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EFFECT OF GALANIN ON THE OPOSSUM LOWER ESOPHAGEAL SPHINCTER

Satish Rattan and Raj K. Goyal

Harvard Digestive Diseases Center, Charles A. Dana Research Institute and Thorndike Laboratory, Division of Gastroenterology, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts 02215

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Summary

The objective of this investigation was to examine the effect of galanin on the resting tone and neurally mediated relaxation of the lower esophageal sphincter (LES) in anesthetized opossums. Galanin caused a dose-dependent rise in the sphincter pressure when given either intra-arterially or intravenously. The D_{50} of intra-arterial galanin was $1.0X10^{-11}$ mols/kg. The rise in the sphincter pressure caused by galanin was not modified by atropine, phentolamine, methysergide, pyrilamine or indomethacin. Furthermore, the contractile response of galanin on the LES was also not affected by tetrodotoxin. The fall in the sphincter pressure in response to vagal efferent stimulation, intramural stimulation or esophageal distension was antagonized by galanin. The results of these studies suggest that, 1) galanin causes sphincter contraction by its direct action on the smooth muscle, and 2) galanin suppresses LES relaxation. These studies suggest that galanin may be an important neuropeptide for the modulation of resting tone and LES relaxation.

Galanin has been recently shown to be present in the central and peripheral nervous system (1-10). In the periphery, the neuropeptide has been shown to be present in the stomach, small intestine and large intestine (2-7). Moreover, it has been shown that it is primarily present in the region of myenteric and submucosal plexus of the gut wall (3,5,7). There is limited information regarding the actions of galanin in gastrointestinal motility. The neuropeptide may cause gut smooth muscle contraction or relaxation. The excitatory action of galanin on the gut is primarily due to its action directly at the smooth muscle (11). The inhibitory actions of galanin could be either due to its direct action at the smooth muscle (12) or indirectly through the inhibition of release of the excitatory neurotransmitter ACh (11,13). In all these studies, the gut tissue was either made to contract by electrical field stimulation or it was spontaneously under phasic contraction. The effect of galanin on gastrointestinal sphincters is not known.

Recently, galanin immunoreactivity has been localized to enteric neurons in the smooth muscle portion of the esophagus including the lower esophageal sphincter (14). The purpose of this investigation was to examine the effects of galanin on the resting LES tone, and LES relaxation mediated by vagal efferent stimulation and esophageal distension.

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Methods

The present studies were performed on 22 adult opossums (*Didelphis virginiana*) of either sex, weighing between 1.8 and 3.5 kg. The animals were anesthetized using pentobarbital sodium (40 mg/kg; intraperitoneally). The animals were then strapped supine on the animal board. The brachial vein was cannulated for administration of various agents.

The respiration was supported via endotracheal intubation with an artificial respirator at 20 strokes/minute; tidal volume was determined from the manufacturer's nomogram (Harvard Apparatus Co., Millis, MA). The vagi were identified in the cervical region and transected.

A specially designed catheter assembly was employed to record lower esophageal sphincter pressures (LESP). The catheters were continuously perfused with bubble-free water through a side hole using a low compliance pneumo-hydraulic capillary system as described before (15). The pressures were recorded on a Beckman Dynograph recorder model R612 (Beckman Instruments, Schiller Park, IL), using Statham P23Db transducers. The catheter assembly was anchored in the sphincter region following laparotomy as described before (15).

The following agents were used: atropine sulfate (Eli Lilly and Co., Indianapolis, IN); galanin (M.W. 3211.03; Peninsula Laboratories, Inc., Belmont, CA); indomethacin (Sigma Chemical Co., St. Louis, MO); methysergide (a gift from Sandoz Pharmaceuticals, Division of Sandoz, Inc., East Hanover, NY); naloxone hydrochloride (Dupont Pharmaceuticals, Wilmington, DE); phentolamine mesylate (Ciba Pharmaceutical Co., Summit, NJ); tetrodotoxin (TTX) (Calbiochem, San Diego, CA). All agents were dissolved or diluted in 0.9% sodium chloride except indomethacin, which was dissolved in 50 mM Tris-HCl buffer (pH 8). The doses of all the agents were expressed on the basis of their salts.

The agents were administered (intra-arterially into the left gastric artery or intravenously into the brachial vein) as a single 20 sec. bolus. The administration of physiological saline volumes by any of the routes had no significant effect on the LESP. The response of galanin was tested before and after the antagonists in the same animals. Thus, each animal served as its own control. The results are expressed as percent and absolute changes in sphincter pressures.

The doses of galanin by intravenous route ranged from $1.6X10^{-11}$ to $1.0X10^{-8}$ mols/kg. No tachyphylaxis to the response of galanin was observed. The doses of different antagonists (administered intravenously) were selected on the basis of previous studies (16). These studies showed that the antagonists given in the doses used here nearly abolished the effect of maximally effective dose of respective agonists. Tetrodotoxin was administered intra-arterially in doses of 5μg/kg at a time at 30 min intervals until the responses to esophageal distension and vagal stimulation were abolished. The effect of galanin was examined during the time of complete obliteration of LES relaxation in response to esophageal distension and vagal stimulation.

In order to examine the influence of galanin on LES relaxation, the responses to vagal stimulation, local intramural stimulation (16,17) and esophageal distension (using 1 to 5cc) were examined. The dose of galanin in these experiments was repeated every 15 min. ($1.0X10^{-9}$ mols/kg; I.v.)

The values are expressed as mean ± SE. The statistical analysis was performed using student's t-test or paired t-test where applicable (18).

Results

Influence of galanin on lower esophageal sphincter pressure:

Intra-arterial (i.a.) administration of galanin caused a rise in sphincter pressure, as shown in FIG. 1. Intravenous (i.v.) administration of the peptide also caused a rise in sphincter pressure, but the dose required to produce a given magnitude of response was larger as compared to that of intra-arterial route.

FIG. 2. summarizes the dose-response curves of i.a. and i.v. administered galanin in causing a rise in LES pressure. The threshold doses for causing rise in LESP with i.a. and i.v. routes are $2.5X10^{-13}$ mols/kg and $1.0X10^{-12}$ mols/kg respectively. The D_{50} (a dose causing 50% of the maximal rise in LESP) is $1.0X10^{-11}$ mols/kg and $1.0X10^{-10}$ mols/kg by i.a. and i.v. routes respectively. The latency of the onset of rise in LESP with intra-arterial administration of galanin in the maximal effective dose of $6.4X10^{-11}$ mols/kg was 14.3±0.9 sec and the duration of the excitatory action of galanin on LES was 488.6±69.8 sec. When administered intravenously, the latency of onset of rise in LESP and its duration of action following maximal effective dose of $1X10^{-9}$ were 39.3±5.2 and 680.0±20.0 sec respectively. Tachyphylaxis to frequent administration of galanin was not observed when the peptide was given either i.a. or i.v.

In order to examine the site of action of galanin on the sphincter, the actions of the peptide administered i.a. in the maximal effective dose ($6.4X10^{-11}$ mols/kg; i.a.) were examined before and after different antagonists.

As seen in TABLE I, cholinergic antagonist atropine, the α-adrenergic antagonist phentolamine, 5-HT excitatory antagonist methysergide or histamine excitatory antagonist pyrilamine, failed to antagonize the excitatory effect of galanin on the sphincter pressure. Naloxone in the doses which antagonized excitatory effect of enkephalins on the sphincter also failed to modify the effect of galanin. Prostaglandin synthesis inhibition by indomethacin treatment also did not modify the excitatory effect of galanin. Moreover, tetrodotoxin which blocks neurally mediated responses also failed to modify the effect of galanin.

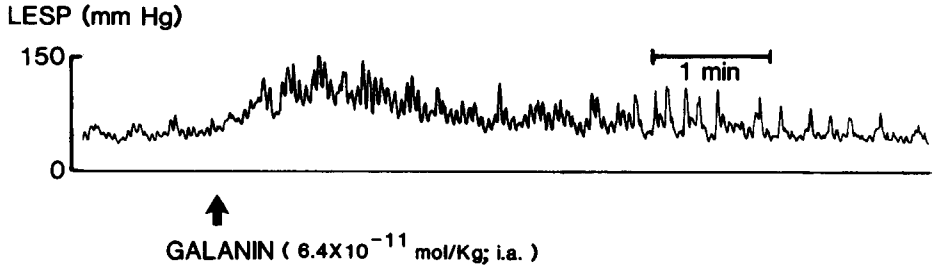

FIG. 1.
A tracing showing an increase in lower esophageal sphincter pressure (LESP) in response to the maximal effective dose of galanin administered intra-arterially.

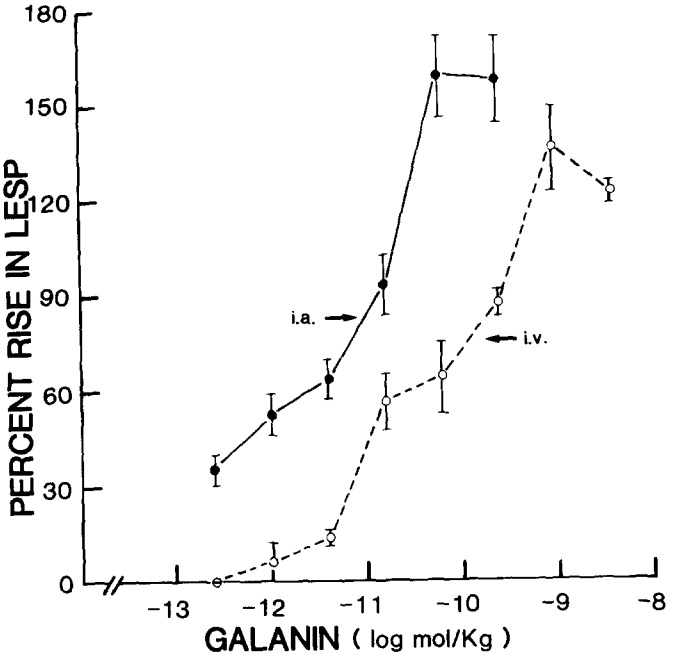

FIG. 2.

Effects of galanin administered either i.a. or i.v. on the resting tone in the lower esophageal sphincter pressures (LESP). Each point represents mean ±SE of 5 observations in five animals, one observation in each animal.

Influence of galanin on the blood pressure and heart rate:

Galanin caused a rise in blood pressure without tachyphylaxis. The i.a. administration of $1.0X10^{-11}$ mols/kg of galanin which caused a significant rise in LESP, caused only minimal rise in blood pressure. However, the D_{50} of i.v. galanin ($1.0X10^{-10}$ which caused 50% of the maximal rise in LESP with galanin) caused a significant rise in blood pressure from 150±8/120±5 to 200±6/158±3 mm Hg of systolic/diastolic blood pressure values ($p<0.05$). The higher doses of galanin caused greater rise in blood pressure. The hypertensive effect of galanin was not associated with tachycardia. The heart rate remained unchanged 187±12 versus 160±15 beats per min ($p>0.05$), with D_{50} doses while higher doses caused bradycardia. These studies are consistent with the view that the hypertensive effect of galanin may be due to vaso-constrictive action of galanin on vascular smooth muscle.

TABLE I

Influence of Different Antagonists on the Rise in LES Pressure Caused by Galanin ($6.4X10^{-11}$ mols/kg)

	no. of observ.	Basal LESP	Final LESP (mmHg)	Rise in LESP (mmHg)	% Rise	p Value
control	5	76±5	184±11	108±7	143±7	>0,05
atropine (30μg/kg)	5	68±5	162±22	94±17	134±14	
control	5	76±5	184±11	108±7	143±7	>0.05
phentolamine (1mg/kg)	5	58±10	137±18	79±9	146±15	
control	5	51±6	122±16	71±10	137±6	>0.05
methysergide (200 μg/kg)	5	46±2	106±7	60±5	130±8	
control	5	70±6	180±9	102±7	144±7	>0.05
indomethacin (10 mg/kg)	5	55±4	133±8	78±8	146±22	
control	5	51±6	122±16	71±10	137±6	>0.05
naloxone (4 mg/kg)	5	58±4	150±26	92±23	153±29	
control	5	75±5	182±11	106±7	142±7	>0.05
tetrodotoxin (5-20 μg/kg)	5	56±2	134±12	78±11	139±17	

* The values in this and subsequent tables represent mean ± SE.

Influence of galanin on LES relaxation:

Vagal efferent stimulation caused frequency-dependent relaxation of the lower esophageal sphincter. As shown in TABLE II, galanin caused a significant increase in the resting LES pressures. Therefore the determination of the relaxation after galanin became somewhat complicated as it differed from the parameters used to express its effect. The residual LES pressure after galanin was significantly higher than the control at all frequencies of vagal stimulation ($p<0.05$). Similarly, percent fall in LESP was significantly reduced with galanin at all the frequencies ($p<0.05$). The absolute values of the fall in pressure showed a biphasic effect: at smaller frequencies of stimulation these values are smaller after galanin, but with larger frequencies of stimulation the values after galanin were larger than in controls. It appears that the overall effect of galanin was to inhibit vagal stimulated sphincter relaxation.

TABLE II

Influence of Galanin ($1.0X10^{-9}$ mol/kg; I.v.) on Fall in LESP by Vagal Stimulation

Vagal stim. (Hz)		No. of observ.	Basal LESP	Residual LESP (mmHg)	Fall in LESP	%Fall in LESP
0.5	control	20	71±4*	51±3*	20±1‡	28±1‡
	galanin	20	133±8	127±8	7±1	6±1
1	control	20	76±2*	46±1*	30±2‡	38±2‡
	galanin	20	128±5	111±4	17±2	13±1
2	control	20	75±6*	36±3*	40±3‡	54±2‡
	galanin	20	129±9	100±7	29±5	22±3
5	control	20	68±2*	14±1*	54±2*	80±1‡
	galanin	20	127±3	60±4	67±3	53±2
10	control	20	73±4*	15±2*	58±3*	80±2‡
	galanin	20	121±3	37±2	84±2	69±1

* Control value is significantly smaller than galanin value ($P<0.05$).
‡ Control value is significantly larger than galanin value ($P<0.05$).
ns Control value is not significantly different from galanin value ($p.0.05$).

Likewise, the fall in LESP in response to esophageal distension was also significantly antagonized by galanin ($p<0.05$; TABLE III).

TABLE III

Influence of Galanin ($1.0X10^{-9}$ mols/kg; I.v.) on Fall in LESP by Esophageal Distension

Eso. dist. (c.c.)		No. of observ.	Basal LESP	Residual LESP (mmHg)	Fall in LESP	% Fall in LESP
1	control	15	68±5*	44±4*	24±3‡	36±2‡
	galanin	15	114±17	99±14	15±5	12±3
2	control	15	67±6*	35±4*	32±3 ns	48±3‡
	galanin	15	119±7	87±10	32±8	27±7
5	control	15	74±5*	28±3*	45±3*	62±2 ns
	galanin	15	134±14	60±7	74±9	55±2

* Control value is significantly smaller than galanin value ($P<0.05$).
‡ Control value is significantly larger than galanin value ($P<0.05$).
ns Control value is not significantly different from galanin value ($P>0.05$).

The sphincter relaxation in response to local intramural stimulation was also significantly antagonized by galanin. The fall in LESP with local stimulation in control experiments was 88.2 ± 1.6 percent (from 38.2 ± 2.8 to 4.5 ± 0.6 mmHg). This response was antagonized to 72.9 ± 2.3 percent (from 77.2 ± 3.6 to 21.0 ± 2.3 mmHg; $p<0.05$; $n = 4$ in four animals). The fall in LESP with isoproterenol (0.025 μg/kg; i.a.) was however not modified by galanin (85.6 ± 4.3 versus 88.3 ± 6.2 percent) ($p>0.05$).

Discussion

These studies show that galanin exerts separate actions at the LES, namely, contraction of the sphincter and inhibition of its relaxation.

LES contraction in response to galanin is due to a direct action on the smooth muscle. This is evidenced by the fact that LES contraction in response to galanin was not modified by antagonists of potential excitatory transmitters and TTX. The direct action of galanin on the LES smooth muscle is similar to its direct contractile actions in the other smooth muscles (11,19). The hypertensive effect of galanin was not associated with tachycardia suggesting that this effect may be due to peripheral vasoconstriction. At no dose level was the relaxation of LES by galanin observed either in the absence or presence of TTX. Thus galanin could not be a noncholinergic, nonadrenergic inhibitory neurotransmitter in the LES as suggested by studies in the dog small intestine (12).

The inhibitory effect of galanin on neurally mediated LES relaxation was difficult to assess because galanin caused marked increase in the resting LESP. Overall, the effect of galanin appears to be inhibition of LES relaxation in response to vagal stimulation, esophageal distention or intramural nerve stimulation. Therefore, this effect which involves all modalities of neurally mediated relaxation may induce inhibition of the release of inhibitory neurotransmitter. This effect was not due to a non-specific action on the muscle, as the inhibitory effect of isoproterenol on the LES was not antagonized by galanin. Recent studies in other laboratories have also shown that galanin modulates the activity of intramural neurons (13,20).

There is some evidence to suggest that galanin may act via separate receptor subtypes to exert its effect on the muscle or the nerves (11). It is not known if the dual excitatory effect of galanin on the sphincter muscle and the inhibitory modulating effect on intramural inhibitory nerves is mediated by separate receptor subtypes.

These effects of galanin on the lower esophageal sphincter may be of physiologic importance as abundant supplies of galanin immunoreactive myenteric neurons and nerve fibers innervating the sphincteric circular muscle have been demonstrated (14).

Acknowledgments

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