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SECONDARY MECHANICAL ALLODYNIA AND HYPERALGESIA DEPEND ON DESCENDING FACILITATION MEDIATED BY SPINAL 5-HT$_4$, 5-HT$_6$ AND 5-HT$_7$ RECEPTORS

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Abstract—In the present study we determined the role of spinal 5-hydroxytriptamine (5-HT) and 5-HT$_{4/6/7}$ receptors in the long-term secondary mechanical allodynia and hyperalgesia induced by formalin in the rat. Formalin produced acute nociceptive behaviors (flinching and licking/lifting) followed by long-term secondary mechanical allodynia and hyperalgesia in both paws. In addition, formalin increased the tissue content of 5-HT in the ipsilateral, but not contralateral, dorsal part of the spinal cord compared to control animals. Intrathecal (i.t.) administration of 5,7-dihydroxytriptamine (5,7-DHT), a serotonergic neurotoxin, diminished tissue 5-HT content in the ipsilateral and contralateral dorsal parts of the spinal cord. Accordingly, i.t. 5,7-DHT prevented formalin-induced secondary allodynia and hyperalgesia in both paws. I.t. pre-treatment (−10 min) with ML-10302 (5-HT$_4$ agonist), EMD-386088 (5-HT$_6$ agonist) and LP-12 (5-HT$_7$ agonist) significantly increased secondary mechanical allodynia and hyperalgesia in both paws. In contrast, i.t. pre-treatment (−20 min) with GR-125487 (5-HT$_4$ antagonist), SB-258585 (5-HT$_6$ antagonist) and SB-269970 (5-HT$_7$ antagonist) significantly prevented formalin-induced long-term effects in both paws. In addition, these antagonists prevented the pro-nociceptive effect of ML-10302, EMD-386088 and LP-12, respectively. The i.t. post-treatment (6 days after formalin injection) with GR-125487, SB-258585 and SB-269970 reversed formalin-induced secondary allodynia and hyperalgesia in both paws. These results suggest that spinal 5-HT, released from the serotonergic projections in response to formalin injection, activates pre- or post-synaptic 5-HT$_{4/6/7}$ receptors at the dorsal root ganglion/spinal cord promoting the development and maintenance of secondary allodynia and hyperalgesia.

Key words: secondary allodynia, secondary hyperalgesia, 5-HT receptors, chronic pain.

INTRODUCTION

Descending facilitation from the brainstem plays a major role in chronic pain. Particularly, rostral ventromedial medulla (RVM) has been associated with this process (Pertovaara et al., 1996; Porreca et al., 2001). RVM sends projections to the spinal cord through the dorsolateral funiculus (Ossipov et al., 2000; Burgess et al., 2002) which releases cholecystokinin, dynorphin, calcitonin gene-related peptide (CGRP) and serotonin (5-hydroxytriptamine, 5-HT), among other neurotransmitters, to promote development and maintenance of long-term nociception (Kovelowski et al., 2000; Gardell et al., 2003; Heinricher and Neubert, 2004; Ambriz-Tututi et al., 2011). It is thought that activation of RVM ON cells, by cholecystokinin, leads to behavioral allodynia and hyperalgesia (Kovelowski et al., 2000; Heinricher and Neubert, 2004; Xie et al., 2005; Marshall et al., 2012) and a time-dependent dynorphin A (Wagner et al., 1993; Laughlin et al., 1997; Malan et al., 2000; Burgess et al., 2002), cholecystokinin (Gustafsson et al., 1998; Xu et al., 2001; Kim et al., 2009) and 5-HT (Wei et al., 2010; Marshall et al., 2012) increase in the spinal cord.

Several studies in rodents suggest that 5-HT modulates nociceptive responses. However, the role of 5-HT in pain processing is complex as it may inhibit and/or facilitate nociceptive transmission depending on the type of nociceptive stimuli and the nature of the 5-HT receptors. In the case of the formalin test, it seems that serotonergic descending inhibition is important to develop primary hyperalgesia while descending facilitation participates in secondary hyperalgesia (Vanegas and Schaible, 2004). Regarding the latter, several studies have demonstrated that the serotonergic

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Abbreviations: 5,7-DHT, 5,7-dihydroxytryptamine creatinine sulfate; 5-HT, 5-hydroxytriptamine or serotonin; CGRP, calcitonin gene-related peptide; CL, contralateral; cyclic AMP, cyclic adenosine monophosphate; DRG, dorsal root ganglion; EMD-386088, 5-chloro-2-methyl-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole hydrochloride; F, formalin; GABA, γ-aminobutyric acid; GR-125487, 5-fluoro-2-methoxy-[1-[2-[(methylsulfonyl)amino]ethyl]-4-piperidinyl]-1H-indole-3-methyl-carboxylate sulfamate; i.t., intrathecal; IL, Ipsilateral; LP-12, 4-(2-diphenyl)-N-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide hydrochloride; ML-10302, 4-amino-5-chloro-2-methoxybenzoic acid 2-(1-piperidinyl)ethyl ester hydrochloride; PKA, protein kinase A; RNAi, shRNA interference; RVM, rostral ventromedial medulla; SB-258585, 4-iodo-N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]benzenesulfonamide hydrochloride; SB-269970, (R)-3-(2-(2-(4-methylpiperidin-1-yl) ethyl) pyrrolidine-1-sulfonyl) phenol hydrochloride; Tph-2, tryptophan hydroxylase-2.

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descending system is involved in the facilitation of the spinal nociceptive transmission (Suzuki et al., 2004; Wei et al., 2010; Leong et al., 2011; Marshall et al., 2012). For instance, spinal nerve ligation leads to a loss (one third) of serotonergic neurons (Leong et al., 2011). The remaining serotonergic neurons seem to facilitate nociception as depletion of spinal 5-HT, *via* intrathecal (i.t.) injection of the serotonergic neurotoxin 5,7-dihydroxytriptamine (5,7-DHT), reduces mechanical allodynia in neuropathic pain models (Oatway et al., 2004; Rahman et al., 2006; Leong et al., 2011) and decreases acute nociceptive behavior in the formalin test (Svensson et al., 2006). Moreover, blockade of the synthesis of 5-HT with a shRNA interference (RNAi) of tryptophan hydroxylase-2 (Tph-2) induces down-regulation of 5-HT in the spinal dorsal horn and attenuates formalin-induced acute nociceptive behavior or nerve injured-induced allodynia and hyperalgesia (Wei et al., 2010). These studies suggest that spinal nerve ligation may switch the serotonergic system from antinociceptive to pronociceptive by promoting apoptosis of some serotonergic neurons in the spinal cord (Leong et al., 2011). Particularly, spinal 5-HT_3 receptors have been associated to the descending facilitatory role of 5-HT (Zeitz et al., 2002; Oatway et al., 2004; Rahman et al., 2006; Wei et al., 2010; Bravo-Hernández et al., 2012; Marshall et al., 2012). However, considering the transduction mechanism, spinal 5-$HT_{2/4/6/7}$ receptors could also have a role in this process. In support of this suggestion, activation of spinal 5-HT_2 (Thibault et al., 2008; Van Steenwinckel et al., 2008; Aira et al., 2010), 5-HT_3 (Oatway et al., 2004) 5-HT_7 (Amaya-Castellanos et al., 2011) receptors are related with nociception in neuropathic pain models. The case of spinal 5-HT_2 (Nakai et al., 2010) and 5-HT_7 (Brenchat et al., 2010, 2012) receptors has been controversial. Furthermore, activation of spinal 5-HT_2 (Kjorsvik et al., 2001; Rahman et al., 2011), 5-HT_3 (Gu et al., 2011), 5-HT_6 (Castañeda-Corral et al., 2009) and 5-HT_7 (Rocha-González et al., 2005) receptors increases acute nociceptive behavior. The role of spinal 5-HT_4 receptors in acute or chronic pain is unknown.

5-$HT_{4/6/7}$ receptors belong to the family of G protein-coupled receptors that positively influence adenylyl cyclase activity (Monsma et al., 1993; Ruat et al., 1993; Boess and Martin, 1994; Ansanay et al., 1995; Hirst et al., 1997) increasing cyclic AMP. In turn, cyclic AMP activates protein kinase A (PKA) and sodium channels leading to depolarization and nociception (Gold et al., 1996; Cardenas et al., 2001). These receptors are localized on the dorsal horn of the spinal cord (Waeber et al., 1994; Gerard et al., 1996; Doly et al., 2005) and dorsal root ganglion (DRG) neurons (Gerard et al., 1996; Pierce et al., 1996; Chen et al., 1998; Wu et al., 2001; Nicholson et al., 2003; Liu et al., 2005) and glial cells (Waeber et al., 1994; Gerard et al., 1996; Hirst et al., 1998; Doly et al., 2005; Mahe et al., 2005). A previous report of our laboratory indicates that peripheral 5-$HT_{4/6/7}$ receptors participate during development and maintenance of secondary allodynia and hyperalgesia induced by formalin (Godínez-Chaparro et al., 2011). However, the role of spinal 5-$HT_{4/6/7}$ receptors in formalin-induced secondary allodynia and hyperalgesia is unknown. Thus, we hypothesized that formalin-induced spinal increase of 5-HT would lead to activation of spinal 5-$HT_{4/6/7}$ receptors which then would contribute to the development and maintenance of long-term secondary allodynia and hyperalgesia. On the basis of above considerations, this work was undertaken to determine the role of serotonergic descending facilitation and spinal 5-$HT_{4/6/7}$ receptors in the development and maintenance of secondary allodynia and hyperalgesia induced by formalin in rats.

EXPERIMENTAL PROCEDURES

Animals

Experiments were performed on adult female Wistar rats (body weight range, 200–220 g). Animals were obtained from our own breeding facilities and had free access to food and drinking water before experiments. All experiments followed Guidelines on Ethical Standards for investigation of Experimental Pain in Animals (Zimmermann, 1983). Additionally, the Institutional Animal Care and Use Committee approved the study (Cinvestav, Mexico City).

Induction of long-term secondary allodynia and hyperalgesia

Rats were briefly immobilized to get open access to the right hind limb. Then, animals received a subcutaneous (s.c.) injection of saline solution or dilute formalin (0.5% or 1%, 50 μL) into the dorsal surface on the right hind paw with a 30-gauge needle (Fu et al., 2001; Ambriz-Tututi et al., 2011). The sixth day was chosen to evaluate nociceptive behaviors because at this time nociceptive behaviors are already established (Ambriz-Tututi et al., 2011). At the end of the experiment the rats were sacrificed in a CO_2 chamber.

Behavioral testing of formalin-induced secondary allodynia and hyperalgesia

Secondary mechanical allodynia and hyperalgesia models were developed by Wiertelak et al. (1994) and Fu et al. (2001) from the formalin test model (Dubuisson and Dennis, 1977). In our study, we used an adaptation of the model previously published by Jolivalt et al., 2006. Briefly, rats were placed in testing cages with a wire mesh bottom and allowed to acclimate for 30 min. Baseline measurements were recorded first. Two von Frey filaments (Stoelting Co., Wood Dale, IL, USA, bending forces of 10 mN [1 g] and 250 mN [26 g]) were applied 10 times in each testing set at the base of the third toe on the plantar surface on both paws. Under normal conditions, a force of 10 mN does not activate cutaneous nociceptors (Leem et al., 1993), and therefore, it does not cause paw withdrawal in normal animals. Accordingly, the occurrence of responses in these rats indicates allodynia. On the other hand, a force of 250 mN or more is considered a noxious stimulus, and hyperalgesia occurs when there is an increased response to this stimulus. Allodynia and hyperalgesia were considered secondary as stimulation with the von Frey filaments were applied to sites different from the formalin injection.