Effect of galanin and galanin antagonists on peristalsis in esophageal smooth muscle in the opossum

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Yamato, Shigeru, Ikuo Hirano, and Raj K. Goyal. Effect of galanin and galanin antagonists on peristalsis in esophageal smooth muscle in the opossum. *Am J Physiol Gastrointest Liver Physiol* 279: G719–G725, 2000.—Galanin, a neuropeptide that is widely distributed in the esophageal nerves, is known to exert a neuromodulatory action in the gut. These studies examined the effect of galanin and galanin antagonists on esophageal peristalsis in anesthetized opossums in vivo. Intraluminal esophageal pressures were recorded at 1, 3, 5, 7, and 9 cm above the lower esophageal sphincter. Esophageal peristaltic contractions were induced by swallow and short- (1-s) and long-train (10-s) vagal stimulation (VS). Galanin (1 nmol/kg) inhibited the amplitude of swallow-induced peristaltic contractions and increased peristaltic velocity by enlarging the latency periods in the upper part of the esophagus and reducing them in the lower part. Galinin nearly abolished esophageal contractions caused by short-train VS at 5 Hz and inhibited the contractions at 10 Hz. Galanin increased latency periods induced by short-train VS with little change in the velocity of peristalsis and reduced the amplitude of both A (cholinergic) and B (noncholinergic) contractions due to long-train VS. However, the decrease in amplitude of B contractions was more marked. Galantide (3 nmol/kg) antagonized the inhibitory action of exogenous galanin on esophageal contractions elicited by short-train VS, but by itself galantide had no significant effect on esophageal contractions. In conclusion, exogenous galanin inhibits the amplitude of swallow-induced peristaltic contractions and converts them into nonperistaltic contractions by inhibiting both the cholinergic and noncholinergic components.

nonperistaltic contractions; cholinergic nerves; nonadrenergic, noncholinergic nerves

GALANIN IS A NEUROPEPTIDE with 29 amino acids (30 in humans) that is widely distributed in the central and peripheral nervous system, including the enteric nervous system (9, 22, 26, 27). In the gut, it binds to synaptosomes as well as to smooth muscle cells (5, 17, 23) and exerts diverse tissue-specific actions (20). Galanin can have an inhibitory effect on myenteric neurons, but it often coexists with other neurotransmitters such as ACh, vasoactive intestinal polypeptide (VIP), and nitric oxide synthase in the nerve endings and acts presynaptically to inhibit the neurotransmitter release (8, 12, 19, 25). On the other hand, galanin may also serve as an excitatory mediator that causes release of other neurotransmitters (4, 18). Galanin can also exert an excitatory or an inhibitory effect on the smooth muscle. For example, galanin causes contraction of the circular muscle but causes relaxation of the longitudinal muscle of guinea pig ileum by stimulating distinct excitatory and inhibitory receptors, respectively (3, 10, 11).

In the esophagus, galanin is localized to myenteric and submucosal neurons and in nerve fibers around the neurons and those innervating esophageal smooth muscle (24). Galanin causes contraction of the lower esophageal sphincter by a direct action and inhibition of relaxation by suppression of the activity of nonadrenergic, noncholinergic (NANC) inhibitory nerves (21). The purpose of these studies was to investigate the effect of galanin on esophageal peristaltic contractions and study the action of galanin antagonists to determine the possible physiological role of galanin in esophageal peristalsis.

MATERIALS AND METHODS

The study protocol was approved by the Animal Studies Subcommittees of Beth Israel Hospital (Boston, MA) and the West Roxbury Veterans Affairs Medical Center (West Roxbury, MA). Studies were performed in anesthetized opossums (*Didelphis virginiana*) weighing 2.3–3.9 kg. After an overnight fast, animals were initially anesthetized with pentobarbital sodium (40 mg/kg ip). Subsequently, α-chloralose (30–50 mg iv) was administered slowly, as needed, to maintain anesthesia. Anesthetized animals were strapped supine on an animal board and maintained at 37°C with a heating pad. The brachial artery was cannulated to monitor blood pressure, and the brachial vein was cannulated for administration of test agents as needed.

Intraluminal esophageal pressures were measured with a catheter assembly consisting of six polyvinyl catheters. Each catheter had a side hole: the five proximal holes were situated 2 cm apart, and the sixth, most distal, hole was 1 cm distal to the fifth so that esophageal pressure was measured at 1, 3, 5, 7, and 9 cm above the lower esophageal sphincter. The outside diameter of the assembly was 5 mm. Each
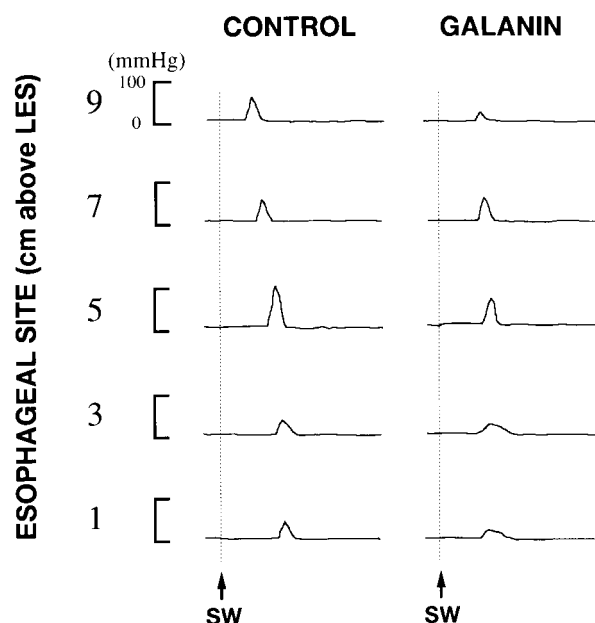
Fig. 1. Manometric tracing of the effect of galanin on swallow-induced esophageal peristalsis. SW, the onset of swallowing. Note that galanin reduced the amplitude of peristaltic contractions. Moreover, galanin increased peristaltic velocity by increasing the latency of contraction in the upper esophagus and decreasing the latency in the lower esophagus. LES, lower esophageal sphincter.

catheter was continuously perfused with bubble-free distilled water using a low-compliance pneumohydraulic system.

Swallowing was induced by stroking the pharynx with a cotton swab. The onset of swallow-induced peristalsis was determined by the onset of spike bursts in the mylohyoid muscle. Mylohyoid electromyography was recorded using a conventional bipolar electrode as described previously (14).

In some animals, the vagi were isolated in the neck and severed, and the peripheral end of one decentralized vagus was used for electrical stimulation with a bipolar electrode. Vagal stimulation of short (1-s) and long (10-s) trains was applied with square-wave pulses of 60 V and 0.5-ms pulse duration at 5, 10, and 20 Hz using a Grass stimulator (model S11; Quincy, MA).

In all animals, control responses were obtained first, followed by responses after galanin. Galanin was dissolved in saline and administered as an intravenous bolus followed by continuous intravenous infusion. Intravenous bolus galanin increased blood pressure in a dose-dependent manner. The increase in mean arterial pressure was $24.4 \pm 4.4\%$, $26.8 \pm 4.0\%$, $40.7 \pm 5.9\%$, and $35.5 \pm 1.5\%$ with doses of 0.1, 0.3, 1, and 3 nmol/kg iv galanin bolus, respectively. Galanin (1 nmol/kg iv) bolus increased mean arterial pressure from 111.3 ± 4.2 to 154.6 ± 4.6 mmHg ($n = 12$, $P < 0.01$). Earlier study (21) showed that this dose of galanin also caused a maximal increase in lower esophageal sphincter pressure in opossum. With intravenous bolus, galanin blood pressure reached a maximal value within 2–3 min and returned to the control level after ~10 min. Therefore, intravenous bolus was combined with continuous infusion of galanin. With the combination of intravenous bolus (1 nmol/kg) followed by intravenous infusion of $1 \text{ nmol} \cdot \text{kg}^{-1} \cdot 2 \text{ min}^{-1}$ of galanin, blood pressure remained stable during the infusion. In these periods, heart rate decreased from 162 ± 4 to 118 ± 2/min ($n = 6$, $P < 0.01$). Galanin antagonists were also administered as intravenous boluses followed by intravenous infusion. When

the effect of galanin antagonist on galanin was examined, galanin and galanin antagonist were administered as intravenous bolus only. The dose of galanin antagonist was three times higher than that of galanin. The galanin antagonist (galantide) only transiently reduced blood pressure, which returned to the control level in 3 to 5 min. Mean arterial pressure was 109 ± 2 and 114 ± 5 mmHg, respectively, before and after galantide.

Statistics

Quantitative data are expressed as means $\pm$ SE. Animals served as their own controls. Statistics of effects of treatments were determined in each animal (3–5 observations in each animal) separately and also cumulatively in all animals. Statistical analysis were performed using paired or unpaired t-tests and ANOVA for multiple comparisons.

Drugs

Galanin and galanin antagonists galantide (M15) and M40 were purchased from Peninsula Laboratories. N^ω-nitro-L-arginine methyl ester (L-NAME), α-chloralose, and atropine sulfate were purchased from Sigma Chemical (St. Louis, MO).

RESULTS

Influence of Galanin on Primary Peristalsis

Esophageal primary peristalsis (P contraction) was produced in response to swallowing induced by pharyngeal stimulation. Figure 1 shows manometric tracing of the effect of galanin on esophageal primary peristalsis. Galanin reduced the amplitude of peristaltic contractions, and it increased peristaltic velocity. Figure 2 shows that the amplitude of contractions caused by swallowing varied from 53.0 ± 8.2 to 101.0 ± 13.2 mmHg at different esophageal sites. Galanin treatment decreased the amplitude of contractions through-
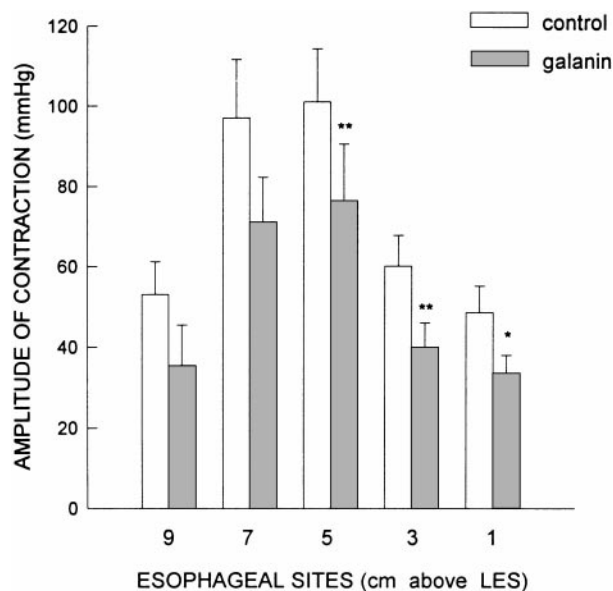

Fig. 2. Effect of galanin on the amplitude of swallow-induced esophageal contractions. Note that galanin reduced the amplitude of the contractions at all sites. The effect of galanin was significant at the distal esophageal sites (*$P < 0.05$, **$P < 0.01$). Bars show means $\pm$ SE of observations in 6 animals.
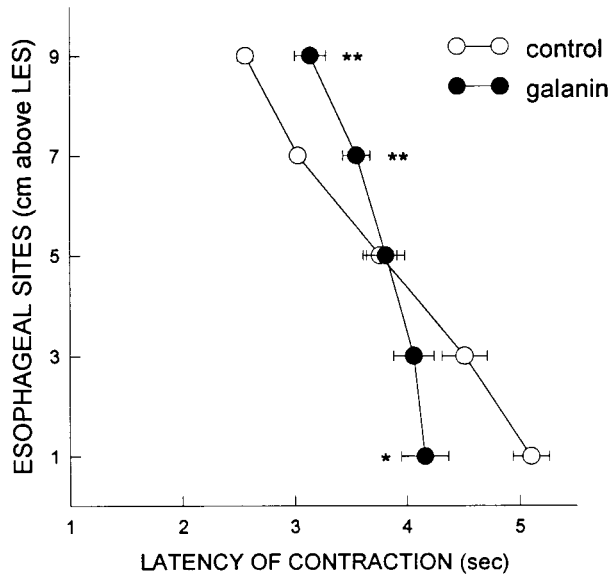
Fig. 3. Effect of galanin on the latencies of swallow-induced contractions. Note that galanin increased the latencies at 9- and 7-cm sites but reduced those at 3- and 1-cm sites ($n = 6$, $*P < 0.05$, $**P < 0.01$). The speed of peristalsis during the control period was 3.0 cm/s, and after galanin treatment it was 7.5 cm/s.

out the esophagus, particularly at the distal sites. At the 5-cm site, galanin decreased the amplitude of contractions from 101.0 ± 13.2 to 76.3 ± 14.2 mmHg ($n = 6$, $P < 0.01$).

Galanin had different effects on the latency of peristaltic contractions at different esophageal sites; it increased latencies at the proximal sites and reduced them at the distal esophageal sites. Figure 3 shows that the latencies of primary peristalsis varied from 2.6 ± 0.1 to 5.1 ± 0.2 s depending on the esophageal sites. Galanin increased the latencies at the 9- and 7-cm sites from 2.6 ± 0.1 to 3.1 ± 0.1 s and from 3.0 ± 0.1 to 3.6 ± 0.1 s, respectively ($P < 0.0\,1$), and it decreased the latencies at the 1-cm site from 5.1 ± 0.2 to 4.2 ± 0.2 s ($P < 0.05$). These modifications of latencies of contractions resulted in an increase in the speed of peristalsis from 3.0 cm/s in control to 7.5 cm/s after galanin treatment.

Influence of Galanin on Esophageal Contractions Caused by Vagal Stimulation

Vagal stimulation produces contractions in the esophageal body due to peripheral mechanisms and excludes the central deglutitive reflex pathways (16). Short trains of vagal stimulation produce esophageal peristaltic contractions (called S contractions) whose latencies, peristaltic velocities, amplitudes, and neurotransmitter mediators vary with the parameters of electrical stimulation (7). Long trains of vagal stimulation (10 s) cause contractions soon after the onset of the stimulus (A contraction) and after cessation of the stimulus (B contraction) (7, 13, 16). The prevalence and quantitative features of A and B contractions also depend on the parameters of electrical stimulation and considerable interspecies variations. Moreover, the

mediators of these two responses are also different; A contractions are cholinergic whereas B contractions are nonadrenergic, noncholinergic in nature (15). B contractions were reported (1, 29) to be mediated by nitric oxide, because a nitric oxide synthase inhibitor abolished B contractions. We examined the effect of galanin on each of these contractions.

Influence on S contractions. Short-train vagal efferent stimulation produced peristaltic contractions. With 1-s train stimulation at 5 Hz, the contractions were almost abolished by galanin treatment (Figs. 4 and 5A). The amplitude of contractions produced by 10 Hz of stimulation were also significantly reduced (Fig. 5B), and latencies of contractions were prolonged at all esophageal sites by $\sim$0.4–1 s ($n = 6$, $P < 0.05$) (Fig. 6).

Influence on A and B contractions. Figure 7 shows the effect of galanin on A and B contractions in response to long-train vagal efferent stimulation at 5 Hz. As shown in Fig. 8A, A contractions with 10-s vagal efferent stimulation at 5 Hz had amplitudes varying from 20.8 ± 6.7 to 102.2 ± 11.4 mmHg, and B contractions had amplitudes varying from 2.0 ± 1.3 to 82.5 ± 22.2 mmHg ($n = 6$). Galanin treatment reduced the amplitude of both A and B contractions; however, the reduction in the amplitude of B contractions was more significant. Galanin completely abolished B contractions in three of six animals. A contractions produced by long-train stimulation had latencies from the onset of stimulus varying from 1.5 ± 0.1 to 2.4 ± 0.2 s. Galanin treatment caused a significant prolongation of the latencies of A contractions at all esophageal sites by $\sim$0.9–1.4 s.
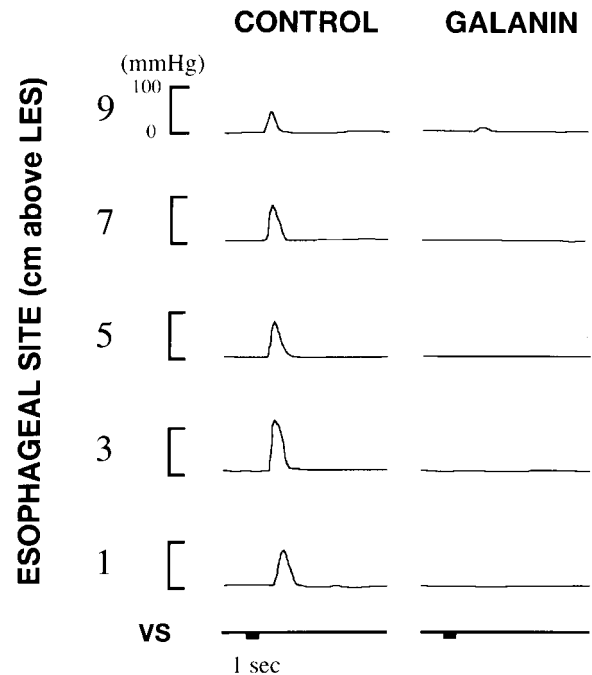

Fig. 4. Effect of galanin on S contractions due to short-train vagal efferent stimulation (VS) at 5 Hz in 1 animal. Note that galanin nearly abolished esophageal contractions due to short-train vagal stimulation at 5 Hz.
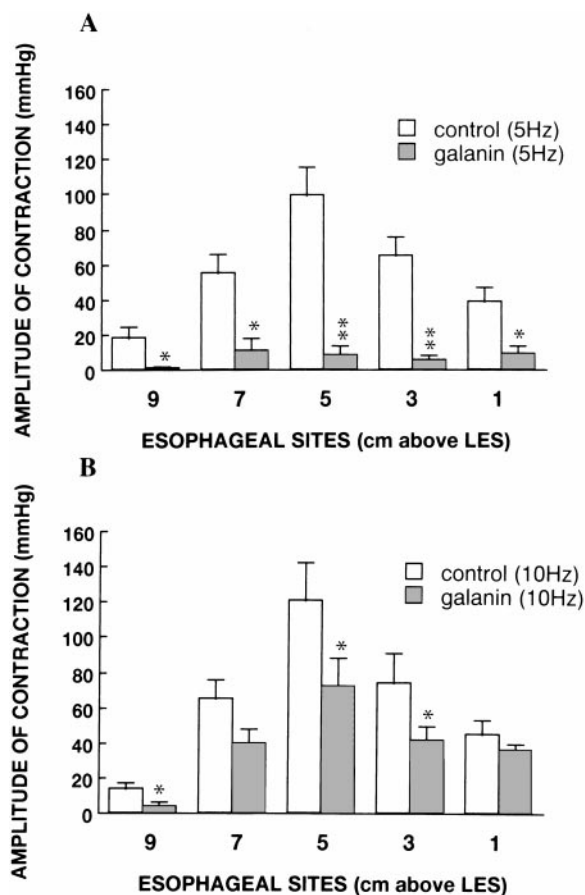
Fig. 5. Effect of galanin on the amplitude of esophageal contractions induced by short-train vagal stimulation. Note that with 5-Hz vagal stimulation, the contractions were nearly abolished at all esophageal sites (*A*). Galanin partially inhibited the contractions with 10-Hz vagal stimulation (*B*). *$P < 0.05$, **$P < 0.01$. Bars are means ± SE of observations in 6 animals. Vagal efferent stimulus parameters: 60 V with 0.5-ms pulse duration for 1-s train.
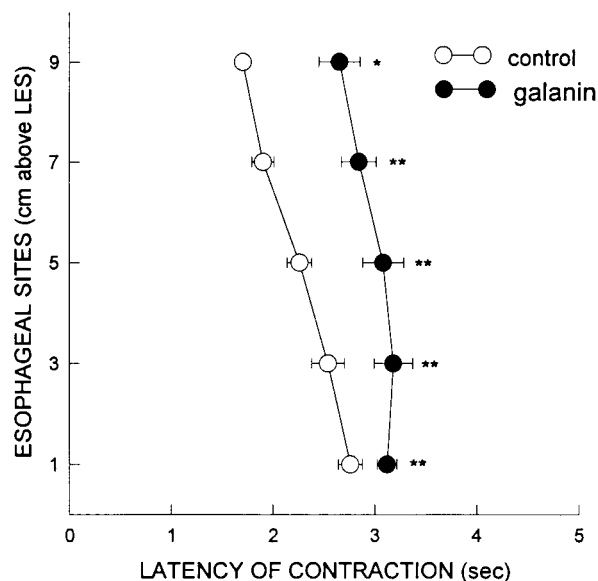

Fig. 6. Effect of galanin on the latencies of esophageal contractions to 1-s vagal stimulation at 10 Hz. Note that galanin increased the latencies of contractions at all esophageal sites ($n = 6$; *$P < 0.05$, **$P < 0.01$).

tions. Galantide slightly increased the amplitude of swallow-induced primary peristalsis and S, A, and B contractions ($n = 4$). However, the increases in the amplitudes were not consistent or significant at all sites (Table 1). Galantide did not modify latency of contractions induced by swallows or vagal stimulation.

DISCUSSION

These studies show that galanin causes a decrease in amplitude of swallow-induced esophageal contractions.

At 10 Hz, galanin also inhibited the amplitudes of A and B contractions (Fig. 8*B*), and it caused a significant prolongation of latency periods of A contractions at all esophageal sites by 0.6–1.1 s (see Fig. 9). Effect of galanin on latencies of B contractions could not be analyzed because many of them were abolished by galanin.

Influence of Galanin Antagonists

M15 (galantide) and M40, at a dose of one to six times that of galanin, were reported (27, 28) to antagonize the effect of galanin. In our studies, M40 (3 nmol/kg iv) reduced the amplitude of S contractions and acted like a galanin agonist rather than an antagonist. On the other hand, M15 (galantide) antagonized the effect of galanin. As shown in Fig. 10, galantide (3 mnol/kg) antagonized the inhibitory effect of galanin on esophageal contractions to short-train vagal efferent stimulation ($n = 3$, $P < 0.01$). To examine a possible inhibitory role of endogenous galanin on esophageal peristalsis, we examined the effect of galantide (3 nmol/kg) to see if it augmented the esophageal contrac-
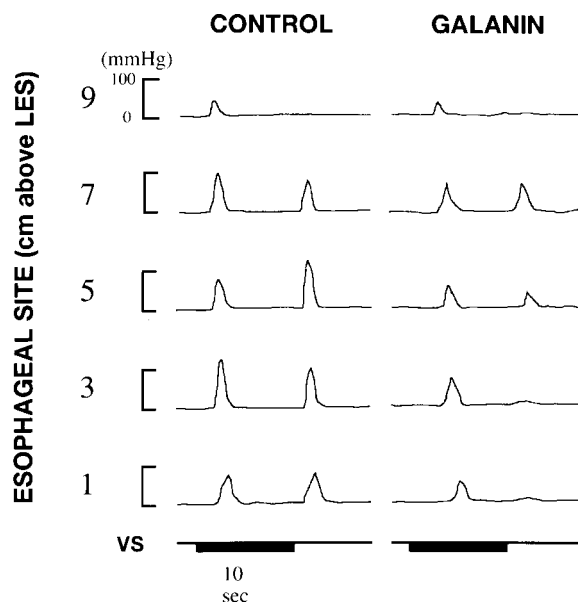

Fig. 7. Influence of galanin on A and B contractions in response to long-train vagal efferent stimulation at 5 Hz in 1 animal. Note that galanin reduced the amplitude of both A and B contractions to long-train vagal stimulation. The reduction of amplitude of B contractions was more significant.
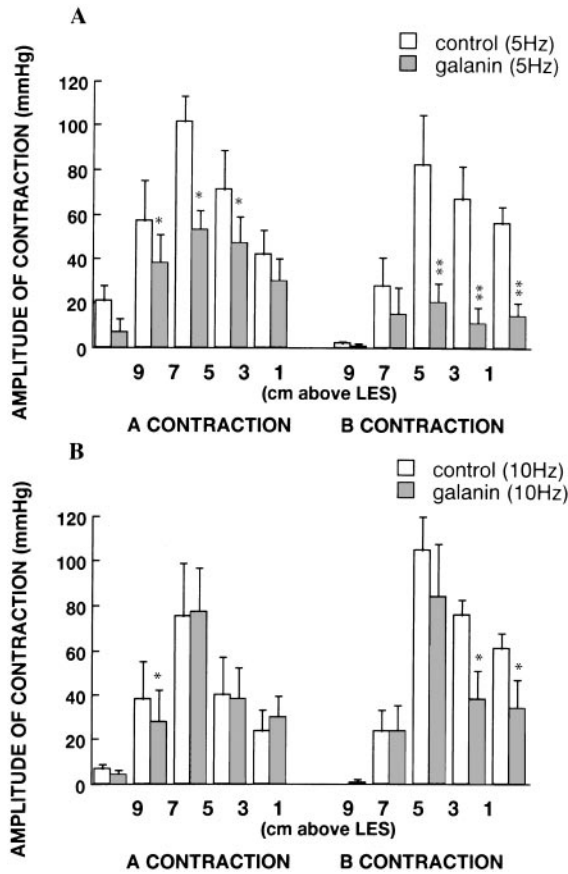
A

B

Fig. 8. Effect of galanin on the amplitude of A and B contractions to 10-s vagal stimulation (5 and 10 Hz). Galanin treatment reduced amplitude of both A and B contractions at 5 Hz; however, the reduction of amplitude of B contractions was more significant (*A*) (*$P < 0.05$, **$P < 0.01$). At 10 Hz, galanin partially inhibited the amplitudes of A and B contractions (*B*). Bars are means ± SE of observations in 6 animals. Vagal efferent stimulus parameters: 60 V with 0.5-ms pulse duration for 10-s train.
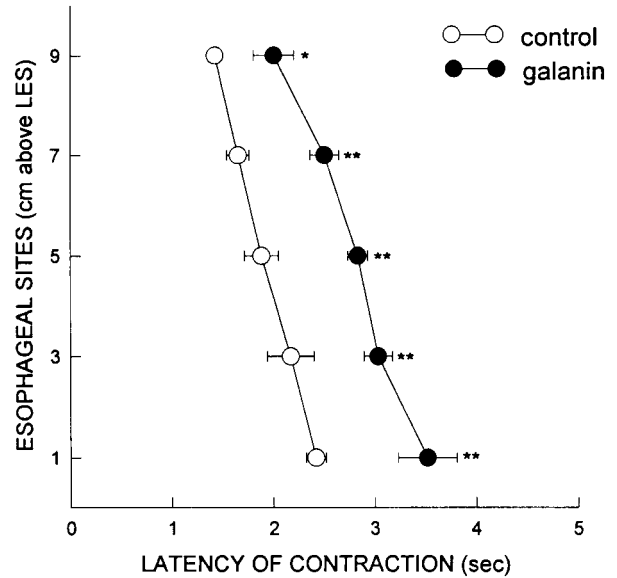

Fig. 9. Effect of galanin on the latencies of A contractions to 10-s vagal stimulation at 10 Hz. Galanin caused a significant prolongation of latencies of A contractions at all esophageal sites by 0.6–1.1 s ($n = 6$; *$P < 0.05$, **$P < 0.01$).

This effect of galanin may be exerted on the central swallowing mechanism or the peripheral motor mechanisms of peristalsis. Galanin reduced the amplitude of both the contractions due to vagal efferent stimulation. These observations suggest that the action of galanin is exerted on the peripheral pathways for peristalsis. Galanin reduced amplitudes of both the A contractions, which are cholinergic as they are atropine sensitive (15), and B contractions, which are rebound contractions due to the inhibitory neurotransmitter nitric oxide (1, 29).

Galanin had opposing effects on the latencies of primary peristalsis at the proximal and the distal sites; it increased the latencies at the proximal sites and decreased them at the distal sites. As a result of these changes, the speed of peristalsis, 3.0 cm/s, increased to 7.5 cm/s. Galanin increased the latencies to vagal efferent stimulation (S and A contractions) at all esophageal sites by ~1 s.

These seemingly confusing effects of galanin on the latencies of the esophageal contractions to swallowing and vagal efferent stimulation are due to differences in the mechanisms of the two types of contractions.

Esophageal contractions involve two overlapping phenomena, a period of inhibition followed by rebound contraction due to NANC nerves and excitation due to cholinergic nerves (7, 13). With vagal efferent stimulation, NANC inhibitory nerves and the cholinergic excitatory nerves are stimulated simultaneously. Under these conditions, the cholinergic component of the contraction precedes the NANC rebound contraction at all
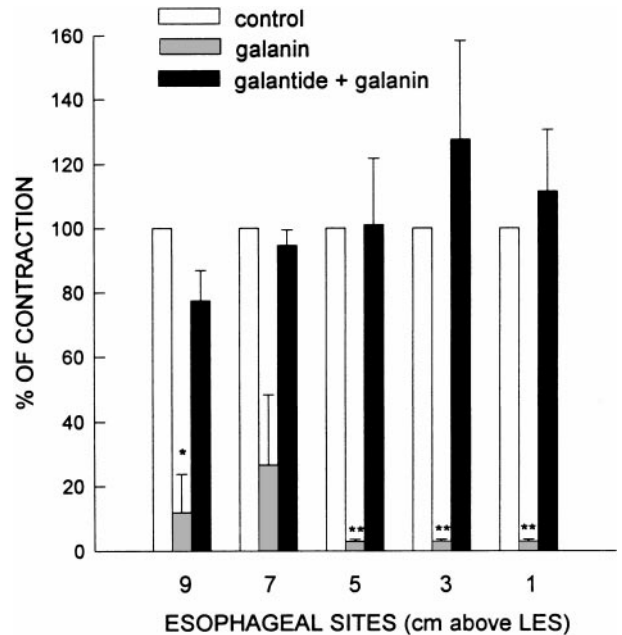

Fig. 10. Effect of galanin and galanin antagonist (galantide) on the amplitudes of contractions to 1-s vagal stimulation at 5 Hz. Galanin (1 nmol/kg) nearly abolished the contractions induced by 1-s vagal stimulation at 5 Hz ($n = 3$; *$P < 0.05$, **$P < 0.01$). Galantide (3 nmol/kg) antagonized the inhibitory effect of galanin.

Table 1. *Effect of galantide on swallow- and vagal stimulation-induced esophageal contraction*

Position Above LES, cm	P Contraction		A Contraction		S Contraction	
	Control	Galantide	Control	Galantide	Control	Galantide
Amplitude, mmHg						
9	44 ± 14	29 ± 7	14 ± 10	7 ± 5	33 ± 18	15 ± 5
7	78 ± 19	68 ± 18	88 ± 13	68 ± 21	80 ± 11	64 ± 20
5	114 ± 71	34 ± 11	101 ± 9	126 ± 11	90 ± 5	117 ± 16
3	70 ± 10	$86 \pm 11*$	92 ± 16	90 ± 9	89 ± 12	87 ± 7
1	56 ± 12	61 ± 14	22 ± 13	50 ± 23	46 ± 11	$69 \pm 12*$
Latency, s						
9	2.4 ± 0.1	2.1 ± 0.2	1.7 ± 0.2	1.6 ± 0.2	1.5 ± 0.1	1.5 ± 0.0
7	2.5 ± 0.2	2.4 ± 0.1	1.6 ± 0.1	1.4 ± 0.2	1.8 ± 0.1	1.5 ± 0.2
5	3.0 ± 0.2	3.0 ± 0.2	1.9 ± 0.1	1.8 ± 0.2	2.0 ± 0.1	1.9 ± 0.1
3	3.7 ± 0.3	3.8 ± 0.3	2.3 ± 0.3	2.1 ± 0.3	2.3 ± 0.2	2.3 ± 0.2
1	4.7 ± 0.3	4.4 ± 0.4	2.3 ± 0.1	2.1 ± 0.1	2.8 ± 0.4	2.4 ± 0.1

Values are means $\pm$ SE of 4 animals. Vagal efferent stimulus parameters were 60 V, 0.5-ms pulse duration, and 10 Hz for 1- and 10-s train. Galantide was administered at 3 nmol/kg. P contraction, swallow induced; A contraction, long train, vagal stimulation induced; S contraction, short train, vagal stimulation induced; LES, lower esophageal sphincter. *$P < 0.05$.

esophageal sites. The net result of cholinergic and NANC nerve inhibition by galanin is to increase latencies of the contractions at all esophageal sites. In contrast to vagal efferent stimulation, swallowing first produces activation of the NANC inhibitory pathways and the activation of the cholinergic excitatory pathway occurs afterward, following a certain delay. Moreover, the interval between the activation of the NANC inhibitory pathway and the cholinergic excitatory pathway increases distally along the esophagus (14). As a result, at the proximal esophageal sites, the cholinergic component of the esophageal contraction precedes the NANC rebound contraction. On the other hand, at the distal sites, the cholinergic component of the contraction overlaps the NANC rebound contraction. The effect of atropine on swallow-induced peristalsis in the opossum was reported by Gilbert and Dodds (15) and that of L-NAME and the combination of L-NAME plus atropine on peristalsis was reported by Yamato et al. (29). L-NAME causes a decrease in the latencies of contractions at distal sites due to suppression of NANC activity so that the contractions occur after a latency of 2–3 s. On the other hand, atropine increases the latencies of contractions, particularly in proximal esophageal sites. Antagonism of both the inhibitory NANC and the excitatory cholinergic nerves by a combination of L-NAME and atropine places the latency gradient midway between that of L-NAME and atropine treatment. With this combination treatment, the latency increased at the proximal sites and decreased at the distal sites so that the swallow-induced contractions occurred almost simultaneously. The effect of galanin on the latencies of swallow-induced contractions was similar to that of the combination of L-NAME and atropine (see Fig. 2). These observations are consistent with the inhibitory effect of galanin on NANC inhibitory and cholinergic excitatory nerves. The effects of the same agents on short-train vagal stimulation-induced esophageal contractions were also reported (1, 29). In contrast to swallow-induced peristalsis, decrease in latencies by L-NAME with vagal stimulation is small and the combination of L-NAME plus atropine prolongs the latencies in all the esophageal sites. The effect of these combinations is also similar to that of galanin (see Fig. 4).

Galanin can act presynaptically to inhibit the synaptic transmission, myenteric neurons, or neuromuscular transmission. Galanin is localized with ACh, VIP, and nitric oxide synthase in the myenteric neurons. It is possible that galanin acts to inhibit the release of cholinergic and noncholinergic neurotransmitters. Further studies are needed to distinguish between these possibilities. The inhibitory action of galanin could also be exerted directly on the smooth muscle. However, such a direct effect on the muscle would not explain the changes in latencies of contraction due to galanin. The possibility that the observed actions of galanin in vivo are mediated indirectly via systemic release of other mediators or hormones cannot be excluded by these studies.

Wiesenfeld-Hallin et al. (28) found that the increase of spinal cord excitability after stimulation of afferents was potentiated by the galanin antagonist M35 and speculated that endogenous galanin is released to suppress nociceptive impulses on intense activation of nociceptors. Our studies show that M15 (galantide) but not M40 is a potent antagonist of the inhibitory action of galanin on esophageal contractions. This may be due to participation of different galanin receptor subtypes in the two responses (3, 20). Galantide, in doses that markedly antagonized the inhibitory action of galanin on the amplitude of esophageal contractions evoked by a short train of vagal stimulation, had no consistently significant effect on esophageal contractions evoked by swallowing or vagal stimulation. These observations suggest that endogenous galanin may not exert a modulatory role on esophageal contractions evoked by swallowing or vagal stimulation. However, further studies using higher concentrations of galantide are needed to fully exclude this possibility.

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