available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Inhibitory effects of somatostatin on the substantia gelatinosa neurons of trigeminal subnucleus caudalis via somatostatin type 2 receptors in juvenile mice

Hua Yin[a,1], *Kyung Eun Lee*[b,1], *Seon Ah Park*[a], *Janardhan P. Bhattarai*[a], *Bong Jik Suh*[b], *Jae Gyu Jeon*[c], *Byung Gook Kim*[d], *Soo Joung Park*[a], *Seong Kyu Han*[a,*]

[a]*Department of Oral Physiology and Institute of Oral Bioscience, School of Dentistry, Chonbuk National University, Jeonju, 561-756, Republic of Korea*

[b]*Department of Oral Medicine, School of Dentistry, Chonbuk National University, Jeonju, Republic of Korea*

[c]*Department of Preventive Dentistry, Institute of Oral Bioscience & BK21 program, School of Dentistry, Chonbuk National University, Jeonju, Republic of Korea*

[d]*Department of Oral Medicine, School of Dentistry, Chonnam National University, Gwangju, Republic of Korea*

ARTICLE INFO

Article history:
Accepted 17 September 2009
Available online 24 September 2009

Keywords:
Somatostatin
Substantia gelatinosa
Trigeminal brainstem subnucleus caudalis
Gramicidin perforated patch clamp

ABSTRACT

The substantia gelatinosa (SG) of the trigeminal subnucleus caudalis (Vc) receives many thin-myelinated Aδ-fiber and unmyelinated C primary afferent fibers and has been implicated in the processing of nociceptive information. Somatostatin (SST) is a neuromodulator in the brain and spinal cord. A number of studies have demonstrated that SST can play a key role in pain modulation at the spinal cord level. However, there is little information available on functional SST receptor expression in the SG neurons of the Vc in mice. This study examined the direct membrane effects of SST and SST receptor type 2 agonist, seglitide (SEG) on the SG neurons of Vc in gramicidin perforated current clamp mode. In addition, SSTR2 mRNA expression was detected on the SG neurons using single cell RT-PCR in juvenile mice. Most SG neurons (37/68, 54%) were hyperpolarized after a bath application of SST. When SST was applied in stages, the second responses (83% of the first response) were less intense than those after the first application suggesting that SSTRs are desensitized by repeated application. The SST-induced hyperpolarizing response was maintained in the presence of TTX (Na^+ channel blocker), AP-5 (NMDA receptor antagonist), CNQX (non-NMDA glutamate receptor antagonist), picrotoxin ($GABA_A$ receptor antagonist) and strychnine (glycine receptor antagonist), respectively, suggesting that SST has direct effects on the postsynaptic SG neurons. SSTR2 mRNA was detected in 11 out of 28 (39%) SG neurons tested. The SST-induced hyperpolarizing effects were mimicked by SEG, a SSTR2 agonist. These results suggest that functional SSTR2 receptors are expressed on the SG neurons of Vc in juvenile mice and can be a potential target for modulating orofacial pain.

* *Corresponding author*. Fax: +82 63 270 4004.
E-mail address: skhan@chonbuk.ac.kr (S.K. Han).

[1] First two authors contributed equally to this work.

0006-8993/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2009.09.070

1. Introduction

The neuropeptide, somatostatin (SST), is synthesized and released in the hypothalamus and transported to the anterior pituitary gland through the portal circulation. SST inhibits the release of growth hormone from the somatotropes and blocks the secretion of prolactin, thyrotropin-stimulating hormone, and adrenocorticotropin hormone (Lamberts, 1988). SST is distributed widely through the peripheral and central nervous system and affects various physiological actions, including sensory information processing (Epelbaum et al., 1994) by binding to the G-protein coupled SST receptors (Schindler et al., 1996; Lahlou et al., 2004).

The substantia gelatinosa (SG) neurons of the trigeminal subnucleus caudalis (Vc), which is also called the medullary dorsal horn, play an important role in orofacial nociceptive transmission (Sessle, 1996). A number of studies have suggested that SST and its receptors are involved in the modulation of orofacial pain. Trigeminal ganglion neurons innervating to the superficial layer of Vc show immunoreactivity to the SST receptors (Ichikawa et al., 2003; Takeda et al., 2007). The SST type 2A receptor is co-expressed with the vanilloid type 1 and calcitonin gene related peptide receptors, which are related to pain processing (Ichikawa et al., 2003). SSTR2 immunoreactivity is also detected in the neuronal cell bodies of SG neurons of the Vc (Ichikawa et al., 2003). Centrally applied SST analogues reduce the number of c-Fos positive neurons at the Vc (Bereiter, 1997). The intrathecal administration of SST has antinociceptive effects in vivo (Mollenholt et al., 1994).

In the spinal dorsal horn, SST inhibits the SG neuronal activities directly by potentiating the voltage dependent K^+ current but is not mediated by the activation of either GABA or glycine receptors affecting the presynaptic mechanism (Jiang et al., 2003). Although several studies have examined the mechanisms of SST-induced inhibition on nociceptive transmission in the spinal dorsal horn, there is no electrophysiological evidence of the SST action on SG neurons of Vc in mice reported thus far. Because mice are a powerful genetically-manipulable experimental model, the data from mice may provide important information on antinociception. Therefore, this study examined the effects of SST and clarified the SST receptor subtype related to the response using the gramicidin-perforated patch clamp technique. Single cell RT-PCR was also performed for the detection of SSTR2 mRNA on SG neurons of the Vc in juvenile mice.

2. Results

2.1. *Somatostatin-mediated responses on SG neurons*

The SG (lamina II) of the Vc was clearly visible as a translucent band, just medial to the spinal trigeminal tract and traveled along the lateral edge of the slice. In total, the gramicidin perforated-patch clamp recordings were obtained from 68 SG neurons (RMP, −67.4±1.43 mV, n=68). In perforated current clamp mode, the bath application of 300 nM SST caused the following: membrane hyperpolarization (Fig. 1A), no response

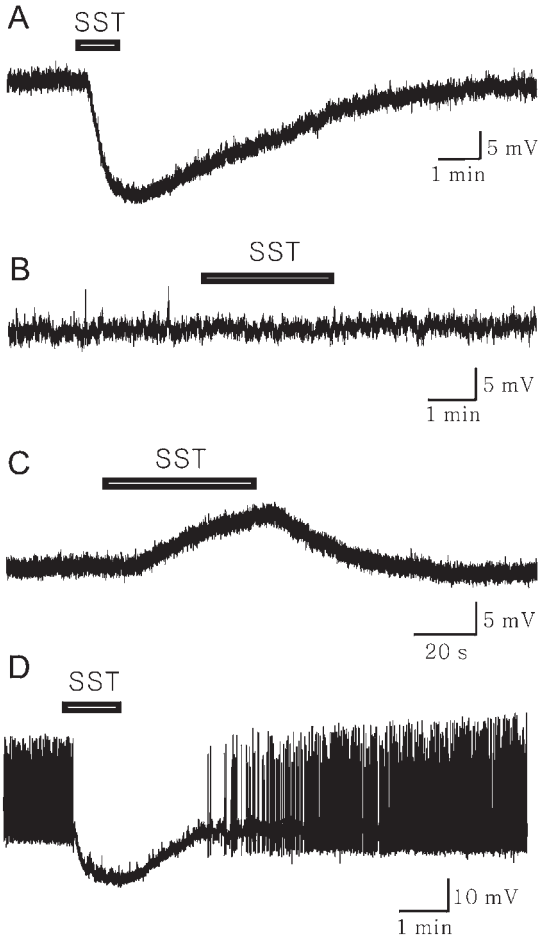

Fig. 1 – Somatostatin (SST) induced various responses on the SG neurons of the Vc in juvenile mice. (A) Gramicidin perforated representative voltage trace depicting hyperpolarization induced by SST (300 nM) applied to a SG neuron. (B) A trace from a SG neuron showing no response to SST (300 nM). (C) A representative trace showing membrane depolarization of a SG neuron. (D) Representative trace showing hyperpolarization by SST in a SG neuron showing spontaneous action potentials. The bars indicate the duration of SST application.

(Fig. 1B), membrane depolarization (Fig. 1C) or membrane hyperpolarization with a decrease in the action potentials in neurons showing spontaneous activity (Fig. 1D). In males, 22 (59.5%) out of 37 SG neurons were hyperpolarized (−9.84±1.36 mV, n=22) by 300 nM SST application and one neuron was depolarized (1.46 mV). However, 14 (48.3%) neurons were not affected by SST. In females, 48.3% (15/31) of the neurons tested were hyperpolarized (-11.4±1.35 mV, n=15) by 300 nM SST application and 2 neurons were depolarized (4.04±0.14 mV, n=2). However, 14 (45.2%) out of 31 neurons were unaffected by SST.

In order to determine whether SG neurons are desensitized by the repeated application of SST, 300 nM SST was applied in stages to the SG neurons showing hyperpolarizing responses. Fig. 2A shows the short-lived and repeatable hyperpolarization by the application of SST. The 2nd response by SST application was less intense than that of the first application. Fig. 2B shows a comparison of the membrane potential