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Serotonergic mechanisms of trigeminal meningeal nociception: Implications for migraine pain

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ABSTRACT

Serotonergic mechanisms play a central role in migraine pathology. However, the region-specific effects of serotonin (5-HT) mediated via multiple types of receptors in the nociceptive system are poorly understood. Using extracellular and patch-clamp recordings, we studied the action of 5-HT on the excitability of peripheral and central terminals of trigeminal afferents. 5-HT evoked long-lasting TTX-sensitive firing in the peripheral terminals of meningeal afferents, the origin site of migraine pain. Cluster analysis revealed that in majority of nociceptive fibers 5-HT induced either transient or persistent spiking activity with prevailing delta and theta rhythms. The 5-HT3-receptor antagonist MDL-72222 or 5-HT1B/D-receptor antagonist GR127935 largely reduced, but their combination completely prevented the excitatory pro-nociceptive action of 5-HT. The 5-HT3 agonist mCPBG activated spikes in MDL-72222-dependent manner but the 5HT-1 receptor agonist sumatriptan did not affect the nociceptive firing. 5-HT also triggered peripheral CGRP release in meninges, which was blocked by MDL-72222.5-HT evoked fast membrane currents and Ca^{2+} transients in a fraction of trigeminal neurons. Immunohistochemistry showed expression of 5-HT3A receptors in fibers innervating meninges. Endogenous release of 5-HT from degranulated mast cells increased nociceptive firing. Low pH but not histamine strongly activated firing. 5-HT reduced monosynaptic inputs from trigeminal Aδ- and C-afferents to the upper cervical lamina I neurons and this effect was blocked by MDL-72222. Consistent with central inhibitory effect, 5-HT reduced CGRP release in the brainstem slices. In conclusion, 5-HT evokes powerful pro-nociceptive peripheral and anti-nociceptive central effects in trigeminal system transmitting migraine pain.

1. Introduction

Migraine is a common neurological disorder which pathophysiology is still poorly understood. For decades, serotonergic mechanisms were supposed to play a key role in migraine pathology (Lance et al., 1967; Dussor, 2014; Hamel, 2007). During migraine attacks, the plasma level of serotonin (5-HT) raises dramatically, whereas between attacks it goes down (Ferrari et al., 1989). Early studies reported the ability of 5-HT to inhibit migraine

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attack (Lance et al., 1967). In general, a low 5-HT level combined with high receptor sensitivity has been proposed as a factor increasing the risk of migraine (Panconesi, 2008). However, because of a wide range of receptors for 5-HT and its region-specific effects on the nociceptive system (Saxena and Ferrari, 1992; Viguier et al., 2013), there are a number of contradictory reports on the role of 5-HT in migraine (Cervantes-Durán et al., 2013; Hamel, 2007).

The 5-HT-caused pain is mediated, at least in part, via activation of perivascular nociceptive fibers innervating extracranial arteries (Koo and Balaban, 2006). 5-HT also has pro-nociceptive action when applied to the dura mater as a component of the inflammatory soup (Strassman et al., 1996; Lukács et al., 2015; Oshinsky and Gomonchareonsiri, 2007). However, the mechanism of the 5-HT-induced pro-nociceptive effect at trigeminal nerve terminals in the meninges remains poorly understood. For example, sumatriptan, one of the most specific anti-migraine agents, is an agonist of the 5-HT1B and 5-HT1D receptors but the site of its action within the nociceptive system still remains to be elucidated (Dussor, 2014). The 5-HT1B receptor is located on vascular smooth muscles and the 5-HT1D receptor on the peripheral and central terminals of the dural afferents (Harriott et al., 2012). Thus, it is reasonable to suggest that both peripheral and central sites represent potential targets for anti-migraine therapy by triptans (Goadsby and Hoskin, 1998; Donaldson et al., 2002). Indeed, Amrutkar et al. (2012) found that sumatriptan inhibited CGRP release both in dura mater and in the brainstem.

The meninges comprising pia and dura mater play one of the major roles in the generation of migraine headaches (Goadsby and Edvinsson, 1993; Strassman et al., 1996; Olesen et al., 2009; Zakharov et al., 2015). Stimulation of the trigeminal ganglia induced release of the migraine mediator CGRP into cranial circulation and this effect was blocked by sumatriptan (Goadsby and Edvinsson, 1993). Many key studies were performed in animal migraine models *in vivo* when meninges were stimulated after sensitization induced by inflammatory compounds and recordings were made either from trigeminal ganglion or from brainstem (Strassman et al., 1996; Goadsby and Hoskin, 1998; Burstein et al., 2005). Meninges have a large number of 5-HT-containing mast cells considered as one of the migraine triggers (Levy, 2009). Degranulation of mast cells *in vivo* induces a long-lasting activation of meningeal nociceptors (Levy et al., 2007). Platelets containing 5-HT in their dense-body granules have also been suggested to contribute to some forms of migraine (Taffi et al., 2005; Borgdorff and Tangelder, 2012; Danese et al., 2014).

The 5-HT3 receptor, the only ionotropic receptor in the extended 5-HT receptor family, mediates excitatory responses in the central and peripheral sensory neurons (Cervantes-Durán et al., 2013; Hicks et al., 2002). There are contradictory data on the functional role of 5-HT3 receptors in the spinal cord, as both pro- and anti-nociceptive effects were reported (Green et al., 2000; Kim et al., 2015; Oatway et al., 2004). About 20% of descending serotonergic terminals make axo-axonal contacts with primary afferents (Zhang et al., 2015), implying their involvement in the presynaptic control of peripheral inputs. In agreement with the pro-nociceptive action of 5-HT, 5-HT3 receptor antagonists are commonly used for pain therapy (Greenshaw and Silverstone, 1997; Sagalajev et al., 2015). However, their mechanisms of action have not been studied in a migraine pain models, which may have its specific physiological properties and neurochemical profile.

Recently, we developed a novel cluster approach to analyze nociceptive discharges in isolated trigeminal fibers innervating cranial meninges, the origin site of migraine pain (Zakharov et al., 2015). This technique overcomes several limitations of the *in vivo* experiments: one can directly record activity in nociceptive terminals under conditions of adequate drug concentration control and without application of concomitant anesthesia.

In the present study, we show that the robust activation of the peripheral nerve terminals by 5-HT is opposed by its inhibitory action on the central terminals, and that both these effects are mediated via the same 5-HT3 receptor.

2. Materials and methods

2.1. Preparations

Experiments were performed in accordance with the European Community Council Directive of September 22, 2010 (2010/63/EEC). Wistar rats from the Animal House of the University of Eastern Finland were housed in cages with controlled temperature, humidity and 12-h light-dark cycle. Food and water were served ad libitum. The protocols were approved by the Animal Care and Use Committee of the University of Eastern Finland. The isolated rat hemiskulls were prepared from adult (5 weeks) male rats as described previously (Zakharov et al., 2015). Briefly, the rats were decapitated after CO_2 inhalation, the skin and muscles were removed from the skull, which was divided into halves by a cut along the sagittal suture. The brain hemispheres were removed without harming the trigeminal ganglia and the meningeal dura mater.

For the whole-cell recordings, laboratory Wistar rats of both sexes (P15-18) were killed by decapitation in accordance with the Portuguese guidelines (Direcção Geral de Alimentação e Veterinária, Ministério da Agricultura) after anesthesia with intraperitoneal injection of Na^+-pentobarbital (30 mg/kg) and a subsequent check for a lack of pedal withdrawal reflexes. The experiments were carried out according to the guidelines laid down by the institution's animal welfare committee (Comissão de Ética do Instituto de Biologia Molecular e Celular). The trigeminospinal complex (the brainstem and the upper cervical spinal cord) with the trigeminal nerve attached was quickly isolated in oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 115, KCl 3, $CaCl_2$ 2, $MgCl_2$ 1, NaH_2PO_4 1, $NaHCO_3$ 25 and glucose 11; (bubbled with 95% O_2/5% CO_2) at room temperature. The pia mater in the spinal segments C1-C2 was locally removed with forceps and scissors, to provide access for the recording pipettes. The isolated trigeminospinal complex was glued with cyanoacrylate adhesive to a plate made of gold (the dorsolateral surface was up) and transferred to the recording chamber. Lamina I neurons in the C1-C2 segment were visualized through the intact white matter using the oblique infrared light-emitting-diode illumination technique (Safronov et al., 2007; Szucs et al., 2009).

Primary cell cultures of trigeminal ganglia of Wistar male rats (P10-12, Animal Center of the University of Eastern Finland, Kuopio, Finland) were prepared as described previously (Fabbretti et al., 2006). In brief, trigeminal ganglia were isolated and ganglion cells dissociated using an enzyme cocktail containing trypsin (0.25 mg/mL) and collagenase type I (760 U/mL) under continuous shaking (850 rpm) at 37 °C for 15 min. Cells were plated on coverslips coated with poly-L-lysine (0.2 mg/ml, P1399, Sigma-Aldrich Co. St. Louis, MO USA) and cultured in F12 medium supplemented with FBS 10% (10270-106; Gibco Invitrogen, Carlsbad, CA, USA) at 37 °C in an atmosphere saturated with 5% CO_2 for 48 h prior to Ca^{2+} imaging.

2.2. Electrophysiology

The hemiskull preparation obtained from Wistar rats was used for the suction electrode recording of activity in the nervus spinosus, which is a part of the mandibular branch of the trigeminal nerve (Schueler et al., 2013). The recording was carried out with a