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Facilitation of reflex swallowing from the pharynx and larynx

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Abstract: To evaluate the cooperative effect of afferent signals from the pharynx and larynx on reflex swallowing, the interactive effect of afferent signals from the pharyngeal branch of the glossopharyngeal nerve (GPN-ph) and superior laryngeal nerve (SLN) was analyzed in detail in urethane-anesthetized rats. The electromyographic activity of the mylohyoid muscle was recorded as an indicator of swallowing activity. The onset latency of reflex swallowing was measured to evaluate the effects of electrical stimulation of these nerves, and found to become shorter following an increase in the GPN-ph and/or SLN stimulus frequency. During simultaneous electrical stimulation of the GPNph and SLN (frequency: 5-10 Hz, intensity: 30 µA, duration: 1.0 ms for each), the onset latency of reflex swallowing became shorter than that for stimulation of each nerve independently. The present findings suggest that spatiotemporal summation of afferent signals from the GPN-ph and SLN results in an increase of motoneuronal activity in the medullary swallowing

center, thus enhancing reflex swallowing. (J Oral Sci 51, 167-171, 2009)

Keywords: glossopharyngeal nerve; superior laryngeal nerve; pharyngeal reflex; deglutition; swallowing.

Introduction

The pharynx and larynx are known to constitute a highly reflexogenic area (1). These regions are specially important for eliciting reflex swallowing following a variety of stimuli. The superior laryngeal nerve (SLN), innervating the laryngeal region, is well known to be the most important afferent nerve for initiation of reflex swallowing (2-8). Animal studies have shown that electrical stimulation of the SLN elicits reflex swallowing (7-13). On the other hand, the role of the glossopharyngeal nerve (GPN) in reflex swallowing is less well understood (2,7). However, mechanical stimulation of the pharyngeal region innervated by the pharyngeal branch of the glossopharyngeal nerve (GPN-ph) evokes reflex swallowing readily (11,14-17). We have previously demonstrated by electrical stimulation of the GPN-ph that it plays a major role in the initiation of reflex swallowing from the pharynx (11). In addition, we showed that electrical stimulation of the lingual branch of

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the GPN (the main trunk to the tongue), known as the taste nerve, had an inhibitory effect on reflex swallowing (11).

The swallowing process is commonly divided into oral, pharyngeal, and esophageal stages, according to the location of the food bolus (18). The speed of food bolus movement to the oropharynx and larynx differs depending on the type of food (solid food or liquid). Chemical or mechanical stimulation of the pharyngeal and laryngeal mucosa is thought to be involved in evoking reflex swallowing (6,11,14-17,19,20), suggesting that inter-activation of afferent signals from the pharynx and larynx plays a crucial role. However, it is still unknown how these afferent inputs from the oropharynx and larynx interact and how they are involved in evoking reflex swallowing.

In the present study, to evaluate the cooperative effect of afferent signals from the pharynx and larynx on reflex swallowing, we analyzed the change in onset latency of the reflex swallowing evoked by electrical stimulation of the GPN-ph and SLN.

Materials and Methods

The experimental protocols were approved by the Intramural Animal Care and Veterinary Science Committee of Niigata University.

Experiments were carried out using 15 male Wistar rats weighing 200 – 400 g. The animals were anesthetized with urethane (1.0 g/kg, i. p.) and laid supine in a stereotaxic frame. Body temperature was maintained at 37°C using a heating pad. A longitudinal midline incision was made in the ventral surface of the neck. The trachea of the animal was cannulated to maintain respiration. Bipolar enamel-coated stainless steel wire electrodes (5 mm in diameter) were inserted into the left mylohyoid muscle, recognized as "obligate muscle" involved in swallowing movements, to record its electromyographic (EMG) activity (10,11,21). Swallowing was identified by the EMG activity of the mylohyoid muscle and by visual observation of laryngeal movement.

Using blunt dissection scissors, the SLN was exposed from the sternothyroid muscle. The GPN was exposed by removal of the digastric muscles and posterior horn of the hyoid bone. These nerves were then dissected free from the surrounding tissue. The lingual branch of the glossopharyngeal nerve (GPN-li) and GPN-ph nerves were isolated and the GPN-ph nerve was transected just distal to the junction of the GPN-li and GPN-ph. Bipolar platinum wire electrodes were fitted onto the central cut end of the GPN-ph and SLN for electrical stimulation.

The GPN-ph and SLN were stimulated individually or simultaneously with a rectangular pulse (frequency: 5 - 50 Hz, intensity: 30μ A, duration: 1.0 ms) to examine the

cooperative effect of afferent signals from the GPN-ph and SLN. The parameter for stimulation of the GPN-ph and SLN was similar to that in our previous study (11).

The onset latency to elicit the first swallowing movement was defined as the time required to evoke the first swallow from the onset of electrical stimulation. The onset latencies to elicit the first swallow during simultaneous stimulation of the GPN-ph and SLN were measured and compared with those for individual stimulation of the GPN-ph or SLN.

Statistical analysis was performed using ANOVA followed by the Newman-Keuls test. Results are presented as mean \pm S.E.M. Differences were considered significant at *P* < 0.05.

Results

Typical reflex swallowing elicited by electrical stimulation of the GPN-ph and/or SLN is shown in Fig. 1. Several swallows were evoked by electrical stimulation (5 Hz, 30 µA, 1.0 ms) of the GPN-ph (Fig. 1A) or SLN (Fig. 1B). In these cases, the latency of the first swallow elicited by GPN-ph stimulation was 2.82 s (Fig. 1A) and that for SLN stimulation was 2.79 s (Fig. 1B). To examine the interactive effect of afferent signals from the GPN-ph and SLN in eliciting reflex swallowing, the GPN-ph and SLN were stimulated simultaneously. The onset latency of the first swallow elicited by simultaneous stimulation of the GPN-ph and SLN (5 Hz, 30 µA, 1.0 ms in each) was 0.71 s in this case (Fig. 1C). The number of reflex swallows was higher for simultaneous stimulation of the GPN-ph and SLN than for stimulation of the GPN-ph or SLN (Fig. 1A, B and C). The onset latency to elicit reflex swallowing was significantly shorter for simultaneous stimulation of the GPN-ph and SLN than for individual stimulation of the GPN-ph or SLN (30 µA, 5 Hz, 1.0 ms).

Mean onset latency of the first swallow was 2.84 ± 0.62 s (n = 15) after GPN-ph stimulation (5 Hz, $30 \,\mu$ A, $1.0 \,m$ s) (Fig. 2A), and that after SLN stimulation (5 Hz, 30 µA, 1.0 ms) was 2.72 ± 0.51 s (n = 15, Fig. 2A). There were no significant differences in the onset latencies of reflex swallowing following stimulation of the GPN-ph and SLN $(5 \text{ Hz}, 30 \,\mu\text{A}, 1.0 \,\text{ms})$. On the other hand, the mean onset latency of reflex swallowing was significantly shorter after simultaneous stimulation of the GPN-ph and SLN $(0.72 \pm 0.32 \text{ s}, n = 15)$ than after individual stimulation of the GPN-ph or SLN (P < 0.01, Fig. 2A). Mean onset latency of the first swallow induced by GPN-ph or SLN stimulation (10 Hz, 30 μ A, 1.0 ms in each) was 1.41 \pm 0.42 and 1.32 ± 0.31 s, respectively (n = 15, in each). There were no significant differences in the mean onset latency of reflex swallowing between stimulation of the GPN-ph and that of the SLN at 10 Hz (Fig. 2B). The mean onset



Fig. 1 Typical examples of EMG recordings from the mylohyoid muscle during swallowing. A and B: successive swallows elicited by electrical stimulation (5 Hz, 30 μ A, 1.0 ms) of the GPN-ph (A) and SLN (B), respectively. C: swallows elicited by simultaneous electrical stimulation of the GPN-ph and SLN (5 Hz, 30 μ A, 1.0 ms for each).



Fig. 2 Relationships between the frequency of electrical stimulation and the latency of the first swallow for stimulation of the GPN-ph or SLN, or simultaneous stimulation of both. A and B: the facilitatory effect on the elicitation of reflex swallowing by electrical stimulation of the GPN-ph and SLN simultaneously (A: 5 Hz, B: 10 Hz). C: mean latency of the first swallow elicited by individual stimulation and simultaneous stimulation of the GPN-ph and SLN (20 – 50 Hz).

latency was significantly shorter during simultaneous stimulation of the GPN-ph and SLN (10 Hz, 30 μ A, 1.0 ms) than that during individual stimulation of the GPN-ph or SLN (0.42 ± 0.22 s, n = 15, *P* < 0.05, Fig. 2B). We also tested the effect of higher-frequency stimulation on reflex swallowing (Fig. 2C), and found that the mean onset latency gradually decreased with the increase in stimulus frequency.

Discussion

The present study analyzed the effect of GPN-ph and/or SLN stimulation at different frequencies $(5 - 50 \text{ Hz}, 30 \mu \text{A},$ 1.0 ms) on the onset latency of reflex swallowing (first reflex swallowing) (Fig. 2C). We observed a significant decrease in the onset latency of reflex swallowing in rats upon simultaneous stimulation of the GPN-ph and SLN at 5 -10 Hz, suggesting that the GPN-ph and SLN afferents are involved in the facilitation of reflex swallowing. On the other hand, neither individual nor simultaneous stimulation of the GPN-ph and SLN at 20 - 50 Hz led to significant changes in the onset latency of reflex swallowing. It has been reported that an increase in the spike frequency in primary afferent fibers causes a strong increase in postsynaptic potentials in spinal cord motoneurons (22,23). The enhancement of postsynaptic potentials in spinal cord motoneurons is thought to be a result of spatiotemporal summation of primary afferent activity. The frequencydependent decrease in the onset latency of reflex swallowing during stimulation of the GPN-ph and/or SLN is followed by an increase of motoneuronal activities in the medullary swallowing center. Together with previous data, our results suggest that temporal summation of the primary afferent activity may occur in the medullary swallowing center following an increase in the frequency of GPN-ph or SLN stimulation (11). Furthermore, significant shortening of the onset latency of reflex swallowing was obvious during simultaneous stimulation of the GPN-ph and SLN, suggesting that spatial summation of primary afferents may occur in the medullary swallowing center. We did not observe any facilitatory effects of reflex swallowing during stimulation of the GPN-ph and/or SLN at a high frequency of more than 20 Hz (Fig. 2C). It is probable that a frequency exceeding 20 Hz may be too high to increase postsynaptic potentials during GPN-ph and/or SLN stimulation.

We used electrical stimulation of the GPN-ph and SLN to evoke reflex swallowing. Therefore, it was difficult to define sensory modalities in the pharynx and larynx. During natural swallowing, a food bolus moves from the oral to the pharyngeal region and the pharyngeal and laryngeal mucosae are simultaneously stimulated chemically and mechanically. Kajii et al. (19) reported that sour taste stimulation of the pharynx and larynx facilitated reflex swallowing in rats. Some previous studies have also described that application of water to the pharynx and larynx evokes reflex swallowing in rats (19), rabbits (6) and humans (20). The spatiotemporal summation of primary afferent signals from the GPN-ph and SLN may be the mechanism underlying the enhancement of reflex swallowing by natural stimulation of the pharynx and larynx.

In conclusion, the present study has revealed that the onset latency of the first swallow decreases during simultaneous stimulation of the GPN-ph and SLN, but not when each of these nerves is stimulated separately. These results suggest that cooperation of the primary afferent signals from the GPN-ph and SLN leads to spatiotemporal summation in the medullary swallowing center, thus facilitating the initiation of reflex swallowing.

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