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Tooth-pulp-evoked rostral spinal trigeminal nucleus neuron activity is inhibited by conditioning sciatic nerve stimulation in the rat: possible role of 5-HT_3 receptor mediated GABAergic inhibition

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Abstract

The purpose of the present study was to determine whether modulation of the trigeminal spinal nucleus oralis (TSNO) neurons related to tooth-pulp (TP)-evoked jaw-opening reflex (JOR) after electrical stimulation of the sciatic nerve (SN) is mediated by the descending serotonergic (5-HT_3) inhibitory system activated by inhibitory GABAergic interneurons. In 30 anesthetized rats, the activity of TSNO neurons (87.5%, 35/40) and all digastric muscle electromyograms (dEMG, $n = 30$) in response to TP stimulation (at an intensity of 3.5 times the threshold for JOR) were inhibited by conditioning stimulation of the SN (5.0 mA × 0.5 ms, 1 Hz, conditioning-test intervals; 50 ms). The inhibitory effects were significantly attenuated after intravenous administration of the 5-HT_3 receptor antagonist ICS 205-930 ($n = 6$). Using multibarrel electrodes, iontophoretic application of ICS 205-930 into the TSNO significantly reduced the SN stimulation-induced inhibition of TP-evoked TSNO neuronal excitation ($n = 6$), and in the same neurons, iontophoretic application of the $GABA_A$ receptor antagonist bicuculline into the TSNO greatly inhibited their effect. On the other hand, we found the expression of 5-HT_3 receptor immunoreactive neurons in the TSNO. These results suggest that SN stimulation may activate the descending serotonergic (5-HT_3) inhibitory system through activation of inhibitory GABAergic interneurons, which inhibit excitatory responses of the TSNO neurons to TP stimulation.

Keywords: Tooth-pulp; Trigeminal spinal nucleus oralis; 5-HT_3 receptor; $GABA_A$ receptor; Iontophoretic application; Sciatic nerve

1. Introduction

The trigeminal spinal nucleus is an important relay station in the transmission of orofacial sensory information, and this nucleus is functionally and anatomically subdivided into three nuclei from the rostral to the caudal: oralis, interpolaris, and caudalis [31,33]. Among them the nucleus oralis conveys information for the nociceptive reflex, e.g., the jaw-opening reflex (JOR) from the orofacial region, including tooth-pulp (TP) [8,34,37,38]. The JOR is a masticatory reflex [20], and the TP-evoked JOR has been considered a pain induced response model if it is evoked by adequate TP stimulation. The majority of sensory neurons in the JOR arc are located in the trigeminal spinal nucleus oralis (TSNO) [13], which projects to the trigeminal motor nucleus of the digastric muscle [23,26,35].

On the other hand, it has been reported that the TP-evoked JOR is depressed by sensory inputs arising elsewhere on the body. For example, electrical stimulation of the saphenous

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nerve inhibits the JOR evoked by TP stimulation in a certain conditioning-test (C-T) paradigm and this inhibition may be mediated by neural pathways involving the periaqueductal gray matter (PAG) and/or nucleus raphe magnus (NRM) [5]. The TP-evoked JOR is known to be inhibited by antinociception pathways mediated by the PAG and/or NRM [27,32,39] as well as by analgesic drugs [8]. It is well known that stimulation of the PAG and NRM inhibits the activity of neurons in both the spinal cord and the trigeminal complex [2,15]. In particular, immunohistochemical studies demonstrated that terminals of serotonin (5-hydroxytryptamine; 5-HT) neurons were observed in the spinal cord as well as in the trigeminal complex originating from in the brainstem nuclei, and that activation of descending serotonergic pathways induced by electrical stimulation of NRM [2,15] inhibits spinal transmission of nociceptive information.

Concerning 5-HT receptors related to the spinal antinociception, different binding sites such as 5-HT_1, 5-HT_2 and 5-HT_3 receptors have been implicated in the analgesic effect of 5-HT [4,29]. In the TP-evoked pain transmission, Tanimoto et al. [40] reported that TP-evoked C1 spinal neurons were inhibited by conditioning stimulation of vagal afferents and this inhibitory effect was attenuated by intravenous administration of a 5-HT_3 receptor antagonist, ICS 205-930, in rats. In addition, Yonehara [43] found that TP stimulation evoked substance P release in the trigeminal spinal nucleus caudalis (TSNC) was decreased by electro-acupuncture and this decrease was antagonized by intravenous administration of ICS 205-930 in rabbits. These reports indicated the possibility that the descending serotonergic system related to 5-HT_3 receptors acts to inhibit TP-evoked nociceptive transmission. Furthermore, in the spinal cord, iontophoretic application of γ-aminobutyric-acid type A ($GABA_A$) receptor antagonists blocks the inhibitory action of a 5-HT_3 receptor agonist applied iontophoretically [1]. This probably implies that endogenously released 5-HT in the spinal cord can activate inhibitory interneurons, which in turn promote the release of GABA. Cropper et al. [7] reported that 5-HT-immunoreactive fibers were found in the TSNO. In addition, there is evidence that 5-HT_3 receptor-immunoreactive neurons were broadly distributed throughout the rat brain and spinal cord, possibly including the trigeminal spinal nucleus [24]. These results lead us to suggest that excitation of the 5-HT_3 receptors located in the TSNO may activate the descending serotonergic inhibitory system through inhibitory GABAergic interneurons.

Thus the question arises as to whether the descending serotonergic inhibitory pathways activated by electrical stimulation of the sciatic nerve (SN) modify the activity of TSNO neurons responding to TP stimulation in rats, but there are no studies examining such an interaction. It has been reported that microiontophoretic method using multibarrel microelectrodes is an effective clue to analyse the local neural circuit [36]. By using two different applications (intravenous and iontophoretic applications) of drugs, we can determine how the drug acts TSNO neurons. Therefore, in the present study, we examined the responses of TP-evoked TSNO neuronal activity to SN stimulation before and after intravenous administration of ICS 205-930 as well as before and after local iontophoretic application of ICS 205-930 or bicuculline in the TSNO. Furthermore, we also examined whether the 5-HT_3 receptor expresses in the TSNO with immunohistochemical technique.

2. Materials and methods

2.1. Animal preparation

The experiments were performed on 30 adult male Wistar rats (270–330 g). All experimental protocols used in this study were approved by the Animal Use and Care Committee at the Nippon Dental University and were consistent with the ethical guidelines of the International Association for the Study of Pain. Each animal was initially anesthetized with sodium pentobarbital (45 mg/kg, i.p.) and maintained with additional doses of 2–3 mg/kg/h as required, through a cannula in the jugular vein. The trachea was cannulated. The rectal temperature was maintained at $37 \pm 0.5\,^{\circ}C$ with a radiant heater. Arterial blood pressure (ABP) was monitored by means of a pressure transducer through a cannula inserted into the femoral artery. The depth of anesthesia was checked by the ABP as well as by the lack of response to paw pinching. All wound margins were covered with a local anesthetic, 2% lidocaine (Xylocaine, Fujisawa Med., Japan), repeatedly throughout the experiment.

2.2. Tooth-pulp stimulation

The method of TP stimulation was similar to that in previous reports [37,38,40]. In brief, bipolar stimulating electrodes made from silver wire (diameter 150 μm, enamel-insulated expect for the tip) were inserted into the pulp of the upper incisors and insulated from the surrounding tissue with dental cement to limit current spread.

2.3. Sciatic nerve stimulation

The right SN was isolated from the connective tissues. The nerve was cut and its proximal portion placed across a pair of silver wires with the cathode proximal. The stimulating electrodes were embedded in a cuff made from a polyethylene tube which was implanted in the muscle around the SN, and the nerve was bathed in warm mineral oil to prevent it from drying as described in the method for electrical stimulation of vagal afferents [38,40].

2.4. Intravenous administration of drugs

We used ICS 205-930 (1.0 and 3.0 mg/kg) (RBI, USA), a 5-HT_3 receptors antagonist, on the basis of a previous report, showing a significant influence on the analgesic effect in the