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BRAIN RESEARCH

Short Communication

Tooth-pulp-evoked rostral spinal trigeminal neuronal excitation is attenuated by the activation of 5-HT_3 receptors via GABAergic interneurons in the rat

Katsuo Oshima[a,*], *Mamoru Takeda*[b], *Takeshi Tanimoto*[b], *Ichiroh Katsuumi*[c], *Shigeji Matsumoto*[b]

[a]*General Dentistry of Univ. Hospital, The Nippon Dental University, School of Dentistry at Tokyo, 2-3-16, Fujimi, Chiyoda-ku, Tokyo 102-8158, Japan*
[b]*Department of Physiology, The Nippon Dental University, School of Dentistry at Tokyo, 1-9-20, Fujimi, Chiyoda-ku, Tokyo 102-8159, Japan*
[c]*Department of Endodontics and Operative Dentistry, The Nippon Dental University, School of Dentistry at Tokyo, 1-9-20, Fujimi, Chiyoda-ku, Tokyo 102-8159, Japan*

ARTICLE INFO

Article history:
Accepted 14 June 2006
Available online 11 July 2006

Keywords:
Tooth-pulp
Pain
Trigeminal spinal nucleus oralis
5-HT_3 receptor
$GABA_A$ receptor

ABSTRACT

The effect of iontophoretic application of the 5-HT_3 receptor agonist, phenylbiguanide (PBG), on the excitation of the trigeminal spinal nucleus oralis (TSNO) neurons to tooth-pulp (TP) stimulation was examined. The PBG application inhibited the TP-evoked TSNO neuronal excitation, and this inhibition was completely blocked by co-application of a $GABA_A$ receptor antagonist, bicuculline. The results suggest that the activation of 5-HT_3 receptors elicits GABA release in the TSNO.

The trigeminal spinal nucleus is a relay station in the transmission of trigeminal sensory information (Sessle, 1987), particularly in which the trigeminal spinal nucleus oralis (TSNO) plays an important role in the processing of nociceptive information from the orofacial region including tooth-pulp (TP), such as the jaw-opening reflex (JOR) (Takeda et al., 1998). With regard to the trigeminal antinociception related to 5-hydroxytryptamine (5-HT) receptors, it has been demonstrated that 5-HT_3 receptors are implicated in the antinociceptive effect (Seo et al., 2002; Takagi and Yonehara, 1998; Tanimoto et al., 2002). We demonstrated that the TP-evoked TSNO neuronal activity was inhibited by conditioning stimulation of the sciatic nerve (SN), and this inhibitory effect was attenuated by iontophoretic application of a 5-HT_3 receptor antagonist or a $GABA_A$ receptor antagonist. In addition, we found that the intense 5-HT_3 receptor immunoreactive cells were broadly distributed in the TSNO (Oshima et al., 2005). In the spinal antinociception, Alhaider et al. (1991) reported that iontophoretic application of a $GABA_A$ receptor antagonist partially blocked the inhibitory effect of 2-methyl 5-HT on NMDA-induced excitation of the dorsal horn neuron activity, suggesting the existence of GABAergic interneurons.

In our previous study (Oshima et al., 2005), the ratio of disinhibition of SN-induced inhibition of TP-evoked TSNO neuronal excitation after intravenous administration of the 5-HT_3 receptor antagonist ICS 205-930 was similar to that seen

* *Corresponding author*. Fax: +81 3 3261 3924.
E-mail address: oshima-k@tky.ndu.ac.jp (K. Oshima).

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doi:10.1016/j.brainres.2006.06.036

after iontophoretic application of ICS 205-930. Furthermore, Takeda et al. (2000) reported that the microiontophoretic application using multibarrel microelectrodes was an effective clue to analyze the local neural circuit. Taken together, these observations show that it is possible that local 5-HT_3 receptor-mediated pathways may play an important role in the TSNO. Thus, the questions arise (1) whether endogenously released 5-HT inhibits TSNO neuronal activity to TP and (2) whether this inhibition is mediated by the activation of GABAergic interneurons. Yet, there are no studies examining these questions. Therefore, in this study, we further examined whether the activation of 5-HT_3 receptors elicits GABA release, inhibiting the TP-evoked neuronal excitation in the TSNO, using the technique with an iontophoretic application.

The experiments were approved by the ethical guidelines of the International Association for the Study of Pain. The experimental procedure was based on our previous reports (Oshima et al., 2005). A total of 5 male Wistar rats (BW. 270–330 g) anesthetized with sodium pentobarbital (45 mg/kg, i.p.) and bipolar stimulating electrodes made from silver wire were inserted into the pulp of the upper incisors. The rats were then placed in a stereotaxic apparatus, and a large occipital craniotomy was performed. The electromyogram (EMG) recorded the digastric muscle as an assessment of the JOR. The intensity of TP stimulation was similar to that in previous reports (stimulus intensity of 3.5×threshold for dEMG). For single-unit activity recordings, a single barrel in the five-barreled glass micropipettes, which was filled with 2% pontamine sky blue with 0.5 M sodium acetate, was inserted into the TSNO (Oshima et al., 2005; Takeda et al., 1998). Of the five lateral barrels of the micropipette, one barrel containing 160 mM NaCl was used for balancing currents to prevent the occurrence of tip polarization artifacts. The remaining barrels contained aqueous solution: bicuculline methiodide (Sigma), a $GABA_A$ receptor antagonist, 5 mM in 160 mM NaCl, pH3.5, ICS 205-930 (Sigma), a 5-HT_3 receptors antagonist, 10 mM in 160 mM NaCl, pH5.0, and phenylubiguanide (PBG) (Sigma), a 5-HT_3 receptors agonist, 10 mM in 160 mM NaCl, pH4.5. The currents for ejecting, retaining and balancing were provided by a constant current unit (Dia Medical, DPI-25, Japan). ICS 205-930 were ejected with 60 nA cationic currents, and bicuculline was ejected with cationic currents of 30 nA. PBG was ejected with cationic currents of 40–80 nA, and 10–25 nA retaining currents were used.

We first determined the effect of the iontophoretic application of PBG (40–80 nA, 5 min) on TSNO activity. Before PBG treatment, the vehicle (the same volume as saline) was administrated into the TSNO and no significant changes in the neuronal activity were observed. We decided upon an approved current (80 nA) and unit number (6 neurons), particularly in which PBG could inhibit TP-evoked TSNO neuronal excitation. Furthermore, we examined the effect of iontophoretic application of ICS 205-930 (60 nA, 5 min) on the PBG application-induced modulation of TP-evoked TSNO neuronal excitation. After confirming the absence of modulated effects of these drugs, in the same TSNO neurons, we also tested the effects of iontophoretic application of bicuculline (30 nA) on the PBG-induced modulation of TP-evoked TSNO neuronal excitation. The effects of ICS 205-930 (60 nA) and bicuculline (30 nA) on the PBG-induced TSNO neuronal modulation were evaluated using the same currents as reported in a previous study (Oshima et al., 2005). The neuronal activity was recorded on a polygraph (NEC-Sanei, 8M14) and stored on magnetic tape for off-line analysis. Trials of 16 responses were summed to construct the poststimulus histogram. Statistical significance of the effects of drugs was calculated using one-way ANOVA. A P value less than 0.05 was considered statistically significant. Data were expressed as means±SEM. After the recording sessions, the rats were deeply anesthetized. Anodal electrolytic lesions (DC, 50 μA for 1 min) were made to mark the recoding sites. The animals were transcardially perfused with 10% formalin. Frozen coronal sections were cut into 30 μm thicknesses and stained with hematoxylin–eosin. The unit location recorded was identified from the blue spots, and electrode tracks were constructed by means of a combination with micromanipulator readings.

Extracellular single unit activity was recorded from 10 neurons in the TSNO. As the stimulus intensity of TP stimulation was increased, TSNO neuronal activities increased proportionally. The threshold of TP stimulation for activation of 10 TSNO neurons was 0.9±0.3 mA, and the onset latency in those neurons during TP stimulation was 5.7±0.5 ms. The average value for conduction velocity was 8.5±0.3 m/s.

As shown in Figs. 1A and B, after iontophoretic application of PBG (40, 60 and 80 nA, 5 min), the TP-evoked TSNO neuronal excitation was inhibited. The inhibition of TSNO neuronal activity following iontophoretic application of PBG was current-dependent (60 and 80 nA, 5 min, $P<0.05$). Of 10 units, 6 units were inhibited by PBG application. But the remaining 4 units were not significantly altered by PBG application. As shown in Fig. 1D, these neurons were located in the dorsal part of the subnucleus oralis.

In 6 TSNO neurons, we also examined whether iontophoretic co-application of a 5-HT_3 receptor antagonist or a $GABA_A$ receptor antagonist attenuates PBG-induced inhibition of the TP-evoked TSNO neuronal excitation. As shown in Figs. 2A–C, the TP-evoked TSNO neuronal excitation was inhibited by PBG application (80 nA, 5 min) and this inhibition was abolished by ICS 205-930 application at 60 nA (5 min) injection currents. After confirming the absence of drug effects, of the same neuron the TP-evoked TSNO neuronal excitation was also inhibited by PBG application (80 nA, 5 min) and this inhibitory effect was abolished by bicuculline at 30 nA (5 min) injection current (Figs. 2D–F). These results are summarized in Fig. 2G. The PBG-induced inhibition of TP-evoked TSNO neuronal excitation was significantly suppressed by iontophoretic co-application of ICS 205-930 or bicuculline ($P<0.05$), but no significant differences were found in their suppressive effects on the TP-evoked TSNO neuronal excitation (Fig. 2G).

It is known that the 5-HT_3 receptor plays a significant role in trigeminal antinociception (Seo et al., 2002; Takagi and Yonehara, 1998; Tanimoto et al., 2002). Immunohistochemical studies have demonstrated that terminals of serotonin neurons were observed in the TSNO (Morales et al., 1998; Oshima et al., 2005), and that activation of descending serotonergic pathways induced by SN-stimulation inhibits transmission of nociceptive information (Oshima et al., 2005). In the present study, the ratio of PBG-induced inhibition of TP-evoked TSNO neuronal excitation was approximately 30.0%,