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Research Report

Functional expression of 5-HT$_7$ receptor on the substantia gelatinosa neurons of the trigeminal subnucleus caudalis in mice

*Eun Ju Yang, Seong Kyu Han, Soo Joung Park**

Department of Oral Physiology and Institute of Oral Bioscience, School of Dentistry, Chonbuk National University, Jeonju, 664-14, 1 Ga, Deokjin-Dong, Jeonbuk 561-756, Republic of Korea

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ABSTRACT

The substantia gelatinosa (SG) of the trigeminal subnucleus caudalis (Vc; medullary dorsal horn) receives and processes orofacial nociceptive inputs, and serotonergic fibers involved in the descending modulation of nociception are more densely distributed in the superficial laminae of the Vc. This study investigated the direct effects of 5-HT$_{1A/7}$ receptor agonist 8-OH-DPAT on SG neurons of the Vc to assess functional expression of the 5-HT$_7$ receptor using gramicidin-perforated patch-clamp in postnatal day (PND) 5-84 male mice. Of the 70 SG neurons tested, bath application of 8-OH-DPAT (30 μM) induced depolarization (n=33), hyperpolarization (n=16) or no response (n=21). In another 10 SG neurons, 8-OH-DPAT in the presence of 5-HT$_{1A}$ receptor antagonist WAY-100635 (1 μM) elicited either depolarization (n=6) or no response (n=4); hyperpolarization was not observed. The 8-OH-DPAT-induced depolarization was significantly blocked by the selective 5-HT$_7$ receptor antagonist SB-269970 (10 μM; n=8), but not by WAY-100635 (1 μM; n=5). The depolarizing effect of 8-OH-DPAT was maintained in the presence of TTX, CNQX, AP5, picrotoxin, and strychnine, indicating direct postsynaptic action of 8-OH-DPAT on SG neurons (n=6). 5-HT$_7$ receptor mRNA was also detected in five of 21 SG neurons by single-cell RT-PCR. The mean amplitude of 8-OH-DPAT-induced depolarization in PND 5-21 mice (n=21) was significantly larger than that in PND 22-84 mice (n=12), although the proportion of SG neurons responding to 8-OH-DPAT by depolarization did not differ significantly between two age groups of mice. These results indicate that 5-HT$_7$ receptors are functionally expressed in a subpopulation of SG neurons of the Vc and activation of 5-HT$_7$ receptors plays an important role in modulating orofacial nociceptive processing in the SG neurons of the Vc.

Abbreviations: 5-HT, 5-hydroxytryptamine; 8-OH-DPAT, (±)-8-hydroxy-2-(di-n-propylamino) tetralin; ACSF, artificial cerebrospinal fluid; AP5, D,L-2-amino-5-phosphonopentanoic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PND, postnatal day; RMP, resting membrane potential; RT-PCR, reverse transcriptase-polymerase chain reaction; SB-269970, (2R)-1-[(3-Hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine hydrochloride; SG, substantia gelatinosa; TTX, tetrodotoxin citrate; Vc, trigeminal subnucleus caudalis; WAY-100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate

*Corresponding author: Fax: +82 63 270 4004.
E-mail address: soopark@jbnu.ac.kr (S.J. Park).

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1. Introduction

The trigeminal subnucleus caudalis (Vc; also called the medullary dorsal horn) is the first relay site in nociceptive transmission from the orofacial region, and is structurally and functionally similar to the spinal dorsal horn (Sessle, 2000). The substantia gelatinosa (SG, lamina II) of the Vc and spinal dorsal horn receives Aδ- and C-primary afferent inputs and has been considered a critical region in the processing and modulation of nociceptive information conveyed to the higher brain centers (Cervero and Iggo, 1980; Todd, 2010).

The serotonergic pathways originating from the rostroventral medulla, including the nucleus raphe magnus, play a major role in the descending modulation of nociception in the Vc (Chiang et al., 1994; Seo et al., 2002; Okamoto et al., 2005, 2007) and the spinal dorsal horn (Bardin, 2011; Bardin et al., 2000; Dogrul et al., 2009; Jeong et al., 2004). Descending serotonergic fibers are more densely distributed in the superficial laminae (i.e. laminae I and II) than in the deeper laminae of the Vc or the spinal dorsal horn, in which serotonin (5-hydroxytryptamine, 5-HT) can either inhibit or facilitate nociceptive transmission depending on the 5-HT receptor subtype activated (Bardin, 2011; Cropper et al., 1984; Li et al., 1997; Millan, 2002). The 5-HT receptors are divided into seven classes (5-$HT_{1\text{-}7}$; Hoyer et al., 2002). Our previous study demonstrated that activation of 5-HT_{1A} and 5-HT_3 receptors induced hyperpolarization and depolarization, respectively, and activation of 5-HT_2 and 5-HT_4 receptors produced either hyperpolarization or depolarization in the SG neurons of the Vc (Yin et al., 2011).

The 5-HT_7 receptor is a G-protein coupled receptor that is the most recently identified 5-HT receptor subtype (Ruat et al., 1993), and has been implicated in pain modulation. For instance, systemically administered 5-HT_7 receptor agonists exert dose-dependent inhibition of capsaicin- or nerve injury-induced mechanical hypersensitivity and/or thermal hyperalgesia, which are blocked by systemic co-administration of the selective 5-HT_7 receptor antagonist in mice (Brenchat et al., 2009, 2010). Immunocytochemical study of 5-HT_7 receptor distribution in the spinal cord has revealed that the 5-HT_7 receptor is mainly localized in the spinal dorsal horn, particularly in the two superficial laminae, and is expressed on primary afferent fibers, intrinsic dorsal horn neurons, and glial cells (Doly et al., 2005; Meuser et al., 2002). Several behavioral studies employing intrathecal administration of 5-HT_7 receptor antagonists or agonists have suggested an antinociceptive role for spinal 5-HT_7 receptors (Brenchat et al., 2012a; Dogrul and Seyrek, 2006; Dogrul et al., 2009; Viguier et al., 2012). In contrast, a pronociceptive role of spinal 5-HT_7 receptors has also been proposed by other studies (Amaya-Castellanos et al., 2011; Godínez-Chaparro et al., 2012; Rocha-González et al., 2005).

Since selective 5-HT_7 receptor agonists are not readily available, the function and mechanism of action of 5-HT_7 receptors in nociceptive processing at the medullary and spinal dorsal horns have not yet been fully elucidated. Garraway and Hochman (2001) reported that 5-HT_7 but not 5-HT_{1A} receptor activation appears to contribute to facilitatory action of (±)-8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) on the dorsal root-evoked synaptic responses of deep dorsal horn neurons in the neonatal rat. Costa et al. (2012) demonstrated that 8-OH-DPAT enhances AMPA receptor-mediated synaptic transmission via postsynaptic 5-HT_7 receptors in the hippocampus. In this study, we examined the effects of 5-$HT_{1A/7}$ receptor agonist 8-OH-DPAT and selective 5-HT_7 receptor antagonist (2*R*)-1-[(3-Hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl] pyrrolidine hydrochloride (SB-269970; Hagan et al., 2000; Lovell et al., 2000) on SG neurons of the Vc to assess functional expression of the 5-HT_7 receptor using the gramicidin-perforated patch-clamp technique in male mice.

2. Results

Using a gramicidin-perforated patch technique, a total of 80 SG neurons were recorded from the Vc of 65 male mice. Of the 70 SG neurons tested, the bath application of 30 μM 8-OH-DPAT alone produced depolarization in 33 (47%) neurons, hyperpolarization in 16 (23%) neurons and no response in 21 (30%) neurons (Fig. 1). The mean resting membrane potential (RMP) and the mean amplitude of the membrane potential change was -60.9 ± 1.3 mV and 5.7 ± 0.5 mV ($n=33$), respectively, in neurons showing a depolarizing response to 8-OH-DPAT,

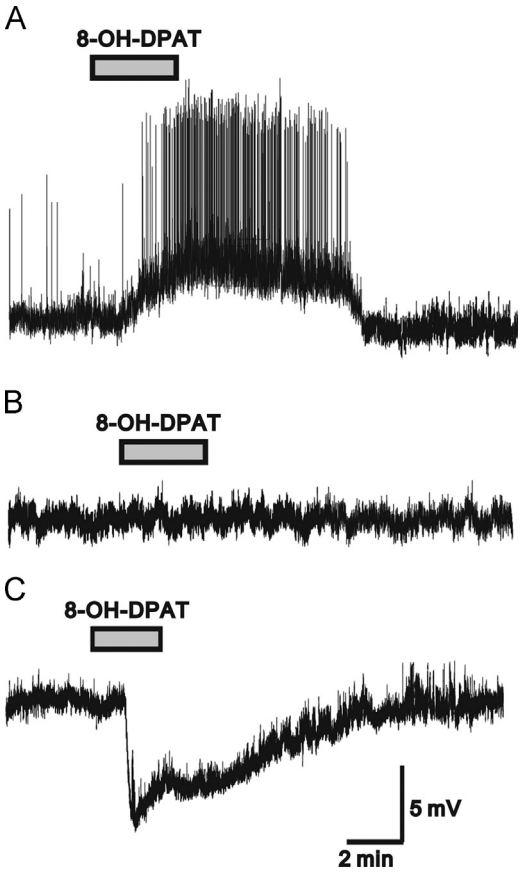

Fig. 1 – Responses of three different substantia gelatinosa (SG) neurons of the trigeminal subnucleus caudalis (Vc) to 8-OH-DPAT in gramicidin-perforated patch recordings under current-clamp mode. Application of 30 μM 8-OH-DPAT alone produced depolarization (A), no response (B), or hyperpolarization (C), respectively. Bars indicate the duration of 8-OH-DPAT application.