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Role of the vestibular nuclear complex in facilitating the jaw-opening reflex following stimulation of the red nucleus

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ABSTRACT

According to our previous studies, stimulation of the red nucleus (RN) facilitates the low-threshold afferent-evoked jaw-opening reflex (L-JOR). It has been reported that the RN projects to the superior (SVN), lateral (LVN) and inferior vestibular (IVN) nuclei. The SVN and the LVN have reciprocal intrinsic connections with the medial vestibular nucleus (MVN). Our previous study demonstrated that stimulation of the vestibular nuclear complex (VN) modulates the L-JOR. These facts suggest that RN-induced facilitation of the L-JOR is mediated via the VN. In the present work we investigated whether electrically induced lesions of the VN, or microinjection of muscimol into the VN, affects RN-induced facilitation of the L-JOR. The L-JOR was evoked by electrical stimulation of the inferior alveolar nerve. The stimulus intensity was 1.2 times the evocation threshold. Lesions of the MVN or the LVN or the SVN, and the muscimol injection into the MVN or the LVN or the SVN, reduced the RN-induced facilitation of the L-JOR. Conversely, lesions of the IVN, and the muscimol injection into the IVN, increased the RN-induced facilitation of the L-JOR. These results suggest that the RN-induced facilitation of the L-JOR is mediated by a relay in the VN.

1. Introduction

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

In our previous papers we reported that stimulation of the red nucleus (RN) facilitates the L-JOR (Satoh et al., 2003, 2013). The effect is probably mediated by an indirect pathway, since there is little evidence for direct projections from the RN to the ventromedial division of the trigeminal motor nucleus (jaw-opening motoneuron pool) (Edwards, 1972; Godefroy et al., 1988; Travers and Norgen, 1983; Yasui et al., 2001). It is possible that an interneuronal link in this pathway is located within the vestibular nuclear complex (VN). The VN consists of four major nuclei: the medial (MVN), lateral (LVN), superior (SVN) and inferior vestibular (IVN) nuclei. Cytoarchitecturally, the MVN is divided into two parts: the parvicellular part (MVNPC) and the magnocellular part (MVNMC) (Paxinos and Watson, 2007). The hypothesis has been made that the RN projects to the bilateral LVN, to the contralateral SVN (Godefroy et al., 1988) and to the contralateral IVN (Edwards, 1972). The SVN has strong reciprocal intrinsic connections with the MVN and the IVN. The LVN is reciprocally related to the MVN and receives inputs from the IVN. These intrinsic connections between individual nuclei suggest an integrative function within the VN (Rubertone et al., 1983). Our previous study demonstrated that stimulation of the LVN, the SVN, the MVNPC and the MVNMC facilitates the L-JOR, and that stimulation of the IVN suppresses the L-JOR (Satoh et al., 2009). Furthermore, antidromic action potentials in the LVN neurons were evoked by electrical stimulation of the RN (Sarkisian and Fanardijian, 1992). Guided by these observations, we test herein our hypothesis that RN-induced facilitation of the L-JOR is mediated via the VN, by examining whether: (1) RN-induced facilitation of the L-JOR is affected by electrically created lesions in the bilateral VN and (2) whether RN-induced facilitation

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of the L-JOR is affected by microinjection of muscimol into the same areas. The present study aims to determine whether the facilitatory effects of RN stimulation on the L-JOR are mediated by the VN.

2. Materials and methods

Our experiments were performed on 62 male Sprague-Dawley rats, weighing 290–416 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 (approval number 146).

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. Lidocaine (2% solution) was injected into the skin to minimize surgical pain before the incisions were made. The trachea and the femoral artery were cannulated. Arterial blood pressure was monitored as an indicator of the condition of the rat during the experiment. Data were collected from an animal when the arterial blood pressure was stable and greater than 70 mmHg. 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. For electrical stimulation of the inferior alveolar nerve (IAN), bipolar stimulating electrodes consisting of silver wires of diameter 0.1 mm, insulated below the tip, were inserted into the right mandibular canal through the mental foramen. Test stimulation (single pulse, 0.1 ms in duration, 1 Hz) was applied to the IAN contralateral to the RN stimulation, so as to evoke the JOR. The intensity of this test stimulation was set at 1.2 times the threshold for evoking the JOR, as in previous studies (Satoh et al., 2013, 2014a,b).

The head of the animal was fixed in a stereotaxic frame using ear bars and an incisal bar. Using a dental drill, parts of the parietal, interparietal and occipital bones were removed to expose the cerebrum and the cerebellum. A bipolar concentric electrode (outer diameter 250 μm) was inserted stereotaxically, vertically toward the left RN. The cerebellum was then removed by suction so as to expose the brainstem. A bipolar concentric electrode was inserted stereotaxically toward the VN bilaterally in 34 rats, at an angle of 5°. The conditioning stimulation (1 pulse, 0.2 ms duration, 1 Hz, 100 μA) was applied to the left RN at a time 10 ms prior to the test stimulation, and contralateral to the side undergoing IAN stimulation. The control JOR responses were recorded, as were the effects induced by stimulation of the RN. After that, bilateral VN lesions were created by the passage of an electric current (20 μA, 2 min). After the lesion had been made, the control JOR and the effects of RN stimulation on the JOR were studied.

A fine stainless pipe (diameter 0.1 mm) was inserted stereotaxically toward the right VN in 28 rats, at an angle of 5°. 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 via polyethylene tubing. The control JOR responses were recorded, and also the effects induced by stimulating the RN at 20 s before administration ceased. At that time 5 nl of muscimol were injected (the duration of the injection was 45 s). The control JOR and the effects of RN stimulation on the JOR at a conditioning-test interval of 10 ms were studied, beginning at 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 20 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 for $P < 0.05$.

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

3. Results

The threshold intensity for eliciting the L-JOR by stimulating the IAN was 21–200 μA (77.1 ± 42.6 μA, mean ± SD, $n = 62$). Conditioning electrical stimulation of the RN facilitated the L-JOR to an extent that was statistically significant (Wilcoxon t-test, $P < 0.05$); see Fig. 1A. The facilitated L-JOR was 233.4 ± 13.0% (mean ± SE, $n = 62$) of the control level. Latency of the control L-JOR evoked by the IAN on the side contralateral to RN stimulation (6.5 ± 0.1 ms, mean ± SE, $n = 62$) decreased significantly when conditioning electrical stimuli were applied to the RN at 10 ms intervals (6.3 ± 0.1 ms, mean ± SE, $n = 62$, Wilcoxon t-test, $P < 0.05$). Fig. 1B plots the latency of the L-JOR on the contralateral side, when conditioning electrical stimuli were applied to the RN, against the facilitation of the L-JOR. A significant correlation exists between the latency of the L-JOR and the facilitation of the L-JOR (rs = 0.37283, Spearman rank correlation coefficient, $P < 0.05$). The latency of the control L-JOR evoked by the IAN on the side ipsilateral to RN stimulation (6.5 ± 0.1 ms, mean ± SE, $n = 62$) decreased significantly when conditioning electrical stimuli were applied to the RN at 10 ms intervals (6.4 ± 0.1 ms, mean ± SE, $n = 62$, Wilcoxon t-test, $P < 0.05$).

3.1. Lesions of the MVN or the LVN or the SVN, and muscimol injection into the MVN or the LVN or the SVN

Before lesions were electrically created in the MVNMC, the RN-induced facilitation of the L-JOR ran at 243.8 ± 37.1% (mean ± SE, $n = 6$) of the control level for a conditioning-test interval of 10 ms (Fig. 2A, left). The electrically created lesions in the bilateral LVN significantly reduced the RN-induced facilitation of the JOR (Wilcoxon t-test with Bonferroni correction, $P < 0.05$). After lesions had been created electrically in the MVNMC, facilitation was down to 161.1 ± 20.2% (mean ± SE, $n = 6$) of the control level for a conditioning-test interval of 10 ms (Fig. 2A, right).

The presence of electrically created lesions in the MVNPC, LVN and SVN significantly reduced the RN-induced facilitation of the L-JOR (Wilcoxon t-test with Bonferroni correction, $P < 0.05$); see Table 1.

Table 1
Effect of stimulation of the RN on the L-JOR before and after the electrical creation of lesions in the MVNPC, the LVN and the SVN. Values are means ± SE. The number of samples is shown in brackets.

Electric lesion sites	Before lesion	After lesion
MVNPC	245.4 ± 75.8% ($n = 6$)	149.2 ± 30.7% ($n = 6$)
LVN	261.9 ± 53.8% ($n = 6$)	161.5 ± 30.7% ($n = 6$)
SVN	232.5 ± 53.5% ($n = 6$)	183.5 ± 30.8% ($n = 6$)