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

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***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 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.**

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I. Introduction

The calcitonin family of peptides comprises five known members: calcitonin (CT[1]), amylin (AMY), two CT gene-related peptides (αCGRP and βCGRP), 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 α-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.

[1] 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*)-quinazolinyl).

TABLE 1
Structures CGRP, amylin, adrenomedullin, and calcitonin

hαCGRP	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
rαCGRP	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
hβCGRP	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
rβCGRP	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 — α-helix

h, human; r, rat; s, salmon.
hAM is the structure of the 15–52 fragment; the N-terminal amino acids are YRQSMNNFQGLRSF. rAM shows the structure of the 13–50 fragment; the N-terminal amino acids are YRQSMNQGSRST.

II. The Calcitonin Gene-Related Peptide Receptors

αCGRP 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 αCGRP, a 37-amino acid peptide. A second CGRP homolog, βCGRP, was subsequently discovered. It differs from human αCGRP by three amino acids and in the rat by one amino acid. βCGRP is encoded by its own unique gene, with high homology to the CT gene (Steenbergh et al., 1985). α- and βCGRP display similar biological activities.

Historically, CGRP receptors have been divided into two classes, $CGRP_1$ and $CGRP_2$. $CGRP_1$ receptors are more sensitive than $CGRP_2$ receptors to the peptide antagonist $CGRP_{8\text{-}37}$ (taken by many workers to mean a pA_2 of 7 or greater) (Dennis et al., 1989; Quirion et al., 1992). On the other hand, the linear CGRP analogs $[Cys(ACM)^{2,7}]$- and $[Cys(Et)^{2,7}]$hαCGRP are more potent agonists at $CGRP_2$ receptors than at $CGRP_1$ (Dennis et al., 1989; Dumont et al., 1997; Moreno et al., 2002).

The $CGRP_1$:$CGRP_2$ 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 $CGRP_2$ receptor is the rat vas deferens (Dennis et al., 1989). Here pA_2 estimates of $CGRP_{8\text{-}37}$, measured with CGRP as agonist, range from below 5.5 (Giuliani et al., 1992) to 6.7 (Longmore et al., 1994). For the prototypical $CGRP_1$ 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

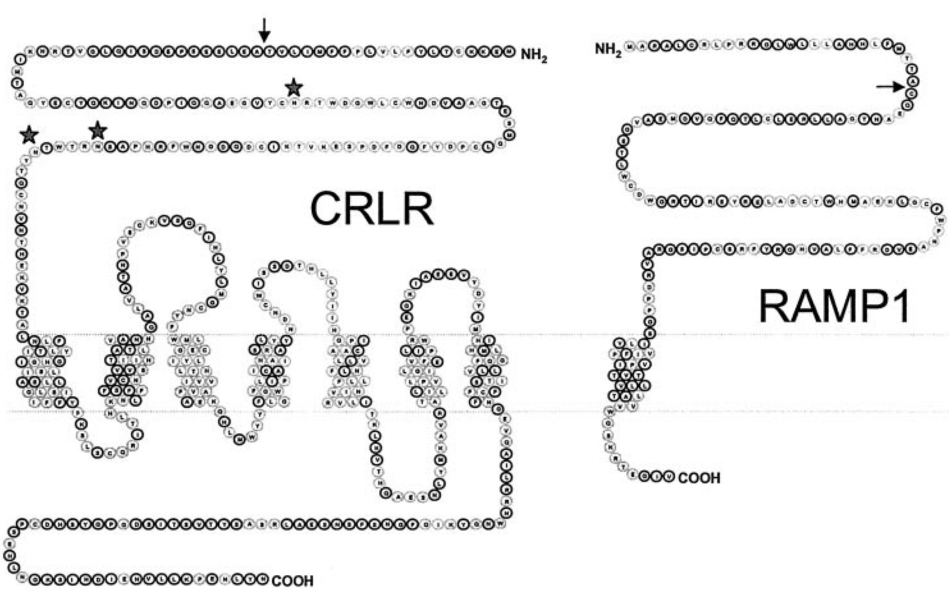

FIG 1. Schematic diagram of human CL receptor (CRLR) and RAMP 1. Stars show glycosylation consensus sites on CL receptor. Residues unique to CL receptor over the human CT receptor, or unique to RAMP1 over RAMP2 or RAMP3 are indicated in bold circles. Arrows show predicted signal peptide cleavage sites.

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}]hαCGRP 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}]hαCGRP and [Cys(Et)^{2,7}]hαCGRP 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; Hilairet et al., 2001a,b). This interaction is also maintained during agonist-mediated internalization of the receptor (Kuwasako 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 (Hilairet 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 $CGRP_{8\text{-}37}$ 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 $CGRP_2$ receptors. It may be that some very labile accessory protein is responsible for promoting a putative $CGRP_2$ receptor form, perhaps by interacting with the CL receptor. The mRNA for the CL receptor is present in rat vas deferens, the prototypical $CGRP_2$ receptor-expressing tissue (Moreno et al., 2002), as well as other tissues showing pharmacology similar to the putative $CGRP_2$ 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 α 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 αCGRP is a potent agonist at the human $CT_{(a)}$ receptor/human RAMP1 complex (EC_{50} of 0.8 nM) (see below for discussion of CT receptor nomenclature). This response is antagonized by salmon calcitonin (8–32) (sCT_{8-32}) but not 100 nM $CGRP_{8\text{-}37}$ (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_{8-32}, 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 $CGRP_2$ 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_{8-32} (which would antagonize complexes containing the CT receptor but not the CL receptor) might be useful in further defining a $CGRP_2$ phenotype.

Currently, there is no molecular correlate for a $CGRP_2$ 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 $CGRP_2$ 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 "$CGRP_2$ 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 (Hilairet 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}I-AM (Hilairet et al., 2001a).

Some biological activities of AM are mediated by CGRP receptors. At these receptors AM behaves as a potent competitor for ^{125}I-αCGRP binding sites, and the production of cAMP induced by both CGRP and AM can be inhibited by $CGRP_{8\text{-}37}$. 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 $CGRP_{8\text{-}37.}$ 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 $CGRP_1$ receptor, sensitive to $CGRP_{8\text{-}37}$, is also observed. These data imply expression of an endogenous CL receptor/RAMP2 AM receptor in RAECs and its conversion into a $CGRP_1$ receptor by competitive interaction of CL receptor with coexpressed exogenous RAMP1.

^{125}I-CGRP and ^{125}I-AM binding studies define tissues that contain CGRP, AM, or mixtures of both receptors. Binding sites labeled by ^{125}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. $AM_{22\text{-}52}$ 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 $AM_{22\text{-}52}$ 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 $AM_{22\text{-}52}$ and $CGRP_{8\text{-}37}$ are selective for the CL receptor/RAMP1 and CL receptor/RAMP2 combinations in

TABLE 2
Pharmacological profile for CL receptor/RAMP complexes

Receptor	αCGRP	βCGRP	$CGRP_{8\text{–}37}$	AM	$AM_{22\text{–}52}$	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
hCL receptor/hRAMP2	*5.9*	*6.5*	*7.1*	*9.2*	*7.8*	NS	Swiss3T3	McLatchie et al., 1998
	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
rCL receptor/rRAMP2	*>7*	*>7*	*>6*	*9.3*	*7*	*>6*	UMR106	Oliver et al., 2001
rCL receptor/hRAMP2	*>6*		*7.1*	*8.8*	*8.5*	*>6*	COS-7	Buhlmann et al., 1999
	>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
rCL receptor/mRAMP2		*6.8*	*7.0*	*8.7*	*8.5*		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
rCL receptor/mRAMP3	*7.7*	*7.7*	*8.4*	*8.2*	*7.8*		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_{50} values measured against ^{125}I-CGRP (normal text) or ^{125}I-adrenomedullin (italics). Displacing ligands are of human for hCL receptor and pCL receptor; otherwise rat. Schneider cells are from Drosophila.

* hαCGRP showed low potency in stimulating cAMP production in the presence of RAMP2 or RAMP3 (pEC_{50} 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_{22-52} 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_1 and AM_2 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 ($CT_{(b)}$ receptor) or absence ($CT_{(a)}$ receptor) of 16 amino acids in the first intracellular loop. Although generally less abundant, the $CT_{(b)}$ receptor is expressed significantly in ovary, placenta, bone marrow, and lung (Kuestner et al., 1994). The human $CT_{(b)}$ receptor and $CT_{(a)}$ 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 $CT_{(a)}$ receptor, the $CT_{(b)}$ receptor is poorly internalized. Experimentally, it also appears that $CT_{(b)}$ receptor has altered coupling to G proteins; loss of Gq-mediated responses and attenuation of Gs-mediated signaling has been observed upon stimulation of $CT_{(b)}$ 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).

TABLE 3
Summary of nomenclature for calcitonin receptor splice variants, referenced within the text

Proposed Nomenclature	Former Nomenclature
$hCT_{(a)}$ receptor	$hCTR_{I1-}$, hCTR2
$rCT_{(a)}$ receptor	rCTR1a
$hCT_{(b)}$ receptor	CTR_{I1+}, hCTR1

h, human; r, rodent. Other splices variants not yet included in this classification are human δ47 (lacking the last 47 amino acids), rat CTR_{E2+} or CTR_{1b} (with a 37-amino acid insert in the second extracellular loop) and rabbit δTM7 (lacking the last transmembrane helix). IUPHAR is currently looking into the nomenclature of splice variants and will publish guidelines in the near future.

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_{I1} 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 $CT_{(a)}$ 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_{1b} or C1b). This interacts only weakly with CT peptides exhibiting poor α-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

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_{8-32}, whereas $CGRP_{8-37}$ or AM_{22-52} 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 ^{125}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 ^{125}I-sCT receptor binding cannot be evaluated. Nonetheless, CT receptors exhibit a general relative potency of sCT $\geq$ porcine CT $\geq$ 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 ^{125}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 ^{125}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 $CGRP_{8-37}$ whereas the "AMY antagonist" AMY_{8-37} 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). ^{125}I-AMY binding sites present as a subset of those labeled with ^{125}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 $CT_{(a)}$ and $CT_{(b)}$ receptors and generate AMY receptors. The $CT_{(b)}$ receptor displays greater capacity to generate RAMP2 AMY receptors than the $CT_{(a)}$ receptor. This may be related to the differential G protein coupling of the two splice variants as well as the level of the $CT_{(a)}$ 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_{50}, 0.3 nM), AMY (IC_{50}, 7.8 nM), CGRP (IC_{50}, 4.7 nM), and to a lesser extent human CT (IC_{50}, 447 nM). CT receptor/RAMP3 shows a pharmacology almost identical to CT receptor/RAMP1 with the exception that the IC_{50} for CGRP is ~40-fold lower (151 nM) (Christopoulos et al., 1999). The responses of these receptors are antagonized by 100 nM sCT_{8-32}, but not by 100 nM $CGRP_{8-37}$ (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_{8-32} 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_{8-32} 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
$hCT_{(b)}$ receptor	**9.45**/6.89	**10.56**/6.93	6.58	6.77	COS-7	Tilakaratne et al., 2000
	10.93/6.78	11.06/**8.12**	10.0/**5.65**	11.08/**5.77**	CHO-P	Tilakaratne et al., 2000
$hCT_{(b)}$ receptor/hRAMP1	6.94	10.98/**9.29**	10.95/**8.43**	**9.93**/7.94	COS-7	Tilakaratne et al., 2000
	9.89/6.95	**11.2**/6.41	11.29/8.66	11.89/**8.64**	CHO-P	Tilakaratne et al., 2000
$hCT_{(b)}$ receptor/hRAMP2	7.95	11.06/**9.33**	10.67/**8.23**	10.87/**7.72**	COS-7	Tilakaratne et al., 2000
	9.63/6.42	**11.83**/8.83	10.65/**7.59**	10.96/**7.7**	CHO-P	Tilakaratne et al., 2000
$hCT_{(b)}$ receptor/hRAMP3	6.87	11.06/9.16	11.03/**8.19**	8.9/7.1	COS-7	Tilakaratne et al., 2000
	10.19/5.95	**11.53**/8.89	11.55/8.2	10.1/6.9	CHO-P	Tilakaratne et al., 2000
$hCT_{(a)}$ receptor	7.81	9.03	6.27	>6	COS-7	Zumpe et al., 1999
			6.3	>6	RAEC	Muff et al., 1999
	11.12/**6.45**	11.96/8.08	6.07	9.94/5.78	CHO-P	Tilakaratne et al., 2000
$hCT_{(a)}$ receptor/hRAMP1	6.92	9.05	8.27	7.87	COS-7	Christopoulos et al., 1999
	7.53	9.51	8.1	7.67	COS-7	Zumpe et al., 1999
			8.5	7.85	RAEC	Muff et al., 1999
	9.79/**5.97**	**12.19**/9.00	11.9/**8.03**	**8.9**/6.03	CHO-P	Tilakaratne et al., 2000
$hCT_{(a)}$ receptor/hRAMP2	8.08	9.89	7.82	5.86	COS-7	Zumpe et al., 1999
	No evidence of coupling; wild-type phenotype				RAEC	Muff et al., 1999
	8.94/5.92	**12.19**/8.54	8.13	7.64	CHO-P	Tilakaratne et al., 2000
$hCT_{(a)}$ 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
	11.11/**6.02**	**12.12**/8.29	11.7/**7.78**	10.96/**6.79**	CHO-P	Tilakaratne et al., 2000

Values represent pIC_{50} values obtained against ^{125}I-AMY or ^{125}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_1, AMY_2, and AMY_3 receptors. Splice variants of the CT receptor can be accommodated in this scheme by use of letters: $AMY_{1(a)}$, $AMY_{1(b)}$, 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) (Hilairet 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}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 diversity 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 $CGRP_2$ 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?
$CGRP_1$	CL receptor + RAMP1	Yes
AM_1	CL receptor + RAMP2	Yes
AM_2	CL receptor + RAMP3	
CT	CT receptor	Yes
AMY_1	CT receptor + RAMP1	Yes, but probably as a mixed population
AMY_2	CT receptor + RAMP2	Yes, but probably as a mixed population
AMY_3	CT receptor + RAMP3	Yes, but probably as a mixed population

Formal naming of the $CGRP_2$ 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 $CGRP_1$ 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/αCGRP	CALCA	SWMain:P01258 SWMain::P06881	11p15.2
βCGRP	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.

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