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Role of the lateral reticular nucleus in suppressing the jaw-opening reflex following stimulation of the red nucleus

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ABSTRACT

We found in a previous study that stimulation of the red nucleus (RN) facilitated the low-threshold afferent-evoked jaw-opening reflex (JOR) and suppressed the high-threshold afferent-evoked JOR. It has been reported that the RN projections to the contralateral lateral reticular nucleus (LRt), and stimulation of the LRt inhibits the nociceptive JOR. These facts suggest that RN-induced modulation of the JOR is mediated via the LRt. We investigated whether electrically induced lesions of the LRt, or microinjection of muscimol into the LRt, affects RN-induced modulation of the 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 either 1.2 (low-threshold) or 4.0 (high-threshold) times the threshold. Electrically induced lesion of the LRt and microinjection of muscimol into the LRt reduced the RN-induced suppression of the high-threshold afferent-evoked JOR, but did not affect the RN-induced facilitation of the low-threshold afferent-evoked JOR. These results suggest that the RN-induced suppression of the high-threshold afferent-evoked JOR is mediated by a relay in the contralateral LRt.

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. The high-threshold afferent-evoked JOR is believed to be evoked by noxious stimulation (Lund and Olsson, 1983; Lund et al., 1984).

The red nucleus (RN) is an oval column of cells extending from the caudal level of the superior colliculus into the caudal diencephalon. Our previous paper found that stimulation of the RN facilitated the low-threshold afferent-evoked JOR, and suppressed the high-threshold afferent-evoked JOR. The facilitation occurred when the conditioning-test interval was 5–20 ms, and the suppression occurred when the conditioning-test interval was 20–40 ms (Satoh et al., 2013). The effect is probably mediated by an indirect pathway, since there is little evidence for direct projections from the RN to the trigeminal motor nucleus (Bernays et al., 1988; Edwards, 1972; Godefroy et al., 1988; Yasui et al., 2001). An interneuronal link in this pathway might be located within the lateral reticular nucleus (LRt). It has been proposed that this region is involved in the mechanisms of analgesia (Liu et al., 1990; Ness et al., 1998), and it receives contralateral projections from the RN (Holstege and Tan, 1988; Hrycyshyn and Flumerelt, 1981; Shokunbi et al., 1986; Rajakumar et al., 1992; Ruigrok, 2004). Furthermore, stimulation of the LRt inhibited the tooth pulp-evoked nociceptive JOR when the conditioning-test interval was 30–60 ms, but did not inhibit the JOR evoked by non-nociceptive stimuli (Sotgiu, 1986). Activities of the spinal trigeminal nucleus oralis (Vo) neurons in response to tooth pulp stimulation were inhibited by the LRt stimulation; this inhibition was greatest at a conditioning-test interval of 20–80 ms (Sotgiu and Bellinzona, 1991). Guided by these observations, we test our hypothesis that RN-induced modulation of the JOR is probably mediated via the LRt, by examining whether: (1) RN-induced modulation of the JOR is affected by electric lesion of the contralateral LRt; and (2) whether RN-induced modulation of the JOR is affected by microinjection of muscimol into the same area. The aim of the present study is to determine whether the modulatory effects of the RN stimulation on the JOR are mediated by the LRt.

2. Material and methods

These experiments were performed on 21 male Sprague–Dawley rats weighing 309–374 g. All animal procedures were carried out in accordance with the National Institute

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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 administered 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 in order to confirm the condition of the rat throughout the experiment. Data were collected from a rat when the arterial blood pressure was more than 70 mmHg and was stable. The 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) contralateral to the RN stimulation, so as to evoke the JOR. The intensity of this test stimulation was set at either 1.2 (low threshold) or 4.0 (high threshold) times the threshold for evoking the JOR.

The head of the animal 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. A bipolar concentric electrode (outer diameter 250 μm) was inserted stereotaxically, vertically toward the left or right magnocellular part of the RN (RMC). This was done because we found in an earlier study that the suppression of the JOR by the RMC stimulation was significantly greater than suppression by the parvicellular part of the red nucleus (RPC) (Satoh et al., 2013). The conditioning stimulation (1 pulse, 0.2 ms duration, 1 Hz, 100 μA) was applied to the RN at 10 or 30 ms prior to the test stimulation, contralateral to the side undergoing IAN stimulation.

The cerebellum was then removed by suction, to expose the brainstem. In 10 rats, a bipolar concentric electrode was inserted stereotaxically toward the LRt contralateral to the RN, and was located at the site where LRt stimulation affects the JOR (1 pulse, 0.2 ms duration, 1 Hz, 100 μA, 30 ms prior to the test high-threshold IAN stimulation). The control JOR responses were recorded, and also the effects induced by stimulation of the LRt. Next, the LRt lesion was created by an electric current (20 μA, 2 min). The control JOR and the effects of RN stimulation on the JOR were tested after the lesion had been made.

In 11 rats, a fine stainless pipe (diameter 0.1 mm) was inserted vertically toward the LRt contralateral to the RN. A fine stainless pipe, attached to a microelectrode and filled with muscimol dissolved in 0.9% saline (5 mM), 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 RN at 100 s before administration ceased. At that point, 0.1 μl of muscimol was injected (injection time 90 s). The control JOR and the effects of RN stimulation on the JOR were tested, beginning from the end of the injection.

The electromyographic responses evoked by stimulating 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 RN stimulation are expressed as a percentage of the control value. Differences were taken as significant when $P < 0.05$.

At the end of each experiment, electrolytic lesions were made by passing negative direct current (20 μA for 90 s) through the RN-stimulating electrodes or microelectrodes attached to a fine stainless pipe used for injection. 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 of the brainstem (50 μm thick) were cut and stained with cresyl violet. The sites of stimulation and injection 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 24–200 μA (68.8 ± 50.4 μA, mean ± SD, $n = 21$). The mean latency of the control JORs evoked by the low and high-threshold afferent-evoked JOR on the side contralateral to the RN stimulation was 6.6 ± 0.1 ms (low) and 6.1 ± 0.1 ms (high); these are mean values ± SE ($n = 21$). There were statistically significant differences in the mean latency between these values (Wilcoxon t-test, $P < 0.05$).

Stimulation of the RN facilitated the low-threshold afferent-evoked JOR, and suppressed the high-threshold afferent-evoked JOR (Wilcoxon t-test, $P < 0.05$). The electrical stimulation sites in the RN are shown in Fig. 1 on diagrams of the coronal sections. All stimulation sites except one were in the RMC.

Electrically induced lesions of the LRt and microinjection of muscimol into the LRt reduced the RN-induced suppression of the high-threshold afferent-evoked JOR (Wilcoxon t-test with Bonferroni correction, $P < 0.05$). They do not affect the RN-induced facilitation of the low-threshold afferent-evoked JOR (Wilcoxon t-test with Bonferroni correction, $P > 0.05$).

3.1. Histological location of electric lesion and muscimol injection sites in the brainstem

The sites of the electrically induced lesion and muscimol injection are shown in Fig. 2 on diagrams of the transverse section of the brainstem. The electric lesion sites that reduced the RN-induced suppression of the high-threshold afferent-evoked JOR were located in the rostrolateral part of the LRt (filled circles), whereas regions dorsal to the LRt or in the caudal part of the LRt were ineffective (filled triangles). The sites that proved effective for muscimol microinjection were also located in the rostral part of the LRt (open circles), whereas the caudal region of the LRt and dorsal region to the LRt were ineffective (open triangles). Fig. 3 shows a photomicrograph of a lesion site in the LRt. The extent of each lesion was 200–250 μm in the rostrocaudal direction, 75–200 μm in the mediolateral direction and 250–375 μm in the dorsoventral direction.

3.2. Electric lesions of the rostral part of the LRt

The RN-induced facilitation of the low-threshold afferent-evoked JOR was 190.4 ± 32.7% (mean ± SE, $n = 7$) of the control level at a conditioning-test interval of 10 ms before electrically induced lesions were created in the LRt (Fig. 4A, left). Electrically induced lesions of the LRt did not significantly influence the RN-induced facilitation of the JOR. Facilitation was 188.9 ± 37.0% (mean ± SE, $n = 7$) of the control level at a conditioning-test interval of 10 ms after electrically induced lesions of the LRt (Fig. 4A, right).