Vagal esophageal receptors in anesthetized dogs: mechanical and chemical responsiveness

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Sekizawa, S.-I., T. Ishikawa, F. B. Sant'ambrogio, and G. Sant'ambrogio. Vagal esophageal receptors in anesthetized dogs: mechanical and chemical responsiveness. J. Appl. Physiol. 86(4): 1231–1235, 1999.—This study was performed to evaluate the characteristics of esophageal receptors in anesthetized and artificially ventilated dogs. The electrical activity of the esophageal afferents was recorded from the peripheral cut end of the cervical vagus nerve. A cuff catheter was inserted into the esophagus at the level of the third tracheal ring and was used to establish the esophageal location of the endings. Most of the receptors were localized in the intrathoracic portion of the esophagus. The majority of the receptors studied (36 of 43) showed a slow adaptation to a maintained stretch of the esophageal wall. Vagal cooling blocked receptor activity at temperatures ranging from 3.5 to 25°C. Twenty-eight of 43 receptors, including 4 rapidly adapting endings (RAR), were challenged with saline, HCl + pepsin (HCl-P; pH 1) and distilled water (8 ml, 37°C). HCl-P solutions specifically stimulated only three receptors; saline or water did not. Five slowly adapting receptors and two RARs were also challenged with topically applied capsaicin; only one RAR was stimulated. To ascertain a possible effect of smooth muscle contraction, 17 receptors were tested with intravenous injections of ACh and/or asphyxia; only 4 were stimulated. These characteristics do not support an important reflexogenic role of the esophagus in response to chemical stimuli.

Methods

Animals, anesthesia, surgical procedure, and recording. After our protocol was approved by the Institutional Animal Care and Use Committee, experiments were carried out on nine healthy mongrel adult dogs of either sex (14.4–17.7 kg). The animals were sedated with ketamine (10 mg/kg im), anesthetized with urethan and α-chloralose (500 and 50 mg/kg iv, respectively), and placed in a supine position on an operating table. The femoral artery and vein of either side were catheterized for monitoring of arterial blood pressure and administration of agents, respectively. The cervical trachea was exposed and cut at the fourth to sixth ring to insert a tracheal cannula with a sidearm. The animal was then paralyzed with doxacurium chloride (2.5 mg iv) and was artificially ventilated through the cannula with a constant-volume type of ventilator (model 607: Harvard Apparatus, Millis, MA). The chest was opened by a median sternotomy, and tidal volume and breathing frequency were adjusted to maintain a constant level of anesthesia (urethan, 0.15 g·kg⁻¹·h⁻¹; α-chloralose, 15 mg·kg⁻¹·h⁻¹; doxacurium, 0.5 mg/h). Pressure in the trachea was measured with a differential pressure transducer (model 16720; Gould, Hato Rey, Puerto Rico) with one port open to the atmosphere. A Foley catheter (22 Fr, 30 cc; Travenol Laboratories, Deerfield, IL) was carefully inserted into the cervical esophagus through a stoma at the level of the third to fourth tracheal ring.

The cervical vagus of either side was exposed and cut. The peripheral cut end of the vagus was placed in a tray filled with warmed paraffin oil and was separated from the surrounding connective tissues by using watchmaker forceps under a binocular microscope. To record the afferent activity from the esophagus, thin filaments separated from the vagal nerve were placed on a pair of platinum electrodes connected to a low-noise DC preamplifier (P15, Grass Instrument, Quincy, MA) and a biophysical amplifier (AM502, Tektronix, Beaverton, OR). Single-unit action potentials were displayed on an oscilloscope in parallel with a loudspeaker. Signals were simultaneously recorded by a thermal-array recorder (TA5000; Gould, Valley View, OH) and were stored at a sampling rate of 20,000 Hz in a personal computer connected to an analog-to-digital converter (WinDAQ/200, Dataq Instruments, Akron, OH).

Experimental protocol. The location of the esophageal receptors was carefully established by inflating the balloon of the Foley catheter, the tip of which had been placed just above the lower esophageal sphincter. The catheter was then pulled cranial until the receptor field was found. The distance from this site and the esophageal stoma was measured to establish the location of the receptor along the esophagus. In addition, the outside of the esophagus and/or the airway was probed to exclude an extravesophageal location of the ending. Each
receptor was then tested with saline (0.9% NaCl unbuffered, pH 6.4), distilled water (pH 7.0), hydrochloric acid-pepsin (HCl-P, pH 1; pepsin, 2 mg/ml), and, in some cases, capsaicin (0.2 mg/ml, pH 6.4). The HCl-P solution is meant to mimic the composition of gastric juice, i.e., that of the refluxate. The capsaicin challenge was meant to establish a possible nociceptive nature of these receptors. The solutions (8 ml, 37°C) were instilled with a homemade catheter, the tip of which was placed in the esophagus in proximity of the receptor site. This catheter had several holes along 4–5 cm of its distal end to allow the irrigation of the esophagus with a fine spray. Some receptors were challenged with an injection of ACh (40 mg/kg iv) and/or 20 s of asphyxia to induce smooth muscle contraction (8). Some filaments were also tested with cold block of conduction to characterize their myelinated or nonmyelinated nature.

Data analysis. Esophageal receptors were classified as slowly adapting (SAR) or rapidly adapting receptors (RAR) on the basis of their discharge pattern during a maintained distension of the esophageal wall induced by inflation of the balloon of the Foley catheter. Discharge frequency of the receptors was counted in 10-s bins for 30 s after the instillation.

Differences within a group were determined through one-way ANOVA for repeated measures, and between-groups comparisons were determined through two-way ANOVA for

Fig. 1. Example of a slowly adapting receptor of canine esophagus (smallest spikes). A.P., action potentials; Ptr, tracheal pressure. This receptor was located in the extrathoracic esophagus. Intravenous injection of ACh, as well as asphyxia (bottom tracings), decreased blood pressure and simultaneously strongly stimulated this receptor. Response to balloon inflation (hatched bar) was blocked at 9°C. Note that the 2 receptors (larger spikes) with respiratory modulation are still active at 10°C.

Fig. 2. Example of a rapidly adapting receptor of the canine esophagus (larger spikes). Note that response to HCl-pepsin is almost the same as that to capsaicin. This receptor was cold blocked at 6.5°C (not shown).
repeated measures. The Dunnett post hoc test was used for multiple comparisons. The level of significance was set at $P < 0.05$. Data are presented as means ± SE.

**RESULTS**

More than 80% of receptors recorded from thin filaments separated from the vagus nerve were identified as nonesophageal receptors and thus were excluded from this study. Forty-three esophageal receptors were recorded: 36 SARs and 7 RARs (Figs. 1 and 2, Table 1). Fourteen SARs and three RARs were spontaneously firing, in most cases with a sparse activity. Thirty-eight of the 43 esophageal receptors recorded were localized: 3 SARs were located in the extrathoracic portion, and 29 SARs and 6 RARs were located in the intrathoracic portion. They were particularly concentrated near both ends of the intrathoracic esophagus (Fig. 3).

Twenty-eight receptors (24 SARs and 4 RARs) were challenged with saline, water, and HCl-P. Twelve were equally stimulated by the three solutions, and only two SARs and one RAR, localized in the distal portion of the intrathoracic esophagus, were stimulated more by HCl-P solution than by saline or water. Figure 4 shows the overall changes in discharge frequency of the 15 receptors stimulated by these challenges. There was no significant difference in the response to each solution ($P > 0.05$).

Five SARs and two RARs were also challenged with capsaicin; one RAR was stimulated to an extent similar to that of HCl-P administration (Fig. 2). Seventeen receptors (16 SARs and 1 RAR) were tested with an iv injection of ACh and/or asphyxia; four SARs were activated (Fig. 1 and Table 1).

Cooling the whole vagus blocked the neural conduction of the 25 receptors tested; all of them were blocked at temperatures ranging from 3.5 to 25°C ($8.7 ± 0.9°C$; Fig. 5).

**DISCUSSION**

In this study, most of the filaments separated from the main trunk of the vagus carried activity that originated from the airway. This suggests a relatively scant afferent innervation of the esophagus. The overwhelming presence of bronchopulmonary receptors, compared with esophageal receptors, is clearly shown in a paper by Mei (see Fig. 3 in Ref. 17).

The electrical activity of vagal esophageal afferents has been investigated in several species, such as dogs (21), cats (4), sheep (5), and ferrets (1). However, these studies were mostly performed to test the mechanosensitivity of these endings, which is possibly associated with the swallowing mechanism. This study was performed mostly to evaluate the chemosensitivity, especially to HCl, of vagal esophageal receptors. Of the 28 receptors tested, only 3 (10.7%) were specifically stimu-

![Fig. 3. Localization of receptors along the esophagus. Intrathoracic esophagus was divided longitudinally into 4 equal portions (1st, 2nd, 3rd, 4th Qtrs) starting from the cranial side. Note: receptors were relatively more concentrated at both ends of the intrathoracic esophagus.](image)

![Fig. 4. Time course of the changes in discharge frequency of esophageal receptors responding to administration of each solution. There is no significant difference among the responses ($n = 15$). Imp, Impulses; HCl-P, HCl-pepsin.](image)

![Fig. 5. Distribution of temperatures needed for blocking esophageal receptor discharge. Note: majority of receptors are cold blocked at temperatures ranging from 5 to 10°C.](image)
lated by HCl-P; i.e., they were more stimulated by HCl-P than by water or saline. None was specifically stimulated by distilled water. Furthermore, only one of seven receptors was stimulated by capsaicin. Overall, our results suggest that esophageal receptors possess a scant chemosensitivity, in contrast to a well-developed mechanosensitivity. In fact, esophageal distension stimulated all receptors tested. Moreover, the overall response to saline, water, and HCl-P was similar. This suggests, once more, that the response was mostly due to the mechanical effect of the instillation rather than to a specific chemical stimulation.

GER is a frequently observed clinical disorder, especially in infants and the elderly (2, 6, 9, 12, 14–16). Reflux of gastric content is also frequently observed in patients with bronchial asthma (2, 12). Although there is agreement on the reflex nature of this bronchoconstriction, there is still debate concerning the site of origin of this reflex: esophagus (10, 18, 22) or airways (3, 19, 23). In the present study, receptors responding to HCl-P were located at the distal end of the intrathoracic esophagus. Thus our results may support the hypothesis that the lower esophagus might play a role as a reflexogenic site in the vagally mediated bronchoconstriction with GER (10, 18). However, considering that only a few receptors were specifically stimulated by HCl-P and that esophageal instillation of the acidic solution could not induce any significant response of airway smooth muscle (see companion paper (12a)), the esophagus does not appear to be an important site for the elicitation of the bronchoconstriction associated with GER. Afferents in other structures, such as the larynx and the proximal portion of the tracheobronchial tree, seem to be more likely candidates for the bronchoconstriction: a response widely recognized to originate from them.

Airways RARs have a high responsiveness to nociceptive stimuli, hence the definition of “irritant” receptors (7). However, in the present study, RARs appear to be scant in number and to have an overall weak chemosensitivity. In fact, only one RAR of the seven tested responded to HCl-P and capsaicin. Thus these results indicate that the characteristics of esophageal RARs are quite different from the characteristics of airway RARs.

To further characterize esophageal receptors, the conduction of the nerve was blocked by cooling the whole vagus nerve. Although this method might not distinguish from which type of fibers (i.e., Aβ-fiber, Aδ-fiber, C-fiber, etc.) each receptor originates, it can indicate their general nature as myelinated or nonmyelinated fibers (20). In the present study, all the receptors tested with cooling were blocked at temperatures >0°C; this suggests a myelinated nature. This is supported by previous results (21), in which the conduction velocity of the esophageal receptors in dogs was found to be >9.3 m/s; this is compatible with myelinated fibers. However, the conduction velocity of the esophageal receptors in cats is lower, ranging from 0.8 to 1.2 m/s, which is compatible with nonmyelinated fibers (4). Thus, marked species differences would seem to exist among animals.

The limited number of esophageal receptors (23.5%) responding to iv ACh and/or asphyxia could be attributed to the partial esophageal denervation (monolateral vagotomy). An additional factor might depend on a different location of the endings within the esophageal wall and thus a different relationship with the esophageal muscles.

Recently, Hamamoto et al. (11) showed that acid infusion into the esophagus caused plasma extravasation in airways through capsaicin-sensitive fibers and neurokinin-1 receptors. Their results may be supported by the present study. In fact, we could record receptors responding to solution that was less acid than that in their study (pH 0), and one receptor was also stimulated by capsaicin solution.

In conclusion, in the dog, the esophagus appears to have a scant vagal innervation, with a preponderance of SARs having a possible role in the mechanisms of deglutition. Moreover, there is a poor response to acidic solutions and other irritants. These receptors have different properties than do vagal airway receptors and do not appear to sustain an important role of the esophagus in response to chemical irritants.

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