J Headache Pain (2005) 6:61–70
DOI 10.1007/s10194-005-0153-6

REVIEW

Pierangelo Geppetti
Jay Guido Capone
Marcello Trevisani
Paola Nicoletti
Giovanni Zagli
Maria Rosalia Tola

CGRP and migraine: neurogenic inflammation revisited

Received: 23 February 2005
Accepted in present form: 23 February 2005
Published online: 8 April 2005

P. Geppetti • J.G. Capone • M.R. Tola
Headache Center, U.O. Neurology,
Department of Neuroscience,
Azienda Università-Ospedale S. Anna,
Ferrara, Italy

P. Geppetti (✉) • M. Trevisani • P. Nicoletti
G. Zagli
Clinical Pharmacology Unit,
Department of Critical Care Medicine and Surgery, University of Florence,
Viale Pieraccini 6, I-50139 Florence, Italy
e-mail: pierangelo.geppetti@unifi.it
Tel.: +39-055-4271329
Fax: +39-055-4271280

Abstract For more than a century neurogenic inflammation has been proposed to have a role in various human diseases. The present review will cover the conceptual steps of the itinerary that has led to the conclusion that neurogenic inflammation is important in migraine. Of particular relevance for the object of this article is the observation that tachykinin-dependent neurogenic inflammatory responses are evident in rodents, but much less pronounced or absent in other mammal species, including man, whereas neurogenic vasodilatation, most likely mediated by CGRP, occurs in most mammalian species and also in man. Recent evidence that a CGRP receptor antagonist was effective in the treatment of migraine attack supports the hypothesis that neurogenic vasodilatation is a major underlying mechanism of migraine.

Key words Calcitonin gene-related peptide • Neurogenic inflammation • Transient receptor potential vanilloid-1 • Migraine • Vasodilatation

Neurogenic inflammation: mechanisms and species differences

The term 'neurogenic inflammation' refers to a series of proinflammatory responses produced by the stimulation of peripheral terminals of a subset of primary sensory neurons and the subsequent release of the neuropeptides, calcitonin gene-related peptide (CGRP) and the tachykinins, substance P (SP) and neurokinin A (NKA) [1]. The neurons that produce inflammation comprise a heterogeneous cell population with A-delta and C fibres, defined as polymodal nociceptors because they sense thermal, chemical and high-threshold mechanical stimuli. These neurons express on their plasma membrane a large panel of excitatory and inhibitory receptors and channels, and some of these signalling proteins have been successfully used as targets for analgesic or anti-inflammatory drugs. Among the channels expressed on peptidergic primary sensory neurons, transient receptor potential vanilloid-1 (TRPV1) [2], activated by the xenobiotic capsaicin, the pungent ingredient of plants of the genus *Capsicum*, is of paramount importance. Capsaicin produces burning pain by stimulating TRPV1 and by releasing sensory neuropeptides causes neurogenic inflammation. However, high concentrations/doses of capsaicin have the ability, after an initial excitatory phase, to desensitise the sensory nerve terminals, thus reducing the transmission of sensory/pain signals and abolishing neurogenic inflammation [3, 4]. This specific feature of capsaicin has greatly contributed to define the role of this subset of sensory nerves in pathophysiological models of human diseases and has been

used as a therapeutic strategy in certain pain and inflammatory conditions [1]. From a large body of evidence accumulated in the last few decades, the general hypothesis has originated that neurogenic inflammation is the underlying mechanism in different human diseases, including migraine and other primary headaches [1].

Neurogenic inflammation occurs mainly at the vascular levels, where in almost every organ of mammals it comprises CGRP-mediated arteriole vasodilatation and tachykinin-mediated plasma protein extravasation and leukocyte adhesion to the venule endothelium. In extravascular tissues, marked differences exist according to tissue and species. Thus, for example, in the airways tachykinins mediate an epithelium-dependent bronchodilatation in the mouse and rat, whereas in guinea pigs and man severe bronchoconstriction is produced [1]. In contrast with the guinea pig, in the urinary bladder of the rat the dilatation of the bladder produced by CGRP is absent, while tachykinin-mediated contraction prevails [1]. More recent evidence obtained with the advent of specific and high affinity antagonists for tachykinin and CGRP receptors have clarified that species-dependent differences in neurogenic inflammation responses exist also at the vascular level. These differences have markedly influenced the process through which the knowledge of the contribution of sensory nerves and neurogenic inflammation in migraine has been developed.

Vascular components of neurogenic inflammation

Biological effects produced by tachykinins are mediated by three different heterotrimeric G-protein coupled receptors, the NK1, NK2 and NK3 receptors. These receptors are associated with $G_{q/11}$ proteins and their stimulation results in the mobilisation of Ca^{2+} ions in the cytosol. Neurogenic plasma protein extravasation occurs at the level of postcapillary venules where SP and NKA, released from sensory nerve terminals, stimulate NK1 receptors on endothelial cells, thus increasing Ca^{2+} mobilisation and activating intracellular contractile elements. The contraction causes the opening of gaps between endothelial cells, a phenomenon that allows the passage of macromolecules, including albumin, into the interstitial space and the formation of inflammatory oedema. Leukocyte adhesion to the venule endothelium, also mediated by NK1 receptor activation [5], follows and potentiates the early inflammatory responses and is accompanied by mast cell degranulation and inflammatory mediator production. Evidence that trigeminal nerve stimulation results in plasma protein extravasation in the meninges of rodents suggested that this mechanism could be important for the clinical manifestation of migraine [6]. The observation that triptans blocked, presumably at a prejunctional site of action, neurogenic plasma extravasation, further corroborated the hypothesis [7]. The discovery of non-peptidic, high affinity and selective NK1 receptor antagonists allowed the design of clinical studies aimed to verify whether NK1 receptor blockade could be beneficial in the migraine attack. However, more than one antagonist with a good pharmacodynamic and pharmacokinetic profile resulted non-effective in clinical trials [8, 9].

A careful critical analysis of the features of neurogenic inflammation in man should have cast doubts regarding the effectiveness of NK1 blockers to ameliorate migraine headaches. Capsaicin administration to human skin causes a remarkable erythema and arteriolar vasodilatation [10], presumably mediated by CGRP release, but there is little or no evidence that capsaicin causes cutaneous plasma extravasation. Whereas in man SP and NKA potently contract the bronchi and the urinary bladder, capsaicin does not. Finally, failure of capsaicin to release SP/NKA from human sensory nerve terminals *in vitro* has been reported [11]. From these findings it may be concluded that if the release of sensory tachykinins occurs in human tissues, this would not be sufficient to produce the biological effects commonly observed in rodents. In contrast, the observations that capsaicin application to the human skin produces a neurogenic flare response, most likely mediated by CGRP [10, 12] and that capsaicin releases CGRP from human tissues *in vitro* [11, 13] have important implications for our current understating of migraine mechanism and the treatment of migraine attack. Mechanistic studies in experimental animals and a recent clinical trial have corroborated the hypothesis that sensory CGRP and neurogenic inflammation have a central role in migraine pathogenesis.

Calcitonin-related peptides

In 1982, it was discovered that alternative processing of RNA transcripts from the calcitonin gene results in the production of distinct mRNAs encoding a 37-amino acid neuropeptide, called CGRP [14]. Whereas the calcitonin mRNA predominates in the thyroid, the specific mRNA for CGRP is abundant in the nervous system [15]. Subsequently, a human form of CGRP was isolated from thyroid tissue of patients with medullary thyroid carcinoma [16]. CGRP is now known to belong to a family that includes the more recently discovered peptides adrenomedullin (AM) and amylin. AM was isolated from human pheochromocytoma cells in 1993 as a 52-amino acid peptide, which was able to stimulate cAMP production in platelets [17]. Endothelial and vascular smooth muscle cells, especially upon stimulation with inflammatory

cytokines, are among the many cell types that can produce AM [18]. The amyloid deposits in the pancreas of non-insulin-dependent diabetics [19] were the source of the 37-amino acid peptide, amylin, or islet amyloid polypeptide (IAPP), which shares some structural homology with CGRP and AM. Amylin is secreted with insulin from pancreatic β-cells after meals, and its major physiological effect is the regulation of glucose metabolism in the opposite manner to insulin with respect to glycogen synthesis and glucose uptake into muscle [20].

CGRP peptides

The two isoforms of CGRP, α and β, are found in most species, differ by one and three amino acids, and exhibit similar functional activities [14, 16]. One of the two forms, αCGRP, encoded by the calcitonin gene, is the more abundant and found in discrete areas of the central and peripheral nervous system. In man, βCGRP, which differs from αCGRP by three amino acids, is primarily located within enteric nerves [21] and the pituitary gland [22]. The nerve growth factor (NGF) is of outmost importance in influencing plasticity of CGRP because it regulates the growth and maintenance of sensory nerve function [23]. The half-life of CGRP in the circulation is ~7–10 min in the human plasma [24]. CGRP metabolism is not regulated by an identified proteolytic pathway and it is probably broken down *via* a number of routes. Efficient cleavage of CGRP into inactive fragments is produced by mast cell tryptase in the skin [25], and matrix metalloproteinase II has the ability to metabolise CGRP and remove its vasodilator activity [26], whereas CGRP is a poor substrate for neutral endopeptidase, so this pathway is probably less important as a route for CGRP degradation in peripheral tissues [27].

CGRP and its receptor: a unique story

In the late 1980s, the existence of two receptors was originally proposed: CGRP1 and CGRP2, with the CGRP1 receptor being the predominant mediator of CGRP effects in the guinea pig or rat atrium, whereas the CGRP2 receptor activity was identified by the CGRP-induced inhibition of electrically evoked twitch responses in the rat vas deferens [28]. $CGRP_{8–37}$, a 30-amino acid fragment of CGRP, behaves as an antagonist and shows a relative selectivity for the CGRP1 receptor [29]. Rat calcitonin receptor-like receptor (CL) was cloned in 1993 [30]. The 7 transmembrane domains human isoform of rat CL was cloned 2 years later and consists of 461 amino acids with 91 and 56% identity to the rat orphan calcitonin receptor-like sequence and the human calcitonin receptor, respectively [31]. Because this receptor did not bind CGRP in the cells studied, it was considered an orphan receptor. However, when the cDNA was expressed in human embryonic kidney 293 (HEK293) cells it exhibited specific, high-affinity binding sites for CGRP and displayed functional properties, including increase in cAMP production, similar to the human CGRP1 receptor [32]. However, because CL expression in COS-7 cells failed to produce a functional receptor, it was concluded that HEK293 cells must also possess a factor essential for the production of a functional receptor. The puzzle was solved after the discovery of the receptor activity-modifying protein (RAMP), a single transmembrane domain protein with 148 amino acids necessarily required to associate with CL to confer receptor activity [33]. Receptors for CGRP/AM that have been cloned and characterised so far consist of a seven-transmembrane G protein-coupled CL receptor in association with one of three single membrane-spanning RAMPs. RAMP1 associated with CL produces a CGRP receptor (CGRP1) that is antagonised by the CGRP antagonist $CGRP_{8–37}$ (Table 1). In particular, it has been shown that

Table 1 Receptors for calcitonin and related peptides

	Calcitonin	Amylin (AMY)	CGRP	Adrenomedullin (AM)
Composition	*CALCR*	AMY-1*: CALCR+RAMP1* AMY-2*: CALCR+RAMP2* AMY-3*: CALCR+RAMP3*	*CALCRL+RAMP1*	AM-1*: CALCRL+RAMP2* AM-2*: CALCRL+RAMP3*
Transduction pathway	G_s/G_q	G_s	G_s/G_q	G_s
Selective agonists	Human CT	AMY	α-CGRP	AM
Selective antagonists	–	–	BIBN4096BS (+++) SB-273779 (+)	AMn_{22-52}
Potency	Salmon CT≥human CT≥AMY, CGRP>AM	Salmon CT≥AMY≥ CGRP>human CT>AM	CGRP>AM≥ AMY≥salmon CT	AM-1: AM>>CGRP> AMY>salmon CT AM-2: AM≥CGRP> AMY>salmon CT

the N-terminus of RAMP1 is the key determinant for CGRP binding, which could be due to the interaction of calcitonin receptor-like receptor with the RAMP1 N-terminus [34]. RAMP2 association with CL results in the AM (AM1) receptor that can be antagonised by the weak AM peptide antagonist $AM_{22–52}$ and the association of RAMP3 with CL produces another AM receptor (AM2).

CGRP receptor antagonists

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*)]), is a competitive non-peptide potent antagonist at the human CGRP1 receptor [35]. Its affinity (K_i, 14.4±6.3 pM) for human CGRP1 receptors in a human neuroblastoma cell line (SK-N-MC cells) was remarkably high. Other antagonists with lower affinities are 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) and SB-273779 [*N*-methyl-*N*-(2-methylphenyl)-3-nitro-4-(2-thiazolylsulfinyl)-nitrobenzanilide]. Compound 1 showed a pK_i of 7.8 in binding experiments on SK-N-MC as compared to 8.9 of $CGRP_{8-37}$ [36]. SB-273779 is selective for the CGRP receptor but is less potent than BIBN4096BS, with a K_i value of 310±40 nM on SK-N-MC cells, and it also exhibited low potency in rat and porcine lungs [37].

Prejunctional modulation of CGRP release

The release of sensory neuropeptides, including CGRP, undergoes a fine tuning by a series of mediators and agents that act at prejunctional receptors/channels on sensory nerve terminals. Evidence indicates a range of G protein-coupled receptors that include those for bradykinin, prostanoids, opioids, 5-hydroxytryptamine ($5\text{-}HT_1$ receptor), histamine (H_3 receptor), neuropeptide Y, somatostatin, vasoactive intestinal polypeptide, purines and galanin. Among the channels the TRPV1 is the best known and it can be activated by noxious temperatures, low extracellular pH and a series of lipid derivatives [2, 38–41]. TRPV1 activity can be regulated by the stimulation of certain G protein coupled receptors (bradykinin and prostaglandins) or tyrosine kinase receptor (NGF) [42, 43]. Other pathophysiologically relevant agents may also regulate TRPV1. The recent observation that ethanol stimulates TRPV1 channels and releases CGRP, by lowering the threshold temperature for channel activation [44], suggests that the ability of alcoholic beverages to trigger the migraine attack may be due to this neurogenic inflammatory mechanism. The presence of excitatory CGRP receptors on sensory neurons, within the dorsal root ganglia, which may act as stimulatory autoreceptors, has been reported recently [45]. Inhibition of CGRP release by α_2-adrenoceptors, located presynaptically on sensory neurons [46], and the ability of CGRP to inhibit the release of norepinephrine from sympathetic nerves [47] in the rat mesentery circulation, suggest that reciprocal interactions can occur between the noradrenergic constrictor system and the sensory (vasodilatatory) system, and are indicative of an important role for CGRP in the regulation of peripheral blood flow.

Arterial relaxation by CGRP

The vascular effects of CGRP appear most pronounced in the microvasculature. CGRP is one of the most potent vasodilator substances identified to date in the microcirculation, with a potency ~10-fold greater than the prostaglandins and 2–3 orders of magnitude greater than other classic vasodilators, including acetylcholine, adenosine, 5-HT and SP. The role of CGRP in the regulation of vascular tone in small vessels is underlined by the observation that CGRP-containing nerves can pass into the vascular smooth muscle layer in smaller arteries. CGRP released at these sites can have profound effects on arteriolar dilatation and on the microvasculature. Although CGRP-containing nerves also innervate venous tissues, its activity on this type of vessels has been less investigated. Current evidence indicates that administration of exogenous CGRP or the release of endogenous CGRP produces an NO- and endothelium-independent relaxation that correlates closely with a rise in intracellular cAMP, activation of protein kinase A (PKA), and activation of K^+ channels (Fig. 1). The involvement of ATP-sensitive potassium channels [48] in the vasodilator mechanism of CGRP has been suggested and subsequently extended to channels sensitive to glibenclamide or charybdotoxin (large-conductance Ca^{2+}-activated K^+ channels) in rat pial arterioles [49].

Exceptions to endothelium-independent relaxation to CGRP occur only in a few tissues, including the rat aorta, where the relaxation to CGRP depends on the presence of an intact endothelium and is attenuated by inhibitors of NO synthase, implying an NO-dependent mechanism [12, 50]. In human internal mammary artery [51] and rat pulmonary artery [52], a similar endothelium-dependent mechanism of relaxation has also been seen. Evidence that CGRP activates phospholipase C in HEK293 cells, lead-

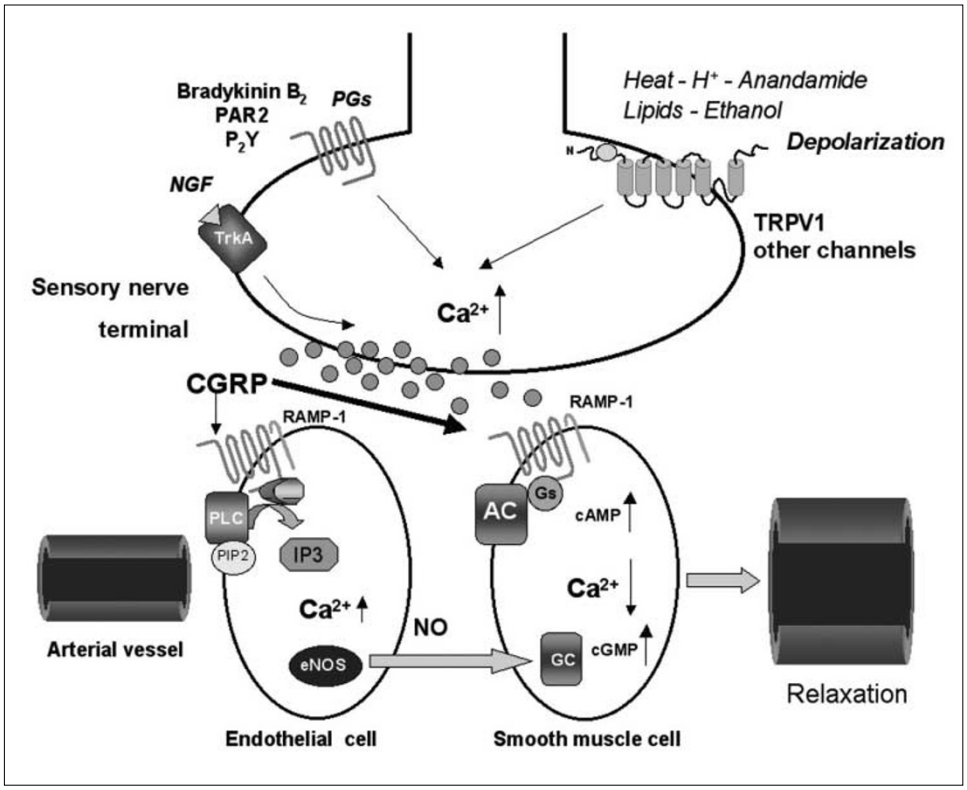

Fig. 1 The drawing depicts the mechanisms by which CGRP is released from the terminal of TRPV1-expressing sensory neurons and produces arterial vasodilatation

ing to an increase in intracellular Ca^{2+} via inositol trisphosphate (IP_3) [53] suggests that this alternative intracellular pathway may be responsible for the CGRP-induced release of NO (Fig. 1). Other peptides of the CGRP family may also produce vascular relaxation. There is evidence that AM acts to relax porcine coronary artery preconstricted with U46619 [54]. The vasodilator effects of amylin appear to be mediated by the CGRP1 receptor as they are blocked by $CGRP_{8–37}$.

Migraine and neurogenic inflammation

Our current comprehension of the migraine mechanism is still incomplete. However, it is possible that genetic abnormalities initiate the alteration of the response threshold to migraine-specific triggers in the brain [55]. Studies using positron emission tomography have shown increased blood flow (an index of neuronal activity) during spontaneous migraine attacks in the cerebral hemispheres (cingulate, auditory and visual suppression areas) and suggest that the process driving the migraine attack and the area susceptible to the migraine triggers may be located in the brain stem [56]. Neural activation eventually results in the dilatation of cranial blood vessels including carotid arteriovenous anastomoses [57]. There is also evidence in experimental animals that the migraine aura, rather than from an ischaemic phenomenon, derives from an initial neuronal dysfunction and is probably the clinical manifestation of a cortical spreading depression [58, 59]. A reflex painful vasodilatation of the cranial blood vessels follows the cerebral oligaemia, and probably derives from activation of the trigeminal perivascular innervation [55, 57].

Stimulation of trigeminal ganglia/sensory nerves is regarded as a necessary step for the process that produces pain and associated symptoms of the migraine attack. Trigeminal neurons may release neuropeptides and cause tachykinin-dependent plasma extravasation in cranial tissues, including the meninges and CGRP-dependent arterial vasodilatation. The hypothesis that neurogenic, tachykinin-mediated plasma protein extravasation could be the underlying mechanism of the migraine attack [6] gained support from the findings that specific antimigraine drugs, including sumatriptan, are able to suppress plasma extravasation produced by antidromic stimulation of trigeminal nerve terminals in the rodent meninges [7]. As mentioned before, this response is entirely mediated by tachykinin NK1 receptors localised on postcapillary endothelial cells. Thus, it was proposed that NK1 receptor antagonists could be beneficial in migraine treatment. However, various clinical trials showed that different NK1 receptor antagonists, including RPR100893 [9], GR205171 [60] or lanepitant [8], were not effective for

the acute treatment of the migraine attack or as pre-emptive treatment of migraine [61]. Although the failure could be due to a variety of pharmacokinetic or other reasons, unrelated to the role of neurogenic plasma extravasation in migraine pathogenesis, it is also possible that tachykinin release, activation of NK1 receptors and their action in neurogenic inflammation do not play any role in this disease.

In contrast, in recent years the hypothesis that the release of CGRP, which dilates cranial blood vessels and stimulates sensory nerve transmission, may have a key role in migraine mechanism, has obtained much attention and experimental support [62]. Trigeminal nuclei as well as non-myelinated trigeminal sensory nerve fibres express an abundant CGRP-like immunoreactivity and cerebral blood vessels are innervated by sensory nerves that store several neuropeptides amongst which CGRP is the most abundant [63]. A Ca^{2+}-dependent and capsaicin-sensitive release of CGRP, but not SP, has been documented from human tissues containing non-trigeminal [13] or trigeminal [11] sensory nerve endings. Furthermore, plasma concentrations of CGRP, but not of SP, were elevated during the headache phase of migraine [64] and cluster headache [65]. There is evidence that intravenous infusion of CGRP produces a migraine-like headache [66], and intravenous infusion of nitric oxide produces a migraine-like headache with an associated increase in plasma CGRP levels [67]. In migraine patients baseline CGRP levels were considerably higher, and the changes in plasma CGRP levels during migraine attacks were significantly correlated with the headache intensity [67].

Action of drugs effective on neurogenic vasodilatation

Ergotamine and triptans have been proposed to abort migraine attacks by diverse mechanisms, including constriction of dilated cranial blood vessels and carotid arteriovenous anastomoses [68], reduction of CGRP release from perivascular trigeminal nerve endings, and inhibition of nociceptive transmission on peripheral and central endings of trigeminal sensory nerves [55]. Similarly to rodents, human trigeminal ganglia/sensory nerves express abundant 5-$HT_{1B/1D}$ receptors [69], thus strengthening the evidence in favour of the presynaptic inhibitory effects of triptans [62]. This effect of triptans results from the stimulation of the heterotrimeric G protein-coupled 5-$HT_{1B/1D}$ receptor that, by inhibiting adenylyl cyclase and cyclic adenosine monophosphate cytosol levels, reduces neural activity, including the neuropeptide release. The principle of inhibiting neuropeptide release from sensory nerve endings by reducing neuronal functioning represents the basis of the antimigraine action of the agonist of the inhibitory adenosine A1 receptor, GR79236 (*N*-[(2-methylphenyl)methyl]adenosine (metrifudil), 2-(phenylamino) adenosine). This compound, stimulating A1 receptors, inhibits neurogenic vasodilatation in rats [70], trigeminal nociception and CGRP release in cats [71], and trigeminal nociception in humans [72]. Pilot clinical studies have reported that GR79236 has antimigraine action, probably due to an inhibitory effect on nociceptive trigeminal neurons [70]. Despite these encouraging results, further studies are required to fully establish the efficacy and safety of adenosine A1 receptor agonists in migraine.

The adverse effects of triptans and the limitation of their use in patients with cardiovascular diseases derive from the ability of this type of drug to maintain the same vasoconstrictor activity. Selective agonists of the 5-HT_{1D} receptor, such as PNU-109291 [(s)-3,4-dihydro-1-ethyl]-*N*-methyl-1H-2-benzopyran-6-carboximide) [73] or 5-HT_{1F} receptors such as LY334370 (4-fluoro-*N*-[3-(1-methyl-4-piperidinyl)-1H-indol-5-yl]-benzamide) [74] have been developed with the idea that they should exert an antimigraine effect by a prejunctional inhibition of neural activity, and should be devoid of any vascular constrictor action. However, PNU-142633 proved to be ineffective in the acute treatment of migraine [75] and LY334370 did show efficacy when used in doses which may interact with 5-HT1B receptors [74, 76]. Thus, further investigation is required to establish whether non-vasoconstrictor triptans or other compounds acting at prejunctional levels of terminals of primary sensory neurons are useful in the treatment of migraine and related headaches.

CGRP antagonists in migraine

The alternative approach to treat the migraine attack, by limiting neurogenic inflammatory vasodilatation, consists in the blockade of CGRP receptor by selective antagonists. The first peptide CGRP receptor antagonist, $CGRP_{8-37}$, proved ineffective in migraine treatment [77]. However, its low potency and very short half-life [29] precluded any firm conclusion on the role of CGRP, its receptor and neurogenic vasodilatation in migraine mechanism. The discovery of the peptoid BIBN4096BS, an antagonist characterised by an exceedingly high affinity for the human CGRP receptor [35], offered evidence in experimental animals that indicated that this drug is a suitable tool to prove the concept that CGRP exerts a pivotal role in the migraine pathogenesis. BIBN4096BS was found to reduce the vasodilatation induced by trigeminal stimulation in marmosets [78], the vasodilator responses induced

by capsaicin in porcine carotid, including carotid arteriovenous anastomotic dilatation, and CGRP-induced porcine carotid vasodilatation and arterial-jugular venous oxygen saturation difference [79]. Thus, BIBN4096BS could produce, at a postjunctional level (by CGRP receptor blockade), effects similar to those that triptans cause by reducing CGRP release, and that result in inhibition of CGRP receptor signalling. Indeed, triptans inhibit trigeminal CGRP release in animal experimental models [55, 63], and clinical data show that sumatriptan normalised the elevated CGRP levels with alleviation of migraine [55] and cluster headache attack [65]. These findings have suggested that BIBN4096BS could be developed as an effective antimigraine drug.

A recent clinical trial has shown that BIBN4096BS is effective in the acute treatment of migraine without significant side effects or intrinsic vasoconstrictor effects [80]. The CGRP receptor antagonist at a dose of 2.5 mg was effective in the treatment of the migraine attack with a response rate of 66% as compared with 27% for placebo. BIBN4096BS also showed significant superiority over placebo in reducing the pain at 2 h and improving the nausea, photophobia, phonophobia, functional capacity and the time to meaningful relief [80]. Side effects were 25% with BIBN4096BS and 12% with placebo. The most frequent side effect was paraesthesia and no serious adverse effect was recorded. Intra- and extracranial vessels and systemic haemodynamics have been studied in man following BIBN4096BS administration. BIBN4096BS had no influence on global or regional cerebral blood flow and did not show any effect on systemic haemodynamics and adverse events were minor [81]. Thus, CGRP-receptor blockade does not seem to affect cerebral or systemic circulation in humans. From this finding it is possible to hypothesise that circulating CGRP in basal condition does exert a tonic vasodilatatory activity and the use of BIBN4096BS or other CGRP receptor antagonists should be without risk of cerebral or systemic vasospasm.

Conclusions

Growing evidence indicates inhibition of CGRP activity as a major avenue in the treatment of the migraine attack. Inhibition of neural activity that produces neuropeptide release from peripheral and central endings of peptidergic trigeminal neurons has been proposed as one of the key antimigraine actions of triptans. However, because these drugs reduce both tachykinin and CGRP release, the identity of the neuropeptide principally involved in migraine pathogenesis remained obscure. The uncertainty regarding the role of one specific neuropeptide mirrors the doubt regarding the component of neurogenic inflammation (plasma extravasation *vs.* arterial vasodilatation) contributing most to migraine pathogenesis. More recent clinical findings reporting the failure of NK1 receptor antagonists and the success of one CGRP receptor antagonist have indicated a major role for CGRP and neurogenic vasodilatation in migraine mechanism. However, waiting for further clinical confirmation of the antimigraine activity of BIBN4096BS or additional CGRP receptor antagonists, several questions remain open, and among them, two are of importance for further development of novel antimigraine therapeutics. Although the possible target of BIBN4096BS is a CGRP receptor expressed on the plasma membrane of vascular smooth muscle of cranial arteries, the precise location (neural *vs.* vascular) of the CGRP receptors that mediate pain and other symptoms of the migraine attack are unknown. The second point of uncertainty deals with the safety profile. Further investigation is, in fact, required to establish whether or not blockade of CGRP receptor is devoid of any possible vasoconstrictor activity in clinical conditions in which the sensory nerve terminal is activated and CGRP released.

Acknowledgements The paper has been supported in part by grants from Consorzio Ferrara Ricerche, Ferrara and by Fondazione DEI-Onlus, Florence, Italy.

References

1. Geppetti P, Holzer P (1996) Neurogenic inflammation. CRC Press, Boca Raton
2. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824
3. Szolcsanyi J (1977) A pharmacological approach to elucidation of the role of different nerve fibres and receptor endings in mediation of pain. J Physiol (Paris) 73:251–259
4. Szallasi A, Blumberg PM (1999) Vanilloid (capsaicin) receptors and mechanisms. Pharmacol Rev 51:159–212
5. Baluk P, Bertrand C, Geppetti P, McDonald DM, Nadel JA (1995) NK1 receptors mediate leukocyte adhesion in neurogenic inflammation in the rat trachea. Am J Physiol 268:L263–L269
6. Markowitz S, Saito K, Moskowitz MA (1987) Neurogenically mediated leakage of plasma protein occurs from blood vessels in dura mater but not brain. J Neurosci 7:4129–4136
7. Moskowitz MA, Buzzi MG (1991) Neuroeffector functions of sensory fibres: implications for headache mechanisms and drug actions. J Neurol 238:S18–S22
8. Goldstein DJ, Wang O, Saper JR, Stoltz R, Silberstein SD, Mathew NT (1997) Ineffectiveness of neurokinin-1 antagonist in acute migraine: a crossover study. Cephalalgia 17:785–790

9. Diener HC (2003) RPR100893, a substance-P antagonist, is not effective in the treatment of migraine attacks. Cephalalgia 23:183–185
10. Simone DA, Ochoa J (1991) Early and late effects of prolonged topical capsaicin on cutaneous sensibility and neurogenic vasodilatation in humans. Pain 47:285–294
11. Geppetti P, Del Bianco E, Cecconi R, Tramontana M, Romani A, Theodorsson E (1992) Capsaicin releases calcitonin gene-related peptide from the human iris and ciliary body in vitro. Regul Pept 41:83–92
12. Brain SD, Williams TJ, Tippins JR, Morris HR, MacIntyre I (1985) Calcitonin gene-related peptide is a potent vasodilator. Nature 313:54–56
13. Franco-Cereceda A (1991) Calcitonin gene-related peptide and human epicardial coronary arteries: presence, release and vasodilator effects. Br J Pharmacol 102:506–510
14. Amara SG, Arriza JL, Leff SE, Swanson LW, Evans RM, Rosenfeld MG (1985) Expression in brain of a messenger RNA encoding a novel neuropeptide homologous to calcitonin gene-related peptide. Science 229:1094–1097
15. Quirion R, Van Rossum D, Dumont Y, St-Pierre S, Fournier A (1992) Characterization of CGRP1 and CGRP2 receptor subtypes. Ann N Y Acad Sci 657:88–105
16. Morris HR, Panico M, Etienne T, Tippins J, Girgis SI, MacIntyre I (1984) Isolation and characterization of human calcitonin gene-related peptide. Nature 308:746–748
17. Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H, Eto T (1993) Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. Biochem Biophys Res Commun 192:553–560
18. Sugo S, Minamino N, Kangawa K, Miyamoto K, Kitamura K, Sakata J, Eto T, Matsuo H (1994) Endothelial cells actively synthesize and secrete adrenomedullin. Biochem Biophys Res Commun 201:1160–1166
19. Cooper GJS, Willis AC, Clark A, Turner RC, Sim RB, Reid KBM (1987) Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients. Proc Natl Acad Sci 84:8628–8632
20. Cooper GJS, Leighton B, Dimitriadis GD, Parry-Billings M, Kowalchuk JM, Howland K, Rothbard JB, Willis AC, Reid KBM (1988) Amylin found in amyloid deposits in human type 2 diabetes mellitus may be a hormone that regulates glycogen metabolism in skeletal muscle. Proc Natl Acad Sci 85:7763–7766
21. Mudderry PK, Ghatei MA, Spokes RA, Jonhs PM, Pierson AM, Hamid QA, Kanse S, Amara SG, Burrik JM, Legon S (1988) Differential expression of [alpha]-CGRP and [beta]-CGRP by primary sensory neurons and enteric autonomic neurons of the rat. Neuroscience 25:195–205
22. Petermann J, Born W, Chang J, Fischer J (1987) Identification in the human central nervous system, pituitary, and thyroid of a novel calcitonin gene-related peptide, and partial amino acid sequence in the spinal cord. J Biol Chem 262:542–545
23. Terenghi G (1999) Peripheral nerve regeneration and neurotrophic factors. J Anat 194:1–14
24. Struthers AD, Brown MJ, Macdonald DW, Beacham JL, Stevenson JC, Morris HR, MacIntyre I (1986) Human calcitonin gene related peptide: a potent endogenous vasodilator in man. Clin Sci (Lond) 70:389–393
25. Brain SD, Williams TJ (1989) Interactions between the tachykinins and calcitonin gene-related peptide lead to the modulation of oedema formation and blood flow in rat skin. Br J Pharmacol 97:77–82
26. Fernandez-Patron C, Stewart KG, Zhang Y, Koivunen E, Radomski MW, Davidge ST (2000) Vascular matrix metalloproteinase-2-dependent cleavage of calcitonin gene-related peptide promotes vasoconstriction. Circ Res 87:670–676
27. Katayama M, Nadel JA, Bunnett NW, Di Maria GU, Haxhiu M, Borson DB (1991) Catabolism of calcitonin gene-related peptide and substance P by neutral endopeptidase. Peptides 12:563–567
28. Dennis T, Fournier A, St Pierre S, Quirion R (1989) Structure-activity profile of calcitonin gene-related peptide in peripheral and brain tissues. Evidence for receptor multiplicity. J Pharmacol Exp Ther 251:718–725
29. Chiba T, Yamaguchi A, Yamatani T, Nakamura A, Morishita T, Inui T, Fukase M, Noda T, Fujita T (1989) Calcitonin gene-related peptide receptor antagonist human CGRP-(8-37). Am J Physiol Endocrinol Metab 256:E331–E335
30. Njuki F, Nicholl CG, Howard A, Mak JC, Barnes PJ, Girgis SI, Legon S (1993) A new calcitonin-receptor-like sequence in rat pulmonary blood vessels. Clin Sci (Lond) 85:385–388
31. Fluhmann B, Muff R, Hunziker W, Fischer JA, Born W (1995) A human orphan calcitonin receptor-like structure. Biochem Biophys Res Commun 206:341–347
32. Aiyar N, Rand K, Elshourbagy NA, Zeng Z, Adamou JE, Bergsma DJ, Li Y (1996) A cDNA encoding the calcitonin gene-related peptide type 1 receptor. J Biol Chem 271:11325–11329
33. McLatchie LM, Fraser NJ, Main MJ, Wise A, Brown J, Thompson N, Solari R, Lee MG, Foord SM (1998) RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. Nature 393:333–339
34. Foord SM, Wise A, Brown J, Main MJ, Fraser NJ (1999) The N-terminus of RAMPs is a critical determinant of the glycosylation state and ligand binding of calcitonin receptor-like receptor. Biochem Soc Trans 27:535–539
35. Doods H, Hallermayer G, Wu D, Entzeroth M, Rudolf K, Engel W, Eberlein W (2000) Pharmacological profile of BIBN4096BS, the first selective small molecule CGRP antagonist. Br J Pharmacol 129:420–423
36. Edvinsson L, Sams A, Jansen-Olesen I, Tajti J, Kane SA, Rutledge RZ, Koblan KS, Hill RG, Longmore J (2001) Characterisation of the effects of a non-peptide CGRP receptor antagonist in SK-N-MC cells and isolated human cerebral arteries. Eur J Pharmacol 415:39–44
37. Aiyar N, Daines RA, Disa J, Chambers PA, Sauermelch CF, Quiniou M-J, Khandoudi N, Gout B, Douglas SA, Willette RN (2001) Pharmacology of SB-273779, a nonpeptide calcitonin gene-related peptide 1 receptor antagonist. J Pharmacol Exp Ther 296:768–775

38. Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Di Marzo V, Julius D, Hogestatt ED (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 400:452–457
39. Hwang SW, Cho H, Kwak J, Lee SY, Kang CJ, Jung J, Cho S, Min KH, Suh YG, Kim D, Oh U (2000) Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. Proc Natl Acad Sci USA 97:6155–160
40. Huang SM, Bisogno T, Trevisani M, Al-Hayani A, De Petrocellis L, Fezza F, Tognetto M, Petros TJ, Krey JF, Chu CJ, Miller JD, Davies SN, Geppetti P, Walker JM, Di Marzo V (2002) An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. Proc Natl Acad Sci USA 99:8400–8405
41. Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D (1998) The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 21:531–543
42. Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, Julius D (2001) Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. Nature 411:957–962
43. Premkumar LS, Ahern GP (2000) Induction of vanilloid receptor channel activity by protein kinase C. Nature 408:985–990
44. Trevisani M, Smart D, Gunthorpe MJ, Tognetto M, Barbieri M, Campi B, Amadesi S, Gray J, Jerman JC, Brough SJ, Owen D, Smith GD, Randall AD, Harrison S, Bianchi A, Davis JB, Geppetti P (2002) Ethanol elicits and potentiates nociceptor responses via the vanilloid receptor-1. Nat Neurosci 5:546–551
45. Segond von Banchet G, Pastor A, Biskup C, Schlegel C, Benndorf K, Schaible H-G (2002) Localization of functional calcitonin gene-related peptide binding sites in a subpopulation of cultured dorsal root ganglion neurons. Neuroscience 110:131–145
46. Kawasaki H, Nuki C, Saito A, Takasaki K (1990) Adrenergic modulation of calcitonin gene-related peptide (CGRP)-containing nerve-mediated vasodilation in the rat mesenteric resistance vessel. Brain Res 506:287–290
47. Kawasaki H, Nuki C, Saito A, Takasaki K (1990) Role of calcitonin gene-related peptide-containing nerves in the vascular adrenergic neurotransmission. J Pharmacol Exp Ther 252:403–409
48. Nelson MT, Huang Y, Brayden JE, Hescheler J, Standen NB (1990) Arterial dilations in response to calcitonin gene-related peptide involve activation of K+ channels. Nature 344:770–773
49. Hong KW, Yoo SE, Yu SS, Lee JY, Rhim BY (1996) Pharmacological coupling and functional role for CGRP receptors in the vasodilation of rat pial arterioles. Am J Physiol Heart Circ Physiol 270:H317–H323
50. Gray DW, Marshall I (1992) Human alpha-calcitonin gene-related peptide stimulates adenylate cyclase and guanylate cyclase and relaxes rat thoracic aorta by releasing nitric oxide. Br J Pharmacol 107:691–696
51. Raddino R, Pela G, Manca C, Barbagallo M, D'Aloia A, Passeri M, Visioli O (1997) Mechanism of action of human calcitonin gene-related peptide in rabbit heart and in human mammary arteries. J Cardiovasc Pharmacol 29:463–470
52. Wisskirchen FM, Burt RP, Marshall I (1998) Pharmacological characterization of CGRP receptors mediating relaxation of the rat pulmonary artery and inhibition of twitch responses of the rat vas deferens. Br J Pharmacol 123:1673–1683
53. Aiyar N, Disa J, Stadel JM, Lysko PG (1999) Calcitonin gene-related peptide receptor independently stimulates 3´,5´-cyclic adenosine monophosphate and Ca2+ signaling pathways. Mol Cell Biochem 197:179–185
54. Yoshimoto R, Mitsui-Saito M, Ozaki H, Karaki H (1998) Effects of adrenomedullin and calcitonin gene-related peptide on contractions of the rat aorta and porcine coronary artery. Br J Pharmacol 123:1645–1654
55. Goadsby PJ, Lipton RB, Ferrari MD (2002) Migraine-current understanding and treatment. N Engl J Med 346:257–270
56. Welch KMA (2003) Concepts of migraine headache pathogenesis: insights into mechanisms of chronicity and new drug targets. Neurol Sci 24:S149–S153
57. De Vries P, Willems EW, Heiligers JPC, Villalon CM, Saxena PR (1998) The antimigraine agent alniditan selectively constricts porcine carotid arteriovenous anastomoses via 5-HT1B/1D receptors. Eur J Pharmacol 351:193–201
58. Leao AA (1951) The slow voltage variation of cortical spreading depression of activity. Electroencephalogr Clin Neurophysiol 3:315–321
59. James MF, Smith JM, Boniface SJ, Huang CLH, Leslie RA (2001) Cortical spreading depression and migraine: new insights from imaging? Trends Neurosci 24:266–271
60. Connor H, Bertin L, Gillies S, Beattie D, Ward P (1998) The GR205171 Clinical Study Group. Clinical evaluation of a novel, potent, CNS penetrating NK1 receptor antagonist in the acute treatment of migraine. Cephalalgia 18:392
61. Goldstein DJ, Offen WW, Klein EG, Phebus LA, Hipskind P, Johnson KW, Ryan RE Jr (2001) Lanepitant, an NK-1 antagonist, in migraine prevention. Cephalalgia 21:102–106.
62. Edvinsson L (2004) Blockade of CGRP receptors in the intracranial vasculature: a new target in the treatment of headache. Cephalalgia 24:611–622
63. Williamson DJ, Hargreaves RJ (2001) Neurogenic inflammation in the context of migraine. Microsc Res Tech 53:167–178
64. Goadsby PJ, Edvinsson L, Ekman R (1990) Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. Ann Neurol 28:183–187
65. Fanciullacci M, Alessandri M, Figini M, Geppetti P, Michelacci S (1995) Increase in plasma calcitonin gene-related peptide from the extracerebral circulation during nitroglycerin-induced cluster headache attack. Pain 60:119–123

66. Lassen LH, Haderslev PA, Jacobsen VB, Iversen HK, Sperling B, Olesen J (2002) CGRP may play a causative role in migraine. Cephalalgia 22:54–61
67. Juhasz G, Zsombok T, Modos EA, Olajos S, Jakab B, Nemeth J, Szolcsanyi J, Vitrai J, Bagdy G (2003) NO-induced migraine attack: strong increase in plasma calcitonin gene-related peptide (CGRP) concentration and negative correlation with platelet serotonin release. Pain 106:461–470
68. Tfelt-Hansen P, De Vries P, Saxena PR (2000) Triptans in migraine: a comparative review of pharmacology, pharmacokinetics and efficacy. Drugs 60:1259–1287
69. Smith D, Hill RG, Edvinsson L, Longmore J (2002) An immunocytochemical investigation of human trigeminal nucleus caudalis: CGRP, substance P and 5-HT1D-receptor immunoreactivities are expressed by trigeminal sensory fibres. Cephalalgia 22:424–431
70. Humphrey PP, Bland-Ward PA, Carruthers AM, Connor HE, Feniuk W, Honey AC, Thomas M (2001) Inhibition of trigeminal nociceptive afferents by adenosine A1 receptor activation: a novel approach towards the design of new anti-migraine compounds. Cephalalgia 21:268–269
71. Goadsby PJ, Hoskin KL, Storer RJ, Edvinsson L, Connor HE (2002) Adenosine A1 receptor agonists inhibit trigeminovascular nociceptive transmission. Brain 125:1392–1401
72. Giffin NJ, Kowacs F, Libri V, Williams P, Goadsby PJ, Kaube H (2003) Effect of the adenosine A1 receptor agonist GR79236 on trigeminal nociception with blink reflex recordings in healthy human subjects. Cephalalgia 23:287–292
73. Ennis MD, Ghazal NB, Hoffman RL, Smith MW, Schlachter SK, Lawson CF, Im WB, Pregenzer JF, Svensson KA, Lewis RA, Hall ED, Sutter DM, Harris LT, McCall RB (1998) Isochroman-6-carboxamides as highly selective 5-HT1D agonists: potential new treatment for migraine without cardiovascular side effects. J Med Chem 41:2180–2183
74. Ramadan NM, Skljarevski V, Phebus LA, Johnson KW (2003) 5-HT1F receptor agonists in acute migraine treatment: a hypothesis. Cephalalgia 23:776–785
75. Gomez-Mancilla B, Cutler N, Leibowitz M, Spierings E, Klapper J, Diamond S, Goldstein J, Smith T, Couch J, Fleishaker J, Azie N, Blunt D (2001) Safety and efficacy of PNU-142633, a selective 5-HT1D agonist, in patients with acute migraine. Cephalalgia 21:727–732
76. Goldstein J, Roon I, Offen W, Phebus LA, Johnson KW, Schaus JM, Vanlaar T, Ferrari MD (1999) Migraine treatment with selective with 5-ht1F receptor agonist (SSOFRA) LY334370. Cephalalgia 19:318
77. Durham PL (2004) CGRP-receptor antagonists-a fresh approach to migraine therapy? N Engl J Med 350:1073–1075
78. Doods H (2001) Development of CGRP antagonists for the treatment of migraine. Curr Opin Investig Drugs 2:1261–1268
79. Kapoor K, Arulmani U, Heiligers JPC, Willems EW, Doods H, Villalon CM, Saxena PR (2003) Effects of BIBN4096BS on cardiac output distribution and on CGRP-induced carotid haemodynamic responses in the pig. Eur J Pharmacol 475:69–77
80. Olesen J, Diener HC, Husstedt IW, Goadsby PJ, Hall D, Meier U, Pollentier S, Lesko LM (2004) Calcitonin gene-related peptide receptor antagonist BIBN 4096 BS for the acute treatment of migraine. N Engl J Med 350:1104–1110
81. Petersen K, Birk S, Lassen L, Kruuse C, Jonassen O, Lesko L, Olesen J (2005) The CGRP-antagonist, BIBN4096BS does not affect cerebral or systemic haemodynamics in healthy volunteers. Cephalalgia 25:139–147