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Themed Section: Secretin Family (Class B) G Protein-Coupled Receptors – from Molecular to Clinical Perspectives

REVIEW

Targeting a family B GPCR/RAMP receptor complex: CGRP receptor antagonists and migraine

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The clinical effectiveness of antagonizing the calcitonin gene-related peptide (CGRP) receptor for relief of migraine pain has been clearly demonstrated, but the road to the development of these small molecule antagonists has been daunting. The key hurdle that needed to be overcome was the CGRP receptor itself. The vast majority of the current antagonists recognize similar epitopes on the calcitonin receptor-like receptor (CLR) and receptor activity-modifying protein 1 (RAMP1). RAMP1 is a relatively small, single, transmembrane-spanning protein and along with the G-protein-coupled receptor CLR comprise a functional CGRP receptor. The tri-helical extracellular domain of RAMP1 plays a key role in the high affinity binding of CGRP receptor antagonists and drives their species-selective pharmacology. Over the years, a significant amount of mutagenesis data has been generated to identify specific amino acids or regions within CLR and RAMP1 that are critical to antagonist binding and has directed attention to the CLR/RAMP1 extracellular domain (ECD) complex. Recently, the crystal structure of the CGRP receptor ECD has been elucidated and not only reinforces the early mutagenesis data, but provides critical insight into the molecular mechanism of CGRP receptor antagonism. This review will highlight the drug design hurdles that must be overcome to meet the desired potency, selectivity and pharmacokinetic profile while retaining drug-like properties. Although the development of these antagonists has proved challenging, blocking the CGRP receptor may one day represent a new way to manage migraine and offer hope to migraine sufferers.

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Abbreviations

AM, adrenomedullin; AMY, amylin; BMS-694153 (R)-4-(8-Fluoro-2-oxo-1,2-dihydroquinazolin-3(4H)-yl)-N-(3-(7-methyl-1H-indazol-5-yl)-1-oxo-1-(4-(piperidin-1-yl)piperidin-1-yl)propan-2-yl)piperidine-1-carboxamide; CGRP, calcitonin gene-related peptide; CLR, calcitonin receptor-like receptor; Compound 1, 4-Ž2-Oxo-2,3-dihydro-benzoimidazol-1-yl.-piperidine-1-carboxylic acid w1-Ž3,5-dibromo-4-hydroxy-benzyl.-2-oxo-2-Ž4-phenyl-piperazin-1-yl.-ethylx-amide; Compound 2, 1-(2,5-Dioxo-3′,4′-dihydro-1′H-spiro[imidazolidine-4,2′-naphthalen]-6′-yl)-3-[(3R)-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]urea; Compound 4, 4-Bromo-N-(3,5-difluorobenzyl)-6-(1,1-dioxido-1,2-thiazinan-2-yl)-3-hydroxypyridine-2-carboxamide; CT, calcitonin; GPCR, G-protein-coupled receptor; MK-3207, 2-[(8R)-8-(3,5-difluorophenyl)-10-oxo-6,9-diazaspiro[4.5]dec-9-yl]-N-[(2R)-2′-oxo-1,1′,2′,3-tetrahydrospiro[indene-2,3′-pyrrolo[2,3-b]pyridin]-5-yl]acetamide; olcegepant, 1-Piperidinecarboxamide, N-[2-[[5-Amino-/-[[4-(4-pyridinyl)-/-piperazinyl]carbonyl]pentyl]amino]-1-[3,5-dibromo-4-hydroxyphenyl)methyl]-2-oxoethyl]-4-(1,4-dihydro-2-oxo-3(2H)-quinazolinyl); PET, positron emission tomography; RAMP, receptor activity-modifying protein; SB-273779, [N-methyl-N-(2-methylphenyl)-3-nitro-4-(2-thiazolylsulfinyl)nitrobenzanilide]; telcagepant, [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]

Introduction

Calcitonin gene-related peptide (CGRP) is a 37-amino acid neuropeptide that has long been postulated to play a role in the pathogenesis of migraine headache. This peptide exerts its biological action through activation of the CGRP receptor, which is a member of the family B (secretin-like) G-protein-coupled receptors (GPCRs). Receptors in this family are characterized by having large N-terminal extracellular regions (between 100 and 160 residues) and are activated by endogenous peptide ligands. There are several attractive drug targets within this family of receptors; however, discovering potent small molecule ligands has proven extremely difficult given the nature of the natural agonists. As a consequence, most compounds in development that target this family tend to be peptidic in nature. Despite these challenges, small molecule antagonists targeting the CGRP receptor have been developed for the treatment of migraine and have shown proof of efficacy in the clinic (Olesen *et al.*, 2004; Ho *et al.*, 2008b).

CGRP and migraine

One of the most well-defined effects of CGRP on biological tissues is in the vasculature where it acts as a potent vasodilator (Brain *et al.*, 1986). The possibility that migraine is a neurovascular disorder was first suggested over 60 years ago by Ray and Wolff who showed that extracranial referred pain resulted from stimulation of meningeal arteries (Ray and Wolff, 1940). CGRP was first postulated to play a role in migraine by Edvinsson in the mid-1980s (Edvinsson, 1985) and since that time, there have been several lines of evidence that implicate CGRP in migraine pathogenesis. Peripheral nociceptors in the vascular smooth muscle are activated by distension of meningeal vessels and carry information to the trigeminal ganglion (Goadsby *et al.*, 2002), and these cerebral vessels are densely innervated by CGRP-expressing nerve endings from the fifth cranial nerve (Uddman *et al.*, 1985). The immunohistological colocalization of the CGRP receptor in cerebral vessels has also been shown in human meningeal blood vessels (Oliver *et al.*, 2002). CGRP has been shown to have vasoactivity in the cerebral vasculature (Jansen *et al.*, 1992) and activation of the trigeminal ganglion leads to an increase in CGRP and cerebral blood flow in humans and cats (Goadsby *et al.*, 1988). A pivotal study by Goadsby found that levels of CGRP are elevated in cranial circulation during a migraine headache (Goadsby *et al.*, 1990). Furthermore, CGRP levels were normalized upon treatment with triptans and this normalization occurred along the same time course as pain relief (Goadsby and Edvinsson, 1993). It has also been shown that intravenous administration of CGRP can induce a migraine-like headache in migraineurs but not in non-migraineurs (Lassen *et al.*, 2002). It should be noted that another study found no increase in cranial CGRP levels during migraine (Tvedskov *et al.*, 2005). This study differed from the earlier Goadsby study as it compared CGRP levels in migraineurs during and between attacks while the Goadsby study compared CGRP levels during a migraine to those in non-migraine controls (Goadsby *et al.*, 1990; Tvedskov *et al.*, 2005). One possible explanation for this discrepancy is that migraineurs have higher CGRP levels even between attacks than non-migraineurs (Fusayasu *et al.*, 2007).

The vascular theory of migraine described earlier was supported by early evidence; however, more recent studies have strongly suggested that migraine is more than just a vascular event. Positron emission tomography (PET) studies in migraine patients with aura have shown that increased cerebral blood flow does not necessarily correlate with headache pain (Olesen, 1990, 1991). Furthermore, infusion of known vasodilators such as vasoactive intestinal peptide causes vasodilation of cranial arteries but does not cause headache in migraineurs (Rahmann *et al.*, 2008). There is evidence suggesting that CGRP could also be involved in migraine pathogenesis through actions in the central nervous system (CNS). The highest levels of CGRP binding in the human brain appear to be in the cerebellum and brainstem (Inagaki *et al.*, 1986) and PET has shown these regions are activated during spontaneous migraine attacks (Weiller *et al.*, 1995; Afridi *et al.*, 2005). In the brainstem, CGRP-immunoreactive fibers are found in regions important for pain transmission such as the spinal trigeminal nucleus, locus coeruleus and the parabrachial nucleus (van Rossum *et al.*, 1997). The cerebellum and brainstem are important for modulation of sensory information from the periphery and it is possible that elevated CGRP levels in these areas could result in defective filtering of information leading to the migraine symptoms of pain, phonophobia and photophobia (Ho *et al.*, 2010).

While the underlying mechanisms of migraine pathogenesis are complex and have yet to be fully elucidated, the early evidence implicating CGRP in this disease was sufficient for pharmaceutical companies to initiate CGRP receptor antagonist programmes. Several small molecule CGRP receptor antagonists have now shown clinical efficacy in humans so the importance of CGRP in migraine has been clearly established (Olesen *et al.*, 2004; Ho *et al.*, 2008a; Diener *et al.*, 2011; Hewitt *et al.*, 2011).

CGRP and its receptor

CGRP belongs to a family of peptides that also includes calcitonin (CT), adrenomedullin (AM) and amylin (AMY), and it is widely expressed in the peripheral and CNS. Two forms of this peptide are present in humans, α-CGRP, which is produced by alternative splicing of the CT gene (Amara *et al.*, 1982), and β-CGRP, which has a separate genetic origin (Alevizaki *et al.*, 1986). These peptides differ by three amino acids and display different expression patterns with α-CGRP highly expressed in primary sensory neurons and β-CGRP in the enteric nervous system (Mulderry *et al.*, 1988; Brain and Grant, 2004).

The CGRP receptor is a multimeric complex consisting of the seven-transmembrane GPCR-designated CT receptor-like receptor (CLR) as well as a single transmembrane protein-designated receptor activity-modifying protein (RAMP) 1 (McLatchie *et al.*, 1998). The discovery of RAMP1 was a result of several attempts at cloning the CGRP receptor. This receptor was thought to be a GPCR because stimulating various tissues and cells with CGRP resulted in cyclic adenosine monophosphate (cAMP) accumulation suggesting a link to

the Gs pathway. However, early attempts to clone the receptor were unsuccessful because it was not known that there was a necessary accessory protein. Eventually, Aiyar and colleagues showed that the orphan receptor CLR was activated by CGRP when transfected into human embryonic kidney (HEK)-293 cells (Aiyar *et al.*, 1996). Earlier attempts to transfect this same receptor into CV-1 in origin, SV40 cells did not show this response to CGRP (Njuki *et al.*, 1993), which led to speculation that there was a cofactor present in HEK-293 cells that resulted in high affinity CGRP binding to this receptor. McLatchie and colleagues published a landmark paper in 1998, which first described the novel family of three single-transmembrane proteins they termed RAMP1, RAMP2 and RAMP3. They first identified RAMP1 by utilizing an expression-cloning strategy using a cDNA library from the neuroblastoma cell line SK-N-MC that was known to respond to CGRP. A single cDNA encoding a 148-amino acid protein was found to elicit a strong CGRP response when expressed in Xenopus oocytes (McLatchie *et al.*, 1998). They showed that co-transfection of CLR with RAMP1 in HEK-293T cells allowed binding of radiolabelled CGRP while expression of either component alone did not. Neither RAMP1 nor CLR were able to be expressed on the cell surface alone suggesting that RAMP1 serves as a chaperone allowing CLR to reach the surface (McLatchie *et al.*, 1998).

RAMPs and receptor pharmacology

The RAMPs share a common structure with a single transmembrane alpha-helix, a large extracellular N-terminus and a short intracellular C-terminus. All three RAMPs have an N-terminal signal sequence that ranges from 26 or 27 residues for RAMP1 and RAMP3 respectively to 42 amino acids in RAMP2. Next, there is an external N-terminal region of roughly 90 residues for RAMP1 and RAMP3 and approximately 103 residues for RAMP2. Finally, all three RAMPs have a transmembrane domain of 22 amino acids and an intracellular C-terminus of nine residues (Hay *et al.*, 2006b).

CLR is able to partner with each of these proteins and this interaction with a specific RAMP is what confers ligand specificity. CLR with RAMP1 forms a CGRP receptor while CLR with either RAMP2 or RAMP3 forms a high-affinity AM receptor (AM_1 and AM_2 respectively) (McLatchie *et al.*, 1998). RAMPs can also form heteromers with other family B GPCRs such as the CT receptor (CTR), vasoactive intestinal polypeptide/pituitary adenylate cyclase-activating peptide receptor, and the parathyroid hormone receptor (Christopoulos *et al.*, 2003). The most well-characterized of these other interactions is with CTR. Expression of CTR alone forms a receptor for CT while CTR partnered with RAMP1, RAMP2 or RAMP3 forms a receptor for AMY (AMY_1, AMY_2, AMY_3 respectively) (Christopoulos *et al.*, 1999; Poyner *et al.*, 2002). The discovery of these receptors followed a similar course as the CGRP receptor. AMY binding was found in tissues where CTR was present; however, transfection of CTR into cell lines did not always result in a high-affinity AMY receptor (Perry *et al.*, 1997). The discovery of the RAMPs' association with CLR showed that it was likely endogenous RAMP expression in different cell lines that resulted in the variation in AMY affinity described in these experiments.

Table 1

Composition and pharmacology of the calcitonin family of receptors (adapted from Hay *et al.*, 2006b)

Receptor	Composition	Rank order of agonist potency
CGRP	CLR + RAMP1	αCGRP > AM ≥ AMY ≥ CT
AM_1	CLR + RAMP2	AM > αCGRP > AMY > CT
AM_2	CLR + RAMP3	AM > αCGRP > AMY > CT
CTR	CTR alone	CT > AMY, αCGRP > AM
AMY_1	CTR + RAMP1	AMY ≥ αCGRP > CT > AM
AMY_2	CTR + RAMP2	Poorly defined
AMY_3	CTR + RAMP3	AMY > αCGRP > AM

Nomenclature conforms to Alexander *et al.* (2011).
CGRP, calcitonin gene-related peptide; AM, adrenomedullin; CT, calcitonin; CTR, CT receptor; AMY, amylin; RAMP, receptor activity-modifying protein.

Table 1 summarizes the molecular composition and pharmacology of the CT family of receptors, which includes CTR, as well as the CGRP, AMY and AM receptors. The intracellular loops of these receptors interact with G-proteins that are predominantly coupled to the Gs pathway in which the second messenger cAMP is produced upon receptor activation. CGRP and AM receptors also partner with an intracellular protein designated receptor component protein, which is required for signal transduction (Evans *et al.*, 2000).

The RAMPs are widely distributed in the body with at least one RAMP being present in nearly every tissue tested (Sexton *et al.*, 2001). RAMP1 is expressed in the heart, uterus, brain, bladder, pancreas, skeletal muscle and gastrointestinal system. High expression of RAMP2 has been shown in the lung, heart, placenta, skeletal muscle and pancreas while RAMP3 is expressed widely in human (Chakravarty *et al.*, 2000; Nagae *et al.*, 2000; Hay *et al.*, 2006b). All three RAMPs have also been shown to be highly expressed in fat tissue but their function is unknown (Nagae *et al.*, 2000; Hay *et al.*, 2006b).

Coexpression of RAMP is necessary for CLR to reach the cell surface. Once on the surface, RAMPs could modulate pharmacology of the receptor either by allosteric modulation of the ligand-binding site on CLR or by defining the ligand-binding pocket by cell surface RAMP–CLR interaction. Radiolabelled CGRP cross-links to proteins equivalent in size to RAMP1 alone, CLR alone and RAMP1/CLR combined suggesting that the ligand makes contact to both components of the CGRP receptor (McLatchie *et al.*, 1998; Aldecoa *et al.*, 2000; Hilairet *et al.*, 2001). Also, the association of CLR with each of the RAMPs is maintained during agonist-induced receptor internalization with CLR/RAMP1 and CLR/RAMP2 being primarily targeted for degradation while CLR/RAMP3 can either be recycled or degraded depending on the cellular context (Kuwasako *et al.*, 2000; Bomberger *et al.*, 2005). Site-directed mutagenesis has also shown that the tryptophan residue at position 84 on RAMP1 is important for agonist potency on both the CGRP and AMY_1 receptor (Gingell *et al.*, 2010; Moore *et al.*, 2010). Finally, ter Haar and colleagues have published the crystal structure of the extracellular portion of

CLR and RAMP1 showing multiple points of contact between the two proteins and a hydrophobic-binding pocket formed at the interface (ter Haar *et al.*, 2010). This structure will be discussed in greater detail later.

Discovery of CGRP receptor antagonists

The first tool to probe CGRP receptor antagonism was the truncated peptide $CGRP_{8-37}$ (Chiba *et al.*, 1989). While this peptide is useful for exploring the pharmacology of the CT family of receptors, it is not highly selective (Poyner *et al.*, 2002) and is unlikely to have suitable physical properties to be a therapeutic for acute migraine treatment. Several pharmaceutical companies have now set out to find small molecule antagonists of the CGRP receptor that would have all the attributes required for a migraine drug. Family B GPCRs have proven to be difficult targets to develop small molecule antagonists against due to the fact that the endogenous ligand is a peptide. An orthosteric antagonist would have to prevent the binding of a much larger molecule that likely interacts with multiple regions of the receptor. Despite the considerable challenges, several pharmaceutical companies have managed to generate potent and selective small molecule antagonists of the CGRP receptor.

The first reported non-peptide antagonist was SB-273779 (Figure 1), developed by SmithKline Beecham (now GlaxoSmithKline), which displayed moderate affinity for the human CGRP receptor with a K_i value of 310 nM (Aiyar *et al.*, 2001). The first potent CGRP receptor antagonist was olcegepant (BIBN4096BS) (Figure 1), which was developed by Boehringer Ingelheim (Doods *et al.*, 2000). Although this compound is extremely potent and selective for the CGRP receptor, it is a relatively large molecule (molecular weight = 870) with low bioavailability, which required it be administered intravenously in the clinic (Olesen *et al.*, 2004; Rudolf *et al.*, 2005). Nevertheless, this compound was tested in humans for the acute treatment of migraine and it showed proof of concept for this mechanism that was a breakthrough for the field (Olesen *et al.*, 2004). Unfortunately, an intravenous drug is not practical for the acute treatment of migraine as this would necessitate a trip to a clinic after a migraine has begun. Migraine is primarily managed on an outpatient basis; therefore, in order to be practical and commercially viable, the drug should be orally bioavailable allowing the patient to take the drug when symptoms of an attack first begin.

Merck & Co. set out to find an orally bioavailable drug by conducting a high-throughput screen, which led to the identification of the benzodiazepine lead compound referred to as compound 2 (Figure 1) (Williams *et al.*, 2006). This compound displayed modest affinity for the human CGRP receptor (K_i = 4.8 μM), but optimization led to the potent molecule telcagepant, which has a K_i of 0.8 nM (Figure 1) (Paone *et al.*, 2007; Salvatore *et al.*, 2008). Telcagepant was the first orally bioavailable CGRP receptor antagonist to be tested in the clinic and it displayed triptan-like efficacy for the acute treatment of migraine (Ho *et al.*, 2008b). Merck later developed another more potent CGRP receptor antagonist designated MK-3207 (Figure 1), which also showed efficacy for acute migraine in a Phase IIb study (Bell *et al.*, 2010; Hewitt *et al.*, 2011).

Bristol–Myers Squibb generated BMS-694153 (Figure 1) which has high affinity for the human (K_i = 13 pM) CGRP receptor. BMS-694153 did not exhibit significant oral bioavailability in monkey or rat and no clinical data are published for this compound (Degnan *et al.*, 2008).

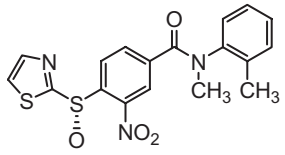
Species selectivity of CGRP receptor antagonists – the role of RAMP1

The species selectivity of small molecule CGRP receptor antagonists is well documented and is summarized in Table 2. Olcegepant demonstrates high affinity for the human and non-human primate CGRP receptors, but exhibited much lower affinity for the receptors from rats, rabbits, dogs and guinea pigs (Doods *et al.*, 2000). A related compound, compound 1 (Figure 1) was likewise shown to be a significantly more potent antagonist on human cerebral arteries than on guinea pig cerebral and porcine coronary arteries (Edvinsson *et al.*, 2001; Hasbak *et al.*, 2001). Therefore, it is not surprising that both small molecule antagonists from Merck & Co. that reached the clinic, telcagepant and MK-3207, also displayed marked species selectivity. Telcagepant displayed a similar affinity for the rhesus receptor, but displayed >1000-fold lower affinity for the rat and dog receptors (Salvatore *et al.*, 2008). MK-3207 is structurally distinct from telcagepant but nonetheless displays approximately 400-fold higher affinity for the human and rhesus CGRP receptors compared with the rat and dog receptors (Salvatore *et al.*, 2010). Bristol–Myers Squibb generated BMS-694153 (Figure 1), which has high affinity for the human and marmoset CGRP receptor, but was inactive on the rat receptor up to 1 μM (Degnan *et al.*, 2008).

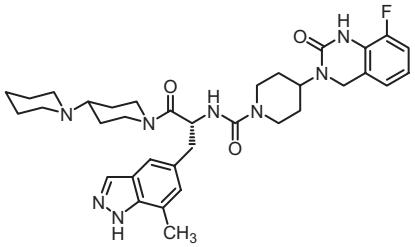
Mallee and colleagues set out to determine which regions of the CGRP receptor are responsible for this species selectivity by expressing mixed species receptor complexes using either human or rat CLR and RAMP1 (Mallee *et al.*, 2002). They found that olcegepant showed similar potency on a receptor consisting of rat CLR and human RAMP1 as the fully human receptor while its potency on human CLR with rat RAMP1 was similar to the rat receptor (Mallee *et al.*, 2002). This finding suggested that this compound's species selectivity is primarily driven by RAMP1 rather than CLR. By using chimeric rat/human RAMP1, they were able to narrow down the region of RAMP1 responsible for species selectivity to residues 66–112. Finally, they showed that changing residue 74 of rat RAMP1 from lysine to tryptophan conferred human-like pharmacology to olcegepant (Mallee *et al.*, 2002). Interestingly, Salvatore *et al.* later identified a structurally distinct compound they designated compound 4 (Figure 1) that displayed similar affinity for the human and rat receptors suggesting this compound binds to a different region of the receptor (Salvatore *et al.*, 2006).

Selectivity of CGRP receptor antagonists

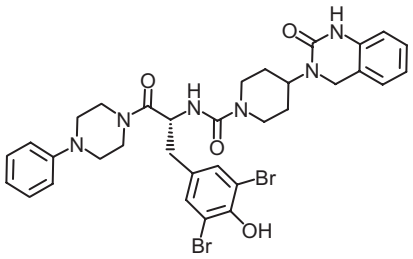
Discovering selective antagonists of the CGRP receptor is difficult because of its heteromeric nature. As described earlier,



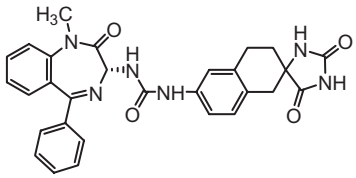
SB-273779



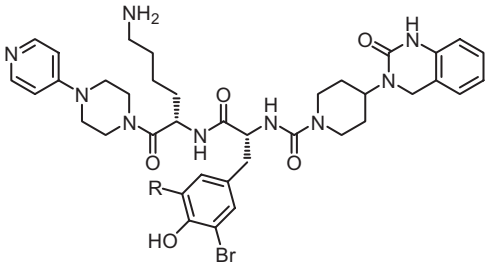
BMS-694153



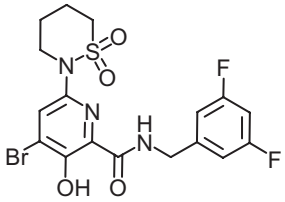
Compound 1



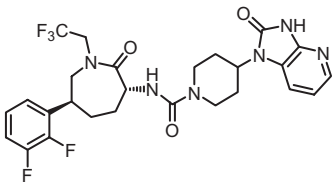
Compound 2



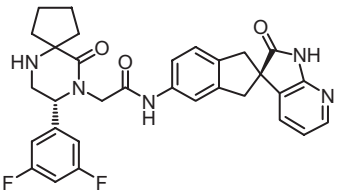
olcegepant (R=Br)
Compound 3 (R=I)



Compound 4



telcagepant



MK-3207

Figure 1

Chemical structures of select CGRP receptor antagonists. SB-273779, [N-methyl-N-(2-methylphenyl)-3-nitro-4-(2-thiazolylsulfinyl)nitrobenzanilide]; BMS-694153 (R)-4-(8-Fluoro-2-oxo-1,2-dihydroquinazolin-3(4H)-yl)-N-(3-(7-methyl-1H-indazol-5-yl)-1-oxo-1-(4-(piperidin-1-yl)piperidin-1-yl)propan-2-yl)piperidine-1-carboxamide; Compound 1, 4-Ž2-Oxo-2,3-dihydro-benzoimidazol-1-yl.-piperidine-1-carboxylic acid w1-Ž3,5-dibromo-4-hydroxy-benzyl.-2-oxo-2-Ž4-phenyl-piperazin-1-yl.-ethylx-amide; Compound 2, 1-(2,5-Dioxo-3′,4′-dihydro-1′H-spiro[imidazolidine-4,2′-naphthalen]-6′-yl)-3-[(3R)-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]urea; olcegepant, 1-Piperidinecarboxamide, N-[2-[[5-Amino-/-[[4-(4-pyridinyl)-/-piperazinyl]carbonyl]pentyl]amino]-1-[3,5-dibromo-4-hydroxyphenyl)methyl]-2-oxoethyl]-4-(1,4-dihydro-2-oxo-3(2H)-quinazolinyl); Compound 4, 4-Bromo-N-(3,5-difluorobenzyl)-6-(1,1-dioxido-1,2-thiazinan-2-yl)-3-hydroxypyridine-2-carboxamide; telcagepant, [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]; MK-3207, 2-[(8R)-8-(3,5-difluorophenyl)-10-oxo-6,9-diazaspiro[4.5]dec-9-yl]-N-[(2R)-2′-oxo-1,1′,2′,3-tetrahydrospiro[indene-2,3′-pyrrolo[2,3-b]pyridin]-5-yl]acetamide.

Table 2

Pharmacology of small-molecule CGRP receptor antagonists

Antagonist	Receptor	Species	Assay	Cell line/tissue	Affinity (nM)	Reference
Olcegepant	CGRP	Human	cAMP	SK-N-MC	0.01 (K_B)*	Doods *et al.* (2000)
			Binding	SK-N-MC	0.01 (K_i)	Doods *et al.* (2000)
			cAMP	COS-7 (transfected)	0.19 (K_B)*	Hay *et al.* (2006a)
			cAMP	COS-7 (transfected)	0.02 (A_2)*	Miller *et al.* (2010)
			Binding	SK-N-MC	0.05 (K_D)	Schindler and Doods (2002)
			cAMP	SK-N-MC	0.11 (A_2)*	Hay *et al.* (2002)
		Marmoset	Binding	Cortex	0.06 (IC_{50})*	Schindler and Doods (2002)
			cAMP	Cortex	0.08 (K_D)	Schindler and Doods (2002)
		Rat	Binding	spleen	3.4 (K_i)	Doods *et al.* (2000)
		Rat	cAMP	L6 cells	0.6 (A_2)*	Hay *et al.* (2002)
	AM_1	Human	cAMP	COS-7 (transfected)	>10 000 (K_B)*	Hay *et al.* (2003)
		Rat	cAMP	Rat 2 cells	>10 000 (A_2)*	Hay *et al.* (2002)
	AM_2	Human	cAMP	COS-7 (transfected)	407 (K_B)*	Hay *et al.* (2006a)
	CTR	Human	cAMP	COS-7 (transfected)	>10 000 (K_B)*	Hay *et al.* (2006a)
	AMY_1	Human	cAMP	COS-7 (transfected)	36 (K_B)*	Hay *et al.* (2006a)
	AMY_3	Human	cAMP	COS-7 (transfected)	≤10 000 (K_B)*	Hay *et al.* (2006a)
Telcagepant	CGRP	Human	cAMP	HEK-293 (stable line)	2.2 (IC_{50})	Salvatore *et al.* (2008)
			Binding	HEK-293 (stable line)	0.8 (K_i)	Salvatore *et al.* (2008)
			Binding	SK-N-MC	0.8 (K_i)	Salvatore *et al.* (2008)
			cAMP	COS-7 (transfected)	0.2 (A_2)*	Miller *et al.* (2010)
			cAMP	HEK-293 (stable line)	0.5 (IC_{50})	Moore *et al.* (2010)
		Rhesus	Binding	Cerebellum	1.2 (K_i)	Salvatore *et al.* (2008)
		Rat	Binding	Brain	1192 (K_i)	Salvatore *et al.* (2008)
		Dog	Binding	Brain	1204 (K_i)	Salvatore *et al.* (2008)
	AM_1	Human	Binding	HEK-293 (transfected)	>100 000 (K_i)	Salvatore *et al.* (2008)
	AM_2	Human	Binding	HEK-293 (transfected)	29 000 (K_i)	Salvatore *et al.* (2008)
	CTR	Human	Binding	HEK-293 (transfected)	>100 000 (K_i)	Salvatore (2009)
	AMY_1	Human	Binding	HEK-293 (transfected)	190 (K_i)	Salvatore (2009)
	AMY_3	Human	Binding	HEK-293 (transfected)	>100 000 (K_i)	Salvatore (2009)
MK-3207	CGRP	Human	cAMP	HEK-293 (stable line)	0.12 (IC_{50})	Salvatore *et al.* (2010)
			Binding	HEK-293 (stable line)	0.02 (K_i)	Salvatore *et al.* (2010)
			Binding	SK-N-MC	0.02 (K_i)	Salvatore *et al.* (2010)
		Rhesus	Binding	Cerebellum	0.02 (K_i)	Salvatore *et al.* (2010)
		Rat	Binding	Brain	10 (K_i)	Salvatore *et al.* (2010)
		Dog	Binding	Brain	10 (K_i)	Salvatore *et al.* (2010)
	AM_1	Human	Binding	HEK-293 (transfected)	16 500 (K_i)	Salvatore *et al.* (2010)
	AM_2	Human	Binding	HEK-293 (transfected)	156 (K_i)	Salvatore *et al.* (2010)
	CTR	Human	Binding	HEK-293 (transfected)	1900 (K_i)	Salvatore *et al.* (2010)
	AMY_1	Human	Binding	COS-7 (transfected)	0.8 (K_i)	Salvatore *et al.* (2010)
	AMY_3	Human	Binding	COS-7 (transfected)	128 (K_i)	Salvatore *et al.* (2010)
BMS-694153	CGRP	Human	Binding	SK-N-MC	0.01 (K_i)	Degnan *et al.* (2008)
		Marmoset	Binding	Brain	0.08(K_i)	Degnan *et al.* (2008)
		Rat	Binding	Brain	>1 000 (K_i)	Degnan *et al.* (2008)
	AM_1	Human	Binding	CHO (transfected)	>5 000 (K_i)	Degnan *et al.* (2008)
	AM_2	Human	Binding	CHO (transfected)	>5 000 (K_i)	Degnan *et al.* (2008)
	CTR	Human	Binding	T47D	>5 000 (K_i)	Degnan *et al.* (2008)
	AMY_1	Human	Binding	CHO (transfected)	>500 (K_i)	Degnan *et al.* (2008)
	AMY_3	Human	Binding	CHO (transfected)	>5 000 (K_i)	Degnan *et al.* (2008)
SB-273779	CGRP	Human	cAMP	COS-7 (transfected)	79 (A_2)*	Miller *et al.* (2010)
			Binding	SK-N-MC	310 (IC_{50})	Aiyar *et al.* (2001)
			cAMP	SK-N-MC	390 (IC_{50})	Aiyar *et al.* (2001)
	AM_1	Human	Binding	HEK-293 (transfected)	1 500 (K_i)	Aiyar *et al.* (2001)
	CTR	Human	Binding	T47D	>3 000 (K_i)	Aiyar *et al.* (2001)
Compound 4	CGRP	Human	Binding	HEK-293 (stable line)	3 500 (K_i)	Salvatore *et al.* (2006)
		Rat	Binding	Brain	6 800 (K_i)	Salvatore *et al.* (2006)
	AM_1	Human	Binding	HEK-293 (stable line)	7 500 (K_i)	Salvatore *et al.* (2006)

*These values were originally reported in log form as pK_B, pIC_{50} or pA_2. Here they have been converted into nM for consistency and ease of comparison.

CGRP, calcitonin gene-related peptide; AM, adrenomedullin; CT, calcitonin; CTR, CT receptor; AMY, amylin; cAMP, cyclic adenosine monophosphate; RAMP, receptor activity-modifying protein.

the CLR component of the CGRP receptor is also a component of the AM receptors while the RAMP1 component is part of the AMY_1 receptor complex. This makes finding a selective molecule more difficult because a compound that binds exclusively to CLR will also likely antagonize AM_1 and AM_2, while a compound that binds to RAMP1 will also antagonize the AMY_1 receptor. The ideal antagonist from a selectivity perspective would be one that makes contact with both components of the CGRP receptor and therefore displays acceptable selectivity against the related receptors. Several of the known CGRP receptor antagonists exhibit this feature.

Table 2 shows the affinities of selected CGRP receptor antagonists for the related CT, AM and AMY receptors. From the species selectivity data described earlier, we know that RAMP1 is important for binding of olcegepant, telcagepant, MK-3207, and presumably BMS-694153. Therefore, it is not surprising that these compounds appear less selective against AMY_1 than the other related receptors (Hay *et al.*, 2006a; Degnan *et al.*, 2008; Salvatore *et al.*, 2008; Salvatore, 2009; Salvatore *et al.*, 2010). All of these compounds show good selectivity against the AM and CT receptors as well as AMY_3 providing further evidence of the crucial role of RAMP1 in binding many CGRP receptor antagonists (Hay *et al.*, 2006a; Degnan *et al.*, 2008; Salvatore *et al.*, 2008; Salvatore, 2009; Salvatore *et al.*, 2010). Compound 4 is again the outlier here as it displays similar affinity for the CLR/RAMP1 containing CGRP receptor and the CLR/RAMP2 containing AM_1 receptor (Salvatore *et al.*, 2006). This again suggests RAMP independent binding and the binding site for compound 4 is discussed further later.

Molecular determinants of antagonist binding

The discovery of the importance of Trp74 of human RAMP1 demonstrated that RAMP1 is critical for antagonist binding. Mutagenesis of the tryptophan at residue 74 of human RAMP1 significantly reduced the potency of telcagepant and either olcegepant or a close analogue designated compound 3 (Table 3) (Hay *et al.*, 2006a; Miller *et al.*, 2010; Moore *et al.*, 2010), consistent with the initial finding that residue 74 is critical for high affinity binding (Mallee *et al.*, 2002). Trp74 of human RAMP1 has also been identified to play a key role in the high affinity binding of MK-3207 (Table 3) (Salvatore *et al.*, 2010). SB-273779 was unaffected by mutation at position 74 suggesting it binds to a different region of the receptor (Table 3) (Miller *et al.*, 2010). A crystal structure of the extracellular region of RAMP1 published by Kusano *et al.* provided additional residues (Arg67, Asp71, Glu78 and Trp84) that could potentially interact with small molecule CGRP receptor antagonists (Kusano *et al.*, 2008). Alanine replacement at Arg67 had a modest effect on the potency of telcagepant but not olcegepant, while replacement at Asp71 and Glu78 had no effect (Moore *et al.*, 2010). Additionally, it has been shown that

Table 3

Potency of CGRP receptor antagonists on the wild type CGRP receptor and mutants of CLR and RAMP1

Antagonist	Mutation	Potency (nM)	Reference
Olcegepant	Wild type	0.02 (A_2)*	Miller *et al.* (2010)
	RAMP1 Trp74Lys	7.4 (A_2)*	Miller *et al.* (2010)
	CLR Met42Ala	1.1 (A_2)*	Miller *et al.* (2010)
	Wild type	0.07 (K_B)*	Hay *et al.* (2006a)
	RAMP1 Trp74Lys	3.9 (K_B)*	Hay *et al.* (2006a)
	RAMP1 Trp74Ala	2.6 (K_B)*	Hay *et al.* (2006a)
Telcagepant	Wild type	0.5 (IC_{50})*	Moore *et al.* (2010)
	RAMP1 Trp74Ala	52 (IC_{50})*	Moore *et al.* (2010)
	RAMP1 Trp84Ala	9.1(IC_{50})*	Moore *et al.* (2010)
	Wild type	0.2 (A_2)*	Miller *et al.* (2010)
	RAMP1 Trp74Lys	51 (A_2)*	Miller *et al.* (2010)
	CLR Met42Ala	162 (A_2)*	Miller *et al.* (2010)
MK-3207	Wild type	0.1 (IC_{50})	Salvatore *et al.* (2010)
	RAMP1 Trp74Ala	2.2 (IC_{50})	Salvatore *et al.* (2010)
SB-273779	Wild type	79 (A_2)*	Miller *et al.* (2010)
	RAMP1 Trp74Lys	78 (A_2)*	Miller *et al.* (2010)
	CLR Met42Ala	83 (A_2)*	Miller *et al.* (2010)

*These values were originally reported in log form as pK_B, pIC_{50} or pA_2. Here they have been converted into nM for consistency and ease of comparison.

CGRP, calcitonin gene-related peptide; AM, adrenomedullin; CLR, calcitonin receptor-like receptor; AMY, amylin; RAMP, receptor activity-modifying protein.

the tryptophan at position 84 is important for the potency of telcagepant and compound 3 as changing this residue to alanine significantly reduces the potency of both molecules (Table 3) (Moore *et al.*, 2010). Interestingly, CGRP potency was also significantly reduced by the Trp84Ala mutation pinpointing for the fist time a residue that is important for both agonist and antagonist binding and, which is consistent with the N-terminal of RAMP1 forming the binding interface with the N-terminal of CLR (Moore *et al.*, 2010). This residue has also been shown to be important for agonist potency at the AMY_1 receptor (Gingell *et al.*, 2010), which strongly indicates this is a key position for agonist binding in two RAMP1 containing receptors, CGRP and AMY_1.

Regions important for antagonist binding to CLR have also been explored. Salvatore and colleagues constructed chimeras of human CLR and CTR and showed that residues 37–63 of CLR are important for the affinity of compound 3, the olcegepant analogue (Salvatore *et al.*, 2006). Alanine mutagenesis subsequently showed that the methionine at residue 42 is important for the potency of both olcegepant and telcagepant (Table 3) with telcagepant much more sensitive to this mutation in CLR, which is consistent with recent structural data described in more detail later (ter Haar *et al.*, 2010; Miller *et al.*, 2010). The summation of this data demonstrates that the extracellular portions of both CLR and RAMP1 are important for binding of high affinity CGRP receptor antagonists like olcegepant and telcagepant. Contact with both CLR and RAMP1 is exactly what was predicted to be necessary to achieve selectivity against the CLR-containing AM_1 and AM_2 receptors and the RAMP1-containing AMY_1 receptor. While this feature may be desirable for an anti-migraine drug, this is not the only way to antagonize the CGRP receptor. The nonselective antagonist compound 4 was found to bind to the seventh transmembrane domain of CLR, likely independent of RAMP1 (Salvatore *et al.*, 2006). This RAMP1 independent binding explains why this compound does not display significant species selectivity or selectivity for the AM_1 receptor (Table 2). While compound 4 provides proof that it is possible to develop an antagonist that binds in regions other than those defined by the olcegepant and telcagepant-like molecules, one must keep in mind that the selectivity profile of each class of molecule may be quite divergent and there is, in all likelihood, a diverse palette of approaches that could be developed to block CGRP from interacting with its receptor.

CGRP receptor structure

Structural studies of GPCRs are notoriously difficult because of their membrane association and lack of abundant protein source for purification. The CGRP receptor poses an additional problem because of its heteromeric nature. Prior to the availability of any structural data on RAMP1, an *ab initio* modeling approach was developed. Using this approach it was suggested that the human RAMP1 N-terminus was comprised of three α-helices (Simms *et al.*, 2006). In order to determine structural information on a GPCR target, one way to proceed is to use the soluble extracellular portion and hope that it retains the physiologically relevant structure of the complete receptor. This is the approach Kusano and colleagues applied to solve the structure of the extracellular domain of human RAMP1. Their RAMP1 construct included the 81 residues from Cys27 through Ser107. The model they derived confirms the trihelical structure suggested by Simms and colleagues and reveals a hydrophobic patch near Trp74 (Kusano *et al.*, 2008). Based on this structure and previous mutagenesis data, it is clear that Trp74 on RAMP1 is part of a small ligand-binding pocket on the solvent-exposed surface of the CLR/RAMP1 complex (Mallee *et al.*, 2002; Kusano *et al.*, 2008). Residues Arg67, Asp71, Trp74 and Glu78 are all part of the α2 helix while residue Trp84 is located on the loop between the α2 and α3 helices with its side-chain oriented in the same direction as Trp74 (Kusano *et al.*, 2008).

This model of the extracellular portion of RAMP1 gave valuable insight into the nature of this receptor component; however, it gave no information on how the complete complex of CLR and RAMP1 is structured. To this end, Koth and colleagues created a construct consisting of both the extracellular portions of human CLR (residues 23–133) and human RAMP1 (residues 26–117). These regions of CLR and RAMP1 were able to form a stable extracellular domain (ECD) complex that was able to compete with the native CGRP receptor on SK-N-MC cells for binding of [^{125}I]CGRP (Koth *et al.*, 2010). The ECD displayed lower affinity for CGRP (IC_{50} = 12 μM) but it was shown to bind the antagonists olcegepant and telcagepant with high affinity (Koth *et al.*, 2010). With this construct in hand, ter Haar *et al.* were able to solve the structure of the ECD complex in an unliganded state as well as in complex with olcegepant or telcagepant (ter Haar *et al.*, 2010).

The model proposed by ter Haar and colleagues shows that CLR contains an alpha helix designated αC1 that is comprised of residues 35–53. This portion of CLR packs perpendicularly against the three alpha helices of RAMP1 and this interaction is stabilized by a number of electrostatic and hydrophobic interactions between αC1 of CLR and helices α2 and α3 of RAMP1 (ter Haar *et al.*, 2010). They found that olcegepant binds over an 18-Å space stretching from Thr122 of CLR, across the interface with RAMP1, and into a hydrophobic-binding pocket formed by the αC1 helix of CLR and the αR2 helix of RAMP1. Residues Trp74 and Trp84 of RAMP1 form the ceiling and back surfaces of this binding pocket and the quinazolinone group of olcegepant interacts with the backbone of CLR Thr122. There are additional interactions with Trp72, Arg38, Phe92, and Asp94 of CLR as well as Arg67 and Asp72 of RAMP1 (ter Haar *et al.*, 2010). Despite being a smaller molecule, telcagepant binds in the same region stretching over that 18-Å space from Thr122 of CLR into the hydrophobic-binding pocket. Trp74 and Trp84 of RAMP1 also frame the binding pocket for telcagepant with the difluorophenyl group extending deep into the hydrophobic pocket making hydrophobic contacts with Met42 of CLR, which explains why this compound was more sensitive to mutagenesis at this position than olcegepant (ter Haar *et al.*, 2010; Miller *et al.*, 2010). Finally, an additional hydrogen bond at CLR Thr122 from the azabenzimidazolone group results in increased binding efficiency of telcagepant (ter Haar *et al.*, 2010). This model is very useful for determining where these antagonists are binding and could potentially be used for rational drug design.

The mechanism of CGRP binding to its receptor is thought to adhere to the two-domain model laid out by Hoare. In this model, the C-terminal of the peptide ligand first binds with high affinity to the extracellular N-terminal of the receptor forming a so-called affinity trap (Hoare, 2005). For the CGRP receptor, the peptide agonist first interacts with the N-terminal of both CLR and RAMP1. This initial high affinity-binding event greatly increases the local concentration of the peptide, which allows the lower affinity interaction of the N-terminal of the peptide with the juxtamembrane region of the receptor to proceed ultimately leading to receptor activation (Hoare, 2005). This phenomenon is nicely demonstrated by the fact that a truncated CGRP peptide missing the first seven amino acids ($CGRP_{8-37}$) is able to bind to the receptor but does not activate cAMP production and in fact behaves as a functional antagonist (Chiba *et al.*, 1989; Maggi *et al.*, 1991). The two-domain model as applied to the CGRP receptor is illustrated in Figure 2. Based on the location of the antagonist-binding pocket in the model by ter Haar and colleagues and the wealth of mutagenesis data it appears small molecules such as telcagepant and olcegepant antagonize the CGRP receptor by preventing the initial high-affinity binding of CGRP thus not allowing the lower-affinity interaction leading to receptor activation (ter Haar *et al.*, 2010).

Clinical studies of CGRP receptor antagonists for the acute treatment of migraine

The ability of a CGRP receptor antagonist to treat an acute migraine attack was established with olcegepant. In the clinical proof of concept study, olcegepant was administered intravenously in a double-blind, placebo-controlled study (Olesen *et al.*, 2004). The primary end points were either the absence of headache or the presence of mild headache 2 h after treatment with olcegepant. Following intravenous administration of olcegepant, 2.5 mg over a 10-min period, 66% of patients experienced a response at 2 h, compared with 27% receiving placebo. A second compound from Boehringer Ingelheim, the orally bioavailable BI 44370 TA, has recently completed Phase II trials. This was a placebo- and active-controlled (eletriptan) trial to assess the safety, tolerability and efficacy of three doses (50, 200 and 400 mg) of BI 44370 TA. In this study, the primary end point of pain-free 2 h was statistically significant for 400 mg BI 44370 TA and eletriptan (40 mg) groups compared with placebo (Diener *et al.*, 2011).

Telcagepant was the first orally available CGRP receptor antagonist to be tested clinically and is currently in Phase III trials. The effectiveness of telcagepant was first established in a randomized, double-blind, placebo- and active-controlled (rizatriptan) Phase IIB study in which doses from 25 to 600 mg were tested (Ho *et al.*, 2008b). Telcagepant doses 300–600 mg were more effective than placebo in treating moderate or severe migraine attacks as measured by the primary end point of pain relief at 2 h. Other end points mirrored the primary end point, including pain freedom and improvement of associated symptoms.

The efficacy of telcagepant (150 and 300 mg) was subsequently studied in a pivotal Phase III acute efficacy migraine trial (Ho *et al.*, 2008a). This was a randomized, double-blind, placebo- and active-controlled (zolmitriptan 5 mg) outpatient study to assess the efficacy and tolerability of telcagepant in patients with an acute migraine attack. Telcagepant 300 mg was more effective than placebo for pain

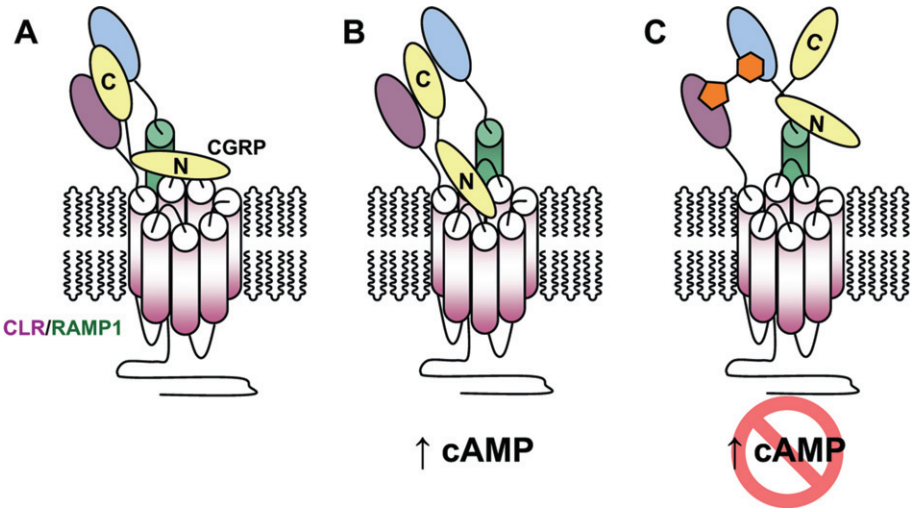


Figure 2

The two-domain binding model applied to the CGRP receptor. A) The C-terminal of CGRP first binds with high affinity to the N-terminal regions of CLR and RAMP1 forming an affinity trap. B) Due to increased local concentration of CGRP, the N-terminal of the peptide is able to interact with the juxtamembrane region of CLR which activates the receptor leading to accumulation of cAMP. C) Antagonists such as telcagepant or olcegepant (in orange) bind to a hydrophobic pocket formed by CLR and RAMP1 preventing the initial CGRP binding event and subsequent receptor activation.

freedom, pain relief and absence of phonophobia, photophobia and nausea. Efficacy of telcagepant 300 mg and 5 mg zolmitriptan were very similar, and both were more effective than telcagepant 150 mg. Telcagepant was generally well tolerated with an adverse event rate similar to placebo. A second large Phase III clinical trial was conducted to confirm the efficacy of telcagepant (Connor *et al.*, 2009). In this study, telcagepant 300 and 150 mg were more effective than placebo on all primary (pain freedom, pain relief, and absence of photophobia, phonophobia and nausea at 2 h post-dose) and the key secondary end point of 2–24 h of sustained pain freedom. In a study to assess the long-term safety and tolerability for the intermittent treatment of acute migraine, telcagepant was used by 640 patients to treat up to eight migraine attacks per month for up to 18 months. In these studies, telcagepant was generally well tolerated and there were fewer of the adverse events commonly associated with triptans such as aesthenia, chest pain, chest tightness and paraesthesia (Ho *et al.*, 2009). However, in 2009, Merck & Co., Inc. announced that they were delaying the filing of the U.S. application for telcagepant (Merck & Co., Inc., 2009). This decision was based upon findings from a Phase II exploratory migraine prophylaxis study in which a small number of patients were found to have elevations in liver transaminases. In this study, patients were treated with twice daily doses of telcagepant 140 or 280 mg for 3 months. The daily dosing regimen in the prophylaxis study was different than the dosing regimen in the Phase III studies in which telcagepant was dosed intermittently.

Conclusions

The drugability of the CGRP receptor remains a significant challenge due to the heteromeric nature of the receptor complex and yet to be defined absolute stoichiometry (Heroux *et al.*, 2007). Our understanding of the structure and function of the CGRP receptor is rapidly progressing with the isolation of the extracellular CLR/RAMP1 complex amenable to crystallography. The crystal structure of the CLR/RAMP1 ECD defines the antagonist-binding pocket and highlights why the development of small molecule antagonists has been, and still remains, a daunting task. The recent structural information also provides confirmation of the proposed antagonist interaction sites delineated by the historical mutagenesis analyses. This review and the majority of the experimental data generated to date have focused on the CLR/RAMP1 ECD domain as the epitope for antagonist binding. Perhaps, the culmination of this data will provide the framework to develop novel chemotypes with which to disrupt the CGRP-receptor interaction via rational drug design, a concept discussed by Archbold *et al.* in a recent review article (Archbold *et al.*, 2011). Ideally, the goal should be the crystallization of the entire receptor complex, which would provide deeper insight into novel ways of targeting this important receptor.

Although there are still many unanswered questions about the exact mechanism linking CGRP and migraine, it is now quite clear that CGRP is not simply a migraine 'biomarker' but is an important player in the underlying pathology of migraine. The advent of small molecule CGRP receptor antagonists offers optimism for an effective new therapy for migraine sufferers.

Conflict of interest

Eric Moore and Christopher Salvatore are employees of Merck & Co., Inc.

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