Nature of the Vagal Inhibitory Innervation to the Lower Esophageal Sphincter

RAJ K. GOYAL and SATISH RATTAN

From the Department of Internal Medicine, University of Texas Health Science Center, Dallas, Texas 75235

ABSTRACT The purpose of the present study was to investigate the nature of the vagal inhibitory innervation to the lower esophageal sphincter in the anesthetized opossum. Sphincter relaxation with electrical stimulation of the vagus was not antagonized by atropine, propranolol, phentolamine, or by catecholamine depletion with reserpine. A combination of atropine and propranolol was also ineffective, suggesting that the vagal inhibitory influences may be mediated by the noncholinergic, nonadrenergic neurons. To determine whether a synaptic link with nicotinic transmission was present, we investigated the effect of hexamethonium on vagal-stimulated lower esophageal sphincter relaxation. Hexamethonium in doses that completely antagonized the sphincter relaxation in response to a ganglionic stimulant, 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP), did not block the sphincter relaxation in response to vagal stimulation at 10 pulses per second, an optimal frequency of stimulation. A combination of hexamethonium and catecholamine depletion was also ineffective, but hexamethonium plus atropine markedly antagonized sphincter relaxation ($P < 0.001$). Moreover, 4-(*m*-chlorophenyl carbamoyloxy)-2-butyltrimethylammonium chloride (McN-A-343), a muscarinic ganglionic stimulant, also caused relaxation of the lower esophageal sphincter. We suggest from these results that: (*a*) the vagal inhibitory pathway to the sphincter consists of preganglionic fibers which synapse with postganglionic neurons: (*b*) the synaptic transmission is predominantly cholinergic and utilizes nicotinic as well as muscarinic receptors on the postganglionic neuron, and; (*c*) postganglionic neurons exert their influence on the sphincter by an unidentified inhibitory transmitter that is neither adrenergic nor cholinergic.

INTRODUCTION

The nature of the vagal inhibitory pathway to the lower esophageal sphincter (LES)[1] (1) is not well defined (2, 3). Conventionally, it is believed that the vagi carry preganglionic fibers (3, 4) which synapse with the postganglionic neurons that are located in the intramural plexuses. The preganglionic fibers are thought to exert their effect by the nicotinic actions of acetylcholine (3, 4). There are three possibilities regarding the nature of postganglionic neurons: (*a*) They exert their influence by the muscarinic actions of acetylcholine. It is thought that although muscarinic receptors on most smooth muscles are excitatory, the sphincter muscle may possess muscarinic receptors which are inhibitory (4–7). (*b*) They exert their influence by the beta-adrenergic receptors on the sphincter muscle (8–10). (*c*) They exert their influence by releasing an unknown inhibitory transmitter (11–14) which is neither adrenergic (11) nor cholinergic (12, 13).

The purpose of the present investigation was to examine the nature of the vagal inhibitory pathway to LES in the anesthetized opossum by studying the influence of different antagonists of the autonomic neurotransmitters on the sphincter response to electrical stimulation of the vagus nerve.

METHODS

Studies were performed in 46 opossums (*Didelphis virginiana*). In this species, the LES, like that in man, is composed of smooth muscle fibers (15). The animals were of either sex and weighed from 2 to 5 kg. They were fasted for 12–16 h before the study and they were anesthetized with sodium pentobarbital. The LES pressures were recorded with an open tip, continuously perfused catheter system, as described previously (16).

The vagi were exposed in the neck and sectioned. The peripheral end of one of the vagi was stimulated using a Grass stimulator (model S48, Grass Instrument Co., Quincy,

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[1] *Abbreviations and trivial names used in this paper:* bethanechol, (2-hydroxypropyl)trimethylammonium chloride carbamate; DMPP, 1,1-dimethyl-4-phenylpiperazinium iodide; hexamethonium, hexamethylenebis[trimethylammonium chloride]; isoproterenol, *dl*-β-(3,4-dihydroxyphenyl)-α-(isopropylamino)ethanol; LES, lower esophageal sphincter; McN-A-343, 4-(*m*-chlorophenyl carbamoyloxy)-2-butyltrimethylammonium chloride; phentolamine, 2-[*N*-(*m*-hydroxyphenyl)-*p*-toluidinomethyl]imidazoline; phenylephrine, 1-*m*-hydroxy-α-[(methylamino)methyl]benzyl alcohol hydrochloride; propranolol, 1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol hydrochloride; PPS, pulses per second; reserpine, 3,4,5-trimethoxybenzoyl methyl reserpate.

TABLE I

Effect of Cholinergic and Adrenergic Antagonism on Vagal-Stimulated LES Pressure

Exp. no.	Vagal stimulation at 10 PPS	No. of observations	Initial pressure*	P	Final pressure*	P	Fall*	P
			mm Hg		*mm Hg*		%	
1	Control	33	31.0±1.4	>0.5	5.7±0.5	>0.1	79.9±2.0	>0.05
	Atropine (30 μg/kg)	36	29.8±1.3		7.2±0.6		74. ±2.1	
2	Control	12	55.2±3.6	>0.05	4.0±0.7	<0.001	92.3±1.5	<0.001‡
	Atropine (1,500 μg/kg)	12	44.6±3.8		24.7±2.2		43.7±3.5	
3	Control	14	40.1±3.5	>0.2	6.3±1.1	>0.9	80.4±4.0	>0.8
	Propranolol (1 mg/kg)	14	34.4±3.4		6.3±1.4		79.1±3.9	
4	Control	9	29.5±1.6	>0.05	5.0±0.2	>0.7	82.4±1.4	>0.9
	Phentolamine (1 mg/kg)	9	26.3±0.7		4.6±0.7		82.4±2.9	
5	Control	13	43.9±3.5	>0.3	2.9±0.4	>0.7	92.6±2.1	>0.7
	Reserpine (3 mg/kg × 2)	14	39.4±3.7		3.0±0.4		91.9±0.9	
6	Control	13	38.4±2.6		4.3±0.5		88.8±0.9	
	Propranolol (1 mg/kg) + Atropine (30 μg/kg)	13	40.1±1.7	>0.6	6.3±1.7	>0.2	88.1±3.7	>0.8

Three to five observations were obtained in each of the three separate animals for each experiment, except exp. 1 which was done in eight animals.

* Mean±SE.

‡ Highly significant.

Mass.). The details of the technique of stimulation are described elsewhere (1). Stimuli of 10 V, with square wave pulses of 0.5 ms duration were applied at 0.25–50 pulses per second (PPS); the train duration was 2–4 s. Stimuli[2] of 0.5 ms at 10 PPS produced maximal relaxation of the LES (1).

Drugs were administered via an intravenous cannula, which was secured in place in one of the systemic veins. Normal saline (2 ml) was used to flush the cannula after each drug administration. Some drugs were administered intra-arterially through a cannula in the esophageal branch of the left gastric artery as described previously (16). The following drugs were used: atropine sulphate (Eli Lilly and Company, Indianapolis, Ind.); DMPP (1,1-dimethyl-4-phenylpiperazinium iodide) (Aldrich Chemical Co., Inc., Milwaukee, Wis.); propranolol (Ayerst Laboratories, New York); phentolamine (Ciba Pharmaceutical Company, Summit, N. J.); isoproterenol (Winthrop Laboratories, New York); phenylephrine (Robinson Laboratory, Inc., San Francisco, Calif.); reserpine (Ciba Pharmaceutical Company); hexamethonium (City Chemical Corp., New York); nicotine sulphate (Sigma Chemical Co., St. Louis, Mo.); McN-A-343 (4-(*m*-chlorophenyl carbamoyloxy)-2-butyltrimethyl +ammonium chloride) (McNeil Laboratories, Inc., Fort Washington, Pa.); bethanechol chloride (Merck Sharp & Dohme, West Point, Pa.); tyramine (Aldrich Chemical Co., Inc.; and tetrodotoxin (Calbiochem, San Diego, Calif.). The drug doses are expressed in terms of their salts.

Separate controls were run for each study because we found considerable variation in the basal sphincter pressure in different animals. The LES relaxation with vagal stimulation was measured in the same animals before and after treatment with the different antagonists. As a rule, only one antagonist or a single combination of antagonists was used in one animal. The doses of the antagonists were so chosen that they antagonized the maximally effective doses of their respective agonists at 30 min after the administration of the antagonists. 30 μg/kg atropine antagonized the effect of 20 μg/kg bethanechol; 1 mg/kg propranolol antagonized the effect of 2.5 μg/kg isoproterenol; 1 mg/kg phentolamine antagonized the effect of 50 μg/kg phenylephrine.

Catecholamine depletion was done with two doses of 3 mg/kg reserpine injected intraperitoneally at 48 and 24 h

[2] The stimulus characteristics are described as measured at the output of the stimulator. These parameters were reproducible within 5% variation. The current strength with the stimulating electrodes in position on the vagus nerve was approximately 2.5 mA at 10 V and the pulse waves were nearly square.

TABLE II

Effect of DMPP on the LES Pressure

Dose	No. of observations‡	Initial pressure*	Final pressure*	Absolute change in pressure*	Percent change in pressure*
μg/kg		*mm Hg*	*mm Hg*	*mm Hg*	%
5	6	35.8±2.2	33.2±5.1	3.2±1.2	7.9±3.5
10	7	28.6±2.9	24.3±3.0	5.0±1.2	13.6±2.9
20	7	25.0±1.5	17.6±2.6	7.4±1.2	28.8±5.1
40	7	29.1±2.8	14.7±3.1	14.4±1.7	51.5±7.1
100	7	30.4±3.5	5.6±1.4	24.8±2.6	82.3±2.7
200	6	38.4±3.8	8.2±2.1	30.2±2.2	81.9±5.5

* Mean±SE.

‡ Two to three observations were obtained in three separate animals.

before the study. The animals reserpinized in this way appeared lethargic, had droopy eyelids, diarrhea, and subnormal temperature, and showed penile erection. The effectiveness of catecholamine depletion was tested with tyramine which acts to release catecholamines from the adrenergic nerves (17). In the nonresperpinized animals, tyramine caused an increase in LES pressure (18). The LES pressure before tyramine was 45.0±4.7 and it increased to 74.2 ±7.8 mm Hg after tyramine ($P < 0.05$; $n = 6$). In reserpinized animals, the LES pressure was 45.0±5.3 before, and 41.2±3.8 mm Hg ($P > 0.05$) after 100 μg/kg tyramine ($n = 5$).

The resting LES pressures were measured just before the onset of the vagal stimulation. The results are expressed both as absolute values, as well as percent changes in pressure.

RESULTS

Influence of cholinergic and adrenergic antagonists on the vagal-stimulated LES relaxation

Influence of atropine. As summarized in Table I, 30 μg/kg atropine produced some inhibition of sphincter relaxation with vagal stimulation, but this was not statistically significant ($P > 0.05$). A very large dose of atropine (1,500 μg/kg), however, caused a significant decrease in the percentage fall in the sphincter pressure with vagal stimulation from 92.3±1.5 to 43.7±3.5% ($P < 0.001$).

Influence of adrenergic antagonists. These results are also summarized in Table I. Propranolol as well as phentolamine pretreatments were ineffective in antagonizing vagal-stimulated sphincter relaxation. Moreover, catecholamine depletion with reserpine also failed to produce a significant influence on the vagal inhibitory response ($P > 0.7$). A combination of propranolol and a small dose of atropine was also ineffective ($P > 0.8$).

Effect of DMPP on LES pressure

A nicotinic stimulant, DMPP, caused a dose-dependent fall in the LES pressure (Table II). The maximal

TABLE III

Influence of Hexamethonium (40 mg/kg-h) on the Frequency Response Curves of the Vagal-Stimulated LES Relaxation in Five Animals

	Initial LES pressure			LES pressure with vagal stimulation					
				Final pressure			Percent fall		
Frequency of stimulation	Control*	Hexamethonium*	*P* value	Control*	Hexamethonium*	*P* value	Control*	Hexamethonium*	*P* value
cycles/s	*mm Hg*			*mm Hg*			%		
0.25	46.7±3.5	40.7±3.9	>0.05	46.7±3.5	40.7±3.9	>0.05	0±0	0±0	
0.5	40.1±4.5	40.3±3.8	>0.05	39.1±3.9	40.3±3.8	>0.05	3.0±1.6	0±0	
1	48.8±3.9	41.1±3.9	>0.05	34.1±3.9	37.8±4.8	>0.05	32.5±4.2	12.9±5.8	<0.001‡
2	46.2±4.1	42.4±3.9	>0.05	14.7±2.0	30.0±4.8	<0.01‡	68.6±4.2	30.6±3.9	<0.001‡
5	44.9±4.1	41.6±3.9	>0.05	7.6±1.4	17.7±4.7	<0.05‡	82.2±3.4	55.9±8.8	<0.05‡
10	44.3±4.2	39.1±3.7	>0.05	7.6±1.1	12.3±3.6	>0.05	82.6±1.9	69.5±7.9	>0.05
20	49.1±4.5	36.7±4.0	<0.05‡	7.1±3.9	11.5±3.8	>0.05	85.2±1.9	71.1±8.1	>0.05
50	47.4±3.9	41.2±4.4	>0.05	5.9±1.2	14.0±4.5	>0.05	86.8±2.2	66.7±10.4	>0.05

These values are mean±SE of 15 observations (3 observations in each animal).

* Mean±SE.

‡ Indicates that the difference is statistically significant.

TABLE IV

Effect of Hexamethonium Alone and in Combination with Other Drugs on the Vagal-Stimulated LES Pressure

Exp.	Vagal stimulation at 10 PPS	No. of observations	Initial pressure*	P	Final pressure*	P	Fall*	P
			mm Hg		*mm Hg*		%	
1.	Control	18	40.3±3.6		5.4±0.7		82.7±2.9	
	Hexamethonium (20 mg/kg)	18	35.0±4.3	>0.3	6.8±0.6	>0.3	75.0±3.5	>0.1
2.	Reserpine (3 mg/kg × 2)	14	39.4±3.7		3.0±0.4		91.9±0.9	
	Hexamethonium (20 mg/kg) + Reserpine (3 mg/kg × 2)	13	40.8±3.6	>0.7	3.6±0.5	>0.3	90.2±1.6	>0.3
3.	Control	16	33.1±3.0		6.5±0.8		76.5±3.6	
	Hexamethonium (20 mg/kg) + Atropine (30 μg/kg)	16	33.4±2.9	>0.9	30.8±3.6	<0.001	9.4±2.5	<0.001‡

Four to six observations were made in each of the three separate animals for each experiment.

* Mean±SE.

‡ Highly significant.

reduction in the sphincter pressure occurred with a dose of 100 μg/kg, which caused 82.3% fall in sphincter pressure. Different doses were administered at 30-min intervals. Administered in this way and in the dose ranges used, DMPP did not exhibit tachyphylaxis. Obvious tachyphylaxis occurred with a dose of 20 mg/kg injected over several minutes, during which time inhibitory effect of 100 μg/kg intravenous or 2.5 μg/kg intra-arterial DMPP was blocked.

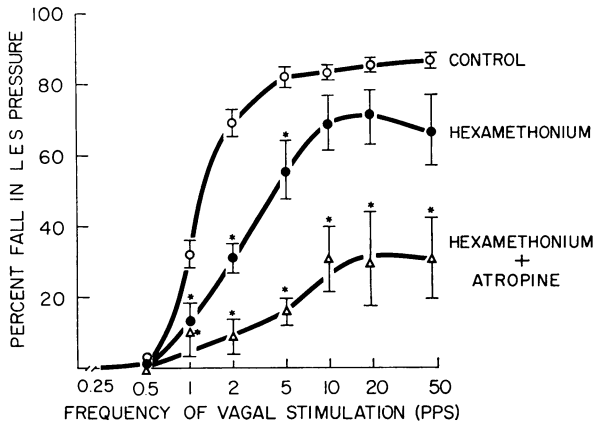

FIGURE 1 Frequency response curves of the effect of vagal stimulation on the percent decrease in LES pressure. Each point represents mean±SE of 15 observations in five animals. Note that hexamethonium (40 mg/kg-h) inhibited sphincter relaxation, which was statistically significant at the lower frequencies of stimulation. However, a combination of hexamethonium and atropine (30 μg/kg) produced a marked statistically significant (indicated by an asterisk) inhibition at all the frequencies of vagal stimulation.

Influence of hexamethonium on LES responses to DMPP and vagal stimulation

Influence of bolus dose of hexamethonium. Hexamethonium in the dose of 20 mg/kg, at 30 min after administration, effectively antagonized the fall in sphincter pressure caused by 100 μg/kg of DMPP. However, it did not influence the LES relaxation with vagal stimulation at 10 PPS. The percent fall in sphincter pressure with vagal stimulation was 82.7±2.9% during the control period and 75.0±3.5% after hexamethonium ($P > 0.1$). Hexamethonium in an 80-mg/kg dose was also ineffective in significantly antagonizing sphincter relaxation with vagal stimulation; the percent fall in pressure was 91.4±0.8% ($n = 4$) during the control period and 87.5±2.5% ($n = 4$) after hexamethonium treatment ($P > 0.05$).

Influence of continuous infusion of hexamethonium on the sphincter response with different frequencies of vagal stimulation. As shown in Table III, hexametho-

TABLE V

Effect of Intra-Arterial McN-A-343 (12.8 μg/kg) on the LES Pressure

Animal identification	Observation no.	Initial pressure	Final pressure at peak relaxation	Fall in pressure	Percent fall
			mm Hg		
45E	(1)	44	6	38	86.4
	(2)	36	4	32	88.9
46E	(1)	42	10	32	76.2
	(2)	40	18	22	55.0
47E	(1)	30	8	22	73.3
	Mean±SE	38.2±2.5	9.2±2.4	29.2±3.1	75.9±6.0

nium infusion (40 mg/kg-h) was effective in significantly antagonizing the sphincter relaxation at the lower frequencies of vagal stimulation. At the frequencies of 2 and 5 PPS, both the final sphincter pressure and the percent fall were significantly modified. Significant antagonism of vagal responses was not observed at frequencies of stimulation of 10 PPS and over. The sphincter relaxation to 100 μg/kg DMPP was abolished during the infusion of hexamethonium.

Influence of hexamethonium plus catecholamine depletion on vagal effects on the LES

As shown in Table IV, hexamethonium administration in animals pretreated with reserpine to deplete them of catecholamine stores (17) did not antagonize LES relaxation. With vagal stimulation at 10 PPS, the LES pressure fell by 90.2±1.6% after hexamethonium plus catecholamine depletion which was not statistically different from the control value ($P > 0.05$).

Influence of hexamethonium plus atropine on vagal-stimulated LES relaxation

Influence of the bolus doses on vagal stimulation at 10 PPS. As shown in Table IV, administration of 20 mg/kg hexamethonium plus 30 μg/kg atropine significantly reduced the vagal-stimulated fall in sphincter pressure from 76.5±3.6% during the control period to 9.4±2.5% after the treatment ($P < 0.001$). The final (residual) sphincter pressure with vagal stimulation was also modified by this combination ($P < 0.001$).

Influence of hexamethonium infusion (40 mg/kg-h) plus atropine (30 μg/kg) on the sphincter response with different frequencies of vagal stimulation. Fig. 1 compared the frequency-response curves of the vagal-stimulated fall in the sphincter pressure during the control period, after hexamethonium alone, and after hexamethonium plus atropine in five animals. Note that hexamethonium plus atropine markedly inhibited the sphincter relaxation at all of the frequencies examined.

Variation among animals. Examination of the responses of the different animals revealed a marked variation. Out of the eight animals examined, the response to vagal stimulation at the maximally effective frequency of 10 PPS was completely blocked by this combination in four, statistically significantly inhibited in two, and only slightly inhibited in the other two animals.

Effect of McN-A-343 on the LES pressure

McN-A-343 is a ganglionic stimulant which is different from other ganglionic stimulants like nicotine or DMPP, as it acts on the muscarinic receptors rather than the nicotinic receptors (4, 19). It is also different from the usual muscarinic agents such as bethanechol which preferentially exerts a powerful effect on the muscarinic receptors on the effector cells (4, 19). McN-A-343 was injected in the esophageal branch of the left gastric artery to avoid other systemic effects of this agent. In the dose 12.8 μg/kg, McN-A-343 caused a brief contraction followed by a prolonged relaxation of the LES (Table V). Intra-arterial injection of DMPP and nicotine also cause LES relaxation (unpublished observations).

To determine the site of inhibitory action of McN-A-343 on LES, we studied the influence of intravenous tetrodotoxin. Tetrodotoxin is a neurotoxin which acts to block conduction of impulses in nerve fibers (20, 21). Tetrodotoxin in the dose of 8–16 μg/kg administered intravenously caused block of sphincter relaxation with vagal stimulation, suggesting blockade of neural activity of the LES. The animals were maintained on respirator and intravenous saline administration. In three animals tetrodotoxin treatment did not significantly alter the basal LES pressure but converted sphincter relaxation caused by intra-arterial administration of 12.8 μg/kg McN-A-343 to sphincter contraction, suggesting that the inhibitory action of McN-A-343 may be mediated by the inhibitory neurons.

The effect of McN-A-343 was not antagonized by hexamethonium. McN-A-343 caused 66.4±4.9% fall in the sphincter pressure ($n = 3$) after 20 mg/kg hexamethonium, which antagonized the inhibitory effect of the nicotinic stimulant, DMPP. The reduction in sphinc-

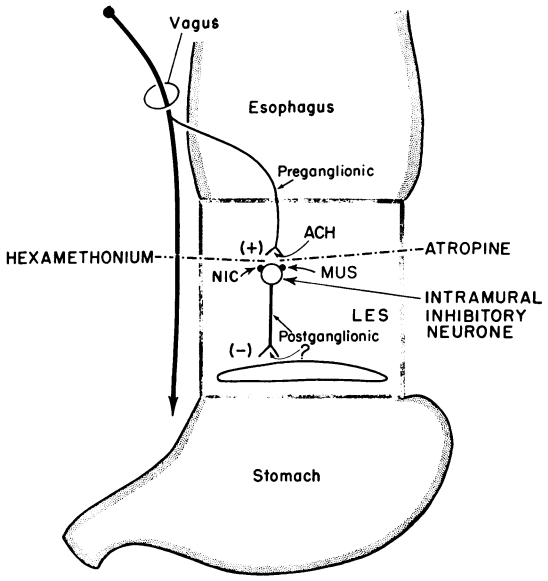

FIGURE 2 Schematic representation of the major vagal inhibitory pathway to the LES. The vagi carry preganglionic neurons which synapse with postganglionic inhibitory neurons. The synaptic transmission involves both nicotinic and muscarinic transmission. Both of these pathways of synaptic transmission are substantial, and only when both of these pathways are blocked, antagonism of synaptic transmission becomes obvious, particularly at the higher frequencies of stimulation. The nicotinic and muscarinic receptors may be present on different or same postganglionic neurons but, as shown in this model, we consider the latter possibility more likely. The postganglionic inhibitory neurons are neither adrenergic nor cholinergic; their neurotransmitter is not known at present.

ter pressure caused by intra-arterial McN-A-343 after hexamethonium treatment was not significantly different from the control value ($P < 0.5$). These observations further indicate that McN-A-343 did not exert its inhibitory influence by the nicotinic receptors. However, in two animals, during DMPP tachyphylaxis, the inhibitory effect of McN-A-343 on the LES was antagonized.

DISCUSSION

The results of these studies are best explained by the model outlined in Fig. 2. The major component of the vagal inhibitory pathway to the LES consists of preganglionic fibers which are cholinergic, and the acetylcholine released by them exerts its influence upon the postganglionic inhibitory neurons through both nicotinic and muscarinic pathways. The postganglionic neurons are neither adrenergic nor cholinergic, and they act by releasing a currently unknown neurotransmitter which acts to inhibit the LES.

The presence of a muscarinic pathway in the parasympathetic ganglia of the gut has not been demonstrated before (19, 22), although several studies in the sympathetic ganglia have shown the existence of such a transmission (23–26). The muscarinic and nicotinic transmission in the vagal pathway cannot be differentiated on the basis of the frequency of stimulation because atropine as reported elsewhere (1) or hexamethonium did not produce any discriminating antagonism of any specific frequency of vagal stimulation. These observations are consistent with the view that the amount of acetylcholine released by the preganglionic fibers in the vagus may be frequency dependent, and that there is considerable redundancy or reserve of these two cholinergic pathways. This is supported by the fact that antagonism of only one pathway does not reveal substantial block in the transmission, particularly at higher frequencies of preganglionic stimulation. Moreover, maximal stimulation of either nicotinic or muscarinic receptors alone produced almost maximal sphincter relaxation. At the higher frequencies of stimulation, even a combination of hexamethonium plus atropine did not abolish the vagal response. This may be related to quantitative interactions between the acetylcholine released and hexamethonium and atropine which are competitive antagonists of acetylcholine. However, one or more of the following possibilities may also play a role: (*a*) Some of the fibers in the vagal pathway to the sphincter may be postganglionic and hence not blocked by the antagonists of synaptic transmission. It has been shown that vagal trunks do carry many postganglionic fibers (27). (*b*) There may be modes of synaptic transmission other than by nicotinic and muscarinic receptors (25, 28–30). Bülbring and Gershon (31) have provided evidence for the participation of 5-hydroxytryptamine in the synaptic transmission in the vagal inhibitory influence to the guinea pig stomach. (*c*) A part of the LES relaxation may be due to antidromic stimulation of the sensory neurons which arise in the LES and may be carried in the vagi. It has been shown that antistimulation of the sensory nerves may cause release of ATP (32) and other purines which may act to inhibit the sphincter muscle (14).

The reasons for the variations among animals, particularly the failure of two animals to show substantial antagonism of vagal responses after treatment with hexamethonium plus atropine is not clear, but it may be related to preponderance of one or more of the alternative mechanisms of vagal-stimulated LES relaxation. Further studies are needed to resolve this problem.

As regards the nature of the postganglionic inhibitory neurons, three possibilities need consideration as follows: First, the conventional view which is currently cited in the textbooks (3, 4) is that they are cholinergic and exert their influence by muscarinic receptors which act to inhibit certain effector organs such as cardiac

conduction system and many regions in the central nervous system (4, 25). These inhibitory effects of acetylcholine are due to a hyperpolarizing effect and may be shown even by the cells which are predominantly excited by it. However, all these inhibitory effects are exclusively muscarinic as they are blocked by small doses of atropine (25). We found, as previously observed (2, 5, 6), that atropine, even in a dose which is over 50 times larger than the antimuscarinic dose, was not able to completely block the vagal response. Yet a small dose of atropine in combination with hexamethonium was able to markedly antagonize the vagal-stimulated sphincter relaxation. Moreover, our studies with McN-A-343 suggest that the muscarinic receptors which participate in transmission of inhibitory influence of the vagus on the LES lie at the synaptic site between the pre- and postganglionic neurons rather than on the sphincter muscle as the fall in sphincter pressure caused by McN-A-343 was abolished by the blockade of axonal transmission by tetrodotoxin. These observations are consistent with the view that sphincter muscles do not possess inhibitory muscarinic receptors (22, 33). The effect of a large dose of atropine can be explained on the basis of its nonspecific inhibitory influence on synaptic transmission (4).

Second, they may be adrenergic neurons which may exert their effect directly on the sphincter muscle by the beta-adrenergic receptors (6, 9, 10, 34), or they may be adrenergic interneurons (35) and exert their influence by inhibiting any excitatory neurons. Our studies show that vagal inhibitory responses were not antagonized by either alpha or beta blockade or by catecholamine depletion with reserpine, suggesing that adrenergic inhibitory pathways are not involved in the vagal-stimulated sphincter relaxation.

Third, they may belong to the family of newly described inhibitory neurons which are neither adrenergic nor cholinergic; their neurotransmitter is not known at present. Burnstock has suggested that ATP or a related nucleotide may be the transmitter, and he has named them as purinergic neurons (14). Presence of nonadrenergic, noncholinergic neurons in the LES has recently been suggested (11–13). Our results are consistent with the view that the postganglionic inhibitory neurons in the vagal pathway to the LES are neither adrenergic nor cholinergic.

The results of our studies may be of considerable physiological and clinical importance, as outlined below:

First, the vagus nerve-LES preparation may provide a model for further studies on synaptic transmission in the inhibitory parasympathetic pathways to the gut. This is because the vagus carries only inhibitory influences to the LES, whereas to most other organs it carries both inhibitory and excitatory influences. Moreover, because of background tonic contraction of the LES, the inhibitory responses to vagal stimulation are easily studied and quantitated. For example, in the previous studies on the nature of synaptic transmission in the vagal inhibitory pathway to organs other than LES, the presence of muscarinic transmission may have been totally overlooked because the tissues and organs had to be pretreated with atropine to reveal the inhibitory action of vagal stimulation (29–31).

Second, the model presented here may explain, at least in part, the possible reasons for conflicting reports of cholinergic agents on the LES (7). This model suggests that cholinergic agents may act indirectly via the intramural inhibitory neuron to cause sphincter relaxation in addition to their direct action on the sphincter muscle which is to cause its contraction. The observed response with any of these agents would be the algebraic sum of their direct and indirect effects. The reported sphincter relaxation with acetylcholine may be due to its indirect action via the inhibitory neurons, which led some observers to postulate the presence of inhibitory muscarinic receptors on the sphincter muscle (4–6, 36).

Third, several morphologic studies have demonstrated that patients with achalasia show lesions in the neurons of Auerbach's plexus (37). These neurons are usually considered to be cholinergic and excitatory. This concept does not satisfactorily explain the abnormality in the LES relaxation which is the primary functional abnormality of the sphincter in achalasia. If a parallel can be drawn from our studies in the opossum, it can be assumed that the intramural neurons which show degeneration are inhibitory in nature. Therefore, lesions in this pathway may cause impairment in LES relaxation. This model would also suggest that the degeneration of the inhibitory neurons may lead to enhanced contraction of the LES with cholinemimetic agents (38) in patients with achalasia due to the loss of the indirect (via the neurons) inhibitory effect of cholinergic agents on the sphincter, leaving their direct stimulatory action on the sphincter muscle unopposed.

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REFERENCES

1. Rattan, S., and R. K. Goyal. 1974. Neural control of lower esophageal sphincter. Influence of vagus nerves. *J. Clin. Invest.* **54**: 899–906.

2. Ingelfinger, F. J. 1958. Esophageal motility. *Physiol. Rev.* **38**: 533–584.
3. Davenport, H. W. 1971. Physiology of the Digestive Trace. Year Book Medical Publishers, Inc., Chicago. 3rd edition. 13–43.
4. Goodman, L. S., and A. Gilman, editors. 1970. Textbook of Pharmacology. Macmillan & Co. Ltd., London, England. 402–441.
5. Clark, C. G., and J. R. Vane. 1961. The cardiac sphincter of the cat. *Gut.* **2**: 252–262.
6. Schenck, E. A., and E. L. Frederickson. 1961. Pharmacologic evidence for a cardiac sphincter mechanism in the cat. *Gastroenterology.* **40**: 75–80.
7. Christensen, J. 1968. Pharmacology of the esophagus. *Handb. Physiol.* **4**:(Sect. 6): 2267–2324.
8. Ellis, F. G., R. Kauntze, and J. R. Trounce. 1960. The innervation of the cardia and lower esophagus in man. *Br. J. Surg.* **47**: 466–472.
9. Misiewicz, J. J., S. L. Waller, P. P. Anthony, and J. W. P. Gummer. 1969. Achalasia of the cardia: pharmacology and histopathology of isolated cardiac sphincteric muscle from patients with and without achalasia. *Q. J. Med.* **38**: 17–30.
10. Youmans, W. B. 1968. Innervation of the gastrointestinal tract. *Handb. Physiol.* **4**(Sect. 6): 1655–1663.
11. Tuch, A., and S. Cohen. 1973. Lower esophageal sphincter relaxation. Studies on the neurogenic inhibitory mechanism. *J. Clin. Invest.* **52**: 14–20.
12. Weisbrodt, N. W., and J. Christensen. 1972. Gradients of contraction in the opossum esophagus. *Gastroenterology.* **62**: 1159–1166.
13. Christensen, J., J. L. Conklin, and B. W. Freeman. 1973. Physiologic specialization at the esophagogastric junction in the three species. *Am. J. Physiol.* **225**: 1265–1271.
14. Burnstock, G. 1972. Purinergic nerves. *Pharmacol. Rev.* **24**: 509–581.
15. Christensen, J., and G. F. Lund. 1969. Esophageal responses to distension and electrical stimulation. *J. Clin. Invest.* **48**: 408–419.
16. Goyal, R. K., and S. Rattan. 1973. Mechanism of the lower esophageal sphincter relaxation. Action of prostaglandin E_1 and theophylline. *J. Clin. Invest.* **52**: 337–341.
17. Bhagat, B. D. 1971. Recent Advances in Adrenergic Mechanisms. Charles C. Thomas, Publisher, Springfield, Ill. 93–96.
18. DiMarino, A. J., and S. Cohen. 1973. The adrenergic control of lower esophageal sphincter function. An experimental model for denervation suprasensitivity. *J. Clin. Invest.* **52**: 2264–2271.
19. Kharkevich, D. A. 1967. Ganglion-blocking and Ganglion-stimulating Agents. Pergamon Press, Inc., Elmsford, N. Y. 225–244.
20. Kao, C. Y. 1960. Tetrodotoxin, saxitoxin and their significance in the study of excitation phenomena. *Pharmacol. Rev.* **18**: 997–1050.
21. Gershon, M. D. 1967. Effects of tetrodotoxin on innervated smooth muscle preparations. *Br. J. Pharmacol. Chemother.* **29**: 259–279.
22. Daniel, E. E. 1968. Pharmacology of the gastrointestinal tract. *Handb. Physiol.* **4**(Sect. 6): 2267–2324.
23. Trelendenburg, U. 1966. Transmission of preganglionic impulses through the muscarinic receptors of the superior cervical ganglion of the cat. *J. Pharmacol. Exp. Ther.* **154**: 426–440.
24. Brown, A. M. 1967. Cardiac sympathetic adrenergic pathways in which synaptic transmission is blocked by atropine sulphate. *J. Physiol. (Lond.).* **191**: 271–288.
25. Krnjevic, K. 1974. Chemical nature of synaptic transmission in vertebrates. *Physiol. Rev.* **54**: 418–540.
26. Volle, R. L. 1969. Ganglionic transmission. *Annu. Rev. Pharmacol.* **9**: 135–146.
27. Schofield, G. C. 1968. Anatomy of muscular and neural tissues in the alimentary canal. *Handb. Physiol.* **4**: (Sect. 6): 1579–1627.
28. Alkadhi, K. A., and R. J. McIsaac. 1973. Non-nicotinic transmission during ganblionic block with chlorisondamine and nicotine. *Eur. J. Pharmacol.* **24**: 78–85.
29. Campbell, G. 1970. Autonomic nerve supply to effective tissues. *In* Smooth Muscle. E. Bulbring, A. F. Brading, A. W. Jones, and T. Tomita, editors. The Williams & Wilkins Co., Baltimore. 451–494.
30. Beani, L., C. Bianchi, and A. Crema. 1971. Vagal non-adrenergic inhibition of guinea pig stomach. *J. Physiol. (Lond.).* **217**: 259–279.
31. Bülbring, E., and M. D. Gershon. 5-hydroxytrypamine participation in the vagal inhibitory innervation of the stomach. *J. Physiol. (Lond.).* **192**: 823–846.
32. Holton, P. 1959. The liberation of adenosine triphosphate on antidromic stimulation of sensory nerves. *J. Physiol. (Lond.).* **145**: 494–504.
33. Daniel, E. E. 1966. Electrical and contractile responses of the pyloric region to adrenergic and cholinergic drugs. *Can. J. Physiol. Pharmacol.* **44**: 951–979.
34. Christensen, J., and E. E. Daniel. 1968. Effect of some autonomic drugs on circular esophageal smooth muscle. *J. Pharmacol. Exp. Ther.* **159**: 243–249.
35. Greengaard, P., D. A. McAfee, and J. W. Kebabian. 1972. On the mechanism of action of cyclic AMP and its role in synaptic transmission. *Adv. Cyclic Nucleotide Res.* **1**: 337–355.
36. Penninckx, F., R. Kerremans, and J. Beckers. 1973. Pharmacological characteristics of the non-striated anorectal musculature in cats. *Gut.* **14**: 393–398.
37. Ellis, F. H., and A. M. Olsen. 1969. Achalasia of the esophagus. *Major Probl. Clin. Surg.* **9**: 15–43.
38. Cohen, B. R., and M. Gruelund. 1971. Cardiospasm in achalasia: demonstration of supersensitivity of the lower esophageal sphincter. *Gastroenterology.* **60**: 769. (Abstr.)