Chapter 16

Calcitonin Gene-Related Peptide and Other Peptides

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Vasoactive peptides can be either stored or synthesized de novo before release from a range of tissues in the brain or from the walls of intracranial vasculature. In this chapter, we concentrate on neuropeptides that are released from perivascular nerves. These include calcitonin gene-related peptide (CGRP), substance P, neurokinin A, nociceptin, somatostatin, and opioids (Table 16-1). The endothelium produces the potent vasoconstrictors endothelin and angiotensin, and dilators such as nitric oxide, prostacyclin, and endothelium-derived hyperpolarizing factors. In addition there are circulating agents; among these the most potent is 5-hydroxytryptamine. The neuronal messengers stored in the intracranial vessels have been reviewed (32) and it was revealed that sympathetic nerves store noradrenaline, neuropeptide Y, and ATP, the parasympathetic fibers nitric oxide, acetylcholine, vasoactive intestinal peptide (VIP), peptide histidine isoleucine, pituitary adenylate cyclase-activating peptide (PACAP), and helodermine. Although VIP is of interest in certain forms of primary headaches, it is mainly the peptides contained in the sensory system, and this has attracted the most excitement during the last few years. A growing body of studies indicate that the distribution and biological activity of these neuropeptides to the areas of the cerebral vasculature and nervous system that are involved in migraine is highly relevant. Research into the precise role and importance of these peptides is often lacking because of the paucity of selective agents that either enhance or block activity or synthesis. However, there have been some major clinical trials that have focused on these peptides in recent years that have, for instance, ruled out a role for substance P, but provided evidence for the involvement of CGRP. This chapter is written in a comparative manner to allow an appreciation of our relative knowledge of the biology of these peptides and their role in migraine.

THE CGRP FAMILY OF PEPTIDES

The expression of mRNA from the calcitonin gene is tissue specific in that CGRP mRNA is predominantly expressed in nerves and calcitonin mRNA in the thyroid (5). The 37 amino acid peptide CGRP belongs to a family that include the more recently discovered peptides adrenomedullin that is primarily produced by non-neuronal tissues, especially vascular tissues and amylin that is mainly produced in the pancreas. They share some structural homology (approximately 25–40%) and also some, but not total, similarities in biological activities (see Brain and Grant [11] for recent review). CGRP is abundant in the body and has a wide distribution throughout the central and peripheral nervous systems. It has a number of biological activities, but the most relevant to migraine are its activities within the nervous and cardiovascular systems. CGRP is an extremely potent and long-lasting vasodilator, that is active at all levels of the cardiovascular system with good evidence for exquisite activity in the cerebral circulation (see Edvinsson [23]).

CGRP is most usually found in unmyelinated sensory C-fibers and myelinated Aδ-fibers that are commonly associated with blood vessels, where perivascular nerves terminate in close association with the vessels. Specific labeling of the C- and Aδ-fibers with wheat germ agglutinin–conjugated horse radish peroxidase and cholera toxin subunit b revealed that the C-fibers are located in lamina 1 and 2, and the Aδ-fibers from mechanoreceptors lay located in lamina 3 and 4 (48). Labeling experiments have revealed that the temporal artery and the superior sagittal sinus differ in their somatotopic organization. CGRP is the most prevalent of the neuropeptides in the sensory fibers. CGRP is commonly colocalized with other peptides in C-fibers, which include substance P (49). There are two forms of CGRP and αCGRP (or CGRPI), encoded by the calcitonin

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TABLE 16-1 Characteristics of Some Peptides Thought to be Involved in the Pathogenesis of Migraine

Neuropeptide	*Amino Acids*	*Activity of Possible Relevance to Migraine*	*Location*	*Receptor Family*	*Modulates Animal Model of Migraine*	*Ligand Effective in Migraine*
CGRP	37	Vasodilator	Sensory nerves	CGRP (CL/RAMP1)	Yes, antagonist (either $CGRP_{8-37}$ or BIBN4096BS) decreases dural blood flow	BIBN4096BS effective in phase II clinical trials
Substance P	11	Vasodilator; pain transmission; plasma extravasation	Sensory nerves	Tachykinin family (NK_1)	Yes, NK_1 antagonist blocks dural plasma extravasation	NK_1 receptor antagonists not effective in acute migraine
Somatostatin	14 (or 28)	Inhibits sensory nerve activity	Sensory nerves and other cells	Somatostatin (sst1–sst5)		Yes octa peptide benefits migraine pain
Opioids (enkephalins, etc.)	Varies	Inhibits sensory nerve activity	Sensory nerves and other cells	Opioid ($\mu\delta\kappa$ and ORL-1)	Yes, inhibits dural plasma extravasation	Yes, but use limited by abusive nature
VIP	28	Vasodilator	Found in range of nerves	VIP (VPAC)	Not known	Not known
Neuropeptide Y	36	Vasoconstrictor	Sympathetic nerves	Y1–Y5	Not known	Not known

gene, that are relevant to the cerebral vasculature. βCGRP (or CGRPII), which has a high structural similarity (90%), is primarily found in the gut and formed from a distinct gene (57).

The distribution of CGRP-containing nerves has been evaluated in detail in the cerebral circulation (23). CGRP is contained in and released from sensory nerves originating in the trigeminal ganglia and innervates cerebral blood vessels (30). CGRP, when given intravenously, acts in a hypotensive manner, but it is generally considered that the major activity of CGRP is local to site of release (12). Plasma levels of CGRP are in the low picomolar level in normal volunteers (26) and migraineurs, but have been shown to increase in blood samples taken from the jugular vein ipsilateral to the attack (27).

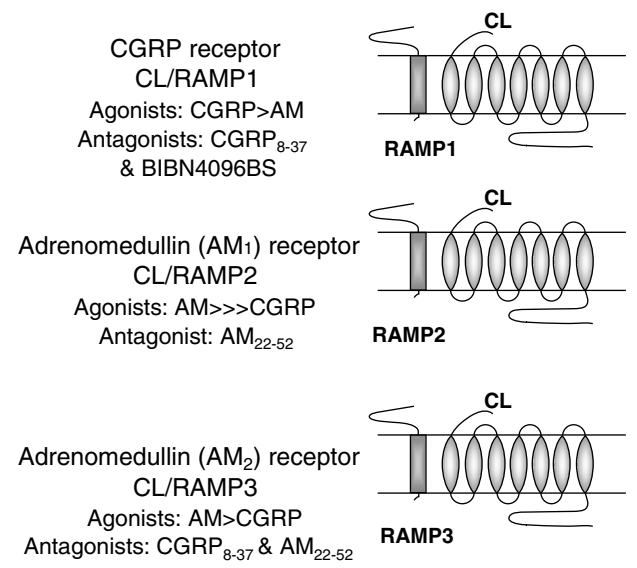

FIGURE 16-1. Summary of the characteristics of the CGRP receptor family.

The release of CGRP occurs in response to nerve stimulation, and this has been studied in tissues in response to chemical, physical, and mechanical stimulation in the laboratory. It is important to note that the TRPV (or VR-1) receptor for capsaicin, the hot extract of chili peppers, exists on the majority of CGRP-containing sensory nerves (13) both in animals and in human trigeminal ganglion (39). The endogenous stimuli for this receptor are under study and include protons, noxious heat, and a range of endogenous mediators, although their relative importance in pathology is unclear (2,3,4). Furthermore, presynaptic/prejunctional receptors on the sensory nerves can modulate CGRP release. These receptors include those for opioids, 5-hydroxytryptamine ($5\text{-}HT_1$ receptor), γ-aminobutyric acid ($GABA_B$ receptor), histamine (H_3 receptor), neuropeptide Y, somatostatin, VIP, purines, and galanin (see below and Maggi [51]).

CGRP receptors were classed as CGRP1 and CGRP2 in the late 1980s, as a consequence of pharmacologic studies. The CGRP1 receptor is considered the important cardiovascular receptor. The 8-37 amino acid fragment of CGRP,

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$CGRP_{8-37}$, is a selective antagonist for this receptor (14). By comparison, the CGRP2 receptor remains poorly defined (60). It is now realized that the CGRP family of receptors consists of the seven transmembrane G protein-coupled calcitonin receptor-like receptor CL with one of three single membrane-spanning receptor activity modifying proteins (RAMPs; 53). This complex is associated with a CGRP-receptor component that is suggested to enhance receptor coupling and activation (25). The RAMP molecule is important for localization of the functional receptor to the cell surface and receptor phenotype, because it influences ligand specificity (65). CL is a G protein-coupled receptor that is important for ligand binding. Three RAMPs (RAMP1, RAMP2, and RAMP3) are known. CL, when presented as a heterodimer with RAMP1 at the cell surface, functions as a CGRP receptor that is antagonized by $CGRP_{8-37}$ and the nonpeptide CGRP antagonist BIBN4096BS (see below). CL with RAMP2 produces an adrenomedullin (AM) receptor that is blocked by the weak AM antagonist $ADM_{22-52.}$ CL with RAMP3 leads to an AM receptor that least is known about. Both CL and the RAMPs have been detected in the human cerebral and meningeal vasculature (59).

The best described, and only potent, small molecule CGRP antagonist described to date has been BIBN4096BS, 1-Piperidinecarboxamide, *N*-[2-[[5-amino-1-[[4-(4-pyridinyl)-1-piperazinyl]carbonyl] pentyl] amino]-1-[(3,5-dibromo-4-hydroxyphenyl)methyl]-2-oxoethyl]-4-(1,4-dihydro-2-oxo-3(2H)-quinazolinyl)-,[R-(R*,S*)]. It is a competitive antagonist with potent properties at the human and marmoset CGRP1 receptor (18). It displays a 200-fold greater affinity in human compared with rodent tissues. RAMP1 governs species selectivity for receptor antagonists, via a single amino acid residue (tryptophan at position 74; see Mallee et al. [52]). A related compound, compound 1, (4-(2-Oxo-2, 3-dihydro-benzoimidazol-1-yl)-piperidine-1-carboxylic acid [1-3,5-dibromo-4-hydroxy-benzyl)-2-oxo-2-(4-phenyl-piperazin-1-yl)-ethyl]-amide) has also been synthesized and studied. It is a weak antagonist of CGRP receptors. It also weakly antagonized CGRP responses in human cerebral and guinea pig basilar arteries (22), but failed to inhibit the vascular relaxation induced by CGRP in porcine tissues (33), where $CGRP_{8-37}$ is an effective antagonist. However, compound 1 acted as a competitive antagonist at the CGRP receptor in human left anterior descending coronary arteries having a pA_2 value similar to that of $CGRP_{8-37}$ (34). This emphasizes the species and tissue selectivity that is now becoming associated with CGRP receptor antagonists. Alternatively, a distinct nonpeptide CGRP1 receptor antagonist that acts across species exists. SB-273779, [*N*-methyl-*N*-(2-methylphenyl)-3-nitro-4-(2-thiazolylsulfinyl)nitrobenzanilide] is selective for the CGRP receptor. The compound is weaker than BIBN4096BS, but does displace radioactive CGRP in rat and porcine lung binding studies (1).

There are multiple mechanisms by which CGRP produces vasodilation that have been previously detailed (9,11). It is accepted that vascular relaxation is mediated via the CGRP1 receptor (CL/RAMP1). This can occur via nitric oxide–dependent endothelium-dependent mechanisms, as has been shown in mouse pial vessels (61), or cAMP endothelium–independent pathways. The latter mechanism is observed in the majority of cerebral vessels studied to date. CGRP acts directly on cerebral vessels to stimulate adenylate cyclase (40). The resulting rise in $[cAMP]_i$ activates protein kinase A, leading to phosphorylation, opening of potassium channels, and relaxation. In rat pial arterioles, vasodilatation to CGRP was inhibited in the presence of glibenclamide or charybdotoxin (a large-conductance Ca^{2+}–activated K^+ channel blocker) (37).

Exogenous CGRP can induce a migraine-like headache (45), but CGRP is not involved in tension-type headaches (6). By comparison, CGRP levels are increased in samples taken from the draining jugular vein during the painful phases of both migraine and cluster headaches (27,28,30). The intracranial extracerebral blood vessels (e.g., middle meningeal artery and its dural arterioles) that supply the dura mater are considered to relax, leading to stimulation of perivascular sensory nociceptive nerve fibers and pain. Mechanisms involved in the release of CGRP are ill-defined and indeed there is evidence for a neuronal site of action for CGRP (63). The small molecule CGRP antagonist BIBN4096BS has been shown to be successful in phase 2 clinical trials (58).

The vasospasm of subarachnoid hemorrhage (SAH) is associated with a reduction in neuronal CGRP (19) and an increase in draining blood levels of CGRP (42). This has led to the suggestion that treatment with CGRP may be beneficial and a preliminary clinical trial with CGRP supported this concept (41); however, a later multicenter clinical trial did not (8). The difference in results may be explained by the potent hypotensive effect of CGRP, which was not carefully monitored in the large clinical trial and hence aggravated the reduction in the cerebral circulation, whereas in the small study, it was carefully monitored in the patients and any drastic drop in blood pressure avoided (42). This suggests that CGRP may be used to counterbalance vasospasm in SAH if adequately monitored. However, it is possible that CGRP gene therapy may have therapeutic potential as gene transfer of recombinant adenoviral preproCGRP in the rabbit can inhibit fatal cerebral vasoconstriction after SAH (64).

SUBSTANCE P

Substance P is a member of the tachykinin family, all of which have a common carboxy-terminal amino acid sequence (50). Substance P shares, in part, a similar distribution to CGRP with which it is colocalized in sensory

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nerves. It is found around blood vessels. Substance P is not only a vasodilator, but also has the ability to increase microvascular permeability. However, there has been some debate about whether endogenous levels of substance P are sufficient to increase microvascular permeability.

There are three tachykinin receptors and the vasoactive properties of the tachykinins are principally mediated via the tachykinin NK_1 receptor. The NK_1 receptor is found throughout the peripheral and central nervous systems. Nonpeptide NK_1 receptor antagonists, such as RPR100893, were found to block plasma protein extravasation within the dura mater stimulated by electrical stimulation of the trigeminal ganglion in guinea pigs (46) and to inhibit *c*-fos expression in the trigeminal nucleus caudalis in response to C-fiber stimulant capsaicin (15). This and other similar studies led to the suggestion that NK_1 antagonists could be beneficial in the treatment of migraine. However, results from clinical studies that utilized RPR100,893 (17) and another antagonist LY303,870 (31) have shown no beneficial effect of these agents in migraine headache. There remains a debate of whether these agents were able to establish a sufficient blockade of NK_1 receptors at the doses used as the agents penetrate the central nervous system relatively poorly.

SOMATOSTATIN

Somatostatin exists as two isoforms, as a tetradecapeptide (somatotropin release-inhibiting factor [SRIF-14]) and as an amino-terminally extended octacosapeptide (SRIF-28). Somatostatin is widely distributed throughout the central nervous system. It is known to inhibit secretion of most hormones (see Moller et al. [56]). In addition, there is a growing belief that SRIF can modulate nervous function and it has been shown to be released from sensory nerves and to inhibit sensory neurogenic inflammation and nociceptive activity (35). There are five somatostatin receptor subtypes (sst_{1-5}) with some structural homology (>50%) between them (56). However, there is some difficulty in determining the contribution of specific receptors in nociception, although it has been suggested that sst_1 and/or sst_4 that comprise the SRIF2 group of receptors may be of importance, in peripheral inflammation, in the rat at least (35). Somatostatin, or more specifically octreotide, SMS201-995, has been shown to be beneficial in the treatment of migraine following subcutaneous administration in a double-blind placebo-controlled trial, with a beneficial effect seen in 75% of migraineurs, compared with 25% of placebo-treated patients (43). It has also been suggested that withdrawal of somatostatin, or repeated exposure to somatostatin, may precipitate a migraine attack, However, a recent study suggests that these techniques are not an effective model for inducing either an acute migraine attack or cluster headache (47).

OPIOIDS

The opioid class of drugs have a long history of use in the treatment of severe pain, for example that associated with cancer. Most of these agents are classified as μ-opioid analgesics, in reference to their activity as pharmacologic agonists of this receptor. Endogenous opioids include the enkephalins, β-endorphin, and dynorphin peptides. There are at least four different receptors that are established as μ, κ, δ, and the opioid-related receptor (ORL-1), also called N/OFQ receptor. The N/OFQ receptor exhibits a high degree of structural homology with conventional opioid receptors, but with a distinct pharmacology (55). All the receptors couple to G-proteins that regulate GTP levels. The enkephalins act via the classic morphine (μ) receptor, in addition to the δ receptor, that can be colocalized and effects modified by the κ receptor. Dynorphins are most potent at the κ receptor. Nociceptin acts at the N/OFQ receptor. Nociceptin immunoreactivity and receptor mRNA has recently been demonstrated to occur in human trigeminal ganglion and to colocalize with CGRP immunoreactivity (38). A role for nociceptin seems to exist also in primary headaches; Ertsey and coworkers 924) observed reduced circulating levels in cluster headache. All the peptides can influence analgesia, although their role in migraine is less well studied. Opioids certainly block nociceptive neurotransmission within the trigeminal nucleus caudalis and inhibit neurogenic dural vasodilation through μ-opioid receptors found on trigeminal sensory fibers that innervate dural blood vessels in anesthetized rats (68). It is generally considered that opioid analgesics may be useful for pain relief in migraine, and butorphanol is known to be effective in migraine (36). However, many physicians consider that opioid analgesics should only be used when other agents cannot be, because of sedative and, more importantly, addictive and tolerance properties. At the moment there is considerable interest in characterizing the subtypes of opioid receptors and it is hoped that this will eventually enable the possibility that opioid ligands with therapeutic efficacy, but without the abuse possibilities, to be developed. Interestingly, it is known that activation of the N/OFQ receptor with nociceptin inhibits neurogenic dural vasodilation induced by electrical stimulation in the rat (7).

VASOACTIVE INTESTINAL PEPTIDE

VIP is a 28-amino acid peptide that coexists with acetylcholine and is also found in a range of nerves throughout the body, especially those associated with sweat glands. VIP has some structural similarities with a range of gastrointestinal peptides (see Delgado et al. [16] for a review). It should be pointed out that VIP occurs in almost 100% of the parasympathetic ganglion neurons (otic and spehnopalatine ganglia) where it is colocalized with

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PACAP (20). VIP has similar potency as a vasodilator as CGRP. It is also known to have modulatory effects in the immune system. The elucidation of the role of VIP has been hampered through lack of availability of selective receptor ligands. However, during cluster headache, levels of VIP measured in the draining jugular vein have been shown to be increased (21). Thus, both in cluster headache and in chronic paroxysmal headache, the VIP level was associated with facial flushing (29), and it was considered that this was caused by activity within the parasympathetic system. Intense activation of the trigeminal vascular system (e.g., superior sagittal sinus) has experimentally demonstrated that the CGRP release occurs in parallel with the VIP release (69). Lesion of the trigeminal system aborts the VIP release, an effect that immediately dries out the sensory system. The VIP and PACAP receptors have been shown in the human cerebral arteries and cranial ganglia (44). Certainly more work is needed to evaluate their role in primary headache disorders.

NEUROPEPTIDE Y

Neuropeptide Y (NPY) is a 36-amino acid peptide that is widely distributed throughout the central and peripheral nervous systems. It is primarily located in perivascular sympathetic nerves. It is a potent vasoconstrictor and found colocalized with noradrenaline (66). It can potentiate the actions of noradrenaline. Neuropeptide Y can influence a wide variety of physiologic processes through a family of receptors already cloned and named Y_1, Y_2, Y_4, Y_5, and Y_6 according to their molecular and pharmacologic activity (54). NPY can act on all Y receptors. There is evidence for the involvement of both Y_1 and Y_2 receptors in the cardiovascular system, although the precise mechanisms and relative importance is unclear. Neuropeptide Y levels are not significantly increased in samples collected from the jugular vein, ipsilateral to the attack (21), but levels of neuropeptide are raised in the cerebrospinal fluid in migraineurs (67). It has been shown by Bloom's group that following sympathetic denervation initial cerebrovascular NPY disappears, but soon returns. This appears to be caused by transcription of this important peptide preferentially in the parasympathetic ganglia. In addition, sympathectomy also results in upregulation of CGRP in the trigeminovascular system (62). Hypothetically enhanced transcription might occur in chronic headache disorders, and hence in such situations a role for NPY might appear.

REFERENCES

1. Aiyar N, Daines RA, Disa J, et al. Pharmacology of SB-273779, a nonpeptide calcitonin gene-related peptide 1 receptor antagonist. *J Pharmacol Exp Ther*. 2001;296:768–775.
2. Akerman S, Kaube H, Goadsby PJ. Anandamide acts as a vasodilator of dural blood vessels in vivo by activating TRPV1 receptors. *Br J Pharmacol*. 2004;142:1354–1360.
3. Akerman S, Kaube H, Goadsby PJ. Vanilloid type 1 receptors (VR1) on trigeminal sensory nerve fibres play a minor role in neurogenic dural vasodilation, and are involved in capsaicin-induced dural dilation. *Br J Pharmacol*. 2003;140:718–724.
4. Akerman S, Kaube H, Goadsby PJ. Anandamide is able to inhibit trigeminal neurons using an in vivo model of trigeminovascular-mediated nociception. *J Pharmacol Exp Ther*. 2003;309:56–63.
5. Amara SG, Jonas V, Rosenfeld MG, et al. Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. *Nature*. 1982;298:240–244.
6. Ashina M, Bendtsen L, Jensen R, et al. Plasma levels of calcitonin gene-related peptide in chronic tension-type headache. *Neurology*. 2000;55:1335–1340.
7. Bartsch T, Akerman S, Goadsby PJ. The ORL-1 (NOP1) receptor ligand nociceptin/orphanin FQ (N/OFQ) inhibits neurogenic dural vasodilatation in the rat. *Neuropharmacology*. 2002;43:991–998.
8. Bell BA. The neuroprotective effect of calcitonin gene-related peptide following subarachnoid hemorrhage. European CGRP in Subarachnoid Haemorrhage Study Group. *Ann N Y Acad Sci*. 1995;765:299–300.
9. Bell D, McDermott BJ. Calcitonin gene-related peptide in the cardiovascular system: characterization of receptor populations and their (patho)physiological significance. *Pharmacol Rev*. 1996;48:253–288.
10. Born W, Fischer JA, Muff R. Receptors for calcitonin gene-related peptide, adrenomedullin, and amylin: the contributions of novel receptor-activity-modifying proteins. *Receptors Channels*. 2002;8:201-209.
11. Brain SD, Grant AD. Vascular actions of calcitonin gene-related peptide and adrenomedullin. *Physiol Rev*. 2004;84:903–934.
12. Brain SD, Williams TJ, Tippins JR, et al. Calcitonin gene-related peptide is a potent vasodilator. *Nature*. 1985;313:54–56.
13. Caterina MJ, Schumacher MA, Tominaga M, et al. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*. 1997;389:816–824.
14. Chiba T, Yamaguchi A, Yamatani T, et al. Calcitonin gene-related peptide receptor antagonist human CGRP-(8-37). *Am J Physiol*. 1989;256:E331–E335.
15. Cutrer FM, Moussaoui S, Garret C, et al. The non-peptide neurokinin-1 antagonist, RPR 100893, decreases *c*-fos expression in trigeminal nucleus caudalis following noxious chemical meningeal stimulation. *Neuroscience*. 1995;64:741–750.
16. Delgado M, Pozo D, Ganea D. The significance of vasoactive intestinal peptide in immunomodulation. *Pharmacol Rev*. 2004;56:249–290.
17. Diener HC, RPR100893 Study Group. RPR100893, a substance-P antagonist, is not effective in the treatment of migraine attacks. *Cephalalgia*. 2003;23:183–185.
18. Doods H, Hallermayer G, Wu D, et al. Pharmacological profile of BIBN4096BS, the first selective small molecule CGRP antagonist. *Br J Pharmacol*. 2000;129:420–423.
19. Edvinsson L, Ekman R, Jansen I, et al. Reduced levels of calcitonin gene-related peptide-like immunoreactivity in human brain vessels after subarachnoid haemorrhage. *Neurosci Lett*. 1991;121:151–154.
20. Edvinsson L, Elsas T, Suzuki N, et al. Origin and co-localization of nitric oxide synthase, CGRP, PACAP, and VIP in the cerebral circulation of the rat. *Microsc Res Tech*. 2001;1:221–228.
21. Edvinsson L, Goadsby PJ. Neuropeptides in the cerebral circulation: relevance to headache. *Cephalalgia*. 1995;15:272–276.
22. Edvinsson L, Sams A, Jansen-Olesen I, et al. Characterisation of the effects of a non-peptide CGRP receptor antagonist in SK-N-MC cells and isolated human cerebral arteries. *Eur J Pharmacol*. 2001;415:39–44.
23. Edvinsson L. Blockade of CGRP receptors in the intracranial vasculature: a new target in the treatment of headache. *Cephalalgia*. 2004;24:611–622.
24. Ertsey C, Hantos M, Bozsik G, et al. Circulating nociceptin levels during the cluster headache period. *Cephalalgia*. 2004;24:280–283.
25. Evans BN, Rosenblatt MI, Mnayer LO, et al. CGRP-RCP, a novel protein required for signal transduction at calcitonin gene-related

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peptide and adrenomedullin receptors. *J Biol Chem*. 2000;275:31 438–443.
26. Girgis SI, Macdonald DW, Stevenson JC, et al. Calcitonin gene-related peptide: potent vasodilator and major product of calcitonin gene. *Lancet*. 1985;2:14–16.
27. Goadsby PJ, Edvinsson L, Ekman R. Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. *Ann Neurol*. 1990;28:183–187.
28. Goadsby PJ, Edvinsson L. Human in vivo evidence for trigeminovascular activation in cluster headache. Neuropeptide changes and effects of acute attacks therapies. *Brain*. 1994;117:427–434.
29. Goadsby PJ, Edvinsson L. Neuropeptide changes in a case of chronic paroxysmal hemicrania—evidence for trigemino-parasympathetic activation. *Cephalalgia*. 1996;16:448–450.
30. Goadsby PJ, Edvinsson L. The trigeminovascular system and migraine: studies characterizing cerebrovascular and neuropeptide changes seen in humans and cats. *Ann Neurol*. 1993;33:48–56.
31. Goldstein DJ, Wang O, Saper JR, et al. Ineffectiveness of neurokinin-1 antagonist in acute migraine: a crossover study. *Cephalalgia*. 1997;17:785–790.
32. Gulbenkian S, Uddman R, Edvinsson L. Neuronal messengers in the human cerebral circulation. *Peptides* 2001;22:995–1007.
33. Hasbak P, Sams A, Schifter S, et al.CGRP receptors mediating CGRP-, adrenomedullin- and amylin-induced relaxation in porcine coronary arteries. Characterization with 'Compound 1' (WO98/11128), a non-peptide antagonist. *Br J Pharmacol*. 2001;133: 1405–1413.
34. Hasbak P, Opgaard OS, Eskesen K, et al. Investigation of CGRP receptors and peptide pharmacology in human coronary arteries. Characterization with nonpeptide antagonist. *J Pharmacol Exp Ther*. 2003;304:326–333.
35. Helyes Z, Than M, Oroszi G, et al. Anti-nociceptive effect induced by somatostatin released from sensory nerve terminals and by synthetic somatostatin analogues in the rat. *Neurosci Lett*. 2000;278:185–188.
36. Hoffert MJ, Couch JR, Diamond S, et al. Transnasal butorphanol in the treatment of acute migraine. *Headache*. 1995;35:65–69.
37. Hong KW, Yoo SE, Yu SS, et al. Pharmacological coupling and functional role for CGRP receptors in the vasodilation of rat pial arterioles. *Am J Physiol*. 1996;270:H317–H323.
38. Hou M, Uddman R, Tajti J et al. Nociceptin immunoreactivity and receptor mRNA in the human trigeminal ganglion. *Brain Res*. 2003;964:179–186.
39. Hou M, Uddman R, Tajti, J, et al. Capsaicin receptor immunoreactivity in the human trigeminal ganglion. *Neurosci Lett*. 2002;330:223–226.
40. Jansen I, Mortensen A, Edvinsson L. Characterization of calcitonin gene-related peptide receptors in human cerebral vessels. Vasomotor responses and cAMP accumulation. *Ann N Y Acad Sci*. 1992;657:435–440.
41. Juul R, Aakhus S, Bjornstad K, et al. Calcitonin gene-related peptide (human alpha-CGRP) counteracts vasoconstriction in human subarachnoid haemorrhage. *Neurosci Lett*. 1994;170:67–70.
42. Juul R, Edvinsson L, Gisvold SE, et al. Calcitonin gene-related peptide-LI in subarachnoid haemorrhage in man. Signs of activation of the trigemino-cerebrovascular system? *Br J Neurosurg*. 1990;4:171–179.
43. Kapicioglu S, Gokce E, Kapicioglu Z, et al. Treatment of migraine attacks with a long-acting somatostatin analogue (octreotide, SMS 201-995). *Cephalalgia*. 1997;17:27–30.
44. Knutsson M, Edvinsson L. Distribution of mRNA for VIP and PACAP receptors in human cerebral arteries and cranial ganglia. *NeuroReport*. 2002;13:1–3.
45. Lassen LH, Haderslev PA, Jacobsen VB, et al. CGRP may play a causative role in migraine. *Cephalalgia*. 2002;2:54–61.
46. Lee WS, Moussaoui SM, Moskowitz MA. Blockade by oral or parenteral RPR 100893 (a non-peptide NK1 receptor antagonist) of neurogenic plasma protein extravasation within guinea-pig dura mater and conjunctiva. *Br J Pharmacol*. 1994;112:920–924.
47. Levy MJ, Matharu MS, Bhola R, et al. Somatostatin infusion withdrawal: a study of patients with migraine, cluster headache and healthy volunteers. *Pain*. 2003;102:235–241.
48. Liu Y, Zhang M, Broman J, et al. Central projections of sensory innervation of the rat superficial temporal artery. *Brain Res*. 2003;966:126-133
49. Lundberg JM, Franco-Cereceda A, Hua X, et al. Co-existence of substance P and calcitonin gene-related peptide-like immunoreactivities in sensory nerves in relation to cardiovascular and bronchoconstrictor effects of capsaicin. *Eur J Pharmacol*. 1985;108:315–319.
50. Maggi CA. The mammalian tachykinin receptors. *Gen Pharmacol*. 1995;26:911–944.
51. Maggi CA. The pharmacology of the efferent function of sensory nerves. *J Auton Pharmacol*. 1991;11:173–208.
52. Mallee JJ, Salvatore CA, LeBourdelles B, et al. Receptor activity-modifying protein 1 determines the species selectivity of non-peptide CGRP receptor antagonists. *J Biol Chem*. 2002;277:14294–14298.
53. McLatchie LM, Fraser NJ, Main MJ, et al. RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. *Nature*. 1998;393:333–339.
54. Michel MC, Beck-Sickinger A, Cox H, et al. XVI International Union of Pharmacology recommendations for the nomenclature of neuropeptide Y, peptide YY, and pancreatic polypeptide receptors. *Pharmacol Rev*. 1998;50:143–150.
55. Mogil JS, Pasternak GW. The molecular and behavioral pharmacology of the orphanin FQ/nociceptin peptide and receptor family. *Pharmacol Rev*. 2001;53:381–415.
56. Moller LN, Stidsen CE, Hartmann B, et al. Somatostatin receptors. *Biochim Biophys Acta*. 2003;1616:1–84.
57. Mulderry PK, Ghatei MA, Spokes RA, et al. Differential expression of alpha-CGRP and beta-CGRP by primary sensory neurons and enteric autonomic neurons of the rat. *Neuroscience*. 1988;25:195–205.
58. Olesen J, Diener HC, Husstedt IW, et al, BIBN 4096 BS Clinical Proof of Concept Study Group. Calcitonin gene-related peptide receptor antagonist BIBN 4096 BS for the acute treatment of migraine. *N Engl J Med*. 2004;350:1104–1110.
59. Oliver KR, Wainwright A, Edvinsson L, et al. Immunohistochemical localization of calcitonin receptor-like receptor and receptor activity-modifying proteins in the human cerebral vasculature. *J Cereb Blood Flow Metab*. 2002;22:620–629.
60. Poyner D, Marshall I. CGRP receptors: beyond the CGRP(1)-CGRP(2) subdivision? *Trends Pharmacol Sci*. 2001;22:223.
61. Rosenblum WI, Shimizu T, Nelson GH. Endothelium-dependent effects of substance P and calcitonin gene-related peptide on mouse pial arterioles. *Stroke*. 1993;24:1043–1047.
62. Schon F, Ghatei M, Allen JM, et al. The effect of sympathectomy on calcitonin gene-related peptide in the rat trigeminovascular system. *Brain Res*. 1985;25:197–200.
63. Storer RJ, Akerman S, Goadsby PJ. Calcitonin gene-related peptide (CGRP) modulates nociceptive trigeminovascular transmission in the cat. *Br J Pharmacol*. 2004;142:1171–1181.
64. Toyoda K, Faraci FM, Watanabe Y, et al. Gene transfer of calcitonin gene-related peptide prevents vasoconstriction after subarachnoid hemorrhage. *Circ Res*. 2000;87:818–824.
65. Udawela M, Hay DL, Sexton PM. The receptor activity modifying protein family of G protein coupled receptor accessory proteins. *Semin Cell Dev Biol*. 2004;15:299–308.
66. Uddman R, Möller S, Nilsson T, et al. Neuropeptide Y Y_1 and neuropeptide Y Y_2 receptors in human cardiovascular tissues. *Peptides*. 2002;23:927–934.
67. Valenzuela RF, Donoso MV, Mellado PA, et al. Migraine, but not subarachnoid hemorrhage, is associated with differentially increased NPY-like immunoreactivity in the CSF. *J Neurol Sci*. 2000;173:140–146.
68. Williamson DJ, Shepheard SL, Cook DA, et al. Role of opioid receptors in neurogenic dural vasodilation and sensitization of trigeminal neurones in anaesthetized rats. *Br J Pharmacol*. 2001;133: 807–814.
69. Zagami AS, Goadsby PJ, Edvinsson L. Stimulation of the superior sagittal sinus in the cat causes release of vasoactive peptides. *Neuropeptides*. 1990;16:69–75.