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Original Paper

Autonomic Responses Associated with Severe Gagging Elicited by Stimulation of the Superior Laryngeal Nerve in Rats

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Abstract

Tactile stimulation of the pharyngo-laryngeal region elicits severe gagging, which is characterized by simultaneous contraction of the costal diaphragm and abdominal muscles. While severe gagging is associated with problems in oral feeding in children, routine dental treatment and gastrointestinal endoscopy, little is known about the neural mechanism of this reflex. In the present study using decerebrate rats, we observed dynamic changes in the activities of the costal and crural diaphragm, abdominal muscles, and infrahyoid muscles, and in pharyngeal and esophageal pressure during severe gagging, and determined the most suitable stimulation of the superior laryngeal nerve (SLN) for induction. High-frequency stimulation of the SLN at 50 Hz (30 μA, 50 pulses) elicited severe gagging in all of the rats. Severe gagging had the following characteristics: 1) simultaneous activation of the costal diaphragm and abdominal muscles, but relaxation of the crural diaphragm; 2) infrahyoid muscle contraction and temporary decrease in pharyngeal pressure; 3) retrograde contraction of esophageal striated muscles; and 4) decrease of blood pressure, which was mediated by a vagal muscarinic pathway. The identification of characteristic changes in respiratory muscles and autonomic responses should assist future studies on the mechanism of severe gagging.

Introduction

The gag reflex is defined, in a narrow sense, as constriction of the pharynx [1, 2]. More traditional descriptions include complex behavioral responses such as lowering of the mandible, forward and downward movement of the tongue, and pharyngeal and velar constriction [2-4]. Furthermore, more severe gagging involves forceful pharyngeal and velar contraction and simultaneous contractions of the costal diaphragm and abdominal muscles (i.e., retching) [2-6].

Severe gagging is considered to prevent a food bolus from being lodged in the pharyngo-laryngeal region and the upper esophagus. A rapid increase in internal gastric pressure is produced by simultaneous contraction of the costal diaphragm and abdominal muscles, which spreads to the esophagus.

Clinical problems related to severe gagging have been reported. Children who were orally deprived

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during infancy cannot have a meal since severe gagging was easily elicited by a food bolus [7]. Sensitive severe gagging prevents dental treatment [8] and gastrointestinal endoscopy [9, 10]. While there have been many clinical reports on severe gagging, little is known about the neural mechanism of this reflex.

In previous human studies, when subjects applied a tactile stimulation to their own oropharyngeal mucosa, severe gagging was elicited in more than 90 % of the subjects [6]. However, it is difficult to elicit the reflex repeatedly and to standardize the intensity of the pressure. Leder [3] reported that tactile stimulation of the oro-pharyngeal region by an examiner elicited severe gagging in only 3 % of normal adult volunteers. Hughes and Wiles [5] reported that touching or stroking of the oro-pharyngeal region by an examiner elicited severe gagging in 25.1 % of healthy adult volunteers. The probability of inducing severe gagging may vary according to the stimulation method and/or the stimulus intensity.

In animal studies, retching in response to emetic stimuli has been well investigated by many researchers [11-15], however, there have been few studies on retching in severe gagging. Beyak et al. [16] observed that electrical stimulation of the superior laryngeal nerve (SLN) induced emesis, which may correspond to severe gagging, in only four of 16 cats. Fukuda and Koga [17] reported that severe gagging could not be induced by SLN stimulation alone, and was observed only when the SLN and abdominal vagal afferent were stimulated simultaneously in dogs. Cats and dogs are not suitable as animal models of severe gagging because induction of severe gagging is difficult.

In rats, Andrew [18] stated that mechanical stimulation of the pharynx elicited the gag reflex, and this reflex is considered to have features similar to those of a single retch [19, 20]. Our previous study using decerebrate rats showed that stimulation of the SLN (20 Hz) occasionally produced severe gagging under the administration of emetic drugs [21]. We reported that severe gagging was elicited by three sets of pulse train stimulation of the SLN at 100 Hz in all rats [22]. In a preliminary study, we found that single pulse train stimulation of the SLN at 50 Hz repeatedly elicited severe gagging without emetic drugs.

Moreover, little is known about the details of the electromyographic activities of muscles and autonomic responses involved in severe gagging. In this study, we observed dynamic changes in the activities of the costal and crural diaphragm, abdominal muscles, and infrahyoid muscles, and in pharyngeal and esophageal pressure during severe gagging, and determined how the frequency and intensity of SLN stimulation could facilitate the induction of this reflex. These physiological features of somatomotor and autonomic responses may assist future studies on the mechanism of severe gagging.

Materials and methods

The experimental procedures were carried out in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Science (Physiological Society of Japan). These procedures were approved by the Institutional Animal Care and Use Committee of Kawasaki University of Medical Welfare (No. 08-018).

Surgical procedure

Experiments were performed on 25 male Sprague Dawley rats, weighing from 320 to 430 g, that were anesthetized by an intra-peritoneal injection of a mixture of urethane (0.7 g/kg) and α-chloralose (0.06 g/kg). When necessary, supplemental doses of a mixture of urethane (0.12 g/kg) and α-chloralose (0.01 g/kg) were given. A midline incision was made on the ventral side of the neck. A stainless steel cannula was inserted into the trachea to maintain the patency of the respiratory tract. Bilateral superior laryngeal nerves (SLNs) were isolated from the surrounding tissue and sectioned near the thyroid cartilage. The central cut end of the unilateral SLN was stimulated with a pulse train using bipolar platinum electrodes.

Decerebration was performed at the precollicular level as follows. The animals were fixed in a stereotaxic frame in the prone position after bilateral external and internal carotid and pterygopalantine arteries were ligated. The dorsal surface of the parietal bones was exposed through an incision along the midline. The bones were removed, the dura was incised and transection was performed at the precollicular level. The brain tissue rostral to the section was removed, and the cranial cavity was loosely packed with cotton balls. A stabilization period of 60 min was allowed after decerebration.

Rats were removed from the frame and then rotated to the supine position. The blood pressure of the femoral artery was monitored. No further anesthetics were given after decerebration. Rectal temperature was maintained at 37-38 °C with a heating pad.

Stimulation and recording

Severe gagging was elicited by electrical stimulation of the SLN with a pulse train (intensity: 5, 10, 20 and 30 μA, frequency: 5, 10, 20 and 50 Hz, number of pulses: 5, 10, 20, 30, 40 and 50, duration: 0.3 ms). The electromyographic activities of the inferior hyoid muscles and abdominal muscles were recorded. In addition, activities of the diaphragm, including the costal and crural parts, were recorded. Investigation revealed that the costal and/or crural parts of the diaphragm contracted when the severe gagging occurred, and the activities of these two parts were recorded separately in an additional experiment. Latex-rubber balloons connected to a pressure transducer were positioned 3 cm (pharynx), 5 cm (upper esophagus) and 9 cm (lower esophagus) from the upper incisors, and inflated with ~0.1 ml of air. All data were digitized using an analog-to-digital converter (PowerLab/16sp; ADInstruments Pty Ltd., Bella Vista, Australia) and stored on a computer. The digitized data were analyzed using Chart ver. 5.0 software program (ADInstruments Pty Ltd., Bella Vista, Australia).

Vagotomy and muscarinic antagonist administration

To investigate the role of parasympathetic nerves in the autonomic response related to severe gagging, bilateral vagotomy was performed in 9 rats. Further, intravenous administration (5 mg/kg) of atropine methyl nitrate (Sigma Chemical Co., St. Louis, MO, U.S.A), which is a muscarinic acetylcholine receptor antagonist, was performed in 8 rats. This drug was dissolved in normal saline solution and administered through the femoral vein.

Assessment of esophageal responses

Esophageal responses were analyzed using the Chart program. Each response was divided into two periods; namely, 2 seconds before the onset of SLN stimulation (P0) and 2 seconds after the onset of SLN stimulation (P1) for quantitative analysis. An esophageal integrated value based on the minimum value between P0 and P1 was calculated for each period. To normalize the data, the ratios of esophageal integrated value between P0 and P1 were used for analyses.

Statistical analysis

All values in the text are presented as means ± standard error (SE). Statistical analysis was performed with Wilcoxon signed-rank test using SPSS statistical analysis software (SPSS Ver. 15, SPSS Inc., Chicago, IL, U.S.A.). Significance level was taken as $p < 0.05$.

Results

Identification of severe gagging induced by superior laryngeal nerve stimulation

The severe gagging characteristically shows synchronous contraction of the diaphragm and abdominal muscles. Figure 1 shows an example of severe gagging elicited by SLN stimulation (50 Hz, 0.3 msec duration, 30 μA), and the following 2 swallowing reflexes. Intra-pharyngeal pressure rapidly increased at the onset of SLN stimulation, and this response was considered to be pharyngeal constriction in the gag reflex (indicated by "G", Fig 1). After the gag reflex, synchronous contractions of the infrahyoid muscles (IH), diaphragm (DIA) and abdominal muscles (ABD) appeared; i.e., severe gagging (indicated by "SG", Figs. 1-3). A decrease in pharyngeal pressure, synchronous increases in the upper (UE) and lower (LE) esophageal pressure and temporary decrease of blood pressure (BP) were observed simultaneously with severe gagging (Fig. 1). After the termination of SLN stimulation, the swallowing reflex was elicited spontaneously (indicated by "S", Fig. 1.). Temporary activation of the infrahyoid muscles and a subsequent rapid increase in pharyngeal pressure were observed, and an increase in internal pressure from the pharynx to the upper and lower esophagus was propagated by orthodromic peristalsis (indicated by arrows, Fig. 1). Inspiratory activities of the diaphragm were temporarily suppressed (swallowing apnea, Fig. 1).

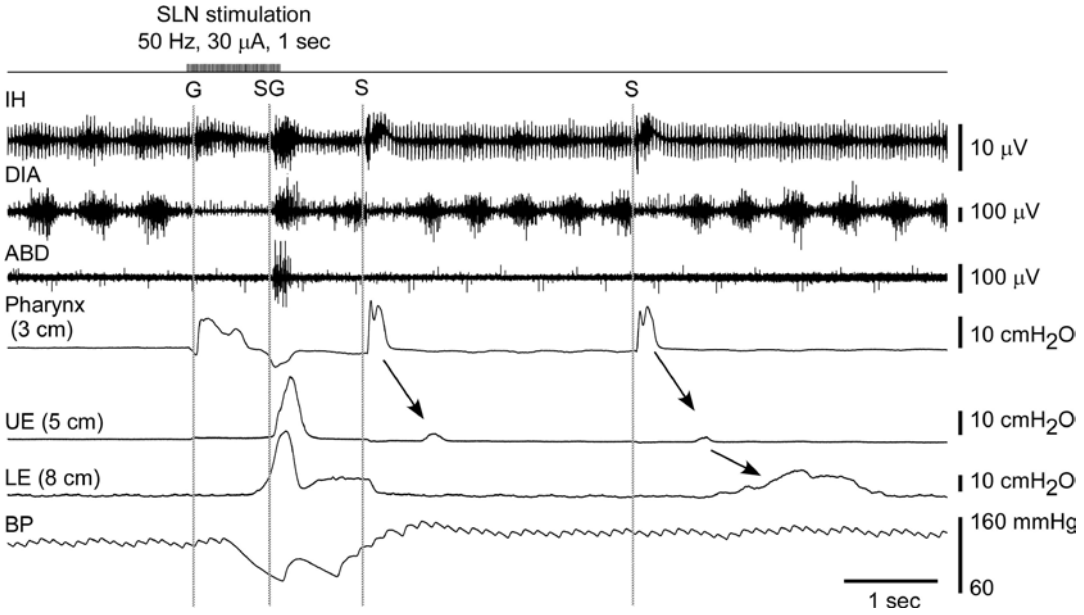

Fig. 1 Dynamic changes in infrahyoid muscles, the diaphragm, abdominal muscles, pharynx, esophagus and blood pressure during severe gagging elicited by SLN stimulation. Electromyographic activities of infrahyoid muscles (IH), diaphragm (DIA) and abdominal muscles (ABD) were recorded. Changes in the internal pressure of the pharynx (3 cm from the upper incisors), upper esophagus (UE, 5 cm from the upper incisors) and lower esophagus (LE, 8 cm from the upper incisors) were recorded. Vertical lines under SG and S indicate the onset of severe gagging and the swallowing reflex, respectively. Arrows show the propagation of the increase in internal pressure from the pharynx to the esophagus. Abbreviations: BP, arterial blood pressure; G, gag reflex; SG, severe gagging; S, swallowing reflex.

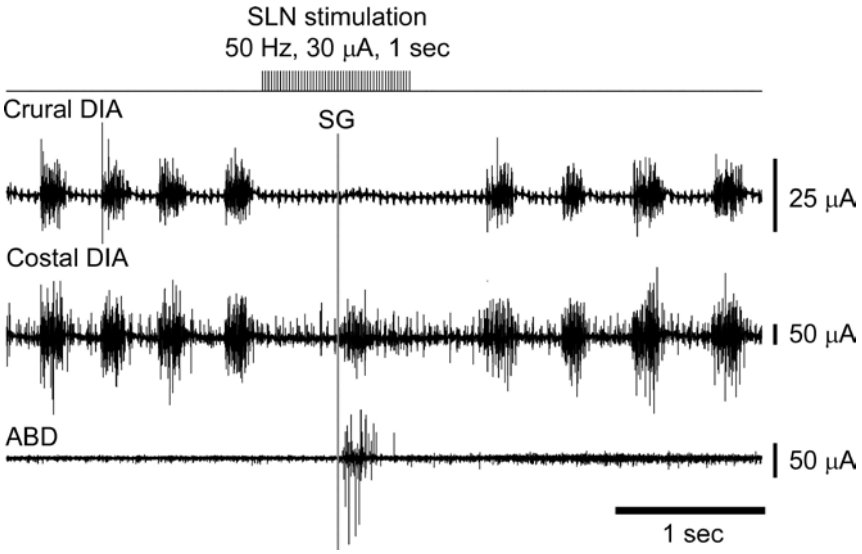

Fig. 2 Responses of the crural and costal diaphragm during severe gagging. The costal diaphragm, but not the crural part, contracted simultaneously with the abdominal muscles.

The crural region of the diaphragm, not the costal region, is known to relax in the expulsive phase of the vomiting reflex [23, 24]. We examined the behaviors of these two distinct muscles during severe gagging. The costal diaphragm was activated synchronously with abdominal muscle activity, whereas the crural diaphragm was silent (Fig. 2).

Stimulus parameters of the SLN for induction of severe gagging

We stimulated the SLN with various stimulation parameters to identify the threshold values at which severe gagging was induced (frequency: 5-50 Hz; number of pulses: 5-50; intensity: 5-30 μA; duration: 0.3 ms) in 8 decerebrate rats. Firstly, to examine the effect of stimulus frequency, electrical stimulation of 5, 10, 20 and 50 Hz at a constant intensity (30 μA) for 1 sec was applied to the SLN. SLN stimulation at 20 Hz elicited severe gagging in 1 rat. However, SLN stimulation at 50 Hz was required for induction of severe gagging in the remaining 7 rats (Table 1, A). Secondly, the effects of stimulus intensity on the induction of severe gagging were investigated at a constant frequency and pulse number (50 Hz, 50 pulses). Severe gagging was elicited by stimulation of the SLN at 5 μA in 1 rat. SLN stimulation at more than 30 μA elicited severe gagging in all of the rats (Table 1, B).

Table 1 Threshold of severe gagging induction

	A frequency (30 μA, 1 sec)	B intensity (50 Hz, 50 pulses)
rat 1	50 Hz	20 μA
rat 2	20 Hz	5 μA
rat 3	50 Hz	10 μA
rat 4	50 Hz	10 μA
rat 5	50 Hz	10 μA
rat 6	50 Hz	30 μA
rat 7	50 Hz	20 μA
rat 8	50 Hz	20 μA

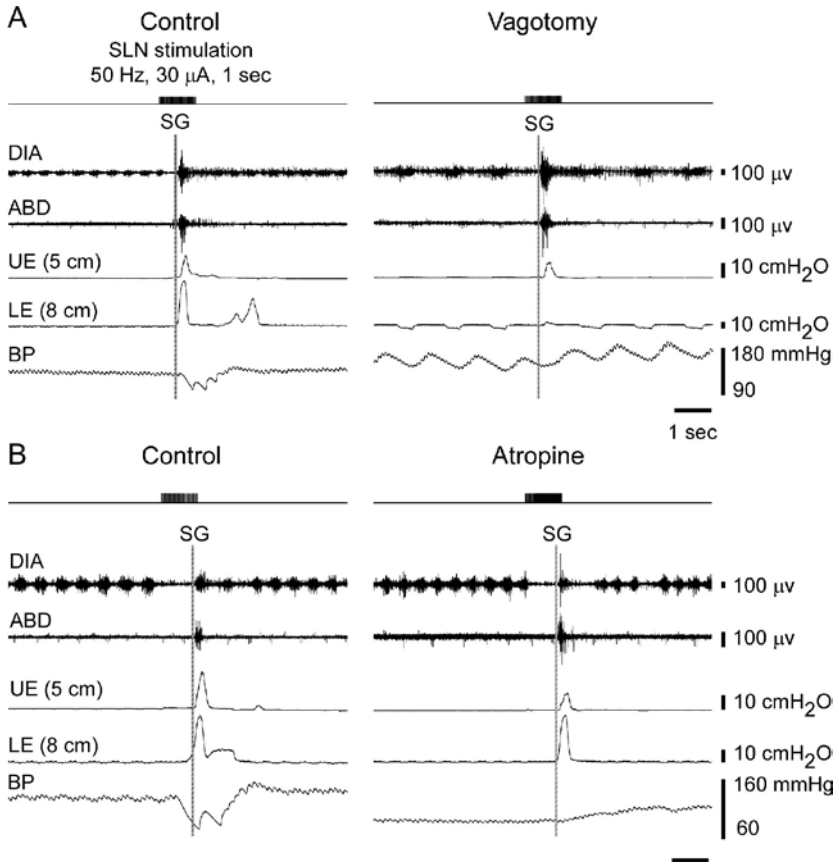

Fig. 3 Effects of vagotomy and atropine on changes in esophageal motility and blood pressure during severe gagging. See text for details.

Participation of vagal nerve in autonomic changes during severe gagging

Autonomic changes associated with severe gagging were observed in this study. In the control recording, a rapid increase in pressure in the upper and lower esophagus and temporary decrease of blood pressure were observed (Fig. 3). The increase in lower esophageal pressure was diminished by cervical vagotomy, whereas upper esophageal pressure was reduced by vagotomy, as shown in Fig. 3A. Figure 3B showed that the increase in upper esophageal pressure was reduced by atropine administration, whereas lower esophageal pressure was not changed. Atropine antagonized decrease of blood pressure associated with severe gagging in all rats (Fig. 3B).

To clarify the role of parasympathetic nerves in these autonomic changes, cervical vagotomy was conducted in 9 rats. The ratios of upper and lower esophageal integrated pressures between P0 and P1 were 20.4 ± 3.48 and 6.84 ± 0.62, respectively (Fig. 4AB). The ratios of upper and lower esophageal integrated pressures between P0 and P1 were significantly diminished to 5.29 ± 1.26 and 0.60 ± 0.15, respectively ($p<0.05$, Fig. 4AB).

To investigate the roles of muscarinic receptors in esophageal contraction associated by severe gagging, administration of atropine (5 mg/kg, i.v.) was conducted in 8 rats. Atropine significantly reduced the increased pressure of the upper esophagus (Fig. 4A), however, it had no obvious effects on contraction of the lower esophagus (Fig. 4B).

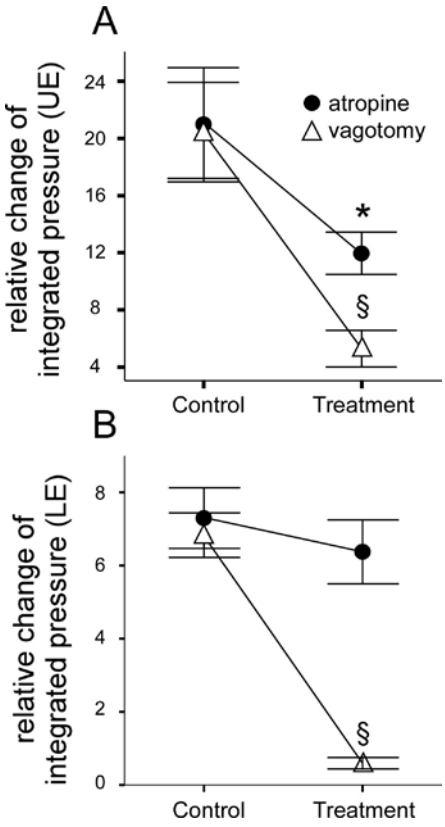

Fig. 4 Relative changes of integrated esophageal pressure associated with severe gagging by vagotomy (§, $p<0.05$, n=9) and atropine administration (*, $p<0.05$, n= 8).

Discussion

Stimulation used to induce severe gagging

In previous studies, the frequency that has been used most often to induce swallowing in rats is between 10 and 30 Hz [25-29]. Furthermore, the stimulus intensity that has been used to induce the swallowing reflex has been reported to be 10-200 μA [26, 28, 29]. There has been no mention of severe gagging in these studies. In the present study, SLN stimulation at 50 Hz and 30 μA elicited severe gagging in all of the rats. Our previous study [22] reported that SLN stimulation at 100 Hz elicited severe gagging in all rats. Thus, stimulation of the SLN at more than 50 Hz was considered to be most effective for induction of severe gagging. High-frequency stimulation of afferent nerves is known to produce temporal summation in secondary neurons. Thus, it seemed that temporal summation in the nucleus of the solitary tract (NST) where the SLN projects is necessary for induction of severe gagging.

Features of severe gagging elicited by SLN stimulation

Diaphragm and abdominal muscles. In the present study, high-frequency stimulation of the SLN elicited synchronous burst activities of the diaphragm and abdominal muscles, which correspond to severe gagging. However, stimulation of the SLN at 10-20 Hz also elicited the cough reflex in decerebrate cats [30, 31]. The main force of the cough reflex is considered to be burst activity of the diaphragm and abdominal muscles. The cough reflex was characterized by an expiratory motor act closely combined with a preceding deep inspiratory motor act [30-32]. In this study, however, no preceding diaphragmatic activity was observed before abdominal burst activities elicited by SLN stimulation. Thus, the synchronous burst activity of the

diaphragm and abdominal muscles did not result in the cough reflex.

Stimulation of the SLN elicited simultaneous activation of the costal diaphragm and abdominal muscles, whereas crural diaphragm activity was not observed (Fig. 2). The somatomotor responses characterized by simultaneous contraction of the diaphragm and abdominal muscles are generally observed during retching and subsequent expulsion in an emetic response. It has been reported that the costal and crural parts of the diaphragm and abdominal muscles synchronously contract in retching, whereas the crural part of the diaphragm relaxes in the expulsion phase [23, 24]. The somatomotor activity patterns of the diaphragm and abdominal muscles in this study were similar to those in the expulsion phase. Relaxation of the crural diaphragm is believed to promote expulsion of the gastric contents. However, rats have a powerful barrier between the esophagus and stomach that would make it difficult to expel the gastric contents [33, 34]. In general, the vomiting and severe gagging have different roles in the defense system. The vomiting reflex mainly prevents toxic compounds from being absorbed in the lower alimentary canal. In contrast, severe gagging prevents foreign materials from blocking the upper alimentary canal. In fact, we have previously reported that SLN stimulation during hypoxia produced burst activities of the diaphragm and abdominal muscles, which was assumed to be severe gagging [21].

Pharynx. A temporary decrease in pharyngeal pressure was observed simultaneously with the SLN-induced severe gagging (Fig. 1). As described above, severe gagging is considered to prevent a bolus from blocking the upper alimentary canal. This may be why relaxation of the pharynx occurs simultaneously with severe gagging.

On the other hand, infrahyoid muscles contract with severe gagging. Groups of hypoid muscles are related to jaw-opening movement. In emetic behavior, the mouth is widely opened by the contraction of jaw-opening muscles and hyoid muscles to expel the gastric contents [23]. In a human study, digastric muscle activity was reported to be associated with severe gagging [6]. Thus, a temporary decrease in pharyngeal pressure with severe gagging may be related to jaw-opening movement.

Esophagus. Lang et al. [35] reported that retrograde contraction of the cervical esophagus was observed during the expulsion phase in dogs. In the present study, an increase in upper esophageal pressure was slightly preceded by an increase in lower esophagus pressure during severe gagging. This pattern of activity in the esophagus seems to be retrograde contraction, which was reported by Lang et al. [35] in dogs. However, they recoded electromyographic activity in the esophagus, whereas in our study we recorded intraesophageal pressure. Therefore, these pressure changes were due to body movement associated with actual severe gagging. Thus, we performed a further study using vagotomy and a neurotransmitter antagonist.

The rat esophagus contains striated as well as smooth muscles over its entire length and electrical stimulation of vagus nerve elicits the contraction of both muscles [36]. Furthermore, it has been reported that the contraction of esophageal smooth muscles elicited by electrical stimulation of the vagus nerve was abolished by administration of the muscarinic acetylcholine receptor antagonist atropine, whereas that of esophageal striated muscles was not abolished [36]. We investigated the effect of atropine administration on esophageal changes associated with severe gagging. Atropine significantly suppressed upper esophageal response, whereas it did not influence lower esophageal response, as shown in Fig. 3B and 4AB. Thus, changes in upper esophageal pressure associated with severe gagging could be partly produced by the contraction of esophageal smooth muscles. However, changes in lower esophageal pressure could be partly produced by the contraction of esophageal striated muscles. Furthermore, the increase in lower esophageal pressure associated with severe gagging disappeared after cervical vagotomy in the present study (Fig.

3A and 4AB), however, the increase in upper esophageal pressure remained even after the vagotomy. The upper esophagus is known to be controlled by the pharyngo-esophageal nerves which are the vagal branch just above the nodose ganglia. Since the SLN merges with the vagus nerve below the nodose ganglia, vagotomy was performed at the cervical level. Thus, upper esophageal contraction with severe gagging remained after cervical vagotomy.

Blood pressure. Temporary decrease of blood pressure was observed following SLN stimulation that induced severe gagging (Fig. 1). However, SLN stimulation below 20 Hz seldom elicited such conspicuous changes in blood pressure. Vagotomy or atropine administration completely eliminated the decrease of blood pressure produced by SLN stimulation (Fig. 3). There have been no reports concerning the changes in blood pressure associated with severe gagging. Faber and Brody [37] reported that stimulation of the SLN produced frequency-dependent reductions in arterial pressure and heart rate. The carotid sinus, aortic depressor nerve and SLN are known to project to the intermediate and caudal portions of the NST in the medulla oblongata. Based on these observations, they concluded that the SLN may constitute a significant projection pathway for the baroreflex. Furthermore, Mendelowitz [38] reported that superior laryngeal neurons directly excite cardiac vagal neurons in the nucleus ambiguus in the medulla oblongata. Thus, the decrease of blood pressure elicited by high-frequency stimulation of the SLN is considered to be the baroreflex via a vagal pathway that involves a muscarinic synapse.

In conclusion, severe gagging had the following characteristics: 1) simultaneous activation of the costal diaphragm and abdominal muscles, but relaxation of the crural diaphragm; 2) infrahyoid muscle contraction and temporary decrease in pharyngeal pressure; 3) retrograde contraction of esophageal striated muscles; and 4) decrease of blood pressure, which was mediated by a vagal muscarinic pathway. This study identified a reliable method of inducing severe gagging and also the physiological features of severe gagging. Based on these results, we will investigate the neural mechanism of severe gagging in a further study.

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