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Role of the red nucleus in suppressing the jaw-opening reflex following stimulation of the raphe magnus nucleus

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ABSTRACT

In a previous study, we found that electrical and chemical stimulation of the red nucleus (RN) suppressed the high-threshold afferent-evoked jaw-opening reflex (JOR). It has been reported that the RN receives bilaterally projection fibers from the raphe magnus nucleus (RMg), and that stimulation of the RMg inhibits the tooth pulp-evoked nociceptive JOR. These facts imply that RMg-induced inhibition of the JOR could be mediated via the RN. The present study first examines whether stimulation of the RMg suppresses the high-threshold afferent-evoked JOR. The JOR was evoked by electrical stimulation of the inferior alveolar nerve (IAN), and was recorded as the electromyographic response of the anterior belly of the digastric muscle. The stimulus intensity was 4.0 (high-threshold) times the threshold. Conditioning electrical stimulation of the RMg significantly suppressed the JOR. A further study then examined whether electrically induced lesions of the RN or microinjection of muscimol into the RN affects RMg-induced suppression of the JOR. Electrically induced lesions of the bilateral RN and microinjection of muscimol into the bilateral RN both reduced the RMg-induced suppression of the JOR. These results suggest that RMg-induced suppression of the high-threshold afferent-evoked JOR is mediated by a relay in the RN.

1. Introduction

The jaw-opening reflex (JOR) is evoked by both innocuous and noxious stimulation of the orofacial region via one or more interneurons (Kidokoro et al., 1968; Sumino, 1971; Thexton, 1973). The JOR can be evoked by electrical stimulation of either the low- or high-threshold afferents of the trigeminal nerve. The low-threshold afferent-evoked JOR is believed to be evoked by innocuous stimulation, and the high-threshold afferent-evoked JOR is believed to be evoked by noxious stimulation (Lund, 1991; Lund and Olsson, 1983; Lund et al., 1984).

The raphe magnus nucleus (RMg) is implicated in the descending analgesic system and in the endogenous opiate-related mechanisms of analgesia (Basbaum and Fields, 1987; Cannon and Liebeskind, 1979). Stimulation of the RMg inhibits the tooth pulp-evoked nociceptive JOR (Iriki and Toda, 1982; Sessle and Hu, 1981; Tanaka and Toda, 1982). The effect is probably mediated by a direct pathway, since there is evidence for direct projections from the RMg to the ventromedial division of the trigeminal motor nucleus (vmMoV, jaw-opening motoneuron pool) (Li et al., 1995). It has also been suggested the effect is mediated indirectly on the other part of the JOR arc (Iriki and Toda, 1982). An interneuronal link in this indirect pathway could be located within the red nucleus (RN). It has been proposed that this region is involved in the development of neuropathic pain (Jing et al., 2009; Li et al., 2008; Wang et al., 2008, 2012; Zhang et al., 2013), and it receives afferent projections from the RMg bilaterally (Bernays et al., 1988). Immunocytochemical studies have found serotoninergic nerve terminals in the RN (Andre et al., 1987; Bosler et al., 1983), suggesting that the serotonin afferent fibers originate from the raphe nuclei (Andre et al., 1987). Our previous papers found that stimulation of the RN suppressed the tooth pulp-evoked nociceptive JOR (Yajima et al., 2012) and suppressed the high-threshold afferent-evoked JOR (Satoh et al., 2013). Suppression occurred when the conditioning-test interval was 20–60 ms. The excitatory interneurons for the JOR are located in the spinal trigeminal nucleus oralis (Vo) (Sumino, 1971). Activities of the Vo neurons were inhibited by applying a short train of stimulation to the RN; inhibition was greatest at a conditioning-test interval of 20–40 ms (Davis and Dostrovsky, 1986). Guided by these observations, we test our hypothesis that RMg-induced suppression of the JOR is probably mediated via the RN by examining whether:(1) RMg stimulation suppresses high-threshold afferent-evoked JOR; (2) RMg-induced suppression of the JOR is affected by electrically induced lesions of the bilateral RN; and (3) whether RMg-induced suppression of the JOR is affected by microinjection of muscimol into the same area. The aim of the present study is

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to examine whether the suppressive effects of RMg stimulation on the JOR is mediated by the RN.

2. Material and methods

These experiments were performed on 21 male Sprague–Dawley rats weighing 297–384 g. All animal procedures were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Laboratory Animal Committee of The Nippon Dental University School of Life Dentistry at Niigata. The rats were initially anesthetized with urethane and α-chloralose (500 mg/kg and 50 mg/kg, respectively; i.p.). Supplemental doses of urethane (50 mg/kg) and α-chloralose (5 mg/kg) were given via a cannula placed into the femoral vein, so as to maintain anesthesia at a level at which no withdrawal reflex was evoked by noxious stimulation of the paw. The trachea and the femoral artery were cannulated. Arterial blood pressure was monitored to confirm the condition of the rat throughout the experiment. The rat was used for data collection when the arterial blood pressure was more than 70 mmHg and was stable. Rectal temperature was maintained at 37 °C using a regulated heating pad.

To record the electromyogram, pairs of Teflon-coated silver wires (diameter 0.1 mm, exposed tip 2.0 mm) were inserted bilaterally into the anterior belly of the digastric muscles. Bipolar stimulating electrodes of silver wire (0.1 mm in diameter), insulated below the tip, were inserted into the left and right mandibular canal through the mental foramen, for electrical stimulation of the inferior alveolar nerve (IAN). Test stimulation was applied to the IAN (single pulse, 0.1 ms in duration, 1 Hz) ipsilateral to the RMg stimulation, so as to evoke the JOR. The threshold intensity for eliciting the JOR by stimulating the IAN was recorded from the electromyogram of the anterior belly of the digastric muscle ipsilateral to the IAN stimulation. The intensity of this test stimulation was set at 4.0 (high threshold) times the threshold for evoking the JOR in order to stimulate sufficiently nociceptive fibers like previous studies (Fukuhara et al., 2011; Satoh et al., 2013, 2014).

The head of the rat was fixed in a stereotaxic frame, using ear bars and an incisal bar. Parts of the parietal, interparietal and occipital bones were removed, using a dental drill to expose the cerebrum and the cerebellum. The cerebellum was removed by suction to expose the brainstem. A bipolar concentric electrode (outer diameter 250 μm) was inserted stereotaxically into the left or right RMg at an angle of 20°. A conditioning stimulation (1 pulse, 0.2 ms duration, 1 Hz, 100 μA) was applied to the RMg of 8 rats. The interval between the conditioning and test stimuli was varied between 5 and 100 ms. The control JOR responses were recorded, and also the effects induced by stimulation of the RMg.

In 5 of 8 rats as described above and also in a further 6 rats, bipolar concentric electrodes were stereotaxically inserted toward the RN bilaterally. The conditioning stimulation (1 pulse, 0.2 ms duration, 1 Hz, 100 μA) was applied to the RMg at 40 ms before the test stimulation, ipsilateral to the side undergoing IAN stimulation. The control JOR responses were recorded, and also the effects induced by stimulating the RMg. Next, bilateral RN lesions were created by the passage of an electric current (20 μA, 2 min). The control JOR and the effects of RMg stimulation on the JOR were tested after the lesions had been made.

In 7 rats, fine stainless pipes (diameter 0.1 mm) were inserted vertically toward the RN bilaterally. A fine stainless pipe, attached to a microelectrode and filled with muscimol dissolved in 0.9% saline (5 mM, Sigma), was attached to a 1 μl Hamilton syringe by polyethylene tubing. The control JOR responses were recorded, and also the effects induced by stimulating the RMg at 100 s before administration ceased. At that point 0.1 μl of muscimol was injected (injection duration 90 s). The control JOR and the effects of RMg stimulation on the JOR at a conditioning-test interval of 40 ms were tested, beginning from the end of the injection.

The electromyographic responses evoked by stimulation of the IAN were amplified (filter bandwidth 10 Hz to 1 kHz) and stored on computer disk. Data were analyzed using a computer and interactive software at a sampling rate of 2 kHz. The magnitude of the JOR was estimated as the peak-to-peak amplitude of electromyograms in the anterior belly of the digastric muscles. The average magnitude of the JOR elicited by 20 test (control) stimuli was compared with that evoked by 20 stimuli in the presence of the conditioning stimulus. The effects of the conditioning RMg stimulation are expressed as a percentage of the control value. Wilcoxon *t*-test was used to test the effects of electrical stimulation of the RMg on the JOR. Wilcoxon *t*-test with Bonferroni correction followed by Frideman's test for a post hoc test was used to test the effects of electric lesion and muscimol injection of the RN. Statistical differences were set at $P < 0.05$ (significant) and $P < 0.01$ (highly significant) level for all statistical tests.

At the end of each experiment, electrolytic lesions were made by passing negative direct current (20 μA for 90 s) through the RMg-stimulating electrodes or through microelectrodes attached to the stainless pipe used for muscimol injection in the RN. The animals were given an additional lethal dose of anesthetic, and the brain was fixed in 10% buffered formalin solution (pH 7.4). Serial coronal sections (60 μm thick) of the brainstem were cut and stained with cresyl violet. The stimulating and injection sites were verified according to a standard atlas (Paxinos and Watson, 2007). The main electrolytic lesion sites were determined as the centers of lesions or muscimol injections. The sizes of electrolytic lesions sites were determined as extent of lesions sites.

3. Results

The threshold intensity for eliciting the JOR by stimulating the IAN was 19–130 μA (62.1 ± 28.8 μA, mean ± SD, $n = 21$). Latency of the control JOR evoked by the IAN on the side ipsilateral to the RMg stimulation (6.1 ± 0.1 ms, mean ± SE, $n = 21$) did not change significantly when conditioning electrical stimuli were applied to the RMg at a conditioning-test interval of 40 ms (6.1 ± 0.1 ms, mean ± SE, $n = 21$, Wilcoxon *t*-test, $P > 0.05$).

3.1. Electrical stimulation of the RMg

The electrical stimulation sites in the RMg are shown in Fig. 1 on diagrams of the coronal sections. Fig. 2 shows a photomicrograph of a stimulus site in the RMg.

Conditioning electrical stimulation of the RMg significantly suppressed the JOR (Wilcoxon *t*-test, $P < 0.05$). Fig. 3A shows suppression of the JOR evoked on the ipsilateral side by stimulation of the RMg. Suppression reached a maximum of 85.6 ± 6.3% (mean ± SE, $n = 8$) of the control level at 30 ms (Fig. 3B, filled triangles). By 60 ms the JOR had returned to the control level.

3.2. Location of electric lesion and muscimol injection sites

Electrically induced lesions of the RN and microinjection of muscimol into the RN reduced the RMg-induced suppression of the JOR (Wilcoxon *t*-test with Bonferroni correction, $P < 0.05$).

Fig. 4 shows the sites of centers of the electrically induced lesion and the sites of centers of muscimol injection on diagrams of the coronal sections of the brainstem. The lesions and injections were successfully located within the RN bilaterally in each rat. The lesions were unsuccessfully located outside of the RN bilaterally. There were not any cases in which one lesion was in the RN and the other was outside it. The electrically created lesion sites that reduced the