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Research Report

Involvement of reticular neurons located dorsal to the facial nucleus in activation of the jaw-closing muscle in rats

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Abstract

The location of excitatory premotor neurons for jaw-closing motoneurons was examined by the use of electrical and chemical stimulation and extracellular single-unit recording techniques in the anesthetized rat. Single-pulse electrical stimulation of the supratrigeminal region (SupV) and the reticular formation dorsal to the facial nucleus (RdVII) elicited masseter EMG response at mean (±SD) latencies of 2.22 ± 0.59 ms and 3.10 ± 1.14 ms, respectively. Microinjection (0.1–0.3 μl) of glutamate (50 mM) or kainate (0.5–100 μM) into RdVII increased masseter nerve activity in artificially ventilated and immobilized rats by 30.2 ± 40.5% and 50.7 ± 46.8% compared to baseline values, respectively. Forty reticular neurons were antidromically activated by stimulation of the ipsilateral trigeminal motor nucleus (MoV). Twenty neurons were found in RdVII, and the remaining 20 neurons were located in SupV, or areas adjacent to SupV or RdVII. Eleven neurons in RdVII responded to at least either passive jaw opening or light pressure applied to the teeth or tongue. Nine neurons responded to passive jaw opening. Five of the nine neurons responded to multiple stimulus categories. A monosynaptic excitatory projection from one neuron in RdVII was detected by spike-triggered averaging of the rectified masseter nerve activity. We suggest that reticular neurons in RdVII are involved in increasing masseter muscle activity and that excitatory premotor neurons for masseter motoneurons are likely located in this area. RdVII could be an important candidate for controlling activity of jaw-closing muscles via peripheral inputs.

Theme: Motor systems and sensorimotor integration
Topic: Spinal cord and brainstem

Keywords: Excitatory premotor neuron; Trigeminal; Masseter; Reticular formation

1. Introduction

The activity of masticatory muscles varies according to the physical properties of food [2,28,38]. The activity of jaw-closing muscles increases with the hardness of food during natural mastication in animals [10,38] and humans [28]. Jaw-closing muscle activity also increases with the hardness of the test material applied between upper and lower molars during cortically induced fictive mastication in anesthetized rabbits [11,25]. These results suggest that in the brain, there is a neuronal circuit responsible for increasing jaw-closing muscle activity based on food properties. This circuit sends the motor command to jaw-closing motoneurons through excitatory last-order premotor neurons. Retrograde axonal tracing studies have shown that premotor neurons for the trigeminal motor nucleus (MoV) were found in the parabrachial, supratrigeminal, and intertrigeminal regions; in the lateral region of the rostral part of the medullary reticular formation; in the dorsal and lateral parts of the caudal portion of the medullary reticular formation; and in the dorsal parts of the principal sensory, oral spinal, and interpolar spinal trigeminal nuclei [6,20,21,24,34,37]. Premotor neurons, which were identified by antidromic

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stimulation of MoV, were also found in these regions [7,13–15,39]. Turman and Chandler [36] showed that glutamatergic premotor neurons, which were most likely excitatory premotor neurons for MoV, were located in these regions. It was suggested that excitatory premotor neurons were located in the parvocellular reticular formation located ventrolateral to the hypoglossal nucleus and in the oral spinal trigeminal nucleus. This suggestion arose from the finding that spike-triggered averaging the intracellular potential of masseter motoneurons (MaMNs) [27] or extracellular field potentials of the masseter motoneuron pool [39]. It is not clear, however, whether activation of premotor neurons in these two areas produces the jaw-closing muscle activities or excitatory premotor neurons are located in only these areas.

The aim of the present study was to investigate the location of excitatory premotor neurons for MaMNs by the use of electrical and chemical stimulation and extracellular single-unit recording techniques in the anesthetized rat. First, we applied single-pulse electrical stimulation systematically to the pons and medulla to find the effective sites to elicit masseter electromyographic (EMG) response at short latency. Second, we examined the effects of microinjection of glutamate or kainate into the effective sites, as revealed by electrical stimulation of masseter nerve activity in artificially ventilated and immobilized rats. Third, we recorded the activities of neurons in the regions as revealed by the above two approaches. The neurons which were antidromically activated from the ipsilateral MoV stimulation were found in the regions, and we examined their responses to intra-oral mechanical stimulation and passive jaw opening. Synaptic connection of some neurons with MaMNs was investigated by averaging the masseter nerve activities after the spike of the neurons. Preliminary results of this study were reported previously in abstract form [32].

2. Materials and methods

2.1. Preparation of animals

All experiments were performed with the approval of the Institutional Animal Care and Use Committee of Showa University. Experiments were performed on 85 adult male Wistar rats weighing 250–350 g. The animals were initially anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg). Atropine sulfate (0.5 mg/kg ip) was then given to reduce tracheal secretions. Heart rate was monitored by electrodes placed over the thoracic cavity. After a standard tracheotomy, animals were artificially ventilated with 1.0–2.0% halothane in the air to maintain the level of anesthesia sufficient to abolish the withdrawal reflex of the hind limb induced by deep pressure to the foot pad during the course of the experiments. A pair of enamel-coated stainless wires (diameter, 80 μm; tip separation, about 1 cm) was inserted into the left masseter muscle to record EMG activities. To minimize contamination in the masseter EMG signal with facial muscle activity, the facial nerve was sectioned under the parotid gland. A heating pad was placed under the abdomen to maintain the core body temperature at ~37 °C. All wound edges were infiltrated with a long-acting local anesthetic (bupivacaine HCl). The animal's head was fixed in the frame of a stereotaxic apparatus. In some experiments, femoral vein cannulation was performed, and animals were paralyzed with sodium pancuronium (3 mg/kg iv). The left masseter nerve was exposed under the zygomatic arch, and a Teflon-coated, platinum iridium fine wire (diameter, 25 μm) was inserted centrally into the nerve to record efferent activities after paralysis. The reference electrode of an enamel-coated stainless steel wire (diameter, 80 μm) was implanted into the left masseter muscle near the masseter nerve.

2.2. Electrical stimulation of the brain stem

The dorsal part of the cerebellum on the left side was exposed for stimulation of the brain stem. A Teflon-insulated tungsten electrode (impedance 800 kΩ at 500 Hz; Unique Medical, TF201-062) was inserted vertically into the brain stem to allow examination of the sites effective for inducing masseter EMG responses with short latencies. A single-pulse stimulation (1 Hz, 1–50 μA, 0.1 ms pulse duration) was systematically applied every 500 μm in the rostrocaudal, mediolateral, and vertical directions. The region investigated was bounded in the rostrocaudal plane by the trigeminal motor nucleus and the obex, respectively. In the mediolateral plane, the area was bounded by the midline and the spinal trigeminal nucleus, respectively. Within this area the entire dorsoventral extent of the brain stem was examined.

2.3. Chemical stimulation of the brain stem

Microinjections of glutamic acid (50 mM) or kainic acid (0.5, 10, and 100 μM) in a volume of 0.1–0.3 μl into the left brain stem were performed using a 1-μl Hamilton syringe (Hamilton, Reno, USA) connected by polyethylene PE-10 tubing to an injector needle (0.25 mm od) 1.0 mm longer than the guide cannula (cf. Canesin et al. [5]). The guide cannula (0.5 mm od) was stereotaxically inserted into the area 1.0 mm upward to the site where the electrical stimulation induced masseter EMG responses. Both drugs were dissolved in saline. Although the facial nerve was sectioned under the parotid gland, we might have failed to cut some branches of the facial nerve. Thus, animals were paralyzed and artificially ventilated, and masseter nerve activity instead of masseter EMG was recorded in order to avoid contamination from the activity of facial muscles. Baseline masseter nerve activity was observed for 1 min before microinjection of glutamate or kainate. The drug was then injected into the brain stem, and resulting changes in the nerve activities were recorded for 10 min.