Neurogenic inflammation in the pathophysiology and treatment of migraine

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Article abstract—The trigeminal nerve transmits headache pain from blood vessels of the pia mater and dura mater. Triggers for this pain are not well understood, but probably are multiple and largely chemical and develop within the brain parenchyma, the blood vessel wall, and the blood itself. These unknown triggers stimulate the trigeminovascular axons, causing pain and releasing vasoactive neuropeptides from perivascular axons. Released neuropeptides activate endothelial cells, mast cells, and platelets to then increase extracellular levels of amines, arachidonate metabolites, peptides, and ions. Hyperalgesia and prolongation of pain develop as a consequence, mediated by products from activated cells and injured tissue. Within postsynaptic brain stem neurons of the trigeminal nucleus caudalis, trigeminovascular activation stimulates the expression of an early immediate response gene c-fos. Both neurogenic inflammation and c-fos expression are blocked by sumatriptan and ergot alkaloids via prejunctional mechanisms involving putative 5-HT receptors closely related to the 5-HT_{1D} subtype on trigeminovascular fibers. The mechanisms of action of sumatriptan and ergot alkaloids decribed herein are unrelated to the nature of the migraine trigger or to the contractile state of vascular smooth muscle.

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Neurogenic inflammation within cephalic tissue, involving vasodilation and plasma protein extravasation, has been proposed as a mechanism in headache pathogenesis.[1-4] This article is a review of the roles of neurogenic inflammation and prejunctional mechanisms in headache pathogenesis and the treatment of vascular headache.

Pathophysiology. Neurogenic inflammation is mediated by the release of the vasoactive neuropeptides substance P,[5] neurokinin A, and calcitonin gene-related peptide (CGRP) from sensory fibers that innervate blood vessels.[6,7] Both the endothelium-dependent vasodilation and the enhanced permeability induced by the tachykinins are mediated by receptors located on the vascular endothelium; dilation produced by CGRP is mediated by receptors on vascular smooth muscle.[8]

Neurogenic plasma protein extravasation. During electrical trigeminal ganglia stimulation or after IV administration of capsaicin, neurogenic plasma protein extravasation develops within the dura mater.[9] This tissue, an important source of headache pain,[10] provides a thick covering for the brain and contains blood vessels with fenestrated capillary endothelia.[11] The dura mater and its attendant blood vessels are innervated by neuropeptide-containing trigeminal and upper cervical sensory nerve fibers;[12,13] perivascular sensory axons, located within the adventitial layer, are unmyelinated and small.

When plasma proteins, such as iodinated albumin or horseradish peroxidase, are administered, leakage from vessels into surrounding tissue can be demonstrated after trigeminal nerve stimulation. This extravasation is markedly attenuated or absent in animals whose perivascular afferent fibers have been destroyed by capsaicin treatment during the neonatal period. In comparison, the systemic administration of substance P or neurokinin A produces the same amount of leakage from dural vessels as do vehicle-treated, electrically stimulated controls. Plasma proteins do not extravasate from the pial circulation under the same conditions, probably because of the blood-brain barrier.

Trigeminal ganglia and electrical stimulation. Recent light and electron microscopy studies have shown that following electrical trigeminal ganglion stimulation, striking changes occur in the appearance of endothelial and mast cells as well as in the platelets surrounding or within postcapillary venules.[14] Platelet aggregates appeared within the lumen on the stimulated side only shortly after stimulation; some were found adhering to the endothelium. Endothelial cells later developed numerous clear cytoplasmic vesicles and vacuoles; the endothelium hypertrophied and microvilli formed. Mast cells also showed signs of secretion

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and degranulation as seen in the loss of normal functorial properties, ultrastructural disintegration of plasma membrane, and loss of electron-dense material within cytoplasmic granules. Following a 5-minute stimulation period, frank degranulation became evident 20 minutes later. All of these changes depended on the presence of small, unmyelinated fibers and were not noted after ganglionic stimulation in animals treated with capsaicin as neonates.

Sensitization and hyperalgesia. In migraine, noxious triggers, which remain to be identified, initiate a complex cascade of events within the meninges that cause both pain through depolarization of primary afferents and long-lasting enhancement of nociceptor activity that leads to sensitization and hyperalgesia.[3,15,16] Neither event, of course, is unique to the meninges or to migraine, and both occur within the skin, joints, and eye, as well as in the urinary and respiratory tracts.[17-19] Important chemical mediators that modulate sensory transduction in affected tissue include potassium, serotonin (5-HT), bradykinin, histamine, prostaglandins, leukotrienes, substance P, and CGRP.[20] In injured tissue—or tissue threatened with injury—the extracellular concentrations of these chemicals increase by release from local cells (potassium, histamine, 5-HT), local synthesis (prostaglandins, leukotrienes, bradykinin), or release from sensory axons (neuropeptides). Hyperalgesia and pain occur as a consequence of the initial trigger and the cellular response to neurogenic inflammation.

Blocking neurogenic plasma protein extravasation. Pharmacologic observations that 5-HT_1 agonists with some selectivity for the D-type receptor block inflammation selectively within the dura mater, support the belief that neurogenic inflammation is especially relevant to vascular headache.[15,21] Vasoconstriction mediated by 5-HT_{1D} receptors on vascular smooth muscle plays a minor role, if any.

Response to sumatriptan and related compounds. Studies have shown that neurogenic plasma protein extravasation from blood vessels in the dura mater can be significantly reduced by drugs such as sumatriptan, 5-carboxamidotryptamine (5-CT), dihydroergotamine (DHE), ergotamine tartrate, and methysergide. The dosage of sumatriptan required to block extravasation is similar to that required to treat acute migraine in humans.[15,22]

The response to these agents has been most consistent with an effect mediated by 5-HT_{1D} receptors or analogous 5-HT_{1B} receptors in rats. Some notable exceptions were found, however. Pretreatment with the 5-HT_1 antagonist metergoline only partially reversed the response to sumatriptan, and methiothepine did not reverse the response at all. The effect of 5-CT was not blocked by either antagonist, and serotonin itself appears inactive in this model. Pretreatment with 5-HT antagonists (pizotifen, ketanserin) or 5-HT_3 antagonists (MDL 7222, ICS 205-930) did not affect leakage, which is consistent with a 5-HT_1 receptor-mediated response.

However, the rank order of effective dosages, or threshold concentrations of 5-HT_{1D} selective compounds, does not correlate with affinities for the same drugs at the 5-HT_{1D} receptor binding site determined by ligand binding in vitro. Hence, emerging pharmacology suggests that a receptor subtype other than 5-HT_{1D} may mediate the antineurogenic inflammatory effects of sumatriptan and DHE. This receptor is present only in intracranial tissue innervated by the trigeminal nerve; the equivalent or higher concentrations of sumatriptan or ergot alkaloid do not block plasma protein extravasation in extracranial cephalic tissue.

Prejunctional mechanisms and inhibited neuropeptide release. Several lines of evidence support the conclusion that 5-HT heteroreceptors are located on neuropeptide-containing unmyelinated C fibers. Prejunctional mechanisms and inhibited neuropeptide release mediate drug blockade of plasma leakage within the dura mater. First, sumatriptan and DHE are inactive when tested against concentrations of substance P or neurokinin A, which cause plasma leakage. Later experiments have found sumatriptan, also, to be inactive against leakage caused by α-methyl 5-HT, a 5-HT_2 agonist that stimulated endothelial cell leakage directly. Extravasation caused by α-methyl 5-HT was blocked by pretreatment with the 5-HT_2 antagonist pimozide. Second, DHE and, to a lesser extent, sumatriptan attenuated the increase in immunoreactive CGRP within sagittal sinus blood (venous effluent) during electrical stimulation of the trigeminal ganglion.[23] This presumably reflects the inhibition of neuropeptide release. Third, sumatriptan and DHE pretreatment block platelet aggregation, endothelial vesicle formation within postcapillary venules, mast cell secretion, and degranulation in the dura mater.[24]

These findings are difficult to reconcile with a mechanism based on activation of postjunctional receptors on vascular smooth muscle, endothelial cells, or sympathetic fibers. Sympathetic fibers were in fact ruled out as a possible mediator by direct experiments showing that sumatriptan inhibits neurogenic plasma extravasation in sympathectomized animals.

Recently, in situ hybridization studies have demonstrated that the 5-$HT_{1D/B}$ gene is expresed within trigeminal ganglia.[25] Moreover, the expressed gene has been amplified using the polymerase chain reaction and oligonucleotide probes complementary to the cDNA prepared from the 5-HT_{1D} receptor message (unpublished data).

Role of 5-HT_1 receptors. Studies involving sumatriptan and endopeptidase 24.11 (enkephalinase), an enzyme that degrades substance P and CGRP, suggest a coupling between the 5-HT receptor and

the blockade of neuropeptide release. When administered after stimulation, the two agents were found to exhibit nearly identical time-dependent effects. They blocked plasma extravasation, even when administered 60 minutes after the 5-minute stimulation period. After terminating the stimulus in our model, neuropeptide release from sensory fibers appeared to continue for some time. Blockade of neuropeptide release from sensory fibers was consistent with the role of 5-HT_{1D} receptors as inhibitors of presynaptic neurotransmitter release within brain and perivascular sympathetic nerve fibers.[24-26] More recent data indicate that α_2, H_3, μ-opioid, and somatostatin receptors may also be located on trigeminovascular fibers and may block neurogenic plasma extravasation.[27]

C-fos expression. C-fos protein, a nuclear phosphoprotein that regulates the transcription rate of target genes, may play a role in the long-term alteration of cellular function.[28-31] This phosphoprotein can be induced in neurons in the dorsal horn of the spinal cord by noxious and non-noxious stimuli.[32-34] Noxious stimuli increase the number of cells expressing c-fos protein within Rexed's laminae (I and II_O), where unmyelinated C fibers terminate and spinothalamic projecting neurons predominate.[34-36]

Continuous or prolonged stimulation evokes the most robust response, and the number of cells containing c-fos protein correlates with stimulus intensity. In the same way, the number of positive cells decreases after analgesic administration. For example, the number of positive cells in the dorsal horn has been correlated with the nociceptive behavioral response observed in rats after formalin has been injected into the hind paw. In the formalin experiments, the number of expressing cells decreased after morphine administration in a dose-dependent, naloxone-sensitive manner.[35,36] C-fos expression provides both spatial and temporal markers of neuronal activation following sensory stimulation.

The trigeminal nucleus caudalis. We have recently studied c-fos expression within the trigeminal nucleus caudalis after instilling autologous blood into the subarachnoid space of rats.[37] The trigeminal nucleus caudalis receives the major synaptic input from the trigeminal nerve and contains neurons that discharge when the meninges are stimulated.[38]

When placed in the subarachnoid space, blood is noxious, as evidenced by the development of severe headache, nausea and vomiting, photophobia, and vasoconstriction in humans. In our model, the injection of blood increased the number of cells expressing the c-fos protein within laminae I and II_O. The number of expressing cells corresponded to the amount of blood injected, and the number of responding cells was maximal 2 hours after injection. After surgical or chemical denervation, the number of expressing cells was reduced by 50% or more. In our model, inhibition of c-fos expression was probably also related to the development of analgesia because pretreatment with morphine decreased the number of expressing cells.

Pretreatment with 5-HT_1 agonists with some selectivity for the B- and D-subtype receptors (and morphine) reduced the number of c-fos–positive cells caused by instilling blood. CP-93 129,[39,40] a selective 5-HT_{1B} agonist, sumatriptan, or dihydroergotamine decreased the number of positive cells significantly in laminae I and II_O, compared with those in vehicle-treated animals.[41] Sumatriptan did not block c-fos expression in the trigeminal nucleus caudalis following formalin application to the nasal mucosa. This means that sumatriptan is fundamentally different from such analgesics as morphine.

Inhibiting c-fos expression. Drug-induced blockade of c-fos expression may well be mediated by receptors on primary afferent (trigeminovascular) fibers. Consistent with this conclusion, we found that chemical or surgical denervation, as well as drugs such as CP-93 129, sumatriptan, and DHE caused a similar pattern of c-fos inhibition within the brain stem. This was seen in the reduced number of expressing cells in the caudalis but not in the nucleus of the solitary tract or area postrema.[40,41] Administering CP-93 129 did not decrease the number of expressing cells below the 50% inhibition in chemically denervated animals, suggesting the importance of extracerebral sites to drug activity.

If the inhibition of c-fos expression within laminae I and II_O correlates with the amelioration of pain, we can infer that constricted blood vessels are not required to alleviate pain by sumatriptan and DHE and, furthermore, that dilation may not be the pain stimulus, or trigger, in vascular headaches. Blood vessel constriction, which occurs after blood enters the subarachnoid space, provides further evidence that 5-HT_{1D}-receptor–mediated vasoconstriction may not be necessary for therapeutic effect.

We recently demonstrated that recurrent spreading depression induces c-fos expression within lamina I, II_O trigeminal nucleus caudalis, as does the intracisternal administration of carrageenan, a proinflammatory agent.[42] C-*fos* expression following spreading depression (SD) was also blocked by prior sectioning of trigeminal meningeal afferents. Sumatriptan did not affect the ability of the brain tissue to initiate and/or propagate SD.[42] Quite remarkably, sumatriptan suppressed c-fos expression in both instances, raising the possibility that 5-HT_{1D} agonists may alleviate pain associated with a wide variety of conditions, including bacterial and viral meningitis and head injury.

Based on the results of these experiments, we suggest that under certain circumstances (eg, migraine), the cortical mantle releases nociceptive molecules and generates the pain signal of migraine. The released molecules collect in the

xtracellular and perivascular space and within nervated draining veins leading to discharge and ctivation of trigeminovascular fibers. The chalnge for the decade ahead is to identify those neurophysiological and metabolic brain events which nticipate and provoke the pain of migrane.

Conclusion. Our data support the formulation hat the actions of sumatriptan and DHE are conected to at least two important events that may liminish pain and sensitization in patients with ascular headache. Sumatriptan and DHE block eural transmission within trigeminovascular bers and, by so doing, gene expression within ostsynaptic brain stem neurons. At the same time, hey block the neuroinflammatory response follow-ng afferent fiber stimulation. The therapeutic pplications of these compounds to other conditions ssociated with meningeal irritation merit further onsideration.

The occurance of c-fos within ipsilateral trigemi-al nucleus caudalis after recurrent spreading epression suggests that the brain can indeed pro-ide a potential source for the pain signal of mi-graine.

For more detailed descriptions of the work dis-ussed in this article, we encourage examination of he original research published in the past 10 ears.[1-3,12-16,21-24,37,38,40,41,43]

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