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# Basic mechanisms of migraine and its acute treatment

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ABSTRACT

Migraine is a neurovascular disorder characterized by recurrent unilateral headaches accompanied by nausea, vomiting, photophobia and phonophobia. Current theories suggest that the initiation of a migraine attack involves a primary event in the central nervous system (CNS), probably involving a combination of genetic changes in ion channels and environmental changes, which renders the individual more sensitive to environmental factors; this may, in turn, result in a wave of cortical spreading depression (CSD) when the attack is initiated. Genetically, migraine is a complex familial disorder in which the severity and the susceptibility of individuals are most likely governed by several genes that vary between families. Early PET studies have suggested the involvement of a migraine active region in the brainstem.

Migraine headache is associated with trigeminal nerve activation and calcitonin gene-related peptide (CGRP) release from the trigeminovascular system. Administration of triptans (5-$HT_{1B/1D}$ receptor agonists) causes the headache to subside and the levels of CGRP to normalize. Moreover, administration of CGRP receptor antagonists aborts the headache. Recent immunohistochemical and pharmacological results suggest that the trigeminal system has receptors for CGRP; further, 5-$HT_{1B/1D}$ receptors, which inhibit the action of CGRP in pain transmission when activated, have been demonstrated. This offers an explanation for the treatment response.

The present review provides an updated analysis of the basic mechanisms involved in the pathophysiology of migraine and the various pharmacological approaches (including 5-$HT_{1B/1D}$ receptor agonists, CGRP receptor antagonists and glutamate receptor antagonists) that have shown efficacy for the acute treatment of this disorder.



**Contents**



*Abbreviations:* ACh, acetylcholine; AChE, acetylcholinesterase; AM, adrenomedullin; ATP, adenosine triphosphate; BBB, blood–brain barrier; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CGRP, calcitonin gene-related peptide; CLR, calcitonin receptor-like receptor; CNS, central nervous system; CNV, contingent negative variation; CSD, cortical spreading depression; CT, calcitonin; DRG, dorsal root ganglia; ERPs, event-related potentials; FHM, familial hemiplegic migraine; GTN, glyceryl trinitrate; 5-HT, 5-hydroxytryptamine serotonin; iNOS, inducible form of nitric oxide synthase; i.v., intravenous route of administration; LC, locus coeruleus; MAPKs, mitogen-activated protein kinases; MMA, middle meningeal artery; MMP-9, matrix metalloproteinase-9; NA, noradrenaline; NKA, neurokinin A; NPY, neuropeptide Y; NO, nitric oxide; NRM, nucleus raphe magnus; PACAP, pituitary adenylate cyclase-activating peptide; PET, positron emission tomography; RAMP, receptor activity-modifying protein; RCP, receptor component protein; SGC, satellite glia cells; SNP, sodium nitroprusside; SP, substance P; TG, trigeminal ganglion; TNC, trigeminal nucleus caudalis; TRPV1, transient receptor potential vanilloid type 1; VIP, vasoactive intestinal peptide.

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## 1. Introduction

Migraine is currently considered a neurovascular disorder characterized by a severe, debilitating and throbbing unilateral headache associated with anorexia, nausea, vomiting, photophobia, phonophobia and/or diarrhea (Headache Classification Subcommittee of the International Headache Society, 2004). This review will focus on updating the pathophysiological mechanisms of migraine, the vascular innervation and involved molecular signals, and illustrate how recent acute pharmacological treatments may act.

## 2. Nature of the perivascular nerves innervating intracranial blood vessels

It is well recognized that the central nervous system (CNS) is devoid of sensory pain receptors; it is only intracranial blood vessels in the dura mater, including the middle meningeal artery, and the large arteries of the circle of Willis that are supplied with sensory nerves and receptors that can respond to thermal, mechanical or distension stimuli. Since intracranial blood vessels are the only source of intracranial pain, and in particular referred pain, the understanding of the vascular innervation by autonomic and sensory nerves is a prerequisite for the understanding of intracranial pain as it occurs in migraine. The intracranial blood vessels are supplied with nerves that emanate from cell bodies in ganglia belonging to the sympathetic, parasympathetic and sensory nervous systems (Fig. 1; Edvinsson & Uddman, 2005). Further, cerebral blood vessels may be innervated by fibers originating within the brain itself (i.e. intrinsic nerve supply; Edvinsson & Krause, 2002).

### 2.1. Sympathetic nervous system

The sympathetic nerves that supply the cerebral blood vessels arise from the ipsilateral superior cervical ganglion, while some nerves that supply the vertebral and basilar arteries originate from the inferior cervical and stellate ganglia. Activation of these fibers results in vasoconstriction, modulation of cerebrovascular autoregulation, reduction of intracranial pressure, and a decrease of cerebrospinal fluid production (Edvinsson & Krause, 2002). The responses are mainly mediated by noradrenaline (NA) and neuropeptide Y (NPY) (Tajti et al., 1999a).

The neurotransmitter contents in the nerve cell bodies are influenced by various factors. For example, activation may increase catecholamine synthesis and NPY mRNA (Hanze et al., 1991), while denervation results in depletion of NA and NPY (Edvinsson et al., 1984). However, after sympathectomy, there is an up regulation of NPY-containing fibers of parasympathetic origin (Bjorklund et al., 1985). Furthermore, there is a significant reduction with age of NPY, VIP, substance P (SP) and CGRP in human cerebral arteries.

Electron microscopic and functional studies have revealed that NA, NPY and adenosine triphosphate (ATP) are co-stored in large dense-cored vesicles (Burnstock, 1990). Sympathetic stimulation induces the release of these transmitters; the stimulus intensity determines the relative contribution of NA and NPY. At resting conditions, little NPY is released; hence, sympathetic vasoconstriction is largely due to activation of adrenoceptors and purinergic receptors, whereas during high sympathetic activity, the contribution of NPY is prominent (Lundberg et al., 1986).

It has been suggested that the small arterioles on the cortical surface are supplied by NA-containing fibers emanating from an intracerebral source such as the locus coeruleus (LC) and/or the hypothalamus. Indeed, destruction of the LC induces a reduction in the number of NA nerve fibers in intracerebral blood vessels (Edvinsson & Krause, 2002). It is tempting to involve such a pathway in coupling neuronal activity to local blood flow regulation (Lou et al., 1987).

### 2.2. Parasympathetic nervous system

The "classical" neurotransmitter in parasympathetic nerves is acetylcholine (ACh), and the cell bodies of the sphenopalatine and otic ganglia contain acetylcholinesterase (AChE). Cerebral blood vessels contain perivascular nerves that display AChE activity (Edvinsson & Krause, 2002; Edvinsson & Uddman, 2005) and choline acetyltransferase (ChAT) (Suzuki et al., 1990). At the ultrastructural level, varicosities that contain numerous small agranular vesicles (which remain after sympathectomy) were considered as parasympathetic nerve terminals (Edvinsson & Krause, 2002; Edvinsson & Uddman, 2005). Since these varicosities occur in close apposition to large dense-cored vesicles in the neuroeffector junction, parasympathetic nerves may interact with sympathetic nerves near cerebrovascular smooth muscle (Edvinsson & Krause, 2002; Edvinsson & Uddman, 2005). In several species, ACh constricts cerebral arteries after endothelium removal, while transmural nerve stimulation predominantly induces relaxation (Lee, 1980). The neurogenic vasodilatation in these preparations is not blocked by atropine and is thus non-cholinergic (Lee, 1980); thus, additional substances seem to be co-released with ACh to mediate dilatation. Several neuromediators inducing cerebral neurogenic vasodilatation have been suggested, including VIP, pituitary adenylate cyclase-activating peptide (PACAP) and nitric oxide (NO). Interestingly, all three seem to mediate a major component of the cerebral vasodilator responses in vivo (Jansen-Olesen et al., 1994) although, in some cases, the potency of PACAP seems to be limited in human isolated blood vessels (Chan et al., 2011a). In fact, NO might be the last link in cholinergic transmission. Another possibility could be that ACh mainly acts prejunctionally to inhibit neurotransmitter release from autonomic nerves (Lee, 2000). The vast majority of parasympathetic fibers to cerebral blood vessels originate from the sphenopalatine and the otic ganglia (Edvinsson et al., 2001).

VIP was the first neuropeptide demonstrated in perivascular nerves around the brain blood vessels (Edvinsson & Krause, 2002; Edvinsson & Uddman, 2005). Other peptides of the VIP family, such as peptide histidine isoleucine (PHI) (Edvinsson & McCulloch, 1985), and its human form PHM (peptide histidine methionine) are seen in nerves that supply cerebral blood vessels (Edvinsson & Krause, 2002). In most species, VIP-containing nerves are most abundant in the circle of Willis and in the major cerebral arteries (Fig. 1). In humans, the supply of VIP-immunoreactive nerves is sparse in both cerebral arteries and veins, while sphenopalatine ganglia show a rich supply of cell bodies containing VIP, PACAP and NO, and express mRNA for NPY $Y_1$ and $VIP_1$ receptors (Uddman et al., 1999).

In some locations, AChE activity and VIP immunoreactivity are found in the same perivascular nerves, suggesting that VIP and ACh coexist in parasympathetic nerves (Gulbenkian et al., 1991). VIP immunoreactivity is localized in large electron-dense secretory vesicles in nerve varicosities, which also contain smaller sized agranular vesicles presumed to represent parasympathetic neurons (Edvinsson et al., 1998a). However, other studies on the cholinergic and VIPergic cerebrovascular innervations have shown that ChAT and VIP immunoreactivity are co-localized in less than 5% of the fibers examined (Miao & Lee, 1990).

PACAP is a vasoactive peptide displaying 68% homology to porcine VIP and is about 1000 times more potent than VIP in stimulating adenylate cyclase activity in cultured rat anterior pituitary cells (Arimura & Shioda, 1995). PACAP-immunoreactivity and PACAP mRNA have been found in the sphenopalatine and otic ganglia (Uddman et al., 1999). Perivascular nerves containing PACAP immunoreactivity are seen in cerebral blood vessels, and PACAP mediates dilatation (Jansen-Olesen et al., 1994). Most of the PACAP-immunoreactive nerves constitute a subpopulation of fibers containing VIP/NOS immunoreactivity as verified by tracing, denervation and co-localization experiments (Edvinsson et al., 2001). VIP/PACAP receptors have been found in human and rodent cerebral vessels and in the sphenopalatine ganglion (Chan et al., 2011a; Csati et al., 2012a).

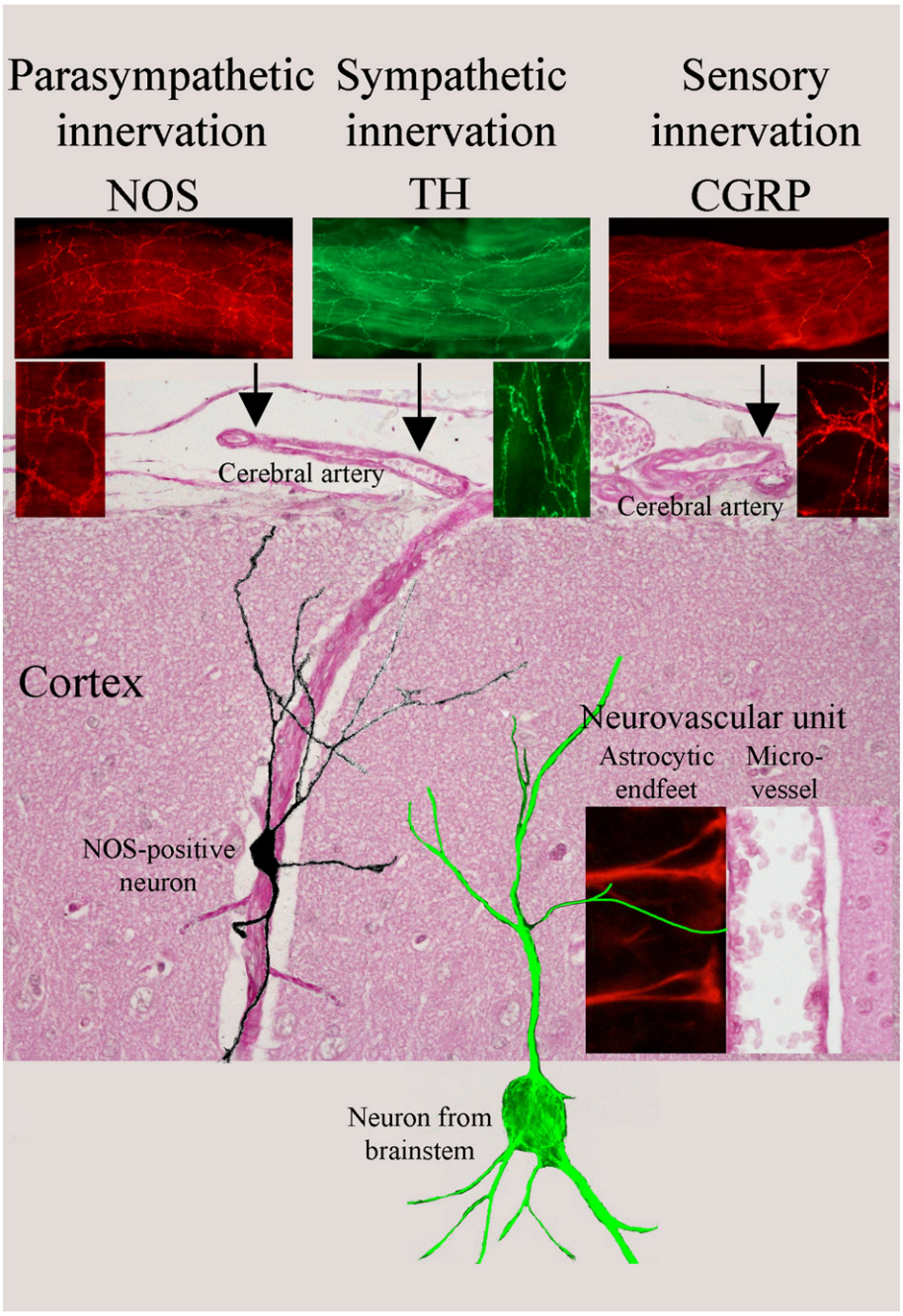


**Fig. 1.** Schematic overview of nerve supply to the cerebral circulation. The nervous supply of the large cerebral arteries belonging to the circle of Willis and the vessels on the surface of the brain and proximal in some penetrating arterioles emanates in the cranial ganglia. The sympathetic fibers store NA (here shown by tyrosine hydroxylase immunohistochemistry, TH) and NPY, and originate in the superior cervical ganglion. The parasympathetic fibers contain VIP, PACAP, NOS and ACh, and originate in the sphenopalatine and otic ganglia. The trigeminal ganglion is the source of intracranial sensory nerves containing CGRP, SP/NKA, PACAP and NOS. Arterioles or microvessels located in the brain parenchyma are supplied with "intrinsic" nerve pathways that originate in the CNS. Cortical microvessels receive NA, 5-HT, Ach, GABAergic and NOS afferents from subcortical neurons from the locus coeruleus, raphe nucleus (shown as brainstem neuron in green), basal forebrain, or local cortical interneurons. The latter is illustrated by NOS containing interneurons in the cortex of rats (Regidor et al., 1993) and humans (Zhang et al., 1995). The "neurovascular unit" depicts yet another intricate mode of control where astrocytic end feet may communicate signaling in the brain to the microcirculation (Hamel, 2006; Attwell et al., 2010).

NO is a highly labile molecule and information on its cellular localization has largely been attained by immunocytochemistry for nitric oxide synthase (NOS). Indeed, not only is NO a candidate for the endothelium-derived relaxing factor, but it also acts as a neurotransmitter (Bredt et al., 1990). However, NO is a non-conventional transmitter since it appears to be released by diffusion rather than exocytosis upon formation, it is not stored in vesicles, and its action is not dependent on the presence of conventional membrane-associated receptors. There is a rich supply of NOS-immunoreactive nerves around cranial blood vessels from several species, including humans (Bredt et al., 1990; Nozaki et al., 1993; Edvinsson et al., 2001). In the human circle of Willis, NOS-containing fibers are relatively sparse and mainly detected in posterior arteries (Nozaki et al., 1993). In rodent and bovine cerebral blood vessels (Nozaki et al., 1993; Gonzalez et al., 1997) NOS-immunoreactive nerves contain both VIP and AChE activity and are considered as parasympathetic nerves originating mainly from the sphenopalatine ganglion (Edvinsson et al., 2001). In fact, Lee (2000) has previously suggested that NO is a primary postjunctional messenger with VIP, and that ACh

rather acts to limit the release of NO via a prejunctional cholinergic receptor.

### 2.3. Sensory nervous system

Most sensory fibers to cranial structures derive from the trigeminal ganglion (TG) and contain several neuromediators including CGRP, SP and neurokinin A (NKA) (Edvinsson, 2011). The cerebrovascular distribution of NKA resembles that of SP, and both substances coexist in cell bodies of sensory ganglia and in perivascular nerves (Dalsgaard et al., 1985). NKA-immunoreactive nerves have a similar distribution to that of SP-containing nerves and are depleted by capsaicin.

CGRP immunoreactive fibers supply the major cerebral arteries and pial arterioles on the cortical surface of several species including humans (Edvinsson et al., 1987a; Fricke et al., 1997); while cerebral arteries of laboratory animals receive a dense supply of CGRP fibers, human cerebral blood vessels contain only a sparse network. Moreover, using double immunogold staining it has been shown that CGRP and SP are co-localized in the same large granular vesicles in both sensory neurons of the TG and in varicosities of perivascular nerves of guinea-pigs and humans. The first studies to show SP containing nerves in the dura mater, meningeal arteries and cerebral blood vessels (Edvinsson et al., 1981) were followed by findings that SP receptor blockers were effective against neurogenic inflammation. Notwithstanding, several clinical studies with different SP receptor blockers showed that they were without effect in migraine (May & Goadsby, 2001). In addition, SP does not seem to play a role in vascular nociception in humans (Holthusen et al., 1997).

In the human TG, CGRP-immunoreactive neurons amount to 50% of all neurons (Eftekhari et al., 2010), whereas SP-immunoreactive neurons are less numerous (18%) (Tajti et al., 1999b). In situ hybridization has revealed that 40% of all nerve cell bodies contain CGRP and CGRP mRNA (Edvinsson et al., 1998a; Tajti et al., 1999b). CGRP and SP are potent vasodilators in vivo and in vitro, with the former being 10–1000 times more potent (Edvinsson et al., 1981; McCulloch et al., 1986; Edvinsson et al., 1987b). Moreover, SP is involved in plasma extravasation in the dura mater during primary headache attacks (Markowitz et al., 1987). While SP (neurokinin-1) receptor antagonists potently inhibit neurogenic plasma extravasation (Shepheard et al., 1993), these compounds have no significant effect in acute migraine attacks (May & Goadsby, 2001). Furthermore, while CGRP is released during migraine headache, SP is not (Goadsby & Edvinsson, 1993; Williamson et al., 1997a). Accordingly, vasodilatation during perivascular stimulation of the middle meningeal artery (MMA) in vivo was blocked by a CGRP receptor antagonist, but remained unaffected by neurokinin-1 agonists or antagonists (Williamson et al., 1997b).

Immunocytochemistry has revealed a minor expression of PACAP not only in parasympathetic, but also in sensory ganglia (Moller et al., 1993; Tajti et al., 1999b), which suggests that PACAP acts as a neuromodulator in the sensory systems (Moller et al., 1993). In human TG, PACAP-containing cell bodies are more numerous than in laboratory animals, amounting to 15%–20% of the neuronal population (Tajti et al., 1999b). Double immunostaining has revealed that PACAP co-localizes with CGRP in some cell bodies in the TG, putatively the small and medium-sized neurons. PACAP dilates cerebral arteries and can increase cerebral blood flow (Jansen-Olesen et al., 1994). Activation of the trigeminovascular system results in release of CGRP and a minor co-release of PACAP into the jugular vein of cats (Zagami et al., 1995). This peptide may, to some extent, participate in antidromic vasodilatation following activation of the trigeminovascular reflex (McCulloch et al., 1986). Indeed, PACAP may play a role in glyceryl trinitrate (GTN)-induced activation of the trigeminovascular system (Markovics et al., 2012). In addition, trigeminovascular activation by GTN or trigeminal ganglion stimulation altered the levels of PACAP in the trigeminal ganglion and the trigeminocervical complex (Tuka et al., 2012). Hence, the increase in both forms of PACAP (PACAP-27 and PACAP-38) in the trigeminal nucleus caudalis (TNC) may play a role in the central sensitization involved in migraine-like headache.

In addition, the opioid peptides endorphins, enkephalins and dynorphins belong to a family of neuropeptides that modulate nociception and inflammation (Olson et al., 1994). Their effects are mediated via three opioid receptor types, termed μ, δ and κ receptors. An additional receptor of this group, namely, the ORL-1 (orphan-like) receptor has been demonstrated (Mollereau et al., 1994), and the identified endogenous ligand was termed nociceptin because of its hyperalgesic properties (Meunier et al., 1995). A reduced circulating nociceptin level has been reported in cluster headache attack periods, and this finding suggested a reduced "break" in the disorder (Ertsey et al., 2004).

Nociceptin behaves like classical opioids with inhibition of cyclic adenosine monophosphate (cAMP) production, activation of inwardly rectifying $K^+$ channels and modulation of a variety of voltage-dependent $Ca^{2+}$ currents (Darland et al., 1998). The action of nociceptin on ion conductance results in reduced neuronal excitability or presynaptic neurotransmitter release (Meunier et al., 1995). Pharmacological studies have shown that nociceptin displays diverse effects on the modulation of pain. In the CNS, nociceptin may be algesic and thus acting opposite to opioid agonists. In the peripheral nervous system there is a substantial overlap of the effects produced by activation of the ORL-1 receptor and the opioid receptors (Giuliani et al., 2000). The bidirectional effects of nociceptin may depend on the activation of either μ-sensitive secondary cells or κ-sensitive primary cells in the nucleus raphe magnus (NRM) (Pan et al., 2000). Nociceptin and its receptors are widely distributed both centrally and peripherally (Ikeda et al., 1998; Calo et al., 2000). Nociceptin immunoreactivity in the superficial dorsal horn is not affected by dorsal root rhizotomy, suggesting that at the spinal level nociceptin is produced by central, rather than primary afferent, neurons (Riedl et al., 1996). Tracing experiments have revealed that the primary trigeminal fibers descend to the superficial layer of the cervical dorsal horn (Marfurt & Del Toro, 1987). About 70% of neuronal cells in TG are nociceptin immunopositive (Hou et al., 2003). Double immunostaining has shown that in the human TG nociceptin is co-localized with CGRP, SP, NOS and PACAP (Hou et al., 2003).

NO has been suggested as an important molecule for initiation of migraine attacks (Olesen et al., 1995). This assumption was based on the early finding that ingestion of GTN resulted in an early headache in healthy volunteers, but in an additional migraine-like attack in migraine patients. The expression of NOS in trigeminal nerves and in TG cell bodies partly supports this suggestion. However, the case for NO is complex as: (i) it can be produced and released from the endothelium (eNOS), from the perivascular nerves (nNOS) and/or synthesized by inducible NOS (iNOS); and (ii) it may activate the guanylate cyclase system in smooth muscle cells. The responses to NO, in turn, result in a decrease in the local intracellular $Ca^{2+}$ level and vasodilatation which may activate the pain sensitive structures around cranial blood vessels (Olesen et al., 1995).

The human TG stores several neurotransmitters such as CGRP, SP, NKA, PACAP, nociceptin and NO, but a functional system requires receptors. Few studies have examined the presence of neuropeptide receptors in this system, but some work in TG has shown the presence of mRNA for: (i) both the NPY $Y_1$ and the NPY $Y_2$ receptors (Tajti et al., 1999b), suggesting a sympathetic modulation on TG function; (ii) $VPAC_1$ receptors, implying a parasympathetic influence on TG activity; and (iii) CGRP and nociceptin receptors (Tajti et al., 1999b; Hou et al., 2003). Moreover, detailed studies have revealed that small to medium sized TG neurons store CGRP, while the CGRP receptor elements, CLR and RAMP1, are found in large TG neurons and in the satellite glial cells (Eftekhari et al., 2010; Eftekhari & Edvinsson, 2011).

Another recent finding showed that the sphenopalatine ganglion contains CGRP positive fibers, indicating a sensory influence on the

parasympathetic system (Csati et al., 2012b). Consistent with this view, clinical studies suggest a link between the sensory trigeminal system and the parasympathetic ganglia (Goadsby & Edvinsson, 1994b). Thus, CGRP is a sensory neuropeptide that plays an important role in vasodilatation and pain transmission in craniocervical structures. Further, we have shown immunoreactivity for CGRP and the CGRP receptor components, the calcitonin receptor-like receptor (CLR) and the receptor activity modifying protein 1 (RAMP1), in human and rat SPG (Csati et al., 2012b) as follows: (i) CGRP fibers in the SPG; (ii) CLR in satellite glial cells (SGCs) and in nerve fibers; and (iii) RAMP1 in many neurons and SGCs. Thus, the two CGRP receptor components together were found in SGCs. Our results suggest a possible sensory influence in parasympathetic cranial ganglia. The sensory CGRP-containing fibers probably originate in the trigeminal ganglion, project to the SPG and act on CGRP receptors on SGCs.

The capsaicin receptor, also known as transient receptor potential vanilloid type 1 (TRPV1 receptor), is an integral membrane protein with homology to a large family of nonselective cation channels responsible for a series of pleiotropic biological responses (Caterina et al., 1997; Nilius et al., 2007). The TRPV1 receptor, co-expressed with SP and CGRP in sensory neurons, is activated not only by capsaicin, but also by noxious heat and acid pH. It may, therefore, be a molecular integrator of those chemical and physical stimuli that elicit pain (Szallasi & Blumberg, 1999). The TRPV1 receptor is easily desensitized by its agonist resiniferatoxin and shows slow recovery (Szallasi & Blumberg, 1992). Capsaicin is an excitatory neurotoxin that can release sensory neurotransmitters and selectively destroys primary afferent neurons expressing the TRPV1 receptor. In addition to its excitatory actions, capsaicin desensitizes the tissue with subsequent anti-nociceptive and anti-inflammatory effects (Bevan & Szolcsanyi, 1990). Within the cranial circulation, capsaicin may activate the trigeminovascular system and release sensory peptides.

Immunohistochemistry and in situ hybridization have revealed expression of TRPV1 receptors in small- to medium-sized neurons both at the mRNA and the protein levels in the dorsal root ganglia (DRG), the TG and the brainstem of rats (Caterina et al., 1997; Guo et al., 1999). In human TG, the TRPV1 receptor was similarly detected (Hou et al., 2002). A low number of human trigeminal neurons were TRPV1 receptor immunopositive (16% of total neuronal cell bodies). This finding suggests that activation of TRPV1 receptors may modulate the release of neurotransmitters.

### 2.4. Intracerebral innervation

In vitro and in situ experiments suggest that brain metabolism produces substances (e.g. $H^+$, $CO_2$, $K^+$, $Ca^{2+}$, adenosine, etc.) that mediate local changes in cerebral blood flow and that accompany neuronal activity. However, these experiments were performed at steady state conditions and in the whole brain or large parts of it; consequently, this view did not seem to account fully for the adaptative responses in vasomotor activity in the microvascular bed. There has been a long quest on whether intracerebral neurons can directly control the intracerebral microcirculation and there are some data to support this hypothesis (Lou et al., 1987). One proposal is that regional changes in brain perfusion are controlled directly by neurons located within the brain parenchyma (Fig. 1). For instance, stimulation of specific brain regions such as the cerebellar fastigial nucleus, the basal forebrain or the brainstem raphe nucleus elicits changes in cerebral blood flow in specific brain areas (Edvinsson & Krause, 2002; Hamel, 2006). These changes in perfusion occur independently of those in glucose metabolism, thus implying that neuronal pathways exert direct effects on the microcirculation. Furthermore, there is a population of neurons the activity of which is related to spontaneous waves of cerebral blood flow in the cerebral cortex, and is suspected to transduce neuronal signals into vasomotor responses (Golanov & Reis, 1996). These observations suggest that the neuronal control of the microvascular bed, in concert with other mechanisms (e.g. ionic gradients and intrinsic endothelial or myogenic vascular responses), is a key determinant in the spatial and temporal adaptation of local perfusion to cellular activity. This implies that brain neurons send projection fibers to microvessels in target regions and that resistance microarterioles (and, possibly, capillaries) can modify their diameters and consequently local blood flow in response to changes in the level of brain neuromediators (Attwell et al., 2010).

Furthermore, early morphological studies have documented the presence of nerve fibers and, occasionally, neuronal cell bodies containing different neuromediators in association with intraparenchymal blood vessels in many areas of the brain (Lou et al., 1987). These perivascular fibers or neuronal perikarya were described as following the contours of blood vessels, apposed to, or literally encircling, the vessel walls. Since adrenergic vesicles remained after bilateral cervical sympathectomy, intracerebral vessels seem to receive an adrenergic innervation of central origin, which has been confirmed by morphological studies (Edvinsson & Krause, 2002; Edvinsson & Uddman, 2005). Accordingly, the LC is the exclusive source of cortical perivascular noradrenergic nerve terminals, and ultrastructural analysis has emphasized the frequent association of these fibers with capillaries and microarterioles (Edvinsson & Krause, 2002; Edvinsson & Uddman, 2005).

A similar morphologic analysis has been performed for central dopaminergic fibers. Although no in vivo studies attest to a primary vascular effect of dopaminergic centers on the local microcirculation, Krimer et al. (1998) have shown: (i) that perivascular application of dopamine in cortical brain slices causes vasoconstriction in about 50% of the microvessels studied; and (ii) the presence of dopaminergic fibers closely associated with intracortical microvessels, such as capillaries, microarterioles and penetrating arteries. In contrast to the relatively minor effect on local perfusion exerted by central noradrenergic neurons, stimulation of the brainstem raphe nuclei (the source of serotonergic nerve fibers throughout the brain) or the ascending serotonergic pathways results in vascular responses in projection areas, such as the cerebral cortex, corresponding primarily to vasoconstriction (Cohen et al., 1996). Histochemical examination has shown intimate associations between serotonergic neuronal processes and intraparenchymal vessels of the raphe nuclei. This innervation of local microvessels appears to embrace all vascular elements — arterioles, capillaries, and venules (Edvinsson et al., 1983). At the ultrastructural level, perivascular nerves containing serotonin (5-hydroxytryptamine; 5-HT) labeled for the 5-HT-synthesizing enzyme tryptophan hydroxylase were associated with capillaries and microarterioles of all sizes, including penetrating arteries (Cohen et al., 1995). Bradley et al. (2002) further supported this view by using confocal imaging and electron microscopy, and showed an intimate association between brainstem serotonergic neurons and the wall of large medullary arteries. Therefore, several findings support the role of neuronally-produced substances in the control of microvascular tone and, thereby, local cerebral blood flow (Lou et al., 1987) including: (i) the presence of frequent and strategically located neurovascular appositions, their region-selective distribution and perivascular proximity in the regions known to modify their local perfusion in response to the stimulation of specific neuronal populations; and (ii) the exceptional positioning of cortical interneurons. Hypothetically, this may provide the anatomical key link between neurons of the brain and the trigeminovascular system which is the central communication for the afferent pain to the brainstem and the central aspects of the migraine symptoms. This view is further supported by Akerman et al. (2011), who suggest that the symptoms observed during migraine attacks derive from diencephalic and brainstem regions.

Another way of connecting the CNS with the cerebral vasculature is within the so-called neurovascular unit, as illustrated in Fig. 1 (Attwell et al., 2010). Indeed, neuronal activation in the CNS seems to link to cerebral microvessels via astrocytes and glutamatergic

signaling. In this system astrocytes are strategically localized to sense glutamatergic synaptic activity over a large area via activation of metabotropic glutamate receptors and subsequent calcium signaling. The astrocyte foot processes can signal to vascular smooth muscle and change vascular tone by prostaglandin pathways and by astrocytic and smooth muscle potassium channels (Koehler et al., 2009). In this brain microenvironment, non-glutamatergic transmitters released from neurons (e.g. NO, VIP and cyclooxygenase-2 metabolites) might modulate the signaling within the neurovascular unit (Fig. 1; Hamel, 2006). It is of course pivotal to understand in the future how this signaling is integrated in the regulation of the brain microcirculation in different conditions. Overactivation in this system may be part of the so-called spreading depression. Indeed, some experimental data support a partial involvement of CGRP in the hyperemic phase of spreading depression (Wahl et al., 1994). However, experimental spreading depression in cats did not elicit release of CGRP (Piper et al., 1993), suggesting at best a minor involvement in spreading depression.

## 3. Possible mechanisms involved in the pathophysiology of migraine

### 3.1. General considerations

Migraine is a neurovascular disorder involving activation of the trigeminovascular system (Edvinsson & Uddman, 2005), with the primary dysfunction located in brainstem centers regulating vascular tone and pain sensation (Link et al., 2008). This activation results in cranial vasodilatation mediated by the release of vasoactive neuropeptides including CGRP, which seems to play a pivotal role in migraine pathophysiology (Villalón & Olesen, 2009). Moreover, the thorough search for more selective and effective antimigraine drugs has led to the development of the triptans (Villalón et al., 2003; Humphrey, 2008) and the gepants (Villalón & Olesen, 2009; Ho et al., 2010), which represent a major therapeutic breakthrough. Interestingly, the antimigraine doses of triptans and gepants are much higher (up to 1000 or 3-log units) than those required to elicit their respective vascular actions in vitro or in vivo. These higher doses may be required to reach specific targets inside the brain, suggesting a central mode of action. However, as discussed elsewhere (Edvinsson et al., 2010; Chan et al., 2011b), the higher plasma concentrations that are required may also be explained by the high plasma protein binding of telcagepant, and the vascular location of the CGRP receptor in the media rather than in the lumen, as well as other factors. On the basis of the above reflections, this section will deal with some aspects of migraine pathophysiology that require in depth analysis, including: (i) what are the possible anatomical structures involved in migraine pathophysiology; (ii) where does the migraine attack start?; (iii) could migraine be considered a channelopathy?; (iv) what are the possible mediators involved in migraine pathophysiology?; and (v) do peripheral and/or central mechanisms play a role in migraine?

### 3.2. What are the possible anatomical structures involved in migraine pathophysiology?

Some of the main brain structures and pathways possibly involved in migraine pathophysiology have been described by a number of quality reviews (Goadsby et al., 2002; Link et al., 2008; Villalón & Olesen, 2009; Gupta & Villalón, 2010; Ho et al., 2010; Edvinsson, 2011). These include: (i) the MMA surrounded by perivascular nerves and escorted by mast cells in the meninges; (ii) large cerebral arteries; (iii) the trigeminal nerve from meningeal and cerebral arteries and from the TG to the TNC; and (iv) TNC conveying the nociceptive signals to higher pain centers, like the thalamus and cortex (Liu et al., 2009). Activation of sensory trigeminal nerves can also release various neuropeptides, including CGRP (Goadsby et al., 1988) which, in turn, activates postsynaptic structures such as the brainstem and produces dilatation of cerebral and meningeal arteries.

Indeed, pain-sensitive structures such as the intracranial blood vessels and the meninges, especially the dura mater, are supplied with sensory nerve fibers by the ophthalmic ramus of the first branch of the trigeminal nerve (Edvinsson & Krause, 2002; Edvinsson & Uddman, 2005). They arise from pseudounipolar neurons located in the TG (Link et al., 2008; Eftekhari et al., 2010), which project onto second order sensory neurons in the TNC in the brainstem and its related extensions down to the C2-level called the trigeminocervical complex (Edvinsson, 2011). From here, a signal is transmitted to the ventroposterior thalamus leading to activation of the frontal cortex, insulae and cingulate cortex, resulting in the experience of pain (Goadsby et al., 2007). In addition, a migraine active region has been pointed out in the brainstem by using positron emission tomography (PET) (Weiller et al., 1995; Bahra et al., 2001). Thus, as suggested by Edvinsson et al. (2011), the trigeminovascular system: (i) seems to be involved at the level of both the brainstem and perivascular nerves; and (ii) transmits nociceptive information to the CNS. Moreover, there is a dense supply of CGRP-containing nerve fibers originating in the first division of the TG that innervates intracranial blood vessels (Link et al., 2008).

Trigeminal release of CGRP from C-fibers produces cranial vasodilatation which, in turn, activates Aδ-fibers that contain the CGRP receptor in the dura mater and the MMA (Edvinsson et al., unpublished). This may result in the release of neurotransmitters (mainly CGRP) that promote a peripheral inflammatory response within the dura and peripheral sensitization of trigeminal nerves. Release of neurotransmitters within the CNS also occurs after trigeminal nerve activation, resulting in central sensitization which is characterized by hyperalgesia and allodynia (see below). Further, the trigeminovascular reflex is probably part of a counter-balancing system (McCulloch et al., 1986), which mediates brain vasodilatation and sustaining of cerebral blood flow. This assures the maintenance of local brain blood flow within normal limits. On the other hand, activation of TG neurons leads to activation of the trigeminocervical complex that projects to the CNS and mediates the central aspects of pain (Goadsby et al., 2007). The massive release of neurotransmitters and vasoactive substances from mast cells, a kind of “inflammatory soup”, may activate the reflex and the local vascular mechanoreceptors (A-fibers) resulting in trigeminal activation.

### 3.3. Where does the migraine attack start?

Although the exact anatomical locus that initiates a migraine attack remains elusive, some cerebral blood flow studies in migraineurs with aura and without aura have shed further light on this issue.

#### 3.3.1. Migraine with aura

In an early study of migraine aura (elicited by intracarotid tracer injections), the changes in cortical blood flow were found to correlate with areas of hypoperfusion which were ipsilateral to the headache and contralateral to the symptoms of the aura; however, no subsequent spreading from the hypoperfusion area could be demonstrated, possibly because these patients had been studied late during the attacks (Olesen et al., 1990). Furthermore, in one subject who suffered a migraine attack during a series of cerebral blood flow measurements with PET (Woods et al., 1994), the headache was associated with bilateral hypoperfusion which started in the occipital lobes and spread anterior into the temporal and parietal lobes; despite the marked flow changes, there were no focal neurological symptoms. This provided a high-resolution evidence of the spreading nature of the hypoperfusion associated with a spontaneous migraine attack. This view was further supported by a study of blood oxygenation level dependent (BOLD) signal changes reflecting the balance between oxygen delivery and oxygen consumption. In one patient, two attacks of induced migraine aura showed an increase in the

mean magnetic resonance (MR) signal (5%) restricted to the occipital cortex contralateral to the visual aura (Hadjikhani et al., 2001). These changes were followed by a decrease in the mean MR signal (by 5%), corresponding to the localized scotomas. The average spread velocity of the cortex hypoperfusion was 3.5 mm/min, being in concert with previous experimental studies (Lauritzen, 1994). Additionally, in three spontaneous migraine attacks that were captured within 20 min of the onset of visual symptoms, the BOLD data revealed increases in the amplitude of the MR signal (Hadjikhani et al., 2001). This evidence supports: (i) cortical spreading depression (CSD) as an initial cortical gray matter hyperemia with a characteristic velocity that is followed by hypoperfusion; and (ii) hypoperfusion, which spreads along the cortical surface at a relatively constant rate, sparing the cerebellum, the basal ganglia and the thalamus, and ultimately spanning the vascular distributions of the four major cerebral arteries (Lauritzen, 1994).

Thus, a plausible explanation for the blood flow changes associated with aura in a migraine attack is that they are the result of CSD. The rate of advance is consistent with the spread of symptoms observed and the decreases in blood flow (Lauritzen, 1994). CSD can move transcallosally to homologous regions of the opposite hemisphere in animals, and transcallosal spread may account for the bilateral headache observed at the onset of the attack (Woods et al., 1994). Moreover, on the basis of the above studies, one could suggest that the migraine aura is evoked by aberrant firing of neurons and related cellular elements rather than by ischemia. In this context, several possibilities can be suggested regarding the events involved in activation of the trigeminovascular reflex (McCulloch et al., 1986) including, among others:

(1) The link between CSD and neurogenic inflammation in the dura mater and, from there, activation of sensory and autonomic reflexes (Bolay et al., 2002). However, the dura mater is an extracerebral structure (separated from the brain by the cerebrospinal fluid and pial and arachnoid connective tissues) nourished by the external carotid artery (Olesen et al., 2006).
(2) The involvement of specific cell bodies projecting from the brainstem to cerebral blood vessels such as the adrenergic and serotonergic efferents from the LC and the raphe nuclei, respectively (Lou et al., 1987; Hamel, 2006). This is further supported by: (i) the association between intracerebral nerves and cerebral blood vessels (Edvinsson et al., 1983; Bradley et al., 2002); and (ii) a direct neurogenic control by intrinsic serotonergic neurons on the cerebral microvascular bed (Cohen et al., 1996). Moreover, there is a link between 5-HT neurons and microarterioles, capillaries and perivascular astrocytes; this is clearer in regions where manipulation of the intrinsic 5-HT neurons elicits uncoupling between flow and metabolism (Cohen et al., 1995, 1996).
(3) A direct effect from local neurons to the cerebral vasculature via astrocytes, as previously postulated (Hamel, 2006; Attwell et al., 2010).
(4) Activation of neurons in the brain, resulting in signaling via brainstem centers to the trigeminocervical complex, and from there to the TG and the cranial vasculature (Goadsby et al., 2002; Edvinsson & Uddman, 2005; Villalón & Olesen, 2009). This may, in turn, induce vasodilatation, dural inflammation and peripheral sensitization.

#### 3.3.2. Migraine without aura

In migraineurs without aura, the situation is more complex (Weiller et al., 1995). During attacks, small increases in blood flow were observed in the cingulate, auditory and visual association cortices, and in brainstem regions. These changes normalized after injection of sumatriptan and induced complete relief from headache, phono- and photophobia. However, these changes could only be significant if the PET data from all nine subjects were normalized, thus being in agreement with previous negative studies with the $^{133}$Xe method which lacks the precision of PET (Olesen et al., 1990). Further support for the importance of a brainstem region was obtained in a patient that developed migraine after administration of GTN. Indeed, Bahra et al. (2001) observed activation in the dorsal rostral brainstem region and hence reproduced the findings observed by Weiller et al. (1995). These authors also observed a neuronally-driven vasodilatation and activation of regions associated with pain processing. In addition, the episodic nature of the disorder suggests the involvement of at least the suprachiasmatic region, possibly associated with the human biological clocks. Interestingly, spontaneous as well as GTN-induced cluster headache attacks are associated with cerebral vasodilatation, interpreted as occurring via a neuronal mechanism (May et al., 2000). Cranial vasodilatation was not considered to be specific to any particular headache syndrome, but generic to cranial neurovascular activation involving both sensory and parasympathetic reflexes as evidenced by the release of CGRP and VIP, respectively (Goadsby & Edvinsson, 1994b). It is noteworthy that while migraine and cluster headache share much in the expression of pain, their underlying initiator mechanisms distinguish them. Thus, it is the CNS triggering or driving process that ultimately characterizes many of the primary headache syndromes. In contrast, PET scans of capsaicin-induced pain showed no hypothalamic activation (Weiller et al., 1995; May et al., 1998). In patients with capsaicin-induced pain, blood flow changes were seen in an area consistent with the cavernous sinus/carotid artery just as there are blood flow changes in these vessels in cluster headache. This implies that activation of the carotid artery does not relate specifically to cluster headache, but rather to a trigeminovascular autonomic reflex; hence, blood flow changes may be epiphenomena of trigeminal activation rather than a part of the disease generation process.

As discussed by Weiller et al. (1995), direct proof for the involvement of brainstem nuclei in migraine comes from PET studies showing: (i) increased local blood flow in brainstem regions (i.e. midbrain and pons regions) during acute attacks; and (ii) brainstem activation which persisted after injection of sumatriptan. These findings support the contention that the pathogenesis of migraine (and the associated emesis) is related to an imbalance in the activity of brainstem nuclei regulating nociception and vascular control. On similar grounds, notwithstanding, it could equally involve activation of the PAG acting as a filter to inhibit the pain (Fields & Basbaum, 1994). The study revealed activation of the dorsal raphe nucleus (DRN) and the LC. It is well known that these centers have a dense supply of serotonergic and adrenergic fibers, respectively. These fibers may evoke vasoconstriction (via 5-HT and catecholamines, respectively) and hence explain the connection with the trigeminovascular reflex. Alternatively, the DRN and LC may send descending fibers to the TNC and DRG (where they act in a gate-control function), with the PAG inhibiting this. However, it is still unclear whether the brainstem findings reveal the origin of the disease or if it is an accompanying activation designed to limit the symptoms of migraine headache.

### 3.4. Could migraine be considered a channelopathy?

Clinical studies have revealed that migraine patients usually have a family history (Goadsby et al., 2002). Indeed, migraine concordance rates are up to two times higher in monozygotic twins than in dizygotic twins for both migraine with and without aura, indicating that genetic factors are important in the susceptibility of a patient to have migraine (Gervil et al., 1999; Ulrich et al., 1999). However, in these two main types of migraine, this familial aggregation cannot be explained by simple Mendelian inheritance patterns. The rare and severe familial hemiplegic migraine (FHM) is the only variety of migraine in which a Mendelian type of inheritance has been clearly established. More solid data exist now for three genes that have been identified in families with FHM. The first gene that was identified is *CACNA1A*, a gene on chromosome 19p13 that encodes the $\alpha_1$

subunit of a voltage-gated P/Q-type $Ca_v2.1$ calcium channel (Ophoff et al., 1996). The demonstration of mutations in the calcium channel gene *CACNA1A* in ~50% of families suffering from FHM (consequently called FHM1) has offered some hope that there is a molecular genetic cause also for the more common types of migraine (Ophoff et al., 1996; Terwindt et al., 1998). Up to now, 21 FHM1 mutations are known (de Vries et al., 2009), leading to a wide spectrum of clinical characteristics, such as cerebellar ataxia (Ducros et al., 1999; Kors et al., 2003), besides the hemiplegic migraine. The second FHM gene that was discovered is *ATP1A2* (FHM2), located on chromosome 1q23 (De Fusco et al., 2003). This gene encodes the $\alpha_2$ subunit of sodium–potassium pumps. Also in this gene, different mutations may induce different phenotypes, including childhood convulsions, epilepsy and permanent mental retardation (de Vries et al., 2009). Over 30 different FHM2 mutations have currently been identified (de Vries et al., 2009). The third FHM gene that was discovered is *SCN1A* (Dichgans et al., 2005), encoding the $\alpha_1$ subunit of neuronal $Na_v1.1$ voltage-gated sodium channels. Mutations in *SCN1A* are well known in epilepsy. Up to now only five FHM3 mutations are known (for review, see de Vries et al., 2009). In still other cases, the disorder is linked to neither site, suggesting the existence of at least one additional locus.

All three known FHM genes do encode for ion channels (*CACNA1A* and *SCN1A*) or an ion pump (*ATP1A2*). FHM1 mutations in the *CACNA1A* gene seem, based on experiments in transgenic knock-in mice, to increase glutamate release in the brain (van den Maagdenberg et al., 2007). The FHM2 mutations in the *ATP1A2* gene could lead to a decreased uptake of $K^+$ and glutamate from the synaptic cleft. Finally, FHM3 mutations in the *SCN1A* gene could lead to neuronal hyperexcitability. Thus, the FHM mutations that are currently known seem to increase levels of $K^+$ and glutamate in the synaptic cleft. The resultant hyperexcitability could lead to CSD (Casucci et al., 2008). These mutations may also lead to an increased activity in the trigeminovascular system, as was recently suggested by increased CGRP release in cultured TG cells of mice expressing one of the FHM1 mutations (Ceruti et al., 2011).

Recently, technical improvements have enabled studies on the genetics of migraine with and without aura in large patient groups. These groups can now be tested in a hypothesis-free manner using a genome-wide association study (GWAS) method. In large cohorts of migraine patients with and without aura, the marker rs1835740 on chromosome 8q22.1 was identified to be associated with migraine (Anttila et al., 2010). This marker is located between the *MTDH* (astrocyte elevated gene 1, *AEG-1*) and *PGCP* (encoding plasma glutamate carboxypeptidase) genes. An expression study indicated that rs1835740 was related to transcript levels of the *MTDH* gene (Anttila et al., 2010). This gene down-regulates the excitatory amino acid transporter 2 (EAAT2), which removes glutamate from the synaptic cleft. Thus, this gene may well fulfill a role in the same pathway as the FHM mutations. More recently, the three additional markers rs2651899, rs10166942 and rs11172113 have been related to migraine, which do encode, respectively, for the *PRDM16*, the *TRPM8* and the *LRP1* genes (Chasman et al., 2011). Whereas a role for the *PRDM16* gene in migraine seems unclear yet, the *TRPM8* gene encodes a sensor for cold and cold-induced burning pain. Indeed, *TRP* receptors could well be involved in migraine (Eid, 2011). Further, the *LRP1* gene encodes a lipoprotein receptor, which is co-localized with and interacts with NMDA glutamate receptors.

Thus, taken together, the current FHM mutations suggest FHM as a channelopathy. Although genetic studies in the more common forms of migraine are still in their early phases and need replication, their results so far also suggest a role for glutamatergic signaling in migraine. As mentioned elsewhere in this review, glia cells are considered increasingly important in the neurovascular coupling, and thus in the pathophysiology of migraine (Carmignoto & Gomez-Gonzalo, 2010). Glia is likely to be an important source of glutamate in the brain (Larrosa et al., 2006). Several antiepileptic drugs, including valproate and gabapentin (with demonstrated activity in migraine prophylaxis), have an effect on astrocytes. Thus, the effects of these drugs in migraine prophylaxis may well be mediated via a modulating effect on ion levels. Changes in glutamatergic signaling may, in turn, interact with levels of CGRP (Chan et al., 2010) as well as 5-HT (Xiao et al., 2008), which are important mediators in migraine, as will be described in the following section.

### 3.5. What are the possible mediators involved in migraine pathophysiology?

Two main mediators have been suggested to be involved in migraine pathophysiology, namely, 5-HT (see Villalón et al., 2003) and CGRP (see Ho et al., 2010). Indeed, this knowledge has led to the development of agents (i.e. the triptans and gepants; see below) effective in the acute treatment of migraine (Villalón et al., 2003; Villalón & Olesen, 2009; Edvinsson & Linde, 2010).

#### 3.5.1. Possible sites of action of the triptans

5-HT was one the first monoamines proposed to be involved in the pathophysiology of migraine since, among other lines of evidence, slow i.v. infusions of 5-HT could abort a migraine attack (see Villalón et al., 2003). Although several side-effects and the need for an i.v. infusion precluded its clinical use, this suggested the existence of a specific 5-HT receptor involved in the relief of migraine that ultimately led to the development of the triptans (see Villalón et al., 2003; Humphrey, 2008). Although their definite mode of action is still under scrutiny, several mechanisms have been proposed, including: (i) inhibitory actions in the CNS, particularly in the TNC; (ii) prejunctional trigeminovascular inhibition at the level of cranial extracerebral arteries, with a corresponding inhibition of CGRP release (Goadsby & Edvinsson, 1994a); and/or (iii) a direct vasoconstrictor action on cranial extracerebral arteries (Goadsby et al., 2002; Villalón et al., 2003). It is undeniable that the latter mechanism of triptans, mediated by the $5\text{-}HT_{1B}$ receptor, is associated with their efficacy in the acute treatment of migraine (Villalón et al., 2003; Chan et al., 2011b).

#### 3.5.2. The role of calcitonin gene-related peptide and possible sites of action of the gepants (calcitonin gene-related peptide receptor antagonists)

CGRP is a potent vasodilator in several species and is expressed throughout the central and peripheral nervous systems (see Ho et al., 2010; Chan et al., 2011b). CGRP is found in: (i) the CNS (particularly in striatum, amygdalae, colliculi and cerebellum), as well as in the vascular wall of intracranial arteries (Arulmani et al., 2004; Link et al., 2008; Villalón & Olesen, 2009); and (ii) primary spinal afferent C and Aδ fibers projecting to the trigeminal nuclear complex in the brainstem (Eftekhari & Edvinsson, 2011). Moreover, in the TNC and at the spinal C1–C2 segments, CGRP acts at second order neurons to transmit pain signals centrally through the brainstem and midbrain to the thalamus and higher cortical pain regions (Goadsby et al., 2007). In addition, components of the functional CGRP receptor complex, such as CLR and RAMP1 have recently been localized on trigeminal neurons, suggesting that they modulate prejunctionally the production of CGRP (Eftekhari et al., 2010; Eftekhari & Edvinsson, 2011). More recently, it was reported that the CGRP containing C-fibers from the TG ends in the lamina I/II, as expected in the human and rat brain stem. The Aδ-fibers from the TG also projected to the same regions, but in separate fibers; thus it is more likely that they interact here with each other via a postjunctional mechanism (Eftekhari & Edvinsson, 2011). Tracing studies have shown that some of the intracranial vessels project to the lamina III/IV (Liu et al., 2003, 2004), but in these regions we did not find any CGRP or CGRP receptor positive fibers in rats or humans (Eftekhari & Edvinsson, 2011).

In the cranial circulation, CGRP is released by perivascular nerves after trigeminal nerve activation, where it induces cranial vasodilatation by binding to the CGRP receptor (Ho et al., 2010). In general, the

trigeminal system provides an important pain-transmitting link from the cranial vasculature to the CNS (Fig. 2). In laboratory animals, the sensory pathway has no resting tonic influence on regional cerebral blood flow or regional cerebral metabolism (Edvinsson et al., 1986; McCulloch et al., 1986), whereas stimulation of the TG increases intracranial blood flow in part via CGRP release (Edvinsson et al., 1998b). In humans, unilateral stimulation of the TG increases bilateral cortical blood flow, slightly more on the stimulated than on the contralateral site (Tran Dinh et al., 1992). Patients under treatment for trigeminal neuralgia are, in addition, noted to flush on the side of stimulation. While the resting levels of CGRP do not differ from control, TG stimulation during operation results in the release of CGRP and SP, a response that is interrupted following cessation of stimulation (Goadsby et al., 1988). During migraine attacks there is a marked increase in the plasma levels of CGRP in the bulbus (confluence of cranial venous blood or external jugular vein; Goadsby & Edvinsson, 1993). At the same time, there is no change in CGRP levels in peripheral blood or in the levels of NPY, VIP or SP in the jugular vein (Table 1). Furthermore, there is no difference between migraine with or without aura, as both result in substantial increases in venous CGRP levels at the same time as the patients exhibit pain (Goadsby & Edvinsson, 1993). A somewhat higher basal level of CGRP has been noted in the cubital fossa vein in migraineurs also outside the attack (see Villalón & Olesen, 2009). Most significantly, Juhasz et al. (2003) provoked migraine attacks by sublingual GTN and collected CGRP samples from the antecubital vein. It was observed that the CGRP concentration increased during the migraine attack and returned to baseline levels after the cessation of migraine (Juhasz et al., 2003). In addition, the raised CGRP levels correlated with the timing of the attack and, most importantly, with the severity of the migraine headache. Consistent with these findings: (i) i.v. administration of CGRP can induce a migraine-like headache in migraine patients (see Villalón & Olesen, 2009); and (ii) following administration of sumatriptan (Goadsby & Edvinsson, 1993), zolmitriptan (Goadsby & Edvinsson, 1994a), avitriptan (Knight et al., 1999) or rizatriptan (Stepien et al., 2003), the CGRP levels returned to control with successful amelioration of the headache. In addition, sumatriptan caused a parallel decrease in plasma CGRP levels and migraine headache during GTN-evoked attacks (Juhasz et al., 2005). The mechanisms behind this reduction in elevated plasma CGRP levels may be due to the presence of 5-$HT_{1B}$ and 5-$HT_{1D}$ receptors expressed on TG cells and fibers (Longmore et al., 1997; Hou et al., 2001) which may, during stimulation, cause inhibition of sensory nerve activity. In this respect, by using neonatal capsaicin treatment in rats (a treatment that destroys primary trigeminal afferents) Hoffmann et al. (2012) have recently suggested that stimulus-induced CGRP release into the external jugular vein blood comes from the primary trigeminal afferents and trigeminal ganglion. In contrast, CGRP released from sources on the abluminal side of the blood–brain barrier (BBB) may be picked up in the cerebrospinal fluid (Edvinsson et al., 2007).

The reason why SP is not released in migraine might be due to a much lower level of SP than of CGRP within the trigeminovascular system to the intracranial vasculature (Tajti et al., 1999b). Direct electrical stimulation of the TG in humans, however, results in co-release of CGRP and SP (Goadsby et al., 1988), possibly because the entire sensory system to the head is activated.

In short, because of the extensive presence of CGRP containing neurons in the trigeminovascular system, the release of CGRP is thought to initiate cranial vasodilatation, thus playing a role in the pathophysiology of migraine (Villalón & Olesen, 2009; Edvinsson & Linde, 2010). Indeed, CGRP receptor antagonists such as olcegepant (Olesen et al., 2004) and telcagepant (Ho et al., 2008, 2010) have been shown to be effective in the acute treatment of migraine. Moreover, olcegepant inhibited trigeminocervical activity evoked by the superior sagittal sinus in the cat (Storer et al., 2004); from these preclinical studies, the central actions of CGRP also seem to participate in the pathophysiology of migraine.

On the basis of the above information, there are at least four sites of action of CGRP (and gepants) that may be relevant in the pathophysiology of migraine (and antimigraine treatment) (Arulmani et al., 2004; Villalón & Olesen, 2009; Edvinsson & Linde, 2010; Ho et al., 2010),

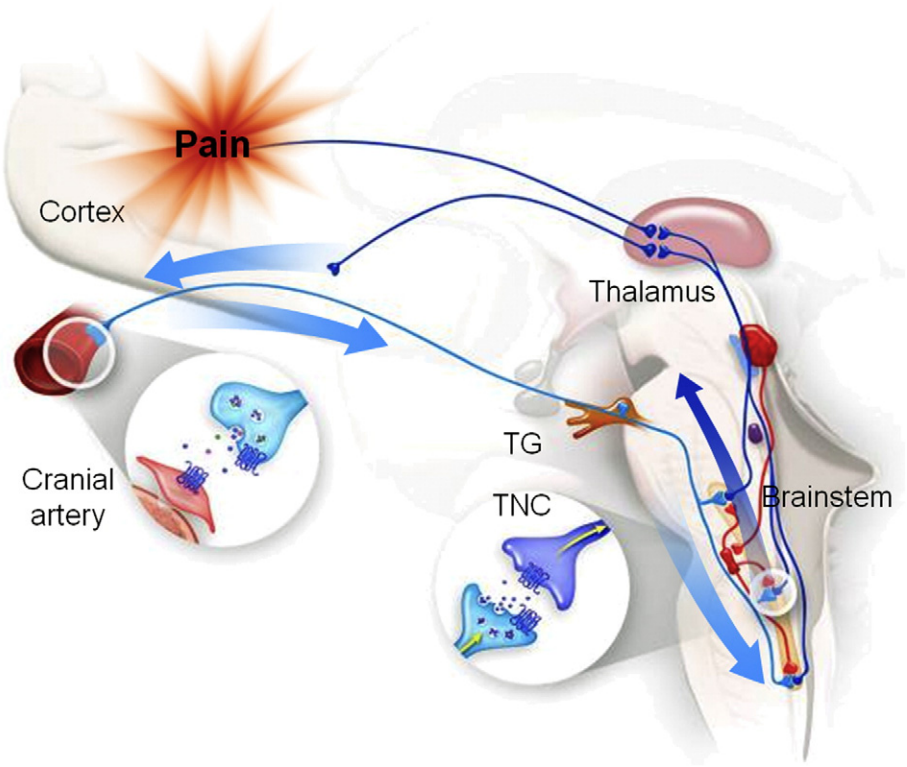


**Fig. 2.** Schematic illustration of some of the brain areas (thalamus, cerebellum, brainstem periaqueductal gray area) that express CGRP and CGRP receptors. In addition, the trigeminovascular pathway (intracranial arteries, trigeminal ganglion and trigeminal nucleus caudalis) contains CGRP fibers and CGRP receptors which may be sites of action for the gepants.

**Table 1**
Overview of changes in perivascular neuropeptide levels occurring in acute attacks of primary headache disorders.
From Edvinsson and Uddman (2005).

| | NPY | VIP | Substance P | CGRP |
|---|---|---|---|---|
| Migraine without aura | ±0 | ±0 | ±0 | ↑ |
| Migraine with aura | ±0 | ±0 | ±0 | ↑ |
| Trigeminal neuralgia | ±0 | ±0 | ±0 | ↑ |
| Cluster headache | ±0 | ↑ | ±0 | ↑ |
| Chronic paroxysmal headache | ±0 | ↑ | ±0 | ↑ |

±0, no change from before headache.
↑ Significant increase in neuropeptide level.

namely: (i) cerebral and extracerebral blood vessels, where activation of CGRP receptors induces a vasodilator response that can be blocked by olcegepant and telcagepant in vitro and in vivo; (ii) dural mast cells, which may be degranulated by CGRP (Ottosson & Edvinsson, 1997) since they contain CGRP receptors (Eftekhari and Edvinsson, unpublished), resulting in the release of histamine, bradykinin, and 5-HT, inter alia, to cause release of cytokines and inflammatory agents involving subsequently neurogenic inflammation; (iii) second-order sensory neurons within brainstem trigeminal nuclei and reciprocal areas at C1–C2, on which postsynaptic CGRP receptors can be blocked by olcegepant and telcagepant; and (iv) TG, where CGRP increases its own synthesis and stimulates the release of NO and several pro-inflammatory cytokines. It has been shown that the small to medium sized neurons in the TG (storing CGRP) may act on large neurons and SGC (which contain the receptor elements CLR and RAMP1) to modulate signaling and/or to elicit a local inflammation (Eftekhari et al., 2010). In keeping with these views, CLR and RAMP1 proteins (which demonstrate functional CGRP receptors) are highly expressed in several tissues/cells, including: (i) the human cerebral vasculature (Oliver et al., 2002; Edvinsson et al., 2010); (ii) cranial dura mater, dural mast cells, TG and presynaptic nerve terminals in the spinal trigeminal nucleus (Eftekhari et al., 2010; Eftekhari & Edvinsson, 2011); and (iii) neurons and glia in the central and peripheral nervous systems, such as second-order neurons and astrocytes (Levy et al., 2004; Morara et al., 2008) as well as TG glial cells (Eftekhari et al., 2010). Furthermore, RAMP1 levels are functionally rate limiting for the actions of CGRP in TG (Zhang et al., 2007); hence, elevated neuronal RAMP1 could potentially sensitize the TG of individuals to the actions of CGRP. These actions would include increased CGRP synthesis and increased neurogenic inflammation, which could potentially help sustain and intensify the nociceptive actions of CGRP in migraine.

### 3.6. Do peripheral and/or central mechanisms play a role in migraine?

Once the trigeminovascular reflex is initiated (resulting in an antidromic activation with the concomitant release of CGRP), the central part of this pathway (i.e. TNC and/or its reciprocal parts at the spinal C1–C2 segments) is also activated (Fig. 2). Indeed, direct stimulation of either the superior sagittal sinus or the TG results in the activation of cells in this region (Goadsby & Edvinsson, 1994a). This phenomenon may be shared by several of the primary headache forms.

#### 3.6.1. How is the trigeminovascular reflex initiated?

Following the identification of the trigeminal vascular pathway and its dependence on neuropeptides, functional studies showed that denervation does not alter the regional cerebral blood flow or cerebral metabolism, the cerebral vascular responses to carbon dioxide or the autoregulation (Edvinsson et al., 1986). However, the vasoconstrictor responses to NA, alkaline pH, $PGF_{2\alpha}$, $BaCl_2$ or subarachnoid blood are modified (Edvinsson et al., 1995). The general picture is that after denervation there is no alteration in the maximum contractile response to any of the above agents, but the time to return to the initial basal tone is markedly prolonged. It is hypothesized that vasoconstriction triggers an antidromic release of sensory neuronal messengers, which results in normalization of the vascular tone. Subsequent studies using antagonists in combination with denervation have shown that CGRP has a significant role in this response (Edvinsson et al., 1995). Moreover, vasodilatation of cortical arterioles by acidic pH is not modified by trigeminal denervation (Edvinsson et al., 1995). Thus, if the primary headache attack involves CSD with subsequent cerebral vasoconstriction, the trigeminal vascular system may have a counterbalancing effect designed to normalize cerebrovascular tone. The activation of this system is noted clinically as an increase in cranial venous outflow of CGRP during the attacks (Goadsby et al., 1988; Goadsby & Edvinsson, 1993, 1994a). Indeed, some experimental studies of CSD showed that CGRP is in part involved in the local dilatation (Piper et al., 1993). In contrast, CSD per se in monkeys did not result in enhanced jugular venous CGRP levels (Piper et al., 1993), which agrees well with patient data (Friberg et al., 1994). If the patient is in a "latent period" (Fanciullacci et al., 1995), then CSD may induce a strong reflex vasoconstriction which activates the trigeminovascular reflex (McCulloch et al., 1986), as seen in acute primary headache attacks (Fanciullacci et al., 1997). The connection may be either functional (as suggested by Bolay et al., 2002) or anatomical (as discussed above in the Intracerebral innervation section; Cohen et al., 1996).

#### 3.6.2. What is the role of the trigeminocervical complex?

The nociceptive input from cerebral blood vessels and the dura mater to the first synapse in the brainstem is transmitted by small-diameter C- and Aδ-fiber afferents of the ophthalmic division of the trigeminal nerve via the TG; this, in turn, is transmitted to nociceptive second-order neurons to the superficial and deep layers of the medullar dorsal horn of the trigeminocervical complex. This system extends from the TNC to the C2–C3 segments. Detailed tracing has revealed the projection of C-fibers to the lamina I/II and IV of the dorsal horn of thin C-fibers and A-fibers, respectively (Liu et al., 2003). A more recent study suggests that the Aδ-fibers mainly end in lamina I/II while other A-fibers (Aβ mechanoreceptor fibers) end in lamina IV (Eftekhari & Edvinsson, 2011). These findings warrant a re-interpretation of some of the results from anatomical and functional studies in this region. In this respect, a rich supply of SP-immunoreactive fibers has been shown in the marginal layer and in the substantia gelatinosa of the subnucleus caudalis of the TNC and the Rexed's laminae I and II of the C1–C2 levels (Uddman et al., 2002). In addition, there is a moderate supply of CGRP and PACAP fibers in these areas (Christiansen et al., 2003).

Migraine attacks involve changes that are characterized by pain and nausea, symptoms that are mediated by the sensory system and by centers in the brainstem. The vascular components of this disorder are mediated via the trigeminal nerve and, accordingly: (i) mechanical or electrical stimulation of the dura mater or of cranial blood vessels reproduces signs of migrainous pain (Edvinsson & Krause, 2002; Edvinsson & Uddman, 2005); and (ii) electrical stimulation of the TG in humans and cats results in increased plasma levels of CGRP and SP in the jugular vein (Goadsby et al., 1988; Goadsby & Edvinsson, 1993). Further, the central structures that process craniovascular pain have partly been mapped; in this respect, stimulation of: (i) the rat TG induces a reduction in the immunoreactivities of CGRP and SP in the TNC, ipsilateral to the stimulated side (Samsam et al., 1999); (ii) the cat superior sagittal sinus leads to increased metabolic activity in the TNC and in the spinal C2 region (Goadsby & Zagami, 1991); and (iii) the MMA, the superior sagittal sinus or the TG in monkeys and cats markedly increases the immediate early gene c-Fos in laminae I and II of the TNC and in the superficial layers of the spinal C1–C2 segments (Kaube et al., 1993; Hoskin et al., 1999). However, the expression of neuropeptides in the brainstem is unaltered during 2 h of superior sagittal sinus stimulation (Christiansen et al., 2003). Interestingly, the c-Fos response is reduced by eletriptan (Knyihar-Csillik et al., 2000). In humans, evidence for a central site of action of the triptans (via $5\text{-HT}_{1B}$ and $5\text{-HT}_{1D}$ receptors) has come from binding

studies that demonstrate their association with the superficial laminae of the TNC and the cervical dorsal horn as well as of the nucleus of the tractus solitarius (Longmore et al., 1997). In addition, a significant amount of 5-$HT_{1F}$ binding sites can be seen (Castro et al., 1997). The 5-$HT_{1F}$ site has been examined using the specific agonists LY334370 (Shepheard et al., 1999) and lasmiditan (Nelson et al., 2010). LY334370 had no contractile effect nor did it inhibit CGRP release, but it blocked the transmission of nociceptive impulses in the TNC (Shepheard et al., 1999). Furthermore, lasmiditan was described to act on the TNC, inhibiting c-Fos expression, but it also reduced dural plasma protein extravasation upon stimulation of the TG (Nelson et al., 2010). This suggests that its antimigraine action could in part be exerted centrally on these nuclei. Moreover, in humans, the immunocytochemical distribution of CGRP, SP and PACAP coincides with the reported localization of 5-$HT_{1B/1D}$ binding sites in the TNC and in particular with the distribution of 5-$HT_{1B/1D}$ receptors (Uddman et al., 2002). Thus, it is suggested that: (i) if the triptans can reach the TNC and the spinal C1–C2 segments they may inhibit the central activity of the sensory trigeminal fibers; and (ii) the role of NO and VIP at this site is minor.

*3.6.3. Is the blood–brain barrier disrupted during migraine?*

Sumatriptan, a hydrophilic triptan, has been regarded either to be incapable of crossing the BBB or to cross it to some extent (Edvinsson & Tfelt-Hansen, 2008). However, the CNS adverse events of sumatriptan in migraineurs and normal volunteers suggest that the drug can cross the BBB in humans. It has previously been discussed whether the BBB may be disrupted (and, consequently, more leaking) during migraine attacks, which then could be responsible for a possible central effect of sumatriptan in migraine (Tfelt-Hansen, 2010). Indeed, a number of studies have demonstrated increased levels of matrix metalloproteinase-9 (MMP-9) (Imamura et al., 2008) or increased MMP activity (Bernecker et al., 2011) in migraine patients, suggesting that the permeability of the BBB is altered. The evidence thus far, however, suggests that there is no need for a breakdown in the BBB to occur in order to explain a possible effect of sumatriptan and the more lipophilic second generation triptans in the CNS (Edvinsson & Tfelt-Hansen, 2008).

*3.6.4. Central sensitization*

Reduced habituation of event-related potentials (ERPs) and enhanced contingent negative variation (CNV) appear to be a unique characteristic of migraine with and without aura (Siniatchkin et al., 2000). Thus, there is evidence of cortical hyperexcitability and lack of habituation to repetitive stimuli in the brain of migraineurs. Furthermore, this phenomenon is normalized by treatment with β-adrenoceptor blockers, calcium channel blockers, triptans and aspirin (Sanchez-Del-Rio et al., 2006). Similarly, hyperexcitability in the occipital (Aurora et al., 1999) and motor (van der Kamp et al., 1996) cortex has also been demonstrated in migraine. In addition, an investigation of trigeminal and olfactory ERPs revealed trigeminal hyperexcitability in migraineurs (Grosser et al., 2000). CNV appears to be under aminergic control (Hansenne et al., 2000) and ERP shows familial similarities, suggesting the involvement of a genetic component. Hence, abnormalities in habituation, which lead to altered states of neuronal excitability, may support a central theory. Moreover, the identification of the genes involved in the regulation of ERPs may be of importance when investigating migraine susceptibility (Sandor et al., 2000). Recently, central sensitization has also been proposed as one of the mechanisms involved in the chronification of migraine (Mathew, 2011).

*3.6.5. Peripheral sensitization*

In many patients, pain of long duration often seems to be associated with a sensitized pain system in which there is facilitated impulse signaling in nociceptive nerves. A new load activating already sensitized fibers in these patients may result in increased pain and in an increased receptive field. These symptoms include hypersensitivity of the face skin or scalp, neck muscle tenderness, and hyperalgesia (Edvinsson & Krause, 2002; Edvinsson & Uddman, 2005). There is increasing evidence that the autonomic and sensory nervous systems can respond to a physical load; indeed, repeated inflammation or injury in experimental animals may result in increased sensitivity for pain in the affected area. Thus, repeated short lasting stimulation of C-fibers in a limb may result in an increased receptive field for the neuron that has been recorded. It is not clear how this happens, but it may be due to an increased sensitivity in peripheral receptors, a facilitated conduction in nociceptive neurons and/or a reduced inhibition of pain via a decrease in the gate control.

Since a migraine attack can be triggered by physiological as well as psychological factors, several hypotheses have emerged regarding the initiation and recurrence of this syndrome, including: (i) activation of peripheral sensory fibers innervating the dura mater and cranial blood vessels; (ii) activation of descending pathways that facilitate processing of pain signals; and (iii) suppression of descending pathways that inhibit processing of pain signals in the spinal cord.

By analyzing the response properties of individual meningeal primary afferents in TG before, during and after exposing the dura to inflammatory agents, it has been shown that: (i) mechanically insensitive neurons became mechano-sensitive after chemical stimulation; and (ii) mechano-sensitive neurons showing minimal response prior to dural chemical stimulation became more sensitive after stimulation (Strassman et al., 1996). In humans, such a mechanical supersensitivity could mediate the throbbing pain of migraine and its worsening during coughing, bending over or other activities. Furthermore, by analyzing the response properties of individual brainstem trigeminal neurons that receive convergent input from the dura before, during and after application of inflammatory agents to the dura, these agents activated dorsal horn neurons (Schepelmann et al., 1999), and sensitized them for up to 10 h (Burstein et al., 1998). The sensitized neurons in the dorsal horn show a significant increase in their response to dural mechanical stimulation and to mechanical or thermal stimulation of cutaneous receptive fields; their response thresholds decrease and their response magnitudes increase. Based on these findings, it was predicted that sensitization that develops in the dorsal horn following application of inflammatory agents will result in intracranial and extracranial sensory hypersensitivities. Interestingly, a large number of humans with migraine have a cutaneous allodynia, ipsilateral to the migraine pain (Burstein et al., 2000).

## 4. Currently available and prospective antimigraine drugs

In acute antimigraine treatment, the ergots ergotamine and dihydroergotamine were the first specific acute antimigraine drugs in use for several decades (Dahlöf & Maassen Van Den Brink, 2012) until the advent of the triptans (Silberstein & McCrory, 2003). Indeed, the triptans represent a considerable advance (Goadsby et al., 2002), but their side-effects (i.e. dizziness, nausea, fatigue, chest symptoms and paresthesia) prevent some patients from using triptans. Furthermore, a number of patients do not respond well to the triptans, whereas triptan monotherapy is ineffective or poorly tolerated in 1 out of 3 migraineurs and in 2 out of 5 migraine attacks (Mathew & Jaffri, 2009).

The subsequent advent of CGRP receptor antagonists (also called "gepants") such as olcegepant (Olesen et al., 2004) and telcagepant (Edvinsson & Linde, 2010; Ho et al., 2010) bodes well for migraineurs who are poor or non-responders to triptan treatment. The gepants, which have an efficacy comparable to triptans, have a better safety and tolerability profile (Edvinsson & Linde, 2010). Interestingly, Bristol Myers Squibb has now added information on clinical trials of another CGRP receptor antagonist (www.clinicaltrials.gov).

Finally, as recently reviewed by Chan et al. (2011b), several glutamate receptor antagonists are also effective in the acute treatment of

migraine, including the mixed AMPA/kainate receptor antagonist LY293558 and the kainate receptor antagonist LY466195.

## 5. Implications, future directions and conclusions

The pathophysiology of migraine is complex and seems to involve the central and peripheral nervous systems, as well as the cranial vasculature. The acutely acting antimigraine treatments that are currently available are all aimed at reversing cranial vasodilatation, while they also may act at a central level. Further, the therapeutic arsenal does not allow us to describe drugs specifically suitable for specific forms of migraine such as, for example, migraine caused by fluctuating hormone levels or migraine due to ion channel mutations. Thus, it is tempting to state that there is still ample space for improving the therapeutic options for the treatment of migraine. There are several avenues that could be explored, for example: (i) inhibition of CGRP release; and (ii) drugs with a direct central action.

## Conflict of interest statement

The authors declare they have no conflict of interest.

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