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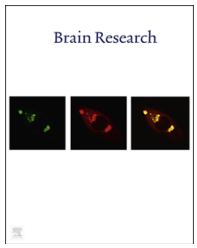

Research Report

Role of spinal 5-HT$_{5A}$, and 5-HT$_{1A/1B/1D}$, receptors in neuropathic pain induced by spinal nerve ligation in rats

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ABSTRACT

Serotonin (5-HT) participates in pain modulation by interacting with different 5-HT receptors. The role of 5-HT$_{5A}$ receptor in neuropathic pain has not previously studied. The purpose of this study was to investigate: A) the role of 5-HT$_{5A}$ receptors in rats subjected to spinal nerve injury; B) the expression of 5-HT$_{5A}$ receptors in dorsal spinal cord and dorsal root ganglia (DRG). Neuropathic pain was induced by L5/L6 spinal nerve ligation. Tactile allodynia in neuropathic rats was assessed with von Frey filaments. Western blot methodology was used to determine 5-HT$_{5A}$ receptor protein expression. Intrathecal administration (on day 14th) of 5-HT (10–100 nmol) or 5-carboxamidotryptamine (5-CT, 0.03–0.3 nmol) reversed nerve injury-induced tactile allodynia. Intrathecal non-selective (methiothepin, 0.1–0.8 nmol) and selective (SB-699551, 1–10 nmol) 5-HT$_{5A}$ receptor antagonists reduced, by ~60% and ~25%, respectively, the antiallodynic effect of 5-HT (100 nmol) or 5-CT (0.3 nmol). Moreover, both selective 5-HT$_{1A}$ and 5-HT$_{1B/1D}$ receptor antagonists, WAY-100635 (0.3–1 nmol) and GR-127935 (0.3–1 nmol), respectively, partially diminished the antiallodynic effect of 5-HT or 5-CT by about 30%. Injection of antagonists, by themselves, did not affect allodynia. 5-HT$_{5A}$ receptors were expressed in the ipsilateral dorsal lumbar spinal cord and DRG and L5/L6 spinal nerve ligation did not modify 5-HT$_{5A}$ receptor protein expression in those sites. Results suggest that 5-HT$_{5A}$ receptors reduce pain processing in the spinal cord and that 5-HT and 5-CT reduce neuropathic pain through activation of 5-HT$_{5A}$ and 5-HT$_{1A/1B/1D}$ receptors. These receptors could be an important part of the descending pain inhibitory system.

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1. Introduction

Serotonin (5-hydroxytryptamine; 5-HT) contributes to a variety of physiological functions including nociception (Bardin, 2011). This agonist produces its effects through a multiplicity of neuronal targets by interacting with different 5-HT receptor subtypes (Hoyer et al., 1994; Barnes and Sharp, 1999). To date, seven different 5-HT receptor families, composed of six distinct heptahelical G-protein-coupled receptors and one ligand-gated ion channel, have been identified (Barnes and Sharp, 1999; Hoyer et al., 2002; Nichols and Nichols, 2008). Their designations include 5-HT_1 (5-$HT_{1A/B/D/E/F}$), 5-HT_2 (5-$HT_{2A/B/C}$), 5-HT_3 (5-$HT_{3A/B/C}$), 5-HT_4, 5-HT_5 (5-$HT_{5A/B}$), 5-HT_6 and 5-HT_7. Some of these subtypes have been cloned, but their function is not well known yet, as is the case for the 5-HT_5 receptor family. There are at least two 5-HT_5 receptor subtypes reported, 5-HT_{5A} and 5-HT_{5B} (Matthes et al., 1993; Grailhe et al., 2001). The genes coding for these receptors have been reported in rodents and human brain. However, the human 5-HT_{5B} gene does not encode a functional protein (Grailhe et al., 2001). The 5-HT_{5A} receptor couples to $G\alpha_i$ protein leading to inhibition of adenylyl cyclase and reduction of intracellular cyclic AMP (Erlander et al., 1993; Hurley et al., 1998; Francken et al., 1998; Thomas et al., 2004) as well as stimulation of inwardly rectifying potassium channels (Grailhe et al., 2001).

5-HT_{5A} receptors are mainly localized in the cerebral cortex, hippocampus and cerebellum (Pasqualetti et al., 1998) as well as the amygdala, caudate nucleus, cerebellum, hypothalamus, substantia nigra, thalamus and spinal cord (Rees et al., 1994; Oliver et al., 2000; Kinsey et al., 2001; Doly et al., 2004; Garcia-Alcocer et al., 2010). Since these receptors have been found in thalamus (Rees et al., 1994), spinal cord (Doly et al., 2004) and dorsal root ganglion (DRG) (Chen et al., 1998; Wu et al., 2001; Liu et al., 2005), we hypothesized that they may have a role in modulating nociceptive behavior. Previously, Doly et al. (2004) predicted an antinociceptive function for these receptors based on their localization in the superficial layers of the spinal dorsal horn. Accordingly, our group reported that intrathecal 5-HT, the 5-HT_1/5-HT_5/5-HT_7 receptor agonist 5-carboxamidotryptamine (5-CT), and the 5-HT re-uptake inhibitor fluoxetine produce antinociception in several inflammatory pain models through activation of 5-HT_{5A} receptors based on antagonism by a selective 5-HT_{5A} receptor antagonist (SB-699551) (Cervantes-Durán et al., 2013; Muñoz-Islas et al., 2014). There is also evidence that spinal 5-HT_{1A} (Colpaert et al., 2004; Aira et al., 2010) and 5-$HT_{1B/1D}$ (Kayser et al., 2002) receptors play an antinociceptive role in neuropathic pain. Thus, the present study set out to determine the participation of spinal 5-$HT_{1A/1B/1D}$ and 5-HT_{5A} receptors in neuropathic pain in rats.

2. Results

2.1. Antiallodynic effect of 5-HT and 5-CT

Spinal nerve ligation diminished withdrawal threshold to values less than 4 g, which was interpreted as tactile allodynia,

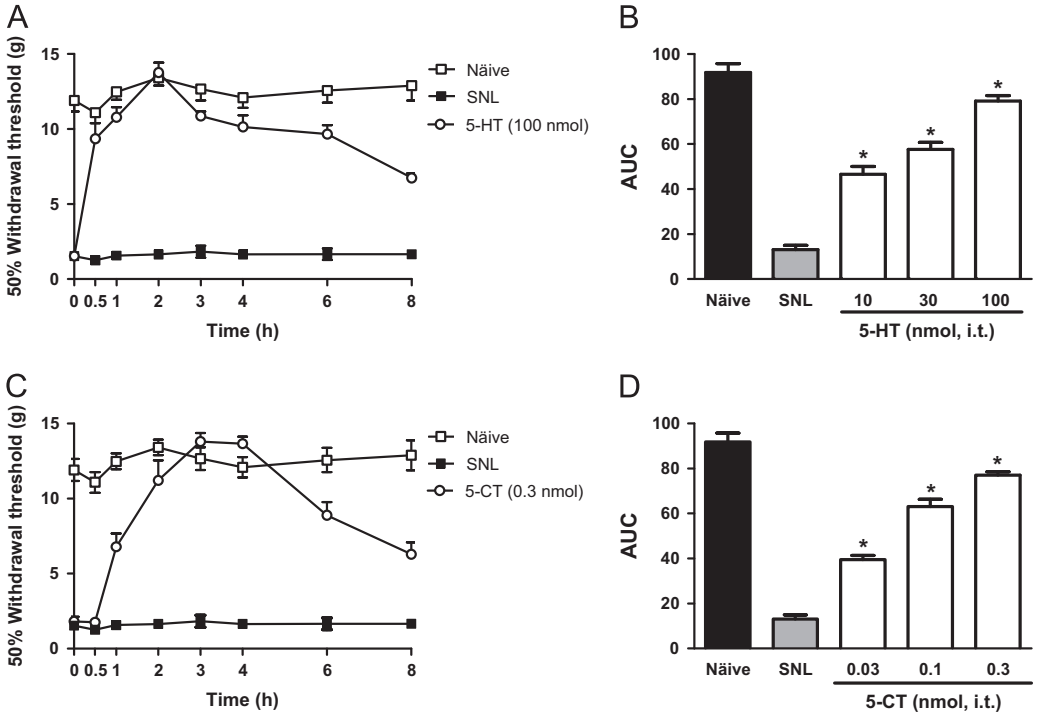

Fig. 1 – Time course of the antiallodynic effect observed after intrathecal injection of serotonin (5-HT, panel A) or 5-carboxamidotryptamine (5-CT, panel C) in rats submitted to L5/L6 spinal nerve ligation (SNL). Withdrawal threshold was determined 14 days after surgery. Antiallodynic effect of 5-HT (10–100 nmol) or 5-CT (0.03–0.3 nmol) observed after intrathecal administration in neuropathic rats. The bars show the area under the curve (AUC) of the 50% withdrawal threshold (panels B and D). The values represent the mean ± S.E.M. of 6 animals. *Significantly different ($p < 0.001$) of SNL group, as determined by one-way ANOVA, followed by the Student-Newman-Keuls test.