Current understanding of meningeal and cerebral vascular function underlying migraine headache

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Abstract

Background: The exact mechanisms underlying the onset of a migraine attack are not completely understood. It is, however, now well accepted that the onset of the excruciating throbbing headache of migraine is mediated by the activation and increased mechanosensitivity (i.e. sensitization) of trigeminal nociceptive afferents that innervate the cranial meninges and their related large blood vessels.

Objectives: To provide a critical summary of current understanding of the role that the cranial meninges, their associated vasculature, and immune cells play in meningeal nociception and the ensuing migraine headache.

Methods: We discuss the anatomy of the cranial meninges, their associated vasculature, innervation and immune cell population. We then debate the meningeal neurogenic inflammation hypothesis of migraine and its putative contribution to migraine pain. Finally, we provide insights into potential sources of meningeal inflammation and nociception beyond neurogenic inflammation, and their potential contribution to migraine headache.

Migraine is a complex, multifactorial neurological disorder affecting about 10% of the adult population worldwide (1). It is the second most prevalent neurological disorder (1) and the first cause of disability in under 50s (2,3). While the exact mechanisms underlying the onset of a migraine attack remain unclear, it is now accepted that the development of the excruciating throbbing headache of migraine requires the initial activation and increased mechanosensitivity (i.e. sensitization) of trigeminal nociceptive afferents that innervate the cranial meninges and their related large blood vessels (4–7). The goal of this review is to summarize current knowledge and understanding of the role that the cranial meninges and their related vasculature and cellular constituents play in the meningeal nociceptive processes underlying the onset of migraine headache.

(i) Anatomical features of the cranial meninges and their associated vasculature

The cranial meninges are comprised of two main distinct layers: The dura mater, or pachymeninx, a thick layer of connective tissue apposing to the cranium, and the leptomeninges. The latter can be further separated into the arachnoid and pia mater. The dura mater can be divided anatomically into three layers, the endosteal (periosteal) layer, the inner meningeal layer, and the dural border cell layer, or subdural neurothelium (8). The dural layers are fused in most places, but separate to form the venous sinuses and at the Falx cerebri. The dural border cell layer is attached to the outer layer of the arachnoid – the arachnoid barrier cell

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layer – by occasional cell junctions, or desmosomes. Under various pathological conditions, such as in the case of subdural hematoma, damage to the dural border cell layer can lead to separation of the dura from the arachnoid and the formation of a "subdural space" (8). The arachnoid layer connects to the thin pia mater via collagen trabeculae to form the subarachnoid space, which is filled with cerebrospinal fluid (CSF). The pia mater, the most inner meningeal layer, abuts a cortical barrier layer made of astrocyte endofoot processes (i.e. the glia limitans, Figure 1(a)). Blood vessels within the subarachnoid space are coated by a pia mater layer (hence the name pial vessels). As arteries penetrate the brain, a single-layered sheath of pial cells
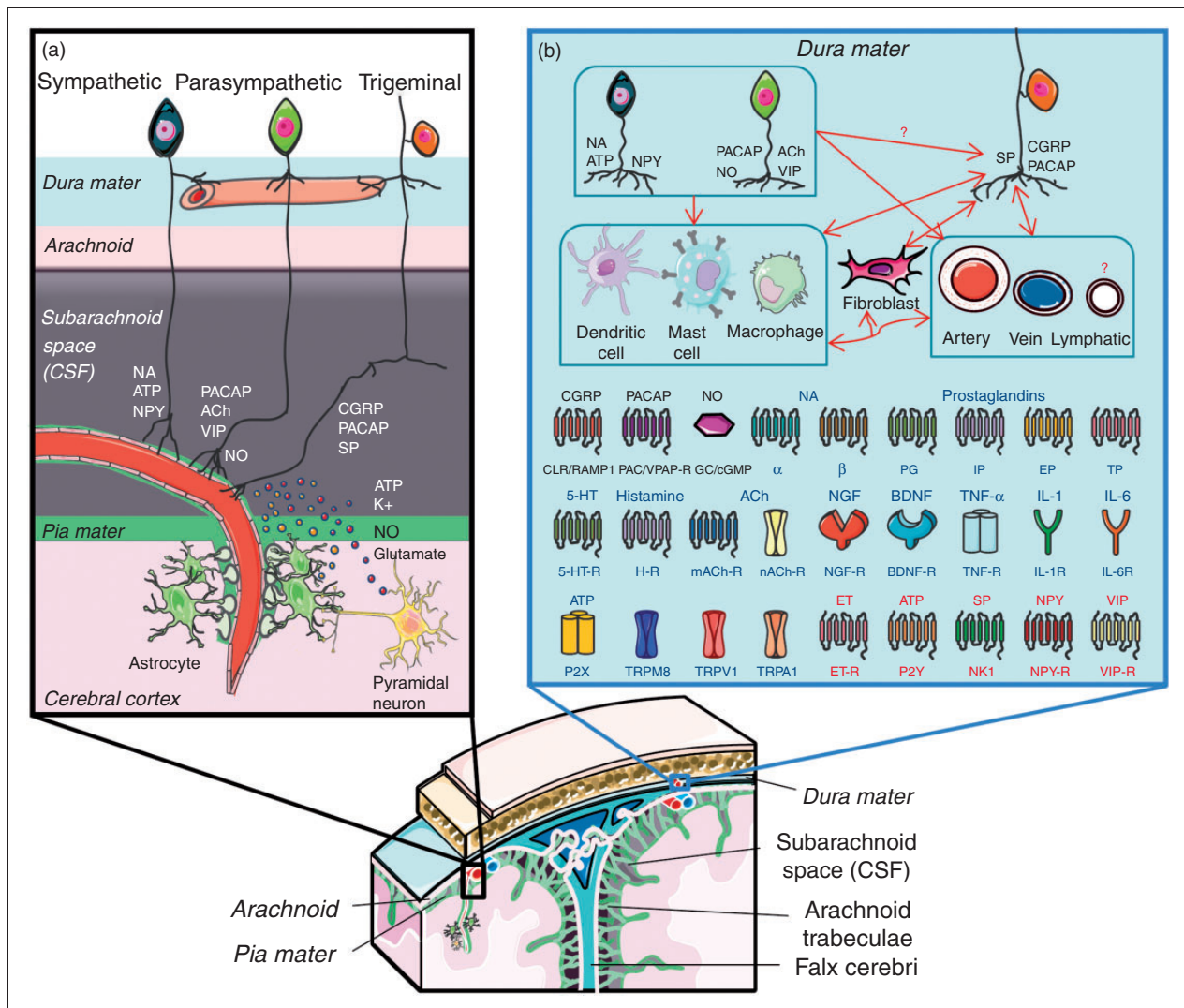
Figure 1. Overview of the cranial meninges and their associated constituents. (a) Schematic illustration of meningeal innervation components in relation to superficial cortical constituents and their mediators (red and yellow, denoting molecules released from neurons and astrocytes respectively), which upon reaching the subarachnoid space via diffusion or bulk flow, could underlie the cortical to meninges signaling in migraine with aura and perhaps also in migraine without aura. (b) Postulated crosstalk between meningeal autonomic and sensory nerves, resident immune cells, fibroblasts and vessels that have been suggested to contribute to meningeal nociception and headache and receptor systems that have been (i) implicated in the genesis of migraine pain in clinical studies (black font), (ii) suggested to activate and/or sensitize the meningeal sensory system based on preclinical studies (blue font), or with questionable contribution to meningeal nociception and headache pain (red font).
CGRP: calcitonin gene-related peptide; PACAP: pituitary adenylate cyclase-activating polypeptide; NO: nitric oxide; NA: noradrenaline; ACh: acetylcholine; NGF: nerve growth factor; BDNF: brain-derived neurotrophic factor; TNF-a: tumor necrosis factor; IL-1: interleukin-1; IL-6: interleukin 6; ATP: adenosine triphosphate; SP: substance P; NPY: neuropeptide Y; VIP: vasoactive intestinal peptide; ET: endothelin.

is maintained for a short distance and separates between the vessel wall and the glia limitans (9,10). Upon entering the cortex, the pial layer that surrounds the arterioles becomes fenestrated. Penetrating veins lack a continuous pial layer (9).

The dura mater is highly vascularized. The arterial supply of the supratentorial dura mater comes primarily from branches of the posterior, middle (the largest), and anterior meningeal arteries, which arise from the occipital, maxillary, and ophthalmic arteries respectively. Meningeal arteries lie predominantly in the endosteal layer. The intracranial middle meningeal artery, which enters the cranium through the foramen spinosum, runs in grooves in the inner table of the calvarium, surrounded almost on three sides by bone (11). A dense capillary network occurs in the inner meningeal layer of the dura. The major dural veins, which run primarily parallel to the path of the meningeal arteries, drain into efferent vessels in the periosteal layer or the dural venous sinuses. The dural venous sinuses absorb CSF from the subarachnoid space via arachnoid granulations. Studies in rodents also identified a dural lymphatic vascular network alongside blood vessels, primarily the middle meningeal artery, superior sagittal sinus, and transverse sinuses (12–15). A recent study in non-human primates and humans has shown that the dural lymphatic system drains macromolecules from the dura (15). In rodents, dural lymphatics drain CSF and parenchymal interstitial fluid (13,14), but whether such lymphatic drainage also occurs in humans remains to be determined (16).

Innervation of the cranial meninges

The cranial meninges are innervated by sensory and autonomic nerves (Figure 1(b)). The sensory innervation of the dura mater originates primarily in the ophthalmic branch of the trigeminal ganglion but also from the mandibular and maxillary branches. Studies on samples obtained from humans, cats, and rodents have demonstrated nerve fibers in the supratentorial dura, along arteries, as well as at the tentorium cerebelli, and the venous sinuses (12,17–20). Using immunohistochemistry in animal and human tissue, axons innervating the cranial dura, cranial arteries and venous sinuses have been shown to express the sensory neuropeptides CGRP (the most abundant) and substance P (19,21–23). Pituitary adenylate cyclase-activating polypeptide (PACAP) was recently found to be co-expressed with CGRP in some dural nerve fibers (24). Glutamate was also identified in trigeminal ganglion cells of rats, albeit primarily in neurons that do not express CGRP (25). While there is no available data to suggest expression of glutamate in meningeal afferents, a recent retrograde tracing study in the mouse identified vesicular glutamate transporter 3 in a subpopulation of small diameter dural afferents (26).

The dura mater is also innervated by autonomic fibers that express PACAP, neuronal nitric oxide synthase (nNOS), vasoactive intestinal polypeptide (VIP), tyrosine hydroxylase (TH), acetylcholine (ACh), and neuropeptide Y (NPY) (18,21–24,27–31). Ultrastructural studies conducted in the rat localized some peptidergic fibers' termination in the vicinity, or walls, of dural blood vessels including lymphatics; axon terminations on collagen bundles in the dural connective tissue have been shown to be more abundant, however (12,18). Within the leptomeningeal, which lack their own blood supply, the cerebral (pial) arteries are the primary sites that receive sensory and autonomic innervations by fibers that originate from sympathetic, parasympathetic, and sensory trigeminal ganglia (Figure 1(a)). Immunohistochemistry studies together with denervation and tracing studies identified trigeminal nerves expressing substance P and CGRP, as well as autonomic nerves that express NPY, VIP, nNOS, ACh, and tryptophan-5-hydroxylase (28,32–39). The sensory and autonomic innervation of cerebral pial vessels is primarily localized to the subarachnoid space and does not follow the vessels when they penetrate the cortex; the loss of pial sheath in the penetrating arteriole is also accompanied by the loss of perivascular innervation. Ultra-structurally, cerebral pial arteries are innervated by peptidergic sensory afferents that terminate either in the outer layer of the adventitia (substance P) or in the inner layer of the adventitia (CGRP), close to arterial smooth muscle cells (40).

Meningeal immune cells

Cytochemical and immunohistochemical studies in naïve rats and mice localized resident immune cells to the dura mater (Figure 1(b)). Macrophages, which express numerous antigens (e.g. CD163, CD68) (41,42) can be found along dural vessels, but also remotely from large vessels. In rodents, resident macrophages that express the chemokine receptor CX3CR1 were also localized to the dura and near pial vessels (43,44). The dura mater also harbors a sizeable population of mast cells (MCs) (19,45–47). Dural MCs are found in the endosteal/periosteal layer and are associated with blood vessels, dural sensory axons expressing CGRP, or substance P, and sympathetic fibers (27,47,48). In the rat, dural MCs can also be found in a distinctive "linear arrays" arrangement (19,46). In the rat, a unique and much denser population of dural MCs is also found immediately caudal to the transverse sinus and medial to the superior cerebellar veins, which

empty into the sinus, on either side of the midline (19). Studies on human dural tissue also identified perivascular MCs in the periosteal dura (49). In rats and mice, there is also a small population of MCs localized to the pia (50,51). Meningeal MCs can be classified based on their histochemical properties, using alcian blue and safranin staining (50,52,53). Using these staining properties, it has been shown that the densities of subsets of dural MCs undergo dynamic changes in female rats during the estrous cycle that are estrogen-dependent (53). Dendritic cells – antigen presenting cells expressing MHC class II and CD11c markers – are localized to the inner layer and connective tissue of the dura, in the arachnoid membrane, and pia mater layer (41,42,44,54,55). Studies in naïve mice demonstrated a small number of T lymphocytes also in the dura, with females showing higher numbers (13,54,55). Memory CD4+ and CD3 T cells can also be found in the subarachnoid space (56,57).

(ii) The meningeal neurogenic inflammation hypothesis of migraine

In vivo electrophysiological studies provided important information about the basic response properties of trigeminal dural afferents (58). Knowledge about the response properties of leptomeningeal afferents is poor, however. Furthermore, knowledge of the endogenous processes that drive the activation and increased sensitivity of both dural and leptomeningeal afferents during a clinically occurring headache attack such as that of migraine is also limited. Tissue injury associated with local inflammation is a major driver of nociceptors' activation, sensitization and pain. However, frank tissue injury or pathology has yet to be demonstrated in migraine (or any other primary headache condition). Nevertheless, a major hypothesis implicates local sterile meningeal inflammation as a key event that mediates the prolonged activation and sensitization of meningeal afferents and the origin of migraine headache (59,60) (see below for further discussion on the contribution of meningeal neurogenic inflammation to meningeal nociception). Numerous clinical findings gathered over the years provided key, yet indirect, support for this inflammatory hypothesis of migraine. Among those are increased levels of inflammatory mediators in the cephalic venous outflow (61,62) and the ability of corticosteroids and non-steroidal-anti-inflammatory drugs to abort migraine pain (63,64). Landmark preclinical studies in rodents provided indirect support for this hypothesis by showing that meningeal afferents are inflammatory sensors and can become persistently activated and sensitized to mechanical stimuli following local stimulation with mediators found in inflammatory exudates (59,65–69).

The origin of such meningeal inflammatory response in primary headaches, and particularly in migraine, remains nevertheless elusive. In their hypothesis paper, Moskowitz and colleagues (70) proposed that "the headache phase of migraine may develop as a result of an abnormal interaction (and perhaps an abnormal release) of vasoactive neurotransmitters from the terminals of the trigeminal nerve with large intracranial and extracranial blood vessels". The meningeal process implicated in this hypothesis was neurogenic inflammation, a peripheral response comprised of (i) increased capillary permeability leading to plasma protein extravasation (PPE), (ii) arterial vasodilatation, and (iii) activation of resident immune cells. Neurogenic inflammation results from activity-dependent release of vasoactive substances, in particular substance P and CGRP from peripheral nerve endings of primary afferent nociceptors: This release occurs through an "axon reflex" process, where action potentials from acutely activated afferents are conducted antidromically and invade peripheral end branches (71). The finding that dural and pial blood vessels are innervated by sensory nerves that express these vasoactive neuropeptides (see above) provided key support for the neurogenic inflammation hypothesis of migraine, which further led to the conceptualization of the trigeminovascular system and its role in migraine headache (72).

Increased meningeal vascular permeability

An early study in animals described the development of meningeal PPE in the dura mater following electrical stimulation of the trigeminal ganglion (73). The subsequent findings that anti-migraine drugs, including ergot alkaloids and triptans, could block this experimental meningeal PPE (74,75) provided additional indirect support for the role of meningeal neurogenic inflammation in migraine headache. The inability to resolve the meningeal tissue and its vasculature in humans using imaging techniques remains a major hurdle in assessing meningeal PPE during migraine headache. However, one imaging study, conducted on a single migraine patient, suggested an increase in meningeal vascular permeability during an attack (76). Current technical improvements in meningeal imaging (15) may be able to provide more clues into this process.

In agreement with studies on non-cranial tissues (77), animal studies also implicated substance P and its neurokinin 1 receptor (NK1-R) in mediating meningeal neurogenic PPE (78,79). However, available data does not support a role for NK-1 signaling in migraine pain. In clinical trials, NK1-R antagonists failed to abort migraine headache (80,81). While such negative data argues against the involvement of substance P signaling and meningeal neurogenic PPE in migraine pain,

the possibility that the doses of NK1-R antagonists used in those studies were suboptimal and thus did not reach biologically-active plasma levels were considered (81,82). The possibility that during migraine, substance P does play a role in mediating a meningeal PPE response, but only during the early stages of the attack, may also be entertained. However, a small study reported the absence of substance P release into the internal jugular vein prior to the onset of the headache phase of a migraine with aura attack induced by intracarotid Xenon-133 injection (83), suggesting lack of substance P involvement in the triggering mechanisms of migraine headache.

Meningeal vasodilatation

Arterial vasodilation – another major characteristic of experimental meningeal neurogenic inflammation – has also been advocated for many years as a key cause of migraine headache. The theory that vasodilatation plays a role in migraine headache was largely based on the early observations of Graham and Wolff (84), who described a close relationship between the decrease in pulsation amplitude of the temporal artery and the decline of headache intensity following treatment with the vasoconstrictor (and antimigraine drug) ergotamine. The later observations that intracranial arteries (mainly the dural branches of the middle meningeal artery) are sensitive to painful stimuli (85) extended the extracranial vascular hypothesis to the intracranial vasculature. Moreover, when distending these arteries, a throbbing headache accompanied with nausea was induced, while neither constriction of the artery lumen nor stimulation of the dura mater 2 mm away from such vessels produced headache pain. These findings have led to the notion that dilatation and distention of intracranial dural meningeal arteries are a major source of migraine headache (86). Thus, it was hypothesized that selective cranial vasoconstrictors would be more efficient and safe antimigraine drugs than ergotamine, which has affinity for a wide array of receptors, including 5-HT and dopamine (87). On this basis, in the beginning of the 1970s, Humphrey et al. aimed at identifying novel antimigraine agents capable of mimicking the beneficial effects of 5-HT without its side-effects (88). As a result, more selective vasoconstrictors of the cranial extra-cerebral circulation were developed, which allowed the identification of the 5-HT_{1B} receptor (at the time of the development designated as 5-HT_1-like receptor) as responsible for this vasoconstriction. Subsequently, one of those agents, the antimigraine drug sumatriptan, has been shown to produce selective cranial vasoconstriction in dogs, and to display much less activity in other vascular beds (89).

In accordance with the hypothesis on which its development was based, migraine-related changes in the middle cerebral artery blood flow, congruent with vasodilation, were reversed by sumatriptan (83). Unfortunately, the vasoconstrictor effects of sumatriptan were not entirely selective for the cranial circulation (i.e. 5-HT_{1B} receptors were also localized in coronary arteries), suggesting that the antimigraine effect of sumatriptan may not be entirely related to meningeal arterial vasodilatation. Whether meningeal vasodilatation plays a causative role in migraine, or is merely an epiphenomenon – a secondary event arising from the activation of intracranial trigeminal afferents and the ensuing meningeal release of vasodilatory neuropeptides – remains a hotly debated subject (90). However, neuroimaging studies have revealed that triptans produce cranial vasoconstriction; this may well contribute to their antimigraine effects (91,92). Assuming that receptor density expression in the cranial vasculature and the trigeminal ganglion have a close similarity, it could indeed be that triptans' antimigraine effects are not only mediated via the blood vessels, but via other structures, such as peripheral nerve endings of meningeal afferents (93) (but see also 94), their trigeminal ganglion cell bodies or central nerve endings in the trigemino-cervical complex (95). According to the "vascular theory", intracranial vasodilatation (but possibly also extracranial) leads to the activation of trigeminal afferents that innervate these vessels, with ensuing headache (96). A key process that could hypothetically mediate the activation of meningeal afferents by arterial vasodilatation is the stimulation of mechanosensitive stretch receptors located within the dilated vessels' walls. While electrophysiological studies indicate that meningeal afferents that innervate the dura mater are mechanosensitive (58,59), anatomical studies in animals suggest that most of the sensory innervation of the dura mater terminates in the connective tissue, far from the vessels (18). Animal studies showing that administration of vasoactive agents, including CGRP and NO, failed to activate afferents with perivascular dural receptive fields (97–99), which further suggests that dural vasodilation alone is not sufficient to activate mechanosensitive meningeal afferents.

As indicated above, the sensory innervation of intracranial pial vessels, which may also be mechanosensitive, terminates in the outer layer of the adventitia (substance P) or in the inner layer of the adventitia (CGRP), close to arterial smooth muscle cells (40); but it is unclear whether pial afferents can be directly activated by arterial dilatation *per se*. The finding of only a slight dilatation of intracranial arteries during migraine attacks that was not reduced by effective treatment with sumatriptan (100) argues against the activation of meningeal afferents in responses to such intracranial vasodilatation in migraine (and see also 101). Moreover, headache provocation studies in

healthy subjects revealed that migraine-like headaches can be induced by oral sildenafil, a phosphodiesterase 5 inhibitor, while no cerebral arterial vasodilation was detected. On the other hand, experimentally-induced headache in migraine patients revealed that the vasodilator adrenomedullin did not induce migraine headaches or changes in mean blood flow velocity of the middle cerebral artery. To properly interpret these studies, it should be taken into account that, due to technical limitations, only extracranial parts of the meningeal vasculature (100) or intracerebral vessels (102,103) were measured and thus potentially do not always exactly reflect changes that occur in the meningeal vasculature (104). Further, although sildenafil is strictly not a vasodilator *per se*, it is quite likely that its phosphodiesterase 5 inhibitory activity may lead to vasodilatation depending on the levels of cGMP in a blood vessel. Indeed, in isolated meningeal arteries from rats or patients undergoing neurosurgery, VIP, SNP, CGRP and PACAP, as well as sildenafil, can promote vasorelaxation. Interestingly, infusion of PACAP38 causes headache and vasodilation in both healthy subjects and migraine patients. In contrast, infusion of VIP in people with migraine evoked a marked cephalic vasodilation, but not a migrainous headache (105), which is also congruent with the notion that a provoked intracranial vasodilation alone is not sufficient to activate meningeal afferents. On the other hand, the above-mentioned discrepancy between VIP and PACAP may also be assigned to the shorter-lasting vasodilatory response evoked by VIP compared to that by PACAP (M Ashina, personal communication). Studies investigating the effects of VIP infusions over a longer period of time may explain whether the discrepancy between VIP and PACAP is due to different pharmacokinetics, or whether provoked vasodilatation is indeed not sufficient to provoke a migraine-like headache. Intriguingly, the receptors mediating dilatation in the meningeal circulation closely resemble many of the receptors expressed on the trigeminal ganglion, hampering a dissection between pure vascular and trigeminal effects. Increased diameter of meningeal vessels, whether dural or pial, may however lead to meningeal tissues' stretching that could activate mechanosensitive afferents, in particular during a sensitized state (59,101,106).

Key studies in rodents have led researchers to suggest that trigeminal nerve endings of activated perivascular meningeal afferents release CGRP, and this is the primary driver of neurogenic meningeal vasodilation in migraine (107). The view that cephalic vasodilatation in migraine is neurogenically mediated received strong support from the findings of Goadsby and colleagues (108,109), who demonstrated elevated levels of CGRP in the extra-cerebral circulation during a migraine attack. While these findings could not be replicated in a later study (110), the validity of its methodology was questioned subsequently (111). Despite the inconclusive findings of increased CGRP levels within the intracranial circulation during a migraine attack (83,112), the finding that sumatriptan normalized the elevated CGRP levels observed in the extra-jugular vein, concomitant with headache relief (109) further promoted the notion that trigeminal release of CGRP and the ensuing cranial neurogenic vasodilatation contribute to migraine headache. Moreover, a human experimental model of neurogenic vasodilation, where capsaicin is applied to a trigeminal (V1) dermatome in the human forehead to release endogenous CGRP (via activation of TRPV1 channels), confirmed that sumatriptan, most likely via 5-HT$_{1D/1F}$ receptors, pre-synaptically inhibits CGRP release from trigeminal nerve endings (113). The key findings that exogenous CGRP infusions could trigger delayed migraine-like headaches accompanied by a unilateral dilatation of the middle meningeal artery, and no dilation of the middle cerebral artery (114,115) suggested a peripheral role for CGRP and its related meningeal vasodilation in migraine headache, especially since CGRP, like substance P, is unlikely to pass readily into the brain, due to its large molecular weight. It should be noted, however, that in chronic migraine an elevated CGRP level was also detected in the CSF (116), pointing to the possibility that CGRP released also from cortical parenchymal cells (117) (or possibly from pial afferents) is also involved in migraine. In a recent preliminary study, peripheral administration of CGRP to mice overexpressing the CGRP receptor subunit RAMP1 in smooth muscle cells (including in vascular smooth muscle cells) promoted light aversion (a rodent behavior suggested to be linked to the photophobia phenomenon of migraine), pointing to the possibility that over-activation of dural arterial cells can promote migraine headache (118). Whether such migraine-related response is mediated via enhanced meningeal vasodilatation or augmented release of algesic mediators from meningeal vascular smooth muscle cells (VSMCs, see also below) remain to be determined. Taken together, the above-mentioned studies demonstrate a complex bidirectional cross-talk between cranial blood vessels and their trigeminal nerve endings in migraine pathogenesis.

Neurogenic activation of meningeal immune cells

Another major feature of neurogenic inflammation is the activation of immune cells (119). Of particular interest to migraine are meningeal MCs (106). Activated MCs are proinflammatory and release a host of pro-nociceptive mediators (120) that can lead to the activation and sensitization of meningeal afferents (66,67,121,122). Clinical studies reported elevated

plasma levels of the MC mediator's histamine, tryptase and TNF-alpha during migraine (61,123,124), supporting the involvement of MCs in migraine. A role for MCs in meningeal neurogenic inflammation was initially supported by the finding that stimulation of the trigeminal ganglion to produce dural PPE also promoted morphological changes in dural MCs suggestive of degranulation (52,125). The anatomical localization of dural MCs in close apposition to terminals of dural afferents that express substance P and CGRP provided indirect support for the ability of trigeminal axon reflex to activate intracranial dural MCs. The activation of MCs' NK1-R is thought to promote their degranulation by substance P (126). However, the presumed lack of involvement of NK-1 signaling in migraine headache suggests that if dural MCs are degranulated in response to meningeal axon reflex, and this process contributes to the headache of migraine (see also below), it is unlikely to involve SP receptor signaling. Release of CGRP from activated meningeal afferents may also promote MC degranulation in experimental animals, although with less potency than substance P (127). In vitro stimulation of rodent's meningeal MCs with CGRP induced 5-HT and histamine release (128,129). The MC degranulating effect of CGRP may be nonetheless rodent specific. Rodent MCs express the required components of the CGRP receptor system, calcitonin receptor-like receptor (CLR) and receptor activity-modifying protein 1 (RAMP1) (24,130,131). Human dural MCs, however, express only RAMP1 (24). Nonetheless, the possibility that CGRP signals via the calcitonin (CTR)/RAMP1 receptor complex (132) in these cells should not be ignored.

Meningeal release of PACAP may also promote MC degranulation (133,134). A recent clinical study demonstrated the expression of the PACAP receptor VPAC1R on human skin MCs (135). Whether PACAP can promote the degranulation of human dural MCs is currently unknown. Meningeal MCs have been shown to become activated after exposure to carbachol (47,136,137), suggesting that activation of meningeal parasympathetic efferents could promote meningeal neurogenic inflammation (138). While cranial parasympathetic activation has been implicated in trigeminal autonomic cephalalgias (139), and may accompany migraine attacks in some patients (140), its role in promoting meningeal nociception and migraine headache is less clear. Taken together, whether meningeal neurogenic inflammation, either mediated by release of sensory or autonomic transmitters, promotes a sufficient dural MC degranulation response that can lead to the activation and sensitization of meningeal afferents with ensuing headache remains to be determined.

Perivascular meningeal macrophages may play a role in meningeal nociception and potentially in headache. Macrophages can release a host of inflammatory and nociceptive mediators including prostaglandins, cytokines, chemokines, and high levels of NO that can act directly, or indirectly, on meningeal afferents to promote their activation and mechanical sensitization (68,69,97,99,121). The finding that systemic administration of the headache and migraine trigger nitroglycerin upregulates proinflammatory cytokines and the inducible (and pro-inflammatory) isoform of nitric oxide synthase (iNOS) in rat dural macrophages, as well as promoting dural inflammation (141), further suggests a role for these immune cells in headache. While functional interactions between sensory or autonomic transmitters, and meningeal macrophages are yet to be defined, in other tissues CGRP rather inhibits proinflammatory macrophage function (142–144). A role for resident DCs and circulating T cells in meningeal neurogenic inflammation remains unknown. While studies in other tissues suggest that CGRP downregulates the expression of the nociceptive cytokine TNF-alpha in DCs, activation of peptidergic nociceptive afferents can drive DC production of the proinflammatory and nociceptive cytokine IL-23 with subsequent tissue PPE (145,146). T cells have been shown to mediate neuropathic pain in rodent models (147), but a role for T cells in acute nociception, such as during episodic migraine attacks, is yet to be demonstrated.

How might meningeal neurogenic inflammation be triggered during migraine?

One critical unknown aspect of the neurogenic inflammation hypothesis of migraine is the identity of the endogenous processes that lead to the initial activation of meningeal afferents and the ensuing release of neuropeptides. One event that has been hypothesized to trigger meningeal neurogenic inflammation is cortical spreading depression (CSD), a wave of neural and glial depolarizations (followed by neuronal silencing) that is thought to underlie the aura phase of migraine (72). In rats and rabbits, induction of a single CSD event gives rise to a brief dilatation of pial arteries and increase in cerebral blood flow (148). The induction of CSD-evoked cortical hyperemia in the mouse (148) (and also in human subjects (149)), however, is less clear. The dilation of pial vessels in response to CSD has been shown to be mediated in part by CGRP receptor signaling (150–152), suggesting a short-lasting activation of peptidergic leptomeningeal afferents. The mechanisms underlying the pial afferent response to CSD are incompletely understood, but presumably involve the release of small nociceptive molecules, such as nitric oxide, potassium ions and ATP in the superficial cortical parenchyma during the passage of the CSD wave, their diffusion or bulk flow into the subarachnoid space and subsequent

action upon leptomeningeal afferent nerve endings (153) (Figure 1(a)). A key part of the meningeal neurogenic inflammation theory of migraine posits that leptomeningeal afferents have additional branches that terminate in the dura mater (Figure 1(a)), and that activation of leptomeningeal afferents can promote the release of proinflammatory peptides also in the dura mater with ensuing local sterile inflammatory responses (154). Anatomical support for this hypothesis comes from rodent studies demonstrating a sizable number of single trigeminal ganglion neurons that project to both the middle cerebral, and middle meningeal arteries, or other dural sites (17), and a small number of dural fibers that issue collateral branches to the pia at the frontal part of the brain (155). The finding of prolonged dural vasodilatation and PPE following a single CSD event that were dependent upon an intact trigeminal nerve and activation of NK1-R (154) provided further support for this theory. In that study, CSD evoked prolonged dilatation of the dural middle meningeal artery that was also dependent on parasympathetic outflow from sphenopalatine ganglion neurons, highlighting the additional contribution of trigemino-parasympathetic reflex to meningeal NI. More recently, an electrophysiological single unit recording in a rat model provided direct evidence for the acute activation of a subpopulation of dural afferents during the passage of the CSD wave (156,157). A direct link between CSD, meningeal neurogenic inflammation and the activation of dural or leptomeningeal afferents, remains to be established. CSD has been shown to promote meningeal MC degranulation (158) and conformational changes in meningeal macrophages, reminiscent of an inflammatory response (44). CSD also leads to arrested migration of meningeal DCs, suggesting inflammatory activation (44). It is unknown, however, whether these inflammatory changes occur in response to the activation of meningeal afferents (i.e. via the release of neuropeptides). Nonetheless, the finding that CSD (which likely contributes to the headache in migraine with aura) leads to meningeal inflammatory changes involving MCs and macrophages, similar to those provoked by other migraine triggers such as GTN and PACAP-38 (which trigger migraine without aura), suggest that meningeal inflammation (whether this involves the release of sensory neuropeptides or not) serves as a common mechanism of migraine headache onset.

Can meningeal neurogenic inflammation actually promote meningeal nociception?

Despite the indirect evidence for meningeal neurogenic inflammation in animal models of migraine, a critical question remains as to whether this event actually contributes to meningeal nociception and headache. Previous studies examined the effect of acute stimulation

of primary afferent neurons that innervate other tissues on the sensitivity of primary afferent nociceptive neurons, but have yielded conflicting data. For example, studies in monkeys (159) and rats (160) have shown that stimulation of cutaneous nociceptive afferents did not subsequently alter their ongoing activity, mechanosensitivity, or heat sensitivity. A study in rabbits, however, reported the development of heat sensitization of nociceptive afferents following antidromic afferent stimulation (161). A rat study that employed local capsaicin stimulation to evoke acute excitation of cutaneous afferents documented a delayed and prolonged increase in the afferents' ongoing activity and mechanosensitivity that were suggested to involve CGRP signaling (162). Finally, we have shown recently that brief meningeal application with potassium levels, similar to that expected during CSD, can lead to prolonged activation of dural afferents (163). The finding that in addition to their acute activation, CSD also gives rise to prolonged activation and enhanced mechanosensitivity of meningeal afferents (156,157,164) may point to neurogenic inflammation as a nociceptive event. Nevertheless, numerous finding suggests meningeal nociception in response to CSD is not directly mediated by neurogenic inflammation. For example, persistent meningeal afferent activation still occurs following excision of the parasympathetic sphenopalatine ganglion (156), whose activation was critical to the CSD-evoked meningeal neurogenic vasodilatation. In addition, persistent meningeal afferent activation following CSD can occur in the absence of acute NI-mediated dural MC degranulation, as was observed in craniotomized animals, a preparation in which the majority of dural MCs are already in a state of degranulation prior to the induction of CSD (165). As indicated above, studies in naive rats suggest that acute activation of meningeal CGRP receptors is not sufficient to activate or sensitize meningeal afferents (98). While there is some evidence in rats and humans for the expression of the CGRP receptors components CLR and RAMP1 in the cytoplasm of some thick myelinated dural axons (24), these receptors were also reported to be localized specifically to the axons' Schwann cells (130). It also remains unclear whether these CLR/RAMP1 expressing dural afferents play a role in meningeal nociception as they are not co-localized with CGRP (24). The possibility that CGRP receptors are preferentially expressed on non-nociceptive, myelinated dural fast A fibers, which comprise about one-third of the myelinated axons in the nerves supplying the dura, and exhibit the highest thresholds and the lowest firing rates, as well as the lowest incidence of mechanosensitivity (12,166) should be considered. Recent data further suggests that acute blockade of meningeal CGRP receptors, using systemic administration of olcegepant (BIBN4096), does not inhibit the prolonged activation

and mechano-sensitization of meningeal afferents, in the wake of CSD, despite diminishing the related cerebral vasodilatation (163). This data is congruent with the observations that CSD does not alter dural CGRP release (167) or leads to CGRP release into the external jugular vein (168). However, the finding that a prolonged sequestering of CGRP, using a monoclonal antibody approach, can inhibit the prolonged activation of slow conducting A-delta afferents following CSD (169) points to the possibility that the basal CGRP level somehow modulates the responsiveness of a subpopulation of meningeal afferents. Such effects are likely to be restricted to the dura mater, as antibodies, due to their large molecular size, are unlikely to gain access to the leptomeningeal space (i.e. CSF). Finally, the finding that acute CGRP receptor blockade can inhibit prolonged meningeal afferent activation in response to their local stimulation with high physiological levels of potassium (163) further suggests that the indirect, pro-nociceptive effect of CGRP, likely mediated through its signaling on non-neural cells and in tandem with other nociceptive factors, may play a role in mediating meningeal nociception. However, whether the anti-migraine prophylactic effects of monoclonal antibodies that target CGRP or its receptor are linked to acute inhibition of dural NI or other, unrelated peripheral effects of CGRP remains unknown.

(iii) Potential sources of meningeal nociception beyond neurogenic inflammation

Activation of meningeal MCs

Beyond neurogenic inflammation, are there other mechanisms that could lead to prolonged activation of meningeal afferents and the ensuing headache? As indicated above, release of MC mediators in the vicinity of meningeal afferents could potentially lead to their activation and sensitization. While not directly related to meningeal neurogenic inflammation, a causative role for MCs in migraine headache was already considered more than 50 years ago (170): In that study, injection of an MC degranulating agent, compound 48/80, into the cranial circulation gave rise to a headache resembling that of migraine. Levy et al. (165) have shown that degranulation of dural MCs, using compound 48/80 as in Sicuteri's study, promoted persistent activation of the majority of meningeal afferents, as well as of nociceptive neurons in the trigeminal nucleus caudalis. This work suggested that extensive dural MC degranulation could serve as a powerful peripheral pro-nociceptive stimulus capable of triggering the activation of the peripheral and central components of the migraine pain pathway. The finding that

activation of dural MCs was also associated with the development of cephalic tactile hypersensitivity (6) provided further indirect evidence for their role in meningeal nociception and migraine headache pain. The identity of non-neurogenic inflammation mechanisms that could lead to dural MC degranulation with ensuing enhanced meningeal nociception are unclear at present. Because there is no blood-dura barrier, it is conceivable that circulating factors, reaching the dural circulation, could lead to the degranulation of a sizable population of dural MCs. The finding that migraine is comorbid with MC-related conditions such as allergies and asthma (171,172) points to a potential link between MC-related events and migraine. The involvement of peripherally-acting MCs in migraine is also congruent with the recent finding that migraine is genetically and environmentally similar to peripheral inflammatory conditions that also involve MCs, such as irritable bowel syndrome and cystitis (173). Finally, it has been suggested that some migraine events may be triggered by various food ingredients acting upon MCs, a notion that gained support from studies showing potent migraine prophylactic action of the MC stabilizing agent cromoglycate in a subset of patients with food-related attacks (174,175). It remains unclear, however, what level of meningeal MC degranulation is actually nociceptive. A relatively low level of dural MC degranulation induced by systemic infusion of the migraine trigger nitroglycerin (141,176) was not sufficient to promote activation of meningeal afferents in a rodent model (99). However, in migraineurs, a higher density of meningeal MCs, potentially due to endocrine changes, such as fluctuation in female sex hormones (53) or increased propensity of these immune cells to become activated in response to a given trigger, could potentially result in a robust proinflammatory that promotes the activation of meningeal afferents and lead to headache.

Beyond vasodilatation: release of algesic mediators from meningeal vascular cells and fibroblasts, and meningeal nociception

Vascular endothelial cells (ECs) and VSMCs are potentially important sources of algesic mediators that can activate and sensitize meningeal afferents. The vascular endothelium has been suggested to promote peripheral enhancement in pain (i.e. hyperalgesia) through the release of endothelin-1 (177) and ATP (178). While a direct role for endothelin-1 in migraine has been questioned based on the lack of efficacy of endothelin receptor antagonism (179), its release from leptomeningeal vessels could potentially trigger CSD (180), and thus indirectly promote meningeal nociception. The genetic association between migraine, endothelin expression

and vascular mechanisms (181,182), as well as with the endothelin A receptor (183), also suggests a rather complex role for endothelin in migraine. Local release of ATP from the meningeal vasculature (but also from other meningeal cells) could promote acute activation of meningeal afferents (157), potentially via activation of afferent P2X2/3 and P2X3 purinergic receptors (184). Prolonged activation and/or sensitization of meningeal afferents mediated by these, and potentially other purinergic receptors localized to meningeal afferent nerve endings, remains, however, to be established. Indirect meningeal nociceptive effects of ATP, promoted by its action upon proinflammatory P2X7 receptors localized to resident meningeal immune cells, such as macrophages (185) may also be of importance to headache and migraine mechanisms. Release of NO and prostacyclin (PGI_2) from the vascular endothelium may lead to meningeal nociception (66,121). The release of COX-2-derived prostanoids, as well as high levels of NO and proinflammatory cytokines, such as IL-1β from VSMCs could activate and sensitize meningeal afferents (68). Finally, the finding that the migraine trigger nitroglycerin, which leads to delayed migraine attacks, evokes a delayed mechanical sensitization of meningeal afferents that depends upon inflammatory signaling (i.e. ERK phosphorylation) in meningeal arteries (99) further points to the role of non-vasodilating meningeal vascular mechanisms in migraine headache.

Recent work suggests that dural fibroblasts, which serve as key building blocks of this meningeal tissue, can release algesic mediators such as IL-6 that can act upon meningeal afferents and promote migraine-like behaviors in rodents (186,187) (Figure 1(b)).

The role of cortical mediators

The finding that CSD, an event that is primarily restricted to the cortex, can lead to the activation and mechanical sensitization of meningeal afferents points to the possibility that algesic factors, such as ATP, prostaglandins and NO that are released into the parenchymal interstitial fluid, gain access to the subarachnoid space, where they can act upon leptomeningeal afferents. The presence of the dura-arachnoid interface layer, however, is likely to impede the passage of these cortical mediators into the cranial dura mater and their nociceptive action on dural afferents. Are there any routes by which cortical-derived algesic factors could reach the dura and interact with its nociceptive afferents? CSF in the subarachnoid space enters the bloodstream in the dural venous sinuses, via arachnoid granulation. Afferents localized to the dural sinus walls, in particular to the sinus lumen (23), could potentially sense these mediators. However, this implies that these afferents also sample venous blood – an unlikely occurrence – since blood itself has been shown to exert a nociceptive effect (188). Alternatively, sensory nerve fiber terminals in the arachnoid granulations (189), which protrude through the dura, may become activated by CSF-derived nociceptive mediators.

(iv) Conclusions and future perspectives

While migraine is considered as a brain disorder, peripheral meningeal components including the sensory innervation of the cranial meninges and immune and vascular cells are likely to play a major role. A better understanding of the brain dysfunction processes and the nature of the cortical to meningeal signaling cascades that promote the activation and increased responsiveness of trigeminal nociceptive afferents is essential for the development of effective antimigraine prophylactic therapies. The finding that, genetically and environmentally, migraine is much closer to immune system diseases such as irritable bowel syndrome and cystitis/urethritis rather than to central nervous system specific conditions (173) further implies that migraine has a peripheral inflammatory origin that is not nervous system-specific.

The concept of neurogenic inflammation undoubtedly had a tremendous impact on migraine research and provided an important roadmap for the development of neuropeptide and receptor driven therapies for migraine. While meningeal neurogenic inflammation continues to be regarded as a causal factor in migraine headache, direct evidence for the occurrence of neurogenic inflammation during migraine and its role in meningeal nociception is limited at best. Future studies may provide better direct evidence for the presence of the various features of meningeal neurogenic inflammation or lack thereof during a migraine attack and, most importantly, whether they constitute active players in driving migraine pain rather than simply epiphenomena.

The advent of monoclonal antibodies against CGRP or its receptor, which are too large in molecular size to cross the blood brain barrier, may serve as a pharmacological tool to decipher the role of peripheral inflammatory mechanisms that involve CGRP-ergic signaling. While the success of treatment with antibodies that curtail CGRP signaling in preventing migraine attacks points to a peripheral, likely meningeal origin of migraine, the failure of such treatments in a subpopulation of patients (190) may point to a larger role for trigeminal leptomeningeal afferents, which cannot be targeted with systemically-administered antibodies, in mediating migraine pain, or to CGRP-independent processes. Thus, the role of other substances, including neuropeptides such as PACAP but certainly also immuno-active substances and numerous meningeal

receptor systems (Figure 1(b)), should be explored and selective pharmacological tools developed. The availability of such tools will help to further unravel the pathophysiology of migraine and thus to ultimately provide a more effective and safe treatment for migraine patients.

Article highlights

- The cranial meninges and their related large blood vessels play a key role in the origin of migraine headaches.
- We provide a critical summary of current understanding of the role that the cranial meninges, their associated vasculature, and immune cells play in meningeal nociception and the ensuing migraine headache.

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