Pharyngeal branch of the glossopharyngeal nerve plays a major role in reflex swallowing from the pharynx

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IT IS WELL KNOWN THAT REFLEX swallowing is mostly elicited from specific areas of the pharynx and larynx (see reviews in Refs. 3, 5, 13, 14). The receptive regions eliciting reflex swallowing have been analyzed with mechanical and chemical stimulation (4, 15, 17, 19–21, 26). In the pharyngeal region, mechanical stimulation is effective in evoking swallowing. The posterior pillars are the regions that are the most reflexogenic to mechanical stimulation in the cat (21). The posterior pharyngeal wall is slightly less reflexogenic. The receptive regions for swallowing in humans are essentially similar to those in animals (17). In addition, in the laryngeal regions, Storey (23, 24) has demonstrated that water stimulation, as well as mechanical stimulation, is effective in evoking swallowing. This finding has been confirmed by several studies (9, 10, 12, 18–20).

The pharyngolaryngeal regions that evoke swallowing are innervated by the glossopharyngeal nerve (GPN), the pharyngeal branch of the vagus nerve (X-ph), and the superior laryngeal nerve (SLN). The laryngeal mucosa is mainly supplied by the SLN, and the pharyngeal mucosa is supplied by a complicated plexus of nerves, namely the pharyngeal plexus. The pharyngeal plexus is mainly formed by the X-ph and the pharyngeal branch of the GPN (GPN-ph). The organization of the plexus varies with the species of animals and even across individuals within a species (5, 8).

Many previous studies that have examined the role of the pharyngolaryngeal region in swallowing have employed electrical stimulation of the peripheral nerves that innervate this area (2, 3, 11, 12, 14). Electrical stimulation of the SLN can readily elicit reflex swallowing (2, 11, 22–24); however, it is much more difficult for stimulation of the GPN to evoke swallowing than it is for stimulation of the SLN to do so (3, 22). This ineffectiveness of the GPN to initiate swallowing is somewhat paradoxical if the GPN innervates the pharynx, because mechanical stimulation of the pharyngeal mucosa is very effective in initiating swallowing (3, 15, 21). Sinclair (22) studied the role of the pharyngeal plexus in the initiation of swallowing in the cat and concluded that the GPN is the primary afferent for swallowing from the pharynx. However, it appears that his results and discussion are somewhat complicated. We can, for instance, find in his results that electrical stimulation of a cephalic X-ph (designated branch 22a by Sinclair) elicits reflex swallowing very easily; the effectiveness of the nerve in the initiation of swallowing is equivalent to that of the SLN. Sinclair’s paper finds, however, that, although electrical stimulation of the GPN elicits reflex swallowing, the GPN is much less effective than branch 22a in this regard. It, therefore, seems that his conclusion is not reasonable.

To resolve the paradox in the initiation of swallowing and to make clear the contradiction in the results of Sinclair (22), the present study was designed to 1)
precisely examine the reflexogenic areas of swallowing in the pharyngolaryngeal region; 2) identify the afferent pharyngeal branch that contributes to swallowing from the pharynx; and 3) investigate the electrophysiological properties of the GPN-ph in initiating swallowing. Rats were used as the experimental animals in this study because the pharyngeal nerve plexus of the rat appears to be relatively simple.

METHODS

Experiments were carried out with the use of 25 male Wistar rats weighing 200–400 g. The animals were anesthetized with urethane (1.0 g/kg ip) and placed in the supine position. Body temperature was maintained at 37°C with 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. Two bipolar platinum wire electrodes were unilaterally fitted onto the central cut end of the GPN-li, GPN-ph, or SLN to stimulate these nerves. The nerves were stimulated by repetitive electrical stimulation with a rectangular pulse (intensity: 0.1–3.0 V, frequency: 1.0–100 Hz, duration: 1.0 ms). The latency for the first swallow was defined as the time required to evoke the first swallow from the onset of electrical stimulation. The time interval between the first and third swallows was also measured. The time interval divided by 2 was considered to be the mean value of the time intervals (mean time interval of swallows). The latencies for the first swallow and the mean time intervals between swallows for the GPN-li and GPN-ph were compared with those of the SLN.

In 5 of 17 rats, electrical stimulation of the GPN-li was applied, in addition to the stimulation of the SLN, so that the contributions of afferent signals from the GPN-li for swallowing induced by the SLN could be examined.

Statistical analysis was performed by using Student’s t-test. Differences with $P < 0.05$ were considered statistically significant.

![Diagram of the pharyngolaryngeal region](Fig. 1. Receptive areas of the pharyngolarynx from which reflex swallowing was induced by mechanical stimulation with a von Frey hair. The most reflexogenic areas were the palatopharyngeal arch, the edge of the soft palate in the pharyngeal region, and the edge of the epiglottis and aryepiglottic fold. The moderately reflexogenic areas included the pharyngeal surface of the epiglottis and the posterior pharyngeal wall. Reflex swallowing was initiated with stimuli of 1.0 mN in the most reflexogenic area and with stimuli of 10 mN in the moderately reflexogenic areas.)
RESULTS

Reflexogenic areas of swallowing in the pharyngolaryngeal region. Reflex swallowing was elicited by mechanical stimulation of the pharyngeal and laryngeal mucosae with a von Frey hair. Reflexogenic areas for swallowing from the pharyngolaryngeal region are shown in Fig. 1. The most effective areas were the palatopharyngeal arch, the posterior edge of the soft palate (an area extending from the palatopharyngeal arch), the edge of the pharyngeal surface of the epiglottis, and the aryepiglottic fold. Mechanical stimulation of these areas elicited swallowing readily with a stimulus of 1.0 mN. In the moderately reflexogenic areas (the pharyngeal surface of the epiglottis and the posterior pharyngeal wall), the swallowing was initiated with a stimulus of 10 mN. It was difficult to evoke any reflex swallowing from the other areas. However, when regions adjoining reflexogenic areas were stimulated with 10, 15, or 20 mN, swallows were occasionally observed, but body movements were sometimes also observed.

Figure 2 illustrates the schematic description of the GPN-li, GPN-ph, and SLN. The GPN-ph originates from the trunk of the GPN and anastomoses with X-ph, forming the pharyngeal plexus. When the SLN was bilaterally sectioned, reflex swallowing from the epiglottis and the aryepiglottic fold was abolished. After the GPN-ph was sectioned, mechanical stimulation failed to elicit reflex swallowing from the posterior pharyngeal wall, the palatopharyngeal arch, or the posterior edge of the soft palate (an area extending from the palatopharyngeal arch). Bilateral sectioning of the GPN-li, as well as of the X-ph, had no effect on the elicitation of swallowing from the pharyngolaryngeal region. These results indicate that the GPN-ph is a primary afferent for reflex swallowing from the pharynx.

Electrophysiological properties of the GPN-ph, SLN, and GPN-li for reflex swallowing. Adequate stimulation of the GPN-ph and of the SLN to elicit swallowing was found to be 0.3–0.7 V and 30–70 Hz at 1.0-ms duration. When both nerves were stimulated with adequate stimulation, reflex swallowing was elicited with short latency (less than ~0.5 s) and was characterized.

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Fig. 2. Diagram showing the pharyngeal branches of the glossopharyngeal nerve (IX) and vagus nerve (X) in the rat. GPN-ph, pharyngeal branch of the glossopharyngeal nerve; GPN-li, lingual branch of the glossopharyngeal nerve; SLN, superior laryngeal nerve; X-ph, pharyngeal branch of the vagus nerve. Arrows indicate the sites to which electrical stimulation was applied.

Fig. 3. Typical examples of electromyographic (EMG) recordings from the mylohyoid muscle during swallowing. A and B: successive swallows elicited by electrical stimulation (0.5 V, 30 Hz, 1.0 ms) of the GPN-ph and SLN, respectively. C: EMG activity during stimulation of the GPN-li (0.5 V, 20 Hz, 1.0 ms). D: swallows elicited by simultaneous electrical stimulation of the SLN (0.5 V, 30 Hz, 1.0 ms) and GPN-li (0.5 V, 40 Hz, 1.0 ms). Note that no swallows were observed in C and that swallowing induced by electrical stimulation of the SLN was delayed and prolonged by electrical stimulation of the GPN-li in D.
by successive swallowing. When both nerves were electrically stimulated (0.3–0.7 V, 1.0 ms) at low (1.0–20 Hz) or high (80–100 Hz) frequency, the stimulation sometimes failed to initiate successive swallows. Electrical stimulation of both nerves at low voltages (0.1–0.2 V) seldom initiated swallowing. Electrical stimulation of the GPN-ph or the SLN at high voltages (0.8–3.0 V) resulted in reflex swallowing, but swallowing was sometimes accompanied by body movements.

A typical example of reflex swallowing elicited by electrical stimulation (0.5 V, 30 Hz, 1.0 ms) of the GPN-ph is shown in Fig. 3A. Several swallows were evoked successively by the stimulation. To compare the effectiveness of GPN-ph stimulation with that of SLN stimulation, electrical stimulation of the SLN was also examined. Stimulation of the GPN-ph elicited sequential swallows similar to those elicited by SLN stimulation (Fig. 3B). The latency of the first swallow for the GPN-ph was 0.38 s, whereas it was 0.22 s for the SLN. The mean time intervals of swallows induced by the GPN-ph and SLN were 0.81 and 0.73 s, respectively (Fig. 3, A and B). Electrical stimulation of the GPN-li failed to evoke reflex swallowing but occasionally induced body movements without swallowing (Fig. 3C). When electrical stimulation was applied simultaneously to both the SLN and GPN-li, the latency of the first reflex swallow was delayed, and the time intervals between swallows were prolonged compared with the reflex swallowing elicited by the SLN alone (Fig. 3, B and D).

The latency of the first swallow and the mean time interval of swallows changed with the frequency of electrical stimulation. The relationships between the stimulus frequency and both the latency of the first swallow and the mean time interval of swallows were obtained by administering electrical stimulation at 0.5 V, 10–70 Hz, and 1.0 ms. The relationships shown in Figs. 4 and 5 were obtained from 17 animals. The latency of the first swallow and the mean time interval between swallows for the GPN-ph and SLN decreased as the frequency of electrical stimulation increased, until the frequency reached ~30 Hz. Once the frequency exceeded 30 Hz, no changes in reflex swallowing were observed. There were no significant differences in effectiveness in the elicitation of swallowing between the GPN-ph and the SLN across the range of frequencies from 10 to 70 Hz.

When electrical stimulation of the GPN-li (0.5 V, 10–100 Hz, and 1.0 ms) was applied in addition to the stimulation (0.5 V, 30 Hz, and 1.0 ms) of the SLN, reflex swallowing was delayed or inhibited, as shown in Fig. 3D. Figure 6 shows the inhibitory effect of the GPN-li on swallowing. When the GPN-li was stimulated simultaneously with the SLN, the latency of the first swallow was 0.57 ± 0.08 s (mean ± SE) at 30 Hz and 1.25 ± 0.11 s at 40 Hz (n = 5), whereas the latency of the first swallow for the SLN alone was 0.38 ± 0.05 s (n = 17). When the GPN-li was stimulated at frequen-
cies >50 Hz, in addition to stimulation of the SLN, body movements were induced without swallowing.

**DISCUSSION**

The present findings, based on mechanical stimulation of the pharyngeal region, showed that the most sensitive areas for reflex swallowing were the palatopharyngeal arch, the posterior pharyngeal wall, and the posterior edge of the soft palate (an area extending from the palatopharyngeal arch). It has been shown that the posterior pillars, posterior pharyngeal wall, and nasopharynx were the most reflexogenic areas for swallowing in cats (21). Pommerenke (17) reported that the tonsillar pillars and the posterior pharyngeal wall were effective in eliciting reflex swallowing in humans. The present findings conformed with those for cats and humans.

Denervation experiments in the present study clearly show that the pharyngeal reflexogenic areas are innervated by the GPN-ph and that the laryngeal reflexogenic areas, such as the pharyngeal surface of the epiglottis and the aryepiglottic fold, are innervated by the SLN, whereas the GPN-li (the main trunk of the GPN) and the X-ph are not associated with the elicitation of swallowing. Sinclair (21) reported that sectioning of the GPN in cats abolished the swallowing reflex in the pharynx in only five out of nine animals. Further sectioning of the X-ph abolished reflex swallowing completely in the rest of the animals. In contrast, the present results indicate that the GPN-ph alone is the afferent for reflex swallowing from the pharynx in rats. The afferent of the X-ph is not associated with the elicitation of swallowing from the pharynx in our denervation experiments. This finding is supported by the previous studies. Anatomic studies demonstrated that afferent fibers are not found in the X-ph in cat and rats (6, 16). Physiological study reported that electrical stimulation of the X-ph does not elicit any cardiovascular responses in rats (7). Therefore, it may be considered that the X-ph consists of efferent fibers.

The present study revealed that electrical stimulation of the GPN-ph was very effective in inducing swallowing. The effectiveness of the GPN-ph in evoking swallowing was almost the same as that of the SLN (Figs. 4 and 5). In Sinclair’s result (22), he reported that electrical stimulation of branch 22a (a cephalic X-ph) was effective in initiating swallowing. It seems that this is because the X-ph has an anastomosis with the GPN-ph, and, therefore, branch 22a consists of both the GPN-ph and X-ph. Previous studies have reported that stimulation of the GPN can elicit swallowing, but the effect is less constant, and swallowing is less readily evoked than in stimulation of the SLN (3, 22). This finding has been seen as somewhat paradoxical. Sinclair (22) stated that electrical stimulation of the GPN (i.e., the main trunk of the GPN, designated GPN19 by Sinclair) elicited reflex swallowing with great difficulty compared with stimulation of the SLN in the cat. The latency of the first swallow and the time required to initiate three successive swallows were much longer when the GPN was stimulated than when the SLN was stimulated. However, in the present study, we showed that the GPN was as effective as the SLN in eliciting swallowing, whereas electrical stimulation of the GPN-li (the main trunk to the tongue) did not evoke swallowing (Fig. 3C).

Several reports have claimed that stimulation of the GPN inhibits the elicitation of swallowing by stimulation of the SLN (1, 25). We also demonstrated that electrical stimulation of the GPN-li inhibited reflex swallowing elicited by stimulation of the SLN (Figs. 3D and 6). These findings suggest that the GPN-li contains the inhibitory fibers for the initiation of swallowing. According to anatomic studies (14), the main trunk of the GPN in cats consists of lingual branches and small pharyngeal branches. It can be assumed, therefore, that, when electrical stimulation was applied to the main trunk of the GPN, the stimulatory effect of the small pharyngeal branch may have been inhibited by the lingual branch. These findings probably explain why electrical stimulation of the GPN elicits reflex swallowing with more difficulty than does stimulation of the SLN.

In conclusion, the present study revealed that the most reflexogenic areas for swallowing in the pharyngeal region in rats were innervated by the GPN-ph and that the effectiveness of the GPN-ph in evoking swallowing by electrical stimulation is comparable to that of the SLN. These results indicate that the GPN-ph, but not the GPN-li, plays a major role in the initiation of reflex swallowing from the pharynx.

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**REFERENCES**