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Neurobiology in Primary Headaches

by

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Abstract

Primary headaches such as migraine and cluster headache are neurovascular disorders. Migraine is a painful, incapacitating disease that affects a large portion of the adult population with a substantial economic burden on society. The disorder is characterised by recurrent unilateral headaches, usually accompanied by nausea, vomiting, photophobia and/or phonophobia. A number of hypothesis have emerged to explain the specific causes of migraine. Current theories suggest that the initiation of a migraine attack involves a primary CNS event. It has been suggested that a mutation in a calcium channel renders the individual more sensitive to environmental factors, resulting in a wave of cortical spreading depression 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. Genom wide scans have been performed in migraine with susceptibility regions on several chromosomes some are associated with altered calcium channel function. With positron emission tomography (PET) a migraine active region has been pointed out in the brainstem. In cluster headache PET studies have implicated a specific active locus in the posterior hypothalamus. Both migraine and cluster headache involve activation of the trigeminovascular system. In support, there is a clear association between the head pain and the release of the neuropeptide calcitonin gene-related peptide (CGRP) from the trigeminovascular system. In cluster headache there is, in addition, release of the parasympathetic neuropeptide vasoactive intestinal peptide that is coupled to facial vasomotor symptoms. Triptan administration, activating the 5-$HT_{1B/1D}$ receptors, causes the headache to subside and the levels of neuropeptides to normalise, in part through presynaptic inhibition of the cranial sensory nerves. These data suggest a central role for sensory and parasympathetic mechanisms in the pathophysiology of primary headaches. The positive clinical trial with a CGRP receptor antagonist offers a new promising way of treatment.

Keywords: migraine, cluster headache, CGRP, VIP, trigeminovascular reflex, autonomic nerves

I. Introduction

The primary headaches include migraine, tension-type headache, cluster headache, other trigeminal autonomic cephalalgias and other primary headaches [146]. Tension-type headache is the most common of these in the general population, however, since little data exist for a neurovascular component we have only described those briefly below [5]. Migraine headaches are ascribed as neurovascular *disorders* which world-wide afflict up to 15-20% of the general population. The socio-economic implications are extensive with considerable impact on productivity and quality of life. In Europe alone it is calculated that 600.000 days of work are lost daily. Migraine, which is the most common type, is characterised by attacks of moderate to severe headache that last for 4 – 72 hrs, often unilateral, pulsating and associated with photophobia/phonophobia and/or nausea/vomiting [147]. In migraine with aura, the headache is preceded by transient focal neurological symptoms, most often contralaterally [82].

Cluster headache is another of the primary headaches; it has a distinct clinic with devastating pain. Some of the features of cluster headache overlap with those of other primary vascular headaches. The pain usually occurs around the eye and is described as retro-orbital or temporal. This implies involvement of the ophthalmic (first) division of the trigeminal nerve. In addition to the pain, there are signs of parasympathetic overactivity, e.g. lacrimation, nasal congestion, and injection of the eye. Short-lasting headaches associated with autonomic symptoms may sometimes be confused with cluster headache. Although the exact causes of the primary headaches remain unknown, some pieces of the pathophysiological puzzle are starting to fall into place, particularly after a series of elegant positron emission tomography (PET) studies [132-134]. During the last 20 years there has been a heated debate whether the primary headaches are neurogenic or vascular in origin. However, current molecular and functional studies suggest a way to incorporate the different aspects into an integrated hypothesis as neurovascular headaches [39,82,156].

In susceptible individuals, changes in environmental or physiological states are known to trigger the migraine headache. Migraine susceptibility has been linked to mechanisms regulating central sensitization. The systems that govern neuronal excitability involve homeostatic mechanisms and intracellular signalling pathways. The demonstration that mutations in the calcium channel gene CACNA1A, in approximately 50% of families suffering from the rare and severe familial hemiplegic migraine (FHM), has offered some hope that there is a molecular genetic cause also of the more common types of migraine [150,188]. However, it is well recognised that the central nervous system (CNS) is devoid of sensory pain receptors and intracranially it is only blood vessels in the dura and the circle of Willis that are supplied with sensory nerves and receptors that can respond to thermal, mechanical or distensional stimuli [147,159].

II. Where does the attack start ?

Some researchers have suggested that migraine is a disease comprised of two main subtypes, migraine with aura and migraine without aura. In the former, the aura is characterized most often by visual field disturbances, but sometimes also by additional somatosensory disturbances. In these patients changes in cortical blood flow correlate with areas of hypoperfusion, but no subsequent spreading from the area of hypoperfusion can be demonstrated, possibly because these patients have been studied late during the attacks. Olesen and colleagues were the first to observe in patients examined early at the onset of induced migraine attacks, a pattern of localized blood flow decrease that spread contiguously over the cerebral cortex [145]. This pattern of “spreading oligemia” or “spreading hypoperfusion” was apparent only in patients who had migraine with aura. The hypoperfusion was ipsilateral to the headache and contralateral to the symptoms of the aura. In one subject who suffered a migraine attack during a series of cerebral blood flow measurements with PET [201] the headache was associated with bilateral hypoperfusion which started in the occipital lobes and spread anteriorly into the temporal and parietal lobes. This provided 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 [94]. These initial changes were followed by a decrease in the mean MR signal (by 5%), corresponding to the localised scotoma. The average velocity of the spread of the hypoperfusion over the cortex was 3.5 mm/min, being in concert with previous experimental studies [121]. In three spontaneous attacks of migraine with aura that were captured within 20 min of the onset of visual symptoms; the BOLD data revealed increases in the amplitude of the MR signal [94].Thus, this study supports previous reports of spreading depression as an initial cortical grey matter hyperaemia with a characteristic velocity that is followed by hypoperfusion. It lends support to studies in animals that the hypoperfusion 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 [121]. A plausible explanation for the blood flow changes seen in association with the aura in a migraine attack is that they are the result of spreading depression - a transient marked reduction in electrical activity in the grey matter which advances across the cortical surface. The rate of advance is consistent with the spread of symptoms observed and is associated with decreases in blood flow [121]. Spreading depression can move transcallosally to homologous regions of the opposite hemisphere in animals, and transcallosal spread may account for the bilaterality observed at the onset of the headache [201]. One conclusion that has been raised from the studies is that the migraine aura is not evoked by ischemia, but evoked by aberrant firing of neurones and related cellular elements. An important question that can be raised is how the event is linked to activation of the trigeminovascular reflex [136]. One tempting way would be to link the cortical spreading depression to neurogenic inflammation in the dura mater and from there activation of sensory and autonomic reflexes [18].However, the dura mater is an extracerebral structure, separated from the brain by e.g. CSF and is nourished by the external carotid artery [146]. Alternatively, specific cell bodies projecting from the brainstem to cerebral vessels such as the extensive adrenergic and serotonergic efferent from nuclei of the locus coeruleus and of the raphe nuclei, respectively, could be involved. In fact there are some data to support this suggestion [19,44,160] showing close association between intracerebral nerve fibers and cerebral blood vessels. This has been

examined at depth subsequently revealing a direct neurogenic control by intrinsic serotonergic (5-HT) neurons on the cerebral microvascular bed [28].There exist close association between the 5-HT neurons and microarterioles, capillaries and perivascular astrocytes; this is more apparent in regions where manipulation of the intrinsic 5-HT neurons elicits uncoupling between flow and metabolism [28,29]-

In patients with migraine without aura, the situation is somewhat more intricate [199]. During attacks, small increases in blood flow were observed in the cingulate, auditory and visual association cortices, and in brain stem regions. These changes normalized after injection of sumatriptan and induced complete relief from headache as well as from phono- and photophobia. However, the changes were small and could only be significant if the PET data from all nine subjects were normalized, thus being by and large in agreement with previous negative studies with the ^{133} xenon method which lacks the precision of PET [145]. Further support for the importance of a brainstem region was obtained in a patient that developed an attack of migraine without aura after glyceryl trinit-ate administration. Bahra and colleagues [13]observed activation in the dorsal rostral brainstem region and hence reproduced those data seen previously by Weiller and colleagues. In addition, the authors observed a neuronally driven vasodilatation and activation of regions associated with pain processing [13,199].

A PET study in patients with attacks of cluster headache and of capsaicin-induced head pain has reported blood flow changes that suggest, in part, a response that is primarily generated by the pain [132,134]. In this study, the anterior cingulate cortex was activated as would be expected, as part of the affective response. Activation was also seen in the frontal cortex, the insulae and the ventroposterior thalamus contralateral to the side of the pain. The only activated area that was particular to cluster headache was the ipsilateral hypothalamus. This region is important in the control of circadian rhythm and can be linked to the neurohormonal imbalance seen in cluster headache. This raised the possibility that the pathophysiology of cluster headache is driven partially or entirely from the CNS. The episodic nature of the disorder suggests involvement of at least the suprachiasmatic region, possibly associated with the human biological clocks. Spontaneous as well as nitroglycerin induced cluster headache attacks were both associated with cerebral

vasodilatation, interpreted as occurring via a neuronal mechanism [133]. Vasodilatation of the cranial vessels was not considered to be specific to any particular headache syndrome, but generic to cranial neurovascular activation involving both sensory and parasympathetic reflex mechanisms as evidenced previously by the release of the sensory neurotransmitter CGRP and the parasympathetic messenger VIP in man [73] and experimentally in animal [74,205].

PET scans of patients with acute attacks of cluster headache demonstrated an unilateral activation in the ipsilateral hypothalamic grey matter [132]. It is likely that the fundamental driving process arises in diencephalic pacemakers. While migraine and cluster headache share much in the expression of the pain, their underlying initiator mechanisms distinguish them. Indeed, 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 or in migraine [134,199] showed no hypothalamic activation. 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 the activation of the carotid artery does not relate specifically to cluster headache, but rather a trigeminovascular autonomic reflex. The flow changes may therefore be epiphenomena of the trigeminal activation, and not part of the disease generation process.

A possible way to link recently documented alterations in the intracranial circulation to the genetic theory is via the observation that genetically defect ion channels may more easily be activated (due to altered membrane potential and/or function) and result in excitation of neurons in situations where they are exposed to excessive stress. Proof for involvement of brainstem nuclei in migraine came from a PET study by Weiller and colleagues [199] and has now been supported by others [13]. During acute attacks, increased local blood flow was observed in brain stem regions (specifically midbrain and pons regions). The brainstem activation persisted after injection of sumatriptan. These findings support the idea 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 the other hand it could equally well be an activation of the PAG acting as a filter to

inhibit the pain [66]. 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. The fibers may evoke vasoconstriction (via catecholamines or 5-HT) and hence explain the connection with the trigeminovascular reflex. Alternatively, the DRN and LC send descending fibers to the trigeminal nucleus caudalis (TNC) and dorsal root ganglia (DRG) where they act in a gate-control function and the PAG acts to inhibit this. Thus, sensory transmission associated with the TNC appears to be regulated by a complex system. It is still unclear whether the brainstem finding reveal the origin of the disease or if it is an accompanying activation designed to limit the symptoms of the migraine headache.

III. The ion channel connection

Clinical studies have revealed that migraine patients usually have a family history [82]. In the two main types of migraine, with aura and without aura, the familial aggregation cannot be explained by simple mendelian inheritance patterns. FHM is the only variety of migraine in which a mendelian type of inheritance has been clearly established. A few years ago a candidate region on chromosome 19 was identified as a gene that encodes an α_1 A subunit of a voltage-gated P/Q-type calcium channel [110,111,150]. FHM with cerebellar signs was subsequently linked to mutations in CACNA1A [15,35,69,150,188,195]. Thus, this type is now called FHM 1 and has been associated with mutations in CACNA1A [150] but in others a second locus has been mapped on chromosome 1 [36,71] and is called FHM 2. In still other cases, the disorder is linked to neither site, suggesting the existence of a third locus, FHM 3 [36]. Eight mutations in CACNA1A have been identified in 18 families affected by hemiplegic migraine and in two patients with sporadic hemiplegic migraine [1,150] CACNA1A is specifically transcribed in cerebellum, cerebral cortex, thalamus, hypothalamus and upper brainstem. The opening and closing of voltage-gated calcium channels are controlled by changes in voltage across the cell membrane and mediate the entry of calcium into the cell. These channels are of critical importance because the gradient between intracellular and extracellular calcium controls neurotransmitter release, neuronal excitation and other neuronal functions. The calcium

channel is present in axons and dendrites, suggesting that it has both presynaptic and postsynaptic roles in modulating cell-to-cell communication.

Several different missense mutations in the CACNA1A gene have been detected in unrelated FHM families [150]. Generally, patients with FHM have missense mutations and these alter the gating properties of the channel [34,150]. The first FHM mutation (R192Q) occurs in a region that is believed to be part of the voltage sensor domain of the calcium channel. The second, most prevalent of the FHM mutations (T666M) is found within the pore-forming hairpin loop of the second domain of the channel. Two other FHM mutations (V714A and I1811L) are located in the transmembrane segments that may influence calcium channel inactivation, thereby blocking calcium transfer. The functional consequences of FHM 1 mutations are now receiving much attention subsequent to producing a knock-in mouse that carry the human FHM 1 R192Q mutation [197]. The researchers found gain-of-function effects that include increased $CA_R2.1$ current density in cerebellar neurons, enhanced neurotransmission at the neuromuscular junction, and a reduced treshold and increased velocity of cortical spreading depression [197]. The data appear to suggest that this mutation may result in increased susceptibility to cortical hyperexcitability and link spreading depression and aura in migraine.

The gene for FHM 2 was recently identified when an Italian research group found two different missense mutations in the ATP A2 gene, coding for the alpha 2 subunit of the $Na^{+,}K^{+}$ - ATPase in two families with pure FHM 2 [33], and this has been confirmed in two Dutch families [198]. This subunit binds sodium, potassium, and ATP, and utilizes ATP hydrolysis to extrude Na^{+} ions. The Na^{+} pumping provides the steep Na^{+} gradient essential for the transport of amino acids and calcium. Hypothetically a mutation like this may result in loss of function which could make the brain more susceptible to spreading depression, however, more experimentation is needed.

IV. Nerves in the walls of intracranial vessels

Since intracranial vessels are the only source for eliciting intracranial pain and in particular referred pain [159] the understanding of the vascular innervation by autonomic and sensory nerves is a prerequisite for the understanding of intracranially pain as it occurs in primary headaches. The intracranial blood vessels are supplied with nerve fibers that emanate from cell bodies in ganglia belonging to the sympathetic, parasympathetic and sensory nervous systems (Figure 1) [91]. In addition, cerebral resistance vessels may be innervated by fibers that originate within the brain itself thereby representing an intrinsic nerve supply [40,55].

Sympathetic nervous system

The sympathetic nerves that supply the cerebral vessels arise mainly from the ipsilateral superior cervical ganglion [142] while some nerve fibers that supply the vertebral and basilar arteries originate from the inferior cervical ganglion and the stellate ganglion [3]. The activation of these fibers results in vasoconstriction, modulation of cerebrovascular autoregulation, reduction of intracranial pressure, and a decrease of cerebral blood volume and cerebrospinal fluid production [55]. The responses are mainly mediated by noradrenaline (NA) and neuropeptide Y (NPY) [48,49], at least 40-50% of the NA-positive cells contain NPY [12,186].

The neurotransmitter content in the nerve cell bodies is influenced by various factors: Activation may increase catecholamine synthesis and NPY mRNA [96] while denervation results in depletion of NA and NPY [49]. However, some time after sympathectomy, there is an upregulation of NPY-containing fibers of parasympathetic origin [17]. Furthermore, there are age-dependent changes in sympathetic neurons; in old rats there is a selective loss of NPY with a concomitant increase of nerve fibers containing vasoactive intestinal peptide (VIP) and calcitonin gene-related peptide (CGRP) around cerebral blood vessels [21] with a significant reduction with age of NPY, VIP, substance P (SP) and CGRP [46].

Electronmicroscopic and functional studies have revealed that NA, NPY and adenosine triphosphate (ATP) are co-stored in large dense-cored vesicles [21]. Stimulation of the sympathetic nerves results in the release of these transmitters, the stimulus intensity

determines the relative contribution of NA and NPY. At resting conditions, little NPY is released, and hence sympathetic vasoconstriction is largely due to adrenoceptor and purinergic receptors whereas in situations of high sympathetic activity, the contribution of NPY becomes prominent [129].

It has been suggested that the small pial vessels on the cortical surface are supplied by NA-containing fibers emanating from an intracerebral source such as the LC and/or the hypothalamus [37,151]. Support for this hypothesis comes from studies showing that destruction of the LC induces a reduction in the number of noradrenergic nerve fibers in intracerebral vessels [55] and that central stimulation of NA neurons in the hypothalamus is associated with an increase in hypothalamic blood flow which is unaffected by superior cervical ganglionectomy or by the β-adrenoceptor antagonist propranolol [163]. It is tempting to involve such a pathway in coupling neuronal activity to local blood flow regulation [127].

Parasympathetic nervous system

The “classical” transmitter in parasympathetic nerves is acetylcholine (ACh) and their cell bodies contain acetylcholinesterase (AChE). Cerebral blood vessels have perivascular nerves that display AChE activity [61,97,181], and are choline acetyltransferase (ChAT) positive [164,181]. At the ultrastructural level, varicosities that contain numerous small agranular vesicles (40-60 nm in diameter) and that remain after sympathectomy are generally presumed to represent cholinergic nerve terminals [61]. These varicosities frequently occur in close apposition to large dense-cored vesicles in the neuroeffector area, thus suggesting that parasympathetic nerves have the potential to interact with sympathetic nerve terminals near cerebrovascular smooth muscle [61,62]. In several species, ACh induces constriction of isolated cerebral arteries when deprived of the endothelium, while transmural nerve stimulation predominantly induces relaxation in the same preparations [123]. The neurogenic vasodilatation in these preparations is not blocked by atropine and is thus non-cholinergic [122,123]. One possible explanation is that additional substances are released together with ACh to mediate dilatation [122,123,164].

Several neuromessengers, which induce cerebral neurogenic vasodilatation, have been suggested. Among these are VIP, pituitary adenylate cyclase activating polypeptide (PACAP) and nitric oxide (NO), which all seem to mediate a major component of the vasodilator responses of isolated cerebral arteries and in vivo as demonstrated by cerebral blood flow measurements [83,107,192]. In fact it has been suggested that NO might be the last link in cholinergic transmission. Another possibility would be that ACh mainly acts prejunctionally to inhibit neurotransmitter release from autonomic nerves [124]. The vast majority of parasympathetic nerve fibers to cerebral vessels originates in sphenopalatine and otic ganglia [51,180].

VIP was the first neuropeptide demonstrated in perivascular nerve fibers around brain vessels [120]. Other peptides of the VIP family, such as PHI (peptide histidine isoleucine) [57], its human form PHM (peptide histidine methionine) are seen in nerve fibers that supply cerebral vessels [40,46,192]. The distribution of VIP-immunoreactive nerve fibers varies between species. In most species, VIP-containing nerves are most abundant in the circle of Willis and the major cerebral arteries. The density of the nerve plexus is highest in the carotid system and diminishes in caudal direction. In man, the VIP-immunoreactive nerve supply is sparse in both cerebral arteries or veins [52]. In the human sphenopalatine ganglion there is a rich supply of cell bodies containing VIP, PACAP and NO [194]. In addition, human sphenopalatine ganglia express mRNA for NPY Y_1 and VIP_1 receptors.

In some locations, AChE activity and VIP immunoreactivity can be seen in the same vascular nerve fibers and this has led to the suggestion that VIP and ACh coexist in parasympathetic nerve endings [92,128]. Furthermore, immunoelectron microscopic studies revealed that, in the human superficial temporal artery, VIP immunoreactivity is localized exclusively to large electron-dense secretory vesicles (diameter 70-100 nm) in nerve terminal varicosities which also contain numerous smaller sized agranular vesicles (diameter 40-60 nm) presumed to represent parasympathetic cholinergic neurons [50]. It should be noted, however, that other studies on the cholinergic and VIPergic cerebrovascular innervation have demonstrated that ChAT and VIP immunoreactivities are co-localised in less than 5% of the fibers examined [138].

Pituitary adenylate-cyclase activating peptide (PACAP) is a vasoactive peptide that displays 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 [4]. PACAP-immunoreactivity and PACAP mRNA have been found in the sphenopalatine and otic ganglia [194]. Perivascular nerve fibers that contain PACAP immunoreactivity can be seen in cerebral blood vessels and PACAP mediates dilatation [107,168,192]. The majority of the PACAP-immunoreactive nerve fibers constitute a subpopulation of fibers containing VIP/NOS immunoreactivity as verified by tracing, denervation and co-localization experiments [47].

NO is a highly labile molecule and information on its cellular localization has largely been attained by immunocytochemistry for nitric oxide synthase (NOS). There is evidence that NO is not only a candidate for the endothelium-derived relaxing factor in the endothelium, but also acts as a neurotransmitter [20]. NO is a non-conventional transmitter, since it appears to be released by diffusion rather than exocytosis upon formation, 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 nerve fibers around cranial blood vessels from several species, including man [14,20,47,83,143,178,189,202]. In the human circle of Willis, NOS-containing fibers are relatively sparse and mainly detected in posterior arteries [143,189]. In rodent and bovine cerebral vessels [14,87,143,178] NOS-immunoreactive nerve fibers contain both VIP and AChE activity and are assumed to represent parasympathetic nerves originating mainly from the sphenopalatine ganglion [47]. Lee has [124] suggested that NO is a primary postjunctional messenger with VIP and that ACh acts to limit the release of NO via prejunctional receptors.

Sensory nervous system

Most sensory fibers to cranial structures derive from the trigeminal ganglion. Histochemical studies have revealed a number of signal substances stored in trigeminal ganglion cells. Based on the sequencing of cDNA for SP, three different SP precursors (α-, β-, and δ-

preprotachykinin A) have been predicted. δ-preprotachykinin produces SP only, whereas proteolytic cleavage of β- and δ-preprotachykinin produces SP [59,106] and a second tachykinin neurokinin A (NKA). The cerebrovascular distribution of NKA resembles that of SP and coexistence of SP and NKA in cell bodies of sensory ganglia and in perivascular nerve fibers have been demonstrated [31,41]. NKA-immunoreactive nerve fibers have a similar distribution to that of SP-containing nerves and are also depleted after capsaicin treatment.

CGRP immunoreactive nerve fibers supply the major cerebral arteries and pial arterioles of the cortical surface of several species including man [43,52,57,58,68,89,91,191,203]. Marked species and regional variations are observed in the density of CGRP-immunoreactive cerebrovascular nerve fibers. While cerebral arteries of laboratory animals receive a dense supply of CGRP fibers, human cerebral vessels contain only a sparse network. In perivascular nerve fibers CGRP is often co-localized with SP/NKA [30,43,47,50-52,179,191]. These results are further supported at the ultrastructural level, where the use of double immunogold staining demonstrated that CGRP and SP are co-localized in the same large granular vesicles (70-150 nm in diameter) in both sensory neurons of the trigeminal ganglion and in varicosities of perivascular nerve fibers of guinea pig and man [30,50,90].

In the human trigeminal ganglion, CGRP-immunoreactive neurones occur in high numbers (40% of all neuronal cells) whereas SP-immunoreactive neurones are less numerous (18%). This agrees well with observations from the cat and the rat in which the relation of CGRP to SP is approximately 3:1 [51,136,187]. In situ hybridisation has revealed that 40% of all nerve cell bodies contain CGRP and CGRP mRNA [50,187]. CGRP and SP are potent vasodilators in vivo and in vitro, the former being 10-1000 times more potent [45,58,59,106,136]. Several studies have suggested that SP is involved in plasma extravasation from postcapillary venules in the dura mater during primary headache attacks [131]. While neurokinin receptor antagonists are potent inhibitors of neurogenic inflammation [155,172,173] clinical studies have shown that these blockers do not have any significant effect in acute migraine attacks [86]. Furthermore, while CGRP is released

during the headache phase of a migraine attack, SP is not [76,79,171,200]. In addition, there are indications that SP does not take part in vascular nociception in man [99]. This view is supported by intravital microscope studies demonstrating that vasodilatation during perivascular stimulation of the middle meningeal artery in vivo was blocked by a CGRP antagonist but unaffected by neurokinin agonists or antagonists [171,200]

Immunocytochemistry has revealed the expression of PACAP not only in parasympathetic but also in sensory ganglia [139,187] which has led to the suggestion that PACAP may act as a neuromodulator in the sensory systems [139]. There is a moderate supply of PACAP immunoreactive nerve fibers in the cat cerebral circulation [192]. In the rat the majority of the PACAP-containing fibers around cerebral blood vessels seems to derive from the sphenopalatine ganglion [47]. In the human trigeminal ganglion, PACAP containing cell bodies are more numerous than in the laboratory animals, amounting to 15% - 20% [187]. Double immunostaining has revealed that PACAP co-localises with CGRP in some cell bodies in the trigeminal ganglion. PACAP dilates cerebral arteries and can increase cerebral blood flow [107,168,192]. Activation of the trigeminovascular system results in co-release of CGRP and PACAP into the jugular vein of the cat [204], a model used in studies of migraine [78]. It is also possible that this peptide may participate in antidromic vasodilatation following activation of the trigemino-vascular reflex [136].

The opioid peptides endorphins, enkephalins and dynorphin belong to a family of neuropeptides with modulator functions on nociception and inflammation [149]. Their effects are mediated through three known types of opioid receptors, termed μ, δ and κ receptors. An additional receptor of this group the ORL-1 (orphan like) receptor has been demonstrated [140], and the identified endogenous ligand was termed nociceptin because of its hyperalgesic properties [137,161]. Recently reduced circulating levels of nociceptin was found in cluster headache attacks and this suggested a reduced “break” in the disorder [63]

At the cellular level, nociceptin behaves like classical opioids with inhibition of cAMP production, activation of inwardly rectifying K^+ channels and modulation of a variety of

voltage-dependent Ca^{2+} currents [32]. The action of nociceptin on ion conduction results in reduced neuronal excitability or presynaptic transmitter release [137]. 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 the opioid agonist. In the peripherial nervous system there is a substantial overlap of the effects produced by activation of the ORL-1 receptor and the opioid receptors [72]. 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 [152]. Previous studies have revealed that nociceptin and its receptor are widely distributed in both the central and the peripheral nervous systems [2,24,105]. Nociceptin immunoreactivity in the superficial dorsal horn is not affected by dorsal root rhizotomy, suggesting that at the spinal cord level nociceptin is produced by central rather by primary afferent neurons [162]. In tracing experiments, it has been demonstrated that the primary trigeminal fibers descend to the superficial layer of the dorsal horn of the cervical spinal cord [130]. About 70% of neuronal cells in trigeminal ganglia are nociceptin immunopositive [103]. Double immunostaining has shown that in the human trigeminal ganglion nociceptin is co-localized with CGRP, SP, NOS and PACAP.

NO has been suggested as an important molecule for initiation of migraine attacks [148]. The expression of NOS in trigeminal nerve cell bodies supports this suggestion. NO released from the endothelium (eNOS) from the perivascular nerves (nNOS), or inducible NOS (iNOS), may activate the guanylate cyclase system in the smooth muscle cells. This results in a decrease in the local intracellular Ca^{++} level, giving rise to vasodilatation which may activate the pain sensitive structures around the cranial vessels [148]. Few trigeminal neurones express NOS in laboratory animals [47,50,143]. In human trigeminal ganglia about 15% of the cell bodies contain NOS [187]. Double immunostaining of the cat trigeminal ganglion has revealed that only few CGRP neurones (less than 5%) are NOS positive [60]. The relative functional role of CGRP and NO in the trigeminal ganglion has been studied in the cat; CGRP blockade markedly attenuates the cerebral blood flow increase following trigeminal nerve activation while NOS blockade is without effect [60] .On the other hand, activation of the parasympathetic nerves results in a NO-dependent flow

increase [83], suggestive of a physiological role for NO in the parasymphatetic vasodilator system.

The human trigeminal ganglion stores several neurotransmitters such as CGRP, SP, NKA, PACAP, nociceptin and NOS, but a functional system requires receptors. Few studies have examined the presence of neuropeptide receptors, however, some work has been done at the mRNA level. Thus, mRNA for both the NPY Y_1 and the NPY Y_2 receptors have been observed in the human trigeminal ganglion [187]. This lends support to the idea of a sympathetic modulatory influence on the function of the trigeminal ganglion. In addition, there is evidence of VIP_1 receptor mRNA in human trigeminal ganglia, which suggests that also parasympathetic nerves may influence the activity in the trigeminal ganglion. In addition CGRP and nociceptin receptor mRNA has recently been shown [42,103,187].

The capsaicin receptor, also known as vanilloid receptor subtype 1 (TRPV1 receptor), is an integral membrane protein with homology to a family of putative store-operated calcium channels [26]. The TRPV1 receptor is activated not only by capsaicin, but also by noxious heat and acid. It has therefore been suggested as a molecular integrator of those chemical and physical stimuli that elicit pain [109,114,183]. The TRPV1 receptor is easily desensitized by its agonist resiniferatoxin and shows slow recovery [184]. Capsaicin is an excitatory neurotoxin that can release sensory neurotransmitters and selectively destroy primary afferent neurons expressing the TRPV1 receptor. The majority of these sensory neurons are small diameter cells. Capsaicin-sensitive neurons are involved in nociception and are responsible for the neurogenic component of the inflammatory response. In addition to its excitatory actions, capsaicin desensitizes the tissue with subsequent anti-nociceptive and anti-inflammatory effects [16]. Within the cranial circulation, capsaicin may activate the trigeminovascular system and release sensory peptides [39,53,108].

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 trigeminal ganglia and the brain stem of the rat [26,93,98,185]. In human trigeminal ganglia the TRPV1 receptor was detected by

immunohistochemistry and RT-PCR [104]. A low number of human trigeminal neurons were TRPV1 receptor immunopositive (16% of total neuronal cell bodies). Double immunostaining showed that 10% of the VR1 receptor immunoreacctive neuronal cells contained (CGRP), 8%SP and 5% NOS which suggests that activation of the VR1 receptor may modulate the release of neurotransmitters in man.

Intracerebral innervation

Metabolically produced substances (e.g. H^+, CO_2, K^+, Ca^{2+}, adenosine) have been proposed to mediate the local changes in cerebral blood flow that accompany neuronal activity. However, they seem not to account fully for the adaptative responses in vasomotor activity in the microvascular bed. There has been a long quest if intracerebral neurons can directly control intracerebral microcirculation. One proposal is that regional changes in brain perfusion are controlled directly by neurons located within the brain parenchyma. 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 [28,29,127]. These changes in perfusion occur independently of those in glucose metabolism, thus implying that neuronal pathways can exert direct effects on the microcirculation. Furthermore, a population of neurons whose activity are related to spontaneous waves of cerebral blood flow has been identified in the cerebral cortex, and is suspected of transducing neuronal signals into vasomotor responses [85]. Together, these observations suggest that the neuronal control of the microvascular bed, which is achieved in concert with other mechanisms (vasoactive metabolic substances, ionic gradients and intrinsic endothelial or myogenic responses within the vessel wall), 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 have the ability to modify their diameters and consequently local blood flow in response to changes in the level of brain neurotransmitters and/or neuromodulators. In addition, there are mechanisms that may not only transmit information to the feeding vessels, but also may redirect blood flow to the areas in demand [55].

Early morphological studies have documented the presence of nerve fibers and, occasionally, neuronal cell bodies containing different neurotransmitters and/or neuromodulators in association with intraparenchymal blood vessels in many areas of the brain [127]. These perivascular fibers or neuronal perikarya were described as following the contours of blood vessels, apposed to or literally encircling the vessel walls. Electron microscopy was employed to study the innervation of intracerebral arterioles and capillaries in which axon terminals abutted their abluminal walls. Adrenergic vesicles remained after bilateral cervical sympathectomy, which leads to the hypothesis that intracerebral vessels receive an adrenergic innervation of central origin. The central innervation of intraparenchymal arterioles seems to be located primarily at branching sites – a strategic location for the control of local blood flow [29,56]. There are now morphologic evidence that nerve fibers of central origin associated with brain intraparenchymal blood vessels belong to the noradrenergic system [182]. Studies have confirmed that the locus coerulens is the exclusive source of cortical perivascular noradrenergic nerve terminals, and ultrastructural analysis has emphasized the frequent association of these fibers not only with capillaries but also with microarterioles [55], and that they functionally have local effects [158].

A similar morphologic analysis has been performed for central dopaminergic fibers. Although no *in vivo* functional studies attest to a primary vascular effect of dopaminergic centers (e.g., mesencephalic ventral tegmental area and substantia innominata) on the local microcirculation, the perivascular application of dopamine in cortical brain slices has been shown to cause vasoconstriction in about 50% of the microvessels studied [119]. These dopaminergic fibers were 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 vasoconstrictions [28,135]. Histochemical examination has shown intimate associations between serotonergic neuronal processes and intraparenchymal vessels. This innervation of local microvessels appears to embrace all vascular elements – arterioles, capillaries, and venules [44]. At the

ultrastructural level, perivascular 5-HT nerve terminals labelled for the 5-HT-synthesizing enzyme tryptophan hydroxylase were associated with capillaries and microarterioles of all sizes, including penetrating arteries [29]. This view was further supported by Bradley and colleagues who showed intimate association between brain stem serotonergic neurons with the wall of large medullary arteries using confocal imaging and electron microscopy [19].

Therefore, the presence and strategical location of neurovascular appositions, their region-selective distribution and perivascular proximity in regions known to modify the local perfusion in response to stimulation of specific neuronal populations, and the exceptional positioning of cortical interneurons provide convincing morphologic arguments for a role of neurally produced substances in the control of microvascular tone and, thereby, local cerebral blood flow [28,127]. This may provide the anatomical link between cerebral neurons and the trigeminovascular system which is the central communication for the afferent pain to the brain stem and the central aspects of the migraine symptoms.

V. Neurotransmitters in primary headaches

Trigeminal ganglion stimulation

The trigeminal system provides an important pain-transmitting link from the cranial vasculature to the CNS. In laboratory animals, the sensory pathway has no resting tonic influence on regional cerebral blood flow or regional cerebral metabolism [58,136] whereas stimulation of the trigeminal ganglion increases intracranial blood flow in part via CGRP release [50,60,81]. In humans, unilateral stimulation of the trigeminal ganglion results in increased bilateral cortical blood flow, slightly more on the stimulated than on the contralateral site [190]. 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, stimulation of the ganglion during operation results in the release of CGRP and SP, a response that is interrupted following cessation of stimulation [78].

Migraine attacks

During migraine attacks there is a marked increase in the plasma levels of CGRP in the bulbus or external jugular vein [79]. At the same time there is no change of CGRP in peripherial blood or in the levels of NPY, VIP or SP in the jugular vein (Table 1). Furthermore, there is no difference between migraine with aura or migraine without aura, both result in substantial increases in venous CGRP levels at the same time as the patients exhibit pain [70,76,79]. A somewhat higher basal level of CGRP has been noted in the cubital fossa vein in migraine patients also outside the attack [9]. Quite importantly Juhasz provoked migraine attacks by sublingual nitroglycerin and collected CGRP samples from the antecubital vein. It was observed that the CGRP concentration increased during the migraine attack and returned to baseline after the cessation of the migraine [113]. In addition, the raised CGRP levels correlated with the timing of the attack and most importantly with the severity (degree of pain) of a migraine headache. On the other hand a limited study measuring neuropeptides in the internal jugular vein was negative [67]. Following sumatriptan [76]or rizatriptan administration [176], the CGRP levels returned to control with successful amelioration of the headache. In addition, sumatriptan caused a parallel decrease in plasma CGRP and migraine headache during nitroglycerin evoked attacks [112]. The mechanisms behind this reduction in elevated plasma CGRP in man may be due to the presence of 5-HT_{1B} and 5-HT_{1D} receptors expressed on the trigeminal ganglion cells and fibers [102,126] which may during stimulation cause inhibition of sensory nerve activity. 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. Direct electrical stimulation of the trigeminal ganglion in man, however, results in co-release of CGRP and SP [78] possibly because here the entire sensory system to the head is activated.

Cluster headache

Patients with episodic cluster headache have been examined during spontaneous attacks of headache to determine the release of neuropeptides [73]. During attacks, the levels of CGRP and VIP are raised in cranial venous blood in all subjects, while no changes are

seen in NPY or SP levels (Table 1). Treatment with oxygen or subcutaneous sumatriptan promptly normalizes the CGRP levels while administration of an opiate does not alter the peptide levels [73]. Activation of the trigeminal pathway results in the release of both CGRP and VIP; this response is blunted by a 5-HT $_{1B/1D}$ agonist [74]. Stimulation of the superior sagittal sinus activates the trigeminovascular pathway and this also results in the release of CGRP and VIP in the external jugular vein [205]. The CGRP and VIP release is abolished by surgical lesion to the trigeminal nerve which suggests that the VIP response is mediated via a brain stem reflex involving the superior salivatory nucleus [205].

Results from patient studies have shown that activation of the trigeminovascular system and the cranial parasympathetic nervous system plays a key role in the acute attacks of cluster headache. It is particularly noteworthy that the release of VIP is in concert with the facial symptoms. The findings agree well with the results of others [64,65] who demonstrated release of CGRP in nitroglycerine-elicited attacks of cluster headache. They also saw that nitroglycerin can elicit an attack of cluster headache with CGRP release only if the patient is in a latent period and thus prone to activation [65]. The finding of elevated levels of both CGRP and VIP in the cranial venous blood during cluster attacks and in experiments on laboratory animals suggests that there is activation of a brainstem reflex, the afferent arc of which is the trigeminal nerve and the efferent the cranial parasympathetic outflow from the VIIth nerve (Figure 2).

Trigeminal neuralgia

There does not seem to be any difference in the resting levels of neuropeptides in cranial venous outflow between patients with trigeminal neuralgia and control subjects [78]. Stimulation of the trigeminal ganglion in conjunction with thermocoagulation causes a marked increase in the external jugular vein levels of CGRP and SP, associated with unilateral facial flushing. After cessation of the stimulation, the peptide levels returns to normal.

Vasoactive substances may be responsible not only for causing or being closely associated with the pain, but also for neurally mediated facial flushing. Thus, local facial

stimulation by tapping a painful trigger point in a patient with a 17-year history of intractable left-sided facial pain led to both unilateral pain and flushing [77]. This was associated with a marked increase in the CGRP level (120%) during the flushing, but there was no change in SP, NPY or VIP levels.

Chronic paroxysmal hemicrania

Chronic paroxysmal hemicrania (CPH) is a relatively well-recognized syndrome that is defined by the International Headache Society operational diagnostic criteria as frequent short-lasting attacks of unilateral pain, usually located in the orbital, supra-orbital or temporal regions that may last for 2-45 minutes. The attack frequency may vary but is usually five or more each day. The pain is associated with prominent autonomic symptoms such as conjunctival injection, lacrimation, nasal congestion, rhinorrhoea, ptosis or eyelid oedema. According to the diagnostic criteria, the attacks should settle rapidly upon treatment with indomethacin.

We have studied one such case and observed that, during the pain, the CGRP level rose by 200% as compared to the level while on indomethacin [75] or in control subjects [73,76,78,79]. The VIP level rose to 32 pmol/L during an attack, dropping to 7 pmol/L upon indomethacin treatment [75]. This shows that attacks of CPH are characterized by activation of both sensory and parasympathetic cranial nerve fibres.

Tension-type headache

Tension-type headache (TTH) is the most common of the primary headaches [146]; increased tenderness of pericranial myofascial tissues is the most prominent finding, but rather little has been done with regard to neuronal messengers. Ashina [7] analyzed plasma CGRP during and after infusion of nitroglycerin in patients with chronic TTH but did not find any change. Furthermore, Bach et al [11] reported normal CGRP levels in CSF of TTH patients. A more detailed work still revealed that both central and peripheral levels of CGRP, VIP, SP and NPY were unaltered in chronic TTH [6,8]. There was no relationship between CGRP levels and muscular factors, however, in eight patients with pulsating pain showed higher circulating CGRP levels [8].

VI. Central mechanisms in headache

Once the trigeminovascular reflex is initiated, resulting in an antidromic activation which involves release of CGRP, the central part of this pathway, the TNC and/or its reciprocal parts at the C1 and C2 levels, are also activated (Figure 3). Experiments in laboratory animals as well as in humans, have shown that direct stimulation of either the superior sagittal sinus or the trigeminal ganglion results in activation of cells in this region [73,84]. This phenomenon may be shared by several of the primary headache forms.

1) How is the trigeminovascular reflex initiated?

Following the identification of the trigeminal vascular pathway and its dependence on neuropeptides [191] 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 [58]. However, vasoconstrictor responses elicited by noradrenaline [136], alkaline pH, $PGF_{2\alpha}$, $BaCl_2$, subarachnoidal blood or capsaicin are modified [53,54]. The general picture is that following denervation there is no alteration in the maximum contractile response to either of the above agents but the time to return to the initial basal tone is markedly prolonged (Figure 3). It is hypothetised that the vasoconstriction triggers an antidromic release of the sensory neuronal messengers, which results in normalization of the vessel tone. Subsequent studies using antagonists in combination with denervations have shown that CGRP has a significant role in this response [53,54]. Vasodilation of cortical arterioles induced by acidic pH is not modified by the trigeminal denervation [54]. Thus, if the primary headache attack involves a spreading wave of depression of cortical neurons with subsequent vasoconstriction of cerebral vessels, the trigeminal vascular system may have a counter-balancing 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 [73,76,78,79]. In an experimental study of spreading depression it was demonstrated that CGRP is in part involved in the local dilatation [157,196]. In contrast, spreading depression per se in

monkeys did not result in enhanced jugular venous CGRP levels [157] which agrees well with patient data [67]. If the patient is in a “latent period” [64], then the spreading depression may induce a strong reflex vasoconstriction which may activate the trigeminovascular reflex [136] as is seen in acute primary headache attacks [65]. The connection may be either functionally as suggested by Bolay [18] or anatomically as discussed above in the section on intracerebral innervation [28].

2) *What is the role of the trigeminocervical complexs?*

The nociceptive input from the cerebral vessels and the dura mater to the first synapse in the brain stem is transmitted by small-diameter Aδ- and C-fiber afferents in the ophthalmic division of the trigeminal nerve via the trigeminal ganglion to nociceptive second-order neurons to the superficial and deep layers of the medullary dorsal horn of the trigeminocervical complex. This system extends from the trigeminal nucleus caudalis to the segments of C2-C3. Detailed tracing has revealed the projection of C-fibers to the LI/II and the LIV of the dorsal horn of thin C-fibers and A-fibers, respectively [125]. To understand the pathophysiology of primary headaches it is essential to identify the regions in the human brain that may process the signs of the disorder. It has been demonstrated that there is a rich supply of SP-immunoreactive fibers in the marginal layer and in the substantia gelantinosa of the subnucleus caudalis of the TNC and the Rexed’s laminae I and II of the C1 and the C2 levels of the human cervical spinal cord [193]. In addition, there is a moderate supply of CGRP and PACAP fibres in these areas while NOS or VIP fibres were not seen [27].

Migraine attacks involve changes that are characterised by pain and nausea, symptoms that are mediated by the sensory system and by centres in the brain stem. The vascular components of the disorder are mediated via the trigeminal nerve. Mechanical or electrical stimulation of the dura mater or of cranial blood vessels reproduces signs of migrainous pain [159]. Electrical stimulation of the trigeminal ganglion in man and cat results in increased plasma levels of CGRP and SP in the jugular vein [78,79]. The central structures that process craniovascular pain have to some degree been mapped. Stimulation of the trigeminal ganglion in the rat induces a reduction in the

immunoreactivities of CGRP and SP in the TNC, ipsilateral to the stimulated side [117,165]. Electrical stimulation of the superior sagittal sinus in the cat leads to increased metabolic activity in the TNC and in the C2 region of the spinal cord [84]. A marked increase of the immediate early gene c-fos in laminae I and II of the TNC and in the superficial layers of the C1 and C2 regions can be seen upon stimulation of the middle meningeal artery, the superior sagittal sinus or the trigeminal ganglion in monkeys and cats [80,101,115]. However, the expression of neuropeptides in the brainstem is unaltered during 2 hours of superior sagittal sinus stimulation [27]. The c-fos response is reduced by anti-migraine drugs, such as triptans [116,118]. In man, evidence for a central site of action of the triptans has come from binding studies that demonstrate their association with the superficial laminae of the caudal part of the TNC and the cervical dorsal horn as well as of the nucleus of the tractus solitarius. In an attempt to characterize the receptors involved it has been suggested that the $5HT_{1B}$ receptors are present in very low concentrations in all these nuclei in man (below 12% of total specific binding), while the $5HT_{1D}$ receptors account for about 50% of the total specific sumatriptan binding [126]. In addition, a significant amount of $5HT_{1F}$ –binding sites can be seen [25,153]. The $5HT_{1F}$ site has been examined using a specific agonist LY334370 [170]. LY334370 had no contractile effect and did not inhibit CGRP release. However, it was observed that LY334370 could block the transmission of nociceptive impulses in the TNC [170]. These data held weight to the proposal that the antimigraine actions could in part be exerted centrally on these nuclei. In man it was revealed that the immunocytochemical distribution of CGRP, SP and PACAP coincides with the reported localisation of the 5-HT $_{1B/1D}$ binding sites in the TNC and in particular with the distribution of 5-HT $_{1B/1D}$ receptor [193]. Thus, it is tempting to suggest that if the triptans can reach the TNC and the C1 and C2 levels they may also here inhibit the activity of the central aspects of the sensory trigeminal fibers. It also suggests that the role of nitric oxide and VIP at this site is minor.

VII. Central sensitization

Reduced habituation of event-related potentials (ERPs) and enhanced contigent negative variation (CNV) appear to be a unique characteristic of migraine with and without aura

[141,175]. Thus, there is evidence of cortical hyperexcitability and lack of habituation to repetitive stimuli in the migraine brain. Furthermore, this phenomenon can be normalised by treatment with beta-blockers, calcium channel blockers, aspirin and 5-HT_1 receptor agonists that show anti-migraine efficacy [174]. Similarly, hyperexcitability in the occipital cortex has also been demonstrated in migraine [10]. In addition, an investigation of trigeminal and olfactory ERPs revealed trigeminal hyperexcitability in migraineurs [88]. CNV appears to be under aminergic control [95] and ERP show familial similarities, suggesting the involvement of a genetic component. Hence, abnormalities in habituation which leads to altered states of neuronal excitability may support a central theory and the identification of the genes involved in the regulation of ERPs may be of importance when investigating in migraine susceptibility [166].

VIII. Peripheral sensitization

In many patients, pain of long duration often seems to be associated with a sensitized pain system in which there is a facilitated impulse signalling in nociceptive nerve fibres. A new load activating already sensitized fibres may in these patients result not only in increased pain but also in an increased receptive field. Such observations have been made by headache researchers since decades; hypersensitivity of the skin of the face or scalp, neck muscle tenderness, and hyperalgesia [169].There is increasing evidence that the autonomic and sensory nervous system have the ability to respond to a physical load. In experimental animals, it has been shown that repeated inflammation or injury may result in increased sensitivity for pain in the affected area. Thus, repeated short lasting stimulation of C-fibres 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 increased sensitivity at peripheral receptors. A facilitated conduction in nociceptive neurons or perhaps a reduced inhibition of pain via a decrease in the gate control may be involved.

It is well known that a migraine attack can be triggered by physiological as well as psychological factors and several hypotheses have emerged regarding the initiation and recurrence of migraine. One theory is that peripheral sensory fibres innervating the dura and cranial blood vessels are activated. Other explanations may be that descending

pathways that facilitate processing of pain signals are activated, or that the descending pathways that inhibit processing of pain signals in the spinal cord are suppressed.
By analyzing response properties of individual meningeal primary afferent neurons in the trigeminal ganglion before, during and after exposing the dura to inflammatory agents it has been shown that mechanically insensitive neurons became mechano-sensitive after chemical stimulation and that mechano-sensitive neurons that showed only minimal response prior to the chemical stimulation of the dura increased after the stimulation [177]. In humans such mechanical supersensitivity could mediate the throbbing pain of migraine and its worsening during coughing or bending over or other physical activities.
By analyzing response properties of individual brainstem trigeminal neurons that receive convergent input from the dura before, during and after application of the inflammatory agent to the dura it has been shown that the inflammatory agent not only activates dorsal horn neurons [38,167], but also sensitizes them for up to 10 h [22]. The sensitized neurons in the dorsal horn show significant increase in their response to mechanical stimulation of the dural receptive fields and to mechanical and thermal stimulation of cutaneous receptive fields; their response thresholds decrease and their response magnitudes increase. Based on these findings it was predicted that the sensitization that develops in the dorsal horn following introduction of the inflammatory agent will result in intracranial as well as extracranial sensory hypersensitivities. Interestingly, it has been seen that a large number of humans with migraine [23] has a cutaneous allydynia on the skin ipsilateral to the migraine pain.

IX. Summary

Current data provide a model in which a central “generator” or an “active region”, different in migraine and in cluster headache, is activated. Following alteration of cerebral blood vessel tone, the trigeminovascular reflex is initiated to counter-balance cerebrovascular constriction in part via release of CGRP and VIP. The study of neuropeptide levels in migraine and cluster headache provides a link between the clinical and the basic research, work that is crucial for the understanding of the pathophysiology. In migraine (with and without aura) marked levels of CGRP indicate activation of the trigeminal system and

normalized by administration of the highly effective triptan antimigraine agents coincident with the relief of the headache.

The activation of TNC provides the central link to nociception, pain development, and associated symptoms (Figure 4). Hypothetically, intense activation of the central pain pathways may involve the superior salivatory nucleus, resulting in parasympathetic VIP release, and manifestation of additional facial symptoms in e.g. cluster headache. A number of possibilities to interact with the sensory system have recently appeared. It was reported that an adenosine A_1 receptor agonist acts prejunctionally to inhibit sensory neurogenic vasodilation, CGRP release and firing of second order neurones in the TNC [100]. Its clinical usefulness is now evaluated. By blocking CGRP receptors post-junctionally, a recently developed CGRP blocker [40] has been found to be effective in acute attacks of migraine [144]. Thus, both in spontaneous cases of migraine and in headache attacks induced by administration of CGRP, the CGRP receptor antagonist BIBN4096 BS was effective without any noticeable side effects establishing a new principle in the acute treatment of migraine [144,154].

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[1] H. Adachi, A. Kume, M. Li, Y. Nakagomi, H. Niwa, J. Do, C. Sang, Y. Kobayashi, M. Doyu, G. Sobue, Transgenic mice with an expanded CAG repeat controlled by the human AR promoter show polyglutamine nuclear inclusions and neuronal dysfunction without neuronal cell death, Hum Mol Genet 10 (2001) 1039-1048.
[2] B. Anton, J. Fein, T. To, X. Li, L. Silberstein, C.J. Evans, Immunohistochemical localization of ORL-1 in the central nervous system of the rat, J Comp Neurol 368 (1996) 229-251.
[3] M.A. Arbab, L. Wiklund, T. Delgado, N.A. Svendgaard, Stellate ganglion innervation of the vertebro-basilar arterial system demonstrated in the rat with anterograde and retrograde WGA-HRP tracing, Brain Res 445 (1988) 175-180.
[4] A. Arimura, S. Shioda, Pituitary adenylate cyclase activating polypeptide (PACAP) and its receptors: neuroendocrine and endocrine interaction, Front Neuroendocrinol 16 (1995) 53-88.
[5] M. Ashina, Neurobiology of chronic tension-type headache, Cephalalgia 24 (2004) 161-172.
[6] M. Ashina, L. Bendtsen, R. Jensen, R. Ekman, J. Olesen, Plasma levels of substance P, neuropeptide Y and vasoactive intestinal polypeptide in patients with chronic tension-type headache, Pain 83 (1999) 541-547.
[7] M. Ashina, L. Bendtsen, R. Jensen, F. Sakai, J. Olesen, Possible mechanisms of glyceryl-trinitrate-induced immediate headache in patients with chronic tension-type headache, Cephalalgia 20 (2000) 919-924.
[8] M. Ashina, L. Bendtsen, R. Jensen, S. Schifter, I. Jansen-Olesen, J. Olesen, Plasma levels of calcitonin gene-related peptide in chronic tension-type headache, Neurology 55 (2000) 1335-1340.
[9] M. Ashina, L. Bendtsen, R. Jensen, S. Schifter, J. Olesen, Evidence for increased plasma levels of calcitonin gene-related peptide in migraine outside of attacks, Pain 86 (2000) 133-138.
[10] S.K. Aurora, Y. Cao, S.M. Bowyer, K.M. Welch, The occipital cortex is hyperexcitable in migraine: experimental evidence, Headache 39 (1999) 469-476.
[11] F.W. Bach, M. Langemark, R. Ekman, J.F. Rehfeld, S. Schifter, J. Olesen, Effect of sulpiride or paroxetine on cerebrospinal fluid neuropeptide concentrations in patients with chronic tension-type headache, Neuropeptides 27 (1994) 129-136.
[12] J. Baffi, T. Gorcs, F. Slowik, M. Horvath, N. Lekka, E. Pasztor, M. Palkovits, Neuropeptides in the human superior cervical ganglion, Brain Res 570 (1992) 272-278.
[13] A. Bahra, M.S. Matharu, C. Buchel, R.S. Frackowiak, P.J. Goadsby, Brainstem activation specific to migraine headache, Lancet 357 (2001) 1016-1017.
[14] C.P. Barroso, L. Edvinsson, W. Zhang, M. Cunha e Sa, D.R. Springall, J.M. Polak, S. Gulbenkian, Nitroxidergic innervation of guinea pig cerebral arteries, J Auton Nerv Syst 58 (1996) 108-114.
[15] S. Battistini, S. Stenirri, M. Piatti, C. Gelfi, P.G. Righetti, R. Rocchi, F. Giannini, N. Battistini, G.C. Guazzi, M. Ferrari, P. Carrera, A new CACNA1A gene mutation in acetazolamide-responsive familial hemiplegic migraine and ataxia, Neurology 53 (1999) 38-43.
[16] S. Bevan, J. Szolcsanyi, Sensory neuron-specific actions of capsaicin: mechanisms and applications, Trends Pharmacol Sci 11 (1990) 330-333.

[17] H. Bjorklund, T. Hokfelt, M. Goldstein, L. Terenius, L. Olson, Appearance of the noradrenergic markers tyrosine hydroxylase and neuropeptide Y in cholinergic nerves of the iris following sympathectomy, J Neurosci 5 (1985) 1633-1640.
[18] H. Bolay, U. Reuter, A.K. Dunn, Z. Huang, D.A. Boas, M.A. Moskowitz, Intrinsic brain activity triggers trigeminal meningeal afferents in a migraine model, Nat Med 8 (2002) 136-142.
[19] S.R. Bradley, V.A. Pieribone, W. Wang, C.A. Severson, R.A. Jacobs, G.B. Richerson, Chemosensitive serotonergic neurons are closely associated with large medullary arteries, Nat Neurosci 5 (2002) 401-402.
[20] D.S. Bredt, P.M. Hwang, S.H. Snyder, Localization of nitric oxide synthase indicating a neural role for nitric oxide, Nature 347 (1990) 768-770.
[21] G. Burnstock, Changes in expression of autonomic nerves in aging and disease, J Auton Nerv Syst 30 Suppl (1990) S25-34.
[22] R. Burstein, H. Yamamura, A. Malick, A.M. Strassman, Chemical stimulation of the intracranial dura induces enhanced responses to facial stimulation in brain stem trigeminal neurons, J Neurophysiol 79 (1998) 964-982.
[23] R. Burstein, D. Yarnitsky, I. Goor-Aryeh, B.J. Ransil, Z.H. Bajwa, An association between migraine and cutaneous allodynia, Ann Neurol 47 (2000) 614-624.
[24] G. Calo, R. Guerrini, A. Rizzi, S. Salvadori, D. Regoli, Pharmacology of nociceptin and its receptor: a novel therapeutic target, Br J Pharmacol 129 (2000) 1261-1283.
[25] M.E. Castro, J. Pascual, T. Romon, C. del Arco, E. del Olmo, A. Pazos, Differential distribution of [3H]sumatriptan binding sites (5-HT1B, 5-HT1D and 5-HT1F receptors) in human brain: focus on brainstem and spinal cord, Neuropharmacology 36 (1997) 535-542.
[26] M.J. Caterina, M.A. Schumacher, M. Tominaga, T.A. Rosen, J.D. Levine, D. Julius, The capsaicin receptor: a heat-activated ion channel in the pain pathway, Nature 389 (1997) 816-824.
[27] T. Christiansen, A. Bruun, Y.E. Knight, P.J. Goadsby, L. Edvinsson, Immunoreactivity of NOS, CGRP, PACAP, SP and VIP in the trigeminal nucleus caudalis and in the cervical spinal cord C1 and C2 of the cat., J. Headache and Pain 4 (2003) 156-163.
[28] Z. Cohen, G. Bonvento, P. Lacombe, E. Hamel, Serotonin in the regulation of brain microcirculation, Prog Neurobiol 50 (1996) 335-362.
[29] Z. Cohen, M. Ehret, M. Maitre, E. Hamel, Ultrastructural analysis of tryptophan hydroxylase immunoreactive nerve terminals in the rat cerebral cortex and hippocampus: their associations with local blood vessels, Neuroscience 66 (1995) 555-569.
[30] M. Cunha e Sa, C.P. Barroso, M.C. Caldas, L. Edvinsson, S. Gulbenkian, Innervation pattern of malformative cortical vessels in Sturge-Weber disease: an histochemical, immunohistochemical, and ultrastructural study, Neurosurgery 41 (1997) 872-876; discussion 876-877.
[31] C.J. Dalsgaard, A. Haegerstrand, E. Theodorsson-Norheim, E. Brodin, T. Hokfelt, Neurokinin A-like immunoreactivity in rat primary sensory neurons; coexistence with substance P, Histochemistry 83 (1985) 37-39.
[32] T. Darland, M.M. Heinricher, D.K. Grandy, Orphanin FQ/nociceptin: a role in pain and analgesia, but so much more, Trends Neurosci 21 (1998) 215-221.
[33] M. De Fusco, R. Marconi, L. Silvestri, L. Atorino, L. Rampoldi, L. Morgante, A. Ballabio, P. Aridon, G. Casari, Haploinsufficiency of ATP1A2 encoding the Na+/K+

pump alpha2 subunit associated with familial hemiplegic migraine type 2, Nat Genet 33 (2003) 192-196.
[34] A. Ducros, C. Denier, A. Joutel, M. Cecillon, C. Lescoat, K. Vahedi, F. Darcel, E. Vicaut, M.G. Bousser, E. Tournier-Lasserve, The clinical spectrum of familial hemiplegic migraine associated with mutations in a neuronal calcium channel, N Engl J Med 345 (2001) 17-24.
[35] A. Ducros, C. Denier, A. Joutel, K. Vahedi, A. Michel, F. Darcel, M. Madigand, D. Guerouaou, F. Tison, J. Julien, E. Hirsch, F. Chedru, C. Bisgard, G. Lucotte, P. Despres, C. Billard, M.A. Barthez, G. Ponsot, M.G. Bousser, E. Tournier-Lasserve, Recurrence of the T666M calcium channel CACNA1A gene mutation in familial hemiplegic migraine with progressive cerebellar ataxia, Am J Hum Genet 64 (1999) 89-98.
[36] A. Ducros, A. Joutel, K. Vahedi, M. Cecillon, A. Ferreira, E. Bernard, A. Verier, B. Echenne, A. Lopez de Munain, M.G. Bousser, E. Tournier-Lasserve, Mapping of a second locus for familial hemiplegic migraine to 1q21-q23 and evidence of further heterogeneity, Ann Neurol 42 (1997) 885-890.
[37] D. Duverger, L. Edvinsson, E.T. MacKenzie, A. Oblin, L. Rouquier, B. Scatton, B. Zivkovic, Concentrations of putative neurovascular transmitters in major cerebral arteries and small pial vessels of various species, J Cereb Blood Flow Metab 7 (1987) 497-501.
[38] A. Ebersberger, M. Ringkamp, P.W. Reeh, H.O. Handwerker, Recordings from brain stem neurons responding to chemical stimulation of the subarachnoid space, J Neurophysiol 77 (1997) 3122-3133.
[39] L. Edvinsson, Migraine & Headache Pathophysiology., Martin Dunitz, London, 1999.
[40] L. Edvinsson, R. Alm, D. Shaw, R.Z. Rutledge, K.S. Koblan, J. Longmore, S.A. Kane, Effect of the CGRP receptor antagonist BIBN4096BS in human cerebral, coronary and omental arteries and in SK-N-MC cells, Eur J Pharmacol 434 (2002) 49-53.
[41] L. Edvinsson, E. Brodin, I. Jansen, R. Uddman, Neurokinin A in cerebral vessels: characterization, localization and effects in vitro, Regul Pept 20 (1988) 181-197.
[42] L. Edvinsson, L. Cantera, I. Jansen-Olesen, R. Uddman, Expression of calcitonin gene-related peptide1 receptor mRNA in human trigeminal ganglia and cerebral arteries, Neurosci Lett 229 (1997) 209-211.
[43] L. Edvinsson, J.R. Copeland, P.C. Emson, J. McCulloch, R. Uddman, Nerve fibers containing neuropeptide Y in the cerebrovascular bed: immunocytochemistry, radioimmunoassay, and vasomotor effects, J Cereb Blood Flow Metab 7 (1987) 45-57.
[44] L. Edvinsson, A. Degueurce, D. Duverger, E.T. MacKenzie, B. Scatton, Central serotonergic nerves project to the pial vessels of the brain, Nature 306 (1983) 55-57.
[45] L. Edvinsson, R. Ekman, I. Jansen, J. McCulloch, R. Uddman, Calcitonin gene-related peptide and cerebral blood vessels: distribution and vasomotor effects, J Cereb Blood Flow Metab 7 (1987) 720-728.
[46] L. Edvinsson, R. Ekman, I. Jansen, A. Ottosson, R. Uddman, Peptide-containing nerve fibers in human cerebral arteries: immunocytochemistry, radioimmunoassay, and in vitro pharmacology, Ann Neurol 21 (1987) 431-437.

[47] L. Edvinsson, T. Elsas, N. Suzuki, T. Shimizu, T.J. Lee, Origin and Co-localization of nitric oxide synthase, CGRP, PACAP, and VIP in the cerebral circulation of the rat, Microsc Res Tech 53 (2001) 221-228.
[48] L. Edvinsson, P. Emson, J. McCulloch, K. Tatemoto, R. Uddman, Neuropeptide Y: cerebrovascular innervation and vasomotor effects in the cat, Neurosci Lett 43 (1983) 79-84.
[49] L. Edvinsson, P. Emson, J. McCulloch, K. Tatemoto, R. Uddman, Neuropeptide Y: immunocytochemical localization to and effect upon feline pial arteries and veins in vitro and in situ, Acta Physiol Scand 122 (1984) 155-163.
[50] L. Edvinsson, S. Gulbenkian, C.P. Barroso, M. Cunha e Sa, J.M. Polak, A. Mortensen, L. Jorgensen, I. Jansen-Olesen, Innervation of the human middle meningeal artery: immunohistochemistry, ultrastructure, and role of endothelium for vasomotility, Peptides 19 (1998) 1213-1225.
[51] L. Edvinsson, H. Hara, R. Uddman, Retrograde tracing of nerve fibers to the rat middle cerebral artery with true blue: colocalization with different peptides, J Cereb Blood Flow Metab 9 (1989) 212-218.
[52] L. Edvinsson, I. Jansen, M. Cunha e Sa, S. Gulbenkian, Demonstration of neuropeptide containing nerves and vasomotor responses to perivascular peptides in human cerebral arteries, Cephalalgia 14 (1994) 88-96.
[53] L. Edvinsson, I. Jansen, T.A. Kingman, J. McCulloch, Cerebrovascular responses to capsaicin in vitro and in situ, Br J Pharmacol 100 (1990) 312-318.
[54] L. Edvinsson, I. Jansen Olesen, T.A. Kingman, J. McCulloch, R. Uddman, Modification of vasoconstrictor responses in cerebral blood vessels by lesioning of the trigeminal nerve: possible involvement of CGRP, Cephalalgia 15 (1995) 373-383.
[55] L. Edvinsson, D. Krause (Eds.), Cerebral Blood Flow and Metabolism, Lippincott, Williams & Wilkins, Philadelphia, 2002.
[56] L. Edvinsson, M. Lindvall, K.C. Nielsen, C. Owman, Are brain vessels innervated also by central (non-sympathetic) adrenergic neurones?, Brain Res 63 (1973) 496-499.
[57] L. Edvinsson, J. McCulloch, Distribution and vasomotor effects of peptide HI (PHI) in feline cerebral blood vessels in vitro and in situ, Regul Pept 10 (1985) 345-356.
[58] L. Edvinsson, J. McCulloch, T. Kingman, R. Uddman, On the functional role of the trigemino-cerebrovascular system in the regulation of cerebral circulation., Elsevier, Amsterdam, 1986, 407-418 pp.
[59] L. Edvinsson, J. McCulloch, R. Uddman, Substance P: immunohistochemical localization and effect upon cat pial arteries in vitro and in situ, J Physiol 318 (1981) 251-258.
[60] L. Edvinsson, H. Mulder, P.J. Goadsby, R. Uddman, Calcitonin gene-related peptide and nitric oxide in the trigeminal ganglion: cerebral vasodilatation from trigeminal nerve stimulation involves mainly calcitonin gene-related peptide, J Auton Nerv Syst 70 (1998) 15-22.
[61] L. Edvinsson, K.C. Nielsen, C. Owman, B. Sporrong, Cholinergic mechanisms in pial vessels. Histochemistry, electron microscopy and pharmacology, Z Zellforsch Mikrosk Anat 134 (1972) 311-325.
[62] L. Edvinsson, C. Owman, N.O. Sjoberg, Autonomic nerves, mast cells, and amine receptors in human brain vessels. A histochemical and pharmacological study, Brain Res 115 (1976) 377-393.

[63] C. Ertsey, M. Hantos, G. Bozsik, K. Tekes, Circulating nociceptin levels during the cluster headache period, Cephalalgia 24 (2004) 280-283.
[64] M. Fanciullacci, M. Alessandri, M. Figini, P. Geppetti, S. Michelacci, Increase in plasma calcitonin gene-related peptide from the extracerebral circulation during nitroglycerin-induced cluster headache attack, Pain 60 (1995) 119-123.
[65] M. Fanciullacci, M. Alessandri, R. Sicuteri, S. Marabini, Responsiveness of the trigeminovascular system to nitroglycerine in cluster headache patients, Brain 120 (Pt 2) (1997) 283-288.
[66] H.L. Fields, A.I. Basbaum, Central nervoussystem mechanisms of pain modulation. In: Wall PD, Melzack R, eds. Textbook of pain., Churchill Livingstone, Edinburgh, 1994.
[67] L. Friberg, J. Olesen, T.S. Olsen, A. Karle, R. Ekman, J. Fahrenkrug, Absence of vasoactive peptide release from brain to cerebral circulation during onset of migraine with aura, Cephalalgia 14 (1994) 47-54.
[68] B. Fricke, M. von During, K.H. Andres, Topography and immunocytochemical characterization of nerve fibers in the leptomeningeal compartments of the rat. A light- and electron-microscopical study, Cell Tissue Res 287 (1997) 11-22.
[69] K.L. Friend, D. Crimmins, T.G. Phan, C.M. Sue, A. Colley, V.S. Fung, J.G. Morris, G.R. Sutherland, R.I. Richards, Detection of a novel missense mutation and second recurrent mutation in the CACNA1A gene in individuals with EA-2 and FHM, Hum Genet 105 (1999) 261-265.
[70] V. Gallai, P. Sarchielli, A. Floridi, M. Franceschini, M. Codini, G. Glioti, A. Trequattrini, R. Palumbo, Vasoactive peptide levels in the plasma of young migraine patients with and without aura assessed both interictally and ictally, Cephalalgia 15 (1995) 384-390.
[71] K. Gardner, M.M. Barmada, L.J. Ptacek, E.P. Hoffman, A new locus for hemiplegic migraine maps to chromosome 1q31, Neurology 49 (1997) 1231-1238.
[72] S. Giuliani, A. Lecci, C.A. Maggi, Nociceptin and neurotransmitter release in the periphery, Peptides 21 (2000) 977-984.
[73] P.J. Goadsby, L. Edvinsson, Human in vivo evidence for trigeminovascular activation in cluster headache. Neuropeptide changes and effects of acute attacks therapies, Brain 117 (Pt 3) (1994) 427-434.
[74] P.J. Goadsby, L. Edvinsson, Joint 1994 Wolff Award Presentation. Peripheral and central trigeminovascular activation in cat is blocked by the serotonin (5HT)-1D receptor agonist 311C90, Headache 34 (1994) 394-399.
[75] P.J. Goadsby, L. Edvinsson, Neuropeptide changes in a case of chronic paroxysmal hemicrania--evidence for trigemino-parasympathetic activation, Cephalalgia 16 (1996) 448-450.
[76] P.J. Goadsby, L. Edvinsson, The trigeminovascular system and migraine: studies characterizing cerebrovascular and neuropeptide changes seen in humans and cats, Ann Neurol 33 (1993) 48-56.
[77] P.J. Goadsby, L. Edvinsson, R. Ekman, Cutaneous sensory stimulation leading to facial flushing and release of calcitonin gene-related peptide, Cephalalgia 12 (1992) 53-56.
[78] P.J. Goadsby, L. Edvinsson, R. Ekman, Release of vasoactive peptides in the extracerebral circulation of humans and the cat during activation of the trigeminovascular system, Ann Neurol 23 (1988) 193-196.

[79] P.J. Goadsby, L. Edvinsson, R. Ekman, Vasoactive peptide release in the extracerebral circulation of humans during migraine headache, Ann Neurol 28 (1990) 183-187.
[80] P.J. Goadsby, K.L. Hoskin, The distribution of trigeminovascular afferents in the nonhuman primate brain Macaca nemestrina: a c-fos immunocytochemical study, J Anat 190 (Pt 3) (1997) 367-375.
[81] P.J. Goadsby, G.A. Lambert, J.W. Lance, Stimulation of the trigeminal ganglion increases flow in the extracerebral but not the cerebral circulation of the monkey, Brain Res 381 (1986) 63-67.
[82] P.J. Goadsby, R.B. Lipton, M.D. Ferrari, Migraine--current understanding and treatment, N Engl J Med 346 (2002) 257-270.
[83] P.J. Goadsby, R. Uddman, L. Edvinsson, Cerebral vasodilatation in the cat involves nitric oxide from parasympathetic nerves, Brain Res 707 (1996) 110-118.
[84] P.J. Goadsby, A.S. Zagami, Stimulation of the superior sagittal sinus increases metabolic activity and blood flow in certain regions of the brainstem and upper cervical spinal cord of the cat, Brain 114 (Pt 2) (1991) 1001-1011.
[85] E.V. Golanov, D.J. Reis, Cerebral cortical neurons with activity linked to central neurogenic spontaneous and evoked elevations in cerebral blood flow, Neurosci Lett 209 (1996) 101-104.
[86] D.J. Goldstein, O. Wang, J.R. Saper, R. Stoltz, S.D. Silberstein, N.T. Mathew, Ineffectiveness of neurokinin-1 antagonist in acute migraine: a crossover study, Cephalalgia 17 (1997) 785-790.
[87] C. Gonzalez, C. Barroso, C. Martin, S. Gulbenkian, C. Estrada, Neuronal nitric oxide synthase activation by vasoactive intestinal peptide in bovine cerebral arteries, J Cereb Blood Flow Metab 17 (1997) 977-984.
[88] K. Grosser, R. Oelkers, T. Hummel, G. Geisslinger, K. Brune, G. Kobal, J. Lotsch, Olfactory and trigeminal event-related potentials in migraine, Cephalalgia 20 (2000) 621-631.
[89] S. Gulbenkian, C.P. Barroso, M. Cunha e Sa, L. Edvinsson, The peptidergic innervation of human coronary and cerebral vessels, Ital J Anat Embryol 100 Suppl 1 (1995) 317-327.
[90] S. Gulbenkian, A. Merighi, J. Wharton, I.M. Varndell, J.M. Polak, Ultrastructural evidence for the coexistence of calcitonin gene-related peptide and substance P in secretory vesicles of peripheral nerves in the guinea pig, J Neurocytol 15 (1986) 535-542.
[91] S. Gulbenkian, R. Uddman, L. Edvinsson, Neuronal messengers in the human cerebral circulation, Peptides 22 (2001) 995-1007.
[92] S. Gulbenkian, A. Valenca, J. Wharton, J.M. Polak, J.F. David-Ferreira, Simultaneous visualization of neuropeptide and acetylcholinesterase nerve subpopulations in the perivascular plexus, Histochem J 23 (1991) 553-558.
[93] A. Guo, L. Vulchanova, J. Wang, X. Li, R. Elde, Immunocytochemical localization of the vanilloid receptor 1 (VR1): relationship to neuropeptides, the P2X3 purinoceptor and IB4 binding sites, Eur J Neurosci 11 (1999) 946-958.
[94] N. Hadjikhani, M. Sanchez Del Rio, O. Wu, D. Schwartz, D. Bakker, B. Fischl, K.K. Kwong, F.M. Cutrer, B.R. Rosen, R.B. Tootell, A.G. Sorensen, M.A. Moskowitz, Mechanisms of migraine aura revealed by functional MRI in human visual cortex, Proc Natl Acad Sci U S A 98 (2001) 4687-4692.

[95] M. Hansenne, W. Pitchot, E. Pinto, P. Papart, M. Ansseau, Serotonergic-1a activity and contingent negative variation, Biol Psychol 52 (2000) 259-265.
[96] J. Hanze, W. Kummer, M. Haass, R.E. Lang, Neuropeptide Y mRNA regulation in rat sympathetic ganglia: effect of reserpine, Neurosci Lett 124 (1991) 119-121.
[97] H. Hara, G.S. Hamill, D.M. Jacobowitz, Origin of cholinergic nerves to the rat major cerebral arteries: coexistence with vasoactive intestinal polypeptide, Brain Res Bull 14 (1985) 179-188.
[98] R.J. Helliwell, L.M. McLatchie, M. Clarke, J. Winter, S. Bevan, P. McIntyre, Capsaicin sensitivity is associated with the expression of the vanilloid (capsaicin) receptor (VR1) mRNA in adult rat sensory ganglia, Neurosci Lett 250 (1998) 177-180.
[99] H. Holthusen, D. Kindgen-Milles, Z.P. Ding, Substance P is not involved in vascular nociception in humans, Neuropeptides 31 (1997) 445-448.
[100] A.C. Honey, P.A. Bland-Ward, H.E. Connor, W. Feniuk, P.P. Humphrey, Study of an adenosine A1 receptor agonist on trigeminally evoked dural blood vessel dilation in the anaesthetized rat, Cephalalgia 22 (2002) 260-264.
[101] K.L. Hoskin, A.S. Zagami, P.J. Goadsby, Stimulation of the middle meningeal artery leads to Fos expression in the trigeminocervical nucleus: a comparative study of monkey and cat, J Anat 194 (Pt 4) (1999) 579-588.
[102] M. Hou, M. Kanje, J. Longmore, J. Tajti, R. Uddman, L. Edvinsson, 5-HT(1B) and 5-HT(1D) receptors in the human trigeminal ganglion: co-localization with calcitonin gene-related peptide, substance P and nitric oxide synthase, Brain Res 909 (2001) 112-120.
[103] M. Hou, R. Uddman, J. Tajti, L. Edvinsson, Nociceptin immunoreactivity and receptor mRNA in the human trigeminal ganglion, Brain Res 964 (2003) 179-186.
[104] M. Hou, R. Uddman, J. Tajti, M. Kanje, L. Edvinsson, Capsaicin receptor immunoreactivity in the human trigeminal ganglion, Neurosci Lett 330 (2002) 223-226.
[105] K. Ikeda, M. Watanabe, T. Ichikawa, T. Kobayashi, R. Yano, T. Kumanishi, Distribution of prepro-nociceptin/orphanin FQ mRNA and its receptor mRNA in developing and adult mouse central nervous systems, J Comp Neurol 399 (1998) 139-151.
[106] I. Jansen, C. Alafaci, J. McCulloch, R. Uddman, L. Edvinsson, Tachykinins (substance P, neurokinin A, neuropeptide K, and neurokinin B) in the cerebral circulation: vasomotor responses in vitro and in situ, J Cereb Blood Flow Metab 11 (1991) 567-575.
[107] I. Jansen-Olesen, P.J. Goadsby, R. Uddman, L. Edvinsson, Vasoactive intestinal peptide (VIP) like peptides in the cerebral circulation of the cat, J Auton Nerv Syst 49 Suppl (1994) S97-103.
[108] I. Jansen-Olesen, A. Mortensen, L. Edvinsson, Calcitonin gene-related peptide is released from capsaicin-sensitive nerve fibres and induces vasodilatation of human cerebral arteries concomitant with activation of adenylyl cyclase, Cephalalgia 16 (1996) 310-316.
[109] S.E. Jordt, D. Julius, Molecular basis for species-specific sensitivity to "hot" chili peppers, Cell 108 (2002) 421-430.
[110] A. Joutel, M.G. Bousser, V. Biousse, P. Labauge, H. Chabriat, A. Nibbio, J. Maciazek, B. Meyer, M.A. Bach, J. Weissenbach, et al., A gene for familial hemiplegic migraine maps to chromosome 19, Nat Genet 5 (1993) 40-45.

[111] A. Joutel, A. Ducros, K. Vahedi, P. Labauge, O. Delrieu, N. Pinsard, J. Mancini, G. Ponsot, F. Gouttiere, J.L. Gastaut, et al., Genetic heterogeneity of familial hemiplegic migraine, Am J Hum Genet 55 (1994) 1166-1172.
[112] G. Juhasz, Sumatriptan causes parelled decrease in plasma calcitonin related peptide (CGRP) concentration and migraine headache during nitroglycerin induced migraine attack, Cephalalgia In press (2004).
[113] G. Juhasz, T. Zsombok, E.A. Modos, S. Olajos, B. Jakab, J. Nemeth, J. Szolcsanyi, J. Vitrai, G. Bagdy, NO-induced migraine attack: strong increase in plasma calcitonin gene-related peptide (CGRP) concentration and negative correlation with platelet serotonin release, Pain 106 (2003) 461-470.
[114] D. Julius, A.I. Basbaum, Molecular mechanisms of nociception, Nature 413 (2001) 203-210.
[115] H. Kaube, K.A. Keay, K.L. Hoskin, R. Bandler, P.J. Goadsby, Expression of c-Fos-like immunoreactivity in the caudal medulla and upper cervical spinal cord following stimulation of the superior sagittal sinus in the cat, Brain Res 629 (1993) 95-102.
[116] E. Knyihar-Csillik, J. Tajti, A.E. Csillik, Z. Chadaide, A. Mihaly, L. Vecsei, Effects of eletriptan on the peptidergic innervation of the cerebral dura mater and trigeminal ganglion, and on the expression of c-fos and c-jun in the trigeminal complex of the rat in an experimental migraine model, Eur J Neurosci 12 (2000) 3991-4002.
[117] E. Knyihar-Csillik, J. Tajti, M. Samsam, G. Sary, P. Buzas, L. Vecsei, Depletion of calcitonin gene-related peptide from the caudal trigeminal nucleus of the rat after electrical stimulation of the Gasserian ganglion, Exp Brain Res 118 (1998) 111-114.
[118] E. Knyihar-Csillik, J. Tajti, M. Samsam, G. Sary, S. Slezak, L. Vecsei, Effect of a serotonin agonist (sumatriptan) on the peptidergic innervation of the rat cerebral dura mater and on the expression of c-fos in the caudal trigeminal nucleus in an experimental migraine model, J Neurosci Res 48 (1997) 449-464.
[119] L.S. Krimer, E.C. Muly, 3rd, G.V. Williams, P.S. Goldman-Rakic, Dopaminergic regulation of cerebral cortical microcirculation, Nat Neurosci 1 (1998) 286-289.
[120] L.I. Larsson, L. Edvinsson, J. Fahrenkrug, R. Hakanson, C. Owman, O. Schaffalitzky de Muckadell, F. Sundler, Immunohistochemical localization of a vasodilatory polypeptide (VIP) in cerebrovascular nerves, Brain Res 113 (1976) 400-404.
[121] M. Lauritzen, Pathophysiology of the migraine aura. The spreading depression theory, Brain 117 (Pt 1) (1994) 199-210.
[122] T.J. Lee, Cholinergic mechanism in the large cat cerebral artery, Circ Res 50 (1982) 870-879.
[123] T.J. Lee, Direct evidence against acetylcholine as the dilator transmitter in the cat cerebral artery, Eur J Pharmacol 68 (1980) 393-394.
[124] T.J.F. Lee, Nitric oxide and the cerebral vascular function, J. Biomed. Sci 7 (2000) 16-26.
[125] Y. Liu, M. Zhang, J. Broman, L. Edvinsson, Central projections of sensory innervation of the rat superficial temporal artery, Brain Res 966 (2003) 126-133.
[126] J. Longmore, D. Shaw, D. Smith, R. Hopkins, G. McAllister, J.D. Pickard, D.J. Sirinathsinghji, A.J. Butler, R.G. Hill, Differential distribution of 5HT1D- and 5HT1B-immunoreactivity within the human trigemino-cerebrovascular system: implications for the discovery of new antimigraine drugs, Cephalalgia 17 (1997) 833-842.
[127] H.C. Lou, L. Edvinsson, E.T. MacKenzie, The concept of coupling blood flow to brain function: revision required?, Ann Neurol 22 (1987) 289-297.

[128] J.M. Lundberg, A. Anggard, J. Fahrenkrug, T. Hokfelt, V. Mutt, Vasoactive intestinal polypeptide in cholinergic neurons of exocrine glands: functional significance of coexisting transmitters for vasodilation and secretion, Proc Natl Acad Sci U S A 77 (1980) 1651-1655.
[129] J.M. Lundberg, A. Rudehill, A. Sollevi, E. Theodorsson-Norheim, B. Hamberger, Frequency- and reserpine-dependent chemical coding of sympathetic transmission: differential release of noradrenaline and neuropeptide Y from pig spleen, Neurosci Lett 63 (1986) 96-100.
[130] C.F. Marfurt, D.R. Del Toro, Corneal sensory pathway in the rat: a horseradish peroxidase tracing study, J Comp Neurol 261 (1987) 450-459.
[131] S. Markowitz, K. Saito, M.A. Moskowitz, Neurogenically mediated leakage of plasma protein occurs from blood vessels in dura mater but not brain, J Neurosci 7 (1987) 4129-4136.
[132] A. May, A. Bahra, C. Buchel, R.S. Frackowiak, P.J. Goadsby, Hypothalamic activation in cluster headache attacks, Lancet 352 (1998) 275-278.
[133] A. May, A. Bahra, C. Buchel, R.S. Frackowiak, P.J. Goadsby, PET and MRA findings in cluster headache and MRA in experimental pain, Neurology 55 (2000) 1328-1335.
[134] A. May, H. Kaube, C. Buchel, C. Eichten, M. Rijntjes, M. Juptner, C. Weiller, H.C. Diener, Experimental cranial pain elicited by capsaicin: a PET study, Pain 74 (1998) 61-66.
[135] D.E. McBean, J. Sharkey, I.M. Ritchie, P.A. Kelly, Evidence for a possible role for serotonergic systems in the control of cerebral blood flow, Brain Res 537 (1990) 307-310.
[136] J. McCulloch, R. Uddman, T.A. Kingman, L. Edvinsson, Calcitonin gene-related peptide: functional role in cerebrovascular regulation, Proc Natl Acad Sci U S A 83 (1986) 5731-5735.
[137] J.C. Meunier, C. Mollereau, L. Toll, C. Suaudeau, C. Moisand, P. Alvinerie, J.L. Butour, J.C. Guillemot, P. Ferrara, B. Monsarrat, et al., Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor, Nature 377 (1995) 532-535.
[138] F.J. Miao, T.J. Lee, Cholinergic and VIPergic innervation in cerebral arteries: a sequential double-labeling immunohistochemical study, J Cereb Blood Flow Metab 10 (1990) 32-37.
[139] K. Moller, Y.Z. Zhang, R. Hakanson, A. Luts, B. Sjolund, R. Uddman, F. Sundler, Pituitary adenylate cyclase activating peptide is a sensory neuropeptide: immunocytochemical and immunochemical evidence, Neuroscience 57 (1993) 725-732.
[140] C. Mollereau, M. Parmentier, P. Mailleux, J.L. Butour, C. Moisand, P. Chalon, D. Caput, G. Vassart, J.C. Meunier, ORL1, a novel member of the opioid receptor family. Cloning, functional expression and localization, FEBS Lett 341 (1994) 33-38.
[141] S. Nagel-Leiby, K.M. Welch, G. D'Andrea, S. Grunfeld, E. Brown, Event-related slow potentials and associated catecholamine function in migraine, Cephalalgia 10 (1990) 317-329.
[142] K.C. Nielsen, C. Owman, Adrenergic innervation of pial arteries related to the circle of Willis in the cat, Brain Res 6 (1967) 773-776.
[143] K. Nozaki, M.A. Moskowitz, K.I. Maynard, N. Koketsu, T.M. Dawson, D.S. Bredt, S.H. Snyder, Possible origins and distribution of immunoreactive nitric oxide

synthase-containing nerve fibers in cerebral arteries, J Cereb Blood Flow Metab 13 (1993) 70-79.
[144] J. Olesen, H.C. Diener, I.W. Husstedt, P.J. Goadsby, D. Hall, U. Meier, S. Pollentier, L.M. Lesko, Calcitonin gene-related peptide receptor antagonist BIBN4096BS for the acute treatment of migraine., N Engl J Med 350 (2004) 1104-1110.
[145] J. Olesen, L. Friberg, T.S. Olsen, H.K. Iversen, N.A. Lassen, A.R. Andersen, A. Karle, Timing and topography of cerebral blood flow, aura, and headache during migraine attacks, Ann Neurol 28 (1990) 791-798.
[146] J. Olesen, P. Tfelt-Hansen, K. Welch, The Headaches, Second Edition, Lippincott, Williams& Wilkins, Philadelphia, 2000.
[147] J. Olesen, P. Tfelt-Hansen, K. Welch, The Headaches, Second Edition, Lippincott, Williams& Wilkins, Philadelphia, 1999.
[148] J. Olesen, L.L. Thomsen, L.H. Lassen, I.J. Olesen, The nitric oxide hypothesis of migraine and other vascular headaches, Cephalalgia 15 (1995) 94-100.
[149] G.A. Olson, R.D. Olson, A.J. Kastin, Endogenous opiates: 1993, Peptides 15 (1994) 1513-1556.
[150] R.A. Ophoff, G.M. Terwindt, M.N. Vergouwe, R. van Eijk, P.J. Oefner, S.M. Hoffman, J.E. Lamerdin, H.W. Mohrenweiser, D.E. Bulman, M. Ferrari, J. Haan, D. Lindhout, G.J. van Ommen, M.H. Hofker, M.D. Ferrari, R.R. Frants, Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4, Cell 87 (1996) 543-552.
[151] C. Owman, L. Edvinsson, Histochemical and pharmacological approach to the investigation of neurotransmiters, with particular regard to the cerebrovascular bed., 1977, pp15-38 pp.
[152] Z. Pan, N. Hirakawa, H.L. Fields, A cellular mechanism for the bidirectional pain-modulating actions of orphanin FQ/nociceptin, Neuron 26 (2000) 515-522.
[153] J. Pascual, C. Del Arco, T. Romon, E. Del Olmo, A. Pazos, [3H]Sumatriptan binding sites in human brain: regional-dependent labelling of 5-HT1D and 5-HT1F receptors, Eur J Pharmacol 295 (1996) 271-274.
[154] K. Petersen, L.H. Lassen, S. Birk, O. J, The effect of the non-peptide CGRP antagonist on human-alphaCGRP induced headache and hemodynamics in healthy volunteers, Cephalalgia 23 (2003) 725.
[155] L.A. Phebus, K.W. Johnson, P.W. Stengel, K.L. Lobb, J.A. Nixon, P.A. Hipskind, The non-peptide NK-1 receptor antagonist LY303870 inhibits neurogenic dural inflammation in guinea pigs, Life Sci 60 (1997) 1553-1561.
[156] D. Pietrobon, J. Striessnig, Neurobiology of migraine, Nat Rev Neurosci 4 (2003) 386-398.
[157] R.D. Piper, L. Edvinsson, R. Ekman, G.A. Lambert, Cortical spreading depression does not result in the release of calcitonin gene-related peptide into the external jugular vein of the cat: relevance to human migraine, Cephalalgia 13 (1993) 180-183; discussion 149.
[158] M.E. Raichle, B.K. Hartman, J.O. Eichling, L.G. Sharpe, Central noradrenergic regulation of cerebral blood flow and vascular permeability, Proc Natl Acad Sci U S A 72 (1975) 3726-3730.
[159] B. Ray, H. Wolff, Experimental studies on headache. Pain sensitive structures of the head and their significance in headache., 1940.

[160] J.F. Reinhard, Jr., J.E. Liebmann, A.J. Schlosberg, M.A. Moskowitz, Serotonin neurons project to small blood vessels in the brain, Science 206 (1979) 85-87.
[161] R.K. Reinscheid, H.P. Nothacker, A. Bourson, A. Ardati, R.A. Henningsen, J.R. Bunzow, D.K. Grandy, H. Langen, F.J. Monsma, Jr., O. Civelli, Orphanin FQ: a neuropeptide that activates an opioidlike G protein-coupled receptor, Science 270 (1995) 792-794.
[162] M. Riedl, S. Shuster, L. Vulchanova, J. Wang, H.H. Loh, R. Elde, Orphanin FQ/nociceptin-immunoreactive nerve fibers parallel those containing endogenous opioids in rat spinal cord, Neuroreport 7 (1996) 1369-1372.
[163] C.M. Rosendorff, D. Mitchell, G. Mitchell, Adrenergic innervation affecting local cerebral blood flow., Pergamon Press, Oxford, 1977, 455-464 pp.
[164] A. Saito, J.Y. Wu, T.J. Lee, Evidence for the presence of cholinergic nerves in cerebral arteries: an immunohistochemical demonstration of choline acetyltransferase, J Cereb Blood Flow Metab 5 (1985) 327-334.
[165] M. Samsam, R. Covenas, R. Ahangari, J. Yajeya, J.A. Narvaez, G. Tramu, Alterations in neurokinin A-, substance P- and calcitonin gene-related peptide immunoreactivities in the caudal trigeminal nucleus of the rat following electrical stimulation of the trigeminal ganglion, Neurosci Lett 261 (1999) 179-182.
[166] P.S. Sandor, J. Afra, A.P. Proietti Cecchini, A. Albert, J. Schoenen, From neurophysiology to genetics: cortical information processing in migraine underlies familial influences--a novel approach, Funct Neurol 15 Suppl 3 (2000) 68-72.
[167] K. Schepelmann, A. Ebersberger, M. Pawlak, M. Oppmann, K. Messlinger, Response properties of trigeminal brain stem neurons with input from dura mater encephali in the rat, Neuroscience 90 (1999) 543-554.
[168] Y. Seki, Y. Suzuki, M.K. Baskaya, T. Kano, K. Saito, M. Takayasu, M. Shibuya, K. Sugita, The effects of pituitary adenylate cyclase-activating polypeptide on cerebral arteries and vertebral artery blood flow in anesthetized dogs, Eur J Pharmacol 275 (1995) 259-266.
[169] G. Selby, J.W. Lance, Observations on 500 cases of migraine and allied vascular headache, J Neurol Neurosurg Psychiatry 23 (1960) 23-32.
[170] S. Shepheard, L. Edvinsson, M. Cumberbatch, D. Williamson, G. Mason, J. Webb, S. Boyce, R. Hill, R. Hargreaves, Possible antimigraine mechanisms of action of the 5HT1F receptor agonist LY334370, Cephalalgia 19 (1999) 851-858.
[171] S. Shepheard, D.J. Williamson, R. Hargreaves, Intravital microscope studies of dural blood vessels in rats, Durity, Martin, London, 1999.
[172] S.L. Shepheard, D.J. Williamson, R.G. Hill, R.J. Hargreaves, The non-peptide neurokinin1 receptor antagonist, RP 67580, blocks neurogenic plasma extravasation in the dura mater of rats, Br J Pharmacol 108 (1993) 11-12.
[173] S.L. Shepheard, D.J. Williamson, J. Williams, R.G. Hill, R.J. Hargreaves, Comparison of the effects of sumatriptan and the NK1 antagonist CP-99,994 on plasma extravasation in Dura mater and c-fos mRNA expression in trigeminal nucleus caudalis of rats, Neuropharmacology 34 (1995) 255-261.
[174] M. Siniatchkin, W.D. Gerber, A. Vein, Clinical efficacy and central mechanisms of cyclandelate in migraine: a double-blind placebo-controlled study, Funct Neurol 13 (1998) 47-56.
[175] M. Siniatchkin, E. Kirsch, P. Kropp, U. Stephani, W.D. Gerber, Slow cortical potentials in migraine families, Cephalalgia 20 (2000) 881-892.

[176] A. Stepien, P. Jagustyn, A. Trafny, K. Widerkiewicz, Effect of rizatriptan on CGRP level in migraine, Cephalalgia 23 (2003) 738.
[177] A.M. Strassman, S.A. Raymond, R. Burstein, Sensitization of meningeal sensory neurons and the origin of headaches, Nature 384 (1996) 560-564.
[178] N. Suzuki, Y. Fukuuchi, A. Koto, Y. Naganuma, K. Isozumi, S. Matsuoka, J. Gotoh, T. Shimizu, Cerebrovascular NADPH diaphorase-containing nerve fibers in the rat, Neurosci Lett 151 (1993) 1-3.
[179] N. Suzuki, J.E. Hardebo, C. Owman, Origins and pathways of cerebrovascular nerves storing substance P and calcitonin gene-related peptide in rat, Neuroscience 31 (1989) 427-438.
[180] N. Suzuki, J.E. Hardebo, C. Owman, Origins and pathways of cerebrovascular vasoactive intestinal polypeptide-positive nerves in rat, J Cereb Blood Flow Metab 8 (1988) 697-712.
[181] N. Suzuki, J.E. Hardebo, C. Owman, Origins and pathways of choline acetyltransferase-positive parasympathetic nerve fibers to cerebral vessels in rat, J Cereb Blood Flow Metab 10 (1990) 399-408.
[182] L.W. Swanson, M.A. Connelly, B.K. Hartman, Ultrastructural evidence for central monoaminergic innervation of blood vessels in the paraventricular nucleus of the hypothalamus, Brain Res 136 (1977) 166-173.
[183] A. Szallasi, P.M. Blumberg, Vanilloid (Capsaicin) receptors and mechanisms, Pharmacol Rev 51 (1999) 159-212.
[184] A. Szallasi, P.M. Blumberg, Vanilloid receptor loss in rat sensory ganglia associated with long term desensitization to resiniferatoxin, Neurosci Lett 140 (1992) 51-54.
[185] A. Szallasi, S. Nilsson, T. Farkas-Szallasi, P.M. Blumberg, T. Hokfelt, J.M. Lundberg, Vanilloid (capsaicin) receptors in the rat: distribution in the brain, regional differences in the spinal cord, axonal transport to the periphery, and depletion by systemic vanilloid treatment, Brain Res 703 (1995) 175-183.
[186] J. Tajti, S. Moller, R. Uddman, I. Bodi, L. Edvinsson, The human superior cervical ganglion: neuropeptides and peptide receptors, Neurosci Lett 263 (1999) 121-124.
[187] J. Tajti, R. Uddman, S. Moller, F. Sundler, L. Edvinsson, Messenger molecules and receptor mRNA in the human trigeminal ganglion, J Auton Nerv Syst 76 (1999) 176-183.
[188] G.M. Terwindt, R.A. Ophoff, J. Haan, M.N. Vergouwe, R. van Eijk, R.R. Frants, M.D. Ferrari, Variable clinical expression of mutations in the P/Q-type calcium channel gene in familial hemiplegic migraine. Dutch Migraine Genetics Research Group, Neurology 50 (1998) 1105-1110.
[189] H. Tomimoto, M. Nishimura, T. Suenaga, S. Nakamura, I. Akiguchi, H. Wakita, J. Kimura, B. Mayer, Distribution of nitric oxide synthase in the human cerebral blood vessels and brain tissues, J Cereb Blood Flow Metab 14 (1994) 930-938.
[190] Y.R. Tran Dinh, C. Thurel, G. Cunin, A. Serrie, J. Seylaz, Cerebral vasodilation after the thermocoagulation of the trigeminal ganglion in humans, Neurosurgery 31 (1992) 658-662; discussion 663.
[191] R. Uddman, L. Edvinsson, R. Ekman, T. Kingman, J. McCulloch, Innervation of the feline cerebral vasculature by nerve fibers containing calcitonin gene-related peptide: trigeminal origin and co-existence with substance P, Neurosci Lett 62 (1985) 131-136.

[192] R. Uddman, P.J. Goadsby, I. Jansen, L. Edvinsson, PACAP, a VIP-like peptide: immunohistochemical localization and effect upon cat pial arteries and cerebral blood flow, J Cereb Blood Flow Metab 13 (1993) 291-297.
[193] R. Uddman, J. Tajti, M. Hou, F. Sundler, L. Edvinsson, Neuropeptide expression in the human trigeminal nucleus caudalis and in the cervical spinal cord C1 and C2, Cephalalgia 22 (2002) 112-116.
[194] R. Uddman, J. Tajti, S. Moller, F. Sundler, L. Edvinsson, Neuronal messengers and peptide receptors in the human sphenopalatine and otic ganglia, Brain Res 826 (1999) 193-199.
[195] K. Vahedi, C. Denier, A. Ducros, V. Bousson, C. Levy, H. Chabriat, M. Haguenau, E. Tournier-Lasserve, M.G. Bousser, CACNA1A gene de novo mutation causing hemiplegic migraine, coma, and cerebellar atrophy, Neurology 55 (2000) 1040-1042.
[196] M. Wahl, L. Schilling, A.A. Parsons, A. Kaumann, Involvement of calcitonin gene-related peptide (CGRP) and nitric oxide (NO) in the pial artery dilatation elicited by cortical spreading depression, Brain Res 637 (1994) 204-210.
[197] A.M. van den Maagdenberg, D. Pietrobon, T. Pizzorusso, S. Kaja, L.A. Broos, T. Cesetti, R.C. van de Ven, A. Tottene, J. van der Kaa, J.J. Plomp, R.R. Frants, M.D. Ferrari, A Cacna1a knockin migraine mouse model with increased susceptibility to cortical spreading depression, Neuron 41 (2004) 701-710.
[198] K.R. Vanmolkot, E.E. Kors, J.J. Hottenga, G.M. Terwindt, J. Haan, W.A. Hoefnagels, D.F. Black, L.A. Sandkuijl, R.R. Frants, M.D. Ferrari, A.M. van den Maagdenberg, Novel mutations in the Na+, K+-ATPase pump gene ATP1A2 associated with familial hemiplegic migraine and benign familial infantile convulsions, Ann Neurol 54 (2003) 360-366.
[199] C. Weiller, A. May, V. Limmroth, M. Juptner, H. Kaube, R.V. Schayck, H.H. Coenen, H.C. Diener, Brain stem activation in spontaneous human migraine attacks, Nat Med 1 (1995) 658-660.
[200] D.J. Williamson, R.J. Hargreaves, R.G. Hill, S.L. Shepheard, Intravital microscope studies on the effects of neurokinin agonists and calcitonin gene-related peptide on dural vessel diameter in the anaesthetized rat, Cephalalgia 17 (1997) 518-524.
[201] R.P. Woods, M. Iacoboni, J.C. Mazziotta, Brief report: bilateral spreading cerebral hypoperfusion during spontaneous migraine headache, N Engl J Med 331 (1994) 1689-1692.
[202] K. Yoshida, T. Okamura, N. Toda, Histological and functional studies on the nitroxidergic nerve innervating monkey cerebral, mesenteric and temporal arteries, Jpn J Pharmacol 65 (1994) 351-359.
[203] J. You, S. Gulbenkian, I. Jansen Olesen, K. Marron, J. Wharton, C.P. Barroso, J.M. Polak, L. Edvinsson, Peptidergic innervation of guinea-pig brain vessels: comparison with immunohistochemistry and in vitro pharmacology in rostrally and caudally located arteries, J Auton Nerv Syst 55 (1995) 179-188.
[204] A.S. Zagami, Edvinsson, L, Hoskin KL, Goadsby PJ, Stimulation of the superior sagittal sinus causes extracranial release of PACAP, Cephalalgia 15 (1995) 109.
[205] A.S. Zagami, P.J. Goadsby, L. Edvinsson, Stimulation of the superior sagittal sinus in the cat causes release of vasoactive peptides, Neuropeptides 16 (1990) 69-75.

Legends to illustrations

Figure 1.

Schematic illustration of the perivascular nerves in intracranial arteries. Sympathetic nerves originate in the superior cervical ganglion and store noradrenaline, ATP and neuropeptide Y. The presynaptic fibres originate in the tract. Parasympathetic nerves have their major origin in otic and spheno- palatine ganglia and store VIP, PACAP, nitric oxide and acetylcholine. Sensory fibers originate mainly in the trigeminal ganglion and store CGRP, substance P, neurokinin A, PACAP and nitric oxide [91]

Figure 2.

Schematic illustration demonstrating the central role of the trigeminovascular system, bridging from intercranial arteries and veins to the trigeminocervical complex with e.g. the trigeminal nucleus caudalis (TNC). Intracranial blood vessels are invested with mechano and sensory Aα- and C, e.g. CGRP, substance P, PACAP, nocicepting inter alia [200]. Tracing with CT_B and WHG .. have determined that the thin C-fibers end in Lanima I while the Aα- end up in Lanima IV of teh brainstem [125]. From these synapses the pain signalling carries up to higher levels and involves e.g. PAG thalamus and cortex.

Figure 3.

Schematic illustration of the trigeminovascular reflex illustrated by local perivascular administration of noradrenaline (NA). The sensory fibers in cerebral vessels sense induced vasoconstriction and release stored neuronal messengers (e.g. CGRP) and quickly normalize tone. After leasioning and the loss of CGRP and substance P the same amount of vasoconstriction is seen, but the time to normalize the vascular tone is markedly prolonged [53,54,58,136].

Figure 4.

Schematic illustration of the involvement of the trigemino-vascular system with the parasympathetic outflow from the superior cervical nucleus to the otic and sphenopalatine ganglion. This pathway is possibly activated upon intense stimulation such as that seen in cluster headache. These attacks are associated with the release of CGRP from the sensory fibers (pain) and also VIP (and other neuronal messengers) from the parasympathetic system (resulting in facial symptoms).

TABLE 1 – Overview of changes in perivascular neuropeptide levels occurring in acute attacks of primary headache disorders

	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