BRAIN RESEARCH 1368 (2011) 91–101

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BRAIN RESEARCH

Research Report

Effects of 5-hydroxytryptamine on substantia gelatinosa neurons of the trigeminal subnucleus caudalis in immature mice

Hua Yin[1], *Seon Ah Park*[1], *Seong Kyu Han*, *Soo Joung Park*[*]

Department of Oral Physiology and Institute of Oral Bioscience, School of Dentistry, Chonbuk National University, Jeonju, Jeonbuk, 561-756, Republic of Korea

ARTICLE INFO

Article history:
Accepted 14 October 2010
Available online 26 November 2010

Keywords:
5-hydroxytryptamine
Trigeminal subnucleus caudalis
Substantia gelatinosa
Gramicidin-perforated patch clamp
Postsynaptic
Single-cell RT-PCR

ABSTRACT

Serotonin (5-hydroxytryptamine, 5-HT) is involved in the descending modulation of nociceptive transmission in the spinal dorsal horn. The trigeminal subnucleus caudalis (Vc; medullary dorsal horn) processes nociceptive input from the orofacial region, and 5-HT-containing axons are numerous in the superficial layers of the Vc. This study examined the actions of 5-HT on the substantia gelatinosa (SG) neurons of the Vc, using gramicidin-perforated patch-clamp recording in brainstem slice preparations from immature mice. In order to clarify the possible mechanisms underlying 5-HT actions in the SG of the Vc, the direct membrane effects of 5-HT and effects of 5-HT receptor subtype agonists were examined. 5-HT induced a hyperpolarization in the majority (64/115, 56%) of the SG neurons tested. Thirty nine (34%) SG neurons showed no response, and 12 (10%) neurons responded with depolarization. The hyperpolarizing response to 5-HT was concentration-dependent (0.1–30 μM; $n=7$), not desensitized by repeated application ($n=22$), and significantly attenuated by Ba^{2+} (K^+ channel blocker; $n=8$). The 5-HT-induced hyperpolarization was maintained in the presence of TTX (Na^+ channel blocker), CNQX (non-NMDA glutamate receptor antagonist), AP5 (NMDA glutamate receptor antagonist), picrotoxin ($GABA_A$ receptor antagonist), and strychnine (glycine receptor antagonist), indicating direct postsynaptic action of 5-HT on SG neurons ($n=7$). The 5-HT-induced hyperpolarizing effects were mimicked by 8-OH-DPAT (5-HT_{1A} receptor agonist) and α-methyl-5-HT (5-HT_2 receptor agonist) and blocked by WAY-100635 (5-HT_{1A} receptor antagonist) and ketanserin (5-HT_2 receptor antagonist). Single-cell RT-PCR also revealed the presence of mRNA for 5-HT_{1A} and 5-HT_{2C} subtypes in the SG neurons. These results suggest that 5-HT acts directly on SG neurons and 5-HT-induced hyperpolarization is mediated, in part, by 5-HT_{1A} receptors and 5-HT_2 receptors, as well as by the activation of K^+ channels, indicating an

* *Corresponding author*. School of Dentistry, Chonbuk National University, 664-14, 1 Ga, Deokjin-Dong, Jeonju, Jeonbuk, 561-756, Republic of Korea. Fax: +82 63 270 4004.
E-mail address: soopark@jbnu.ac.kr (S.J. Park).

Abbreviations: ACSF, artificial cerebrospinal fluid; AP5, D,L-2-amino-5-phosphonopentanoic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; EC_{50}, effective concentration producing half-maximal response; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; mCPBG, 1-(m-chlorophenyl)-biguanide; RT-PCR, reverse transcriptase-polymerase chain reaction; SG, substantia gelatinosa; TTX, tetrodotoxin citrate; Vc, trigeminal subnucleus caudalis; WAY-100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate; 5-HT, 5-hydroxytryptamine; 8-OH DPAT, (±)-8-hydroxy-2-(di-n-propylamino) tetralin

[1] The first two authors contributed equally to this study.

0006-8993/$ – see front matter
doi:10.1016/j.brainres.2010.10.050

important role for 5-HT in the modulation of orofacial nociceptive processing at the level of the SG of the Vc in mice.

1. Introduction

The substantia gelatinosa (SG, lamina II) of the spinal dorsal horn receives nociceptive inputs from thin myelinated Aδ- and unmyelinated C-primary afferent fibers and has been considered a critical site for the modulation of nociceptive transmission to the ascending sensory pathway neurons (Cervero and Iggo, 1980; Todd, 2002). The trigeminal subnucleus caudalis (Vc; also called the medullary dorsal horn) is structurally and functionally similar to the spinal dorsal horn and is the major relay site of orofacial nociceptive information from the periphery to the higher centers in the brain (Sessle, 2000). In the trigeminal sensory nuclear complex of the rat, the density of the distribution of 5-HT-immunoreactive fibers and axon terminals was much higher in the superficial laminae of the Vc than in the deeper laminae of the Vc or other subnuclei (Cropper et al., 1984; Li et al., 1997). The descending serotonergic pathway from the rostral ventromedial medulla, particularly the nucleus raphe magnus to the Vc, as well as the spinal dorsal horn, plays an important role in the modulation of nociceptive transmission (Chiang et al., 1994; Millan, 2002; Suzuki et al., 2004).

Serotonin (5-hydroxytryptamine, 5-HT) acts on multiple receptor subtypes that are classified into seven groups (5-$HT_{1–7}$; Hoyer et al., 2002). All of the 5-HT receptors are G-protein-coupled receptors, except for the 5-HT_3 receptor, which is a ligand-gated ion channel. 5-HT can produce either antinociceptive or pronociceptive effects depending on which 5-HT receptor subtype is activated and the nature of the nociceptive stimulus at the level of the spinal cord (Bardin et al., 1997, 2000; Millan, 2002; Jeong et al., 2004) and the Vc (Seo et al., 2002; Okamoto et al., 2005, 2007), although conflicting results have been reported even with regard to the same receptor subtype. Among several subtypes of 5-HT receptors, 5-HT_1, 5-HT_2, and 5-HT_3 receptors have been identified in the Vc (Pompeiano et al., 1992; Tecott et al., 1993; Morales et al., 1998; Cornea-Hébert et al., 1999; Fonseca et al., 2001; Miquel et al., 2002; Doly et al., 2004b). Previous anatomical studies have suggested that 5-HT receptors are located postsynaptically on cell bodies and dendrites or presynaptically on axons and nerve terminals in the Vc (Pompeiano et al., 1992; Cornea-Hébert et al., 1999; Miquel et al., 2002).

In the rat and guinea pig, 5-HT produces hyperpolarization or outward current in the majority of superficial dorsal horn neurons in the spinal cord or in the Vc, a process that may be mediated by 5-HT_{1A} receptors (Grudt et al., 1995; Lu and Perl, 2007; Abe et al., 2009). 5-HT also increases the frequency of spontaneous inhibitory postsynaptic potentials in some SG neurons of the Vc (Grudt et al., 1995) and induces a presynaptic suppression and/or a facilitation of excitatory glutamatergic transmission in the superficial dorsal horn of the rat (Hori et al., 1996; Ito et al., 2000; Jennings et al., 2004).

Although several studies have examined the mechanisms of 5-HT-induced inhibitory effects on nociceptive transmission in the spinal and medullary dorsal horns, electrophysiological evidence of the 5-HT effect on SG neurons of the Vc in mice has not yet been well studied. In this study, we examined the effects of 5-HT and its receptor subtype agonists on SG neurons and determined if 5-HT acts directly on postsynaptic neurons located in the SG to clarify the site of action of 5-HT in the Vc of mice, using the gramicidin-perforated patch clamp technique. Single-cell reverse transcriptase-polymerase chain reaction (RT-PCR) was also performed to examine whether or not SG neurons express 5-HT receptor subtype mRNA.

2. Results

Gramicidin-perforated patch recordings were made from a total of 115 SG neurons in the current-clamp mode. The mean resting membrane potential of SG neurons was –60.6±2.1 mV (n=115).

2.1. 5-HT–induced responses in SG neurons

The application of 30 μM 5-HT resulted in a hyperpolarization in 64 (56%) of the 115 SG neurons (Fig. 1A). The mean resting membrane potential and the mean amplitude of the hyperpolarization were –60.2±3.3 mV and –10.1±0.8 mV, respectively. Thirty-nine (34%) of the 115 neurons were not affected by 5-HT (Fig. 1B), and the mean resting membrane potential

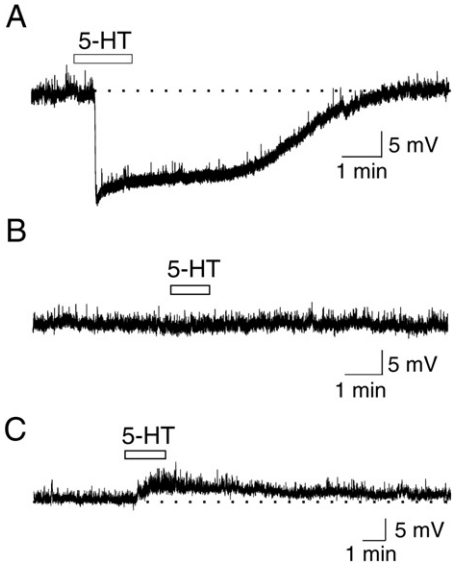

Fig. 1 – Responses of three different substantia gelatinosa (SG) neurons of the trigeminal subnucleus caudalis (Vc) to 5-HT in gramicidin-perforated patch recordings under current-clamp mode. Application of 30 μM 5-HT produced hyperpolarization (A), no response (B), or depolarization (C), respectively. Bars indicate the duration of 5-HT application.