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THE INHIBITORY ACTION OF THE ANTIMIGRAINE NONSTEROIDAL ANTI-INFLAMMATORY DRUG NAPROXEN ON P2X3 RECEPTOR-MEDIATED RESPONSES IN RAT TRIGEMINAL NEURONS

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Abstract—Enhanced nociceptive firing in trigeminal ganglion neurons is a likely reason for migraine pain. In experimental migraine-like conditions induced by the calcitonin gene-related peptide (CGRP), P2X3 receptors abundantly expressed in trigeminal neurons are highly responsive to the excitatory action of extracellular ATP. In this study, we tested whether naproxen, a common antimigraine medicine, could affect the function of P2X3 receptors in the presence or absence of the algogen nerve growth factor (NGF), the level of which is elevated in patients with chronic migraine. We used calcium imaging and patch clamp recordings from rat trigeminal neurons, which were activated by a relative specific P2X3 agonist α,β-meATP or by high potassium-induced depolarization. In the absence of NGF, naproxen dose-dependently (0.1–1 mM) reduced intracellular calcium transients elicited by α,β-meATP. Naproxen also led to a slight, but significant, reduction in calcium transients induced by potassium ions, indicating the involvement of voltage-gated calcium channels. The inhibitory action of 1 mM naproxen was enhanced after NGF pretreatment, suggesting that P2X3 receptors in sensitized neurons are more susceptible to inhibition by high doses of this nonsteroidal anti-inflammatory drug (NSAID). Using patch clamp recordings from HEK293 cells expressing P2X3 receptors, we tested the direct action of naproxen on P2X3 receptor-mediated membrane currents. In clinically relevant concentrations of 0.5 mM, naproxen produced a use-dependent blocking effect on ATP receptors. Kinetic analysis suggests that naproxen inhibited P2X3 receptors via facilitation of fast desensitization, which determines current decay in the continuous presence of the agonist. In summary, we present a novel fast mechanism for the antimigraine action of naproxen, which can act in synergy with the cyclooxygenase inhibition to attenuate headaches.

Key words: ATP, P2X3 receptor, trigeminal neurons, naproxen, calcium imaging, patch clamp.

Naproxen is one of most commonly used medicines for treatment of migraine attack. In combination with sumatriptan, naproxen has been suggested as the most efficient medicine for acute treatment of migraine (Brandes et al., 2007). Naproxen is a nonsteroidal anti-inflammatory drug (NSAID) belonging to a group of profens that are nonselective inhibitors of both types 1 and 2 cyclooxygenase (COX-1/2) (Duggan et al., 2010). The main mechanism of its analgesic action is thought to be the inhibition of COX-dependent synthesis of proinflammatory algogenic prostaglandins and oxygenated endocannabinoids (Kozak et al., 2004). However, NSAIDs are reported to have several other effects within the nociceptive system. For example, the NSAIDs diclofenac and ibuprofen have been found to inhibit acid-sensing ion channels in hippocampal interneurons (Dorofeeva et al., 2008) and in sensory neurons (Voilley et al., 2001).

Naproxen has been shown to inhibit trigeminal neuronal firing, which is activated through the *in vivo* application of "inflammatory soup" onto the dura mater (Jakubowski et al., 2007; Levy et al., 2008). However, the mechanism of the inhibitory action on neuronal firing by this COX inhibitor remains unexplained.

Extracellular ATP is the one of the most potent algogens that can initiate pain signaling via membrane P2X2/3 or P2X3 receptors expressed in sensory neurons (North, 2004). ATP-gated P2X3 receptors have recently been implicated in migraines (for review see Giniatullin et al., 2008 and Burnstock et al., 2011). The responsiveness of P2X3 receptors in rat trigeminal neurons could be largely enhanced by the migraine mediator calcitonin gene-related peptide (CGRP) (Fabbretti et al., 2006) consistent with "purinergic" mechanisms of migraine pain (Giniatullin et al., 2008). The powerful algogen nerve growth factor (NGF), which is elevated in patients with chronic migraine (Jang et al., 2011), also strongly potentiates P2X3 receptor-mediated responses in trigeminal neurons via protein kinase C-dependent phosphorylation (D'Arco et al., 2007).

In the current study, we show that the P2X3 receptor represents one of the targets for the depressant action of naproxen in pain signaling.

EXPERIMENTAL PROCEDURES

Cell cultures and transfection

The trigeminal ganglion neurons of P10–12 male rats were prepared for culture, as previously described (Simonetti et al., 2006; Mazzuca et al., 2011). All efforts were made to minimize the number of animals used and their suffering. Briefly, neurons were plated on coverslips coated in poly-L-lysine (0.2 mg/ml) and cultured for 1–2 days at 37 °C in an atmosphere containing 5% CO_2.

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Abbreviations: CGRP, calcitonin gene-related peptide; COX, cyclooxygenase; HEK, human embryonic kidney cells; NGF, nerve growth factor; NSAID, nonsteroidal anti-inflammatory drug; VGCCs, voltage-gated calcium channels; α, β-meATP; α, β-methylene-ATP.

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