

# International Union of Pharmacology. XXXII. The Mammalian Calcitonin Gene-Related Peptides, Adrenomedullin, Amylin, and Calcitonin Receptors

DAVID R. POYNER, PATRICK M. SEXTON, IAN MARSHALL, DAVID M. SMITH, REMI QUIRION, WALTER BORN, ROMAN MUFF, JAN A. FISCHER, AND STEVEN M. FOORD

*Pharmaceutical Sciences Institute, Aston University, Birmingham, United Kingdom (D.R.P.); Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne, Victoria, Australia (P.M.S.); Department of Pharmacology, University College London, London, United Kingdom (I.M.); AstraZeneca Pharmaceuticals, Alderley Edge, Cheshire, United Kingdom (D.M.S.); Douglas Hospital Research Centre and Department of Psychiatry, McGill University, Verdun, Quebec, Canada (R.Q.); Research Laboratory for Calcium Metabolism, Departments of Orthopedic Surgery and Medicine, University of Zurich, Klinik Balgrist, Zurich, Switzerland (W.A.B., R.M., J.A.F.); and GlaxoSmithKline, Medicines Research Centre, Stevenage, Hertfordshire, United Kingdom (S.M.F.)*

This paper is available online at <http://pharmrev.aspetjournals.org>

Abstract .....	233
I. Introduction .....	234
II. The calcitonin gene-related peptide receptors .....	235
A. Additional cofactors or receptors .....	237
B. Tissue factors .....	237
C. Contribution of more than one receptor to pA <sub>2</sub> values .....	237
III. The adrenomedullin receptors .....	237
IV. The calcitonin receptors .....	239
V. The amylin receptors .....	240
VI. Receptor modulation .....	241
A. Expression of mRNA encoding calcitonin-like receptor, calcitonin receptor, and receptor activity modifying proteins .....	242
VII. Receptor effector mechanisms .....	242
VIII. The evolution of receptor activity modifying proteins, the calcitonin peptide family, and its receptors .....	243
IX. Conclusions and recommendations .....	243
References .....	244

**Abstract**—The calcitonin family of peptides comprises calcitonin, amylin, two calcitonin gene-related peptides (CGRPs), and adrenomedullin. The first calcitonin receptor was cloned in 1991. Its pharmacology is complicated by the existence of several splice variants. The receptors for the other members of the family are made up of subunits. The calcitonin-like receptor (CL receptor) requires a single transmembrane domain protein, termed receptor activity modifying protein, RAMP1, to function as a CGRP receptor. RAMP2 and -3 enable the same CL receptor to behave as an adrenomedullin receptor. Although the calcitonin receptor does not require RAMP to

bind and respond to calcitonin, it can associate with the RAMPs, resulting in a series of receptors that typically have high affinity for amylin and varied affinity for CGRP. This review aims to reconcile what is observed when the receptors are reconstituted *in vitro* with the properties they show in native cells and tissues. Experimental conditions must be rigorously controlled because different degrees of protein expression may markedly modify pharmacology in such a complex situation. Recommendations, which follow International Union of Pharmacology guidelines, are made for the nomenclature of these multimeric receptors.

## I. Introduction

The calcitonin family of peptides comprises five known members: calcitonin (CT<sup>1</sup>), amylin (AMY), two CT gene-related peptides ( $\alpha$ CGRP and  $\beta$ CGRP), and adrenomedullin (AM) (Table 1). Although homology at the level of the primary sequence is weak, there are stronger relationships between the secondary structures of the peptides. They all have a six-amino acid ring structure (seven for CT) close to their N termini, formed by an intramolecular disulfide bond. This is followed by a region of potential amphipathic  $\alpha$ -helix, and they all are C terminally amidated.

The peptides are widely distributed in various peripheral tissues as well as in the peripheral and central nervous system (CNS) and induce multiple biological effects including potent vasodilatation (CGRP and AM), reduction in nutrient intake (AMY), and decreased bone resorption (CT). Due to their structural similarities, the peptides share some biological activities, suggesting that they interact with similar G protein-coupled receptors (GPCRs). However, there is clear evidence to support unique biological activities and so distinct receptors. Receptors have been characterized, *in vivo* and *in vitro*, on the basis of pharmacological responses and radioligand binding studies. The cDNA encoding a porcine CT receptor was cloned in 1991 (Lin et al., 1991), with human (Gorn et al., 1992; Kuestner et al., 1994) and rat receptors (Albrandt et al., 1993; Sexton et al., 1993) cloned soon after. The CT receptor belongs to the “family B” of GPCRs, which typically recognize regulatory peptides such as parathyroid hormone, secretin, glucagon, and vasoactive intestinal polypeptide (Sexton, 1999). The rat and human calcitonin receptor-like receptors were identified shortly after (Chang et al., 1993; Njuki et al., 1993; Flühmann et al., 1995). They show 50 and 54% overall identity with the respective calcitonin receptors. Historically, this protein has been abbreviated to “CRLR”; however, following IUPHAR guidelines (Ruffolo et al., 2000), it will be referred to in this article as the “CL receptor”. (Although this follows the convention adopted in the field, since it does not bind any known ligand by itself, it would be strictly correct to refer to it as the “CL protein”.)

Initially, the CL receptor was considered an orphan receptor. Evidence for CGRP receptor function of the CL receptor was first obtained by Aiyar et al. (1996) who

observed CGRP binding and CGRP-dependent cAMP accumulation in a single subclone of HEK293 cells stably transfected with the hCL receptor encoding cDNA. The important breakthrough came when an expression cloning approach demonstrated that the CL receptor required a single transmembrane domain protein, termed receptor activity modifying protein, RAMP1, to function as a CGRP receptor (McLatchie et al., 1998) (Fig. 1). The RAMP family of proteins comprises three members, RAMP1, -2, and -3. RAMP2 and -3 enable the same CL receptor to behave as an AM receptor. The RAMPs share a common topological organization but less than 30% sequence identity. They are intrinsic membrane proteins (predicted sizes:  $M_r$  14,000–17,000) with an extracellular N terminus of ~100 amino acids, a single transmembrane domain, and a short intracellular domain (10 amino acids) (Muff et al., 2001; Sexton et al., 2001). The CT receptor does not require RAMP to bind and respond to CT, but it can associate with the RAMPs. This results in a series of receptors that typically have high affinity for AMY, and varied affinity for CGRP (Christopoulos et al., 1999; Muff et al., 1999).

Two related members of “family A” GPCRs, RDC1 and L1/G10D, have been considered as receptors for CGRP and AM, respectively (Kapas et al., 1995; Kapas and Clark, 1995). This was unexpected because although RDC1 and G10D are homologous to each other, they are very different from the CT and CL receptors. In fact, several attempts have been made to further characterize RDC1- and L1/G10D-induced CGRP and AM responses without success (Kennedy et al., 1998; McLatchie et al., 1998; Tong et al., 2000). These receptors are now considered “orphans” once more.

Detailed studies of the pharmacology displayed by CT and CL receptors, expressed with or without all three RAMPs, have now been reported by several laboratories (Bühlmann et al., 1999; Christopoulos et al., 1999; Fraser et al., 1999; Muff et al., 1999; Aldecoa et al., 2000; Husmann et al., 2000; Leuthäuser et al., 2000; Tilakaratne et al., 2000; Zumpe et al., 2000; Aiyar et al., 2001; Oliver et al., 2001). They have revealed receptors that bind and respond to CT, CGRP, AM, and AMY. In view of the structural similarity between the CT family of peptides, it is not surprising that cross-reactivity between them at their cognate receptors has been demonstrated. Because two GPCR proteins and three RAMPs can reconstitute receptors for the whole CT family of peptide ligands, the situation is even more complex. In addition, at least some cells appear to express more than one RAMP and there is evidence to support the regulation of RAMP expression at the mRNA level.

This review aims to reconcile what is observed when the receptors are reconstituted *in vitro* with the properties they show in native cells and tissues. The Nomenclature Committee of the IUPHAR subcommittee on calcitonin receptors also makes recommendations for the nomenclature of these receptors.

<sup>1</sup> Abbreviations: CT, calcitonin; CL, CT-like; CGRP, CT gene-related peptide; sCT, salmon CT; hCT, human CT; CTR, CT receptor; AMY, amylin; AM, adrenomedullin; CNS, central nervous system; GPCR, G protein-coupled receptor; IUPHAR, International Union of Pharmacology; HEK, human embryonic kidney; RAMP, receptor activity modifying protein(s); RCP, receptor component protein; CHO, Chinese hamster ovary; RAEC, rabbit aortic endothelial cell; UTR, untranslated region; BIBN4096BS, 1-piperidinecarboxamide, *N*-[2-[[5-amino-1-[[4-(4-pyridinyl)-1-piperazinyl]carbonyl]pentyl]amino]-1-[[3,5-dibromo-4-hydroxyphenyl)methyl]-2-oxoethyl]-4-(1,4-dihydro-2-oxo-3(2*H*)-quinazoliny].

TABLE 1  
Structures CGRP, amylin, adrenomedullin, and calcitonin

h $\alpha$ CGRP	A C D T A T C V T H R L A G L L S R S G G V V K N N F V P T N V G S K A F
r $\alpha$ CGRP	S C N T A T C V T H R L A G L L S R S G G V V K D N F V P T N V G S E A F
h $\beta$ CGRP	A C N T A T C V T H R L A G L L S R S G G M V K S N F V P T N V G S K A F
r $\beta$ CGRP	S C N T A T C V T H R L A G L L S R S G G V V K D N F V P T N V G S K A F
hAMY	K C N T A T C A T Q R L A N F L V H S S N N F G A I L S S T N V G S N T Y
rAMY	K C N T A T C A T Q R L A N F L V R S S N N L G P V L P S T N V G S N T Y
hAM	G C R F G T C T V Q K L A H Q I Y Q F T D K D K D N V A P R N K I S P Q G Y
rAM	G C R F G T C T M Q K L A H Q I Y Q F T D K D K D G M A P R N K I S P Q G Y
hCT	C G N L S T C M L G T Y T Q D F N K F H T F P Q T A I G V G A P
sCT	C S N L S T C V L G K L S Q E L H K L Q T Y P R T N T G S G T P

Disulfide bond

 $\alpha$ -helix

h, human; r, rat; s, salmon.

hAM is the structure of the 15–52 fragment; the N-terminal amino acids are YRQSMNMFQGLRSF. rAM shows the structure of the 13–50 fragment; the N-terminal amino acids are YRQSMNQGSRSST.

## II. The Calcitonin Gene-Related Peptide Receptors

$\alpha$ CGRP was cloned from the gene encoding CT (Amara et al., 1982). Alternate splicing of the CT gene leads to the production, especially in nervous tissues, of  $\alpha$ CGRP, a 37-amino acid peptide. A second CGRP homolog,  $\beta$ CGRP, was subsequently discovered. It differs from human  $\alpha$ CGRP by three amino acids and in the rat by one amino acid.  $\beta$ CGRP is encoded by its own unique gene, with high homology to the CT gene (Steenbergh et al., 1985).  $\alpha$ - and  $\beta$ CGRP display similar biological activities.

Historically, CGRP receptors have been divided into two classes, CGRP<sub>1</sub> and CGRP<sub>2</sub>. CGRP<sub>1</sub> receptors are more sensitive than CGRP<sub>2</sub> receptors to the peptide antagonist CGRP<sub>8-37</sub> (taken by many workers to mean a pA<sub>2</sub> of 7 or greater) (Dennis et al., 1989; Quirion et al., 1992). On the other hand, the linear CGRP analogs

[Cys(ACM)<sup>2,7</sup>]- and [Cys(Et)<sup>2,7</sup>]h $\alpha$ CGRP are more potent agonists at CGRP<sub>2</sub> receptors than at CGRP<sub>1</sub> (Dennis et al., 1989; Dumont et al., 1997; Moreno et al., 2002).

The CGRP<sub>1</sub>:CGRP<sub>2</sub> classification has provided a conceptual framework for the development of CGRP pharmacology. However, progress in the field has been hampered by lack of suitable selective drugs (Marshall and Wisskirchen, 2000). The prototypical tissue expressing the CGRP<sub>2</sub> receptor is the rat vas deferens (Dennis et al., 1989). Here pA<sub>2</sub> estimates of CGRP<sub>8-37</sub>, measured with CGRP as agonist, range from below 5.5 (Giuliani et al., 1992) to 6.7 (Longmore et al., 1994). For the prototypical CGRP<sub>1</sub> receptor expressing tissue (guinea pig left atrium), the range is 6.9 to 7.7 (Dennis et al., 1990; Mimeault et al., 1991). In both tissues, there is at least a 10-fold spread of values. Although a meta-analysis of these data sets confirms that there is a significant difference, the variation demonstrates that any individual

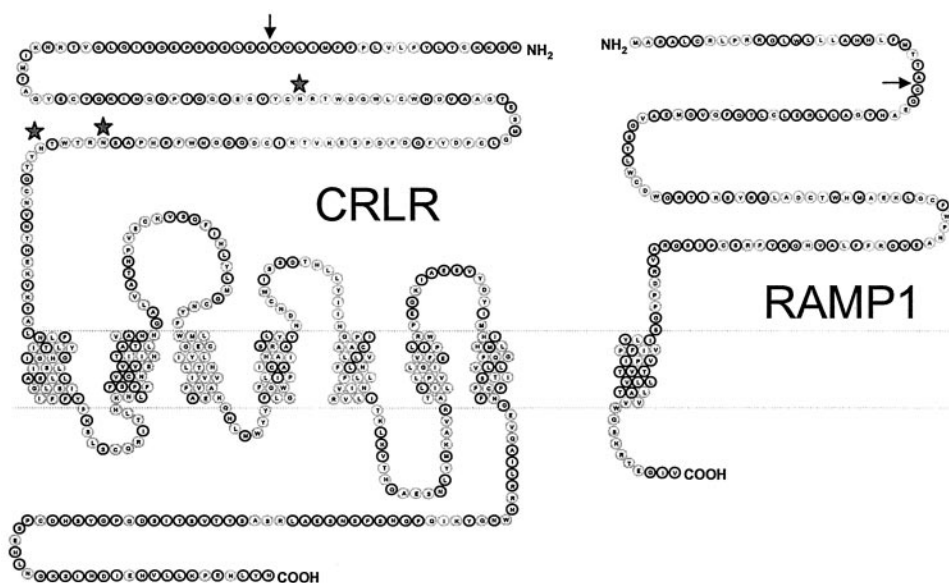


FIG 1. Schematic diagram of human CL receptor (CRLR) and RAMP 1. Stars show glycosylation consensus sites on CL receptor. Residues unique to CL receptor over the human CT receptor, or unique to RAMP1 over RAMP2 or RAMP3 are indicated in bold circles. Arrows show predicted signal peptide cleavage sites.

$pA_2$  value reported for the antagonist must be treated with caution. Values in the range of 6.5 to 7.5 are likely to be particularly hard to interpret under the  $CGRP_1$ :  $CGRP_2$  classification. A wide range of  $pA_2$  values have been reported for  $CGRP_{8-37}$  in different preparations; in the rat for example: 8.0 in kidney, 7.9 in cerebral artery, 7.6 in mesenteric artery, 6.9 in pulmonary and coronary arteries, 6.6 in caudal artery, 6.5 in uterus, 6.1 in vas deferens, 5.9 in internal anal sphincter, <6 in colon, and <5 in thoracic aorta (Poyner and Marshall, 2001). Although it is difficult to conceive that a single receptor subtype can account for such a wide range of  $pA_2$  values, there do not appear to be any natural breaks in the data.

Recently, several nonpeptide antagonists have been introduced; the best characterized is BIBN4096BS (although, strictly, this is a low molecular weight antagonist because BIBN is a highly modified peptide). It shows a marked selectivity for human over rat receptors (Doods et al., 2000), and it is more potent than  $CGRP_{8-37}$  in both species (Doods et al., 2000; Wu et al., 2000). Recent work has revealed that the relative affinity of BIBN4096BS is related to the species of origin for the RAMP1, rather than the receptor protein. Subsequent chimeric protein and point mutation analysis have identified tryptophan 74 of RAMP1 as a key residue for the higher affinity of human CGRP receptors (Mallee et al., 2002).

The status of the  $CGRP_2$ -selective agonists is unclear. Agonists are not the tools of choice to define receptor subtypes. Waugh et al. (1999) reported that  $[Cys(ACM)^{2,7}]h\alpha CGRP$  is a partial agonist in the porcine coronary artery; this could explain its apparent selectivity in other systems. Wu et al. (2000) have shown that  $[Cys(ACM)^{2,7}]h\alpha CGRP$  and  $[Cys(Et)^{2,7}]h\alpha CGRP$  have similar potencies in both the rat atria and vas deferens and apparently act through an adrenomedullin-activated receptor, even though  $CGRP_{8-37}$  and BIBN4096BS demonstrate heterogeneity in the receptors responding to CGRP in these preparations.

In summary, there is good evidence for heterogeneity among receptors that respond to CGRP in tissues, derived from data with antagonists. Although  $CGRP_{8-37}$  is not easy to use, the limited data available from nonpeptide antagonists broadly support the conclusions that were based on this compound. The "selective" nonpeptide antagonists show promise, although as yet they have been little used. Questions remain about the extent of the heterogeneity of CGRP receptors, particularly whether the  $CGRP_2$  receptors are a homogeneous group, what the boundaries of this group are in terms of antagonist affinity, and whether CGRP is the endogenous ligand for all the receptors that have been described as belonging to this category.

There is good evidence for the presence of CL receptor/RAMP1 complexes on the surfaces of cells. Coimmunoprecipitation experiments carried out in total cell extracts revealed stable interactions between RAMP1 and

both the immature (58 kDa) and mature (66 kDa) glycosylated forms of CL receptor. Whole cell coimmunoprecipitation and confocal microscopy indicated that the interaction was maintained once the receptor reached the cell surface (Leuthäuser et al., 2000; Hilaiet et al., 2001a,b). This interaction is also maintained during agonist-mediated internalization of the receptor (Kuwasaki et al., 2000). The covalent cross-linking of an ~85-kDa complex [composed of one CL receptor (66 kDa) and one RAMP1 molecule (17 kDa)], using the membrane impermeable bis(sulfosuccinimidyl) suberate ( $BS^3$ ), further demonstrated that the interaction has a 1:1 stoichiometry. Cross-linking experiments with  $^{125}I$ -CGRP also showed that both the CL receptor/RAMP1 complex and RAMP1 were radiolabeled, indicating that: 1) RAMP1 and CL receptor remain associated upon CGRP binding; and 2) residues within RAMP1 lie close to, or may be part of, the CGRP binding pocket. These data demonstrate the existence of a meta-stable complex between the RAMP1 and CL receptor, which is maintained upon activation of the receptor by CGRP (Hilaiet et al., 2001a,b).

The receptor formed by the heterodimerization of CL receptor and RAMP1 in recombinant systems is similar enough to that observed for the  $CGRP_1$  receptor in native tissues and cell lines to suggest that the combination occurs naturally and has physiological significance. The  $pA_2$  for  $CGRP_{8-37}$  for human CL receptor expressed with human RAMP1 in HEK293 cells has been reported as 7.6 (Aiyar et al., 1996). This value correlates well with those obtained for the CGRP receptor in cell lines such as SK-N-MC (7.5–8.7) (Muff et al., 1992; Longmore et al., 1994; Entzeroth et al., 1995; Zimmermann et al., 1995; Poyner et al., 1998) and in human cerebral artery (7.7) (Edvinsson et al., 2001). Data are not yet available for the antagonist affinities of the rat CL receptor/rat RAMP1 complex, but the rat CL receptor/human RAMP1 complex has a  $pA_2$  of 7.5 (calculated from the data of Leuthäuser et al., 2000). Thus, in the rat and human, there are good grounds for assuming that the majority of receptors with a  $pA_2$  for  $CGRP_{8-37}$  in excess of 7 correspond to CL receptor/RAMP1. There are areas where CL receptor encoding mRNA is poorly expressed or apparently absent, e.g., in the cerebellum, but which show high levels of CGRP binding (Dotti-Sigrist et al., 1988; Flühmann et al., 1997). Although this may simply reflect problems of trying to equate protein with mRNA expression, if workers find a  $CGRP_1$  phenotype in these tissues, they should exercise due caution in assuming that it is necessarily CL receptor/RAMP1. In species where no data on the CL receptor are available, such as the guinea pig, receptors with a  $pA_2$  for  $CGRP_{8-37}$  in excess of 7 are usually considered to be  $CGRP_1$ ; pragmatically, it seems reasonable for this practice to continue.

Nevertheless, caution should be exercised in recombinant systems because different degrees of protein ex-

pression may markedly modify pharmacology in such a complex situation. This particularly applies to experiments where RAMPs and receptors from different species are used.

There is evidence that the CGRP receptor complex might interact with another protein. A cytosolic protein, RCP (receptor component protein), was identified on the basis of its ability to potentiate the CGRP-mediated responses in *Xenopus laevis* oocytes in much the same way as was RAMP1 (although the two proteins show no similarity) (Luebke et al., 1996). RCP appears to be required for NIH-3T3 cells to show CGRP responsiveness. If mRNA encoding RCP is complexed with antisense oligonucleotides, there is a reduction in NIH-3T3 cell CGRP responsiveness (with appropriate controls being unaltered). Some immunoprecipitation data imply a direct interaction between CL receptor and RCP (Evans et al., 2000). CGRP receptors can be reconstituted through the expression of CL receptor/RAMP1 in *Xenopus* oocytes or insect cells without exogenous RCP (McLatchie et al., 1998; Aldecoa et al., 2000). However, this is not to say that RCP has no role; it may be that these systems express endogenous RCP. Recent work has shown the reconstitution of CGRP and AM receptors in the yeast *Saccharomyces cerevisiae* through the coexpression of RAMPs with CL receptor (Miret et al., 2001). Yeast may also share an RCP homolog, but it is more distantly related again. It will take more work to define the role of RCP.

Although many receptors responding to CGRP are likely to correspond to CL receptor/RAMP1, these are unlikely to explain the behavior of those found, for example, in the rat vas deferens or aorta or cell lines such as the human-derived Col 29 cells (Cox and Tough, 1994). What might explain these data? Several alternatives include additional receptors or RAMPs, additional tissue factors, or the contribution of more than one receptor to the  $pA_2$  values for CGRP<sub>8-37</sub> that have been determined.

#### A. Additional Cofactors or Receptors

As the human genome sequencing project refines its data, it will soon become apparent whether another GPCR will be discovered to account for the observations suggesting the existence of CGRP<sub>2</sub> receptors. It may be that some very labile accessory protein is responsible for promoting a putative CGRP<sub>2</sub> receptor form, perhaps by interacting with the CL receptor. The mRNA for the CL receptor is present in rat vas deferens, the prototypical CGRP<sub>2</sub> receptor-expressing tissue (Moreno et al., 2002), as well as other tissues showing pharmacology similar to the putative CGRP<sub>2</sub> type, e.g., porcine coronary artery (Rorabaugh et al., 2001). A possible contributor may be G proteins, as recent work with CT and AMY receptors has indicated that modulation of the level and type of G protein  $\alpha$  subunit can profoundly influence phenotype (Tilakaratne et al., 2000; Smyth et al., 2001). Another

potential cofactor is the RCP (see above, Moreno et al., 2002).

#### B. Tissue Factors

A number of workers have considered that tissue-specific factors such as proteolysis (of ligand, CL receptor, or RAMP1) may play a role in the apparent CGRP receptor heterogeneity (e.g., Longmore et al., 1994; Rorabaugh et al., 2001). However, others have found that peptidase inhibitors make little difference to CGRP pharmacology (Tomlinson and Poyner, 1996; Wisskirchen et al., 1998).

#### C. Contribution of More than One Receptor to $pA_2$ Values

The complexes of CL receptor with RAMP2 and -3 as well as the various CT receptor/RAMP complexes all generate receptors that interact with CGRP (see above). Little work has been done on the pharmacology of the majority of these complexes but there are some potentially relevant data. Human  $\alpha$ CGRP is a potent agonist at the human CT<sub>(a)</sub> receptor/human RAMP1 complex (EC<sub>50</sub> of 0.8 nM) (see below for discussion of CT receptor nomenclature). This response is antagonized by salmon calcitonin (8–32) (sCT<sub>8-32</sub>) but not 100 nM CGRP<sub>8-37</sub> (Leuthäuser et al., 2000). This complex is probably better considered an AMY receptor than a CGRP receptor, but without use of an appropriate antagonist such as sCT<sub>8-32</sub>, this would not be apparent (see below). Aiyar et al. (2001) have reported that porcine CL receptor expressed with either human RAMP2 or RAMP3 shows high-affinity CGRP binding but the functional pharmacology is closer to that of adrenomedullin receptors. Given that CGRP<sub>2</sub> receptor-expressing tissues may sometimes respond to amylin and AM (e.g., Poyner et al., 1999; Wisskirchen et al., 1999), these other complexes may mediate some of the effects of CGRP. Use of sCT<sub>8-32</sub> (which would antagonize complexes containing the CT receptor but not the CL receptor) might be useful in further defining a CGRP<sub>2</sub> phenotype.

Currently, there is no molecular correlate for a CGRP<sub>2</sub> receptor. As noted above, it is possible that the class represents more than one pharmacologically distinct entity. Indeed, some workers have noted “atypical” CGRP receptors (Wisskirchen et al., 1998; Esfandyari et al., 2000), although frequently these are all considered as being CGRP<sub>2</sub> receptors; a tendency that might obscure real differences. Furthermore, CGRP may not be the endogenous ligand for all of these receptors. Given these problems, it is recommended that authors use the term “CGRP<sub>2</sub> receptor” with care.

### III. The Adrenomedullin Receptors

AM is a peptide that was isolated in 1993 from human pheochromocytomas (Kitamura et al., 1993). Human AM is 52 amino acids long. The N-terminal 12 amino

acids can be removed with little change in potency (Lin et al., 1994). The general pharmacology of AM has been reviewed elsewhere (Hinson et al., 2000).

CL receptor and RAMP2 or -3 reconstitute AM receptors that show the pharmacological characteristics of the native receptors (Table 2). RAMP2 and RAMP3 enable CL receptor to generate AM receptors that are pharmacologically similar. The biochemistry of the CL receptor/RAMP2 or the CL receptor/RAMP3 complexes suggests they form in the endoplasmic reticulum and remain tightly associated as they are delivered to the cell surface (Hilairret et al., 2001b), and this is supported by confocal microscopic data (Kuwasako et al., 2000). Only the maturely glycosylated forms of the CL receptor/RAMP2 or CL receptor/RAMP3 complexes at the cell surface appear to bind  $^{125}\text{I}$ -AM (Hilairret et al., 2001a).

Some biological activities of AM are mediated by CGRP receptors. At these receptors AM behaves as a potent competitor for  $^{125}\text{I}$ - $\alpha$ CGRP binding sites, and the production of cAMP induced by both CGRP and AM can be inhibited by CGRP<sub>8-37</sub>. Because it is common for the CL receptor to be coexpressed with combinations of RAMPs, it is likely that CGRP and AM receptors are coexpressed in the same cells.

However, there are activities of AM that are clearly mediated by a unique AM receptor because they cannot be antagonized by CGRP<sub>8-37</sub>. Examples are AM-induced vasodilatation in the guinea pig pulmonary artery, tachycardia, hypotensive effects in Long-Evans rats, and the control of aldosterone production by the rat adrenal (Rebuffat et al., 2002). A cellular model of a unique AM receptor is provided by a rabbit aortic endothelial cell (RAEC) line and the neuroblastoma/glioma NG108-15 hybrid cell line (Zimmermann et al., 1996;

Muff et al., 1998). Upon transfection of RAECs with RAMP1 encoding cDNA, expression of a CGRP<sub>1</sub> receptor, sensitive to CGRP<sub>8-37</sub>, is also observed. These data imply expression of an endogenous CL receptor/RAMP2 AM receptor in RAECs and its conversion into a CGRP<sub>1</sub> receptor by competitive interaction of CL receptor with coexpressed exogenous RAMP1.

$^{125}\text{I}$ -CGRP and  $^{125}\text{I}$ -AM binding studies define tissues that contain CGRP, AM, or mixtures of both receptors. Binding sites labeled by  $^{125}\text{I}$ -AM bind CGRP and AMY with over 200-fold lower affinities compared with AM (Poyner et al., 1999). Furthermore, AM binding sites in the CNS have a unique distribution compared with those for CGRP or AMY. Thus, the binding data strongly suggests that CGRP and AM interact with distinct sites (Owji et al., 1995; Coppock et al., 1996; Zimmermann et al., 1996; Upton et al., 1997; Juaneda et al., 2000). AM pharmacology would be greatly facilitated by the availability of selective antagonists. AM<sub>22-52</sub> behaves as a competitive antagonist and blocks a variety of AM activities. It has been reported to selectively antagonize AM in cell lines, e.g., NG108-15 neuroblastoma cells (Zimmermann et al., 1996). However, in vivo it seems much less effective (Santiago et al., 1995; Champion et al., 1997). In the vas deferens, it was active by itself in inhibiting electrically stimulated contractions, and whereas it could additionally antagonize the response to AM, it had similar effects on the responses to CGRP and to some extent, AMY. Any lack of a specific effect of AM<sub>22-52</sub> in contrast to some other reports might argue for heterogeneity among AM receptors. Recent studies of the reconstituted CGRP and AM receptors in yeast suggest AM<sub>22-52</sub> and CGRP<sub>8-37</sub> are selective for the CL receptor/RAMP1 and CL receptor/RAMP2 combinations in

TABLE 2  
Pharmacological profile for CL receptor/RAMP complexes

Receptor	$\alpha$ CGRP	$\beta$ CGRP	CGRP <sub>8-37</sub>	AM	AM <sub>22-52</sub>	AMY	Cell	References
hCL receptor/hRAMP1	10	11	9.5	8.1	6.4	NS	Swiss3T3	McLatchie et al., 1998
	10.3		9	7.8			HEK293	Aiyar et al., 1996
	9.6	9.8	9.1	6.8			HEK293	Aiyar et al., 2001
rCL receptor/rRAMP1	8.9	9.3	8.2	8.8	>6	7.8	UMR106	Oliver et al., 2001
rCL receptor/hRAMP1	8.3	8.4	8.9	>7	>6	>6	COS-7	Buhlmann et al., 1999
	7.9	8.3	8.8	6.9	>6	>6	UMR106	Buhlmann et al., 1999
	8.2	8.3	8.8	6.9			Schneider	Aldecoa et al., 2000
rCL receptor/mRAMP1	8.4	8.2	8.8	6.8			COS-7	Husmann et al., 2000
pCL receptor/hRAMP1	10	9.8	9.18	7.7			HEK293	Elshourbagy et al., 1998
	9.5	10.2	9.1	8.2			HEK293	Aiyar et al., 2001
	5.9	6.5	7.1	9.2	7.8	NS	Swiss3T3	McLatchie et al., 1998
hCL receptor/hRAMP2	6.3	6.8	7.2	9.1	7.7	NS	HEK293	Fraser et al., 1999
		7.7	>5	8.5	7.0		HEK293	Aiyar et al., 2001
	>7	>7	>6	9.3	7	>6	UMR106	Oliver et al., 2001
rCL receptor/rRAMP2	>6		7.1	8.8	8.5	>6	COS-7	Buhlmann et al., 1999
rCL receptor/hRAMP2	>6	7.0	7.4	9.5	8.6	>6	UMR106	Buhlmann et al., 1999
	6.7	6.7	7.0	9.1	7.9		Schneider	Aldecoa et al., 2000
		6.8	7.0	8.7	8.5		COS-7	Husmann et al., 2000
rCL receptor/mRAMP2							COS-7	Husmann et al., 2000
pCL receptor/hRAMP2*	8.6/7.5	8.8/7.2	7.7/6.2	9.0/8.6	7.14		HEK293	Aiyar et al., 2001
hCL receptor/hRAMP3	6.5	7.1	7.3	8.3	7.1		HEK293	Fraser et al., 1999
	6.8	7.3	7.3	8.9	7.3		HEK293	Aiyar et al., 2001
	7.7	7.7	8.4	8.2	7.8		COS-7	Husmann et al., 2000
rCL receptor/mRAMP3							COS-7	Husmann et al., 2000
pCL receptor/hRAMP3*	8.3/7.0	9.2/7.5	8.2/>5	9.4/9.2	7.2		HEK293	Aiyar et al., 2001

r, rat; m, mouse; p, pig. Values represent pIC<sub>50</sub> values measured against  $^{125}\text{I}$ -CGRP (normal text) or  $^{125}\text{I}$ -adrenomedullin (italics). Displacing ligands are of human for hCL receptor and pCL receptor; otherwise rat. Schneider cells are from *Drosophila*.

\*  $\alpha$ CGRP showed low potency in stimulating cAMP production in the presence of RAMP2 or RAMP3 (pEC<sub>50</sub> values of 6.8 and 6.5) in spite of high affinity binding.

that system (Miret et al., 2001). It may be that the problem with determining the selectivity of these peptides is the complexity of the native systems in which they are evaluated. Caution is clearly needed, particularly with AM<sub>22-52</sub> because it has only been used in comparatively few studies, and it is important to determine its specificity in native (and hopefully simple) systems.

The ability of CL receptor/RAMP2 and CL receptor/RAMP3 to respond with high affinity to AM implies the existence of two molecularly distinct AM receptors. On this basis, it is recommended that these are referred to as AM<sub>1</sub> and AM<sub>2</sub> receptors. However, their pharmacological properties are poorly defined, and further revision cannot be ruled out.

#### IV. The Calcitonin Receptors

CT protects the skeleton by direct inhibition of osteoclastic bone resorption. Less well characterized are its actions on the kidney, gastrointestinal tract, reproductive system, and CNS (Sexton et al., 1999). A porcine CT receptor was originally cloned by Lin et al. (1991). The CT receptor, although it is the receptor most similar to the CL receptor, does not require RAMP coexpression for cell surface localization. The CT receptor, expressed alone in all cells studied to date, is efficiently transported to the cell surface.

Numerous alternatively spliced transcripts of the human CT receptor gene have been described (Table 3). In contrast, only one transcript of the CL receptor has been reported. The best characterized splice variant of the human CT receptor differs by the presence (CT<sub>(b)</sub> receptor) or absence (CT<sub>(a)</sub> receptor) of 16 amino acids in the first intracellular loop. Although generally less abundant, the CT<sub>(b)</sub> receptor is expressed significantly in ovary, placenta, bone marrow, and lung (Kuestner et al., 1994). The human CT<sub>(b)</sub> receptor and CT<sub>(a)</sub> receptor isoforms have also been referred to as hCTR1 and hCTR2, respectively (Gorn et al., 1995), with the numbering reflecting the order in which they were discovered. There is little difference between the ability of these two forms to recognize peptide ligands. However, unlike the CT<sub>(a)</sub> receptor, the CT<sub>(b)</sub> receptor is poorly internalized. Experimentally, it also appears that CT<sub>(b)</sub> receptor has altered coupling to G proteins; loss of Gq-mediated re-

sponses and attenuation of Gs-mediated signaling has been observed upon stimulation of CT<sub>(b)</sub> receptor (Moore et al., 1995). Another splice variant leads to an in-frame stop codon within the first intracellular loop (Moore et al., 1995) and corresponds to a presumably nonfunctional protein. Two alternate splicing events have been reported to occur within the 5'-untranslated region (UTR) of the mRNA. One of these is entirely confined within the UTR and thus does not change the protein (Nishikawa et al., 1999). The second transcript encodes a new in-frame AUG and therefore a presumed extension of the receptor protein by 18 amino acids. If translation from the upstream site occurs, the receptor "signal peptide" may still be cleaved at the original site, and so receptor function would be maintained (Gorn et al., 1995). An additional alternate splicing variant has a predicted truncation of 47 amino acids at the amino terminus (with splicing out of the initial AUG). The modified receptor is presumably translated from the AUG at amino acid 48. This deletion appears to have minimal effects on expression and phenotype and does not appear to be a common transcript (Albrandt et al., 1995; Ho et al., 1999).

For the purpose of establishing receptor nomenclature for CT receptors, we propose that only the major human splice variants that yield different functional proteins be considered. By convention these would be designated by lower case letters (a, b), rather than designating them by 1 or 2 (more usually reserved for receptor subtypes). Splicing of the receptors is complex and may potentially lead to proteins with more than one structural change. On this basis, as cleavage of the signal peptide is likely (as has been established for at least one of the class II receptors; Dong et al., 2000), variants around the translation start codon are currently not classified. Similarly, neither are those variants that affect only the UTR of the receptor classified. Finally, the hCT<sub>11</sub> receptor variant with a stop codon is also excluded from classification (as it is not likely to be functional).

Distinct splice variants occur also in rodents and rabbits. The most common splice variant in rodents is similar to CT<sub>(a)</sub> receptor. Alternate splicing leads to insertion of a 37-amino acid insert into the first extracellular loop 1 of the receptor (known historically as CTR<sub>1b</sub> or C1b). This interacts only weakly with CT peptides exhibiting poor  $\alpha$ -helical secondary structure, leading to a marked decrease in response to human CT, but less effect on salmon CT (Albrandt et al., 1993; Sexton et al., 1993; Houssami et al., 1994, 1995; Yamin et al., 1994). These receptors have been characterized in native tissue membranes and their distribution mapped in the CNS (Nakamuta et al., 1990; Hilton et al., 1995). In rabbits, alternate splicing may lead to deletion of the exon encoding transmembrane domain 7. There is a consequent decrease in affinity of salmon and human CT, being greater for salmon CT such that the two peptides become equipotent, along with a loss of receptor-mediated

TABLE 3

Summary of nomenclature for calcitonin receptor splice variants, referenced within the text

Proposed Nomenclature	Former Nomenclature
hCT <sub>(a)</sub> receptor	hCTR <sub>11-</sub> , hCTR2
rCT <sub>(a)</sub> receptor	rCTR1a
hCT <sub>(b)</sub> receptor	CTR <sub>11+</sub> , hCTR1

h, human; r, rodent. Other splices variants not yet included in this classification are human  $\delta$ 47 (lacking the last 47 amino acids), rat CTR<sub>E2+</sub> or CTR<sub>1b</sub> (with a 37-amino acid insert in the second extracellular loop) and rabbit  $\delta$ TM7 (lacking the last transmembrane helix). IUPHAR is currently looking into the nomenclature of splice variants and will publish guidelines in the near future.

mobilization of intracellular calcium (Shyu et al., 1996). To date, an equivalent receptor has not been characterized in native tissue. Neither the rodent nor the rabbit isoforms appear to be expressed in man (Kuestner et al., 1994). In the absence of clear IUPHAR guidelines for dealing with splice variants, it is not yet possible to propose any official nomenclature recommendations for any of the above forms.

Calcitonin receptors in man and rodents have highest affinity and efficacy for CT peptides, with relatively lower affinity interaction with CGRP, AMY, and AM. CT receptor-mediated responses can be antagonized by sCT<sub>8-32</sub>, whereas CGRP<sub>8-37</sub> or AM<sub>22-52</sub> are essentially ineffective. Unlike CGRP peptides, which display a high degree of evolutionary structural conservation, there is considerable variance in amino acid sequence of CT peptides from different species. These can be divided into three structural classes that exhibit sequence conservation: 1) teleost/avian such as sCT; 2) artiodactyl such as porcine CT; and 3) human/rodent CT. Although <sup>125</sup>I-human CT binds reversibly to its receptor, salmon CT binds irreversibly, resulting in sustained cAMP accumulation (Fischer et al., 1981; Nicholson et al., 1987; Hilton et al., 2000). The affinity therefore of <sup>125</sup>I-sCT receptor binding cannot be evaluated. Nonetheless, CT receptors exhibit a general relative potency of sCT ≥ porcine CT ≥ hCT > AMY, CGRP, AM. Salmon CT and hCT have similar efficacy at human CT receptors, with hCT having ~3- to 10-fold lower affinity in competition binding studies. At the rat CT receptor, hCT is ~10-fold weaker in stimulating cAMP accumulation and is 100- to 1000-fold weaker in competing for <sup>125</sup>I-sCT binding.

## V. The Amylin Receptors

AMY is a 37-amino acid peptide isolated from amyloid deposits of human insulinoma and the pancreas of type 2 diabetic patients (Cooper et al., 1987). Circulating levels of AMY are raised in response to meal ingestion, and the peptide potently inhibits gastric emptying and gastric acid secretion, and opposes the metabolic actions of insulin in skeletal muscle (Hoppener et al., 2000). Furthermore, knockout of the AMY gene leads to weight gain in mice, suggesting an important physiological role for the peptide in weight control. The mice also show reduced pain perception (Gebre-Medhin et al., 1998).

Early reports suggested that AMY was acting via a CGRP receptor to induce its biological effects. It was shown to compete for specific <sup>125</sup>I-CGRP binding sites in the rat liver and skeletal muscle as well as guinea pig and pig liver. The activity of AMY in ventricular cardiomyocytes and the vasodilatation of microvessels was antagonized by CGRP<sub>8-37</sub> whereas the "AMY antagonist" AMY<sub>8-37</sub> was without effect. So, what is the evidence for AMY specific receptors? AMY can bind to a site that has a very distinctive pharmacology, having a high affinity for sCT and somewhat lower affinity for CGRP (Beaumont et al., 1993; Sexton et al., 1994; Veale et al.,

1994; Beaumont et al., 1995). <sup>125</sup>I-AMY binding sites present as a subset of those labeled with <sup>125</sup>I-sCT in the mammalian brain suggest that sCT (or its analogs) binds to an AMY receptor. This AMY binding site discovered in the CNS was termed the C3 binding site (Sexton et al., 1988). It is likely to represent a receptor through which AMY mediates certain biological effects (Beaumont et al., 1995).

AMY receptors can be reconstituted in cellular systems by coexpressing the CT receptor with RAMPs. These AMY receptors have similar and high affinities for sCT and AMY and lower affinities for CGRP. In this respect, the CT receptor/RAMP combination represents a similar AMY receptor to that characterized in native tissues (Table 4) (Chen et al., 1997). However, there are several possible combinations between the variants of the CT receptor and the RAMPs. In cellular systems, all three RAMPs will interact with CT<sub>(a)</sub> and CT<sub>(b)</sub> receptors and generate AMY receptors. The CT<sub>(b)</sub> receptor displays greater capacity to generate RAMP2 AMY receptors than the CT<sub>(a)</sub> receptor. This may be related to the differential G protein coupling of the two splice variants as well as the level of the CT<sub>(a)</sub> receptor/RAMP2 AMY receptor (Tilakaratne et al., 2000).

Much like CL receptor/RAMP complexes, different CT receptor/RAMP complexes form receptors with distinct pharmacologies. The CT receptor/RAMP1 complex possesses high affinities for sCT (IC<sub>50</sub>, 0.3 nM), AMY (IC<sub>50</sub>, 7.8 nM), CGRP (IC<sub>50</sub>, 4.7 nM), and to a lesser extent human CT (IC<sub>50</sub>, 447 nM). CT receptor/RAMP3 shows a pharmacology almost identical to CT receptor/RAMP1 with the exception that the IC<sub>50</sub> for CGRP is ~40-fold lower (151 nM) (Christopoulos et al., 1999). The responses of these receptors are antagonized by 100 nM sCT<sub>8-32</sub>, but not by 100 nM CGRP<sub>8-37</sub> (Leuthäuser et al., 2000). Although not extensively investigated, AMY receptors with varying affinity for CGRP have been observed in competition binding studies in rat brain. These may arise from CT receptor interaction with different RAMPs (van Rossum et al., 1994).

Do these different AMY receptors have physiological relevance? This is difficult to answer because of a lack of selective pharmacological agents. Salmon CT<sub>8-32</sub> probably has the greatest discrimination between AMY and CGRP receptors, >100-fold, but it does not effectively discriminate between AMY and CT receptors. AC187, a chimeric peptide antagonist derived from sCT<sub>8-32</sub> and AMY, has similar properties. Native peptides such as AMY, hCT, or CGRP have much higher specificity. They label predominantly single sites, but the use of nonselective and high-affinity antagonists can highlight any binding they might have to other sites (Aiyar et al., 1995; Owji et al., 1995). Biphasic displacement curves can be explained within the existing CT receptor/RAMP or CL receptor/RAMP combinations without need to evoke novel subtypes.



TABLE 4  
Pharmacological profile of human receptors in the presence and absence of human RAMPs

Receptor	hCT	sCT	rAMY	hαCGRP	Cell	References
hCT <sub>(b)</sub> receptor	<b>9.45/6.89</b>	<b>10.56/6.93</b>	6.58	6.77	COS-7	Tilakaratne et al., 2000
	10.93/6.78	11.06/ <b>8.12</b>	10.0/ <b>5.65</b>	<b>11.08/5.77</b>	CHO-P	Tilakaratne et al., 2000
hCT <sub>(b)</sub> receptor/hRAMP1	6.94	10.98/ <b>9.29</b>	10.95/ <b>8.43</b>	<b>9.93/7.94</b>	COS-7	Tilakaratne et al., 2000
	9.89/6.95	<b>11.2/6.41</b>	11.29/8.66	<b>11.89/8.64</b>	CHO-P	Tilakaratne et al., 2000
hCT <sub>(b)</sub> receptor/hRAMP2	7.95	11.06/ <b>9.33</b>	10.67/ <b>8.23</b>	<b>10.87/7.72</b>	COS-7	Tilakaratne et al., 2000
	9.63/6.42	<b>11.83/8.83</b>	10.65/ <b>7.59</b>	<b>10.96/7.7</b>	CHO-P	Tilakaratne et al., 2000
hCT <sub>(b)</sub> receptor/hRAMP3	6.87	11.06/9.16	11.03/ <b>8.19</b>	8.9/7.1	COS-7	Tilakaratne et al., 2000
	<b>10.19/5.95</b>	<b>11.53/8.89</b>	11.55/8.2	10.1/6.9	CHO-P	Tilakaratne et al., 2000
hCT <sub>(a)</sub> receptor	7.81	9.03	6.27	>6	COS-7	Zumpe et al., 1999
			6.3	>6	RAEC	Muff et al., 1999
hCT <sub>(a)</sub> receptor/hRAMP1	11.12/ <b>6.45</b>	11.96/8.08	6.07	9.94/5.78	CHO-P	Tilakaratne et al., 2000
	6.92	9.05	8.27	7.87	COS-7	Christopoulos et al., 1999
hCT <sub>(a)</sub> receptor/hRAMP2	7.53	9.51	8.1	7.67	COS-7	Zumpe et al., 1999
			8.5	7.85	RAEC	Muff et al., 1999
hCT <sub>(a)</sub> receptor/hRAMP3	9.79/ <b>5.97</b>	<b>12.19/9.00</b>	11.9/ <b>8.03</b>	<b>8.9/6.03</b>	CHO-P	Tilakaratne et al., 2000
	8.08	9.89	7.82	5.86	COS-7	Zumpe et al., 1999
hCT <sub>(a)</sub> receptor/hRAMP3	No evidence of coupling; wild-type phenotype				RAEC	Muff et al., 1999
	8.94/5.92	<b>12.19/8.54</b>	8.13	7.64	CHO-P	Tilakaratne et al., 2000
hCT <sub>(a)</sub> receptor/hRAMP3	7.71	9.68	8.2	6.82	COS-7	Zumpe et al., 1999
			8.40	6.59	RAEC	Muff et al., 1999
	<b>11.11/6.02</b>	<b>12.12/8.29</b>	<b>11.7/7.78</b>	<b>10.96/6.79</b>	CHO-P	Tilakaratne et al., 2000

Values represent pIC<sub>50</sub> values obtained against <sup>125</sup>I-AMY or <sup>125</sup>I-sCT. Where two sites were detected, the sites highlighted in bold were present at >60%.

As with adrenomedullin receptors, it is recommended that the molecularly distinct complexes of CT receptor and the RAMPs are known as AMY<sub>1</sub>, AMY<sub>2</sub>, and AMY<sub>3</sub> receptors. Splice variants of the CT receptor can be accommodated in this scheme by use of letters: AMY<sub>1(a)</sub>, AMY<sub>1(b)</sub>, etc. However, unknown tissue factors clearly play an important part in determining the pharmacological properties of any given CT receptor/RAMP combination (Table 4). Thus, this classification may be subject to further refinement.

## VI. Receptor Modulation

The RAMPs provide a mechanism whereby a cell could dynamically change its sensitivity from one peptide to another. This would occur if RAMP and receptors were in equilibrium at the cell surface or if receptors are synthesized, associated with RAMP, and recycled at a rapid rate. To date the RAMPs have all been localized to the endoplasmic reticulum (unless coexpressed with CT or CL receptors) (Hilairret et al., 2001b).

Coexpression of the CL receptor (either from the native gene or by a recombinant route) generates receptors, depending on the proportion of the CL receptor associated with each RAMP. Not all the RAMPs appear to associate equally with the CL receptor. SK-N-MC cells respond to CGRP rather than AM, yet the cells express human RAMP1 and RAMP2 encoding mRNA at similar levels (Fraser et al., 1999). Either RAMP1 and -2 are translated with different efficiencies or RAMP1 has a dominant activity. This does not mean that RAMP1 is dominant in every system. When mouse RAMPs were expressed with the rat CL receptor, each produced the expected phenotypes. But, the cotransfection of RAMP1 plus -2 caused a mutual decrease in either the CGRP or AM binding, respectively, suggesting that RAMP1 and -2 have a similar level of interaction with the rodent CL

receptor. In contrast, the cotransfection of RAMP1 or RAMP2 with RAMP3 in COS cells, led to similar levels of binding to those seen with RAMP3 alone, suggesting that, in the cells, RAMP3 has the greatest affinity for the rodent CL receptor (Husmann et al., 2000). The high-affinity interaction of RAMP3 with the CL receptor is consistent with the data from rabbit aortic endothelial cells that express endogenous RAMP2 and most likely a rabbit CL receptor (Muff et al., 1998). Transfected human RAMP3 reduced human RAMP1-induced CGRP responses. In cells expressing transfected rat CL receptor and endogenous RAMP2, AM binding was reduced by 70% (Muff et al., 2001). RAMP3 may therefore compete with RAMP1 and RAMP2 to reveal receptors for an as yet unknown ligand with low affinity for CGRP and AM. These data illustrate how species differences in receptors and RAMP affect the somewhat different human and rodent pharmacology. The most conserved RAMP is RAMP3, with ~85% identity between human compared with rat and mouse sequences. RAMP1 shows ~71% identity whereas RAMP2 only shows ~65% identity (Sexton et al., 2001).

The CT receptor is expressed without the need for RAMP, but the CT receptor readily associates with these proteins to form AMY and AMY/CGRP receptors (Christopoulos et al., 1999; Leuthäuser et al., 2000; Tilakaratne et al., 2000). The CT receptor transports RAMP1 and probably other RAMPs to the cell surface. The association between the RAMP and the CT receptor remains to be quantitated. There is evidence for competition between different transfected RAMPs. Furthermore, there is the added complexity of CT receptor splice variants as well as the cellular complement of RAMPs. Endogenous RAMPs have been documented in over five cell lines, and they are present in essentially all tissues examined. Important variables are expression levels of

the receptors, the ability of the RAMP to interact with the receptors, and certain "ill defined" constituents, perhaps the RCP, G proteins, and other elements remaining to be discovered.

#### A. Expression of mRNA Encoding Calcitonin-Like Receptor, Calcitonin Receptor, and Receptor Activity Modifying Proteins

RAMPs are widely expressed in tissues and cell lines, with moderate levels of at least one RAMP encoding mRNA in each of the tissues studied so far. The CT receptor gene product and CL receptor are expressed at relatively low levels, whereas mRNA encoding RAMPs are abundant.

The mRNAs encoding the CL and CT receptor gene products are expressed at low levels in many tissues but predominantly in the lung and hypothalamus (Njuki et al., 1993). mRNA encoding the CL receptor was detected in many tissues but particularly in the lung, heart, and spinal cord. In the rat fetus, CL receptor is expressed in the lung, midgut and rectum, placenta, blood vessels, and liver, but 6 days after birth it is predominantly present in the lung (Flühmann et al., 1995).

mRNA encoding all three RAMPs are similarly expressed in heart, fetal heart, skeletal muscle, the gastrointestinal tract, spinal cord, the urogenital tract, and the mammary, salivary, pituitary, adrenal, and thyroid glands. RAMP1 and RAMP3 transcripts predominate in the brain, pancreas, and gonads where RAMP2 expression is low. RAMP2 and -3 transcripts predominate in the placenta, lung, trachea, immune system, kidney, and in the fetus where RAMP1 expression is low. RAMP3 mRNA is expressed in the liver to a greater extent than that encoding RAMP1 and -2 (Sexton et al., 2001). Overall, the tissue distributions of CL receptor, RAMP2, and RAMP3 transcripts are more similar to each other than they are to RAMP1. The tissue expression of RAMPs and CL receptor is related to the endogenous receptor phenotype as revealed by radioligand binding (Chakravarty et al., 2000). An important correlation was observed between RAMP1 and CGRP binding, and RAMP2 and AM binding. A correlation was established between the levels of CL receptor encoding mRNA and both binding sites.

Recent work has demonstrated that RAMP mRNA expression is dynamically regulated in some pathophysiological situations and/or as a result of drug treatment. An example is the unilateral urethral obstruction model of rat renal fibrosis where RAMP1 mRNA expression in kidney is increased up to 13-fold, levels of RAMP2 are increased 3-fold, and levels of RAMP3 are unaltered. At day 14, CL receptor mRNA is also increased, but AM mRNA expression remained unchanged. In a mouse model of sepsis, there are tissue-dependent changes in the expression of specific RAMPs. In the lungs, there is a small decrease in RAMP1 mRNA, whereas RAMP2 and CL receptor mRNA became undetectable in the face

of a large increase in RAMP3 mRNA expression. In the spleen, modest decreases are seen in RAMP1 and RAMP2 mRNA expression, but there is a large increase in RAMP3 expression. In contrast, in the heart and thymus, where small decreases in RAMP1 and RAMP2 mRNA are also seen, RAMP3 mRNA is unaltered or decreased (Ono et al., 2000). The strong up-regulation of RAMP3 mRNA under conditions where CL receptor expression is reduced implies either that RAMP3 acts through an unknown receptor or that it may have novel receptor-independent actions. In the rat left coronary ligation model of ischemic heart failure, the level of RAMP2 mRNA expression is increased in both ischemic and nonischemic left ventricle, although larger changes are seen in the ischemic left ventricle. In this same model, AM mRNA is increased, again particularly in the ischemic left ventricle, along with increases in  $^{125}\text{I}$ -AM binding in the region of the left ventricle adjacent to the ischemia. This up-regulation of the paracrine AM response mechanism may be protective in heart failure (Shimosawa et al., 2002).

Frayon et al. (2000) have provided evidence for glucocorticoid regulation of RAMP1 (but not RAMP2) mRNA expression in vascular smooth muscle cells derived from human coronary arteries. They speculate that, in vivo, these vessels are responsive to CGRP, whereas isolated cell preparations of vascular smooth muscle cells or RAECs exhibit higher RAMP2 expression and AM receptor phenotypes.

Such dynamic regulation of RAMP expression had earlier been proposed as an attractive hypothesis for the regulation of CGRP or AM responsiveness, wherein changes in RAMP expression may desensitize a cell to CGRP and sensitize it to AM. Such effects appear important in those pathologies that are associated with altered levels of CGRP or AM, such as migraine, septic shock, pain, muscular denervation, and ischemia.

RAMP expression in cell lines is a complicated issue because expression can vary considerably between one clone of cells and another. HEK293T cells generally show low levels of RAMP1 and RAMP2 expression (McLatchie et al., 1998), although these may be up-regulated in some clones. No endogenous RAMPs can be found in COS-7 and CHO-P cells by Northern analysis, although RAMP1 and RAMP3 can be detected by reverse transcription-polymerase chain reaction (Tilakaratne et al., 2000). Rat UMR106 and rabbit aortic endothelial cells show endogenous RAMP2 (Muff et al., 1999; Sexton et al., 2001) but not RAMP1.

## VII. Receptor Effector Mechanisms

There is no evidence so far that CGRP and AM activate different second messenger pathways. Both peptides increase intracellular cAMP in many systems but not all. CGRP activates calcium and potassium channels

via pertussis toxin-sensitive G proteins, but comparable studies have not been performed with AM (Main et al., 1998).

The calcitonin receptor is linked to the stimulation of adenylate cyclase and phospholipase C, but alternate coupling to phospholipase D and inhibition of adenylate cyclase (at high agonist concentration) have also been observed (Purdue et al., 2002). AMY stimulates the formation of cAMP as the principal mediator in skeletal muscle (Pittner et al., 1994; Beaumont et al., 1995).

### VIII. The Evolution of Receptor Activity Modifying Proteins, The Calcitonin Peptide Family, and Its Receptors

There are precedents for single transmembrane proteins regulating GPCRs but, so far, the RAMPs are unique in defining both cell-surface expression and pharmacology. Nina A enables the expression of opsins in the eyes of the fruit fly *Drosophila melanogaster*. Because Nina A has homology to cyclophilins, it may enable the receptors to fold (Baker et al., 1994). More recently, ODR4 was identified as controlling the expression of olfactory receptors in the worm *Caenorhabditis elegans* (Dwyer et al., 1998). ODR4 appears to have functional homologs in higher species since mammalian olfactory receptors are difficult to express, but it has a different topology to Nina A and the RAMPs. Perhaps a better precedent is the involvement of the low-density lipoprotein receptor-related proteins in the response of frizzled receptors to their ligands, the Wnts (Winklbauer et al., 2001). Frizzled receptors show significant similarities to the family B receptors, of which CT and CL receptors are examples (Barnes et al., 1998). A fundamental role for RAMPs in GPCR signaling seems unlikely. Most aspects of GPCR signaling are conserved in mammals, fish, insects, worms, and yeast (e.g., G protein subunits, G protein receptor kinases and regulator of G protein signaling proteins). However, so far RAMPs have only been identified in mammals. To date, no other GPCRs have been reported to have their pharmacology altered by RAMPs.

The evolution of the RAMPs within the vertebrates may provide some clues to their physiological role. Which evolved first, the RAMPs enabling receptor diver-

sity or the CT family of peptides? CGRP is a phylogenetically ancient peptide conserved from fish to man. The same applies to CT, but the peptides are less well conserved. AM-like immunoreactivity and specific AM responses have been shown in amphibians and fish, but the genes encoding AM-like peptides have not been reported as yet. The existence of AMY in lower vertebrates is less clear. Neither is it clear when the CT receptor and CL receptor proteins first appeared. The CT receptor appears to diverge more rapidly between species but this has been attributed to the diversity of CT peptides (Suzuki et al., 2000). The existence of the RAMPs in *Xenopus* is inferred by the differential ability of *Xenopus* oocytes and *Xenopus* melanophores to express CL receptor (Armour et al., 1999). The completion of the first simple chordate and fish genomes should provide additional information. There are *Drosophila* receptors within the family B similar to CT and CL (Harmar, 2001).

### IX. Conclusions and Recommendations

Receptors for CT, CGRP, AM, and AMY have been well characterized in a number of tissues and cell lines. There is evidence for the existence of receptor subtypes in native tissues. Much of the pharmacology observed in native tissues can be reconstituted in recombinant systems, but not all (Tables 2–4). The consolidation and explanation of this large amount of data will prove an exciting challenge made all the more appealing because of the discovery of novel nonpeptide antagonists and the potential they hold as both therapeutic and pharmacological tools.

The recommendations of the subcommittee for receptor nomenclature are found in Tables 3 and 5. These follow the IUPHAR guidelines (Ruffolo et al., 2000) on naming the receptor after the endogenous ligand for which it has the highest affinity (although for the AMY receptors, data on rat amylin have been used because that for human amylin is currently unavailable). As the molecular nature of the CGRP<sub>2</sub> receptor is unclear, it has not been included in Table 6, although this will be reviewed in due course. As the terms for the individual receptor components (i.e., CT receptor, CL receptor, RAMP1, -2, and -3) are well established, it is suggested

TABLE 5

Summary of the established receptors for the calcitonin family of peptides with their recommended names, their molecular constituents, and the state of receptor characterization within native tissues; referenced within the text.

Recommended Name	Molecular Constituents	Characterized in Native Tissues?
CGRP <sub>1</sub>	CL receptor + RAMP1	Yes
AM <sub>1</sub>	CL receptor + RAMP2	Yes
AM <sub>2</sub>	CL receptor + RAMP3	
CT	CT receptor	Yes
AMY <sub>1</sub>	CT receptor + RAMP1	Yes, but probably as a mixed population
AMY <sub>2</sub>	CT receptor + RAMP2	Yes, but probably as a mixed population
AMY <sub>3</sub>	CT receptor + RAMP3	Yes, but probably as a mixed population

Formal naming of the CGRP<sub>2</sub> receptor has been delayed until its molecular composition can be identified. In the event that this independent existence of this subtype cannot be confirmed, then the CGRP<sub>1</sub> receptor will simply become the CGRP receptor.

TABLE 6  
Genomics of the CT/CGRP/AM/AMY family and their receptors

Receptor/Peptide	HUGO gene Name	Accession Number	Human Chromosomal Location
CT receptor	CALCR	SWMain:P30988	7q21.3
CL receptor	CALCRL	SWMain:Q16602	2q32.1
RAMP1	RAMP1	SWMain:O60894	2q37.3
RAMP2	RAMP2	SWMain:O60895	17q21.2
RAMP3	RAMP3	SWMain:O60896	7p13
CT/ $\alpha$ CGRP	CALCA	SWMain:P01258	11p15.2
		SWMain::P06881	
$\beta$ CGRP	CALCB	SWMain:P10092	11p15.2
AM	ADM	SWMain:P35318	11p15.2
AMY	IAPP	SWMain:P10997	12p12.1

HUGO, human genome organization; IAPP, islet amyloid polypeptide.

The CALCA, CALCB, and ADM genes are within a 5-megabase region on chromosome 11p15, with CALCA and CALCB adjacent to each other. This is almost certainly the result of gene duplication.

that these remain in use. In time, it may also be necessary to revise the composition of receptors to take account of RCP; however, there is not enough data to do this at present.

#### REFERENCES

- Aiyar N, Baker E, Martin J, Patel A, Stadel JM, Willette RN, and Barone FC (1995) Differential calcitonin gene-related peptide (CGRP) and amylin binding sites in nucleus accumbens and lung: potential models for studying CGRP/amylin receptor subtypes. *J Neurochem* **65**:1131–1138.
- Aiyar N, Disa J, Pullen M, and Nambi P (2001) Receptor activity modifying proteins interaction with human and porcine calcitonin receptor-like receptor (CRLR) in HEK-293 cells. *Mol Cell Biochem* **224**:123–133.
- Aiyar N, Rand K, Elshourbagy NA, Zeng ZZ, Adamou JE, Bergsma DJ, and Li Y (1996) A cDNA encoding the calcitonin-gene-related peptide type-1 receptor. *J Biol Chem* **271**:11325–11329.
- Albrandt K, Brady EMG, Herich J, Moore CX, and Beaumont K (1993) Molecular cloning of two receptors from rat brain with high affinity for salmon calcitonin. *FEBS Lett* **325**:225–232.
- Albrandt K, Brady EMG, Moore CX, Mull E, Sierzega ME, and Beaumont K (1995) Molecular cloning and functional expression of a third isoform of the human calcitonin receptor and partial characterization of the calcitonin receptor gene. *Endocrinology* **136**:5377–5384.
- Aldecoa A, Gujer R, Fischer JA, and Born W (2000) Mammalian calcitonin receptor-like receptor/receptor activity modifying protein complexes define calcitonin gene-related peptide and adrenomedullin receptors in *Drosophila* Schneider 2 cells. *FEBS Lett* **471**:156–160.
- Amara SG, Jonas V, Rosenfeld MG, Ong ES, and Evans RM (1982) Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. *Nature (Lond)* **298**:240–244.
- Armour SL, Foord SM, Kenakin T, and Chen WJ (1999) Pharmacological characterization of receptor-activity-modifying proteins (RAMPs) and the human calcitonin receptor. *J Pharmacol Toxicol Methods* **42**:217–24.
- Baker E, Colley N, and Zuker C (1994) The cyclophilin homolog NinaA functions as a chaperone, forming stable complex in vivo with its protein target rhodopsin. *EMBO J* **13**:4886–4895.
- Barnes MR, Duckworth DM, and Beeley LJ (1998) Frizzled proteins constitute a novel family of G protein-coupled receptors, most closely related to the secretin family. *Trends Pharmacol Sci* **19**:399–400.
- Beaumont K, Kenney MA, Young AA, and Rink TJ (1993) High affinity amylin binding sites in rat brain. *Mol Pharmacol* **44**:493–497.
- Beaumont K, Pittner RA, Moore CX, Wolfe-Lopez D, Prickett KS, Young AA, and Rink TJ (1995) Regulation of muscle glycogen metabolism by CGRP and amylin: CGRP receptors not involved. *Br J Pharmacol* **115**:713–715.
- Bühlmann N, Leuthäuser K, Muff R, Fischer JA, and Born W (1999) A receptor activity modifying protein (RAMP)2-dependent adrenomedullin receptor is a calcitonin gene-related peptide receptor when coexpressed with human RAMP1. *Endocrinology* **140**:2883–2890.
- Chakravarty P, Suthar TP, Coppock HA, Nicholl CG, Bloom SR, Legon S, and Smith DM (2000) CGRP and adrenomedullin binding correlates with transcript levels for calcitonin receptor-like receptor (CRLR) and receptor activity modifying proteins (RAMPs) in rat tissues. *Br J Pharmacol* **130**:189–195.
- Champion HC, Santiago JA, Murphy WA, Coy DH, and Kadowitz PJ (1997) Adrenomedullin-(22-52) antagonizes vasodilator responses to CGRP but not adrenomedullin in the cat. *Am J Physiol* **272**:R234–R242.
- Chang CP, Pearce RV, O'Connell S, and Rosenfeld MG (1993) Identification of a seven transmembrane helix receptor for corticotrophin-releasing factor and sauvagine in mammalian brain. *Neuron* **11**:187–1195.
- Chen WJ, Armour S, Way J, Chen G, Watson C, Irving P, Cobb J, Kadwell S, Beaumont K, Rimele T, and Kenakin T (1997) Expression cloning and receptor pharmacology of human calcitonin receptors from MCF-7 cells and their relationship to amylin receptors. *Mol Pharmacol* **52**:1164–1175.
- Christopoulos G, Perry KJ, Morfis M, Tilakaratne N, Gao Y, Fraser NJ, Main MJ, Foord SM, and Sexton PM (1999) Multiple amylin receptors arise from receptor activity-modifying protein interaction with the calcitonin receptor gene product. *Mol Pharmacol* **56**:235–242.
- Cooper GJ, Willis AC, Clarke A, Turner RC, Sim RB, and Reid KB (1987) Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients. *Proc Natl Acad Sci USA* **84**:8628–8632.
- Coppock HA, Owji AA, Bloom SR, and Smith DM (1996) A rat skeletal muscle cell line (L6) expresses specific adrenomedullin binding sites but activates adenylyl cyclase via calcitonin gene-related peptide receptors. *Biochem J* **318**:241–245.
- Cox HM and Tough IR (1994) Calcitonin gene related peptide receptors in human gastrointestinal epithelia. *Br J Pharmacol* **113**:1243–1248.
- Dennis TB, Fournier A, Cadiuex A, Pomerleau F, Jolicœur FB, and Quirion R (1990) hCGRP8-37, a calcitonin gene-related peptide antagonist revealing CGRP receptor heterogeneity in brain and periphery. *J Pharmacol Exp Ther* **254**:123–128.
- Dennis TB, Fournier A, St Pierre S, and Quirion R (1989) Structure-activity profile of calcitonin gene-related peptide in peripheral and brain tissues. Evidence for receptor multiplicity. *J Pharmacol Exp Ther* **251**:718–725.
- Dong M, Asmann YW, Zang M, Pinon DI, and Miller LJ (2000) Identification of two pairs of spatially approximated residues within the carboxyl terminus of secretin and its receptor. *J Biol Chem* **275**:26032–26039.
- Doods H, Hallermayer G, Wu D, Entzeroth M, Rudolf K, Engel W, and Eberlein W (2000) Pharmacological profile of BIBN4096BS, the first selective small molecule CGRP antagonist. *Br J Pharmacol* **129**:420–423.
- Dotti-Sigrist S, Born W, and Fischer JA (1988) Identification of a receptor for calcitonin gene-related peptides I and II in human cerebellum. *Biochem Biophys Res Commun* **151**:1081–1087.
- Dumont Y, Fournier A, St-Pierre S, and Quirion R (1997) A potent and selective CGRP<sub>2</sub> agonist, [Cys(Et)<sup>2,7</sup>]hCGRP  $\alpha$ : comparison in prototypical CGRP<sub>1</sub> and CGRP<sub>2</sub> in vitro bioassays. *Can J Physiol Pharmacol* **75**:671–676.
- Dwyer ND, Troemel ER, Sengupta P, and Bargmann CI (1998) Odorant receptor localization to olfactory cilia is mediated by ODR-4, a novel membrane-associated protein. *Cell* **93**:455–466.
- Edvinsson L, Sams A, Jansen-Olese I, Tajti J, Kane SA, Rutledge RZ, Koblan KS, Hill RG, and Longmore J (2001) Characterisation of the effects of a non-peptide CGRP receptor antagonist in SK-N-MC cells and isolated human cerebral arteries. *Eur J Pharmacol* **415**:39–44.
- Elshourbagy NA, Adamou JE, Swift AM, Disea J, Mao J, Ganguly S, Bergsma DJ, and Aiyar N (1998) Molecular cloning and characterization of the porcine calcitonin gene-related peptide receptor. *Endocrinology* **139**:1678–1683.
- Entzeroth M, Doods HN, Wieland HA, and Wiennen W (1995) Adrenomedullin mediates vasodilation via CGRP1 receptors. *Life Sci* **56**:19–25.
- Esfandiyari T, Macnaughton WK, Quirion R, St Pierre S, Junien JL, and Sharkey KA (2000) A novel receptor for calcitonin gene-related peptide (CGRP) mediates secretion in the rat colon: implications for secretory function in colitis. *FASEB J* **14**:1439–1446.
- Evans BN, Rosenblatt MI, Mnayer LO, Oliver KR, and Dickerson IM (2000) CGRP-RCP, a novel protein required for signal transduction at calcitonin gene-related peptide and adrenomedullin receptors. *J Biol Chem* **275**:31438–31443.
- Fischer JA, Sagar SM, and Martin JB (1981) Characterization and regional distribution of calcitonin binding sites in the rat brain. *Life Sci* **29**:663–671.
- Flühmann B, Lauber M, Lichtensteiger W, Fischer JA, and Born W (1997) Tissue-specific mRNA expression of a calcitonin receptor-like receptor during fetal and postnatal development. *Brain Res* **774**:184–192.
- Flühmann B, Muff R, Hunziker W, Fischer JA, and Born W (1995) A human orphan calcitonin receptor-like structure. *Biochem Biophys Res Commun* **206**:341–347.
- Fraser NJ, Wise A, Brown J, McLatchie LM, Main MJ, and Foord SM (1999) The amino terminus of receptor activity modifying proteins is a critical determinant of glycosylation state and ligand binding of calcitonin receptor-like receptor. *Mol Pharmacol* **55**:1054–1059.
- Frayon S, Cueille C, Gnidehou S, de Vernejoul MC, and Garel JM (2000) Dexamethasone increases RAMP1 and CL receptor mRNA expressions in human vascular smooth muscle cells. *Biochem Biophys Res Commun* **270**:1063–1067.
- Gebre-Medhin S, Mulder H, Zhang Y, Sunder F, and Betsholtz C (1998) Reduced nociceptive behavior in islet amyloid polypeptide (amylin) knockout mice. *Brain Res Mol Brain Res* **63**:180–183.
- Giuliani S, Wimalawansa SJ, and Maggi CA (1992) Involvement of multiple receptors in the biological effects of calcitonin gene-related peptide and amylin in rat and guinea-pig preparations. *Br J Pharmacol* **107**:510–514.
- Gorn AH, Lin HY, Yamin M, Auron PE, Flannery MR, Tapp DR, Manning CA, Lodish HF, Krane SM, and Goldring SR (1992) Cloning, characterization and expression of a human calcitonin receptor from an ovarian carcinoma cell line. *J Clin Invest* **90**:1726–1735.
- Gorn AH, Rudolph SM, Flannery MR, Morton CC, Weremowicz S, Wang TZ, Krane SM, and Goldring SR (1995) Expression of two human skeletal calcitonin receptor isoforms cloned from a giant cell tumor of bone. The first intracellular domain modulates ligand binding and signal transduction. *J Clin Invest* **95**:2680–2691.
- Harmar AJ (2001) Family-B G-protein coupled receptors. *Genome Biol* **2**:3013.1–3013.10.
- Hilaret S, Belanger C, Bertrand J, Laperriere A, Foord SM, and Bouvier M (2001a) Agonist-promoted internalization of a ternary complex between calcitonin receptor-like receptor, receptor activity-modifying protein 1 (RAMP1) and beta-arrestin. *J Biol Chem* **276**:42182–42190.
- Hilaret S, Foord SM, Marshall FH, and Bouvier M (2001b) Protein-protein interaction and not glycosylation determines the binding selectivity of heterodimers between the calcitonin receptor-like receptor and the receptor activity-modifying proteins. *J Biol Chem* **276**:29575–29581.
- Hilton JM, Chai SY, and Sexton PM (1995) In vitro autoradiographic localisation of the calcitonin receptor isoforms, C1a and C1b, in rat brain. *Neuroscience* **69**:1223–1237.
- Hilton JM, Downton M, Houssami S, and Sexton PM (2000) Identification of key

- components in the irreversibility of salmon calcitonin binding to calcitonin receptors. *J Endocrinol* **166**:213–226.
- Hinson JP, Kapas S, and Smith DM (2000) Adrenomedullin, a multifunctional regulatory peptide. *Endocr Rev* **21**:138–167.
- Ho HH, Gilbert MT, Nussenzeig DR, and Gershengorn MC (1999) Glycosylation is important for binding to human calcitonin receptors. *Biochemistry* **38**:1866–1872.
- Hoppener JW, Ahren B, and Lips CJ (2000) Islet amyloid and type 2 diabetes mellitus. *N Engl J Med* **343**:411–419.
- Houssami S, Findlay DM, Brady CL, Martin TJ, Epand RM, Moore EE, Murayama E, Tamura T, Orlowski RC, and Sexton PM (1995) Divergent structural requirements exist for calcitonin receptor binding specificity and adenylate cyclase activation. *Mol Pharmacol* **47**:798–809.
- Houssami S, Findlay DM, Brady CL, Myers DE, Martin TJ, and Sexton PM (1994) Isoforms of the rat calcitonin receptor: consequences for ligand binding and signal transduction. *Endocrinology* **135**:183–190.
- Husmann K, Sexton PM, Fischer JA, and Born W (2000) Mouse receptor-activity-modifying proteins 1, -2 and -3: amino acid sequence, expression and function. *Mol Cell Endocrinol* **162**:35–43.
- Juaneda C, Dumont Y, and Quirion R (2000) The molecular pharmacology of CGRP and related peptide receptor subtypes. *Trends Pharmacol Sci* **21**:432–438.
- Kapas S, Catt KJ, and Clark AJ (1995) Cloning and expression of cDNA encoding a rat adrenomedullin receptor. *J Biol Chem* **270**: 25344–25347.
- Kapas S and Clark AJ (1995) Identification of an orphan receptor gene as a type 1 calcitonin gene-related peptide receptor. *Biochem Biophys Res Commun* **217**:832–838.
- Kennedy SP, Sun D, Oleynek JJ, Hoth CF, Kong J, and Hill RJ (1998) Expression of the rat adrenomedullin receptor or a putative human adrenomedullin receptor does not correlate with adrenomedullin binding or functional response. *Biochem Biophys Res Commun* **244**:832–837.
- Kitamura K, Kangawa K, Kawamoto M, Ichikii Y, Nakamura S, Hisa Yuki M, and Eto T (1993) Adrenomedullin, a novel hypotensive peptide isolated from human pheochromocytoma. *Biochem Biophys Res Commun* **192**:553–560.
- Kuestner RE, Elrod RD, Grant FJ, Hagen FS, Kuijper JL, Matthews SL, Sheppard PO, Stroop SD, Thompson DL, Whitmore T, et al. (1994) Cloning and characterization of an abundant subtype of the human calcitonin receptor. *Mol Pharmacol* **46**:246–255.
- Kuwasako K, Shimekake Y, Masuda M, Nakahara K, Yoshida T, Kitaura M, Kitamura K, Eto T, and Sakata T (2000) Visualization of the calcitonin receptor-like receptor and its receptor activity-modifying proteins during internalization and recycling. *J Biol Chem* **275**:29602–29609.
- Leuthäuser K, Gujer R, Aldecoa A, McKinney RA, Muff R, Fischer JA, and Born W (2000) Receptor-activity-modifying protein 1 forms heterodimers with two G-protein-coupled receptors to define ligand recognition. *Biochem J* **351**:347–351.
- Lin B, Gao YG, Chang JK, Heaton J, Hyman A, and Lipton H (1994) An adrenomedullin fragment retains the systemic vasodepressor activity of rat adrenomedullin. *Eur J Pharmacol* **260**:1–4.
- Lin HY, Harris TL, Flannery MS, Aruffo A, Kaji EH, Gorn A, Kolakowski LF, Lodish HF, and Goldring SR (1991) Expression cloning of an adenylate cyclase-coupled calcitonin receptor. *Science (Wash DC)* **254**:1022–1024.
- Longmore J, Hogg JE, Hutson PH, and Hill RG (1994) Effects of two truncated forms of human calcitonin-gene related peptide: implications for receptor classification. *Eur J Pharmacol* **265**:53–59.
- Luebke AE, Dahl GP, Roos BA, and Dickerson IM (1996) Identification of a protein that confers calcitonin gene-peptide responsiveness to oocytes using a cystic fibrosis transmembrane conductance regulator assay. *Proc Natl Acad Sci USA* **93**: 3455–3460.
- Main MJ, Brown J, Brown S, Fraser NJ, and Foord SM (1998) The CGRP receptor can couple via pertussis toxin sensitive and insensitive G proteins. *FEBS Lett* **441**:6–10.
- Mallee JJ, Salvatore CA, LeBourdelle B, Oliver KR, Longmore J, Koblan KS, and Kane SA (2002) RAMP1 determines the species selectivity of non-peptide CGRP receptor antagonists. *J Biol Chem* **277**:14294–14298.
- Marshall I and Wisskirchen FM (2000) The classification of CGRP, amylin and adrenomedullin receptors: CGRP '98 consensus view, in *The CGRP Family: CGRP, Amylin and Adrenomedullin*. (Poyner DR, Marshall I, and Brain SD eds) pp 13–24, Eurekacom, Texas.
- McLatchie LM, Fraser NJ, Main MJ, Wise A, Brown J, Thompson N, Solari R, Lee MG, and Foord SM (1998) RAMPs regulate the transport and ligand specificity of the calcitonin receptor-like receptor. *Nature (Lond)* **393**:333–339.
- Mimeault M, Fournier A, Dumont Y, St-Pierre S, and Quirion R (1991) Comparative affinities and antagonistic potencies of various human calcitonin gene-related peptide fragments on calcitonin gene-related peptide receptors in brain and periphery. *J Pharmacol Exp Ther* **258**:1084–1090.
- Miret JJ, Rakhilina L, Silverman L, and Oehlen B (2001) Functional expression of heteromeric calcitonin gene-related peptide and adrenomedullin receptors in yeast. *J Biol Chem* **277**: 6881–6887.
- Moore EE, Kuestner RE, Stroop SD, Grant FJ, Matthews SL, Brady CL, Sexton PM, and Findlay DM (1995) Functionally different isoforms of the human calcitonin receptor result from alternative splicing of the gene transcript. *Mol Endocrinol* **9**:959–968.
- Moreno MJ, Terron JA, Stanimirovic DB, Doods H, and Hamel E (2002) Characterization of calcitonin gene-related peptide (CGRP) receptors and their receptor-activity-modifying proteins (RAMPs) in human brain microvascular and astroglial cells in culture. *Neuropharmacology* **42**:270–280.
- Muff R, Born W, and Fischer JA (2001) Adrenomedullin and related peptides: receptors and accessory proteins. *Peptides* **22**:1765–1772.
- Muff R, Bühlman N, Fischer JA, and Born W (1999) An amylin receptor is revealed following co-transfection of a calcitonin receptor with receptor activity modifying protein-1 or -3. *Endocrinology* **140**:2924–2927.
- Muff R, Leuthäuser K, Bühlman N, Foord SM, Fischer JA, and Born W (1998) Receptor activity modifying proteins regulate the activity of a calcitonin gene-related peptide receptor in rabbit aortic endothelial cells. *FEBS Lett* **441**:366–368.
- Muff R, Stangl D, Born W, and Fischer JA (1992) Comparison of a calcitonin gene-related peptide receptor in a human neuroblastoma cell line (SK-N-MC) and a calcitonin receptor in a human breast carcinoma cell line (T47D). *Ann N Y Acad Sci* **657**:106–116.
- Nakamura H, Orlowski RC, and Epand RM (1990) Evidence for calcitonin receptor heterogeneity: binding studies with nonhelical analogs. *Endocrinology* **127**:163–169.
- Nicholson GC, Moseley JM, Yates AJP, and Martin TJ (1987) Control of cyclic adenosine 3',5'-monophosphate production in osteoclasts: calcitonin-induced persistent activation and homologous desensitization of adenylate cyclase. *Endocrinology* **120**:1902–1908.
- Nishikawa T, Ishikawa H, Yamamoto S, and Koshihara Y (1999) A novel calcitonin receptor gene in human osteoclasts from normal bone marrow. *FEBS Lett* **458**: 409–414.
- Njuki F, Nicholl CG, Howard A, Mak JC, Barnes PJ, Gargis SI, and Legon S (1993) A new calcitonin-receptor-like sequence in rat pulmonary blood vessels. *Clin Sci (Lond)* **85**:385–388.
- Oliver KR, Kane SA, Salvatore CA, Mallee JJ, Kinsey AM, Koblan KS, Keyvan-Fouladi N, Heavens RP, Wainwright A, Jacobson M, et al. (2001) Cloning, characterization and central nervous system distribution of receptor activity modifying proteins in the rat. *Eur J Neurosci* **14**:618–628.
- Ono Y, Okano I, Kojima M, Okada K, and Kangawa K (2000) Decreased gene expression of adrenomedullin receptor in mouse lungs during sepsis. *Biochem Biophys Res Commun* **271**:197–202.
- Owji AA, Smith DM, Coppock HA, Morgan DGA, Bhogal R, Ghatel MA, and Bloom SR (1995) An abundant and specific binding site for the novel vasodilator adrenomedullin in the rat. *Endocrinology* **136**:2127–2134.
- Pittner RA, Albrandt K, Beaumont K, Gaeta LS, Koda JE, Moore CX, Rittenhouse J, and Rink TJ (1994) Molecular physiology of amylin. *J Cell Biochem* **55**: 19–28.
- Poyner DR and Marshall I (2001) CGRP receptors: beyond the CGRP(1)-CGRP(2) subdivision. *Trends Pharmacol Sci* **22**:223–223.
- Poyner DR, Soomets U, Howitt SG, and Langel U (1998) Structural determinants for binding to CGRP receptors expressed by human SK-N-MC and Col-299 cells: studies with chimeric and other peptides. *Br J Pharmacol* **124**:1659–1666.
- Poyner DR, Taylor GM, Tomlinson AE, Richardson AG, and Smith DM (1999) Characterization of receptors for calcitonin gene-related peptide and adrenomedullin on the guinea-pig vas deferens. *Br J Pharmacol* **126**:1276–1282.
- Purdue B, Tilakaratne N, and Sexton PM (2002) Molecular pharmacology of the calcitonin receptor. *Recept Channels*, in press.
- Quirion R, Van Rossum D, Dumont Y, St-Pierre S, and Fournier A (1992) Characterization of CGRP1 and CGRP2 receptors. *Ann N Y Acad Sci* **657**:88–105.
- Rebuffat P, Forneris ML, Aragona F, Ziolkowska A, Malendowicz LK, and Nussdorfer GG (2002) Adrenomedullin and its receptors are expressed in the zona glomerulosa of human adrenal gland: evidence that ADM enhances proliferation and decreases apoptosis in cultured ZG cells. *Int J Mol Med* **9**:119–124.
- Rorabaugh BR, Scofield MA, Smith DD, Jeffries WB, and Abel PW (2001) Functional calcitonin gene-related peptide subtype 2 receptors in porcine coronary arteries are identified as calcitonin gene-related peptide subtype 1 receptors by radioligand binding and reverse transcription-polymerase chain reaction. *J Pharmacol Exp Ther* **299**:1086–1094.
- Ruffolo RR, Humphrey PPA, Watson SP, and Spedding M (2000) Revised NC-IUPHAR recommendations for nomenclature of receptors, in *The IUPHAR Compendium of Receptor Characterization and Classification*, 2nd ed, pp 7–8, IUPHAR Media, London, UK.
- Santiago JA, Garrison EA, and Purnell WL (1995) Comparison of responses to adrenomedullin and adrenomedullin analogues in the mesenteric vascular bed of the cat. *Eur J Pharmacol* **272**:115–118.
- Sexton PM (1999) Recent advances in our understanding of peptide hormone receptors and RAMPs. *Curr Opin Drug Discov Dev* **2**:440–448.
- Sexton PM, Albiston A, Morfis M, and Tilakaratne N (2001) Receptor activity modifying proteins. *Cell Signal* **13**:73–83.
- Sexton PM, Findlay DM, and Martin TJ (1999) Calcitonin. *Curr Med Chem* **6**:1067–1093.
- Sexton PM, Houssami S, Hilton JM, O'Keefe LM, Center RJ, Gillespie MT, Darcy P, and Findlay DM (1993) Identification of brain isoforms of the rat calcitonin receptor. *Mol Endocrinol* **7**:815–821.
- Sexton PM, McKenzie JS, and Mendelsohn FAO (1988) Evidence for a new subclass of calcitonin gene-related peptide binding-site in rat-brain. *Neurochem Int* **12**:323–325.
- Sexton PM, Paxinos G, Kenney MA, Wookey PJ, and Beaumont K (1994) In vitro autoradiographic localization of amylin binding sites in rat brain. *Neuroscience* **62**:553–567.
- Shimosawa T, Shibagaki Y, Ishibashi K, Kitamura K, Kangawa K, Kato S, Ando K, and Fujita T (2002) Adrenomedullin, an endogenous peptide, counteracts cardiovascular damage. *Circulation* **105**:106–111.
- Shyu JF, Inoue D, Baron R, and Horne WC (1996) The deletion of 14 amino acids in the seventh transmembrane domain of a naturally occurring calcitonin receptor isoform alters ligand binding and selectively abolishes coupling to phospholipase C. *J Biol Chem* **271**:31127–31134.
- Smyth K, Tilakaratne N, Morfis M, Christopoulos A, Chen WJ, and Sexton PM (2001) Influence of the G $\alpha$  protein subtype and expression level on receptor phenotypes generated from calcitonin receptor and RAMP interaction. *Proc AS-CPT* **9**:75.
- Steenbergh PH, Hoppener JW, Zandberg J, Lips CJ, and Jansz HS (1985) A second human calcitonin/CGRP gene. *FEBS Lett* **22**:403–407.
- Suzuki N, Suzuki T, and Kurokawa T (2000) Cloning of a calcitonin gene-related peptide receptor and a novel calcitonin receptor-like receptor from the gill of flounder, *Paralichthys olivaceus*. *Gene (Amst)* **244**:81–88.
- Tilakaratne N, Christopoulos G, Zumpe E, Foord SM, and Sexton PM (2000) Amylin

- receptor phenotypes derived from human calcitonin receptor/RAMP coexpression exhibit pharmacological differences dependent on receptor isoform and host cell environment. *J Pharmacol Exp Ther* **294**:61–72.
- Tomlinson AE and Poyner DR (1996) Multiple receptors for calcitonin gene-related peptide and amylin on guinea pig ileum and vas deferens. *Br J Pharmacol* **117**:1362–1368.
- Tong Y, Dumont Y, Van Rossum D, Clark AJL, Shen SH, and Quirion R (2000) In *The CGRP Family: CGRP, Amylin and Adrenomedullin* (Poyner DR, Marshall I, and Brain SD eds) pp 179–186, R. G. Landes Bioscientific, Austin, TX.
- Upton PD, Austin C, Taylor GM, Nandha KA, Clark AJ, Ghatei MA, Bloom SR, and Smith DM (1997) Expression of adrenomedullin (ADM) and its binding sites in the rat uterus: increased numbers of binding sites and ADM messenger ribonucleic acid in 20-day old pregnant rats compared with nonpregnant rats. *Endocrinology* **138**:2508–2514.
- van Rossum D, Menard DP, Fournier A, St-Pierre S, and Quirion R (1994) Autoradiographic distribution and receptor binding profile of [<sup>125</sup>I]Bolton Hunter-rat amylin binding sites in the rat brain. *J Pharmacol Exp Ther* **270**:779–787.
- Veale P, Bhogal R, Morgan D, Smith D, and Bloom S (1994) The presence of islet amyloid polypeptide/calcitonin gene-related peptide/salmon calcitonin binding-sites in the rat nucleus-accumbens. *Eur J Pharmacol* **262**:133–141.
- Waugh DJ, Bockman CS, Smith DD, and Abel PW (1999) Limitations in using peptide drugs to characterize calcitonin gene-related peptide receptors. *J Pharmacol Exp Ther* **289**:1419–1426.
- Winklbauer R, Medina A, Swain RK, and Steinbeisser H (2001) Frizzled-7 signalling controls tissue separation during *Xenopus* gastrulation. *Nature (Lond)* **413**:856–860.
- Wisskirchen FM, Burt RP, and Marshall I (1998) Pharmacological characterization of CGRP receptors mediating relaxation of the rat pulmonary artery and inhibition of twitch responses of the rat vas deferens. *Br J Pharmacol* **123**:1673–1683.
- Wisskirchen FM, Gray DW, and Marshall I (1999) Receptors mediating CGRP-induced relaxation in the rat isolated thoracic aorta and porcine isolated coronary artery differentiated by h(alpha) CGRP<sub>(8-37)</sub>. *Br J Pharmacol* **128**:283–292.
- Wu D, Eberlein W, Rudolf K, Engel W, Hallermayer G, and Doods H (2000) Characterisation of calcitonin gene-related peptide receptors in rat atrium and vas deferens: evidence for a [Cys(Et)(2,7)]hCGRP-preferring receptor. *Eur J Pharmacol* **400**:313–319.
- Yamin M, Gorn AH, Flannery MR, Jenkins NA, Gilbert DJ, Copeland NG, Tapp DR, Krane SM, and Goldring SR (1994) Cloning and characterization of a mouse brain calcitonin receptor complementary deoxyribonucleic acid and mapping of the calcitonin receptor gene. *Endocrinology* **135**:2635–2643.
- Zimmermann U, Fischer JA, Fre K, Fischer AH, Reinscheid RK, and Muff RA (1996) Identification of adrenomedullin receptors in cultured rat astrocytes and in neuroblastoma X glioma hybrid cells. *Brain Res* **724**:238–245.
- Zimmermann U, Fischer JA, and Muff R (1995) Adrenomedullin and calcitonin gene-related peptide interact with the same receptor in cultured human neuroblastoma SK-N-MC cells. *Peptides* **16**:421–424.
- Zumpe ET, Tilakaratne N, Fraser NJ, Christopoulos G, Foord SM, and Sexton PM (2000) Multiple ramp domains are required for generation of amylin receptor phenotype from the calcitonin receptor gene product. *Biochem Biophys Res Commun* **267**:368–372.