ELSEVIER

Modulating receptor function through RAMPs: can they represent drug targets in themselves?

Patrick M. Sexton[1], David R. Poyner[2], John Simms[1], Arthur Christopoulos[1] and Debbie L. Hay[3]

[1] Drug Discovery Biology Laboratory, Monash Institute of Pharmaceutical Sciences and Department of Pharmacology, Monash University, Victoria 3800, Australia
[2] School of Life and Health Sciences, Aston University, Birmingham B4 7ET, UK
[3] School of Biological Sciences, University of Auckland, Symonds Street, Auckland, New Zealand

G protein-coupled receptors (GPCRs) are successfully exploited as drug targets. As our understanding of how distinct GPCR subtypes can be generated expands, so do possibilities for therapeutic intervention via these receptors. Receptor activity-modifying proteins (RAMPs) are excellent examples of proteins that enhance diversity in GPCR function. They facilitate the creation of binding pockets, controlling the pharmacology of some GPCRs. Moreover, they have the ability to regulate cell-surface trafficking, internalisation and signalling of GPCRs, creating novel opportunities for drug discovery. RAMPs could be directly targeted by drugs, or advantage could be taken of unique RAMP/GPCR interfaces for generating highly selective ligands.

Introduction

It is generally well accepted that the largest group of 'druggable' proteins is represented by a handful of cell-surface receptor superfamilies. Of these, G protein-coupled receptors (GPCRs) constitute the largest single grouping, yet the number of actively targeted GPCRs still represents only a small fraction of the total number of GPCRs that could be effectively exploited therapeutically. These receptors, which signal predominantly through heterotrimeric G proteins, comprise ~2% of the human genome and represent the major signalling system in cells. Mammalian GPCRs are further subdivided into three groups: Family A, the largest family, which contains receptors for prototypical neurotransmitters and hormones such as the biogenic amines; Family B, incorporating peptide hormone receptors such as calcitonin (CT), glucagon and secretin receptors; and Family C, including receptors for small molecules such as glutamate, GABA and calcium. GPCR-based signalling complexes engender enormous diversity and amplification in cell signalling processes and this is likely to account for the ubiquitous distribution of GPCRs across all cell types. Moreover, it is not surprising that many major disease states, such as CNS disorders, metabolic disorders and cardiovascular disease, often involve aberrations in GPCRs or their signalling pathways [1].

Increasing evidence indicates that GPCRs, like many other signalling proteins, can form oligomeric protein arrays and that these are crucial to many aspects of GPCR function, including cellular trafficking, compartmentalisation and signalling, regulation and even ligand recognition. It is thought that, for many GPCRs, constitutive dimers or oligomers act as the core functional unit, but the GPCR, either as a monomer, or oligomeric complex, may also interact with a diverse range of other proteins that regulate their function. These interactions create novel opportunities for drug discovery and development. An exemplar model for protein–protein modulation of GPCR function is the receptor activity-modifying protein (RAMP) family.

RAMPs are a family of three type I transmembrane proteins initially shown to regulate the glycosylation, transport and pharmacological phenotype of the, then orphan, CT receptor-like (CL, Box 1) receptor (CLR) [2]. In the absence of RAMPs, the CLR is principally retained intracellularly, however, RAMP interaction with the receptor leads to terminal glycosylation of the receptor and transit of the complex from the ER/Golgi to the cell surface. Similarly RAMPs, in the absence of an interacting receptor partner, are relatively poorly expressed at the cell surface and are

Corresponding author: Hay, D.L. (dl.hay@auckland.ac.nz)

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2008.12.009

BOX 1

Glossary, including receptor abbreviations

AM—adrenomedullin
AM2—adrenomedullin 2; intermedin
CT—calcitonin
CGRP—calcitonin gene-related peptide
CaS—calcium-sensing receptor
CLR—calcitonin receptor-like receptor
$CT_{(a)}$—calcitonin receptor; insert negative splice variant (lacking a 16 amino acid insert in the first intracellular loop)
$CT_{(b)}$—calcitonin receptor; insert positive (containing a 16 amino acid insert in the first intracellular loop)
GHRH—growth hormone-releasing hormone receptor
GLP-1—glucagon-like peptide 1 receptor
GPCR—G protein-coupled receptor
NSF—N-ethylmaleimide-sensitive factor
PTH1—parathyroid hormone 1 receptor
PTH2—parathyroid hormone 2 receptor
RAMP—receptor activity-modifying protein
RCP—receptor component protein
VPAC1—vasoactive intestinal peptide/pituitary adenylate cyclase-activating polypeptide type 1 receptor

translocated in complex with the receptor to the cell surface [2,3]. In addition to this chaperone role, RAMPs in stable complex with the CLR are required for the expression of phenotype, whereby RAMP1/CLR is a CT gene-related peptide (CGRP) receptor, while RAMP2/CLR and RAMP3/CLR exhibit distinct adrenomedullin (AM) receptor phenotypes (see below) [4–7]. Thus, the discovery of RAMPs revealed a novel mechanism for producing phenotypic diversity in receptor response. Subsequently, RAMPs were shown to interact with the related CT receptor, and each RAMP/CT receptor complex displayed a distinct amylin receptor phenotype [8,9].

For the CT receptor and CLR, strong interaction occurs for all three RAMPs. These receptors are family B peptide hormone receptors, and subsequent analysis of other members of this receptor family for RAMP interaction, utilizing an assay of translocation of RAMPs to the cell surface, revealed additional RAMP-receptor partners with different degrees of specificity in the RAMP-receptor interaction. Like the CT receptor and CLR, the VPAC1 receptor interacted with all three RAMPs, whereas the PTH1 and glucagon receptors interacted specifically with RAMP2, and the PTH2 receptor specifically with RAMP3 [3]. No measurable translocation of RAMPs was seen, however, with the VPAC2, GHRH, GLP-1 or GLP-2 receptors, although this does not exclude the potential for weak or non-trafficking interactions between the receptors and RAMPs. With the exception of the VPAC1/RAMP2 interaction (see below), little is known of the potential role of RAMPs in the function for these receptors. More recently, a chaperone role for RAMPs 1 and 3, but not RAMP2, in the trafficking of the Family C, calcium sensing receptor (CaS receptor) has been reported [10], illustrating potential for RAMP modulation of receptors outside of Family B GPCRs. Although individual RAMPs are differentially expressed, the protein family is very broadly distributed throughout cells and organs of the body, beyond the localisation of known receptor partners (reviewed in [6,11,12]), providing significant scope for additional RAMP-interacting GPCRs to be discovered. The full extent of RAMP interaction with other GPCRs, however, is yet to be elucidated.

Thus, RAMPs have the potential to interact with, and modulate the function of many GPCRs (Figure 1). This offers access to novel strategies for drug development at these highly tractable drug targets.

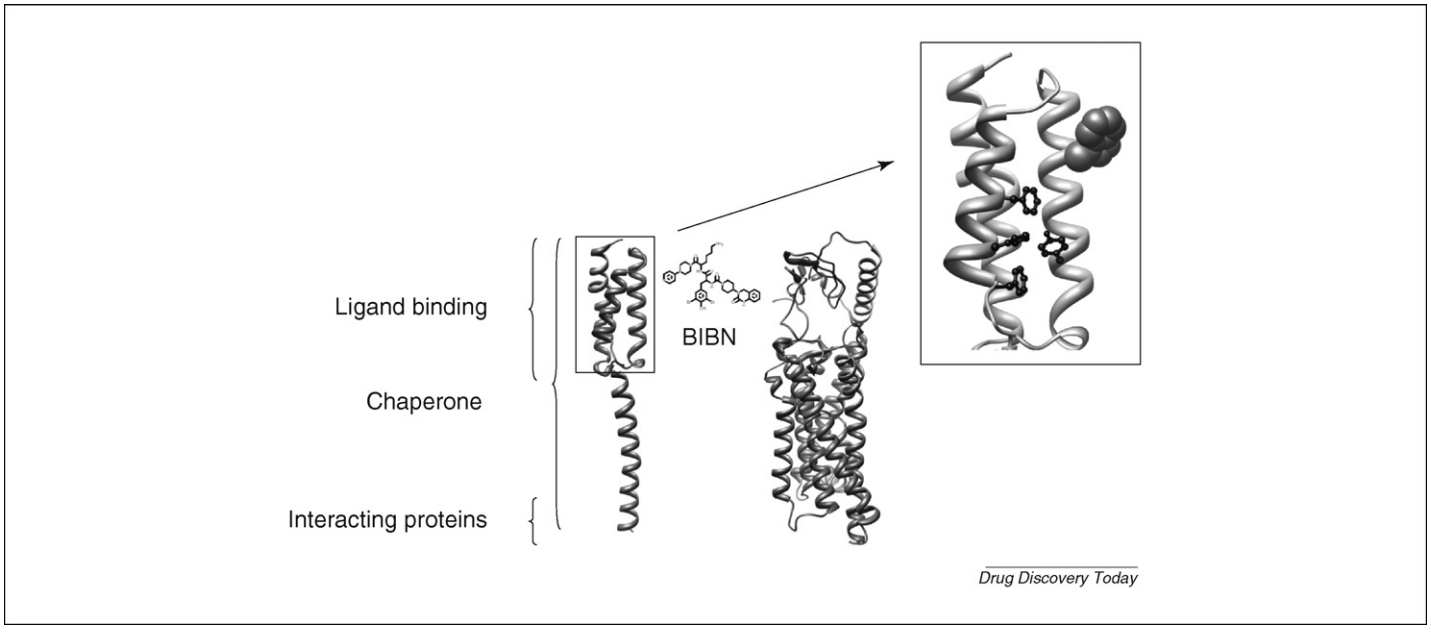

FIGURE 1

The multiple functions of RAMPs. Single transmembrane RAMPs interact with 7 transmembrane domain GPCRs to modify trafficking, both in a chaperone role and owing to the interaction of proteins with their intracellular C termini. RAMPs also modulate GPCR pharmacology and signalling. For the CGRP receptor (RAMP1/CLR), the RAMP is also key for high affinity binding of small molecule antagonists such as BIBN4096 (BIBN). Inset is the N-terminal domain of RAMP1 illustrating the positions of amino acids Tyr^{66}, Phe^{93}, His^{97}, Phe^{101} (stick representation) that have been implicated in the interface between RAMP1 and the CLR and also the position of Trp^{74} (space-filling representation) that is crucial for the binding of small molecule CGRP receptor antagonists such as BIBN.

RAMP-regulated GPCR pharmacology

The most well-studied consequence of RAMP interaction with GPCRs is their ability to alter pharmacology; they function as pharmacological switches for the CT peptide family [7]. This family comprises CT, amylin, CGRP, AM and AM2 (intermedin). These peptides have many biological activities and some of their receptors are validated pharmaceutical targets for diseases including diabetes, migraine (see below) and osteoporosis. As indicated above, the receptors for these peptides are two Family B GPCRs, the CT receptor and the CLR. CLR by itself will bind no known endogenous ligand and is only poorly expressed at the cell surface. When paired with RAMP1, this complex is the CGRP receptor. On the contrary, two pharmacologically distinct subtypes of AM receptor are formed from complexes of RAMP2/CLR (AM_1 receptor) or RAMP3/CLR (AM_2 receptor). The AM_2 receptor may also recognise CGRP with reasonable affinity, depending on the species from which the receptor components have been derived [5]. Similarly, the CGRP receptor has moderately high affinity for AM [7]. The CT receptor without RAMP is a conventional Family B GPCR and is sufficient for CT binding. RAMPs are not required for cell surface expression of this receptor, allowing for the co-existence of RAMP/CT complexes with the well-characterised CT receptor phenotype, which has low affinity for amylin when expressed alone [7]. The CT receptor expressed with RAMP1, 2 or 3 respectively, gives three subtypes of high-affinity amylin ($AMY_{1–3}$) receptor. Several CT receptor splice variants have been reported; these include the insert negative ($CT_{(a)}$) and positive ($CT_{(b)}$; 16 additional amino acids in the first intracellular loop) isoforms of the receptor. In essence, their interaction with RAMPs generates a large number of possible amylin receptor subtypes. The discovery of AM2 and its interaction with these receptors [13–15] is a relatively recent discovery, as is the identification of pro-CT as another potential endogenous ligand of the CGRP receptor [16]. Thus, the complexity of this system is considerable with RAMPs enabling substantial pharmacological diversity from only two GPCR genes. It is unclear whether this phenomenon is unique to this peptide family; no other RAMP pairings with GPCRs have been shown to cause changes in pharmacological phenotype. There may be novel combinations, however, which may yet reveal that this is more widespread than the current literature suggests.

There are two chief mechanisms by which RAMPs may influence GPCR pharmacology; they might directly contribute binding epitopes to the relevant ligands or could act indirectly by altering the conformation of the GPCR. In practice, it is challenging to differentiate between these. Nevertheless, there is strong experimental support for interactions between the long N-termini of the RAMP and the CT receptor or CLR determining the specific pharmacology of each receptor complex [4,6,17–22]. It is conceivable that the interface between these proteins generates the unique structures required for the binding of selective ligands. Amino acid 74 in RAMP1 and RAMP3 appears particularly influential in determining AM and small molecule antagonist affinity (see below and Figure 1).

RAMP-regulated receptor signalling

There is increasing evidence that RAMPs can play a role in the signalling profile of receptors. This has been most extensively studied for RAMP/CT receptor-derived AMY receptors, where early data identified receptor isoform-dependent and cell background-dependent differences in the ability of RAMP2 to create a high affinity AMY phenotype. In COS-7, and rabbit aortic endothelial cells, RAMP2 only weakly engenders amylin binding from the $CT_{(a)}$ receptor isoform, but strongly induces an AMY phenotype when co-expressed with the $CT_{(b)}$ receptor isoform [8,9,23]. The presence of the 16 amino acid insert in the first intracellular domain of the CT receptor leads to loss of Gq-mediated signalling and impaired receptor internalisation [24]. By contrast, co-expression of either receptor isoform with RAMP2 in CHO-P cells led to strong induction of AMY phenotype [23]. These data suggested that a component of the cellular background, potentially G proteins, could modulate RAMP/CT receptor interaction. Chimeras of RAMP1 and RAMP2 where the intracellular C-terminus was exchanged demonstrated that CGRP potency for cAMP formation tracked with the C-terminal RAMP region, despite the binding affinity tracking with the RAMP N-terminal domain, indicating that the RAMP C-terminus played a direct role in signalling [20]. Consistent with this, RAMP C-terminal truncation decreased amylin binding, an effect that could be reversed, at least partially, by overexpression of Gαs [25], suggesting that the RAMP C-terminus played a direct role in G protein coupling. Further work revealed a preferential coupling of AMY_1 and AMY_3 receptors to Gs vs. Gq, relative to $CT_{(a)}$ receptors expressed alone (Figure 2). Furthermore, Gα subtypes differentially modulated ^{125}I-amylin binding to RAMP/$CT_{(a)}$ complexes where Gs overexpression increased binding of RAMP2/$CT_{(a)}$ complexes and RAMP3/$CT_{(a)}$ complexes and Gq selectively increased binding at RAMP3/$CT_{(a)}$ complexes [26]. Thus, these data support a direct role for RAMPs, via their C-terminal domain, in the G-protein interaction profile of AMY receptors, potentially in an individual RAMP-dependent manner. In contrast to this action at AMY receptors, RAMPs do not appear to be directly involved in G protein coupling of RAMP/CLR complexes [25]. This may be owing to the important role of CGRP receptor-component protein (RCP) in CGRP and AM receptor signalling [27]. RCP is an intracellular peripheral membrane protein that is associated with RAMP/CLR complexes and is crucial for Gs-mediated cAMP signalling of CLR-based CGRP and AM receptors [27]. To date, there is no evidence for a role for RCP in the signalling of other RAMP/GPCR complexes.

Modulation of receptor signalling by RAMPs has also been observed for the VPAC1 receptor, where overexpression of RAMP2 caused a selective augmentation of phosphoinositide hydrolysis (presumably downstream of Gq) without altering cAMP formation [3]. It was speculated, however, that this effect may have been due to changes in compartmentalisation of the RAMP2/VPAC1 complex, rather than a direct effect on G protein coupling. The effect of RAMPs on the signalling profile of other interacting receptors has not been investigated. Nonetheless, these data identify the potential for selective modulation of receptor signalling through targeting of the RAMP/receptor interface.

RAMP-regulated receptor trafficking

One of the original proposed functions for RAMPs was to act as chaperones, to promote cell-surface expression of the CLR [2]. All three RAMPs can associate with this receptor in the endoplasmic reticulum and promote terminal glycosylation in the Golgi. The CaS receptor requires co-expression of RAMPs 1 or 3 (but not

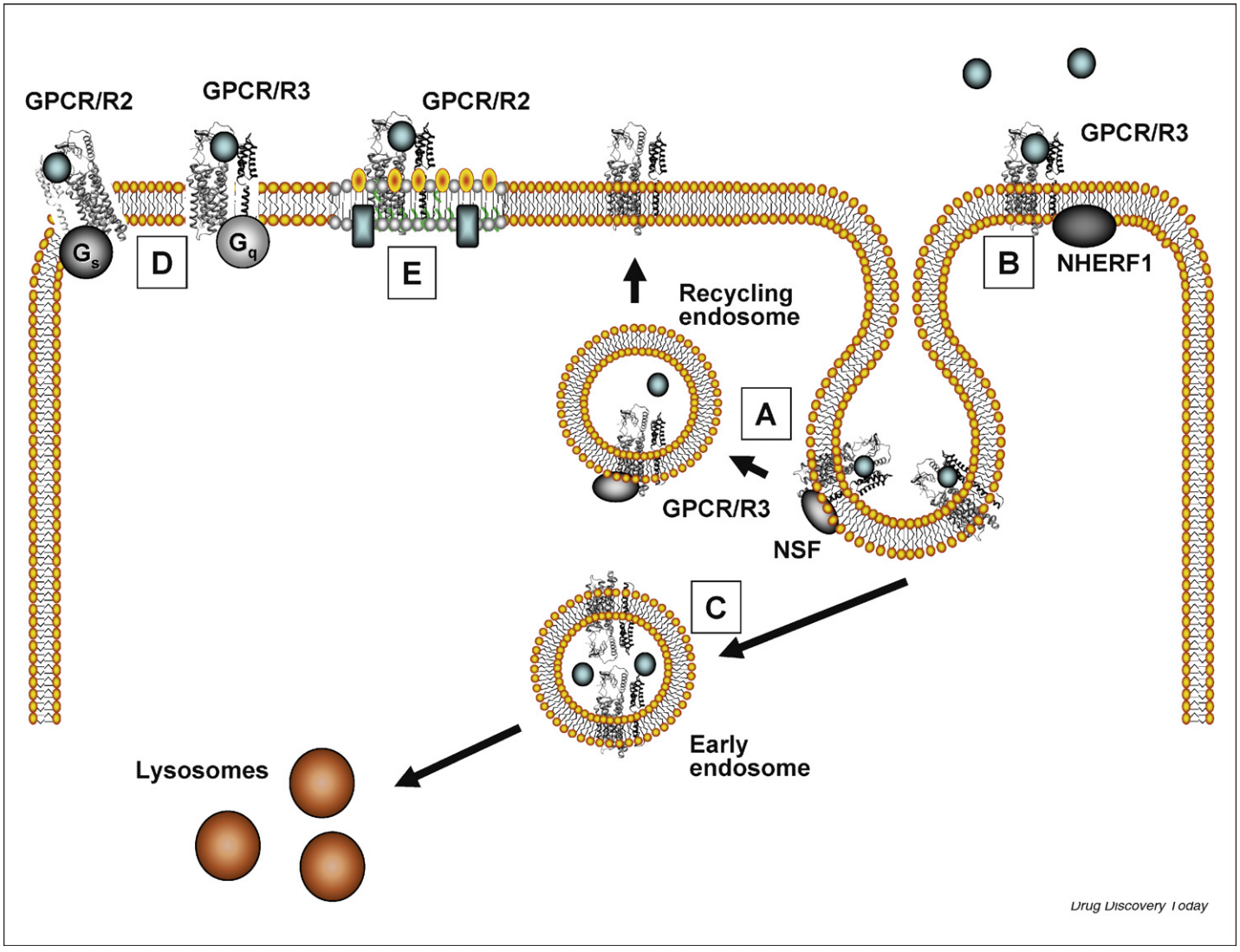

FIGURE 2

The association of RAMPs with GPCRs may lead to alteration in trafficking and signalling properties of the receptors. In the case of CL/RAMP3, the PDZ domain present in RAMP3 allows the association of this complex with accessory proteins that alter the trafficking of the receptor. Where NSF is present **(A)**, the receptor complex is recycled rapidly back to the cell surface following ligand-stimulated internalisation. When NHERF1 is present **(B)** this receptor complex is, however, retained at the cell surface and does not undergo internalisation. In the absence of accessory proteins, or when associated with either RAMP1 or RAMP2, the receptor complexes can be shuttled to lysosomal degradation **(C)** when high concentrations of ligand are present. RAMP association with GPCRs has also been speculated to modify signalling of the receptors, either through a direct effect on G protein coupling **(D)**, as has been suggested for CT/RAMP complexes or by moving RAMP/receptor complexes into or out of signalling microdomains (**E**), as has been speculated for the VPAC1 receptor. These latter properties remain speculative, however, as definitive evidence for these effects is not yet available.

RAMP2) for trafficking to the cell surface [10]. The mechanism of action seems similar to that for the CLR, in that the RAMPs deliver the receptor from the endoplasmic reticulum to the Golgi, thereby promoting glycosylation. The effect of RAMPs on the expression of other receptors is little explored and it would not be surprising if there were significant cell background-dependent differences.

There is now a significant body of evidence that suggests that RAMPs can modulate receptor trafficking during downregulation, where the RAMP/CLR complex is internalised with β-arrestin [28]. The RAMP is in a position to influence the extent and fate of the internalised receptor (Figure 2). It has been reported that RAMP2-containing AM_1 receptors show greater internalisation than RAMP3-containing AM_2 receptors when expressed in HEK293 cells, although the mechanism behind this is unclear [29]. Indeed, although the C-termini of RAMPs contain potential phosphorylation and ubiquitinylation sites, there is no evidence so far of covalent modification during desensitisation and internalisation [30,31]. The C-terminus is, however, important in other respects. The final four amino acids in human RAMP3, DTLL, form a type-1 PDZ1 recognition site. In rat and mouse, the final three amino acids (RLL) form a PDZ-like domain. This has significant consequences for the RAMP3-containing AM_2 receptor. In some HEK293 cells, challenge with AM normally leads to receptor internalisation followed by degradation. In cells expressing N-ethylmaleimide-sensitive factor (NSF), however, the NSF can interact with the PDZ domain and direct the receptor back to the cell surface for recycling (Figure 2). NSF expression has no effect on CGRP or AM_1 receptors, which contain RAMPs lacking the PDZ domain [32].

Likewise, expression of the Na^+/H^+ exchanger regulatory factor-1, which can also bind to the RAMP3 PDZ domain, blocks internalisation of AM_2 receptor but not that of the CGRP or AM_1 receptors [33]. It has also been noted that the C-terminus of RAMP3 contains a dileucine motif, frequently involved in protein–protein recognition [29].

Consequences of changes in RAMP expression/activity

There is now abundant evidence for regulation of RAMP expression, be this in disease, in response to drugs/hormones or physiologically [34,35]. Many of these studies, including those reporting RAMP regulation in heart failure, hypertension and renal failure have been reviewed [11]. More recently, knockout mouse models for each RAMP gene have been developed, allowing new insight into the function of these proteins. For example, RAMP2 and RAMP3 knockout mice have revealed distinct roles for these two proteins, despite them both forming receptors for AM [36]. While deletion of the RAMP2 gene results in a lethal phenotype, probably resulting from abnormalities in the blood and lymphatic vasculature, RAMP3 knockout mice are viable, with no obvious phenotype until old age when they are lighter than their wild-type counterparts [36–38]. Mice in which RAMP1 expression is disrupted have a distinct phenotype, exhibiting hypertension and a dysregulated immune response [39].

From the perspective of drug discovery, there are two pertinent studies for discussion in this review. A mouse model with overexpression of human RAMP1 in the nervous system has established that this protein might be functionally limiting for the neuropeptide CGRP. Thus, mice with ~2-fold increase in RAMP1 mRNA in the brain/trigeminal ganglia were sensitised to CGRP-induced plasma extravasation, a measure of neurogenic inflammation [40]. Similarly, a transgenic mouse model with overexpression of RAMP2, primarily in smooth muscle, was sensitised to the vasodilatory actions of AM [41]. These mice displayed enhancement of AM-induced decreases in blood pressure, indicating increased sensitivity to AM. Both of these examples serve to illustrate how alterations in the expression of RAMP could have profound consequences on responsiveness to endogenous ligands or drugs that act at RAMP-receptor complexes. In turn, this has implications for the onset and progression of disease and also its treatment.

Naturally, these phenotypes have so far been discussed in the context of the best-characterised cognate ligands and receptors, the CT family peptides and receptors. However, the potentially broader roles of RAMPs, as previously discussed, should also be considered when attempting to understand the consequences of modulating the expression of these proteins. An important consideration in interpreting RAMP protein expression data is the poor quality of antibodies that are often used in these studies. While several RAMP antibodies are commercially available, the authors (unpublished observations) and others have noted issues with the specificity of these antibodies [42].

The potential of RAMPs as drugs targets

In principle, RAMPs can be utilised as drug targets either directly themselves, or the complex between the RAMP and its target receptor could be targeted. A drug acting at a RAMP/receptor complex could interact with both the RAMP and the receptor. If the RAMP caused significant structural perturbation of the receptor, yielding a conformation different from that of the receptor expressed alone, then this novel structure by itself might be sufficient for selective drug binding. While most interest is likely to focus on agents targeted to the extracellular portions of the RAMP/receptor complex, drugs that bound to the C-terminus of the RAMP might alter receptor trafficking or signalling profiles (see above). In addition, agents that prevented RAMP-receptor association in the endoplasmic reticulum could prevent the appearance of such complexes at the cell surface. Preventing protein–protein interactions with low molecular weight drugs, as required for the latter two options, is a challenging problem, but the potential should be considered.

Theoretically, interactions between RAMPs and receptors could also be allosterically promoted to enhance cell-surface expression. This could be important in the case of naturally occurring, disease-causing mutations of GPCRs, where defective cell-surface expression contributes to the phenotype. The CaS receptor provides a relevant example [43]. Since the effects of allosteric modulators may depend on the nature of the specific protein–protein interaction that is being modified [44], there is scope for such drugs to be specific for individual RAMP/GPCR complexes.

There may also be scope for targeting RAMPs with RNAi therapeutics or antibodies. As RAMPs are present at the cell surface as part of a receptor complex, however, it may be challenging to find suitable epitopes that could be targeted with antibody-based therapies. This may explain why it has been so difficult to generate antibodies that are suitable as experimental tools (see above).

The ability to translate any such drugs into viable therapeutics, however, may be limited if the principle epitope for binding is the RAMP. Broad disruption of RAMP interactions may have unforeseen consequences owing to the widespread distribution of the proteins and our limited understanding of the breadth of potential RAMP function. Nonetheless, RAMP-targeted drugs, if they can be developed, would provide great utility in understanding RAMP function and could be empirically examined for therapeutic potential.

Although the future of therapeutics that target the RAMP itself is uncertain, there is no doubt that RAMP/receptor complexes can be successfully targeted. This is illustrated by the development of CGRP antagonists, in particular BIBN4096 (Olcegepant) and, most recently, MK0974 (Telcagepant) [6,45–49]. These have been developed for the treatment of migraine headache, the pathology of which involves CGRP. The role of this peptide and the rationale for the use of CGRP antagonists in migraine treatment have recently been reviewed [48,50]; it is the mechanism behind the selectivity of these compounds that is of relevance to this article. BIBN4096 was the first of these to be developed, arising from a high throughput screening campaign and subsequent lead optimisation. BIBN4096 has sub nanomolar affinity for primate CGRP receptors; at rodent receptors the affinity is considerably lower (~250-fold) [46,47]. The exchange of a single amino acid (lysine/tryptophan) between rat and human RAMP1 is sufficient to switch this affinity [17,46]. Interestingly, the properties of the amino acid residue at this position also impart pharmacological specificity; BIBN4096 has virtually no affinity for RAMP2 and is a weak antagonist at RAMP3-based AM receptors (these RAMPs have glutamic acid at this position). Sensitivity to BIBN4096 can,

however, be enhanced at the RAMP3-based AM_2 receptor by the incorporation of the crucial tryptophan residue [5,17,51]. MK0974 appears to have a similar mechanism of interaction with CGRP receptors; this compound also has RAMP1-dependence, resulting in profound species and pharmacological selectivity [45]. Nevertheless, the nature of the receptor is also important to the affinity of RAMP1-dependent CGRP antagonists. Comparison of RAMP1/CLR vs. RAMP1/$CT_{(a)}$ complexes (~55% amino acid homology in the GPCR component) revealed that BIBN4096 was ~300-fold more effective at the CLR-based CGRP receptors [17]. Furthermore, studies with the BIBN4096-like Compounds 1 and 3 using CLR/$CT_{(a)}$ chimeras indicated that the extreme N-terminus of the CLR (residues 37–63) is a crucial determinant of BIBN4096 affinity [52]. Thus, the interface between these proteins probably provides the binding pocket for these antagonists and is responsible for their selectivity (Figure 1). By contrast, compounds that interact predominantly with CLR are non-selective [52].

It is interesting that both BIBN4096BS and MK0974 are relatively large molecules in comparison with most drugs; this may arise because they need to interact with epitopes on both the RAMP and the receptor. Perhaps as a consequence of this, BIBN4096 is an allosteric antagonist with slow kinetics [17,48]. It remains to be established if the large size is a common feature for all agents directed against RAMPs. Of course, these RAMP/GPCR complexes need not only be targeted with small molecules; the native peptides themselves could be formulated as drugs and there is considerable scope for using modified peptides. For example, Symlin (Pramlintide) is a modified form of amylin and is marketed for the treatment of diabetes.

What lessons for drug design can be learnt from the CGRP receptor antagonists? Unfortunately, there is currently a lack of solid information on the structure of RAMP/receptor complexes. For family B GPCRs alone, a consistent pattern has developed for how ligands bind to such receptors where (among other things) the C-terminus of the ligand makes multiple contacts with the extreme N-terminus of the extracellular domain of the receptor [53]. The extreme N-terminus of the CLR (residues 23–60), substituted into the equivalent sequence in the PTH1 receptor, was able to induce an association between the chimeric receptor and RAMP1 [54], suggesting it is crucial for RAMP recognition. If this portion of the CLR is also key to binding CGRP, then it becomes easy to see how RAMP association might modify ligand binding. Of course, this model is less compelling for those GPCRs where RAMP association does not modify pharmacology and cannot be applied to the interaction of RAMPs with the Family C CaS receptor. Thus, there are likely to be multiple interaction sites between GPCRs and RAMPs, depending on the nature of the GPCR. A further complication is the receptor:RAMP stoichiometry. Early cross-linking studies suggested that there was a 1:1 complex between RAMP1/CLR [55]. A recent study using bioluminescence resonance energy transfer elegantly illustrated that the minimum stoichiometry was, however, 2 CLR:1 RAMP1 [56], nonetheless, their data did not preclude 2 monomeric RAMPs binding individually to the CLR dimer. This issue remains unresolved.

A recent publication has shed light on the structure of RAMP1 and possibly a common fold for the family as a whole [57]. The structure revealed three α-helical domains and also confirmed the presence of three disulphide bonds between cysteines 1–5, 2–4 and 3–6. Interestingly, the crystal structure validated a previously predicted disulphide bonding pattern and *ab initio* model for RAMP1 [58], such that the RMSD between the two structures is 2.2 Å for all Cα atoms including loop regions (1.86 Å Cα RSMD for helical regions). The crystal structure has also revealed the location of residues that have been shown to be important for CGRP or AM binding (see [6] for review); however, it is often unclear if these are required directly in formation of the peptide binding sites or whether they act indirectly to promote receptor-RAMP interactions. Further work to elucidate the structure of a RAMP in complex with the N-terminus of a GPCR would be valued as it would be anticipated that such a complex would have numerous crevices that could be exploited for drug targeting.

The precedent for selective targeting of RAMP-receptor complexes has been set with the CGRP receptor antagonists. An interesting question is whether new drugs should be targeted at individual RAMP-receptor complexes or RAMPs alone. The former would clearly have the greatest specificity and the current agents all belong to this category. Agents directed at RAMPs alone potentially would have much broader actions. Whether this would bring advantages or disadvantages is currently an open question as we do not know the significance of most RAMP-receptor interactions.

Conclusion

While there is little solid information available as yet on how drugs can be designed to target RAMP/receptor complexes or RAMPs by themselves, there are grounds for optimism; the CGRP receptor antagonists provide proof of principle. Such agents provide novel therapeutic opportunities. New data indicate that, in addition to receptors, RAMPs may also interact with other proteins such as tubulin [59]. Although, the physiological significance of this observation for RAMP1 is currently unknown, it may provide a mechanism for compartmentalisation of RAMP-receptor complexes or potentially novel receptor-independent functions. Furthermore, the scope for RAMP splice and sequence variants has been little explored. There appears to be considerable scope for exploiting RAMPs as drugs targets, both in themselves and in association with their receptor partners.

References

1 Ellis, C. (2004) The state of GPCR research in 2004. *Nat. Rev. Drug Discov.* 3, 577–626

2 McLatchie, L.M. *et al.* (1998) RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. *Nature* 393, 333–339

3 Christopoulos, A. *et al.* (2003) Novel receptor partners and function of receptor activity-modifying proteins. *J. Biol. Chem.* 278, 3293–3297

4 Fraser, N.J. *et al.* (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

5 Hay, D.L. *et al.* (2003) CL/RAMP2 and CL/RAMP3 produce pharmacologically distinct adrenomedullin receptors: a comparison of effects of adrenomedullin22-52, CGRP8-37 and BIBN4096BS. *Br. J. Pharmacol.* 140, 477–486

6 Hay, D.L. *et al.* (2006) GPCR modulation by RAMPs. *Pharmacol. Ther.* 109, 173–197
7 Poyner, D.R. *et al.* (2002) International Union of Pharmacology. XXXII. The mammalian calcitonin gene-related peptides, adrenomedullin, amylin, and calcitonin receptors. *Pharmacol. Rev.* 54, 233–246
8 Christopoulos, G. *et al.* (1999) Multiple amylin receptors arise from receptor-activity-modifying-proteins interaction with the calcitonin receptor gene product. *In Mol. Pharmacol.* 56, 235–242
9 Muff, R. *et al.* (1999) An amylin receptor is revealed following co-transfection of a calcitonin receptor with receptor activity modifying proteins-1 or -3. *Endocrinology* 140, 2924–2927
10 Bouschet, T. *et al.* (2005) Receptor-activity-modifying proteins are required for forward trafficking of the calcium-sensing receptor to the plasma membrane. *J. Cell Sci.* 118, 4709–4720
11 Udawela, M. *et al.* (2004) The receptor activity modifying protein family of G protein coupled receptor accessory proteins. *Semin. Cell Dev. Biol.* 15, 299–308
12 Sexton, P.M. *et al.* (2001) Receptor activity modifying proteins. *Cell. Signal.* 13, 73–83
13 Takei, Y. *et al.* (2004) Identification of novel adrenomedullin in mammals: a potent cardiovascular and renal regulator. *FEBS Lett.* 556, 53–58
14 Roh, J. *et al.* (2004) Intermedin is a calcitonin/calcitonin gene-related peptide family peptide acting through the calcitonin receptor-like receptor/receptor activity-modifying protein receptor complexes. *J. Biol. Chem.* 279, 7264–7274
15 Hay, D.L. *et al.* (2005) Pharmacological discrimination of calcitonin receptor: receptor activity-modifying protein complexes. *Mol. Pharmacol.* 67, 1655–1665
16 Sexton, P.M. *et al.* (2008) Procalcitonin has bioactivity at calcitonin receptor family complexes: potential mediator implications in sepsis. *Crit. Care Med.* 36, 1637–1640
17 Hay, D.L. *et al.* (2006) Determinants of 1-piperidinecarboxamide, N-[2-[[5-amino-l-[[4-(4-pyridinyl)-l-piperazinyl]carbonyl]pentyl]amino]-1-[(3,5-dibromo-4-hydroxyphenyl)methyl]-2-oxoethyl]-4-(1,4-dihydro-2-oxo-3(2H)-quinazolinyl) (BIBN4096BS) affinity for calcitonin gene-related peptide and amylin receptors—the role of receptor activity modifying protein 1. *Mol. Pharmacol.* 70, 1984–1991
18 Zumpe, E.T. *et al.* (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
19 Fitzsimmons, T.J. *et al.* (2003) The extracellular domain of receptor activity-modifying protein 1 is sufficient for calcitonin receptor-like receptor function. *J. Biol. Chem.* 278, 14313–14320
20 Udawela, M. *et al.* (2006) Distinct receptor activity-modifying protein domains differentially modulate interaction with calcitonin receptors. *Mol. Pharmacol.* 69, 1984–1989
21 Steiner, S. *et al.* (2002) The transmembrane domain of receptor-activity-modifying protein 1 is essential for the functional expression of a calcitonin gene-related peptide receptor. *Biochemistry* 41, 11398–11404
22 Qi, T. *et al.* (2008) Identification of N-terminal receptor activity-modifying protein residues important for CGRP, adrenomedullin and amylin receptor function. *Mol. Pharmacol.* 74, 1059–1071
23 Tilakaratne, N. *et al.* (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
24 Moore, E.E. *et al.* (1995) Functionally different isoforms of the human calcitonin receptor result from alternative splicing of the gene transcript. *Mol. Endocrinol.* 9, 959–968
25 Udawela, M. *et al.* (2006) A critical role for the short intracellular C terminus in receptor activity-modifying protein function. *Mol. Pharmacol.* 70, 1750–1760
26 Morfis, M. *et al.* (2008) Receptor activity modifying proteins differentially modulate the G protein-coupling efficiency of amylin receptors. *Endocrinology* 149, 5423–5431
27 Prado, M.A. *et al.* (2001) The role of the CGRP-receptor component protein (RCP) in adrenomedullin receptor signal transduction. *Peptides* 22, 1773–1781
28 Kuwasako, K. *et al.* (2000) Visualization of the calcitonin receptor-like receptor and its receptor activity-modifying proteins during internalization and Recycling. *J. Biol. Chem.* 275, 29602–29609
29 Kuwasako, K. *et al.* (2006) Functions of the cytoplasmic tails of the human receptor activity-modifying protein components of calcitonin gene-related peptide and adrenomedullin receptors. *J. Biol. Chem.* 281, 7205–7213
30 Cottrell, G.S. *et al.* (2007) Post-endocytic sorting of calcitonin receptor-like receptor and receptor activity-modifying protein 1. *J. Biol. Chem.* 282, 12260–12271
31 Hilairet, S. *et al.* (2001) 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
32 Bomberger, J.M. *et al.* (2005) Novel function for receptor activity-modifying proteins (RAMPs) in post-endocytic receptor trafficking. *J. Biol. Chem.* 280, 9297–9307
33 Bomberger, J.M. *et al.* (2005) Receptor activity-modifying protein (RAMP) isoform-specific regulation of adrenomedullin receptor trafficking by NHERF-1. *J. Biol. Chem.* 280, 23926–23935
34 Phelps, E. *et al.* (2005) Parathyroid hormone induces receptor activity modifying protein-3 (RAMP3) expression primarily via 3′, 5′-cyclic adenosine monophosphate signaling in osteoblasts. *Calcif. Tissue Int.* 77, 96–103
35 Yue, W. *et al.* (2007) Frequent inactivation of RAMP2, EFEMP1 and Dutt1 in lung cancer by promoter hypermethylation. *Clin. Cancer Res.* 13, 4336–4344
36 Dackor, R. *et al.* (2007) Receptor activity-modifying proteins 2 and 3 have distinct physiological functions from embryogenesis to old age. *J. Biol. Chem.* 282, 18094–18099
37 Fritz-Six, K.L. *et al.* (2008) Adrenomedullin signaling is necessary for murine lymphatic vascular development. *J. Clin. Invest.* 118, 40–50
38 Ichikawa-Shindo, Y. *et al.* (2008) The GPCR modulator protein RAMP2 is essential for angiogenesis and vascular integrity. *J. Clin. Invest.* 118, 29–39
39 Tsujikawa, K. *et al.* (2007) Hypertension and dysregulated proinflammatory cytokine production in receptor activity-modifying protein 1-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 104, 16702–16707
40 Zhang, Z. *et al.* (2007) Sensitization of calcitonin gene-related peptide receptors by receptor activity-modifying protein-1 in the trigeminal ganglion. *J. Neurosci.* 27, 2693–2703
41 Tam, C.W. *et al.* (2006) Enhanced vascular responses to adrenomedullin in mice overexpressing receptor-activity-modifying protein 2. *Circ. Res.* 98, 262–270
42 Zhao, Y. *et al.* (2006) Differential expression of components of the cardiomyocyte adrenomedullin/intermedin receptor system following blood pressure reduction in nitric oxide-deficient hypertension. *J. Pharmacol. Exp. Ther.* 316, 1269–1281
43 Pidasheva, S. *et al.* (2005) Impaired cotranslational processing of the calcium-sensing receptor due to signal peptide missense mutations in familial hypocalciuric hypercalcemia. *Hum. Mol. Genet.* 14, 1679–1690
44 Leach, K. *et al.* (2007) Allosteric GPCR modulators: taking advantage of permissive receptor pharmacology. *Trends Pharmacol. Sci.* 28, 382–389
45 Salvatore, C.A. *et al.* (2008) Pharmacological characterization of MK-0974 [N-[(3R,6S)-6-(2,3-Difluorophenyl)-2-oxo-1-(2,2,2-trifluoroethyl)azepan-3-yl]-4-(2-oxo-2,3-dihydro-1H-imidazo[4,5-b]pyridin-1-yl)piperidine-1-carboxamide], a potent and orally active calcitonin gene-related peptide receptor antagonist for the treatment of migraine. *J. Pharmacol. Exp. Ther.* 324, 416–421
46 Mallee, J.J. *et al.* (2002) Receptor activity-modifying protein 1 determines the species selectivity of non-peptide CGRP receptor antagonists. *J. Biol. Chem.* 277, 14294–14298
47 Doods, H. *et al.* (2000) Pharmacological profile of BIBN4096BS, the first selective small molecule CGRP antagonist. *Br. J. Pharmacol.* 129, 420–423
48 Doods, H. *et al.* (2007) CGRP antagonists: unravelling the role of CGRP in migraine. *Trends Pharmacol. Sci.* 28, 580–587
49 Ho, T.W. *et al.* (2008) Efficacy and tolerability of MK-0974 (telcagepant), a new oral antagonist of calcitonin gene-related peptide receptor, compared with zolmitriptan for acute migraine: a randomised, placebo-controlled, parallel-treatment trial. *The Lancet* 372, 2115–2123
50 Benemei, S. *et al.* (2007) Pain pharmacology in migraine: focus on CGRP and CGRP receptors. *Neurol. Sci.* 28, S89–S93
51 Hay, D.L. *et al.* (2002) A comparison of the actions of BIBN4096BS and CGRP8-37 on CGRP and adrenomedullin receptors expressed on SK-N-MC, L6, Col 29 and Rat 2 cells. *Br. J. Pharmacol.* 137, 80
52 Salvatore, C.A. *et al.* (2006) Identification and pharmacological characterization of domains involved in binding of CGRP receptor antagonists to the calcitonin-like receptor. *Biochemistry* 45, 1881–1887
53 Pioszak, A.A. and Xu, H.E. (2008) Molecular recognition of parathyroid hormone by its G protein-coupled receptor. *Proc. Natl. Acad. Sci. U.S.A.* 105, 5034–5039
54 Ittner, L.M. *et al.* (2005) The N-terminal extracellular domain 23–60 of the calcitonin receptor-like receptor in chimeras with the parathyroid hormone receptor mediates association with receptor activity-modifying protein 1. *Biochemistry* 44, 5749–5754
55 Hilairet, S. *et al.* (2001) 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
56 Heroux, M. *et al.* (2007) Functional calcitonin gene-related peptide receptors are formed by the asymmetric assembly of a calcitonin receptor-like receptor homo-oligomer and a monomer of receptor activity-modifying protein-1. *J. Biol. Chem.* 282, 31610–31620
57 Kusano, S. *et al.* (2008) Crystal structure of the human receptor activity-modifying protein 1 extracellular domain. *Protein Sci.* 17, 1907–1914
58 Simms, J. *et al.* (2006) Characterization of the structure of RAMP1 by mutagenesis and molecular modeling. *Biophys. J.* 91, 662–669
59 Kunz, T.H. *et al.* (2007) Interaction of receptor-activity-modifying protein1 with tubulin. *Biochimica et Biophysica Acta (BBA)—General Subjects* 1770, 1145–1150