Abstract

Migraine is a common neurological disorder that is associated with an increase in plasma calcitonin gene-related peptide (CGRP) levels. CGRP, a neuropeptide released from activated trigeminal sensory nerves, dilates intracranial blood vessels and transmits vascular nociception. Therefore, it is propounded that: (i) CGRP may have an important role in migraine pathophysiology, and (ii) inhibition of trigeminal CGRP release or CGRP-induced cranial vasodilatation may abort migraine. In this regard, triptans ameliorate migraine headache primarily by constricting the dilated cranial blood vessels and by inhibiting the trigeminal CGRP release. In order to explore the potential role of CGRP in migraine pathophysiology, the advent of a selective CGRP receptor antagonist was obligatory. The introduction of di-peptide CGRP receptor antagonists, namely 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 breakthrough in CGRP receptor pharmacology and can be used as a tool to investigate the role of CGRP in migraine headaches. Preclinical investigations in established migraine models that are predictive of antimigraine activity have shown that BIBN4096BS is a potent CGRP receptor antagonist and that it has antimigraine potential. Indeed, a recently published clinical study has reported that BIBN409BS is effective in treating acute migraine attacks without significant side effects. The present review will discuss mainly the potential role of CGRP in the pathophysiology of migraine and the various treatment modalities that are currently available to target this neuropeptide.

Keywords: Antimigraine drug; BIBN4096BS; CGRP; Migraine

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1. Introduction

Migraine is a highly prevalent neurovascular disorder that affects a significant proportion of the adult population worldwide; it represents an enormous socio-economic burden to the individual as well as to the society and affects the quality of life (Ruiz de Velasco et al., 2003). A migraine attack is characterised by a severe, debilitating headache associated with nausea, vomiting, photophobia and/or phonophobia (Silberstein, 2004). Although the precise mechanisms behind migraine still remain elusive, the pathophysiology of migraine is currently based on the trigeminovascular system with the associated headache resulting from pain produced in intracranial blood vessels (De Vries et al., 1999; Villalón et al., 2002). These intracranial blood vessels are innervated by trigeminal sensory nerves that store several neuropeptides, including substance P, calcitonin gene-related peptide (CGRP) and neurokinin A (Edvinsson, 2004). Interestingly, plasma levels of CGRP, but not of other neuropeptides, are elevated in the external jugular vein during the headache phase of migraine and these levels are normalised by triptans in parallel with amelioration of headache (Goadsby et al., 2002b). Therefore, it is now generally accepted that a clear association exists between CGRP and migraine (Edvinsson, 2004), and CGRP is considered as a biological marker of trigeminovascular activation (Edvinsson, 2004; Goadsby et al., 2002b).

Last decade showed an uptrend in CGRP research and its notable role in migraine pathophysiology (Doods, 2001; Edvinsson, 2004). Amongst other lines of evidence, what stands out are: (i) the wide expression of CGRP immunoreactivity in the cranial vasculature as well as in the trigeminal ganglia (Edvinsson et al., 2002; van Rossum et al., 1997); and (ii) an increase in CGRP release during the headache phase of migraine (Goadsby et al., 2002b). Hence, it is to be expected that inhibition of CGRP release and/or its effects could be a novel approach in migraine therapy. However, this assumption could not be demystified because of the lack of availability of suitable agents, but with the recent advent of BIBN4096BS (1-piperidinecarboxamide, N-[2-[[5-amino-1-[[4-(4-pyridinyl)-1-piperazinyl]carbonyl]pentyl] amino]-1-{3,5-dibromo-4-hydroxyphenyl} methyl]-2-o xoethyl]-4-{1,4-dihydro-2-oxo-3(2H)-quinazolinyl}]-, [R- (R*,S*)]-), a selective CGRP receptor antagonist (Doods et al., 2000), this is now clearly possible. The present review is therefore devoted to discuss the putative role of CGRP in migraine pathogenesis and to describe the various treatment modalities that are currently available to target this neuropeptide. To provide a relevant background, a brief discussion of CGRP and its receptors will also be considered in this review.

2. Calcitonin gene-related peptide

2.1. Introduction and discovery

The calcitonin family of peptides consists of at least five members, namely calcitonin, amylin, CGRP (two forms: α-CGRP and β-CGRP) and adrenomedullin (Poyner et al., 2002). CGRP is a 37-amino acid neuropeptide, which was first identified in 1983 in rats as being generated by alternative splicing of the calcitonin/CGRP gene (Rosenfeld et al., 1983). In 1984, based on the sequence of rat α-CGRP, a similar peptide (human α-CGRP) was demonstrated in a
human medullary carcinoma (Morris et al., 1984). A second form of CGRP (β-CGRP) with high sequence homology to α-CGRP, but not derived from the same gene, was subsequently identified (Amara et al., 1985; Hoppener et al., 1985; Steenbergh et al., 1985). The rat α-CGRP differs from rat β-CGRP by one amino acid and the human β-CGRP differs by three amino acids from homologous human α-CGRP (Wimalawansa, 1996); the α- and β-form of CGRP are very similar in their biological activities (Poyner and Marshall, 2001). α-CGRP is primarily seen in sensory neurons, whereas β-CGRP is predominantly found in the enteric nervous system and in the human pituitary gland (Wimalawansa, 1996).

2.2. Structure of α-CGRP

All species variants of α-CGRP have 37 amino acids, constituted as a single polypeptide chain (Poyner et al., 2002). As shown in Fig. 1, the structure of α-CGRP comprises: (i) an N-terminal disulfide bridge between positions 2 and 7 (Cys2 and Cys7), (ii) a well-defined α-helix between residues 8 and 18, (iii) either a β or a γ-turn in the region of residues 19–21, and (iv) a phenylalanylamide C-terminus in the regions of residues 28 and 30, and also in 32 and 34 (Conner et al., 2002). CGRP shares ~50% homology in its sequence of amino acids with adrenomedullin and has some homology with amylin (Poyner et al., 2002; Wimalawansa, 1996).

2.3. Structure–activity relationships

The intact peptide is required for the full biological activity of a CGRP molecule (Conner et al., 2002). In this respect, they reported that: (i) the N-terminal loop (disulfide-bonded loop; see Fig. 1) is principally involved in triggering the signal transduction and receptor activation, (ii) removal of the first seven amino acids engenders CGRP(8–37), an antagonist, which binds with high affinity to CGRP receptors, (iii) the amphipathic α-helix (residues 8–18) plays an important role in the binding of the molecule to the receptor and its deletion causes approximately 100-fold loss of affinity, (iv) the residues of 19–27 are necessary as a spacer or hinge region and removal of this segment causes 10-fold decrease in the affinity of CGRP(8–37), indicating its significance in binding, and (v) the C-terminal region is requisite for the peptide to assume the right conformation in the interaction with its receptor.

2.4. Distribution and localisation

CGRP is widely distributed in the peripheral and central nervous systems as well as in the cardiovascular system (Wimalawansa, 2001). In the periphery, CGRP is abundantly present in the posterior horn cells. In primary sensory ganglia, CGRP is often co-stored with substance P, whilst in motor neurons it is co-stored with acetylcholine (Wimalawansa, 1996).

In the cardiovascular system, CGRP-containing nerve fibres are more abundant around the arteries than around the veins (Bell and McDermott, 1996); in the arterial system, they are predominantly seen in the junction of the adventitia and media (Wimalawansa, 2001). Moreover, CGRP-containing nerve fibres are more abundant in atra than in ventricles; within the right atrium, they are localised in the sinoatrial node, the ativoventricular node and the specialised conduction system (Wimalawansa, 2001). In addition, the myocardium is less densely innervated than the epicardium, endocardium or pericardium (Wimalawansa, 2001).

In the periphery, CGRP-containing nerve fibres are often associated with smooth muscles such as: (i) most parts of the gastrointestinal tract, including the excretory ducts of the parotid gland, over the epithelium of the fundic glands of stomach, endocrine cells of the duodenum and ileum and some myenteric ganglia, (ii) lungs, (iii) thyroid gland (close to C cells), (iv) splenic vein and sinusoids, (v) human skin, and (vi) pituitary gland (Hagner et al., 2002a,b,c).

2.5. Physiological functions of CGRP

The wide distribution of CGRP-containing nerve fibres and its receptors in the body suggests that CGRP plays an important role in the modulation of physiological functions. For example, in the cardiovascular system, CGRP: (i) increases heart rate, force of cardiac contraction, coronary blood flow and microvascular permeability (Kaygisiz et al., 2003; Saetrum Opgaard et al., 2000; Wimalawansa, 2001); (ii) mediates cardioprotective effects through preconditioning induced by brief ischaemia (Brzozowski et al., 2004; Li

![Fig. 1. Structure of human α-CGRP. Reproduced with permission, from Conner et al. (2002). © The Biochemical Society.](image-url)
and Peng, 2002); and (iii) produces vasodilatation in capacitance blood vessels and regulates vascular tone and angiogenesis (Wimalawansa, 2001).

In the central nervous system, CGRP modulates the motor, sensory and integrative systems (van Rossum et al., 1997). Accordingly, CGRP: (i) modulates pain perception (Powell et al., 2000; van Rossum et al., 1997), and (ii) potentiates in general the excitatory actions by enhancing the release of substance P (Oku et al., 1987) as well as of excitatory amino acids from primary afferent fibres (Kangrga et al., 1990; van Rossum et al., 1997), (iii) acts as a neurotrophic factor, e.g. increases the synthesis of acetylcholine receptors (Rossi et al., 2003). In efferent nerve fibres, CGRP co-exists with acetylcholine-containing neurons thereby modulating the release of as well as the response to acetylcholine (Rossi et al., 2003).

Additional biological functions mediated by CGRP include: (i) regulation of the pituitary hormone secretion, (ii) release of pancreatic enzymes, (iii) control of gastric acid secretion; (iv) thermoregulation, (v) decrease in food intake, (vi) antagonism of some actions of insulin, (vii) growth-factor like functions, (viii) bone remodelling, and (ix) prevention of complications during pregnancy (Wimalawansa, 1996; Yallampalli et al., 2002).

2.6. CGRP receptors

2.6.1. Classification and pharmacological characterisation

Based on functional studies using the C-terminal fragment of α-CGRP, α-CGRP(8–37) and linear CGRP analogues, [Cys(ACM)2,7]h-CGRP [[acetamidomethyl-cysteine2,7] human α-CGRP] and [Cys(Acm)2,7]h-CGRP [[acetamidomethyl-cysteine2,7] human α-CGRP], CGRP receptors are classified into CGRP1 and CGRP2 subtypes (Table 1) (Poyner et al., 2002). Experimental evidence has shown that α-CGRP(8–37) behaves as a more potent antagonist on CGRP-induced responses in guinea pig atra (high affinity, pA2=7–8) than in those induced in rat vas deferens (low affinity, pA2=5.5–6.5) (Poyner et al., 2002). In contrast, the linear CGRP analogues [Cys(Acm)2,7] and [Cys(Et)2,7]h-CGRP have higher affinity for the rat vas deferens than for the guinea pig atra (Poyner et al., 2002). Based on this evidence, it was proposed that the CGRP-induced responses are mediated by CGRP1 receptors in the guinea pig atra and by CGRP2 receptors in the rat vas deferens (Poyner et al., 2002).

BIBN4096BS (Table 1) is now considered a valuable tool to characterise CGRP receptor subtypes (Doods et al., 2000). The fact that this antagonist showed a 10-fold higher affinity for CGRP receptors in the rat left atrium as compared to the rat vas deferens supports the existence of CGRP receptor subtypes in these two tissues (Wu et al., 2000). Interestingly, this study evidenced the presence of two CGRP-like receptor subtypes in rat vas deferens, namely: (i) the CGRP2 receptor, and (ii) a “novel” receptor that displays a low efficacy for CGRP and that is selectively stimulated by [Cys(Et)2,7]h-CGRP and amylin, and can be blocked with high affinity by BIBN4096BS (Wu et al., 2002). In addition, binding studies clearly demonstrated that BIBN4096BS has a very high affinity on human neuroblastoma cells, which possess the CGRP1 receptor subtype (Schindler and Doods, 2002). Moreover, BIBN4096BS also revealed additional functional differences between the actions of α-CGRP and β-CGRP in the pig left anterior descending coronary artery and in the cerebral basilar artery, indicating the existence of different CGRP receptor subtypes (Wu et al., 2002). Notwithstanding the above findings, the molecular nature of both CGRP receptor subtypes (mainly the CGRP2 subtype) remains far from clear and final demonstration must come from their respective cloning and the development of highly selective agonists and antagonists.

2.6.2. Receptor structure

The calcitonin receptor-like receptor, a G protein coupled receptor (GPCR; family B) forms the basic receptor protein...
for CGRP and adrenomedullin receptors (McLatchie et al., 1998). Though CGRP and adrenomedullin bind with calcitonin receptor-like receptor, the receptor specificity is being determined by a single transmembrane domain protein, termed as the receptor activity modifying protein (RAMP) (McLatchie et al., 1998). The RAMPs (148–175 amino acids in size) are cleavable signal peptides, with a relatively large N-terminal extracellular domain, one transmembrane spanning domain and nine amino acid intracellular C-terminal domains (Fitzsimmons et al., 2003). The RAMPs have been localised in the endoplasmic reticulum and they are required to: (i) facilitate the intracellular translocation of the calcitonin receptor-like receptor-maturing protein and its insertion into plasma membranes (McLatchie et al., 1998), (ii) express calcitonin receptor-like receptor on the cell surface and thereby determine the relative affinity of this receptor for CGRP and adrenomedullin (Foord and Marshall, 1999), and (iii) modulate the pharmacology of the given calcitonin receptor-like receptor by providing a mechanism whereby a cell changes its sensitivity from one receptor to another receptor (Mallee et al., 2002). Three RAMPs have been identified in the human tissues, namely RAMP1, RAMP2 and RAMP3 (Poyner et al., 2002). Co-expression of calcitonin receptor-like receptor with RAMP1 reveals CGRP receptors (Fig. 2), whereas co-expression of calcitonin receptor-like receptor with RAMP2 and RAMP3 forms adrenomedullin receptors (Poyner et al., 2002). The mechanism of action of RAMPs in CGRP/ adrenomedullin binding is not clear, but in chimaeric RAMPs, it has been shown that 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 N-terminus (Foord et al., 1999). Similarly, in the human RAMP1, the extracellular domain of RAMP1 is sufficient for a normal calcitonin receptor-like receptor association and efficacy, while the specific sequences of the transmembrane domain contribute to CGRP affinity and specificity (Fitzsimmons et al., 2003). Moreover, in humans, substitution of tryptophan at position 74 with lysine (as found in rat RAMP1) confers a low affinity and vice versa; this suggests that important determinants for small molecule antagonists are located in RAMP1 (Mallee et al., 2002). In addition to RAMPs, the CGRP receptor complex requires another chaperone protein named as the “receptor component protein” to function optimally (Evans et al., 2000). This receptor component protein is a 148-amino acid intracellular polypeptide that is required for G protein-coupled signal transduction at CGRP receptors (Prado et al., 2002). The receptor component protein is well-expressed in CGRP-responsive tissues and its expression correlates with the biological efficacy of CGRP in vivo (Evans et al., 2000).

The structure of the CGRP₂ receptor subtype is unclear and little has been done thus far to characterise the structural requirements of CGRP to bind to CGRP₂ receptors (Poyner et al., 2002). The linear CGRP analogues [Cys(Acm)²⁷]- and [Cys(Et)²⁷]-h-CGRP have been used to classify CGRP₂ receptor subtypes, but an agonist may not definitively characterise a receptor (Waugh et al., 1999). In isolated large porcine coronary arteries, CGRP receptors have low affinity for hα-CGRP₈₋₃⁷ and [Cys(ACM)²⁷]-h-CGRP causes relaxation of this tissue (Waugh et al., 1999). These functional studies established large porcine coronary arteries as a model for studying the CGRP₂ receptor (Waugh et al., 1999). In contrast to this, several lines of evidence do not support that the CGRP₁ and CGRP₂ receptor subtypes are different proteins that represent independent receptors, namely: (i) identification of mRNA encoding calcitonin receptor-like receptor and RAMP1 in the rat vas deferens (Moreno et al., 2002b) as well as in the porcine coronary artery (Rorabaugh et al., 2001), (ii) CGRP₁ and CGRP₂ receptor-selective ligands do not discriminate between CGRP receptors in porcine coronary arteries (Rorabaugh et al., 2001), (iii) porcine CGRP₁ receptors that have been transfected into HEK-293 cells (Rorabaugh et al., 2001), and (iv) peptidase inhibitors make little difference to CGRP pharmacology (Poyner et al., 2002). Interestingly, a recent investigation has characterised CGRP receptor subtypes and they have identified three CGRP₈₋₃⁷-insensitive receptors following co-expression of RAMPs (RAMP1, RAMP2 and RAMP3) with calcitonin receptor 2 (Kuwasaki et al., 2004). Amongst the three receptors, calcitonin receptor 2/RAMP1 is most sensitive to the two linear analogues, suggesting that it could be classified as a CGRP₂ receptor; but further work is necessary to confirm the structure, activity and effects of the CGRP₂ receptor in different tissues (Kuwasaki et al., 2004).}

**2.6.3. Distribution and binding**

**2.6.3.1. Nervous system.** Both high and low binding sites for CGRP have been reported in the central nervous system (Morara et al., 2000; Segond von Banchet et al., 2002). In dorsal root ganglion and other neurons, CGRP receptors co-exist with receptors for other neurotransmitters and neuro-

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**Fig. 2. Schematic representation of the CGRP₁ receptor showing interactions of receptor activity modifying protein (RAMP1) with calcitonin receptor-like receptor and a receptor component protein (RCP). This complex is tightly coupled to Gₛ to promote cAMP production (Kapoor, 2003).**
modulators, such as substance P, noradrenaline, neuropeptide Y, vasoactive intestinal peptide, etc. (Ohtori et al., 2002; van Rossum et al., 1997). Moreover, in the non-adrenergic and noncholinergic fibres and in the coronary vessels, CGRP receptors co-exist with receptors for tachykinins and substance P, respectively (Ursell et al., 1991; Wiesenfeld-Hallin et al., 1984).

2.6.3.2. Cardiovascular system. The highest density of CGRP binding sites are present in the heart and in blood vessels (Wimalawansa, 2001). Regardless of the species, the density of the CGRP binding sites in atria invariably exceeds that of ventricles (Chang et al., 2001). Autoradiographic studies in the hearts of rats (Chang et al., 2001), guinea pigs and humans (Coupe et al., 1990) have shown the highest density of CGRP binding sites in the coronary arteries, coronary veins and in the heart valves, while a lower density is found in the coronary arterioles and endocardium (Wimalawansa, 2001). These findings substantiate the relevance of CGRP receptors in regulating blood flow as well as inotrope and chronotropic effects in the heart (Poyner et al., 2002; Saetrum Opgaard et al., 2000).

2.6.3.3. Other tissues. CGRP receptors are also abundantly present in the thyroid gland, gastrointestinal tract, parotid gland, adrenals, pituitary, exocrine pancreas, kidneys, bones, skin and skeletal muscles (Hagner et al., 2002a,b,c; Rossi et al., 2003; Wimalawansa, 1996).

2.6.4. Signal transduction mechanisms

The CGRP-induced vascular responses are mediated by both endothelium-dependent and endothelium-independent mechanisms (Wimalawansa, 1996). In the endothelium-dependent pathway (e.g. rat thoracic aorta, pulmonary and renal arteries in rats as well as brachial artery in humans), CGRP activates adenylyl cyclase thereby increasing cyclic adenosine monophosphate (cAMP) levels. This increase in cAMP levels activates the enzyme nitric oxide synthase, which in turn, increases the level of nitric oxide. Nitric oxide acts on the smooth muscle cells by activating guanylyl cyclase with an ensuing production of cyclic guanosine monophosphate (cGMP) leading to smooth muscle relaxation (de Hoon et al., 2003; Wimalawansa, 1996). On the other hand, in the endothelium-independent pathway (e.g. human skeletal muscle artery), CGRP bypasses the endothelium and directly binds to CGRP receptors on the smooth muscle cells, activating adenylyl cyclase; this, in turn, increases cAMP levels leading to vascular relaxation (Wimalawansa, 1996). Interestingly, blood vessels such as the rat basilar and superior mesenteric arteries show both endothelium-dependent and endothelium-independent mediated mechanisms (Wimalawansa, 1996). CGRP also acts indirectly by stimulating protein kinase A that activates K ATP channels (in rabbit arterial smooth muscle) (van Rossum et al., 1997). Evidently, CGRP-induced responses involve multiple second messengers, including cAMP, nitric oxide-cGMP and K+ channels (Springer et al., 2004; van Rossum et al., 1997). Notwithstanding, irrespective of the second messengers involved, the final common pathway for CGRP-induced vasorelaxation depends ultimately upon the decrease in intracellular calcium (Wimalawansa, 1996).

2.7. Therapeutic potentials of CGRP receptor ligands

2.7.1. CGRP receptor agonists

In view that CGRP is a potent vasodilator agent, CGRP receptor agonists may be used in, for example, coronary heart disease and myocardial ischaemia (CGRP relieves arterial vasospasm). In patients with congestive cardiac failure, CGRP increases cardiac output and decreases blood pressure, without altering heart rate (Bell and McDermott, 1996). Moreover, CGRP receptor agonists can be used as antiarrhythmic agents because they reduce the degree of arterioventricular blockade and protect against ventricular fibrillation (Bell and McDermott, 1996). Furthermore, the potential therapeutic usefulness of CGRP receptor agonists in the treatment of hypertension is of interest. Indeed, an experimental rat model for hypertension demonstrated a significant decrease in CGRP contents in perivascular nerves and an impaired vascular sensitivity to CGRP. Consistent with these findings, infusions of β-CGRP in hypertensive patients significantly decreased blood pressure (Wimalawansa, 2001).

Other potential clinical applications of CGRP receptor agonists include: (i) Raynaud’s syndrome, (ii) peripheral vascular diseases (thromboembolism or diabetic vascular disease), (iii) subarachnoid haemorrhage, (iv) nerve and neuromuscular regeneration, (v) erectile dysfunction, (vi) pulmonary hypertension, (vii) pre-eclamptic toxaemia and preterm labour, and (viii) venous stasis ulcer (Ackermann et al., 2002; Bivalacqua et al., 2001; Ellington et al., 2002; Knerr et al., 2002; Qing and Keith, 2003).

2.7.2. CGRP receptor antagonists

Several lines of evidence have shown that an inappropriate release of CGRP is a potential causative factor in several diseases including, amongst others: (i) migraine (discussed below), (ii) inflammation (as meningitis), (iii) cardiogenic shock associated with sepsis, and (iv) thermal injury (Hoffmann et al., 2002; Wimalawansa, 1996). Moreover, other studies have demonstrated that CGRP receptor antagonists can be used in the treatment of insulin resistant type II diabetes mellitus (e.g. antagonism of insulin effects, which is comparable to that of amylin) (Wimalawansa, 1996).

In view that CGRP and substance P containing nerve fibres are abundantly seen in atopic dermatitis and nummular eczema, CGRP receptor antagonists may dampen
the associated inflammatory response, neurogenic inflammation and/or pain transmission (Jarvikallio et al., 2003).

3. CGRP and migraine

Migraine is a common chronic, paroxysmal neurobiological disorder that ranks among the world’s most disabling medical disorders (Silberstein, 2004). Migraine prevalence varies with age (peaking between 35 and 55 years) and it is more common in women than in men (Bigal et al., 2004). Migraine attacks can be triggered by several factors, including environmental, genetic, dietary and hormonal changes; with respect to the latter, migraine attacks are more frequent during menstruation and are often relieved by pregnancy (Blau, 1992; Lipton, 2000).

The characteristic features of migraine include: (i) recurrent attacks of unilateral headache, which can be severe, throbbing and pulsatile in nature, (ii) anorexia, nausea and vomiting, and (iii) autonomic dysfunction (Barbanti et al., 2003; Peroutka, 2004; Silberstein, 2004). In some 20–30% of the migraineurs, the headache is preceded by a complex of focal neurological symptoms known as aura (Goadsby et al., 2002b; Silberstein, 2004). Based on the presence or absence of aura symptoms, migraine is formally classified as: (i) migraine with aura (classical migraine), and (ii) migraine without aura (common migraine; more commonly seen). Migraine headache usually occurs in the early morning, typically lasts for 4–72 h and it has a striking feature of freedom from headache between the attacks (Olesen, 2004).

3.1. Migraine pathophysiology

Although elusive for a long time, the underlying pathophysiological bases of migraine have undergone significant progress in the last decade. The current theories basically suggest that migraine may involve dilatation of cranial blood vessels including carotid arteriovenous anastomoses (De Vries et al., 1999; Villalón et al., 2002) and activation of the trigeminovascular system (Goadsby et al., 2002b). Based on clinical features, three distinct phases of migraine can be discerned, namely, a trigger, an aura (when present) and a headache phase (Goadsby et al., 2002b; Villalón et al., 2002).

3.1.1. Trigger phase including premonitory symptoms

It has been proposed that genetic abnormalities may be responsible for altering the response threshold to migraine-specific triggers in the brain (Goadsby et al., 2002b). The process driving the pathogenesis of the migraine attack and the area susceptible to the migraine triggers may be located in the brain stem; indeed studies using positron emission tomography detected increased blood flow (an index of neuronal activity) during spontaneous migraine attacks in the cerebral hemispheres (cingulate, auditory and visual association cortices) and in the brain stem (Weiller et al., 1995). The brain stem activation persisted even after the injection of sumatriptan, which induced complete relief from headache and phono- and photophobia, suggesting that the brain stem activation is a fundamental part of the migraine trigger (Weiller et al., 1995). The subsequent events following the trigger phase leading to the symptoms observed during the aura and headache phases can be explained on the basis of the neurovascular hypothesis (Villalón et al., 2002).

3.1.2. Aura phase

Research into the mechanisms underlying the aura phase of migraine has fascinated a large number of investigators for several centuries; up to 15–30% of migraine sufferers experience aura symptoms that last for 5–60 min before the onset of headache (Olesen, 2004; Welch, 2003). Once the brain stem gets activated by unknown triggers (see Fig. 3), there is a decrease in the regional cerebral blood flow, possibly following a wave of cortical spreading depression (Lauritzen, 2001; Spierings, 2004). Cortical spreading depression is a wave of transient intense spike activity that spreads along the cortex slowly (at rates between 2 and 6 mm/min), which may lead to a long lasting neuronal

![Fig. 3. Diagram showing the pathophysiology of migraine headache and the therapeutic targets of acutely acting antimigraine drugs. These drugs are believed to owe their antimigraine efficacy to direct vasoconstriction of dilated cranial blood vessels (1), inhibition of trigeminally induced cranial vasodilatation (2), plasma protein extravasation (3) and/or central neuronal activity (4). Figure taken from De Vries et al. (1999) and Villalón et al. (2002). TNC, trigeminal nucleus caudalis.](image-url)
suppression (Welch, 2003). This decrease in the cerebral blood flow usually begins in the occipital lobe, but this reduction enlarges and may involve the whole hemisphere (Lauritzen, 2001). Many clinicians believe that the migraine aura is due to a neuronal dysfunction rather than ischaemia and it is probably the clinical manifestation of a cortical spreading depression (James et al., 2001).

3.1.3. Headache phase

Although the underlying concept of migraine has been refined over the years by elegant functional neuroimaging procedures, the mechanisms by which the aura transduces into headache still remains inscrutable (Welch, 2003). However, the cerebral oligoemia is subsequently followed by a reflex painful vasodilatation of the cranial blood vessels, including arteriovenous anastomoses (see Fig. 3), probably due to activity changes in the nerves that innervate these blood vessels (De Vries et al., 1999; Villalón et al., 2002). Therefore, migraine pain is due to activation of nociceptors of the pain producing structures in concert with reduction in the function of endogenous pain-control pathways (Goadsby et al., 2002b). This noceptive information from the blood vessels is conveyed to central neurons in the trigeminal sensory nucleus that, in turn, relays the pain signals to higher centres where headache pain is perceived. In addition, stimulation of trigeminal nerves may also release CGRP; this would reinforce the already existing vasodilatation, relaying the nociceptive impulses to the central nervous system (De Vries et al., 1999; Villalón et al., 2002; Welch, 2003).

3.2. CGRP and its role in migraine pathophysiology

3.2.1. CGRP: a biological marker for migraine

As mentioned above, migraine headache is closely associated with the activation of the trigeminovascular system. Hence, stimulation of trigeminal ganglia/sensory nerves in several species (including humans) leads to the release of CGRP, which dilates cranial blood vessels and stimulates sensory nerve transmission (Edvinsson, 2004). Moreover, CGRP-like immunoreactivity is abundantly expressed in trigeminal nuclei as well as in non-myelinated trigeminal sensory nerve fibres (Welch, 2003). Thus, it is clear that the cerebral vasculature is preferentially innervated by CGRP-containing sensory nerves (Edvinsson, 2004).

With a better acquaintance of the organisation of sensory nerves, which innervate intracranial blood vessels, one can explore their involvement in migraine by analysing neurotransmitter release in cranial outflow (Edvinsson, 2004). Based on this notion, clinical studies were designed to measure plasma neurotransmitter concentrations during migraine attacks (Edvinsson, 2004; Goadsby et al., 2002b). Interestingly, plasma concentrations of CGRP in the jugular venous blood, but not of other neuropeptides, was elevated during the headache phase of migraine (Goadsby et al., 2002b). Furthermore, in migraine patients: (i) a strong correlation was found between plasma CGRP concentrations and migraine headache (Juhasz et al., 2003), (ii) intravenous (i.v.) infusion of CGRP produced a migraine-like headache (Lassen et al., 2002), (iii) baseline CGRP levels were considerably higher (Juhasz et al., 2003), and (iv) the changes in plasma CGRP levels during migraine attacks significantly correlated with the headache intensity (Juhasz et al., 2003).

Recent investigations have shown that nitric oxide, a potent vasodilator implicated in migraine headache (Lassen et al., 2003; Thomsen and Olesen, 2001), has a strong correlation with CGRP (Juhasz et al., 2003). Migraineurs are supersensitive to nitric oxide (Thomsen and Olesen, 2001) and the vascular effects of nitric oxide are partly mediated by CGRP released from trigeminal nerve fibres, while at the level of the trigeminal system, nitric oxide synthase coordinates with nitric oxide production to release CGRP from trigeminal nerve fibres (Akerman et al., 2002). Furthermore, i.v. infusion of nitric oxide produces a migraine-like headache with an associated increase in plasma CGRP levels (Juhasz et al., 2003). Hence, the above lines of evidence support the contention that the trigeminal CGRP release is a reliable marker for migraine that can be measured in a venous blood sample (Juhasz et al., 2003); accordingly, the decrease in this marker seems to be highly predictive of antimigraine activity in humans (Edvinsson, 2004).

3.2.2. CGRP: a novel target for antimigraine therapy

Though the treatment modalities for acute migraine headaches are plentiful, the triptans still remain as the drugs of choice (Villalón et al., 2002). Triptans are 5-HT1B/1D/1F receptor agonists, which abort migraine attacks by at least two main mechanisms, namely: (i) constriction of dilated cranial arteries, including arteriovenous anastomoses via the stimulation of 5-HT1B receptors (De Vries et al., 1999; Villalón et al., 2002), and (ii) inhibition of CGRP release as well as of noceptive transmission on peripheral and central trigeminal sensory nerves via 5-HT1D receptors (Goadsby et al., 2002b). Moreover, not only does CGRP dilates the cranial blood vessels, but it also transmits vascular nociception (Edvinsson, 2004). Therefore, it is reasonable to propose that inhibition of trigeminal CGRP release or antagonism of CGRP receptors may represent a novel approach in treating migraine headaches (Durham, 2004; Edvinsson, 2004). Either strategy would ultimately result in the prevention of cranial vasodilatation, as clearly demonstrated for essentially all acute antimigraine agents, such as the triptans and ergot derivatives (Villalón et al., 2002).

3.2.2.1. Inhibition of CGRP release. Cerebral blood vessels are innervated by sensory nerves that store several neuropeptides amongst which CGRP is the most abundant (Williamson and Hargreaves, 2001). As mentioned above, one of the mechanisms by which triptans alleviate migraine
is probably inhibition of CGRP release (Goadsby et al., 2002b). Moreover, human trigeminal ganglia/sensory nerves express abundant 5-HT_{1B/1D} receptors (Edvinsson, 2004; Smith et al., 2002), thus strengthening the evidence in favour of the presynaptic inhibitory effects of triptans (Edvinsson, 2004). On this basis, it is clear that the triptans represent a significant advance in migraine therapy; notwithstanding, they may not provide adequate relief to some patients (Tfelt-Hansen et al., 2000) and have limitations due to their potential coronary side effects (MaassenVanDenBrink et al., 1998, 2000). Therefore, the crucial improvement in antimigraine therapy would seem to be the development of an antimigraine agent with no cardiovascular side effects, but still capable of inhibiting the trigeminal CGRP release (Goadsby et al., 2002b). In this context, several novel approaches have been proposed, namely: (i) selective agonists at 5-HT_{1D} receptors, such as PNU-109291 ([3S]-3,4-dihydro-1-ethyl-N-methyl-1H-2-benzopyran-6-carboxamide) (Ennis et al., 1998) and PNU-142633 ([3S]-3,4-dihydro-1-[2-[4-[4-aminocarbonyl]-phenyl]-1-piperazinyl][ethyl]-N-methyl-1H-2-benzopyran-6-carboxamide) (McCall et al., 2002); and (ii) selective 5-HT_{1F} receptor agonists such as LY344864 (N-[3-(dimethylamino)-2,3,4,9-tetrahydro-1H-carbazol-6-yl]-4-fluorobenzamide) and LY334370 (4-fluoro-N-[3-[1-methyl-4-piperidinyl]-1H-indol-5-yl]-benzamide) (Ramadan et al., 2003). These developments are very significant because, unlike the triptans (5-HT_{1B/1D} receptor agonists), 5-HT_{1D} and 5-HT_{1F} receptor agonists are devoid of contractile effects on coronary and cerebral blood vessels (Bouchelet et al., 2000; McCall et al., 2002); in addition, these receptors may have a presynaptic role, such as inhibiting CGRP release and central nociception (Ramadan et al., 2003; Sheppard et al., 1999; Villalón et al., 2002). However, PNU-142633 proved to be ineffective in the acute treatment of migraine (Gómez-Mancilla et al., 2001), whilst LY334370 did show some efficacy when used in doses which may interact with 5-HT_{1B} receptors (Goldstein et al., 1999; Ramadan et al., 2003). Though clinical studies have shown that LY334370 is effective in treating migraine headaches without coronary side effects (Ramadan et al., 2003), dosing of 5-HT_{1F} receptor agonists clouds the outcomes (Goldstein et al., 1999). Therefore, on the role of 5-HT_{1F} receptor populations in experimental migraine models are warranted (Goadsby and Clasey, 2003).

Likewise, several lines of evidence have shown that α_2-adrenoceptor subtypes and adenosine A_1 receptors inhibit CGRP release and trigeminal nociception; these findings may prove vital in the development of novel antimigraine compounds (Goadsby et al., 2002b; Willems et al., 2003). Indeed, in vivo studies have clearly demonstrated that a selective adenosine A_1 receptor agonist, GR79236 (N-[2-methylphenyl]methyladenosine (metrifudil), 2-(phenylamino) adenosine), inhibits: (i) neurogenic vasodilatation in rats (Humphrey et al., 2001), (ii) trigeminal nociception as well as CGRP release in cats (Goadsby et al., 2002a), and (iii) trigeminal nociception in humans (Giffin et al., 2003). Taken together, these findings suggest that GR79236 may have antimigraine potential (Goadsby et al., 2002b). In fact, results from pilot clinical studies have reported that GR79236 has antimigraine action, probably due to an inhibitory effect on nociceptive trigeminal neurons (Humphrey et al., 2001), but more clinical studies are indispensable. Furthermore, stimulation of presynaptic α_2-adrenoceptors mediate antinociceptive effects and inhibit the expression and release of CGRP (Hargreaves et al., 2003; Shi et al., 2000; Supowit et al., 1998); hence, selective agonists of α_2-adrenoceptor subtypes may also have potential antimigraine usefulness; in fact, these compounds also produce carotid vasoconstriction (Willems et al., 2003). Other inhibitors of CGRP release may include agonists (civimide) or antagonists (capsazepine) at capsaicin vanilloid receptors as well as agonists at cannabinoid receptors (anandamide); these agents seem to be have potential therapeutic usefulness in preclinical (Akerman et al., 2003; 2004) and clinical (Diamond et al., 2000) investigations for migraine therapy.

### 3.2.2.2. Antagonism of CGRP receptors.

Several studies suggest that CGRP containing cell bodies are abundant in the human cranial blood vessels (Edvinsson, 2004; Uddman et al., 1999). Moreover, neurogenic vasodilatation induced by trigeminal ganglion/nerve stimulation was inhibited by the truncated fragments of CGRP, such as CGRP_{8-37} (Williamson and Hargreaves, 2001). These findings indicate that CGRP has a potential role in cranial vasodilatation, a pathognomonic feature of migraine (Edvinsson, 2004). Moreover, i.v. infusion of CGRP in migraineurs produces migraine-like headaches (Lassen et al., 2002). Therefore, based on the excellent correlation between CGRP release and migraine headache (Goadsby et al., 2002b), a specific CGRP receptor antagonist that could abolish CGRP-induced cranial vasodilatation, may be of potential usefulness in acute migraine therapy (Edvinsson, 2004). However, CGRP_{8-37} proved ineffective in migraine treatment (Durham, 2004) due to its low potency and short half-life (Chiba et al., 1989). Nevertheless, these studies have provided the evidence that blockade of CGRP receptors by non-peptide molecules could be beneficial in migraine therapy (Durham, 2004).

An important breakthrough in the field of CGRP receptors is the development of potent CGRP receptor antagonists (Fig. 4), namely: (i) BIBN4096BS (Doods et al., 2000), (ii) 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) (Hasbak et al., 2003), and (iii) SB-(+)-273779 (2,3,6,7-tetrahydro-1’-methyl-5’-[2’-methyl-4’-(5-methyl-1,2,4-oxadiazol-3-yl) biphenyl-4’-carboxyl] fur [2,3-f] indole-3-spiro-4’-piperidine hydrochloride) (Aiyar et al., 2001). BIBN4096BS demonstrates extremely high affinity for human CGRP receptors expressed in SK-NM-C cells.
Although two other CGRP receptor antagonists have been introduced, BIBN4096BS shows >1000-fold higher receptor affinity than that described for other compounds (Doods, 2001). In view of its potent antagonist properties and selectivity at CGRP receptors, BIBN4096BS was subsequently evaluated for its antimigraine potential. In the in vivo animal models, which are predictive of antimigraine activity, BIBN4096BS clearly attenuated: (i) the vasodilatation induced by trigeminal stimulation in marmosets (Doods et al., 2000), (ii) capsaicin-induced porcine carotid vasodilator responses, including carotid arteriovenous anastomotic dilatation (see Fig. 5) (Kapoor et al., 2003a), and (iii) \( \alpha \)-CGRP-induced porcine carotid vasodilatation and arterial–jugular venous oxygen saturation difference (Fig. 6) (Kapoor et al., 2003b).

These findings gain additional weight within the context of antimigraine mechanisms when considering that during migraine there is a dilatation of carotid arteriovenous anastomoses, which results in the narrowing of arterial–jugular venous oxygen saturation difference (Saxena, 1987). In addition to its antimigraine potential, BIBN4096BS has no effect on the baseline systemic and regional haemodynamics in porcine and rat cardiovascular models, reinforcing its cardiovascular safety (Arulmani et al., 2004b; Kapoor et al., 2003b). These findings suggest that BIBN4096BS could be developed as an effective antimigraine compound with little cardiovascular side effects (Arulmani et al., 2004b).

Recently, clinical trials with BIBN4096BS have shown its efficacy in the acute treatment of migraine without significant side effects or intrinsic vasoconstrictor effects (Olesen et al., 2004).

Similar to BIBN4096BS, Compound 1: (i) displaced \(^{125}\)I-CGRP from SK-N-MC cells (human neuroblastoma cell line), (ii) antagonized the CGRP-induced increase in...
cAMP production in SK-N-MC cells with pA2 values of ~8 nM, and (iii) produced a parallel rightward shift on the concentration–response curve to CGRP in isolated human cranial arteries with a pA2 value of 10 nM (Edvinsson, 2004). A third CGRP receptor antagonist, SB-(+)-273779, inhibited the CGRP binding to SK-N-MC cells, reduced CGRP-induced adenyl cyclase activity and displayed no significant affinity for other receptors including those for calcitonin, endothelin, angiotensin II and catecholamines (Aiyar et al., 2001). In addition, SB (+)-273779 antagonized CGRP-mediated: (i) stimulation of intracellular Ca$^{2+}$ in recombinant CGRP receptors in HEK-293 cells; (ii) vasodilatation in rat pulmonary artery; and (iii) decrease in blood pressure in anaesthetised rats (Aiyar et al., 2001).

Table 2 shows the apparent p$K_B$ values for various CGRP receptor antagonists on different cell lines and different tissues.

### 3.3. Pre- and post-junctional modulation of CGRP: implications for migraine treatment

The hypothesis underlying the development of antimigraine compounds is based on the involvement of vascular or neural components in migraine (Fusco et al., 2003). It is well known that migraine pathogenesis involves the activation of trigeminal nerves, which may release CGRP that, in turn, promotes neurogenic inflammation (Goadsby et al., 2002b) and cranial vasodilatation (Edvinsson, 2004; Villalón et al., 2002). Although analgesics and non-steroidal anti-inflammatory drugs are used as a first line of treatment for migraine headache, triptans are considered as a gold standard (Saxena and Tfelt-Hansen, 2000). As described above, triptans abort migraine attacks by several mechanisms, including: (i) constriction of dilated cranial blood vessels and carotid arteriovenous anastomoses (Saxena and Tfelt-Hansen, 2000), and (ii) inhibition of CGRP release as well as of nociceptive transmission on peripheral and central trigeminal sensory nerves (Goadsby et al., 2002b).

Several studies have reported that inhibition of trigeminal CGRP release may underlie the therapeutic efficacy of triptans (Goadsby et al., 2002b). Indeed, several findings in animal experimental models have shown that triptans inhibit trigeminal CGRP release (Goadsby et al., 2002b; Williamson and Hargreaves, 2001); this is further strengthened by clinical data showing that sumatriptan normalised the elevated CGRP levels with alleviation of migraine headache (Goadsby et al., 2002b). In marked contrast, however, it has been shown that compounds that exclusively inhibit neurogenic inflammation (e.g. tachykinin NK1 receptor antagonists) (Williamson et al., 1997) and the trigeminovascular system (e.g. 5-HT1D receptor agonists) (Gómez-Mancilla et al., 2001) are ineffective in acute migraine treatment. Therefore, it is not yet clear whether the inhibition of trigeminal CGRP release per se is an

### Table 2

<table>
<thead>
<tr>
<th>CGRP receptor antagonist</th>
<th>CGRP(8-37)</th>
<th>BIBN4096BS</th>
<th>Compound 1</th>
<th>SB-(+)273779</th>
</tr>
</thead>
<tbody>
<tr>
<td>SK-N-MC cells</td>
<td>7.49a</td>
<td>10.47b</td>
<td>7.7c</td>
<td>6.41d</td>
</tr>
<tr>
<td>L6 cells</td>
<td>7.8e</td>
<td>9.25e</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Col 29</td>
<td>6.48a</td>
<td>9.98e</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Human coronary vessels</td>
<td>7.3b</td>
<td>10.4b</td>
<td>7.1f</td>
<td>n.d.</td>
</tr>
<tr>
<td>Porcine coronary vessels</td>
<td>6.2g</td>
<td>7.23 (distal)b</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Human cerebral vessels</td>
<td>7.7bh</td>
<td>10.1i</td>
<td>8.1e</td>
<td>n.d.</td>
</tr>
<tr>
<td>Bovine cerebral vessels</td>
<td>6.75i</td>
<td>7.5i</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Human temporal artery</td>
<td>6.55i</td>
<td>10.1i</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

n.d., not determined.

- a Data taken from Poyner et al. (1998).
- b Data taken from Edvinsson et al. (2002).
- d Data taken from Aiyar et al. (2001).
- e Data taken from Hay et al. (2002) and Howitt and Poyner (1997).
- f Data taken from Hasbak et al. (2003).
- g Data taken from Wisskirchen et al. (1999).
- h Data taken from S. Gupta et al. (unpublished).
- i Data taken from Verheggen et al. (2002).
- j Data taken from Moreno et al. (2002a).
important mechanism behind the therapeutic efficacy of antimigraine agents. Certainly, the above effect of triptans (inhibition of trigeminal CGRP release) may be secondary to the alleviation of headache produced by cranial vasoconstriction (Arulmani et al., 2004a). Accordingly, it is tempting to suggest that vasoconstriction of cranial blood vessels, including arteriovenous anastomoses, is the most important effect of the acutely acting antimigraine drugs available thus far. This suggestion gains weight when considering that: (i) sumatriptan, which poorly penetrates the central nervous system, did not have any effect on capsaicin-induced CGRP release (see Fig. 7), while potently constricting arteriovenous anastomoses (Arulmani et al., 2004a), (ii) the 5-HT1B/1D receptor agonists, alniditan and IS159, which have little affinity for 5-HT1F receptors, are effective in aborting acute migraine attacks due to vasoconstrictor effects via 5-HT1B receptors (De Vries et al., 1998; Goadsby and Classey, 2003), and (iii) BIBN4096BS is reported to be effective in migraine based on its antagonism of CGRP gene-related peptide 1 receptor antagonist (J. Pharmacol. Exp. Ther. 296, 768–775).

4. Conclusions

A large body of evidence indicates that CGRP plays an important role in the pathophysiology of migraine and this provides an excellent opportunity to integrate basic and clinical research for a better understanding of this multi-faceted disorder. The encouraging results obtained in a “proof of concept” study with BIBN4096BS, administered i.v., in the acute treatment of migraine holds significant promise to suggest that orally effective CGRP receptor antagonists will become available in the not too distant future. An important advantage of CGRP antagonists over the triptans can be their use in patients with coronary artery disease. Moreover, migraine research is beginning to be focussed on the development of preventive medications and it would be worthwhile to explore whether inhibition of CGRP synthesis, release or its effects may reduce the frequency of migraine attacks. It is evident that further investigation to understand migraine pathophysiology will continue in the emerging post-triptan era, ultimately providing new hope to patients whose migraine attacks presently remain inadequately controlled.

References


