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Putative role of 5-HT_{2B} receptors in migraine pathophysiology

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Abstract

Objective: In this review we attempt to characterize the acute and chronic role of 5-HT_{2B} receptors with regard to meningeal nociception in animal experiments and clinical data targeting migraine therapy.

Background: Migraine is a common disabling neurovascular primary headache disease, the pathomechanism of which is still unclear. Serotonin (5-HT) and its receptors might play an important role in some aspects of migraine pathogenesis. The ability of the unselective 5-HT_{2B} receptor agonist m-chlorophenylpiperazine to induce migraine attacks in migraine sufferers, the high affinity of prophylactic antimigraine drugs to this receptor and its expression in migraine-relevant structures like the dura mater argue for a role of 5-HT_{2B} receptors in the pathogenesis of migraine attacks.

Methods: For this review, the relevant databases such as PubMed, MEDLINE®, Cochrane Library and EMBASE, respectively, were searched to December 2015 using the keywords "migraine, 5-HT_2, trigeminal, neurogenic inflammation, nitric oxide, nitroxyl, vasodilatation, plasma protein extravasation" and combinations thereof.

Conclusion: Our literature review suggests an important role of 5-HT_{2B} receptor activation in meningeal nociception and the generation of migraine pain.

Keywords

5-HT_{2B} receptor, vasodilatation, NO dependent, CGRP, plasma protein extravasation

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Introduction

Serotonin (5-HT) and its receptors play an important role in migraine research. The concentration of the 5-HT metabolite, 5-hydroxyindole acetic acid (5-HIAA), has been found increased in urinary excretion during migraine attacks and could thus serve as a biomarker of migraine (1). 5-HT is released from platelets by compounds such as fenfluramine, while reserpine or 5-HT reuptake inhibitors (zimeldine and femoxetine) may increase the frequency of migraine attacks (2,3). During migraine attacks the 5-HT concentration is decreased in platelets and subsequently increased in the blood plasma (4–6). Brewerton et al. reported that administration of m-chlorphenylpiperazine (mCPP), a metabolite of the antidepressant tradozone and nefazodone, with different affinities to 5-$HT_{2B/2C}$ receptors and other serotonin, adrenergic, dopamine and muscarine cholinergic receptors, leads to delayed "typical migraine headache" in migraineurs and unspecific headaches in healthy individuals (7). Experimental data show that particularly 5-HT_{2B} receptors play a role in functions that may be associated with the pathophysiology of headaches. Acute activation of 5-HT_{2B} receptors by mCPP led to nitric oxide (NO)-dependent plasma protein extravasation (PPE) in the dura mater of guinea pigs (8,9) and activation of neurons in the trigeminal nucleus caudalis (TNC) of rats (10). Therefore it has been assumed that meningeal 5-HT_{2B} receptors are involved in early steps of migraine pathogenesis (11). Clinical evidence for a role of 5-HT_2-receptors in migraine is provided by the fact that various prophylactic agents (methysergide or pizotifen) are 5-HT_2 receptors antagonists.

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Structure and localization of 5-HT_{2B} receptors

The 5-HT_{2B} receptor was first described by Vane in the rat gastric fundus, where it is responsible for the contraction of the longitudinal muscle cell layer (12). In 1992, 5-HT_{2B} receptors were first cloned from rats and mice (13,14) followed by cloning of the human 5-HT_{2B} receptor (15,16). The receptor protein consists of 481 amino acids (16) and has a 45% homology with 5-HT_{2A} and a 42% homology with the 5-HT_{2C} receptor protein (17). The occurrence of the 5-HT_{2B} receptor is ubiquitous. It occurs with a particularly high rate in liver and kidney, while a low expression rate was detected in pancreas and spleen (18). Likewise it is expressed in lung arterial endothelial cells, where it is responsible for the development of pulmonary hypertension (19). In the rat brain 5-HT_{2B} receptors are slightly expressed in neurons located in the cerebellum, the posterior hypothalamus, the lateral septum and the medial amygdala but these data, which are derived from immunohistochemical staining (IHS) (20), are considered controversial. Other studies using IHS and molecular biological methods showed expression in motoneurons (21), in rat spinal cord and in dorsal root ganglion (DRG) (22–24). The subcellular localization of the receptor in blood vessels is not evident from the literature; however, it has been suggested that it is localized on the luminal side of vascular endothelial cells (11).

An important function in the context of the 5-HT_{2B} receptor is the activation of nitric monoxide synthase (NOS), which promotes cleavage of the guanidino group from the amino acid arginine and other intermediate steps of NO synthesis (25). According to experiments in cell cultures, NOS seems to be coupled to the 5-HT_{2B} receptor through a PDZ-domain of the c-terminus (26). The link between the receptor and NOS in vivo is still unclear, and direct measurements in vivo are difficult because of the low number of 5-HT_{2B}-positive cells (27).

Location of the receptor in structures relevant for migraine

Schmuck et al. succeeded in preparing RNA transcripts from migraine-relevant structures such as the dura mater (11). The receptor in the dura mater could be detected in endothelial cells and it was also found weakly expressed in smooth muscle cells. Lin et al. detected messenger RNA (mRNA) of the 5-HT_{2B} receptor by in situ hybridization in trigeminal ganglion (TG) neurons of mice. A specific function of the receptor in the TG is not yet known but it can be assumed to be transported along peripheral and central processes of trigeminal afferents (23).

The acute activation of 5-HT_{2B} receptors leads to neurogenic inflammation

Components of meningeal neurogenic inflammation, particularly vasodilatation and plasma protein extravasation (PPE) induced by the release of neuropeptides from primary sensory nerve terminals, have frequently been observed in animal experiments as parameters of meningeal nociception (28). Although these changes are not directly responsible for the headaches, they may reflect peripheral pathophysiological events associated with migraine pain. In a study on guinea pigs, acute intravenous administration of 1 μg/kg mCPP induced PPE in dura mater (determined with intravenous Evans blue application), which could be inhibited by selective 5-HT_{2B} receptor antagonists (LY53857, LY215840; both 10 μg/kg), whereas selective inhibition of the 5-HT_{2C}-receptor (using the antagonist LY310898) had no influence on the PPE (9). It should be mentioned that, in addition to different 5-HT receptors, mCPP interacts also with other neurotransmitter receptors such as adrenergic, dopamine and muscarine cholinergic receptors (for overview see Hamik and Peroutka (29) and Martin and Martin (10)).

Schmitz et al. showed similar results with the same preparation using a new substance (BF-1) for blocking PPE. BF-1 has very high affinity for the 5-HT_{2B} receptor ($pki = 8.63 \pm 0.24$, SD) with only minor affinity to the 5-HT_{2C} receptor ($pki = 7.64$) and the histamine 1 (H1) receptor ($pki = 7.81$). According to the authors these low affinities for the 5-HT_{2C} receptor and the H1 receptor preclude unspecific effects, which appeared to be more prominent with older substances, e.g. pimethixene (1-methyl-4-(9H-thioxanthen-9-ylidene) piperidine ($pki = 10.14$ for H1 receptors and 8.42 for 5-HT_{2C} receptors) (8). The inhibition of 5-HT_{2B} receptors with the compound RS-127445, a selective, high-affinity ($pki = 9.5$), orally bioavailable 5-HT_{2B} receptor antagonist (30), was shown to inhibit the mCPP-induced PPE as well as c-fos expression in the rat trigeminal nucleus caudalis (TNC) evoked by capsaicin (31). Schmuck et al. has also shown that the activation of 5-HT_{2B} receptors by the unspecific agonist dihydroergotamine (DHE) (10^{-9} to 10^{-6} mol/l) causes vasodilatation of isolated cerebral arteries in the pig. The DHE-induced relaxation could be blocked by pizotifen (10^{-6} mol/l), a prophylactic antimigraine drug (11). As a limitation for this association it should be mentioned that pizotifen also has an antihistamine (H_1-receptor blocking) and weak anticholinergic action, which could be involved in the prophylactic effect (32–34). However, taking together the above results, it seems most likely that it is the 5-HT_{2B} receptor that is involved in formation of the major components of neurogenic

inflammation, vasodilatation and PPE evoked by mCPP. The neurogenic inflammation is induced by the release of substance P, which is mainly responsible for the PPE, and calcitonin gene-related peptide (CGRP), which mediates vasodilatation. Substance P binds to the neurokinin receptor 1 (NK-1), which is localized on endothelial cells, and thereby induces the formation of gaps in the endothelium (ca. 0.5–1.5 μm) allowing plasma proteins to diffuse into the perivascular tissue, e.g. demonstrated in the rat trachea (35). PPE has been studied extensively in different tissues including the dura mater (36). In an electron microscopic study of dural blood vessels, Dimitriadou found that the electrical-induced PPE is not caused by an increased number of endothelial gaps but rather by an increased number of pinocytotic vesicles (37). Hunfeld et al. observed an mCPP-induced PPE that was associated with increased transcellular transport of dissolved substances (e.g. horseradish peroxidase, HRP) in endothelial cells with no damage of fenestrae or tight junction integrity in mice dura mater (38). Other studies showed both transcellular and paracellular transport through the endothelial junction and clefts (39). However, the precise mechanism of PPE in the dura mater and whether it is important for the emergence of migraine remains questionable, because blockade of the PPE via NK-1 receptor antagonists turned out to be ineffective in migraine treatment (40,41).

The extent of vasodilatation is routinely measured by parameters such as an increased vessel diameter or increased blood flow (42,43). Furthermore, substance P and CGRP released from meningeal afferents may be involved in the activation and degranulation of dural mast cells, which may express receptors for both neuropeptides (44). Through the release of pro-inflammatory substances such as histamine and cytokines from mast cells, the neurogenic inflammation of the dura mater may be aggravated (45).

Massive mast cell degranulation (by compound 48/80) has been shown to activate primary meningeal afferents and second-order neurons in the TNC (46). However, it is questionable if the neuropeptides as weak mast cell activators can induce such an effect, and it is unclear which substances released from mast cells are capable of activating primary afferents. One candidate, histamine, activated only a very small proportion of meningeal afferents in an in vitro study (47). In experimental and clinical studies administration of histamine caused dilatation of cerebral arteries and induced typical migraine headache, which could be inhibited by blockade of H1 receptors (48,49). However, inhibition of H1 receptors blocked only the "histamine-induced headache" but was ineffective in prophylactic migraine treatment (4,50). This may support the view that 5-HT, which is another substance concentrated in mast cell granules, may be more important as a "natural player" in the generation of migraine attacks (11).

In summary, the observations that 5-HT_{2B} receptor agonists like mCPP induce plasma extravasation as the main element of neurogenic inflammation in the dura mater and increase c-fos expression in the TNC argue for a role of 5-HT_{2B} receptors in events associated with the generation of trigeminal activity and possibly migraine pain (9,10).

5-HT_{2B} receptor-induced neurogenic inflammation depends on NO synthesis

Kalkman postulated in 1994 that activation of "5-HT_{2C}-like" receptors can provoke migraine attacks because the activation of these receptors leads to NO release, which may be a key event in triggering migraine (2). Fozard already postulated this hypothesis as early as 1975 but was not sure which of the 5-HT receptors can induce the release of NO. It should be noted also that the activation of other receptors, e.g. H1 receptors, leads to NO release (51). Recent studies focused on elucidating the role of NO and its specific actions. The peripheral activation of 5-HT_{2B} receptor leads to formation of NO (11). In a study with acute 5-HT_{2B} receptor activation, administration of the NO synthase inhibitor L-NAME caused inhibition of dural PPE in the guinea pig (9). NO is known to activate the soluble guanylate cyclase (sGC) in smooth vascular muscle cells resulting in an increase in cyclic guanosyl monophosphate (cGMP) (52). By this way NO causes relaxation of smooth muscle resulting in vasodilatation of arterial blood vessels and increased blood flow (53). Recent experiments indicate alternative, possibly even more potent, vasodilatory mechanisms. In the presence of hydrogen sulfide (H_2S), a product of the condensation of cysteine with homocysteine to cystathionine, catalyzed by enzymes like cystathionine β-synthase (CBS) (54), NO can be metabolized to nitroxyl (HNO). HNO is a potent agonist of TRPA1 receptor channels, which upon their opening induce an influx of Ca^{2+} (55,56). In TRPA1 expressing peptidergic sensory neurons, this mechanism is mainly responsible for the release of neuropeptides like CGRP (56). CGRP is regarded as the most potent vasodilator of intracranial arteries (57), hence this mechanism caused strong vasodilatation and blood flow increase in the rat cranial dura mater (58).

In the TG, NO may be involved in a neuron-glia crosstalk. In TG cell cultures, NO donors caused CGRP promoter activity and secretion (59). Conversely, CGRP treatment increased glial iNOS expression and NO release from TG satellite cells (60). This could lead to a vicious circle if CGRP-releasing neurons are surrounded by NO-producing satellite cells; however, it is yet uncertain if this crosstalk takes place in the intact TG in vivo. Communication between CGRP-releasing and NO-

producing neurons in the TG also seems possible. Glycerol trinitrate (nitroglycerin, GTN), a substance directly activating the sGC (61), which is long known to induce vascular headaches such as delayed migraine attacks in migraineurs (62), caused upregulation of CGRP, CGRP receptor components and neuronal NO synthase (nNOS) in rat TG neurons (63,64).

Similar signaling mechanisms may take place in the superficial laminae of the TNC, where the central terminals of nociceptive trigeminal afferents synapse onto second-order neurons. CGRP released from such terminals acts as a neuromodulator facilitating synaptic transmission (65). Multiple neurons in all spinal layers seem to express nNOS (66) and potentially produce NO as a "retrograde transmitter" that facilitates neurotransmitter release from presynaptic terminals (67).

Taken together, several lines of evidence are suggestive of 5-HT_{2B} receptor-dependent NO production, which may be involved in the pathogenesis of nociceptive processes of migraine pain. These changes are likely based on gene expression of components like nNOS and CGRP receptor proteins and may therefore be very slow. Hence a therapeutic approach making use of these mechanisms can be expected to be successful rather in a prophylactic manner than in an acute intervention. To test hypotheses of such long-term drug actions beyond cell cultures, there is a need for new models that represent the complexity of expression changes in the TG and the respective effects in peripheral and central trigeminovascular tissues.

The chronic inhibition of 5-HT_{2B} receptors may be prophylactic in migraine

Antagonists of the 5-HT_{2B} receptor, methysergide, eyproheptadine and pizotifen, are effective in the prophylactic treatment of migraine (34,68), whereas ketanserin, which lacks affinity for 5-HT_{2B} or 5-HT_{2C} receptors, has no such prophylactic effect. The above substances act not only on 5-HT_{2B} but also on 5-HT_{2C} receptors, leaving the question open which of the receptor subtypes is responsible for the prophylactic effect.

Prophylactic migraine drugs, e.g. methysergide, can significantly reduce migraine frequency but this compound must be taken for a longer period (three to four weeks) to achieve a therapeutic effect (69). Accordingly, in an animal model in rats, Saito et al. found that chronic but not acute treatment with methysergide inhibited PPE in the dura mater (70). The authors speculated that the difference between acute and chronic administration is the accumulation of the active metabolite, methylergotamine, as the actually effective drug.

Schaerlinger et al. found in an in vitro system (transfected human 5-$HT_{2B/2C}$ in $LMTK^-$ cells) that the long-term use of dihydroergotamine (DHE) leads to desensitization of 5-HT_{2B} receptors but not 5-HT_{2C} receptors (71). In transfected Chinese Hamster Ovary (CHO)-K1 cells, among all 5-HT_2 receptor subtypes, the 5-HT_{2B} receptors underwent the highest degree of desensitization to chronic 5-HT exposure (72). Moskowitz postulated in 1992 that methysergide and its metabolite methylergotamine inhibit the release of CGRP from perivascular sensory nerves (73). Though vasodilatation is no longer regarded as the key mechanism in migraine pain generation, limiting CGRP release, as it is achieved by 5-$HT_{1B/D}$ agonists (triptans), is closely associated with an antimigraine effect. In a recent study for the first time mice could be made sensitive to 5-HT_{2B} receptor agonists by chronic hypoxia, a model that may demonstrate the potential importance of this receptor in chronic migraine processes. The authors postulated that four weeks' hypoxia induced a shift from a "non-migraineur" to a "migraineur-like" state, which was shown to depend on chronic activation of 5-HT_{2B} receptors. The mechanism for this shift could not be explained by an increased expression of 5-HT_{2B} receptors or other proteins involved. Learning more about the underlying cellular mechanisms of this phenomenon is demanding and could explain why the exclusively chronic treatment with 5-HT_{2B} receptor antagonists leads to a reduction of migraine attacks (38).

In conclusion, in search of a preventive antimigraine drug based on 5-HT_2 receptor inhibition, it is desirable to find a highly selective 5-HT_{2B} receptor antagonist that readily binds to the receptor and does not depend on an effective metabolite generated in the body. Second, such a receptor antagonist should have no or minimal activity at the 5-HT_{2C} receptor to reduce central side effects, e.g. psychedelic and hallucinogenic actions (74). Clinical studies are awaited to prove this concept.

Article highlights

- 5-HT_{2B} receptors are expressed at a low rate in a variety of tissues including blood vessels of the cranial dura and in the trigeminal ganglion.
- Activation of 5-HT_{2B} receptors causing neurogenic inflammation via nitric oxide synthesis indicates their possible involvement in migraine pathophysiology.
- Inhibition of 5-HT_{2B} receptors may contribute to a prophylactic effect in migraine.

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