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ANTI-HYPERALGESIC EFFECTS OF ANTI-SEROTONERGIC COMPOUNDS ON SEROTONIN- AND CAPSAICIN-EVOKED THERMAL HYPERALGESIA IN THE RAT

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Abstract—The peripheral serotonergic system has been implicated in the modulation of an array of pain states, from migraine to fibromyalgia; however, the mechanism by which serotonin (5HT) induces pain is unclear. Peripherally released 5HT induces thermal hyperalgesia, possibly via modulation of the transient receptor potential V1 (TRPV1) channel, which is gated by various noxious stimuli, including capsaicin. We previously reported in vitro that 5HT increases calcium accumulation in the capsaicin-sensitive population of sensory neurons with a corresponding increase in proinflammatory neuropeptide release, and both are antagonized by pretreatment with $5HT_{2A}$ and $5HT_3$ antagonists, as well as the anti-migraine drug sumatriptan. In the current study, we extended these findings in vivo using the rat hind paw thermal assay to test the hypothesis that peripheral 5HT enhances TRPV1-evoked thermal hyperalgesia that can be attenuated with $5HT_{2A}$ and $5HT_3$ receptor antagonists, as well as sumatriptan. Thermal hyperalgesia and edema were established by 5HT injection (0.1–10 nmol/100 μl) into the rat hind paw, and the latency to paw withdrawal (PWL) from noxious heat was determined. Rats were then pretreated with either 5HT before capsaicin (3 nmol/10 μl), the $5HT_{2A}$ receptor antagonist ketanserin or the $5HT_3$ receptor antagonist granisetron (0.0001–0.1 nmol/100 μl) before 5HT and/or capsaicin, or the $5HT_{1B/1D}$ receptor agonist sumatriptan (0.01–1 nmol/100 μl) before capsaicin, and PWL was determined. We report that 5HT pretreatment enhances TRPV1-evoked thermal hyperalgesia, which is attenuated with local pretreatment with ketanserin, granisetron, or sumatriptan. We also report that peripheral 5HT induced a similar magnitude of thermal hyperalgesia in male and female rats. Overall, our results provide in vivo evidence supporting an enhancing role of 5HT on TRPV1-evoked thermal hyperalgesia, which can be attenuated by peripheral serotonergic intervention. Published by Elsevier Ltd on behalf of IBRO.

Key words: hyperalgesia, 5HT, 5HT receptors, granisetron, ketanserin, sumatriptan.

It is estimated that up to 56 million American adults, or about 28% of the adult population, experience some form of persistent pain (Brennan et al., 2007). Persistent pain, such as migraine and fibromyalgia, is often poorly managed and is an expensive burden for both taxpayers and the healthcare system. It is becoming increasingly clear that the neurotransmitter serotonin (5HT) plays a modulatory role in various acute and persistent pain states (Hargreaves and Shepheard, 1999; Ernberg et al., 2000b, 2006; Herken et al., 2001; Parada et al., 2001; Sommer, 2004). Therapeutics targeting the 5HT system are currently being examined in clinical trials for their ability to treat migraine (Ferrari et al., 2001; Chen and Ashcroft, 2008), fibromyalgia (Häuser et al., 2009), and irritable bowel syndrome (Cremonini et al., 2003). However, despite the successful development of pain therapies targeting the 5HT system, the precise peripheral mechanisms of 5HT in pain states remain unclear.

The vast majority of 5HT in the mammalian body is located in peripheral tissues where 5HT is actively taken up and released with other chemical mediators by platelets, mast cells, and immune cells (Sommer, 2004). 5HT levels are increased during inflammation occurring in rats after thermal injury (Sasaki et al., 2006), in humans with joint movement pain (Kopp, 1998) and in human muscle associated with pain and allodynia (Ernberg et al., 2000b,c). Peripheral 5HT administration evokes inflammation and hyperalgesia in both humans (Babenko et al., 2000; Ernberg et al., 2000b,c, 2006) and animals (Taiwo and Levine, 1992; Tokunaga et al., 1998; Okamoto et al., 2002), as well as pruritogenic effects (Jinks and Carstens, 2002; Akiyama et al., 2010) that may play a role in hyperalgesia. Inflammation in the rat hind paw induces a dose-dependent increase in peripheral 5HT levels (Nakajima et al., 2009), and formalin-induced nociception is attenuated by local administration of 5HT receptor antagonists (Parada et al., 2001). Administration of $5HT_{2A}$ and $5HT_3$ agonists mimic 5HT-induced hyperalgesia in rats (Tokunaga et al., 1998; Obata et al., 2000; Ohta et al., 2006), whereas antagonists reduce hyperalgesia (Tokunaga et al., 1998; Obata et al., 2000; Sasaki et al., 2006). On the other hand, $5HT_{1B/1D}$ receptor agonists significantly attenuate neurogenic inflammation (Carmichael et al., 2008) and hyperalgesia (Bingham et al., 2001; Nikai et al., 2008) presumably by inhibiting release of neuropeptides (Moskowitz and Buzzi, 1991). Together, these studies indicate that the complexity of the peripheral 5HT system in various pain states may be due, in part, to activation of a broad range of 5HT receptor subtypes. However, it is possible that these various 5HT receptor subtypes may regulate

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Abbreviations: ANOVA, analysis of variance; DMSO, dimethyl sulfoxide; ipl, intraplantar; OVX, ovariectomized; PWL, paw withdrawal latency; TRPV1, transient receptor potential V1 channel; 5HT, serotonin.

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common transduction systems, leading to convergence of peripheral 5HT pain mechanisms.

The transient receptor potential V1 channel (TRPV1) plays a critical role in inflammatory pain states by mediating sensory neuron activation by chemical and thermal stimuli (Caterina et al., 1997, 2000; Davis et al., 2000; Voets et al., 2004) and factors released during inflammation sensitize TRPV1 to enhance hyperalgesia (Cesare et al., 1999; Pingle et al., 2007). Importantly, there is mounting evidence of a role of 5HT in modulating TRPV1 function. 5HT reportedly increases sensory neuron membrane excitability to thermal stimuli and enhances capsaicin- and heat-evoked currents (Sugiuar et al., 2004; Ohta et al., 2006), whereas 5HT depletion attenuates visceral pain with a corresponding reduction in TRPV1 activation (Qin et al., 2010). 5HT enhances intracellular calcium accumulation in capsaicin-sensitive sensory neurons (Ohta et al., 2006; Loyd et al., 2011) and enhances capsaicin-evoked calcitonin gene-related peptide (CGRP) release, whereas $5HT_{2A}$ and $5HT_3$ antagonists, as well as the anti-migraine drug sumatriptan, attenuate 5HT enhancement of capsaicin-evoked CGRP release (Loyd et al., 2011).

Despite the growing evidence of a modulatory role of 5HT on TRPV1 function, it remains unclear whether 5HT enhances TRPV1-evoked thermal hyperalgesia *in vivo* or whether targeting peripheral 5HT receptors can reduce capsaicin-evoked thermal hyperalgesia. We hypothesized that 5HT enhances TRPV1-evoked thermal hyperalgesia that can be attenuated with $5HT_{2A}$ and $5HT_3$ receptor antagonists, as well as the $5HT_{1B/1D}$ agonist sumatriptan. Using behavioral assays in the rat hind paw, we (1) established 5HT-evoked thermal hyperalgesia and edema, (2) evaluated whether 5HT pretreatment enhanced TRPV1-evoked thermal hyperalgesia, and (3) determined whether the $5HT_{2A}$ receptor antagonist ketanserin, the $5HT_3$ receptor antagonist granisetron, or the $5HT_{1B/1D}$ receptor agonist sumatriptan treatment could reduce 5HT- and TRPV1-evoked thermal hyperalgesia.

EXPERIMENTAL PROCEDURES

Subjects

A total of 118 adult (250–350 g) intact male, 34 intact female, and 32 ovariectomized (OVX) female Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA, USA) were used in these experiments. Rats were housed for at least 5 days before study with *ad libitum* access to food and water. These studies were performed in compliance with the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio and conform to federal guidelines and guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain. All efforts were made to reduce the number of animals used and to minimize any possible suffering.

Drugs

Drugs (Sigma-Aldrich, St. Louis, MO, USA) were dissolved and stored at 4 °C in stock concentration form and diluted in buffered saline (experimental vehicle) immediately before use, except serotonin hydrochloride which was both dissolved and diluted immediately before each use. Capsaicin (TRPV1 agonist) was dissolved in ethanol stock, with final dilutions containing <0.5% ethanol. Serotonin hydrochloride, sumatriptan succinate ($5HT_{1B/1D}$ receptor agonist) and granisetron hydrochloride ($5HT_3$ receptor antagonist) were dissolved in double-distilled water. Ketanserin (+)-tartrate salt ($5HT_{2A}$ antagonist) was dissolved in dimethyl sulfoxide (DMSO). Ritanserin ($5HT_{2A}$ antagonist) was dissolved in saline containing 5% DMSO and 5% Tween.

Behavioral testing

Paw withdrawal latencies to a noxious thermal stimulus were determined using the paw thermal stimulator (Univ. California San Diego, San Diego, CA, USA). Rats were placed in a clear Plexiglas box resting on an elevated glass plate maintained at 30 °C. Following acclimation, a radiant beam of light was positioned under the hind paw, and the average time over three trials for the rat to remove the paw from the thermal stimulus was electronically recorded in seconds as the paw withdrawal latency (PWL). The intensity of the beam was set to produce basal PWL's of approximately 10–12 s. A maximal PWL of 20 s was used to prevent excessive tissue damage due to repeated application of the thermal stimulus. All studies were conducted by an observer blind to the experimental condition.

Serotonin-evoked thermal hyperalgesia. To determine 5HT-induced thermal hyperalgesia in male and female rats, 5HT (0.01–10 nmol/100 μl; n=6–10 each) or saline vehicle was administered by intraplantar (ipl) injection using a 30-gauge syringe into the hind paw immediately following collection of basal PWL measures. PWL levels were then reassessed at 15 min and 30 min following injections based on previous studies (Sufka et al., 1992; Taiwo and Levine, 1992; Tokunaga et al., 1998). An additional group of male rats received pretreatment with ketanserin (0.0001–0.1 nmol/100 μl), granisetron (0.0001–0.1 nmol/100 μl), or saline vehicle 15 min before 5HT (10 nmol/100 μl) injections (n=6–8/group). In a separate group, 0.1 nmol/100 μl ketanserin or 0.1 nmol/100 μl granisetron (n=6 each) was injected into the contralateral hind paw before 5HT injections to confirm a peripheral action of these drugs. In addition, a preliminary group of 12 male rats were used to determine the optimal methodology for administration of 5HT injections followed by behavioral testing. Six rats received brief inhalation anesthesia (Isoflurane; Butler Animal Health Supply, Dublin, OH, USA), and six rats were placed under brief restraint in an individual plastic restrainer (Braintree Scientific, Braintree, MA, USA) during ipl injection. Based on the results, all studies were then carried out using brief restraint for injections. The opposite hind paw of rats receiving peripherally restricted compounds were used in additional experiments within 1–2 weeks to reduce the total number of animals used, except with animals receiving isoflurane.

Capsaicin-evoked thermal hyperalgesia. 5HT (0.1 or 10 nmol/100 μl; n=6 each) was injected ipl to the hind paw 10 min before capsaicin (3 nmol/10 μl). The time course was chosen as 5HT-evoked thermal hyperalgesia peaks at 10–15 min, and the dose of capsaicin was chosen to permit analysis of potential-increased reduction in PWL based on previous studies (Gilchrist et al., 1996; Patwardhan et al., 2006). In a separate group of animals, ketanserin (0.1 nmol/100 μl), granisetron (0.1 nmol/100 μl), or saline was injected into the same hind paw 15 min before 5HT and capsaicin (n=6/group). Additional groups received ketanserin (0.1 nmol/100 μl), granisetron (0.1 nmol/100 μl), sumatriptan (0.1 nmol–1 nmol/100 μl), or ritanserin (100 nmol/100 μl) before capsaicin alone to detect a potential effect of these drugs on capsaicin-evoked thermal hyperalgesia (n=6/group). Ritanserin (100 nmol/100 μl; n=5) was also given alone to determine effects on basal PWL.