGRP has been reviewed extensively (Fischer and Born, 1985; Zaidi et al., 1987; Breimer et al., 1988). Several excellent reviews covering the peripheral haemodynamic effects of CGRP have been published previously with accent on molecular biology and localization (McEwan et al., 1989b), the regulation of human cardiovascular haemodynamics (Preibisz, 1993) and clinical applications (Shulkes, 1993). In the present overview, the peripheral haemodynamic effects of CGRP have been summarized in the light of more recent findings, and emphasis has been placed on the emerging evidence concerning less well documented actions of the peptide such as the regulation of inotropy, chronotropy and microvascular permeability and novel effects, such as the regulation of angiogenesis in the vasculature and hypertrophic effects in the myocardium, and the protection of the myocardium against ischaemia-reperfusion injury. The development and use of analogues and fragments of CGRP have been fundamental in the recent advances made in characterization of CGRP receptor subtypes and their physiological significance. Therefore, in this review, the criteria for the classification of subtypes of CGRP receptors is described to provide a basis for discussion of the characterization of CGRP receptors in the cardiovascular system. In view of the close structural similarity of CGRP to amylin and salmon calcitonin, the present knowledge about the actions of each of these peptides in the cardiovascular system has been compared and contrasted, where appropriate, with those of CGRP. The major focus of this article, however, is to assess what is known about the receptor interactions and cellular mechanisms by which the peptide exerts its various effects on the vasculature and myocardium. Until comparatively recently, most of these investigations were conducted in multicellular preparations in vitro. However, the progression toward the use of homogenous populations of cardiovascular cells such as cardiomyocytes, endothelial cells and vascular smooth muscle cells for the study of receptor pharmacology and receptor-effector coupling mechanisms represents a major advance in that it enables the direct effects of the peptide in a given cell type to be dissected from those in the tissue as a whole. In this article, we will review the evidence for (*a*) regional heterogeneity in the magnitude, endothelial-dependence and cellular mechanisms associated with the action of CGRP in the peripheral and coronary vasculature and for (*b*) variation between atrial and ventricular myocardium with respect to the signal transduction cascades activated in response to CGRP. Finally, our conclusion is based on examining what understanding exists regarding the involvement of

Abbreviations: NANC, nonadrenergic noncholinergic; CGRP, calcitonin gene-related peptide; hCGRP, human CGRP; rCGRP, rat CGRP; CT, calcitonin; cAMP, cyclic adenosine monophosphate; mRNA, messenger ribonucleic acid; CNS, central nervous system; ACM, acetamidomethyl cysteine; NO, nitric oxide; IP, standard nomenclature for the subtype of prostanoid receptor at which prostacyclin (PGI_2) is an agonist; L-NAME, N^G-nitro-l-arginine methyl ester; cGMP, cyclic guanosine monophosphate; ATP, adenosine triphosphate; 5-HT, serotonin.

CGRP in the various physiological and pathophysiological states of the cardiovascular system and the rationale for therapeutic intervention.

II. Discovery

Alternative tissue-specific processing of primary messenger ribonucleic acid (mRNA) from the calcitonin gene (α gene) of rats generates two distinct peptides, calcitonin (rCT) and calcitonin gene-related peptide (rαCGRP) (Rosenfeld et al., 1983; Amara et al., 1984, 1985). CGRP is expressed predominantly in the nervous system and calcitonin in the thyroid gland. The existence of a structurally similiar peptide in humans was first demonstrated by isolation of human CGRP-α (hαCGRP) from a human medullary thyroid carcinoma (Morris et al., 1984). Another CT/CGRP gene (β gene), thought to have arisen by exon duplication, and generating the alternative β-forms of the peptide, rβCGRP and hβCGRP, was subsequently identified in rats and humans, respectively (Steenbergh et al., 1985; Alevizaki et al., 1986). There is no evidence for the expression of a second form of CT mRNA from the β-gene; although a CT-like sequence is present in the β-gene, it is unlikely that this is expressed because no alternative splice acceptor site has been identified (Steenbergh et al., 1986; Alevizaki et al., 1986). Although these α and β genes are almost exclusively, or predominantly, expressed as either CT or CGRP in a given tissue, co-expression has been demonstrated recently in bronchopulmonary neuroendocrine cells of rats (Shimosegawa and Said, 1991).

III. Structure of Calcitonin Gene-Related Peptide and Related Peptides

The amino acid sequences of the various forms of CGRP presently known are depicted in figure 1. CGRP is a polypeptide that contains 37 amino acids constituted as a single chain. The peptide is highly conserved in different mammalian species, between which 26 amino acids are homologous (Collyear et al., 1991). The β-forms differ from the corresponding α-forms by one and three amino acids in rats and humans, respectively (Morris et al., 1984; Steenbergh et al., 1985; Amara et al., 1985; Peterman et al., 1987; Wimalawansa et al., 1990). The structure of hαCGRP comprizes an N-terminal disulfide bonded loop (amino acids 2 to 7), a well defined α-helix (amino acids 8 to 18) and a turn type conformation (amino acids 19 to 21) that leads into an area of predominantly disordered structure before terminating in a carboxy-terminal amide group (Breeze et al., 1991). Comparison of the structure of hβCGRP indicates that the conformation adopted by each form of the peptide is virtually identical.

CT is single chain polypeptide containing 32 amino acids and also possesses a disulfide bonded loop conformation at the amino terminus and a proline amide structure at the carboxy terminus. CTs can be classified into three groups on the basis of their primary structures, which are: (*a*) the artiodactyl group (pig, sheep, cow); (*b*) primate/rodent group; (*c*) teleostean and avian group (salmon, eel, chicken). Homology is high within groups but low between groups. In addition to hCT, rCT and bCT, isolated from the respective thyroid glands, these mammals have been shown to possess salmon CT-like peptides (sCTs) (Fischer et al., 1983; Perez-Cano et al., 1982; Henke et al., 1985). $rCGRP_{1-37}$ and sCT_{1-32} have 30% similarity in their amino acid sequences (figs. 1 and 2), whereas rCT and hCT have only 23% homology with $rCGRP_{1-37}$. Alignment of amino terminal loop structures and of hydrophilic carboxyl terminal amides of sCT and CGRP reveals closer secondary structural similarities between these two peptides.

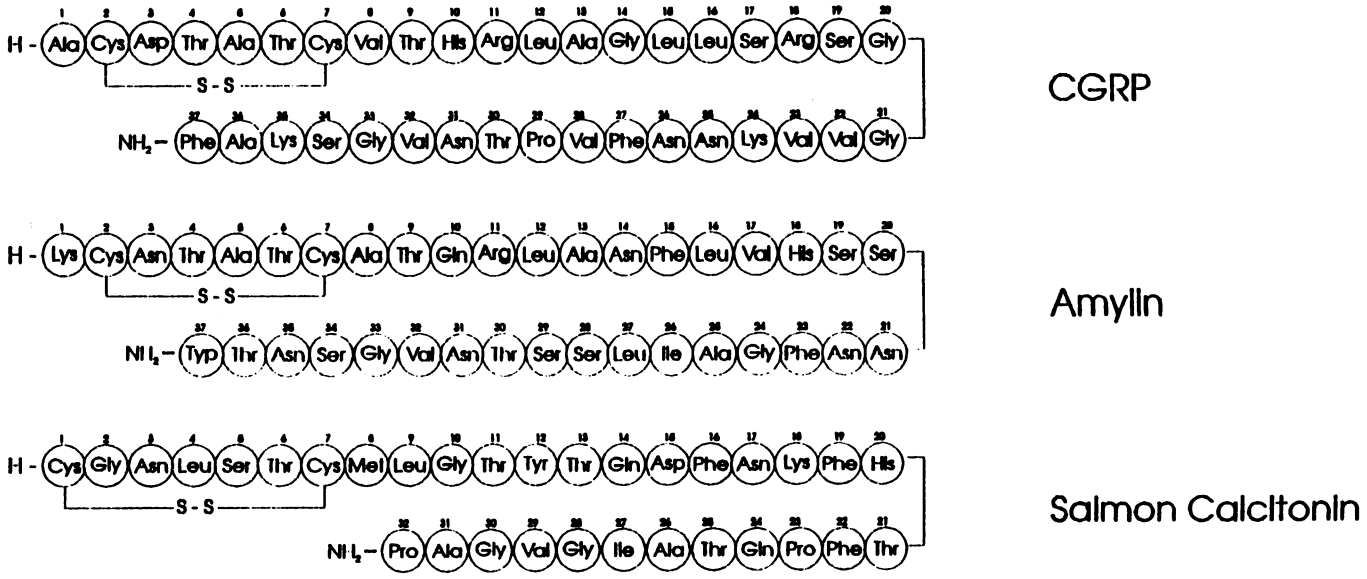
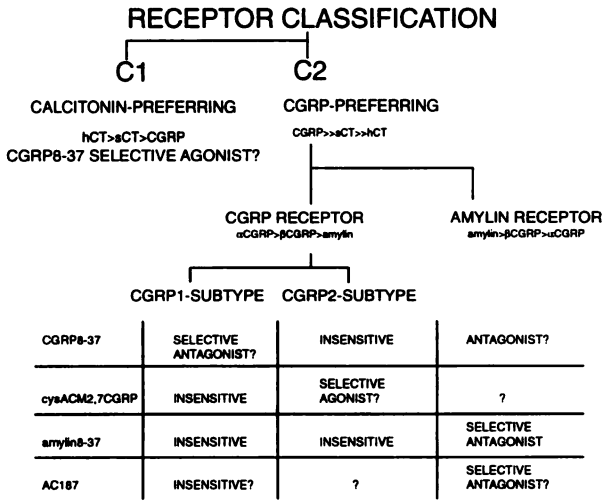


FIG. 1. Primary structures of hαCGRP, salmon calcitonin and human amylin.



CGRP8-37	SELECTIVE ANTAGONIST?	INSENSITIVE	ANTAGONIST?
cysACM2,7CGRP	INSENSITIVE	SELECTIVE AGONIST?	?
amylin8-37	INSENSITIVE	INSENSITIVE	SELECTIVE ANTAGONIST
AC187	INSENSITIVE?	?	SELECTIVE ANTAGONIST?

FIG. 2. Criteria proposed for the classification of receptors for the calcitonin/CGRP family of peptides.

CGRPs share 46 to 50% homology in their sequence of amino acids with the recently discovered peptide, amylin (fig. 1) (Rink et al., 1993), isolated from amyloid deposits present in pancreas of patients with noninsulin-dependent diabetes mellitus (Cooper et al., 1987, 1989; Chantry et al., 1991). These peptides are identical in length and have two homologous posttranslational modifications; a C terminal amide and an intramolecular disulphide bond (Cooper et al., 1988; Rink et al., 1993). CGRPs also have some homology in their sequence of amino acids with adrenomedullin, a novel peptide derived from human phaechromocytoma tissue, which contains 52 amino acids (Kitamura et al., 1993).

IV. Distribution

CGRP is extensively distributed throughout the brain and spinal cord, often coexisting in the same population of neurons with other neurotransmitters (Gibson et al., 1984; Lundberg et al., 1985; Zaidi et al., 1987). Within the peripheral nervous system, CGRP is present in the sensory ganglia, often costored with substance P. Together with substance P, CGRP-rich nerve fibres form part of the primary afferent nervous system, comprising capsaicin-sensitive A(δ) and C fibre afferent nerves, and "type B" medium-sized cells (McCullough et al., 1986; Uddman et al., 1986; Zaidi et al., 1987). Lower amounts of CGRP are synthesized in motor neurons in coexistence with acetylcholine (Rosenfeld et al., 1983; Takami et al., 1985; Mora et al., 1989; Popper and Micevych, 1989; Uchida et al., 1990) and in autonomic sympathetic ganglionic fibres (Zaidi et al., 1987). In contrast to most other CGRP-containing neurons that contain CGRP-α, the β-form of the peptide is found in neurons of the enteric nervous system (Mulderry et al., 1985b, 1988; Self et al., 1985; Grunditz et al., 1986). Immunoreactivity to CGRP-α is present in thyroid C cells and released from the thyroids of newborn rats in vitro (Cooper et al., 1985). Indeed, the highest concentration of CGRP-α outside the central nervous system (CNS) is found in the thyroid (Wimalawansa et al., 1987). Human pituitary is an extremely rich source of the β-form of the peptide (Petermann et al., 1987; Jonas et al., 1985). β-cells of pancreatic islets are the major site of amylin biosynthesis. There is, however, evidence for the production of amylin in tissues of the gut (Ohtsuka et al., 1993; Mulder et al., 1994), lung and CNS albeit at markedly lower concentrations than in the pancreas (Cooper, 1994).

A. The Cardiovascular System

1. The systemic vasculature. CGRP-immunoreactive nerve fibres are distributed widely in the cardiovascular system and generally are more numerous around arteries than veins. Varicose and smooth CGRP-immunoreactive fibres are seen at the junction of the adventitia and the media and passing into the muscle layer (Holzer, 1988). Human blood vessels generally display a lower density of CGRP-immunoreactive fibres than those of guinea pigs and rats (Wharton and Gulbenkian, 1987). CGRP-immunoreactive nerve fibres are particularly abundant in the superior mesenteric (Holzer, 1988), renal, femoral (Edvinsson et al., 1989c), cerebral (Hanko et al., 1985; Uddman et al., 1985) and carotid arteries, in pial (cortical) arterioles (McCullouch et al., 1986) and in the abdominal aorta, including its bifurcation (Mulderry et al., 1985a; McCullough et al., 1986). Such fibres are comparatively less abundant in the aortic arch and thoracic aorta. Smaller arteries in the respiratory, gastrointestinal (Holzer and Guth, 1991) and genito-urinary tracts (Uddman et al., 1986), and small arterioles in skin (Dalsgaard et al., 1989) possess numerous CGRP-immunoreactive fibres. High density CGRP-innervation, occurring as a dense varicose mesh with occasional penetrating branches, is found in the inferior vena cava and in renal and femoral veins.

2. The heart. CGRP coexists with substance P and other tachykinins in nerves innervating the hearts of several mammals, including rats and guinea pigs (Mulderry et al., 1985a) and humans (Rechardt et al., 1986; Franco-Cereceda et al., 1987c; Opgaard et al., 1995), but at a much lower density than in major blood vessels (Lundberg et al., 1985). Consistently higher amounts of CGRP-immunoreactivity are found in atria than in ventricles (Mulderry et al., 1985a; Lundberg et al., 1985; Ishikawa et al., 1987; Franco-Cereceda et al., 1987c). Within atria, CGRP-immunoreactivity is localized especially in the sino-atrial node, atrio-ventricular node and specialized conduction system (Mulderry et al., 1985a; Saito et al., 1986, 1987). Myocardium is much less densely innervated than epicardium, endocardium and pericardium (Mulderry et al., 1985a; Ishikawa et al., 1988; Wimalawansa and MacIntyre, 1988). Cardiac valves receive a moderate innervation, as do coronary blood vessels (Opgaard et al., 1995), especially those supplying the right atrium (Gibbens et al., 1985; Gulbenkian et al., 1990). CGRP-immunoreactivity is found

only rarely within the ventricles of pigs (Miyauchi et al., 1988), rats (Mulderry et al., 1985a; Ishikawa et al., 1987) and guinea pigs (Shoji et al., 1987; Franco-Cereceda et al., 1987c), often in association with the coronary vasculature (Uddman et al., 1986). In right and left ventricular walls and interventricular septum, CGRP-immunoreactive fibres run parallel to coronary arteries and their collaterals. From this perivascular plexus, some nerve fibres extend into the myocardium and run beside individual muscle fibres, forming a nerve plexus that is particuliarly well developed within papillary muscles. CGRP-immunoreactivity has also been identified in nerve fibres present in subepicardial ganglia of rats (Forsgren et al., 1990). Overall, a similiar distribution pattern is found in canine hearts (Sugiyama et al., 1989; Ursell et al., 1991a, 1991b; Quebbeman et al., 1993). In this species, however, there is a perinatal peak in the density of CGRP-immunoreactivity that declines subsequent to the sparse pattern of distribution found in adult hearts, indicating that CGRP may have a developmental role in the canine heart.

3. Plasma. During the last few years, considerable efforts have been made in developing and validating assay procedures for the assessment of the circulating concentration of CGRP. CGRP circulates in the plasma of rats (Zaidi et al., 1985) and humans (Zaidi et al., 1986; Struthers et al., 1986). In the plasma of healthy humans, the concentrations of the peptide have been found to be undetectable in a few studies (Edbrooke et al., 1985; Kraenzlin et al., 1985), to average 2 to 35 pM in most (Girgis et al., 1985; Stevenson et al., 1986; Schifter, 1989; Edvinsson et al., 1989a; Tang et al.; 1989; Oder-Cederloef et al.; 1989; Transforini et al., 1991), and to reach a mean value of 94 pM in a group of subjects selected in a wide range of blood pressures (Hvarfner et al., 1988). Such values are less than, and do not correlate with, the concentrations of CT in plasma (Girgis et al., 1985). The immunochemical heterogeneity of the CGRP molecule and the specificity of the antisera used for the radioimmunoassay might account for discrepancies in the reported levels of tissue or circulating CGRP under experimental conditions. Thyroidectomy reduces the concentration of CGRP in plasma by 30 to 70% in old but not in young rats (Zaidi et al., 1986), whereas colchicine, which blocks transport of the peptide along nerve axons and its subsequent release from nerve terminals, causes a dramatic fall in the concentration of CGRP in plasma, regardless of age (Zaidi et al., 1985). This indicates that, although circulating CGRP reflects mainly the overspill of peptide from perivascular nerves, the thyroid, in rats at least, makes an increasingly important contribution with advancing age. The peptide circulates in humans with an underlying circadian rhythm (Transforini et al., 1991; Wimalwansa, 1991). Concentrations of circulating CGRP in human plasma have been found to decrease (Hvarfner et al., 1988) or remain unchanged (Schifter, 1989) with advancing age. The concentrations of the peptide are elevated during pregnancy (Stevenson et al., 1986), although there is evidence both for (Hvarfner et al., 1988) and against (Schifter, 1989) a higher concentration of CGRP in the plasma of healthy nonpregnant females than in males. Conflicting findings may reflect the need to consider variation in the circulating concentrations of CGRP during different phases of the menstrual cycle (Valdermarsson et al., 1990). It is uncertain whether CGRP circulating in plasma has a hormonal function. However, it is apparent that after intravenous infusion, CGRP can reach appropriate target tissues from plasma (Fischer and Born, 1985; Gennari and Fischer, 1985).

Amylin-like immunoreactivity has been measured in circulating plasma by a variety of radioimmunoassays, most of which use rabbit anti-amylin antiserum and an extraction and concentration procedure to increase sensitivity (Nakazoto et al., 1989; Hulst et al., 1994). The concentrations of amylin detected in the plasma of healthy humans range generally from 1 to 10 pM in fasting individuals (Hartter et al., 1991; Koda et al., 1992; Bretherton-Watt et al., 1993), although a value of 40.5 pM has been reported in one study (Kautzky-Willer and Thomaseth, 1994), and from 5 to 20 pM upon postprandial stimulation or upon oral or intravenous administration of glucose (Hartter et al., 1991; van Jaarsveld et al., 1993). These values have generally been lower than those reported in rodents (30 to 100 pM) (Gill and Yen, 1991; Rink et al., 1993). Evidence from studies in animal models of diabetes support a mainly pancreatic origin of circulating amylin. It is unclear at present whether the lower reported concentrations in humans are a product of variations between assays e.g., human amylin has a greater tendency than rat amylin to aggregate and adhere to surfaces, or whether there are real differences in the biology of amylin between different groups of mammals.

B. The Actions of Capsaicin

Capsaicin, a chemical irritant extracted from hot chili peppers, initially depolarizes and leads subsequently to the selective degeneration of thin unmyelinated Aδ and C sensory nerve fibres. This compound was shown to induce the release of CGRP from the spinal cords of rats in a calcium-dependent manner (Saria et al., 1986). Concentrations of CGRP in plasma rise dramatically but transiently after administration of capsaicin in adult rats (Zaidi et al., 1985). Capsaicin induces a profound (88 to 99%) long-term loss of CGRP and a parallel loss of the costored peptide, substance P, from the cardiovascular system of adult rats and guinea pigs (Wharton et al., 1986). This loss is associated with depletion of CGRP-immunoreactivity from sensory nerve fibres innervating the myocardium and coronary vessels (Lundberg et al., 1985; Wimalawansa, 1993). There is conflicting evidence concerning whether administration of capsaicin to neonatal rats alters significantly the concentration of CGRP

present in the cardiovascular system in adult life (Mulderry et al., 1985a; Wimalawansa, 1993). The more varied responses to capsaicin observed in neonatal rats may reflect an ability to regenerate sensory afferent neurons.

C. Metabolism of Calcitonin Gene-Related Peptide

The half-life of CGRP in mammalian plasma is approximately 10 minutes (Struthers et al., 1986). Many target cells for CGRP contain a cell surface enzyme, neutral endopeptidase (enkephalinase). Although this enzyme can cleave and so inactivate CGRP, the weak substrate-enzyme interaction, predicted from available kinetic data, indicates that CGRP is not the preferred substrate for this enzyme (Katayama et al., 1991). Other substrates include substance P, neurotensin and neurokinin-A. The ability of CGRP to compete with substance P for degradation may explain the potentiation by CGRP of several biological actions of substance P at concentrations at which CGRP itself is devoid of activity. A tryptase enzyme, derived from mast cells of the human lung, and the widely distributed enzyme, chymotrypsin, can also metabolize substance P and CGRP (Greves et al., 1989). It is probable that more specific enzymes responsible for the selective degradation of CGRP remain to be discovered.

V. Classification of Receptors for Calcitonin Gene-Related Peptide

Using tissue autoradiography and radioligand binding techniques performed on crude suspensions of membranes, CGRP binding sites, varying in their affinity for the peptide, have been detected in a wide spectrum of tissues. Because of their close structural similarities, CGRP and CT often recognize identical populations of binding sites (Goltzman and Mitchell, 1985; Wohlwend et al., 1985; Yamaguchi et al., 1988). Consequently, binding sites, identified initially in the CNS (Goltzman and Mitchell, 1985) and subsequently in the periphery (Seitz and Cooper, 1987; Seifert et al., 1985; Vanvalen et al., 1990), were classified on the basis of their relative affinity for these two peptides, into CT-preferring (or C_1), CGRP-preferring (or C_2) and nonselective (or C_3) receptors possessing high affinity for both peptides. Because more than one type of receptor may coexist in the same tissue (Goltzman and Mitchell, 1985; Seitz and Cooper, 1987; Henke et al., 1985), it is often difficult to elucidate the direct actions of each peptide at its particular receptor.

The C-terminal fragment, $CGRP_{8-37}$, despite lacking the disulphide bonded-loop structure contained in amino acids 1 to 7, (Chiba et al., 1989; Dennis et al., 1990), retains the high affinity of the native peptide. $CGRP_{8-37}$ is a full antagonist at CGRP-preferring receptors in rat liver and guinea pig ileum (Yamaguchi et al., 1988; Chiba et al., 1989; Dennis et al., 1990), although it possesses some efficacy at CT-preferring receptors in porcine kidney (Chiba et al., 1989; Aiyar et al., 1991). On the basis of its action as a selective partial agonist at CT-preferring receptors, and as a selective antagonist at CGRP-preferring receptors, it was proposed that $CGRP_{8-37}$ would be a useful agent for the classification of receptors for this family of peptides (Chiba et al., 1989). Similiar findings have been reported for the shorter fragments, $CGRP_{11-37}$ (Mimeault et al., 1991), $CGRP_{12-37}$ (Dennis et al., 1989), $CGRP_{19-37}$, $CGRP_{23-37}$ (Rovero et al., 1992) and Tyr-0 $CGRP_{28-37}$ (Maton et al., 1990; Chakder and Rattan, 1990) in a number of tissues. Although these fragments possess markedly less affinity than $CGRP_{8-37}$, they retain selective antagonism at CGRP-preferring receptors. Subsequent identification of CGRP-preferring receptors in rat vas deferens preparations at which $CGRP_{8-37}$ was much less able to antagonize the actions of CGRP has necessitated further subdivision of CGRP-preferring receptors into the $CGRP_1$ subtype, at which $CGRP_{8-37}$ behaves as a full antagonist (pA2 value = 7 to 8) and the $CGRP_2$ subtype, at which $CGRP_{8-37}$ is considerably less active (pA2 value $\leq$ 6.2) (Dennis et al., 1989; Mimeault et al., 1991). The fact that such fragments act as rather potent antagonists in certain tissues suggests that it is critical to study the metabolism of CGRP to determine whether some of these fragments can be generated in vivo.

Reduction of the disulphide bond, by substitution of the hydrogen atoms of amino acids at positions 2 and 7 by acetamidomethyl moieties, destroys the N-terminal ring structure of CGRP and yields the linear analogue, Cys acetamido-methyl cysteine ACM 2,7 CGRP (Cys ACM 2,7 CGRP). It has been proposed that this analogue is a selective agonist at the $CGRP_2$ receptor subtype, at which the analogue possesses only moderately less affinity than the native peptide, while possessing greatly reduced affinity at the $CGRP_1$ receptor subtype (Seifert et al., 1985; Dennis et al., 1989). The high affinity of Cys ACM 2,7 CGRP and, to a lesser extent, of cyclo-Asp^2Lys^7 CGRP, an analogue in which the side chains of the substituted asparagine residue in position 2 and lysine residue in position 7 are linked together by an amide bond, for recognition of binding sites for CGRP in membrane homogenates prepared from the brain and spleen of rats provides further evidence that the disulphide bridge between amino acids 2 and 7 is not essential for binding of CGRP to its receptor (Dennis et al., 1989).

Binding sites possessing almost equal affinity for CGRP and salmon CT have been identified in the nucleus accumbens of rats (Sexton et al., 1988; Dennis et al., 1991). Binding of the native peptides to these binding sites can be displaced by both $CGRP_{8-37}$ and Cys ACM 2,7 CGRP. These findings are clearly inconsistent with the elementary classification outlined above (from the beginning of Section V.).

Other evidence also suggests that subtypes of CGRP-preferring receptors exist: e.g., salmon CT, does not compete for binding to CGRP binding sites present in nu-

cleus accumbens, lateral bed nucleus of stria terminalis and central amygdaloid nucleus but does in other regions of the CNS such as cortex, striatum and brainstem, indicating interregional variation in the properties of these CGRP receptors (Dennis et al., 1990; Chatterjee and Fisher, 1991). In addition, Cys ACM 2,7 CGRP is equipotent to the native peptide in displacing radiolabeled hαCGRP from binding sites in rat brain homogenates, but displays only half the potency of CGRP in displacing the radioligand from binding sites located on splenic membranes of rats (Dennis et al., 1989).

The existence of receptors selective for amylin against CGRP is uncertain. Although amylin cross-reacts at CGRP-preferring receptors in a number of tissues including brain, skeletal muscle and liver (Chantry et al., 1991; Galeazza et al., 1991; Silvestre et al., 1993; Young et al., 1993; Zhu et al., 1991), and with very high affinity at receptors selective for salmon CT against CGRP in the nucleus accumbens (Rink et al., 1993), the metabolic effects exerted by amylin in skeletal muscle may be mediated by a population of receptors distinct from either CGRP-preferring or CT-preferring receptors (Kreutter et al., 1993). In addition, Chinese hamster ovary-KI cells possess a population of receptors at which amylin exhibits greater affinity than CGRP, and at which $CGRP_{8-37}$ is devoid of antagonistic activity (Beaumont et al., 1995). It was proposed recently that the novel compound, AC187, may be a selective antagonist at these amylin-preferring receptors (Young et al., 1994), whereas AC137, which contains proline substitutions at positions 25, 28 and 29 to limit self-aggregation, has been developed as a potent synthetic analogue of human amylin (Gottleib and Kolterman, 1994). There is also preliminary evidence for cross-reactivity of CGRP and amylin at receptors for adrenomedullin in certain tissues (Owji et al., 1995). This is based on the displacement by CGRP and amylin of ^{125}I-adrenomedullin from binding sites in the heart but not lung.

Although multiple subtypes of receptors for this family of peptides do exist, consistent pharmacological criteria for their classification remain to be established (fig. 2). It is also uncertain why two highly similiar forms of CGRP (α and β) display tissue-specific expression in a mutually exclusive manner. hβCGRP displaces radiolabeled hαCGRP binding from membrane homogenates in brain and spleen tissue of rats more potently than hαCGRP, rαCGRP and rβCGRP (Sigrist et al., 1986; Dennis et al., 1989). By contrast, rαCGRP and rβCGRP are equipotent as relaxants of strips of colonic smooth muscle of rats in vitro (Spokes et al., 1987), and there is no evidence that the receptors that mediate the responses to each peptide in rat vas deferens (Butler et al., 1993) and oppossum internal anal sphincters (Chakder and Rattan, 1990) can be differentiated in terms of their sensitivity to $CGRP_{8-37}$. However, the existence of selectivity of each form of CGRP for distinct subtypes of receptor cannot be excluded at present (Wimalawansa et al., 1990). Cross-linking of binding sites by radiolabeled peptides, followed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), has confirmed the existence of distinct CGRP-preferring and CT-preferring receptors. There is substantial heterogeneity in published estimates of the molecular weights of CGRP binding sites that vary between 13 and 400 kDa, although the majority fall within the range of 60 to 70 kDa (Aiyar et al., 1991; Hirata et al., 1988; Foord and Craig, 1987; Sigrist et al., 1986; Chantry et al., 1991). The amylin binding site isolated from porcine nucleus accumbens has been characterized as a 66 kDa protein (Wimalawansa et al., 1993). No sequence data of any of these proteins have been published. At present, it is uncertain whether such variation (*a*) is a consequence of the limited proteolysis of receptors during purification, (*b*) reflects their variable glycosylation between tissues, or (*c*) genuinely indicates the heterogeneity of CGRP-preferring, CT-preferring and amylin-preferring receptors.

CGRP-preferring receptors are coupled to production of the second-messenger substance, cyclic adenosine monophosphate (cAMP), in a wide range of tissues (Takami et al., 1986; Michelangeli et al., 1986; McEwan et al., 1989a; Osborne and Barnett, 1991). In addition, CGRP stimulates weakly the production of cAMP by interacting with CT-preferring receptors located in the kidneys of rats and pigs (Wohlwend et al., 1985; Goltzman and Mitchell, 1985; Fischer and Born, 1985; Aiyar et al., 1991) and in the CNSs of rats (Goltzman and Mitchell, 1985) and humans (Fischer and Born, 1985). Coupling of CGRP-mediated effects to production of cAMP is not, however, universal. CGRP-preferring receptors that are not coupled to production of this second-messenger substance are present in the cerebellum and spinal cord of rats (Goltzman and Mitchell, 1985; Henke et al., 1985) and humans (Fischer and Born, 1985; Tschopp et al., 1985) and in the adrenal glands of rats (Goltzman and Mitchell, 1985). Receptors for salmon CT that are not coupled to the production of cAMP have also been found in the CNSs of rats and humans (Fischer and Born, 1985). A population of receptors for CGRP that are coupled to phosphatidylinositol turnover has been detected in skeletal muscle (Laufer and Changeux, 1989).

A. Cardiovascular Receptors

Studies of the regional distribution in rats in vivo indicate that the greatest densities of CGRP binding sites are present in the heart and within the intima and media layers of blood vessels (Sigrist et al., 1986; Wimalawansa et al., 1987). Within the systemic circulation, the highest densities of binding sites have generally been found in peripheral, mesenteric, femoral and carotid arteries and in caval veins (Wimalawansa and MacIntyre, 1988) and in the spleen (Sigrist et al., 1986); however, lower densities are present in the major ves-

sels (Nakamata et al., 1986), renal arteries and pulmonary arteries and veins (McCormack et al., 1989). Binding sites corresponding to proteins of molecular weight approximately 60 kDa and displaying equal affinity for rCGRPα and hαCGRP and greatly reduced affinity for CT, have been localized on isolated vascular smooth muscle cells from rats (Hirata et al., 1988) and bovine aortic endothelial cells (Hirata et al., 1988). CGRP displaces binding of ^{125}I-adrenomedullan from binding sites present in cultured rat vascular smooth muscle cells (Eguchi et al., 1994).

Binding sites of high affinity for CGRP have been found in crude membrane preparations of atrial and ventricular tissue (table 1). In atria of rats, a single homogeneous population of receptors with high affinity (K_d = 0.085 to 1.5 nM) has been detected at which rCGRPα and hαCGRP bind with equal potency (Spokes et al., 1987; Yoshizaki et al., 1987; Wimalawansa and MacIntyre, 1988). Receptors possessing high affinity for CGRP are present in suspensions of crude membranes and soluble membrane fractions prepared from ventricular tissue of pigs (Miyauchi et al., 1988) and rats (K_d = 34 pM) (Yoshizaki et al., 1987) and in both atrial and ventricular tissue obtained from human hearts (Henke et al., 1987). In contrast, receptors possessing low affinity (K_d = 59 nM) for the peptide have been observed only in a few preparations (Yoshizaki et al., 1987). Regardless of species, the density of binding sites for CGRP in the atria invariably exceeds that in the ventricles (Sigrist et al., 1986; Henke et al., 1987). hαCGRP and hβCGRP display similar binding characteristics and appear to recognize the same populations of receptors (Henke et al., 1987; Wimalawansa and MacIntyre, 1988). The fragments, $CGRP_{8-37}$ and $CGRP_{9-37}$, displace radiolabeled CGRP from its binding sites with greater potency than CGRP itself in membrane suspensions prepared from the vas deferentia and CNS of rats, but with similar potency to CGRP in atrial preparations (Mimeault et al., 1991). These data provide further evidence in support of receptor heterogeneity and also indicate that the presence of an intact disulphide bridge may not be critical for binding of the CGRP molecule and, in some tissues, might even be detrimental for optimal binding (Dennis et al., 1990). Salmon, but not human, CT often displays limited cross-reactivity at CGRP-preferring binding sites (Sigrist et al., 1986; Henke et al., 1987). There is evidence that radiolabeled rCGRPα binding is more sensitive to displacement by sCT in atrial than in splenic membranes, which may indicate that subtle differences exist between the populations of receptors present in these tissues (Sigrist et al., 1986).

Binding proteins of molecular weight 70 kDa and 120 kDa have been detected in porcine coronary arteries and cardiac muscles (Sano et al., 1988; Miyauchi et al., 1988). It is possible that the larger of these estimates may reflect coupling of receptor proteins to G-proteins

TABLE 1

Binding data for CGRP receptors in various cardiovascular tissues

Tissue	K_d values (^{125}I-CGRP)	Reference
Rat atrial membranes	1.5 nM	Wang and Fiscus, 1989
	85.2 pM	Yoshizaki et al., 1987
Rat ventricular membranes	34.3 pM, 59 nM	Yoshizaki et al., 1987
Rat neonatal cardiomyocyte membranes	41 pM	Chatterjee et al., 1991
Guinea pig cardiac membranes	600–850 pM	Coupe et al., 1990
Porcine cardiac membranes	50.4 pM	Miyauchi et al., 1988
Human coronary arteries	670 pM	Coupe et al., 1990
Rat aortic vascular smooth muscle	120 nM	Hirata et al., 1988
Bovine aortic endothelial cells	260 nM	Hirata et al., 1988
Guinea pig atrial membranes		
hαCGRP$_{8-37}$	670 pM	Quirion et al., 1992
	2.3 nM	Mimeault et al., 1991
hαCGRP	2.0 nM	Mimeault et al., 1991
hαCGRP$_{9-37}$	2.3 nM	Mimeault et al., 1991
hαCGRP$_{10-37}$	9.0 nM	Mimeault et al., 1991
hαCGRP$_{11-37}$	20 nM	Mimeault et al., 1991
Rat atrial membranes		
rαCGRP	5.0 nM	Sigrist et al., 1986
Neonatal rat cardiomyocytes		
hαCGRP	250 pM	Chatterjee et al., 1991
hαCGRP$_{8-37}$	800 nM	Chatterjee et al., 1991
amylin	65 nM	Chatterjee et al., 1991
Porcine cardiac membranes		
hαCGRP	850 pM	Miyauchi et al., 1988
Porcine coronary arteries		
hαCGRP	2.3 nM	Sun et al., 1993
hβCGRP	1.2 nM	Sun et al., 1993

(molecular weight ≤ 50 kDa) rather than evidence for the existence of multiple receptor proteins.

Autoradiographic investigations, performed in hearts of rats (Sigrist et al., 1986), guinea pigs and humans (Coupe et al., 1990) have indicated that the greatest density of binding of CGRP is localized in coronary arteries, veins and heart valves, and, to a lesser extent, in the coronary arterioles and endocardium. Myocardium, fibrous tissue, nervous tissue and fat cells are poorly labeled. Within coronary arteries, binding sites for CGRP are restricted almost entirely to the intima and media layers (Ursell et al., 1991a). Higher densities of specific CGRP binding sites are present in small intramyocardial compared with large epicardial coronary arteries (Sun et al., 1993). Such findings have fueled speculation that binding sites previously detected in crude membrane preparations are predominantly derived from the tissues of the coronary vasculature, especially in the ventricle, because coronary vasculature is abundant in this tissue. Alternatively, high nonspecific binding in ventricular myocardium might obscure a sparse density of receptors, perhaps restricted to a specialized region. G-protein-coupled receptors of high affinity (41 pM) have been detected recently within membranes prepared from cardiomyocytes isolated from neonatal rats (Chatterjee et al., 1991) that had been maintained in culture for 3 to 4 days before study. However, it was not clarified whether these cardiomyocytes were exclusively atrial or ventricular in origin.

VI. Physiological and Pharmacological Actions of Calcitonin Gene-Related Peptide in the Cardiovascular System

In rats, intracerebroventricular administration of CGRP evokes a transient elevation of the mean arterial blood pressure and sustained tachycardia, coupled with a sustained elevation in the concentration of catecholamines present in plasma (Fisher et al., 1983). This indicates that the sympathetic nervous system has a role in these centrally induced actions of the peptide. In contrast, intravenous administration of CGRP in rats (Fisher et al., 1983), rabbits (Anand et al., 1991) and humans (Gennari and Fischer, 1985; Struthers et al., 1986; Franco-Cereceda et al., 1987b) causes significant reduction in the mean arterial blood pressure and total peripheral resistance, coupled with a more sustained tachycardia. At maximally effective concentrations of CGRP, the hypotensive effects persist despite activation of the renin-angiotensin pressor mechanism (Kurtz et al., 1988; 1989). In addition, the effects of CGRP are not diminished in the presence of selective antagonists at adrenoceptors and histaminergic receptors (Marshall et al., 1986a), but are attenuated by $CGRP_{8-37}$ and $CGRP_{12-37}$, and mimicked, albeit at greatly reduced potency, by Cys ACM 2,7 hCGRP and cyclo-2,7-Asp^2Lys^7 hCGRP (Donoso et al., 1990).

Intravenous administration of amylin in rats, rabbits and humans is associated with a transient decrease in mean arterial blood pressure, tachycardia and enhanced renal blood flow; amylin is approximately 100-fold less effective on a molar basis than CGRP (Brain et al., 1990; Young et al., 1993). The haemodynamic effects elicited by amylin in rats in vivo are antagonized by $CGRP_{8-37}$ (Gardiner et al., 1991b).

A. Effects on the Vasculature

1. Peripheral vasodilation. The relative contributions of different vascular beds to the hypotensive action of CGRP depends on the dose of the peptide administered. Vasodilation in the hindquarter and common carotid regions, together with mesenteric vasoconstriction, predominate at high doses (Gardiner et al., 1989a, 1991a, c). As a consequence, blood flow is redistributed in favour of the skin and brain at the expense of the gastrointestinal tract (Jager et al., 1990). Infusion of CGRP at a concentration that evokes only marginal changes in the mean arterial blood pressure can give some indication of the regionally selective effects of the peptide. Inability to demonstrate regionally selective changes in blood flow in response to infusion of CGRP at nonhypotensive concentration in rats, prompted Di-Pette et al. (1989) to conclude that CGRP is a general as opposed to a regionally selective vasodilator. Although small elevations in flow have been observed in response to this peptide, notably in the vasculature serving the stomach, spleen, skin and carotid bed (Marshall et al., 1988; Bratveit et al., 1991), it is unclear to what extent these effects are because of local regulatory responses induced by the slight decline in mean arterial blood pressure. Changes in regional vascular resistances are easier to detect and can be more accurately measured. Intrarenal infusion of CGRP into anaesthetized dogs (Villarreal et al., 1988), at concentrations that do not induce systemic hypotension, increase renal blood flow and glomerular filtration rate.

Additional supportive evidence for the existence, and regional selectivity, of the vasodilatory action of CGRP is provided by studies conducted in situ, or in vitro using isolated blood vessel strips (table 2). Topical application of CGRP causes vasodilation of cortical arterioles of cats (McCullough et al., 1986). CGRP causes vasodilation in isolated, perfused kidneys of rats, thereby raising glomerular filtration rate and filtered fraction (Kurtz et al., 1989; Edwards and Trizna, 1990). Vasorelaxation in response to CGRP has been demonstrated in a number of human blood vessels in vitro, including skeletal muscle arteries (Pernow, 1989), omental arteries and veins (Edvinsson et al., 1985b), pulmonary arteries and veins (McCormack et al., 1989), cerebral arteries (Mejia et al., 1988; Uddman and Edvinsson, 1989) and small intracerebral arterioles and venules (Edwards et al., 1991). CGRP relaxes preconstricted rat splenic strips in vitro

TABLE 2

EC_{50} values for various responses to CGRP, analogs and structurally-related peptides in cardiovascular tissues

Peptide	EC_{50} value	Reference
Myocardial tissues		
Guinea pig left atria (positive inotropic response)		
rαCGRP	6.3 nM	Mantelli et al., 1992
rαCGRP	9.9 nM	Giuliani et al., 1992
rβCGRP	5.1 nM	Giulaini et al., 1992
amylin	158 nM	Giuliani et al., 1992
hαCGRP	6.0 nM	Ishikawa et al., 1988
hαCGRP	14.7 nM	Dennis et al., 1989
[Ala^1,$(CH_2NH)Cys^2$]CGRP	11.0 nM	Dennis et al., 1989
[Tyr^0]hαCGRP	74.0 nM	Dennis et al., 1989
CysACM^{2,7}hαCGRP	>710 nM	Dennis et al., 1989
cyclo^{2,7}Asp^2Lys^7hCGRP	>1.67 μM	Dennis et al., 1989
Guinea pig right atria (positive inotropic response)		
hαCGRP	50 nM	Franco-Cereceda and Lundberg, 1985
hαCGRP	7.6 nM	Dennis et al., 1989
[Ala^1,$(CH_2NH)Cys_3$]CGRP	6.0 nM	Dennis et al., 1989
[Tyr^0]hαCGRP	282 nM	Dennis et al., 1989
CysACM^{2,7}hαCGRP	>710 nM	Dennis et al., 1989
cyclo^{2,7}Asp^2Lys^7hCGRP	>1.67 μM	Dennis et al., 1989
Guinea pig right atria (positive chronotropic response)		
hαCGRP	50 nM	Franco-Cereceda and Lundberg, 1985
Rat left atria (positive inotropic response)		
hαCGRP	3.3 nM	Wang and Fiscus, 1989
Rat right atria (positive inotropic response)		
hαCGRP	120 nM	Sigrist et al., 1986
Rat right atria (positive chronotropic response)		
hαCGRP	70 nM	Sigrist et al., 1986
hαCGRP	4.5 nM	Wang and Fiscus, 1989
Rat right atria (accumulation of cAMP)		
hαCGRP	3.0 nM	Sigrist et al., 1986
Neonatal rat cardiomyocytes (accumulation of cAMP)		
hαCGRP	10 nM	Fisher et al., 1988
Guinea pig atrial cardiomyocytes (stimulation of ICa)		
hαCGRP	12.8 nM	Ono and Giles, 1991
Rat ventricular cardiomyocytes (positive inotropic response)		
hαCGRP	31 pM	Bell and McDermott, 1994
CysACM^{2,7}hαCGRP	6 nM	Bell and McDermott, 1994
amylin	216 pM	Bell and McDermott, 1995
Blood Vessels		
Relaxation of pre-contracted vessels in vitro		
Rat intracerebral arterioles		
hαCGRP	3.9 nM	Edwards et al., 1991
Human skeletal muscle arteries		
hαCGRP	590 pM	Franco-Cereceda et al., 1987
hβCGRP	370 pM	Franco-Cereceda et al., 1987
Feline pial arteries		
hαCGRP	8 nM	Edvinsson et al., 1985
Feline middle cerebral arteries		
hαCGRP	9.6 nM	McCullough et al., 1986
rαCGRP	790 pM	Saito et al., 1989
hαCGRP	2.5 nM	Saito et al., 1989
rβCGRP	630 pM	Saito et al., 1989
hβCGRP	1.0 nM	Saito et al., 1989
Human pial (cerebral) arteries		
rαCGRP	250 pM	Jansen et al., 1992
hαCGRP	125 pM	Jansen et al., 1992
rβCGRP	50 pM	Jansen et al., 1992
hβCGRP	500 pM	Jansen et al., 1992
Guinea pig cerebral arteries		
rαCGRP	1.2 nM	Jansen, 1991
hαCGRP	3.2 nM	Jansen, 1991
rβCGRP	2.5 nM	Jansen, 1991
hβCGRP	6.3 nM	Jansen, 1991
Human coronary vein		
hαCGRP	4.2 nM	Opgaard et al., 1995
Accumulation of cyclic AMP		
Human umbilical vein endothelial cells		
hαCGRP	190 nM	Crossman et al., 1987
Rat aortic vascular smooth muscle cells		
hαCGRP	1 nM	Kubota et al., 1985
hαCGRP	250 nM	Hirata et al., 1988
rαCGRP	250 nM	Hirata et al., 1988
Bovine aortic endothelial cells		
hαCGRP	250 nM	Hirata et al., 1988
rαCGRP	250 nM	Hirata et al., 1988
Rat intracerebral arterioles		
hαCGRP	8 nM	Edwards et al., 1991

(Sigrist et al., 1986) and may be involved in filling of the spleen in vivo to regulate volume of circulating blood.

Gardiner and colleagues (1989b) have undertaken a series of studies aimed at characterizing the regional haemodynamic effects of α and β variants of CGRP. Although intravenous infusion of hβCGRP in conscious, unrestrained rats caused a dose-dependent vasodilation of the renal and hindquarters vascular beds in a manner similar to the response to hαCGRP, at intermediate doses of the peptide, hαCGRP elicited significantly greater haemodynamic effects than hβCGRP. A number of studies have addressed the relative potency of α and β variants of rat and human CGRP in vitro or in situ using tissue obtained from the mesenteric vasculature (Marshall et al., 1986a) and renal vasculature of rats (Castelluci et al., 1993), middle cerebral arteries of cats (Saito et al., 1989), basilar arteries of guinea pigs (Nilsson et al., 1992), human pial arteries (Marshall, 1989) and small arteries obtained from human skeletal musculature (Franco-Cereceda et al., 1987b). At present, however, there is no consensus regarding the ability of CGRP receptors within the peripheral vasculature to discriminate between various forms of the peptide.

Using a regional haemodynamic approach, Gardiner and colleagues (1991a) observed that $CGRP_{8-37}$ was an effective antagonist of hαCGRP in vivo. In this study, it was demonstrated that the fragment had no significant effects per se on mean arterial blood pressure, heart rate or common or internal carotid haemodynamics, but caused a significant (although reversible) inhibition of the vasodilation of the carotid arteries by hαCGRP in conscious Long Evans rats. $CGRP_{8-37}$ also antagonizes CGRP-induced vasodilation of small arterioles and venules from cremaster muscles (Kim et al., 1995), renal arteries (Chin et al., 1994), intracerebral arterioles (Edwards et al., 1991), basilar arteries (Kitazano et al., 1993) and thoracic aortae of rats (Hao et al., 1994). It has been suggested on the basis of differential sensitivity to $CGRP_{8-37}$, that hαCGRP and hβCGRP act via different receptors because hαCGRP-mediated, but not hβCGRP-mediated, dilation of cerebral blood vessels from guinea pigs and humans is antagonized by $CGRP_{8-37}$ (Jansen, 1992; Jansen et al., 1992). Similar findings have been reported in the microvascular circulation of rabbit skin, in which $CGRP_{8-37}$ is a more potent antagonist of vasodilation induced by hαCGRP than by hβCGRP (Hughes and Brain, 1991). By contrast, there is no evidence that receptors that mediate the responses to each peptide in rabbit hepatic and canine basilar arteries can be differentiated in terms of their sensitivity to $CGRP_{8-37}$ (Butler et al., 1993).

Studies in which the peptide was infused intravenously indicate that amylin is approximately 100-fold less potent as a vasodilator than CGRP (Brain et al., 1990), which suggests that these effects of amylin may be mediated by CGRP-preferring receptors (Chantry et al., 1991). The vasodilator activity of amylin displays a marked regional dependence and is most pronounced in the renal vascular bed (Brain et al., 1990; Gardiner et al., 1991a). However, unlike CGRP, amylin evokes only moderate increases in hindquarters and mesenteric blood flow. Amylin is at least an order of magnitude less potent than CGRP as a vasodilator of the renal arterial bed of rats in vitro and is sensitive to the antagonistic action of the fragment, $CGRP_{8-37}$ (Castellucci et al., 1993). Furthermore, amylin has significantly reduced efficacy in this preparation compared with CGRP. These findings are consistent with the interaction by amylin, albeit with markedly reduced potency and efficacy, at $CGRP_1$ receptors in the renal vasculature.

Infusion of adrenomedullin in conscious rats (Gardiner et al., 1995) causes dose-dependent hypotension and tachycardia, accompanied by vasodilation of the renal, mesenteric and hindquarters vascular beds. In contrast, hypotension and tachycardia in response to CGRP are accompanied by only transient vasodilation of these vascular beds in vivo. In addition, the actions of CGRP are antagonized by the fragment, $CGRP_{8-37}$, whereas those of adrenomedullin are not. Adrenomedullin dilates the pulmonary vasculature of cats in vivo with a potency greater than CGRP and amylin, but with a shorter duration of action (Dewitt et al., 1994). In contrast, the vasodilator response evoked by adrenomedullan in the mesenteric vasculature of rats in vitro is inhibited by $CGRP_{8-37}$ (Nuki et al., 1993). There is at present, therefore, no consensus about whether the vasodilatory effects of adrenomedullan reflect the ability of the peptide to interact with $CGRP_1$-receptors in the vasculature or, alternatively, are mediated by a distinct population (or populations) of receptors selective for adrenomedullin.

2. Coronary vasodilation. Potent vasodilatory effects of CGRP on the coronary circulation in vivo, as evidenced by measurements of coronary flow, coronary resistance, myocardial flow and radiographic measurement of the diameter of coronary vessels, have been demonstrated in several species, including humans (McEwan et al., 1986), pigs (Tippens et al., 1988), dogs (Joyce et al., 1990b) and rats (Bratveit et al., 1991). CGRP lowers the perfusion pressure of isolated hearts of rats, rabbits (Holman et al., 1986; Marshall et al., 1986b) and dogs (Sugiyama et al., 1989), which is indicative of a direct action on the coronary vasculature, independent of systemic reflexes activated in vivo in response to reduction in mean arterial blood pressure. rαCGRP has been reported to be equipotent to (Marshall et al., 1986b) or more potent than (Holman et al., 1986) hαCGRP in reducing perfusion pressure of isolated hearts of rats and rabbits. CGRP-induced relaxation of isolated rings of coronary arteries has been demonstrated in rats and guinea pigs (Prieto et al., 1991), cows (Greenberg et al., 1987), pigs (Shoji et al., 1987; Beny et al., 1989) and humans (Franco-Cereceda and Rudehill, 1989). Proximal epicardial and distal intramyocardial

arteries of rats are more sensitive to the vasorelaxant effects of rαCGRP than to hαCGRP (Prieto et al., 1991). CGRP has also been found to be a potent vasodilator of precontracted human epicardial coronary veins in vitro (Opgaard et al., 1995)

3. Permeability of the microvasculature. A neurogenic component may be provided by CGRP that may act synergistically with other mediators in the production of inflammatory hyperaemia, characterized by local oedema and increased microvascular permeability (Brain et al., 1986). Injection of CGRP into the volar surface of the human forearm elicits a rapidly developing and sustained local reddening that is dispersed laterally via lymphatic vessels. This action is similiar to, but more sustained than, the characteristic "wheal and flare response" induced by histamine and can be inhibited by the nonsteroidal anti-inflammatory agent, aspirin (Fuller et al., 1987). Topical administration of CGRP also induces intense dilation and enhanced permeability of the microvasculature serving the cheek pouch of hamsters and skin of rabbits, (Brain et al., 1985) and the upper airways of ferrets (Webber et al., 1991). Investigation of the effects of CGRP on vasodilation of the skin of rabbits has indicated that the three different forms of synthetically available CGRP (rαCGRP, hαCGRP and hβCGRP) are equipotent (Brain et al., 1986). After intradermal injection into rabbits, amylin increases blood flow and potentiates bradykinin-induced oedema formation with a potency approximately 100-fold less than that of CGRP (Brain et al., 1990). In the cutaneous microvasculature of rabbits (Brain et al., 1993), rats and hamsters (Hall et al., 1995), the response to CGRP is abolished by the $CGRP_1$-selective antagonist, $CGRP_{8-37}$. CGRP has no effect on plasma protein extravasation per se, but when administered concurrently can potentiate oedema induced by inflammatory mediators such as bradykinin and platelet-activating factor. The potentiation of tissue oedema by CGRP is probably related to arteriolar dilation causing an increased perfusion pressure at the level of the capillary and venule where other mediators have already increased permeability. Indeed, CGRP actually reduces leaky cerebral venular permeability in rats, indicating that the peptide may function to restore and maintain the blood-brain barrier in vivo (Easton and Fraser, 1993). CGRP also enhances interleukin-1-induced adhesion of neutrophils to endothelial cells from human umbilical veins by increasing expression of E-selectin (Rangnekar et al., 1994). This response is sensitive to antagonism by the fragment, $CGRP_{8-37}$, and is not mimicked by CT. This novel action of the peptide indicates that CGRP may regulate the infiltration of leukocytes at local sites by modulating cytokine induction of the expression of adhesion molecules.

4. Angiogenesis. CGRP promotes the proliferation of endothelial cells isolated from human umbilicial veins (Haegerstrand et al., 1990). Although CGRP does not stimulate directly the proliferation of endothelial cells isolated from human ileac arteries, veins and microvasculature, the peptide promotes migration of endothelial cells into wounded monolayers of human ileac microvascular endothelium; this migration is considered to be an initial stage in angiogenesis (Carter et al., 1993). CGRP inhibits proliferation of cultured bovine aortic smooth muscle cells (Fiscus, 1993), indicating that another function of CGRP, which may be especially important in large conduit vessels such as aorta and coronary arteries, could be to inhibit proliferation of vascular smooth muscle cells and thereby provide an important physiological counterbalance to the mitogenic effects of other mediators. These mitogenic properties of CGRP have implications for the formation of new vessels during physiological and pathophysiological events such as ischaemia, inflammation and wound healing. Application of CGRP improves the survival of surgical flaps of ischaemic tissue, partly by promoting the synthesis of new blood vessels within the tissue (Kjartansson and Dalsgaard, 1987; Knight et al., 1993).

B. Chronotropic Effects

Tachycardia in response to intravenous administration of CGRP has been demonstrated in rats (Fisher et al., 1983; Gardiner et al., 1989a, 1991a), rabbits (Anand et al., 1991), dogs (Sugiyama et al., 1989) and humans (Gennari and Fischer, 1985; Struthers et al., 1986; Franco-Cereceda et al., 1987b). This action is resistant to blockade of adrenoceptors in rats (Marshall et al., 1986b) and humans (Gennari and Fischer, 1985). In rats, tachycardia occurs in response to nonhypotensive doses of the peptide (Di-Pette et al., 1989), which is consistent, therefore, with a direct chronotropic action of CGRP in the heart. However, intracoronary infusion of CGRP in open-chest anaesthetized dogs, while causing significant increases in coronary and myocardial blood flow, does not alter heart rate. Direct injection of CGRP into canine epicardial sites containing parasympathetic ganglia attenuates the negative chronotropic effects of vagal stimulation of the sino-atrial node. However, infusion of CGRP into the sinus node artery of surgically sympathectomized and parasympathectomized dogs does not affect impulse initiation (Rigel, 1988). Thus, in this species, CGRP may act indirectly as a neuromodulator in the epicardial ganglia, rather than directly as a neurotransmitter in atrial myocardium. Indeed, CGRP does not exert a positive chronotropic action in trabeculae muscles isolated from canine atria (Rigel, 1988). In contrast, there is evidence in support of a direct chronotropic action of CGRP in the atria of other species. CGRP elevates heart rate in isolated perfused hearts of rats (Holman et al., 1986) and guinea pigs (Marshall et al., 1986a; Franco-Cereceda and Lundberg, 1988), but not of rabbits (Holman et al., 1986). This chronotropic action is independent of the fall in perfusion pressure induced by the coronary vasodilator action of CGRP, because a chronotropic response is not elicited by another coronary

vasodilator, nitroprusside (Marshall et al., 1986b). CGRP exerts a positive chronotropic effect in isolated spontaneously beating right atria of rats (Sigrist et al., 1986; Marshall et al., 1986a; Wang and Fiscus, 1989) and guinea pigs (Franco-Cereceda and Lundberg, 1985, 1988; Marshall et al., 1986b; Saito et al., 1986), and in cardiomyocytes isolated from the hearts of neonatal rats (Fisher et al., 1988).

C. Inotropic Effects

Intravenous infusion of CGRP enhances cardiac output in rabbits (Anand et al., 1991) and humans (Gennari and Fischer, 1985; Struthers et al., 1986; Franco-Cereceda et al., 1987a), but not in rats (Bratveit et al., 1991). Intracoronary infusion of the peptide into open-chested anaesthetized dogs, while increasing coronary and myocardial blood flow, does not alter left ventricular wall thickness and cardiac index (Rigel et al., 1988). In humans, intravenous infusion of CGRP reduces both the period of pre-ejection and duration of electromechanical systole. These inotropic effects, but not the accompanying chronotropic and hypotensive effects, are antagonized by the β-adrenoceptor antagonist, labetalol, which indicates that the inotropic action of CGRP is secondary to reflex activation of the sympathetic nervous system in response to the peripheral vasodilation induced by this peptide. Assessment of the direct action of a neuropeptide upon ventricular myocardium in vivo is, therefore, complicated by the comprehensive array of peripheral haemodynamic reflexes that are operational. In addition, such studies do not indicate whether the inotropic response is because CGRP is functioning as a neurotransmitter or neuromodulator in the heart, or whether the peptide gains access to the ventricular myocardium via the coronary circulation.

The several reports concerning the inotropic action of the peptide in the mammalian heart in vitro have provided conflicting results. CGRP does not alter contractile force in isolated perfused hearts of rats, rabbits (Holman et al., 1986) and dogs (Sugiyama et al., 1989). However, CGRP induces a sustained increase in the contractile force in isolated perfused hearts of guinea pigs, by an action that is resistant to the β-adrenoceptor antagonist, metoprolol (Franco-Cereceda and Lundberg, 1985). Studies using intact hearts perfused in vitro with the coronary vasculature intact also fail to provide information as to whether CGRP has a direct action on the ventricle, or an indirect action that is secondary to coronary vasodilation. The influence of the coronary vasculature is absent in strips of cardiac muscle perfused in vitro and in suspensions of isolated cardiomyocytes.

CGRP exerts a potent positive inotropic action in isolated atria of rats (Satoh et al., 1986; Sigrist et al., 1986; Wang and Fiscus, 1989; Ishikawa et al., 1987) and guinea pigs (Ishikawa et al., 1988; Franco-Cereceda and Lundberg, 1985, 1988) (table 2). Positive inotropic effects of CGRP have also been demonstrated in pieces of tissue isolated from the right atria of human hearts (Franco-Cereceda et al., 1987a; Du et al., 1994), but are absent in isolated atrial trabeculae of dogs (Rigel et al., 1988). The positive inotropic action is resistant to blockade by hexamethonium, an inhibitor of ganglionic transmission, and by selective antagonism of adrenoceptors, histaminergic, serotonergic and muscarinic cholinoceptors, and to inhibition of synthesis of prostaglandins (Franco-Cereceda and Lundberg, 1985; Satoh et al., 1986; Sigrist et al., 1986; Ishikawa et al., 1987, 1988; Wang and Fiscus, 1989). Additional evidence that the inotropic action of CGRP in atria is not mediated by the sympathetic nervous system is provided by the failure of the peptide to stimulate the efflux of catecholamines from atria of guinea pigs (Franco-Cereceda and Lundberg, 1985).

There is substantial evidence, derived from investigations of the functional effects of peptide fragments and analogues in vitro, that the positive myotropic effects of CGRP in atrial tissues are mediated by $CGRP_1$-receptors, which do not discriminate between naturally occurring forms of the peptide. The α and β variants of CGRP are equiactive as positive inotropic agents in the atria of rats (Spokes et al., 1987; Franco-Cereceda and Lundberg, 1988) and humans (Franco-Cereceda et al., 1987b). In addition, rαCGRP and hαCGRP are equiactive as positive inotropic agents in the atria of rats (Marshall et al., 1986b). In guinea pigs, however, rαCGRP is ten-fold more potent as a positive chronotropic agent than as a positive inotropic agent, whereas hαCGRP is equipotent for both effects (Marshall et al., 1986b). These data indicate that differences may exist between the receptors and/or receptor-effector coupling mechanisms associated with the positive inotropic and chronotropic responses to CGRP, respectively.

Generally, the structure-activity profiles of the various analogues and fragments of CGRP are fairly similar in right and left atrial tissue. However, important differences have been observed between these two tissues and preparations of rat vas deferentia (Dennis et al., 1989; Quirion et al., 1992), tissue which is enriched with $CGRP_2$-receptors. Rupture of the disulphide bridge of the CGRP molecule drastically reduces the agonist activity of the peptide in the former, but not the latter, tissue. The linear analogue, Cys ACM 2,7 hCGRP is virtually inactive as a positive inotropic and chronotropic agent in the atria of guinea pigs in vitro, but retains the full intrinsic activity of CGRP, albeit with significantly reduced potency, at $CGRP_2$-receptors in the vas deferentia of rats (Dennis et al., 1989). A tyrosine residue is often added at position 0 of the CGRP molecule to facilitate radiolabeling of the peptide for use in binding experiments. Tyr0 hαCGRP displays reduced potency in cardiac preparations, but is equipotent to CGRP in preparations of vas deferentia (Dennis et al., 1989). These results provide further evidence for receptor heterogeneity and, perhaps more importantly, indi-

cate that iodinated Tyr0 hαCGRP might not be an appropriate radioligand with which to characterize receptors for CGRP in certain tissues. In vivo, inactivation of CGRP is thought to occur through endopeptidases. In the analogue, $Ala^1CH_2NH\ Cys^2$ hαCGRP, the peptide bond is substituted by a pseudopeptide bond between Ala^1 and Cys^2 to produce a more stable peptide that is less susceptible to degradation. This analogue is marginally more potent than CGRP in all preparations in which it has been tested, and retains the full efficacy of the native peptide both as a myotropic agent and for the inhibition of twitch responses in vas deferentia. This approach may be promising, therefore, in the design of stable agonists at CGRP receptors for in vivo administration. N terminal fragments of CGRP, including cyclo-^{2,7} $hCGRP_{1-7}COOH$, cyclo-^{2,7} $hCGRP_{1-8}COOH$, cyclo-^{2,7} $hCGRP_{2-7}COOH$ and cyclo-^{2,7} $hCGRP^{2-8}COOH$, are inactive in all preparations in which they have been tested (Dennis et al., 1989). Such findings confirm the importance of the C terminal part of the CGRP molecule in the maintenance of appropriate receptor recognition. The ability of cyclo-^{2,7} $hCGRP_{1-7}COOH$ to antagonize weakly the positive chronotropic response to CGRP in spontaneously beating right atria of guinea pigs, although it fails to antagonize the positive inotropic response to the peptide in electrically driven left atria of guinea pigs, remains unexplained and may provide further evidence that subtle differences exist between the populations of receptors mediating the inotropic and chronotropic effects of the peptide, respectively.

Many studies have focused on the antagonistic potential of the fragment, $CGRP_{8-37}$ (table 3). $CGRP_{8-37}$ is a potent competitive antagonist of the inotropic and chronotropic actions of CGRP in atrial preparations ($pA_2 > 7.0$), but is at least ten-fold less potent as a competitive antagonist of CGRP at $CGRP_2$-receptors located in vas deferentia of rats ($pA_2 < 6.2$). These data are compatible with receptors for CGRP present in atrial tissues being of the $CGRP_1$-subtype (Mimeault et al., 1991; Quirion et al., 1992). The fragment, $CGRP_{9-37}$, is at least as potent ($pA_2 > 7.0$) as $CGRP_{8-37}$, whereas $CGRP_{10-37}$ is less potent ($pA_2 = 6.6$), and $CGRP_{11-37}$ is significantly less potent ($pA_2 = 6.1$) as a competitive antagonist of the actions of CGRP in atrial tissues (Mimeault et al., 1991). $CGRP_{9-37}$ also antagonizes the actions of CGRP weakly at $CGRP_2$-receptors present in preparations of rat vas deferentia with a potency similar to $CGRP_{8-37}$ ($pA_2 = 6.2$), whereas shorter fragments are almost completely ineffective as antagonists in this preparation (Mimeault et al., 1991; Quirion et al., 1992). These data indicate that the threonine residue in position 9 of the peptide might be critical for recognition of putative CGRP receptor subtypes, whereas the valine residue in position 8 is not so important. The presence of the histidine residue in position 10 of the peptide may also be important to ensure adequate affinity at CGRP receptors, because $CGRP_{11-37}$ displays a significant loss of affinity in all preparations (Mimeault et al., 1991). In contrast, the presence of an arginine residue in position 11 may not be involved directly in the maintenance of adequate affinity

TABLE 3
pA_2 values for various CGRP fragments in cardiovascular tissues

Fragment	pA_2 value	Reference
Guinea pig left atria (positive inotropic response)		
$CGRP_{8-37}$	6.81	Rovero et al., 1992
	7.66	Mimeault et al., 1991
	6.42–6.95	Giuliani et al., 1992
$CGRP_{9-37}$	7.70	Mimeault et al., 1991
$CGRP_{10-37}$	6.68	Mimeault et al., 1991
$CGRP_{11-37}$	6.15	Mimeault et al., 1991
$CGRP_{12-37}$	6.03	Quirion et al., 1992
$CGRP_{19-37}$	5.39	Rovero et al., 1992
$CGRP_{23-37}$	4.81	Rovero et al., 1992
Guinea pig right atria (positive inotropic response)		
$CGRP_{8-37}$	7.22	Mimeault et al., 1991
$CGRP_{9-37}$	7.04	Mimeault et al., 1991
$CGRP_{10-37}$	6.15	Mimeault et al., 1991
$CGRP_{11-37}$	6.14	Mimeault et al., 1991
$CGRP_{12-37}$	6.09	Quirion et al., 1992
Rat ventricular cardiomyocytes (positive inotropic response)		
$CGRP_{8-37}$	7.95	Bell and McDermott, 1994
Rat intracerebral arterioles (relaxation)		
$CGRP_{8-37}$	6.70	Edwards et al., 1991
Guinea pig cerebral blood vessels (relaxation)		
$CGRP_{8-37}$	7.12	Jansen, 1991
Feline middle cerebral arterioles (relaxation)		
$CGRP_{8-37}$	7.14	Jansen et al., 1992
Human cerebral blood vessels (relaxation)		
$CGRP_{8-37}$	6.70	Jansen et al., 1992

because $CGRP_{12-37}$ is equiactive (pA_2 = 6.1) to $CGRP_{11-37}$ as an antagonist of CGRP in the atria of guinea pigs (Dennis et al., 1989; Mimeault et al.; 1991). $CGRP_{19-37}$ is a major product of the metabolic breakdown of CGRP by endopeptidases in the CNS and spinal cord (Sakurada et al., 1991). This observation raises the possibility that some C-terminal fragments that are biologically active as antagonists may have a modulatory influence upon the action of the parent peptide in vivo. However, $CGRP_{19-37}$ and shorter C-terminal fragments, including $CGRP_{23-37}$, retain very weak antagonistic activity (apparent pA_2 values < 5.4) at CGRP receptors in the atria of guinea pigs (Rovero et al., 1992).

CGRP stimulates a potent positive inotropic response in thin strips of false tendon obtained from the ventricles of pigs (Miyauchi et al., 1988), but does not alter the force of contraction in isolated papillary muscle strips obtained from left or right ventricles of rats (Sigrist et al., 1986; Ishikawa et al., 1987) or guinea pigs (Ishikawa et al., 1987), in superfused trabecular strips obtained from canine ventricles (Rigel et al., 1988) or in ventricular trabeculae obtained from nondiseased human hearts (Du et al., 1994). Such differences may indicate greater impedence to diffusion of the peptide into, and nonspecific binding within, papillary muscles as a consequence of their greater thickness as compared with strips of 'false-tendon' muscles. The use of single cardiomyocytes in mechanical studies would be expected to overcome some of the problems associated with multicellular preparations. Single cells need not be affected by preload and extrinsic afterload or barriers to diffusion. Also, such preparations should not be contaminated by other cell types or nerve terminals, and are not specialized for impulse generation or propagation and hence twitch only when stimulated electrically. CGRP stimulates an increase in the strength of contraction of cardiomyocytes isolated from the hearts of neonatal rats (Fisher et al., 1988). This action is characterized by an increase in the amplitude and velocity of cell edge motion, which has similiar temporal-dependence to the accompanying chronotropic effect of the peptide in these cells. However, these authors were unable to quantify this inotropic response and did not clarify whether these cells were exclusively atrial or ventricular in origin. Quantification of cell shortening in isolated cardiomyocytes is a relatively novel experimental technique in which the properties of the contractile system measured are different from those obtained by measurement of isometric force recorded in cardiac muscle strips (Kruegar et al., 1980; Powell et al., 1980; Tung, 1986). The information provided by determining the parameters of cell shortening however can be compared and contrasted usefully with that obtained from multicellular preparations (Meyer et al., 1987; Piper et al., 1989). CGRP exerts a potent positive contractile response directly in ventricular cardiomyocytes isolated from the hearts of adult rats (Bell and McDermott, 1994a). This response is mediated by receptors of the $CGRP_1$-subtype, because the analogue, Cys ACM 2,7 CGRP, is approximately 200-fold less potent than the native peptide, and the response to CGRP is antagonized by the fragment, $CGRP_{8-37}$ (pA2 value = 7.95) (Bell and McDermott, 1994a) (fig. 3). Salmon CT is devoid of activity in this preparation. CGRP does not alter the amplitude of cellular contraction elicited in cardiomyocytes isolated from the hearts of adult rabbits and from samples of ventricular tissue obtained from patients undergoing surgery for coronary artery disease or at the time of mitral valve replacement (Anand et al., 1991). However, the quality of cardiomyocytes isolated from such specimens of human myocardium is often a cause for concern.

Amylin interacts at the population of $CGRP_1$-receptors coupled to positive contractile response in rat ventricular cardiomyocytes (Bell and McDermott, 1995b) because amylin possesses approximately 20-fold lower potency than CGRP, and the responses to each peptide are antagonized significantly by the competitive antagonist at $CGRP_1$-receptors, $CGRP_{8-37}$, but not by the selective antagonist at amylin-preferring receptors, $amylin_{8-37}$. However, amylin may be a partial agonist (rather than possessing full efficacy at this receptor population) because amylin possesses moderately lower efficacy than CGRP as a stimulant of cellular contraction. The peptide, when present at maximally effective concentration, enhances the action of low concentrations of CGRP, at which fewer receptors would presumably be occupied by the full agonist, and attenuates the action of high concentrations of CGRP by occupying receptors that would otherwise be available to the full agonist. These data are in agreement with those obtained by van Rossum and coworkers (1994) who reported that amylin is a partial agonist at $CGRP_1$-receptors in isolated atrial preparations of guinea pigs but a full agonist at $CGRP_2$-receptors in the vas deferens of this species.

VII. Calcitonin Gene-Related Peptide Receptor-Effector Coupling Mechanisms

A. In Peripheral Vasculature

The potency of the vasorelaxant effect of CGRP, and the requirement of this action on the presence of intact endothelium, has a marked regional variation. There is an absolute requirement for endothelium in thoracic aortae of rats (Brain et al., 1985; Kubota et al., 1985; Grace et al., 1987; Gray and Marshall, 1992a), renal arteries of rats (Elhawary and Pang, 1995) and human cerebral arteries (Marshall, 1989). CGRP-induced vasorelaxation is endothelium-independent in cerebral arteries of cats (Edvinnsson et al., 1985a; Saito et al., 1989), splenic arteries of pigs and skeletal muscle arteries of humans (Pernow, 1989), mesenteric arteries of rabbits (Nelson et al., 1990) and rats (Amerini et al., 1993), gastric, splenic and hepatic arteries of rats (Bratveit et al., 1991), pulmonary arteries of guinea pigs

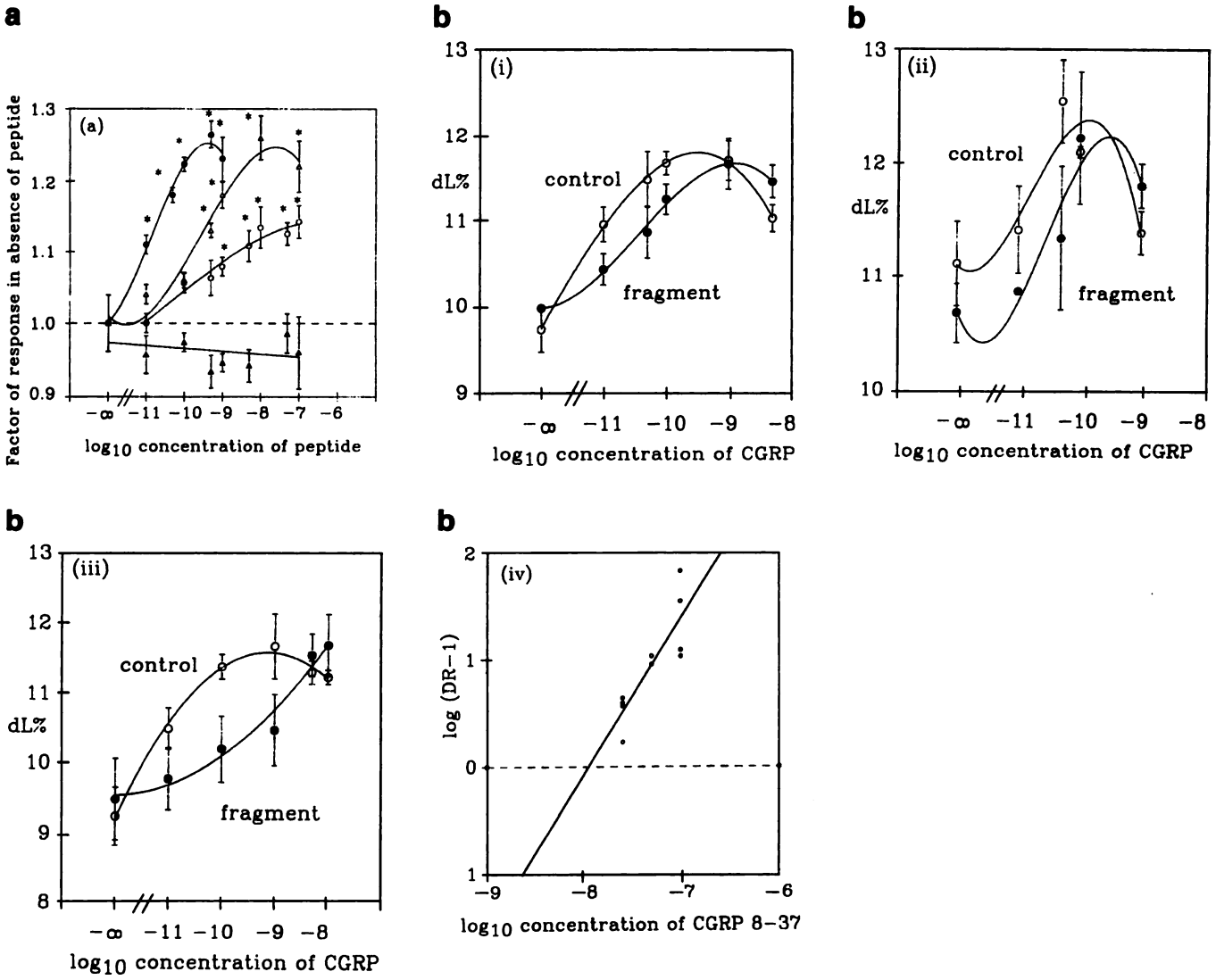


FIG. 3. Pharmacology of CGRP receptors in cardiovascular tissues: rat ventricular cardiomyocytes as a model to illustrate basic principles of receptor classification. (*a*) Concentration-dependence of the effects of hαCGRP (●), [Cys,ACM$^{-2,7-}$]hαCGRP (△), human amylin (○) and salmon calcitonin (▲) on amplitudes of cellular contraction elicited in adult mammalian ventricular cardiomyocytes. Cardiomyocytes, isolated from the hearts of adult rats, were incubated with each peptide for 3 minutes before stimulation at 0.5 Hz. Contractile response, expressed as maximum shortening, is a factor of the response in the absence of peptide (1). Data are mean ± standard error of the mean of five heart cell preparations. *Significant variation between responses elicited in the absence and presence of peptide ($p < 0.05$). (*b*) contractile responses of cardiomyocytes elicited with the fragment, $CGRP_{8-37}$, a selective agonist at $CGRP_1$ receptors. Effect of $CGRP_{8-37}$ (25, 50, 100 nM) on contractile response relation for CGRP (panels *i* to *iii*). Cardiomyocytes, with and without $CGRP_{8-37}$ added, were incubated with CGRP for 3 minutes before stimulation at 0.5 Hz. Contractile response, expressed as maximum shortening, is a percentage (dL%) of prestimulus cell length (L). Other experimental conditions were as described in figure *3a*. Data are mean ± standard error of the mean of two to four heart cell preparations. Schild Plot of these data (panel *iv*).

(Maggi et al., 1990) and human pulmonary arteries and veins (McCormack et al., 1989). Some vessels, such as the external ileac arteries of pigs (Samuelson and Jernbeck, 1991), basilar arteries of rats (Kitazono et al., 1993) and superior mesenteric arteries of rats (Bratveit et al., 1991), possess both an endothelium-dependent and an endothelium-independent vasorelaxant response to CGRP.

Binding of a vasorelaxant peptide to an endothelial receptor is generally thought to trigger synthesis and release of nitric oxide (NO) from L-arginine, or less commonly, of prostacyclin from arachadionic acid. The unique chemistry of NO allows this molecule, with the capacity to diffuse rapidly into nearby cells, to activate the cytosolic enzyme, guanylate cyclase, thereby stimulating the formation of cyclic guanosine monophosphate (cGMP). This nucleotide, by virtue of its ability to activate protein kinase G, can stimulate Ca-adenosine triphosphate (ATP)ases, reducing $[Ca^{2+}]_i$ in vascular smooth muscle and causing vasorelaxation (Popescu et al., 1985). A related series of events is thought to occur after stimulation of adenylate cyclase in vascular

smooth muscle. The precise biochemical details about how these two cyclic nucleotide systems interact cooperatively in the control of smooth muscle tone is not yet clear.

At present, there is no consensus regarding the involvement of NO and cGMP in those vessels in which CGRP induces endothelium-dependent relaxation. In rings of rat thoracic aorta, preconstricted with noradrenaline, the endothelium-dependent vasorelaxant response to CGRP is inhibited by haemoglobin, which binds NO released from endothelial cells, and by N^G-monomethyl-L-arginine, which inhibits the synthesis of NO from the terminal guanidino nitrogen of L-arginine. The inhibitory effect of the latter compound can be reversed by L-arginine but not by D-arginine; this implies a common synthetic pathway for NO and the endothelium-derived relaxant factor released in response to CGRP (Gray and Marshall, 1992b; Hao et al., 1994). This conclusion is supported by a study by Fiscus and coworkers (1994), who reported that nitroglycerin, an exogenous NO donor, potentiated the vasorelaxant response to CGRP in this tissue. Similarly, the endothelium-dependent component of the vasorelaxant response to CGRP in porcine external ileac arteries is reduced in the presence of the NO inhibitor, gossypol (Samuelson and Jernbeck, 1991).

Marshall and coworkers (Marshall, 1992; Gray and Marshall, 1992a) have also reported that CGRP stimulated accumulation of cGMP in rings of rat thoracic aortae with intact endothelium and that accumulation of cGMP and the concomitant vasorelaxant response to CGRP were abolished by methylene blue, an inhibitor of cytosolic guanylate cyclase and by inhibitors of NO synthase. This is at variance with a study by Grace and coworkers (1987) who found that vasorelaxation of rat thoracic aortae by acetylcholine and sodium nitroprusside was accompanied by a parallel increase in cGMP in vascular smooth muscle, whereas that produced by CGRP was not accompanied by the accumulation of cGMP either in the absence or presence of endothelium, prompting these authors to conclude that the endothelium-derived relaxant factor released in response to CGRP is different to that released by acetylcholine. Because CGRP does not stimulate the accumulation of cGMP in vascular myocytes from rat thoracic aortae (Kubota et al., 1985), or incidentally in bovine endothelial cells (Hirata et al., 1988), it might be anticipated that receptors for CGRP are not coupled to guanylate cyclase per se and that an increase in cGMP in vascular smooth muscle would only occur in the presence of intact endothelium.

The synthesis and release of prostacyclin in response to CGRP has been demonstrated in endothelial cells from human umbilical veins (Crossmann et al., 1987) but does not occur in bovine aortic endothelial cells (Hirata et al., 1988). It should be noted, however, that endothelial cells from microvessels have a quite different profile of prostaglandin production from those derived from large vessels (Charo et al., 1985). The inhibitor of prostaglandin biosynthesis, indomethacin, does not attenuate the endothelium-dependent vasorelaxant responses to CGRP in porcine external ileac arteries (Samuelson and Jernbeck, 1991) in vitro and skin arterioles of rabbits in vivo (Brain et al., 1985). Gray and Marshall (1992a) reported that endothelium-dependent relaxation of rat thoracic aortae in vitro in response to CGRP was not attenuated in the presence of another inhibitor of prostaglandin biosynthesis, ibuprofen. Although it has been suggested that endothelium-dependent vasorelaxation is mediated, at least in part, by the release of prostacyclin in rat renal arteries and arterioles (Villareal et al., 1988a), this has not been substantiated by Wisskirchen and Marshall (1994), who found that endothelium-dependent vasorelaxation in rat renal and pulmonary arteries in response to CGRP but not sodium nitroprusside was antagonized by the inhibitor of NO synthesis, L-N^G-nitro-L-arginine and by the calmodulin antagonist, calmidozaliam, and was therefore mediated by calcium-dependent activation of NO within endothelial cells.

It is possible that incomplete removal of the endothelium in endothelium-denuded vessels, or damage to the endothelial cell layer in vessels defined as having intact endothelium, might have contributed to some of the discrepancies between studies in which the role of endothelium-derived relaxant factors and the accumulation of cGMP in the vasorelaxant responses to CGRP was investigated. Progress has also been hampered by the lack of specificity or membrane permeability of many inhibitors of prostaglandin biosynthesis and protein kinase G and the lack of availability of selective antagonists at prostacyclin (IP) receptors.

In many vascular preparations, there is a strong correlation between CGRP-induced relaxation and the elevation in intracellular cAMP (Wang et al., 1991). CGRP stimulates the accumulation of cAMP in cultured vascular smooth muscle cells from rat thoracic aortae (Kubota et al., 1985; Hirata et al., 1988). However, a causal role for this cyclic nucleotide in endothelium-dependent vasorelaxation in response to CGRP has not been established. Marshall and coworkers (Marshall, 1992; Gray and Marshall, 1992a) reported that accumulation of cAMP in rings of rat thoracic aortae occurred only in the presence of intact endothelium and was not affected by inhibition of NO synthase, indicating that the accumulation of this nucleotide might precede, and might play a causal role in, the synthesis of NO in endothelial cells. Alternatively, it is possible that binding of CGRP to endothelial receptors might stimulate the release from the endothelium of a mediator other than NO that is capable of stimulating the accumulation of cAMP in vascular smooth muscle. Although the release of prostacyclin from endothelial cells isolated from human umbilical veins is associated with the accumulation of

cAMP (Crossman et al., 1987), bradykinin elicits a significantly greater release of prostacyclin from these cells in the absence of the accumulation of cAMP, indicating that this cyclic nucleotide might not be necessary for the release of prostacyclin. In addition, although prostacyclin stimulates the accumulation of cAMP in rat thoracic aortae (Gorman et al., 1977), vasorelaxation in response to prostacyclin has not been established in this tissue (Gray and Marshall, 1992b). Binding sites for CGRP are present in vascular smooth muscle cells from rat thoracic aortae (Hirata et al., 1988), and CGRP stimulates the accumulation of cAMP in these cells with a sensitivity, concentration-dependence and amplitude (Kubota et al., 1985) such that it would seem likely this cyclic nucleotide acts to mediate some action of CGRP in vascular smooth muscle. However, rat aortic smooth muscle does not relax in direct response to CGRP in the absence of endothelium (Kubota et al., 1985). It might be that, although the accumulation of cAMP does not alter smooth muscle tone in response to CGRP in endothelium-denuded vessels, this nucleotide might contribute to the vasorelaxant effect of CGRP when superimposed on a modest accumulation of cGMP in endothelium-containing tissues. Accumulation of cAMP in response to CGRP is also associated with endothelium-dependent vasodilation in human cerebral arteries (Jansen et al., 1992), endothelium-independent vasorelaxation of feline middle cerebral and pial arteries (Edvinsson et al., 1985a), and relaxation of rat splenic muscle strips (Sigrist et al., 1986). However, a causal role for cAMP in the vasorelaxant responses to CGRP in these tissues has not been established. In further studies designed to provide evidence for a causal relationship between the accumulation of cAMP and endothelium-dependent and independent vasorelaxation induced by CGRP, the use of a selective, membrane-permeable antagonist of cAMP, such as Rp-cAMPS, would be advantageous. Although the use of a homogenous population of isolated vascular smooth muscle cells, maintained in culture, would be useful in the investigation of the direct vasorelaxant response to CGRP in those vessels in which the action of the peptide is independent of endothelium, the coculturing of vascular smooth muscle cells and endothelial cells would be of value in the elucidation of the role of cyclic nucleotides such as cGMP and cAMP, and of the release of endothelial-derived factors such as NO and prostacyclin, in which the vasorelaxant response to CGRP is dependent on the presence of intact endothelium.

The membrane potential of the smooth muscle cell might be an important determinant of the magnitude of a vasorelaxant response. Evidence of the direct hyperpolarizing effect of CGRP in vascular smooth muscle is emerging; this effect might account, at least in part, for endothelium-independent vasorelaxation elicited by the peptide in a number of vessels. CGRP causes hyperpolarization of smooth muscle cells of rabbit mesenteric arteries by activating an outward potassium channel current (Nelson et al., 1990). Membrane hyperpolarization in response to CGRP is inhibited in the presence of glibenclamide, a blocker of ATP-sensitive potassium channels, in mesenteric arteries of rats and rabbits (Nelson et al., 1990; Tenney, 1990). The vasorelaxant effect of CGRP is reduced in the presence of glibenclamide in rabbit mesenteric arteries (Nelson et al., 1990), rabbit opthalmic arteries (Zschauer et al., 1991, 1992) and rat mesenteric arteries (Tenney, 1990) but not in human mammary arteries and saphenous veins (Boyle and Brown, 1991), guinea pig basilar arteries (Nilsson et al., 1991) and porcine vesical arteries (Persson et al., 1991) in vitro. Vasorelaxation of rat basilar arteries in vivo in response to CGRP, administered by topical application via a cranial window, is antagonized by $CGRP_{8-37}$ and reduced significantly by glibenclamide, indicating that vasodilation in response to CGRP is mediated by $CGRP_1$-preferring receptors which, in these vessels, are coupled primarily to the activation of ATP-sensitive potassium channels in vascular smooth muscle. A causal role for cAMP in CGRP-mediated activation of ATP-sensitive potassium channels has not been established, but is possible (Edwards et al., 1991).

Noradrenaline-induced contraction of the tail arteries of rats in vitro has been attributed to an elevation in $[Ca^{2+}]_i$ as a consequence of the combination of enhanced entry of calcium ion across the sarcolemma and the mobilization of calcium ion from intracellular stores. CGRP does not inhibit the contractile responses to potassium chloride or noradrenaline when calcium ion is added in increasing concentration to a calcium-deficient buffer, indicating that CGRP has no effect on calcium entry across the sarcolemma. However, CGRP inhibits noradrenaline-induced contraction of these vessels, incubated in calcium-free buffer, in a concentration-dependent manner, indicating that the relaxant effect of CGRP in rat tail arteries might be accounted for by the inhibition of the release of calcium ion from intracellular stores (Kline and Pang, 1988).

At present, therefore, there is no consensus regarding the receptor-effector coupling mechanisms mediating either endothelium-dependent (fig. 4) or endothelium-independent (fig. 5) vasodilation in response to CGRP. The existence of qualitative variations in the inherent properties of the endothelium and vascular smooth muscle, and in populations of receptors for CGRP and their associated receptor-effector coupling mechanisms between different vascular beds, often within the same species, might account for the conflicting experimental results obtained in such studies. Perhaps of greater significance is the fact that such variations might be expected to be advantageous in the development of receptor-selective analogues and antagonists of CGRP designed to target specific regions of the vasculature.

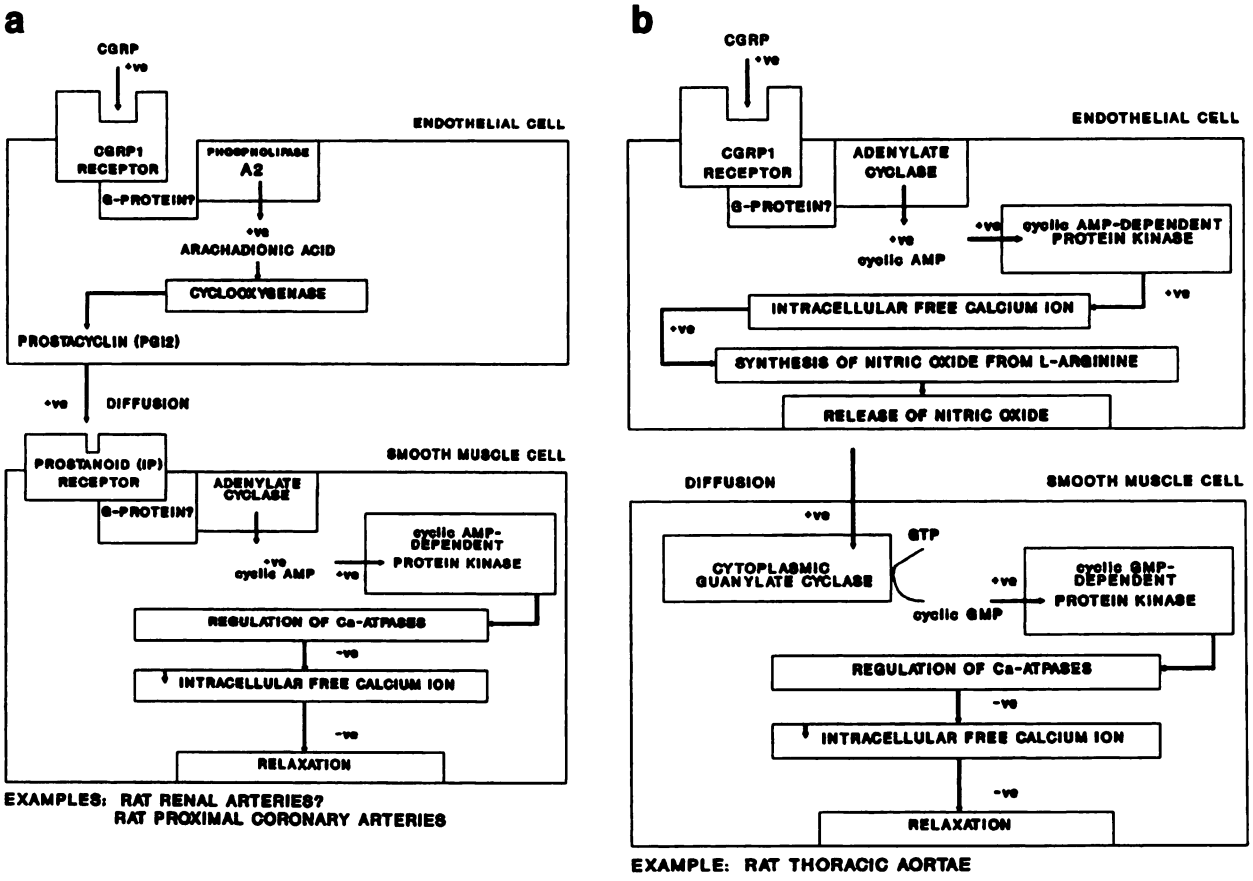


FIG. 4. Overview of the signal transduction mechanisms implicated in vasorelaxation in response to CGRP in the presence of intact endothelium. Binding of CGRP to an endothelial receptor is generally thought to trigger synthesis and release of NO from L-arginine (*a*), or less commonly, of prostacyclin from arachadionic acid (*b*). These molecules then diffuse to adjacent smooth muscle cells, in which they stimulate the accumulation of the cyclic nucleotides, cGMP (*a*) and cAMP (*b*), respectively.

B. In Coronary Vasculature

The effect of CGRP on the coronary circulation is nonuniform, exhibiting a regional variation not only in terms of magnitude, but also receptor pharmacology and endothelium-dependence. In humans, CGRP causes much greater changes in diameter of epicardial coronary arteries from the circumflex and mid-distal left anterior descending regions than from the proximal left anterior descending region (McEwan et al., 1986). $CGRP_{8-37}$ inhibits CGRP-induced relaxation of rings of large but not of small coronary arteries of pigs (Fawlkes et al., 1991), indicating that subtle differences exist in the vasodilator profile of CGRP with respect to the size of the vessel. CGRP-induced vasorelaxation is endothelium-independent in bovine (Greenberg et al., 1987), porcine (Shoji et al., 1987) and human coronary arteries (Thom et al., 1987; Franco-Cereceda and Rudehill, 1989) and distal intramyocardial arteries of rats (Prieto et al., 1991), but endothelium-dependent in proximal coronary arteries of rats (Prieto et al., 1991). Prostacyclin, but not NO, has been implicated as a mediator of endothelium-dependent vasorelaxation in the proximal coronary arteries of rats, because the vasorelaxant effect of the peptide, elicited in vitro in vessels with intact endothelium, is abolished by indomethacin but not by methylene blue (Prieto et al., 1991). Endothelium-independent relaxation elicited by CGRP in segments of porcine coronary arteries precontracted with $PGF_{2\alpha}$ is associated with, and preceded by, the accumulation of cAMP, but not of cGMP (Shoji et al., 1987). However, a causal role for cAMP in the vasorelaxant response to CGRP has not been established. In endothelium-dependent proximal epicardial, but not in endothelium-independent distal intramyocardial, coronary arteries of rats, the extent of the vasorelaxant effect of CGRP depends on the type of vasoconstrictor used and on the level of tone induced in the vessel (Prieto et al., 1991). This indicates that the membrane potential of vascular smooth muscle cells might play an important role in the determination of the magnitude of the vasorelaxant response to CGRP in these vessels. However, the failure of glibenclamide to inhibit the vasorelaxant response to the peptide in both proximal epicardial and distal intramyocardial coronary arteries of rats excludes an involvement of ATP-sensitive potassium channels in both endothelium-dependent and endothelium-independent vasodilation of the coronary vasculature in this species (Prieto et al., 1991). In rats, at least, proximal epicardial vessels might represent a

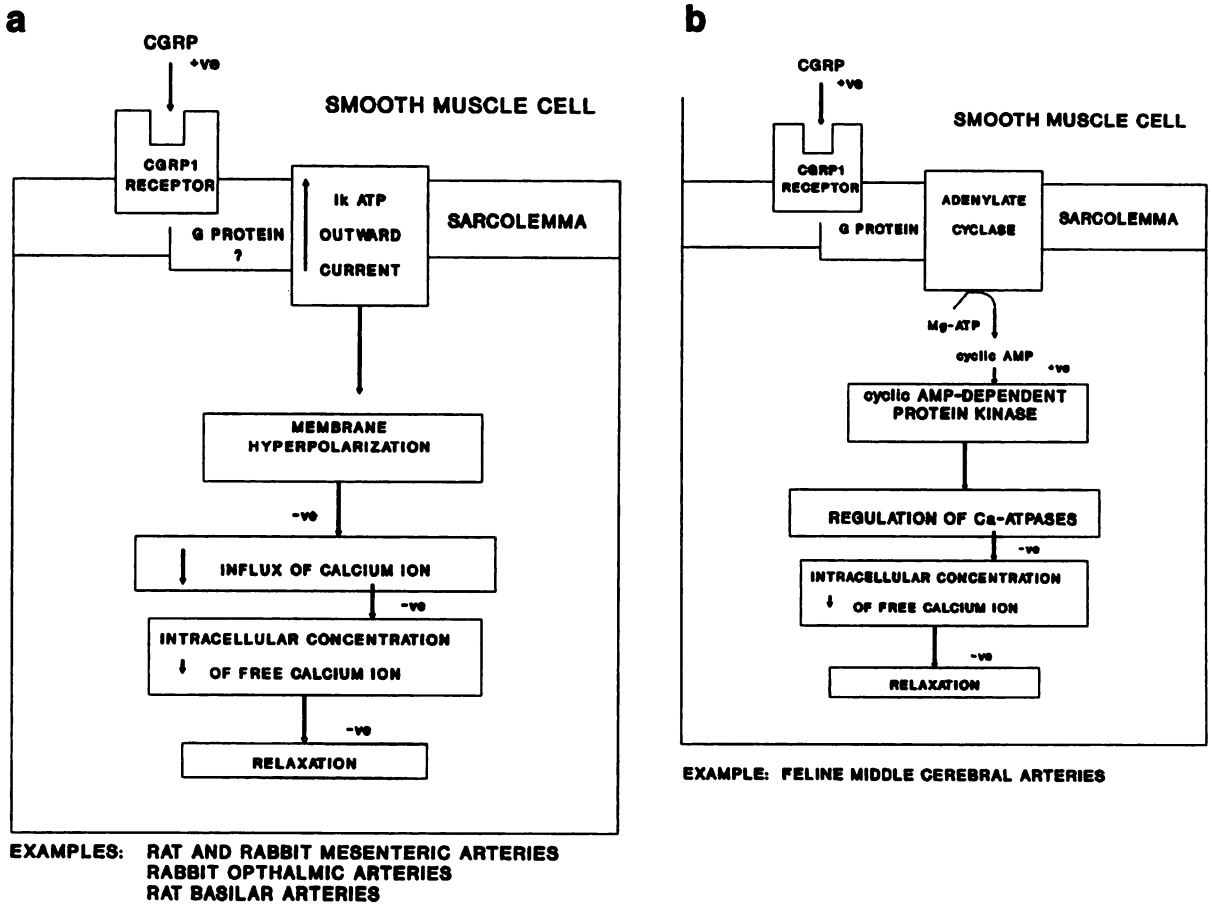


FIG. 5. Overview of the signal transduction mechanisms implicated in vasorelaxation in response to CGRP in the absence of endothelium. Binding of CGRP to a receptor on the vascular smooth muscle cell is generally thought to trigger the activation of an I_{kATP} current, causing membrane hyperpolarization (*a*) or stimulating the accumulation of cAMP (*b*). The activation of either of these mechanisms causes a reduction in the intracellular concentration of free calcium ion in the smooth muscle cell.

transition between endothelium-dependent actions of CGRP in the aorta (Kubota et al., 1985) and endothelium-independent actions in distal intramyocardial vessels.

The luminal surface of the mammalian ventricle is lined by 'endocardium,' which consists of a monolayer of closely apposed endothelial cells overlying a thin layer of connective tissue. Interest in this much neglected area of research has grown since the demonstration that endocardial-endothelium influences contractility of the subjacent myocardium by releasing chemical mediators in a manner analogous to the regulation of smooth muscle tone by vascular endothelium (Smith et al., 1991; Henderson et al., 1992). Because CGRP mediates endothelium-dependent vasodilation in a number of vessels, and a very high density of binding sites for CGRP are present in the endocardium (Sigrist et al., 1986; Coupe et al., 1990), the actions of the peptide in this region of the heart should be explored.

C. In Myocardial Tissues

1. Second-messenger substances. There is indirect evidence both for and against a causal role for cAMP as a mediator of the myotropic effects of CGRP. The inotropic and chronotropic actions of the peptide in isolated atrial muscle preparations of rats (Wang and Fiscus, 1989) and guinea pigs (Franco-Cereceda and Lundberg, 1988; Ishikawa et al., 1988) are potentiated by 3-isobutyl-1-methylxanthine but not by the less potent and less selective phosphodiesterase inhibitor, theophylline (Satoh et al., 1986). Forskolin, a direct activator of adenylate cyclase, potentiates the inotropic and chronotropic effects of CGRP in these species (Wang and Fiscus, 1989; Mantelli et al., 1992), but prolonged pre-incubation with this agent does not desensitize these actions of CGRP (Mantelli et al., 1992). Wang and Fiscus (1989) have suggested that, in rats at least, the receptors and/or postreceptor mechanisms mediating the inotropic effect of CGRP in electrically driven left atrial preparations might differ from those mediating the chronotropic effects of the peptide in spontaneously beating right atrial preparations, because forskolin potentiates the positive inotropic response of CGRP markedly but the positive chronotropic response only slightly in these preparations. Cholera toxin, which irreversibly ribosylates G_s proteins (Murakami and Yasuida, 1986), does not mod-

ify the inotropic effect of CGRP, despite antagonizing the response to isoprenaline in left atria of guinea pigs (Mantelli et al., 1992). CGRP stimulates the production of cAMP in crude membrane suspensions prepared from the atria of rats (Ishikawa et al., 1987; Wang and Fiscus, 1989) and guinea pigs (Ishikawa et al., 1988). However, CGRP does not stimulate the production of this second-messenger substance in crude membrane suspensions prepared from the ventricles of these species (Ishikawa et al., 1987, 1988). Accumulation of a second-messenger substance in membrane suspensions or crude homogenates prepared from whole heart or isolated atria or ventricles indicates but does not in itself establish that the second-messenger substance is localized in cardiomyocytes. This information can be obtained by the use of purified suspensions of intact cardiomyocytes. Although CGRP has been shown to stimulate production of cAMP in cultured cardiomyocytes prepared from the hearts of neonatal rats (Fisher et al., 1988; Chatterjee et al., 1991), it has not been clarified whether these cells were exclusively atrial or ventricular in origin. CGRP does not stimulate production of cAMP in ventricular cardiomyocytes isolated from the hearts of adult rats (Bell and McDermott, 1994) (table 4). The inability of a neuropeptide to stimulate accumulation of cAMP in a given preparation should be viewed with caution, because small, localized increases in the amount of cAMP might not be detected by the bioassay used, although such amounts might be sufficient to trigger biochemical events associated with cellular contraction. Equally, accumulation of cAMP within cardiomyocytes indicates but does not in itself provide conclusive evidence that this second-messenger substance is linked in a causal manner to the contractile responses elicited in these cells, even though there might be strong temporal and concentration-dependent correlation between the two parameters (Warbanow and Wollenberger, 1982). The use of Rp-cAMPS, a membrane-permeable selective antagonist of cAMP, has confirmed that CGRP stimulates cellular contraction by a cAMP-independent mechanism in rat ventricular cardiomyocytes (Bell and McDermott, 1994b).

The contractile coupling mechanism associated with CGRP receptors in ventricular cardiomyocytes has not been determined. CGRP does not alter basal or agonist-stimulated accumulation of cGMP in rat ventricular cardiomyocytes (Bell and McDermott, 1994a). In addition, CGRP, present at a maximally effective concentration (1 nM), when combined with isoprenaline or low, but not high, concentrations of extracellular calcium ion, elicits a greater increase in contractile amplitude than that elicited by these stimuli, respectively, in the absence of peptide (Bell and McDermott, 1994a). These data indicate that CGRP might increase the intracellular concentration of free calcium ion by a mechanism that differs, at least in part, from that stimulated by isoprenaline. Indeed, CGRP stimulates a modest increase in $[Ca^{2+}]_i$ in Fura-2 loaded cells, as measured by microspectrofluorimetry (McDermott, unpublished observations). To produce an increase in $[Ca^{2+}]_i$, CGRP might either promote transarcolemmal calcium influx or enhance release of calcium ion from reticular stores. It is possible that coupling of CGRP receptors to phospholipase C stimulates accumulation of inositol triphosphates and activation of PKC, and therefore, release of reticular stores of calcium and regulation of sarcolemmal sodium-calcium exchange. Indeed, activation of protein kinase C in response to CGRP has been demonstrated in rat ventricular cardiomyocytes, and the hypertrophic effects exerted by CGRP in these cells are reduced in the presence of the protein kinase C-selective inhibitor, bisindoylmalemide (Bell et al., 1995).

TABLE 4

Comparison of receptor-effector coupling mechanisms associated with the activation of CGRP receptors in atrial and ventricular cardiomyocytes

	Atrial myocyte	Ventricular myocyte
Effects		
Positive inotropy	Yes[1,2,3]	Yes[4,5]
Positive chronotropy	Yes[2,3]	not known
Protection against myocardial iscaemia	Yes[6]	Yes[6]
Growth-regulatory effects	not known	Yes[7]
Receptor pharmacology		
$CGRP_{8-37}$	antagonist[2] ($pA_2 > 7.0$)	antagonist[5] ($pA_2 = 7.95$)
cysACM[2,7]CGRP	agonist[2] (low potency)	agonist[5] (low potency)
Amylin	partial agonist[8]	partial agonist[9]
Second-messenger substances		
Activation of G-protein	Yes[10]	not known
cAMP	Yes[1,3]	No[1,5]
Activation of protein kinase A	not known	No[11]
cyclic GMP	not known	No[5]
Activation of protein kinase C	not known	Yes[7]
Electrophysiology		
Inward calcium current (ICa)	Yes[10,12]	not known
Outward potassium current (Ik_{mACh})	Yes[12,13]	not known
Activation of L-type calcium channel	Yes[10,12,14,15]	No[15,16]
Activation of Na/Ca exchange mechanism	Yes[14]	not known

Data are from the following references: (1) Ishikawa et al., 1987; (2) Dennis et al., 1989; (3) Fisher et al., 1988; (4) Miyauchi et al., 1988; (5) Bell and McDermott, 1994a; (6) Ren et al., 1993; (7) Bell et al., 1995; (8) van Rossum et al., 1994; (9) Bell and McDermott, 1995a; (10) Ono and Giles, 1991; (11) Bell and McDermott, 1994b; (12) Ono et al., 1989; (13) Kim, 1991; (14) Satoh et al., 1986; (15) Nakajima et al., 1991; (16) Bell and McDermott, 1995a.

2. Electrophysiological effects of calcitonin gene-related peptide in the heart. CGRP increases the amplitude and prolongs the duration of action potentials elicited at a level of 50% repolarization in isolated left atria

of guinea pigs, concomitantly with a positive inotropic effect and increased velocity of relaxation (Ishikawa et al., 1988). In addition, CGRP induces "slow response" action potentials in partially depolarized isolated atria of guinea pigs (Ishikawa et al., 1988). Because CGRP does not change the upstroke velocity of action potentials elicited in this tissue, an influence of the peptide on voltage-dependent sodium channels is unlikely. The observed alteration in the duration of action potentials has been attributed to an action of CGRP either on inward calcium currents or on outward potassium currents.

In atria of rats, verapamil, a selective antagonist at L-type calcium channels, inhibits the inotropic action of CGRP only when the peptide is present at low concentration. Ouabain inhibits, and tetrodotoxin potentiates, the inotropic action of CGRP only when the peptide is present at high concentration (Satoh et al., 1986). Both of these agents modulate indirectly the sodium-calcium exchange mechanism. The inotropic effect of CGRP in this tissue is completely inhibited when ouabain and verapamil are present together. This suggests that a low concentration of CGRP stimulates the influx of calcium ion mainly through L-type calcium channels, whereas the sodium-calcium exchange mechanism makes a significant contribution to the influx of calcium ion only when the peptide is present at a higher concentration. CGRP increases the inward calcium current in cardiomyocytes isolated from the atria of bullfrogs and rabbits dramatically, but transiently (Ono et al., 1989). In cardiomyocytes isolated from the atria of guinea pigs, a similiar but more sustained effect has been observed (Ono and Giles, 1991); however, in this species, CGRP prolongs the duration of action potentials, whereas in bull frogs, the peptide shortens the duration of action potentials. This difference might explain why, when I_{Ca} is fully activated by isoprenaline, CGRP, present at high concentration, can strongly inhibit this current in atrial cardiomyocytes of bull frogs but not of guinea pigs. In both species, the voltage characteristics of calcium influx in response to CGRP are compatible with an action of the peptide at L-type rather than T-type calcium channels. These CGRP-activated currents are sensitive to antagonism by $CGRP_{8-37}$ (Nakajima et al., 1991) and are enhanced by guanosine 5′-O-3′-thiotriphosphate ($GTP\gamma S$) and partially suppressed by guanosine 5′-O-2-thiodiphosphate ($GDP\beta S$) (Ono and Giles, 1991). In addition, previous application of cAMP or forskolin abolishes CGRP-induced influx of calcium ion. This indicates an involvement of both G-proteins and cAMP in the generation of these currents in response to the peptide (table 4). However, CGRP does not influence L-type calcium channel current in cardiomyocytes isolated from ventricles of guinea pigs (Nakajima et al., 1991). Similarly, the contractile response to CGRP elicited in ventricular cardiomyocytes isolated from the hearts of adult rats is not antagonized by the selective antagonists at L-type calcium channels, diltiazem or verapamil (Bell and McDermott, 1995).

When applied at low concentration, CGRP accelerates activation and retards deactivation of the delayed rectifier current, I_k, in atrial cardiomyocytes of bull frogs, but these effects are apparent in atrial cardiomyocytes of guinea pigs only when the peptide is present at higher concentrations (Ono and Giles, 1991). These effects persist even in the presence of complete inhibition of I_{Ca}. In cardiomyocytes isolated from the atria of neonatal rats, CGRP, when present at high concentrations, activates muscarinic-gated potassium channels (Kim, 1991). This evokes an inward rectifying potassium current that might exert a small opposing action on the overall stimulatory response to CGRP, and thus serves as a protective mechanism when the peptide is present at high concentration. CGRP is without effect on the other known inward rectifying potassium currents, I_{k1} and I_{kATP}, and on the transient outward current, I_{to}.

VIII. Evidence in Support of a Role for Calcitonin Gene-Related Peptide as a Neurotransmitter in the Cardiovascular System

A. The Actions of Capsaicin Indicate a Physiological Role for Neuronally Released Calcitonin Gene-Related Peptide in the Heart and Vasculature

Transmural nerve stimulation of isolated right atria of guinea pigs induces a biphasic positive chronotropic response (Saito et al., 1986). Inhibition of the rapid phase of this response, which is abolished by surgical sympathectomy or application of reserpine or atenolol, unmasks a slow phase of the chronotropic response. This slow phase persists in the presence of hexamethonium or selective antagonism at muscarinic cholinoceptors, histamine or 5-HT receptors, but is absent in animals pretreated systemically with tetrodotoxin or capsaicin. This suggests an involvement of NANC transmitters in the generation of the slow phase of this chronotropic response (Goto et al., 1987). Capsaicin induces a sustained tachycardia accompanied by a transient negative inotropic response that is succeeded by a small, but sustained, positive inotropic effect in isolated perfused hearts of guinea pigs (Franco-Cereceda and Lundberg, 1985, 1988), and elicits a positive inotropic response in atria of rats (Sigrist et al., 1986). $CGRP_{8-37}$ strongly suppresses the increase in heart rate induced by infusion of capsaicin in guinea pigs (Satoh et al., 1993). Capsaicin does not affect the contractility of isolated perfused hearts of dogs and rabbits (Toda et al., 1972). Chemical sympathectomy does not inhibit the contractile action of capsaicin in isolated perfused hearts of guinea pigs (Lundberg et al., 1984). However, previous administration of capsaicin in vivo abolishes the chronotropic action, but not the inotropic action, of capsaicin observed in this tissue in vitro. This indicates that, although capsaicin might stimulate the release of a neu-

rotransmitter from sensory nerves to exert a chronotropic action in atria, the irritant might exert a direct depressant action on ventricular contractility. In agreement with this finding, capsaicin has been shown to exert an inhibitory effect on the upstroke velocity of the action potential in papillary muscles of guinea pigs. Positive inotropic and chronotropic effects of capsaicin have been demonstrated in isolated atria of guinea pigs (Fukada and Fumijiwara, 1969; Molnar et al., 1969; Lundberg et al., 1984) but are absent in humans (Franco-Cereceda et al., 1987a). In atrial preparations of guinea pigs, however, capsaicin has little or no effect on action potential upstroke velocity (Franco-Cereceda et al., 1988). Capsaicin does not alter the contractility of isolated ventricular muscle strips of rats (Sigrist et al., 1986) and pigs (Miyauchi et al., 1988).

Capsaicin stimulates a calcium-dependent outflow of CGRP and neurokinin A from isolated perfused hearts of guinea pigs (Franco-Cereceda et al., 1987d, 1988, 1989, 1991). Recent studies suggest that capsaicin interacts with a specific receptor site located on the cell membrane of sensory nerves, thereby activating a receptor-operated cation channel, leading to an elevation in the concentration of free calcium ion intracellularly and, consequently, to the release of CGRP (Franco-Cereceda et al., 1991). Immunoreactivities to CGRP, substance P and neurokinin A coexist in capsaicin-sensitive sensory nerves in the hearts of guinea pigs (Papka et al., 1981; Lundberg et al., 1985; Saito et al., 1986; Franco-Cereceda et al., 1987b, 1988). Substance P does not affect cardiac contractility (Franco-Cereceda and Lundberg, 1985, 1988), whereas neurokinin A exerts small, nonsignificant, negative inotropic and chronotropic actions in isolated atria and ventricles from this species (Franco-Cereceda and Lundberg, 1988). In addition, substance P and neurokinin A, in contrast to CGRP, do not induce any changes in contractility in pieces of electrically driven human right atrial tissue (Franco-Cereceda et al., 1987a). The positive contractile response to capsaicin in atria of guinea pigs is similiar in magnitude and duration to that of CGRP (Sigrist et al., 1986; Franco-Cereceda and Lundberg, 1988) and is absent after the establishment of tachyphylaxis in the response to CGRP. Local release of CGRP from primary sensory afferent neurons may, therefore, underlie the stimulatory effects of capsaicin on cardiac contractility. The absence of a positive inotropic response to capsaicin in isolated ventricular muscle strips of pigs (Miyauchi et al., 1988), despite the positive inotropic action of CGRP in this species, has been attributed to either poor innervation of this tissue by NANC nerves or corelease of several other neurotransmitters with a mutually antagonistic action to that of CGRP. Similarly, the absence of a positive inotropic response to capsaicin in isolated pieces of electrically paced auricle of human right atrial tissue might indicate that few CGRP-containing nerves penetrate between individual atrial myocytes in humans; alternatively, this might be caused by depletion of endogenous peptides from these nerves during electrical pacing experiments or as a consequence of the influence of anaesthesia upon C-fibre afferent nerves during surgery (Franco-Cereceda et al., 1987a).

Depletion of CGRP and substance P from the NANC innervation to the coronary vasculature of rats after administration of capsaicin results in a significant reduction in basal coronary flow that is reversible upon the administration of exogenous CGRP (Yaoita et al., 1994). In contrast to CGRP, substance P-induced relaxation has been demonstrated in only a few distal intramyocardial coronary arteries in rats in vitro, and is absent in the majority of these vessels, and in proximal epicardial coronary arteries of this species (Prieto et al., 1991). Ruthenium red, which exerts an antagonistic action upon calcium ion and thus prevents the active release of neurotransmitter substances, inhibits the vasorelaxant action of capsaicin in proximal epicardial and distal intramyocardial coronary arteries of rats (Prieto et al., 1991). This indicates that capsaicin elicits specific active release of CGRP from sensory nerve endings supplying coronary arteries. Indeed, CGRP release has been demonstrated in human epicardial coronary arteries in vitro (Franco-Cereceda, 1991). The endothelium-independent vasodilator response to capsaicin in human coronary arteries is not influenced by tachyphylaxis to substance P, which, in contrast to CGRP, elicits only a small, endothelium-dependent vasodilator response in this tissue (Franco-Cereceda and Rudehill, 1989).

Similar studies conducted in the peripheral vasculature indicate a role for endogenous CGRP in NANC-mediated vasodilatory responses. In isolated, perfused, mesenteric arterial beds of rats, pretreated with guanethidine and precontracted with methoxamine, stimulation of the periarterial nerves elicited a frequency-dependent and endothelium-dependent vasodilation that was slow in onset, sustained, reversible and resistant to pharmacological intervention with propanolol and atropine, but sensitive to tetrodotoxin and capsaicin (Kawasaki et al., 1988). This NANC vasodilatory response was abolished by antiserum to CGRP (Han et al., 1990a), by desensitization of receptors for CGRP, and by $CGRP_{8-37}$ (Han et al., 1990b). In addition, stimulation of the periarterial nerves in this preparation elicits a three-fold increase in the amount of CGRP released from the tissue; this increase is attenuated markedly in the presence of capsaicin or tetrodotoxin (Fujimori et al., 1987; Manzini et al., 1991). Intragastric administration of capsaicin dilates gastric submucosal arterioles in rats; this is markedly reversed by intravenous infusion of the inhibitor of NO synthesis, N^G-nitro-L-arginine methyl ester (L-NAME), and by submucosal infusion of L-NAME of $CGRP_{8-37}$ (Chen and Goth, 1995). The latter findings indicate that both NO and CGRP are released locally at the submucosal level. Submucosal application of CGRP induces a dose-dependent dilation of gastric submucosal

arterioles that is significantly attenuated, but to a much lesser extent, by L-NAME. These data indicate that endothelium-derived NO is released in response to CGRP, but this is not the only source of submucosal NO released in response to capsaicin. Capsaicin-induced dilation of arterioles and venules supplying cremaster striated muscles in situ (Kim et al., 1995), and the microvasculature of rabbit skin in vivo (Hughes and Brain, 1991; Brain et al., 1993) are also antagonized by $CGRP_{8-37}$. Electrical field stimulation of bovine intraocular anterior ciliary arteries in vitro, in the presence of phentolamine, propanolol and atropine, elicits an endothelium-independent vasorelaxation that is unaffected by tachyphylaxis to substance P, but is attenuated in vessels pretreated with capsaicin or $CGRP_{8-37}$ (Wiencke et al., 1993).

B. Evidence that Endogenous Calcitonin Gene-Related Peptide is Present at Physiologically Relevant Concentrations

Studies such as those outlined above indicate that endogenous CGRP might be actively released from the NANC primary afferent supply to the cardiovascular system in response to appropriate stimuli and might participate in the regulation of blood vessel tone. Although the concentrations of exogenous CGRP required to elicit significant vasorelaxant effects in some blood vessels in vitro is not always compatible with the low picomolar concentrations of the peptide present in mammalian plasma in vitro, it is probable that localized concentrations of the peptide are much higher in regions receiving a prominent innervation with CGRP-immunoreactive nerve fibres. In the superior mesenteric arteries of rats (Kawasaki et al., 1988) and coronary arteries of guinea pigs (Goto et al., 1992), a high concentration of CGRP immunoreactivity in the sensory innervation of these vessels is correlated with the potent vasodilatory responses to exogenous CGRP. However, in rat femoral arteries and veins, which contain relatively few CGRP-immunoreactive nerve fibres, exogenous CGRP elicits only a weak vasorelaxation (Edvinsson et al., 1989c).

The source of CGRP in vivo for the responses demonstrated in ventricular myocardium in vitro remains uncertain. CGRP-immunoreactive nerve fibres are present only rarely within mammalian ventricles, where these fibres are found predominantly in association with the coronary vasculature. It might be that a sufficient amount of CGRP is released from this limited nervous supply to reach the majority of ventricular cardiomyocytes at significant concentrations. The source of CGRP that circulates in the plasma of rats at picomolar concentrations is thought to mainly represent an overspill from the cardiovascular primary afferent nerves. Although such concentrations are compatible with the concentration of the peptide required to elicit inotropic and hypertrophic effects in ventricular cardiomyocytes isolated from the hearts of adult rats (Bell and McDermott, 1994a; Bell et al., 1995a), it is not clear whether the peptide could reach the ventricular myocardium from the plasma at similar concentrations under normal physiological conditions.

C. Evidence that Endogenous Feedback Mechanisms Regulate the Release of Calcitonin Gene-Related Peptide from the Cardiovascular Sensory Nerve Supply

There is evidence that adrenergic neurotransmitters exert a neuromodulatory influence upon neurotransmission in CGRP-containing nerves. In rat mesenteric resistance vessels, neuronally released noradrenaline modulates CGRP-mediated vasodilation by inhibiting the release of CGRP via presynaptic α_2-adrenoceptors located on primary sensory afferent neurons (Kawasaki et al., 1990a). Neuropeptide Y, a vasoconstrictor peptide colocalized with noradrenaline in sympathetic perivascular nerves, inhibits dilation of mesenteric resistance vessels induced by perivascular nerve stimulation. Neuropeptide Y has no effect by itself on vasodilation in response to bolus infusion of exogenous CGRP but might modulate release of CGRP from CGRP-containing nerves in these vessels (Kawasaki et al., 1991). On the other hand, CGRP-containing nerves, by releasing CGRP postsynaptically, inhibit noradrenergic nerve-mediated vasoconstriction in rat mesenteric resistance vessels (Kawasaki et al., 1990b). Therefore, it has been proposed that adrenergic and CGRP-containing nerves regulate the tone of mesenteric resistance vessels by reciprocal interaction. Nuki and coworkers (1994) have recently provided additional evidence in support of a physiological role for CGRP as a neurotransmitter in the peripheral vasculature: these authors found that CGRP-containing vasodilator nerves supplying the mesenteric arteries of rats are endowed with presynaptic CGRP receptors that regulate the release of CGRP from the nerves by a negative feedback mechanism.

Compelling evidence was provided in a recent study by Portaluppi and coworkers (1993) that CGRP has a physiological role in the regulation of vascular tone and blood pressure. In eight healthy subjects, after assumption of an upright posture, a rapid rise in the concentration of CGRP in the plasma was observed concomitantly with the expected increases in plasma noradrenaline, aldosterone and renin-angiotensin. Infusion of angiotensin II also caused dose-dependent increases in the concentrations of CGRP and aldosterone in plasma in parallel with the rise in blood pressure. These data demonstrate that modification of the concentration of CGRP in plasma is part of the normal responses to postural and vasomotor changes. These authors (Transforini et al., 1994) have also reported that a pharmacologically induced enhancement of cholinergic tone results in an increase in CGRP in plasma, indicating that the cholinergic system might act as a positive modulator for the release of CGRP. However, the available data do not indicate that there is a tonic cholinergic influence

responsible for the secretion of CGRP under physiological conditions because the release of the peptide was unaltered in the presence of the muscarinic receptor antagonist, pirenzepine.

Serotonin (5-HT) elicits a capsaicin-sensitive positive inotropic response in the atria of guinea pigs in vitro. Application of 5-HT into isolated perfused hearts of guinea pigs induces an efflux of CGRP-immunoreactivity that is attenuated if the tissue is pretreated with capsaicin or selective antagonists at 5-HT_3 receptors. These results indicate that 5-HT, by stimulating 5-HT_3 receptors located on primary sensory afferents, thereby causing membrane depolarization and neurotransmitter release, might generate a sensory impulse leading to reflex cardiovascular responses including tachycardia and hypotension (Tramontana et al., 1993).

IX. Pathophysiology and Possible Therapeutic Applications in Diseases of the Cardiovascular System

A. Hypertension

Because of its role as a vasodilator peptide, it might be expected that the concentration of CGRP would be elevated in the plasma of hypertensive patients to compensate for the excessive vasoconstrictor activity associated with hypertension. There is, however, no consensus on the relationship between the concentration of CGRP in the plasma and elevated blood pressure. In spontaneously hypertensive rats, used as a model of human essential hypertension, the concentration of CGRP has been found to be higher (Zaidi et al., 1991) or lower (Tang et al., 1989) than normotensive controls. Similarly, the concentration of CGRP in the plasma of patients with essential hypertension is reported to be increased (Masuda et al., 1992), decreased (Edvinsson et al., 1989b; Jian et al., 1989; Tang et al., 1989) or unchanged (Schifter et al., 1991; Edvinsson et al., 1992). In hypertensive patients with high urinary sodium, CGRP concentrations are significantly higher than in the plasma of hypertensive patients with low sodium excretion (Resnick, 1989; Resnick et al., 1989). In a single study, the concentration of amylin was reported to be significantly higher in the plasma of patients with essential hypertension (83.1 pM) than in normotensive controls (40.5 pM) (Kautsky-Willer and Thomaseth, 1994). However, in this study, the control values were much greater than the consensus of 1 to 20 pM. Conflicting findings might be explained by variation in the sampling methods and radioimmunoassays used or might reflect differences caused by the heterogeneity, severity and duration of the hypertensive state, the degree of end organ damage, or the treatment regimen. It has been established in studies conducted in spontaneously hypertensive rats that the content of CGRP in the perivascular nerves is significantly lower than in normotensive control animals (Lewis et al., 1990; Kawasaki et al., 1990c; Kawasaki and Takasaki, 1992) and that the vascular sensitivity to CGRP is impaired (Westfall et al., 1990; Amerini et al., 1994). The neuronal content of mRNA coding for CGRP is significantly decreased in the dorsal root ganglia, but not in cardiac or brain tissue, of spontaneously hypertensive rats compared with normal animals (Supowit et al., 1993). It is uncertain whether a decrease in vasodilator activity of CGRP-containing nerves contributes to the development and maintenance of essential hypertension or reflects the exhaustion of the CGRP content of the perivascular nerve supply with increasing severity and duration of the disease. Further studies designed to investigate the content of CGRP and the regulation of populations of receptors for the peptide in a range of blood vessels obtained from appropriate animal models of essential hypertension and normotensive controls would be useful. Nevertheless, based on circulating levels, there is little evidence in humans for the hypothesis that a deficit of CGRP-induced vasodilation has a causal role in the pathogenesis of essential hypertension. Because CGRP is a potent vasodilator and it is possible that the content of this peptide might be depleted in the perivascular nerves of patients with severe hypertension, it is perhaps surprising that there have been few reports concerning the administration of exogenous CGRP for the treatment of hypertension. Sustained intravenous infusion of hβCGRP into one kidney-one clip dogs, used as a model of essential hypertension, increases heart rate and decreases mean arterial blood pressure, coupled with increases in renal blood flow and glomerular filtration rate (Verburg et al., 1989). However, these beneficial effects are offset by reduction in sodium and potassium excretion caused by activation of compensatory mechanisms such as the sympathetic nervous system, renin-angiotensin system and perhaps a direct action of the peptide on the kidney tubule (Lappe et al., 1987). A single intravenous injection of hβCGRP into 7 patients with essential hypertension lowered mean arterial blood pressure and elevated heart rate significantly. However, within 2 hours, mean arterial blood pressure had returned to pretreatment levels (Jian et al., 1989). This might reflect metabolism of the peptide and activation of compensatory reflexes. It will be necessary to conduct further large-scale clinical trials incorporating a range of doses and treatment regimens and defining more precisely the hypertensive state before application of CGRP can be advocated in the treatment of essential hypertension. The use of hαCGRP rather than hβCGRP might be warranted in such studies because the α form of the peptide predominates in the cardiovascular system, and because of the emerging evidence that subtypes of CGRP-preferring receptor might differ in their sensitivity to the two forms of the peptide. However, the inconvenience of intravenous infusion and the possible mitogenic side effects of prolonged infusion of the peptide might limit the usefulness

of the therapeutic application of CGRP in essential hypertension.

The concentration of CGRP is also elevated in the plasma of patients with hypertension secondary to phaeochromocytoma or primary aldosteronism (Masuda et al., 1992). A marked decrease in mean arterial blood pressure and in the concentration of CGRP in plasma is observed after adrenalectomy, indicating that, in these patients, the elevation in the circulating concentration of the peptide could be a compensatory reaction to elevated mean arterial blood pressure.

B. Septic and Hypotensive Disorders

It might be expected that such a potent hypotensive agent would play a role in hypotension and diminished vascular responsiveness characteristic of septic and endotoxic shock. The concentration of CGRP is elevated significantly (7.5-fold), and mean arterial blood pressure, cardiac index and left ventricular stroke work are reduced in the plasma of septic patients and experimental animal models of the disease (Wang et al., 1988, 1991a, 1992a; Joyce et al., 1990a; Arden et al., 1994). CGRP might function in the pathogenesis of circulatory shock because infusion of $CGRP_{8-37}$ can alleviate tachycardia and hypotension in endotoxic rats (Huttemeier et al., 1993). Vascular beds that have become restricted by neural mechanisms as a result of hypotension might release CGRP from their prominent sensory innervation in response to specific stimuli such as hypoxia and ischaemia, as well as to increasing amounts of inflammatory mediators such as bradykinin, histamine and prostaglandins, all of which are released in circulatory shock, rather than caused by a direct action of endotoxin on the release of CGRP from sensory nerve terminals per se (Huttemeier et al., 1993). In support of this conclusion, application of (*a*) dexamethasone, an anti-inflammatory glucocorticoid, (*b*) histamine antagonists, and (*c*) indomethacin, an inhibitor of prostaglandin synthesis, reduce the release of CGRP into the plasma during experimental endotoxemia in rats (Wang et al., 1991a; 1992). Although the gastrointestinal tract has frequently been demonstrated to be an important target organ in the pathogenesis of septic shock, there is no evidence that the portal circulation is the major source of circulating CGRP during the pathogenesis of septic shock (Wang et al., 1991a). It would appear that the release of CGRP from vascular beds, although improving local blood flow, would exacerbate the hypotensive situation and oppose the compensatory release of vasoconstrictor substances such as noradrenaline and angiotensin. However, elevated concentrations of CGRP might exert important overall beneficial effects on the circulation as a whole, such as enhanced coronary blood flow and, therefore, improved cardiac performance in severe hypotension. It is possible that excessive and sustained release of CGRP into the circulation during prolonged and severe haemorrhagic hypotension could contribute to the eventual decompensation of peripheral resistance vessels. There is evidence that CGRP might contribute to the substantial production of NO that occurs in the vasculature during septic shock by enhancing expression of inducible NO synthase, which in turn would account, at least in part, for the collapse of the vascular system (Schini-Kerth et al., 1994).

C. Raynaud's Syndrome

Despite application of vasodilators such as iloprost, an analogue of prostacyclin (McHugh et al., 1988), and the calcium channel antagonist, nifedepine, the management of patients with Raynaud's syndrome, characterized by severe episodic peripheral vascular insufficiency, remains problematic. Chronic digital pain, ulceration, infection and gangrene are major problems that, if not responsive to conventional medical management, might culminate in surgical amputation. There is evidence that CGRP is deficient in digital cutaneous perivascular nerves of patients with Raynaud's syndrome (Bunker et al., 1990). However, the concentration of CGRP in the plasma of patients with primary Raynaud's phenomenon is not elevated significantly when compared with healthy age- and sex-matched controls (Mydral et al., 1994). Intravenous infusion of CGRP significantly dilated compromised digital cutaneous vasculature, as measured by laser Doppler flowmetry, and promoted healing of ulcers in four of five patients with severe Raynaud's syndrome secondary to connective tissue disease (Bunker et al., 1993). It is uncertain why ulcers should heal and remain in remission after apparently transient improvements in blood flow in response to CGRP, because other vasodilators, e.g., iloprost, which also promote transient improvements in blood flow, have no long-term beneficial effects upon the healing of ischaemic lesions. It could be that the beneficial effects of CGRP are associated with the angiogenic properties of the peptide. The inconvenience of intravenous infusion of CGRP might limit its routine use. Further clinical trials, designed to explore the effects of various doses of the peptide and a range of treatment regimens in a larger number of patients, coupled with studies conducted in appropriate animal models of the disease in vitro to investigate the cellular basis of the actions of the peptide in healthy and diseased cutaneous vasculature, are warranted.

D. Subarachnoid Haemorrhage

The first line of treatment for subarachnoid haemorrhage is surgery to prevent rebleeding. However, this might exacerbate cerebral ischaemia and vasospasm. Given the potent vasodilator effects of CGRP on cerebral blood vessels (Uddman and Edvinsson, 1989) and that the peptide can increase cerebral blood flow without increasing blood pressure, administration of CGRP is a logical therapeutic strategy. Indeed, a preliminary study indicated that infusion of CGRP could improve coma

score, used as an index of neurological function, without gross haemodynamic disturbance (Johnston et al., 1990). However, in a subsequent randomized trial, CGRP was infused at progressively increased concentrations in patients with neurological defects after intracranial aneurysm undergoing surgery for subarachnoid haemorrhage. Only one-third of the group completed the treatment with the peptide, with hypotension being the main reason for cessation (European CGRP in Subarachnoid Haemorrhage Study Group, 1992). During the maximum infused dose of the peptide, mean systolic and diastolic blood pressure decreased significantly, and heart rate increased significantly. The neurological defects, according to the World Federation of Neurological Surgeons' Scale, improved with no adverse effects in 9 of 15 patients after infusion of CGRP. Although CGRP failed to show significant beneficial effects over standard treatments such as hypervolaemia and hypertension, the ability of CGRP to exert a direct action on the cerebral vasculature and the temporary postoperative improvement in cerebral blood flow merits further studies with more prolonged application and different doses and routes of delivery of the peptide, perhaps in combination with existing treatments designed to induce hypervolaemia and hypertension. Holland and coworkers (1994) have provided preliminary evidence, obtained from a study conducted in rats in vivo, that the intrathecal route is effective for the administration of CGRP and avoids the onset of systemic hypotension associated with the intravenous infusion of the peptide. The activation of protein kinase C is thought to be a critical component in spastic constriction associated with the pathogenesis of cerebral vasospasm after subarachnoid haemorrhage. There is preliminary evidence, obtained from studies conducted in rabbit basilar arteries in vitro, that CGRP can modulate the vasoconstrictor response associated with activation of protein kinase C (Sutter et al., 1995). Further studies are, therefore, warranted to identify the interaction CGRP might have with other components of the vasospastic response and to characterize the mechanisms through which the peptide elicits vasodilation, to better understand the potential usefulness of targetting CGRP-mediated vasorelaxation as a strategy for the treatment of cerebral vasospasm after subarachnoid haemorrhage.

E. Cluster Headache Attack

Cluster headache is a typical example of a vascular headache that presents with cycles of frequent attacks alternating with attack-free periods. The pathophysiological mechanism(s) responsible for cluster headache are poorly defined, although well documented vascular changes indicate that intra- and extracranial vessels are among the anatomical structures involved. Recent experiments have demonstrated that unmyelinated nociceptive trigeminal fibres provide the major source of sensory innervation for cranial blood vessels and contain neuropeptides such as CGRP and substance P (Uddman et al., 1985). These neurons convey nociceptive impulses to the brain and, at the same time, might corelease CGRP and substance P from the peripheral nerve endings, thus evoking a variety of effects collectively known as 'neurogenic inflammation' (Holzer, 1988). Nitroglycerin-provoked cluster headache can reproduce attacks that are indistinguishable from spontaneously occurring attacks and, therefore, represent a suitable model of the pathophysiological condition. CGRP-like immunoreactivity is elevated in blood collected from the external jugular vein homolateral to the pain side in patients during an acute period of nitroglycerin-induced cluster headache and is further elevated at the peak of a provoked attack (Fanciullacci et al., 1995). In addition, electrical stimulation of the trigeminal ganglion causes an ipsilateral increase in facial skin blood flow in rats in vivo by a mechanism that is markedly attenuated by the selective antagonist at $CGRP_1$ receptors, $CGRP_{8-37}$ (Escott et al., 1995). Taken together, these data indicate that CGRP might be involved in the activation of the trigeminovascular system during the active period of cluster headache.

F. Coronary Heart Disease

Spasm of coronary arteries might occur in association with atheromatous disease, in isolation, or in combination with so-called variant or Prizmetal's angina (McEwan et al., 1986). Areas of coronary artery with atheroma, taken from grossly noninfarcted tissue obtained from hearts removed at the time of transplantation in patients with end-stage ischaemic heart disease and containing a mixture of normal, stenosed and occluded vessels, contain significantly decreased numbers of binding sites for CGRP (Coupe et al., 1990), whereas upregulation of binding sites for the peptide is observed in areas immediately adjacent to the atheroma (Sun et al., 1993). The lowest concentrations of CGRP are detected in human plasma between 3 to 6 a.m., a time-interval that correlates with a high incidence of cardiovascular episodes such as cerebral and coronary thrombosis and might indicate that deficiency of the peptide or its receptors could contribute to the occlusion of already diseased arteries. Infusion of exogenous CGRP dilates stenosis of epicardial coronary arteries and improves workload to ischaemia (Ludman et al., 1991; Uren et al., 1993). At variance with these findings, the inability of previous infusion of CGRP to prevent coronary arterial spasm in patients with variant angina, indicates that CGRP is not involved with the pathophysiology of this disease. However, CGRP can partially relieve spasm, and so the application of a stable CGRP analogue with a long duration of action might be useful in the treatment of other forms of angina, in which less dramatic changes in coronary vasomotor tone occur. In response to arterial occlusion, coronary collateral vessels undergo extensive

growth to provide an alternative blood supply to the dependent myocardium. CGRP might act as an endogenous vasodilator capable of increasing coronary collateral blood flow to dependent myocardium (Quebbeman et al., 1993). The possibility that CGRP could play a role in the angiogenesis of coronary collateral vessels should also be considered. However, the potential growth-regulating actions of CGRP also has important implications for already-diseased coronary arteries. Although the ability of CGRP to inhibit the proliferation of vascular smooth muscle cells might retard the progression of atherosclerosis, the potential of CGRP to stimulate mitogenesis in endothelial cells might contribute to cellular proliferation and intimal hyperplasia, leading to stenosis. For this reason, prolonged or repeated infusion of CGRP might not be useful in the treatment of coronary arterial spasm.

G. Myocardial Ischaemia

It is well known that myocardial ischaemia is associated with activation of cardiac C-fibre afferent nerves. In isolated Langendorff-perfused hearts of guinea pigs, total stop-flow ischaemia and moderate acidosis evoke active, calcium-dependent release of CGRP immunoreactivity from capsaicin-sensitive nerve terminals (Franco-Cereceda et al., 1987d; 1989). The actual mechanism for ischaemia-induced release of CGRP-immunoreactivity from sensory nerves in the heart remains unclear but might be multifactorial and depend on the formation of a variety of intermediate substances such as adenosine and prostaglandins as well as changes in the extracellular concentration of calcium ions (Franco-Cereceda et al., 1994). Exogenous CGRP reduces the severity and delays the occurrence of ischaemic reperfusion-induced arrhythmia characterized by ventricular tachycardia and fibrillation after experimentally induced temporary occlusion of the coronary artery of rats in vivo (Zhang et al., 1994). CGRP, released in response to increased metabolic demand and global myocardial ischaemia, might serve not only to increase coronary blood flow to myocardial tissue but might also exert direct effects on myocardial cells. There is evidence to indicate that CGRP is directly cardioprotective during hypoxic-reoxygenation by mechanisms not simply accounted for by the peptide's vasodilatory effect on the coronary vasculature. CGRP reduces the release of creatinine phosphokinase and glutamine-oxalacetic transaminase, used as markers of myocardial injury, from cardiomyocytes isolated from the hearts of neonatal rats and subjected to experimental hypoxic-reoxygenation (Ren et al., 1993). These cardioprotective effects might be associated with the peptide's ability to reduce calcium overload and loss of magnesium ion and with effects on anti-lipid peroxidation and stabilization of the cell membrane.

H. Myocardial Infarction

Acute myocardial infarction is another situation in which there is demand for counteraction of coronary vasoconstriction but in a more urgent form. An almost two-fold increase in CGRP-immunoreactivity has been detected in the plasma of patients with acute myocardial infarction within 24 hours after admission (Mair et al., 1990), indicating that CGRP might be released in response to ischaemia associated with myocardial infarction or because of constriction of peripheral vessels in response to reduced cardiac output.

I. Congestive Heart Failure

Congestive heart failure is characterized by reduction in cardiac output caused by impaired myocardial contractility. In patients with severe congestive heart failure (New York Heart Association Classes III and IV, ejection fraction $< 35\%$, treated with digoxin and diuretics and/or angiotensin-converting enzyme inhibitors), the concentration of CGRP-immunoreactivity in plasma is mildly, but not significantly, elevated compared with healthy subjects or patients with mild congestive heart failure (New York Heart Association Classes I and II) (Edvinsson et al., 1990). CGRP is not elevated in the plasma of rats with congestive heart failure induced experimentally by ligation of the coronary artery (Helin et al., 1994). Although intravenous infusion of the peptide enhances cardiac performance in patients with congestive heart failure (Gennari et al., 1990; Shekar et al., 1991), it is uncertain whether the beneficial enhancement of cardiac output can be attributed to a direct action of CGRP on the heart or to the reduction in afterload that occurs in response to the vasodilator-induced reduction in systemic and pulmonary vascular resistance (Anand et al., 1991). However, infusion of CGRP at a concentration chosen to limit the hypotensive and chronotropic actions of the peptide reduces the ratio of the pre-ejection period to the left ventricular ejection time and shortens QT interval while enhancing left ventricular shortening index ratio in these patients (Gennari et al., 1990). The substantial improvement of myocardial contractility upon infusion of low doses of CGRP, which does not occur to any significant extent upon infusion of digoxin, and the absence of tachyphylaxis to the peptide coupled with the limitation of hypotensive side effects and therefore of activation of the neurohormonal axis, indicates that prolonged ($\geq$ 24 hour) intravenous infusion of CGRP might represent a novel strategy for the treatment of congestive heart failure.

J. Myocardial Hypertrophy

In view of the pathophysiological complications in heart function caused by cardiac hypertrophy in adult mammals, there has been increasing interest in studies designed to investigate the growth of the ventricular myocardium. At a cellular level, myocardial hypertrophy

is based on the increase in mass, not an increase in the number of myocardial cells, because adult cardiomyocytes do not undergo cell division. Hypertrophying cardiomyocytes also exhibit an altered pattern of gene expression. The synthesis of several proteins that are abundant in the fetal but not the adult state of development is again induced. CGRP and amylin exert hypertrophic effects directly on ventricular cardiomyocytes isolated from the hearts of adult rats and maintained in short-term (24-hr) serum-free primary culture providing mechanical quiescence (Bell et al., 1995). These effects (*a*) are caused by de novo protein synthesis because total content of cellular RNA and incorporation of L-^{14}C phenylalanine into cellular protein were also increased, (*b*) are mediated by a common population of $CGRP_{8-37}$ sensitive, $CGRP_1$-receptors at which amylin binds with lower potency, and (*c*) are associated with a fetal shift in gene expression, characterized by the specific induction of creatine kinase and the β-isoform of myosin heavy chain. Conditions leading to the development and maintenance of myocardial hypertrophy in vivo include increased mechanical loading of myocardial cells and abnormal elevations, either localized or in plasma, in the concentrations of growth-regulating factors. As discussed above, there is no consensus, however, about whether the concentrations of amylin and CGRP are elevated in the plasma of patients with essential hypertension. Although this disease is associated, at least in part, with the onset and maintenance of ventricular hypertrophy, a causal role for CGRP and amylin in the development of myocardial hypertrophy in vivo has not been established, and, for amylin at least, the concentrations at which the hypertrophic effects of the peptide are observed in adult rat ventricular cardiomyocytes in vitro are appreciably greater than those found in the plasma of patients with essential hypertension. Studies designed to investigate the correlation between the concentrations of amylin and CGRP in the plasma of patients with precisely defined essential hypertension and noninvasive indices of myocardial hypertrophy in vivo are warranted. The content of CGRP in the local nerve supply to, and the density of CGRP binding sites within, hypertrophying myocardium could also be compared and contrasted usefully with those found in nonhypertrophied myocardium, using appropriate animal models such as spontaneously hypertensive rats.

X. Conclusions

In conclusion, it is apparent from the growing body of experimental, autoradiographical and immunohistochemical evidence that CGRP is distributed widely within the mammalian cardiovascular system and exerts a complex array of effects on the vasculature and myocardium. According to existing criteria for the classification of receptors for this peptide, the results obtained are generally compatible with the presence of $CGRP_1$-receptors throughout the cardiovascular system. At present, however, there is no consensus regarding the relative potency of the various synthetically available variants of CGRP. In addition, amylin and adrenomedullin have been shown to mimic some of the effects of CGRP by virtue of the ability of these peptides to interact at receptors for CGRP in certain tissues. The existing criteria for the classification of $CGRP_1$ and $CGRP_2$-receptors may, therefore, require adaptation to accommodate the findings obtained with α and β and species-specific variants of CGRP, and with structurally similar peptides such as calcitonin, amylin and adrenomedullan. It is anticipated that the application of molecular biological techniques will facilitate the characterization of receptor binding proteins and might establish the existence of receptor subtypes within the cardiovascular system and, therefore, provide the key to understanding differences between the actions of these peptides in different cell types.

At present, the physiological role of CGRP is only partially understood. In addition to the well established vasodilator and myotropic effects of the peptide, CGRP might exert a subtle influence upon the growth and development of the vasculature and myocardium under physiological and pathophysiological conditions and might be directly cardioprotective during periods of myocardial ischaemia. Although the pathophysiological role of CGRP in many diseases of the cardiovascular system has not been established and remains, at best, poorly understood, there is evidence, obtained from preliminary clinical trials, that CGRP might have therapeutic potential in the treatment of conditions such as Raynaud's syndrome, subarachnoid haemorrhage, angina and congestive heart failure. Although the mitogenic effects of CGRP might have a beneficial application in wound healing and the growth of new blood vessels into ischaemic tissue, such effects might be detrimental in already stenosed vessels. The ability of CGRP to exert potentially detrimental mitogenic and trophic effects, coupled with the inconvenience of intravenous infusion, might necessitate a progression toward the local application of receptor-selective analogues with enhanced stability and a precisely defined target site and mechanism of action. Indeed, preliminary evidence for the existence of marked regional variation in the receptor-effector coupling mechanisms associated with CGRP in the cardiovascular system offers exciting opportunities for the separation of therapeutically desirable effects from adverse effects and for targeting specific regions of the heart and vasculature. The more widespread acceptance and use of purified populations of isolated cardiovascular cells as experimental models that can be applied to the study of receptor pharmacology and cellular mechanisms in the cardiovascular systems of healthy mammals, and the eventual progression to the use of similar models to investigate the role of CGRP in the pathogenesis of various disease states, will

be of considerable influence in the development of novel strategies for therapeutic intervention.

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