Different responsiveness of excitatory and inhibitory enteric motor neurons in the human esophagus to electrical field stimulation and to nicotine

Asensio A. González,1,* Ricardo Farré,1,* and Pere Clavé1,2
1 Fundación de Gastroenterología Dr. Francisco Vilardell, 08025 Barcelona; and 2 Department of Surgery, Hospital de Mataró, 08304 Mataró, Spain

Submitted 23 December 2003; accepted in final form 3 March 2004

González, Asensio A., Ricardo Farré, and Pere Clavé. Different responsiveness of excitatory and inhibitory enteric motor neurons in the human esophagus to electrical field stimulation and to nicotine. Am J Physiol Gastrointest Liver Physiol 287: G299–G306, 2004. First published March 11, 2004; 10.1152/ajpgi.00534.2003.—To compare electrical field stimulation (EFS) with nicotine in the stimulation of excitatory and inhibitory enteric motoneurons (EMN) in the human esophagus, circular lower esophageal sphincter (LES), and circular and longitudinal esophageal body (EB) strips from 20 humans were studied in organ baths. Responses to EFS or nicotine (100 μM) were compared in basal conditions, after 100 μM G-nitro-L-arginine (L-NNA; 100 μM), and after 100 μM L-NNA and apamin (1 μM). LES strips developed myogenic tone enhanced by TTX (5 μM) or L-NNA. EFS-LES relaxation was abolished by TTX, unaffected by hexamethonium (100 μM), and enhanced by atropine (3 μM). Nicotine-LES relaxation was higher than EFS relaxation, reduced by TTX or atropine, and blocked by hexamethonium. After L-NNA, EFS elicited a strong cholinergic contraction in circular LES and EB, and nicotine elicited a small relaxation in LES and no contractile effect in EB. After L-NNA and apamin, EFS elicited a strong cholinergic contraction in LES and EB, and nicotine elicited a weak contraction amounting to 6.64 ± 9.20%/H11006 and nicotine elicited a weak contraction amounting to 6.64 ± 9.20%/H11006 and 9.20%/H11006/L-NNA and apamin, nicotine did not induce any response. Inhibitory EMN tonically inhibit myogenic LES tone and are efficiently stimulated both by EFS and nicotinic acetylcholine receptors (nAChRs) located in somatodendritic regions and nerve terminals, releasing nitric oxide and an apamin-sensitive neurotransmitter. In contrast, although esophageal excitatory EMN are efficiently stimulated by EFS, their stimulation through nAChRs is difficult and causes weak responses, suggesting the participation of nonnicotinic mechanisms in neurotransmission to excitatory EMN in human esophagus.

nicotinic receptors; inhibitory neurotransmitters; nitric oxide; apamin-sensitive neurotransmission; human esophageal motility

The two main physiologic motor events in the human esophagus are the swallowing-induced primary peristalsis and lower esophageal sphincter (LES) relaxation and the transient LES relaxation that causes physiological gastroesophageal reflux and allows belching. Stimuli triggering both motor events are integrated in the central nervous system (CNS), conveyed to the dorsal motor nucleus of the vagus, which contains the preganglionic neurons, and travel along a few vagal efferent fibers to a huge number of enteric motor neurons (EMN) located in the myenteric plexus (MP) (4, 16). The esophageal MP is a network of interconnected ganglia that have, in addition, integrative functions independent of the CNS that determine the peristaltic nature of the esophageal body contraction and LES relaxation and modulate LES resting tone (4, 26). The esophageal EMN have their cell bodies in the myenteric ganglia, and their motor axons travel in small nerve bundles penetrating the longitudinal and circular smooth muscle layers (4, 16). Efforts during recent years have been directed at characterizing the final neurotransmitters involved in the terminal motor pathways of EMN to the esophageal smooth muscle, but little is known about the physiology of the neuronal circuitry that directly controls esophageal peristalsis, LES tone, and LES relaxation. In vitro studies on human esophageal strips using direct activation of EMN by electrical field stimulation (EFS) have shown that LES relaxation is mainly caused by nitric oxide (NO) released from inhibitory EMN, whereas esophageal contraction is mainly caused by ACh released from excitatory EMN acting on muscarinic receptors located in the smooth muscle (21, 31).

The vagal efferent fibers synapse with EMN in myenteric ganglia, and the overall consensus is that vagal fibers can stimulate both inhibitory and excitatory EMN (16). ACh released by vagal fibers in the LES of guinea pigs acts on inhibitory motoneurons through nicotinic and muscarinic receptors (15), although in most animals, a serotoninergic antagonist is also required to completely antagonize ganglionic transmission in this pathway (34). Nevertheless, there is some controversy over the existence and nature of the vagal input to the excitatory EMN, because they appear to lack direct vagal nicotinic inputs in the rat (6) and guinea pig (49) LES. Some enteric motor reflexes caused by distension or mucosal stimulation are fully blocked, whereas others are only reduced by nicotinic acetylcholine receptor (nAChR) antagonists (36, 39, 40). There are few data available on the receptors and interneurons involved in neural transmission from vagal pathways and interneurons to EMN in the smooth muscle of the human esophagus.

The aim of the present study was to characterize, in vitro, the effect of stimulation with nicotine on intrinsic excitatory and inhibitory EMN in the smooth muscle of the human esophagus and to compare it with the effect of direct stimulation with EFS.

METHODS

Preparations. Twenty specimens including part of the gastric fundus, the gastroesophageal junction (GEJ), and the EB were obtained from eight patients with esophageal cancer and 12 organ donors (female/male = 1.2, mean age = 56.3 ± 4.5 yr). The study protocol

* A. A. González and R. Farré contributed equally to the work.

Address for reprint requests and other correspondence: P. Clavé, Dept. of Surgery, Hospital de Mataró, C/Círera s/n, 08304 Mataró, Spain (E-mail: pclave@teleline.es).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
had been previously approved by the institutional review board of the Hospital de la Santa Creu i Sant Pau, that of the Hospital de Mataró, and that of the Hospital Clinic i Provincial (Barcelona, Spain). The specimens were opened following the lesser curvature of the stomach, the transitional line was marked with sutures, and the mucosa was resected. Consecutive 3-mm-wide strips, including the circular muscle, the longitudinal muscle, and the MP, were cut parallel to the circular muscle fibers. Four to six consecutive strips were mainly obtained from the left (sling) side of the GEJ in each specimen starting 3 mm apart from the node of the transitional line (30); and four to six circular strips were obtained from the EB, 6–9 cm above the transitional line. In five specimens, full-thickness longitudinal strips were also obtained from the EB, 6–9 cm above the transitional line. Strips were always obtained from macroscopically noninvaded regions.

Procedures. Studies were started within the 18-h period after resection. Final strips measuring 10 mm in length were placed in 25-ml organ baths containing Krebs solution constantly bubbling with 5% CO₂ in O₂ (5, 41). Changes in tension of the strips were measured 25-ml organ baths containing Krebs solution constantly bubbling with also obtained from the EB, 6–9 cm above the transitional line. In five specimens, full-thickness longitudinal strips were also obtained from the EB, 6–9 cm above the transitional line. Strips were always obtained from macroscopically noninvaded regions.

The effect of nicotine (Sigma) was evaluated on circular EB strips precontracted with TTX (5 × 10⁻⁶ M), or atropine. The effect of nicotine was studied on 100 (μM) transformed the on relaxation at the end of the stimulus in 21.54 ± 2.20 g. Maximal relaxation in upper LES strips was observed at 10 Hz (3.54 ± 0.45 g). Maximal relaxation in upper LES strips was observed at 3 Hz (3.35 ± 0.41 g; differences were not significant with regard to lower LES, Fig. 2A), and maximal off contraction (1.60 ± 0.25 g) was observed at 3 Hz (Fig. 2B; n = 22, N = 11). EFS-induced relaxation was unaffected by HEX (100 (μM), was completely blocked by TTX (5 (μM), and was significantly enhanced by atropine (Fig. 3). In all LES strips, l-NNA (100 (μM) transformed the on relaxation into an on contraction during EFS (Fig. 1A), which was also frequency dependent and of higher amplitude than the off contraction (Fig. 2B) and fully abolished by atropine (3 (μM; Fig. 2B; n = 7, N = 3). A slight off relaxation following l-NNA and atropine appeared in five of seven of these strips (Fig. 1A); this off relaxation was fully blocked by TTX (5 (μM). Nicotine relaxed LES strips in a concentration-dependent manner (Fig. 2C). Mean relaxation induced by the highest concentration tested (100 (μM) was 4.44 ± 0.68 g (n = 14, N = 7), significantly higher than that obtained by 10 Hz EFS (2.32 ± 0.41, n = 15, N = 7, P < 0.01). HEX (100 (μM) completely blocked the maximal response to nicotine. In contrast, TTX (10 (μM) significantly decreased but did not block the relaxation induced by nicotine by 38 ± 7.6%, and atropine significantly inhibited the nicotine-induced relaxation by 88.45 ± 11.55% (n = 5, N = 2; Fig. 3). l-NNA (100 (μM) abolished the relaxation induced by nicotine at concentrations <30 μM; at 100 μM, nicotine induced a slow relaxation accounting for 0.54 ± 0.19 g (P < 0.05; Figs. 1B and 2C). Nicotine (100 (μM) did not elicit any contraction in circular LES strips in the presence of l-NNA (100 (μM). After simultaneous blockade with l-NNA and atropine, EFS induced a strong cholinergic on contraction (6.28 ± 1.65 g at 10 Hz, n =
Effect of EFS and nicotine on circular and longitudinal EB strips. Thirty-eight circular strips taken from the EB of 11 specimens did not develop active resting tone. EFS induced two types of contractions on circular EB strips. During the application of the stimulus, 50% of the strips responded with slight mono- or multiphasic on contractions, and briefly after cessation of the stimulus, a more prominent off contraction emerged (Fig. 5A) in all strips. The amplitude of both contractions was frequency dependent, and off contractions were higher than on contractions (P < 0.05; Fig. 6A). Atropine (3 μM) fully abolished on contractions; in contrast, it only partially antagonized off contractions (Fig. 6A). L-NNa (100 μM) transformed the response to EFS into a frequency-dependent monophasic on contraction (Fig. 5A) of higher amplitude than the off contraction (17.02 ± 1.48 vs. 4.73 ± 4.04 g at 20 Hz, P < 0.05), which was totally blocked by atropine (3 μM) at 0.5–20 Hz (Fig. 6B).

Nicotine (20–100 μM) did not elicit any mechanical response in EB circular strips at rest (n = 4, N = 2). In EB strips precontracted at 5.3 ± 0.6 g by 2 μM carbachol, nicotine (100 μM) induced an immediate relaxation (Fig. 5B), decreasing the carbachol-induced tone by 82.2 ± 5.8% (n = 6, N = 2). The relaxation induced by nicotine was completely blocked by HEX (100 μM) and antagonized by pretreatment with 100 μM L-NNa, but a remaining relaxation of 0.83 ± 0.3 g (P < 0.05) on the carbachol-induced tone was still elicited (Fig. 5B). After simultaneous blockade with L-NNa and apamin, EFS induced a strong cholinergic contraction (16.56 ± 1.57 g at 20 Hz) and nicotine elicited a slight cholinergic contraction (1.49 ± 1.02 g, n = 9, N = 3) that was 9.20 ± 5.51% of that induced by EFS (Fig. 7B).

Fig. 1. Tracings showing the effect of electrical field stimulation (EFS; 10 Hz, pulses indicated below tracings) or nicotine (NIC; 100 μM; dots above tracings) on lower esophageal sphincter (LES) strips. A: EFS-induced responses (“on” relaxation, “off” contraction); L-NNa transformed this response into an on contraction (middle) that was fully blocked by atropine: following Nω-nitro-arginine (L-NNa) and atropine, a slight off relaxation appeared. B: NIC-induced LES relaxation (left) was inhibited but not blocked by L-NNa. C: EFS induced a strong cholinergic contraction during simultaneous blockade by L-NNa and apamin (APA); in this experimental setting, NIC induced a slight cholinergic contraction.

8, N = 3) in all LES strips; in contrast, in the same experimental setting, nicotine only elicited a slight contraction (0.27 ± 0.15 g, n = 8, N = 3) amounting to 6.64 ± 3.19% of that induced by EFS on the same strips (Fig. 4).

Effect of EFS and nicotine on circular and longitudinal EB strips. Thirty-eight circular strips taken from the EB of 11 specimens did not develop active resting tone. EFS induced two types of contractions on circular EB strips. During the application of the stimulus, 50% of the strips responded with slight mono- or multiphasic on contractions, and briefly after cessation of the stimulus, a more prominent off contraction emerged (Fig. 5A) in all strips. The amplitude of both contractions was frequency dependent, and off contractions were higher than on contractions (P < 0.05; Fig. 6A). Atropine (3 μM) fully abolished on contractions; in contrast, it only partially antagonized off contractions (Fig. 6A). L-NNa (100 μM) transformed the response to EFS into a frequency-dependent monophasic on contraction (Fig. 5A) of higher amplitude than the off contraction (17.02 ± 1.48 vs. 4.73 ± 4.04 g at 20 Hz, P < 0.05), which was totally blocked by atropine (3 μM) at 0.5–20 Hz (Fig. 6B).

Nicotine (20–100 μM) did not elicit any mechanical response in EB circular strips at rest (n = 4, N = 2). In EB strips precontracted at 5.3 ± 0.6 g by 2 μM carbachol, nicotine (100 μM) induced an immediate relaxation (Fig. 5B), decreasing the carbachol-induced tone by 82.2 ± 5.8% (n = 6, N = 2). The relaxation induced by nicotine was completely blocked by HEX (100 μM) and antagonized by pretreatment with 100 μM L-NNa, but a remaining relaxation of 0.83 ± 0.3 g (P < 0.05) on the carbachol-induced tone was still elicited (Fig. 5B). After simultaneous blockade with L-NNa and apamin, EFS induced a strong cholinergic contraction (16.56 ± 1.57 g at 20 Hz) and nicotine elicited a slight cholinergic contraction (1.49 ± 1.02 g, n = 9, N = 3) that was 9.20 ± 5.51% of that induced by EFS (Fig. 7B).

Figure 2. A: frequency-dependent relaxation curves induced by EFS in upper [number of strips (n) = 12, number of specimens (N) = 4] and lower (n = 11, N = 5) LES strips showing similar profiles. B: L-NNa changed the on relaxation to a frequency-dependent on contraction during EFS, which was fully blocked by atropine (ATR; n = 12, N = 6). C: NIC induced a dose-dependent relaxation (n = 5, N = 3). After NO blockade by L-NNa, a residual but significant relaxation was still induced by 100 μM NIC (n = 4, N = 3, *P < 0.05).
Longitudinal EB strips did not develop active resting tone. EFS induced a frequency-dependent on contraction on longitudinal EB strips (Fig. 5D) reaching 14.03 ± 2.52 g at 10 Hz (Fig. 6C). Atropine (3 μM) significantly reduced the amplitude of longitudinal contractions (from 90.2 ± 9.8% at 10 Hz to 94.39 ± 3.21% at 70 Hz; Fig. 6C) and evoked the appearance of a residual low-amplitude off contraction following EFS (Fig. 5D). Nicotine (20–100 μM) did not elicit any mechanical response in longitudinal EB strips at rest (n = 5, N = 3). After l-NNA and apamin, EFS also induced a strong cholinergic contraction amounting to 6.64% of that induced by EFS and showing less efficient stimulation of excitatory LES EMN through nAChRs (*P < 0.05).

DISCUSSION

We have found that inhibitory EMN in the human esophagus are easily and efficiently stimulated both by EFS and by nAChRs located in somatodendritic regions as well as in nerve terminals. In contrast, although esophageal excitatory EMN can be efficiently stimulated by EFS even at basal conditions, its stimulation through nAChRs is very difficult, requiring full blockade of inhibitory motor pathways and causing a weak response, suggesting the participation of nonnicotinic mechanisms in ganglionic neurotransmission to excitatory esophageal body (EB) strips to EFS (20 Hz, pulses indicated below tracings) or to NIC (100 μM, dots above tracings). A: left: control response including on contraction, latency, and off contraction. Middle: l-NNA transformed the response to EFS into a monophasic on contraction that was totally blocked by ATR (right). B: NIC induced a relaxation in circular EB strips precontracted with carbachol. l-NNA antagonized, but did not block, the relaxation induced by NIC (middle). C: during simultaneous nitrergic and purinergic blockade, EFS induced a strong on contraction and NIC elicited a slight cholinergic contraction in circular EB strips (right). D: longitudinal EB strips responded to EFS with an on contraction that was antagonized by ATR, and a minor off contraction appeared (middle). NIC did not induce any effect. E: in longitudinal strips pretreated by l-NNA and APA, EFS induced an on contraction (middle) and NIC did not induce any effect (right).
contractions (43) or preganglionic during stimulus without direct activation of muscle (5, 35, 41). Indeed, the fall in LES pressure in humans occurs in the CNS Altogether, these findings strongly suggest that the cholinergic response induced in healthy humans (7, 24), and hyoscine butylbromide, a muscarinic antagonist that does not atropine. However, the effect of atropine suggests the absence of an EMN tonically inhibit LES resting tone and that NO is the main but not the only inhibitory neurotransmitter released by inhibitory EMN, suggesting a minor role for an apamin-sensitive neurotransmitter in the human esophagus.

The human LES is constituted by the sling muscle on the angle of His and the clasp component in lesser curvature, which develop greater active tone. This explains the asymmetry of LES pressure in vivo (30). Due to our method of dissection, we obtained more sling strips than clasplike fibers, thus influencing our resting LES tone values. Our results show a sharp reduction in LES resting tone following 10 min exposure to the calcium-free buffer, further confirming that human LES tone is main myogenic and depends on extracellular calcium (43). On the other hand, the relative neurogenic contribution to LES tone may vary according to the species of animal. Previous studies on human LES strips failed to demonstrate any effect of TTX (30); however, in vivo, NO synthase inhibitors increased LES resting pressure in animal (1, 47, 48) and human (18) studies. In our study, the similarity of the increase in LES tone induced by TTX and L-NNa suggests that, in humans, NO tonically released from inhibitory EMN continuously inhibits the myogenic LES resting tone. The lack of effect of atropine suggests the absence of an intrinsic cholinergic contribution to LES tone in vitro, agreeing with previous in vitro studies on the human LES (30). In vivo, atropine reduced LES pressure in healthy humans (7, 24), and hyoscine butylbromide, a muscarinic antagonist that does not cross the blood-brain barrier, did not alter LES pressure (22). Altogether, these findings strongly suggest that the atropine-induced fall in LES pressure in humans occurs in the CNS rather than at peripheral sites.

In our study, EFS activated excitatory and inhibitory EMN during stimulus without direct activation of muscle (5, 35, 41, 43) or preganglionic fibers, because the neurotoxin TTX blocks and the ganglionic blocker Hex did not affect EFS-induced responses. We used full-thickness esophageal preparations that included the MP to compare these well-characterized neural responses induced by EFS in human esophagus (3, 30) with those induced by nicotinic stimulation of EMN. Most esophageal EMN have single-process Dogiel type 1 morphology and the soma located in myenteric ganglia. In the LES of opossums, inhibitory EMN are larger and with longer projections than the excitatory EMN, and the proportion of local excitatory and inhibitory EMN is similar and ~50% (2). In the human intestine, circular inhibitory EMN also have larger cell bodies and larger projections (3.2 mm) than the excitatory EMN (2 mm) (28), and EMN to longitudinal muscle were smaller than those to the circular muscle (29, 46). On the basis of these morphological data, we inferred that our human esophageal circular preparations also contain a significant number and a similar proportion of both local excitatory and inhibitory EMN. Immunohistochemical and functional studies have shown that nAChRs are located in somatodendritic regions of EMN in humans.

The human LES is constituted by the sling muscle on the angle of His and the clasp component in lesser curvature, which develop greater active tone. This explains the asymmetry of LES pressure in vivo (30). Due to our method of dissection, we obtained more sling strips than clasplike fibers, thus influencing our resting LES tone values. Our results show a sharp reduction in LES resting tone following 10 min exposure to the calcium-free buffer, further confirming that human LES tone is main myogenic and depends on extracellular calcium (43). On the other hand, the relative neurogenic contribution to LES tone may vary according to the species of animal. Previous studies on human LES strips failed to demonstrate any effect of TTX (30); however, in vivo, NO synthase inhibitors increased LES resting pressure in animal (1, 47, 48) and human (18) studies. In our study, the similarity of the increase in LES tone induced by TTX and L-NNa suggests that, in humans, NO tonically released from inhibitory EMN continuously inhibits the myogenic LES resting tone. The lack of effect of atropine suggests the absence of an intrinsic cholinergic contribution to LES tone in vitro, agreeing with previous in vitro studies on the human LES (30). In vivo, atropine reduced LES pressure in healthy humans (7, 24), and hyoscine butylbromide, a muscarinic antagonist that does not cross the blood-brain barrier, did not alter LES pressure (22). Altogether, these findings strongly suggest that the atropine-induced fall in LES pressure in humans occurs in the CNS rather than at peripheral sites.

In our study, EFS activated excitatory and inhibitory EMN during stimulus without direct activation of muscle (5, 35, 41, 43) or preganglionic fibers, because the neurotoxin TTX blocks and the ganglionic blocker Hex did not affect EFS-induced responses. We used full-thickness esophageal preparations that included the MP to compare these well-characterized neural responses induced by EFS in human esophagus (3, 30) with those induced by nicotinic stimulation of EMN. Most esophageal EMN have single-process Dogiel type 1 morphology and the soma located in myenteric ganglia. In the LES of opossums, inhibitory EMN are larger and with longer projections than the excitatory EMN, and the proportion of local excitatory and inhibitory EMN is similar and ~50% (2). In the human intestine, circular inhibitory EMN also have larger cell bodies and larger projections (3.2 mm) than the excitatory EMN (2 mm) (28), and EMN to longitudinal muscle were smaller than those to the circular muscle (29, 46). On the basis of these morphological data, we inferred that our human esophageal circular preparations also contain a significant number and a similar proportion of both local excitatory and inhibitory EMN. Immunohistochemical and functional studies have shown that nAChRs are located in somatodendritic regions of EMN in humans.

The human LES is constituted by the sling muscle on the angle of His and the clasp component in lesser curvature, which develop greater active tone. This explains the asymmetry of LES pressure in vivo (30). Due to our method of dissection, we obtained more sling strips than clasplike fibers, thus influencing our resting LES tone values. Our results show a sharp reduction in LES resting tone following 10 min exposure to the calcium-free buffer, further confirming that human LES tone is main myogenic and depends on extracellular calcium (43). On the other hand, the relative neurogenic contribution to LES tone may vary according to the species of animal. Previous studies on human LES strips failed to demonstrate any effect of TTX (30); however, in vivo, NO synthase inhibitors increased LES resting pressure in animal (1, 47, 48) and human (18) studies. In our study, the similarity of the increase in LES tone induced by TTX and L-NNa suggests that, in humans, NO tonically released from inhibitory EMN continuously inhibits the myogenic LES resting tone. The lack of effect of atropine suggests the absence of an intrinsic cholinergic contribution to LES tone in vitro, agreeing with previous in vitro studies on the human LES (30). In vivo, atropine reduced LES pressure in healthy humans (7, 24), and hyoscine butylbromide, a muscarinic antagonist that does not cross the blood-brain barrier, did not alter LES pressure (22). Altogether, these findings strongly suggest that the atropine-induced fall in LES pressure in humans occurs in the CNS rather than at peripheral sites.

In our study, EFS activated excitatory and inhibitory EMN during stimulus without direct activation of muscle (5, 35, 41, 43) or preganglionic fibers, because the neurotoxin TTX blocks and the ganglionic blocker Hex did not affect EFS-induced responses. We used full-thickness esophageal preparations that included the MP to compare these well-characterized neural responses induced by EFS in human esophagus (3, 30) with...
myenteric ganglia (36), and nAChRs that cause transmitter release by action potential-independent mechanisms have also been described in nerve terminals of excitatory (9, 36) and inhibitory (39) EMN. In our study, nicotine-induced LES relaxation was partly antagonized by higher concentrations of TTX than those that fully blocked EFS-relaxation (9, 36). Our data suggest the presence of nAChRs in the soma as well as in the nerve terminals of human LES inhibitory EMN, providing strong evidence for the functional integrity of our preparation, which contains the circular as well as the longitudinal muscular layer and all the components of MP including myenteric ganglia and EMN rather than being merely a muscle-axon preparation (41, 43). Our results further confirm that NO is the main mediator for EFS-relaxation in the human LES (31) and suggest that one or more minor inhibitory neurotransmitters are released, because an off relaxation sensitive to TTX appeared in our study following nitrergic blockade (44). Our study also agrees with previous studies that showed a relaxant effect through stimulation of nAChRs by nicotine or dimethylphenylpiperazinium in human LES strips (3, 38), also mainly mediated by NO. Immunomorphological studies (25, 37, 45) on the human esophagus found that most nitrergic EMN also contain VIP, galanin, and PACAP; and VIP, PACAP, and adenosine relaxed human LES strips in functional studies (25, 42). We recently found nitrergic and apamin-sensitive inhibitory cotransmission on porcine LES (8), very similar to that found by Yuan et al. (49) on the opossum. In our study on porcine LES, apamin inhibited the relaxation induced by ATP or PACAP (8), and ATP has been proposed as the mediator for the apamin-sensitive component in the opossum LES (49). What we found in the present study on human LES is indirect evidence for an apamin-sensitive inhibitory conneurotransmitter, because nicotine induced a relaxation following nitrergic blockade and a small contraction following simultaneous blockade with L-NNa and apamin.

We compared the responses induced by direct stimulation of EMN by EFS with those induced through stimulation of nAChRs. In our human study, LES stimulation by nicotine in basal conditions caused a more intense relaxation than that induced by EFS, both being LES inhibitory responses of comparable magnitude and suggesting high efficiency of stimulation of inhibitory EMN. Simultaneous blockade of inhibitory neurotransmitters by L-NNa and apamin was required in opossum LES to show focal EFS-induced stimulation of excitatory EMN (49). This was not the case in our study because a significant number of circular LES strips responded in basal conditions to EFS with off contractions and circular EB strips responded with both on and off frequency-dependent contractions (35, 41, 43). The on contraction was abolished by atropine, clearly showing its origin in cholinergic EMN. The latency depended on activation of inhibitory EMN, and it was fully blocked by NO synthesis inhibitors, as shown in other studies (4, 31). In contrast, the off contraction was only partly antagonized by atropine (4, 20, 35, 41), suggesting the participation of a second noncholinergic mechanism, such as rebound depolarization (27), or a second excitatory neurotransmitter, such as tachykinins (19). We explored the effect of nicotine on circular LES and EB circular strips at basal conditions and following blockade of NO synthesis representing the majority of inhibitory innervation. We expected nicotine would strongly stimulate nAChRs on cholinergic EMN and make the strips contract, but it had no effect. However, EFS easily induced a strong stimulation of excitatory EMN in both human LES and EB under the same experimental conditions during nitrergic blockade. In addition, our experimental design included the comparison between EFS and nAChRs stimulation of EMN during simultaneous blockade by L-NNa and apamin in a third set of experiments. To enhance our observations, the comparisons between EFS and nAChRs-mediated responses were always performed in pairs, each strip acting as its own control. In this experimental setting, stimulation of nAChRs induced a slight contraction in LES and EB accounting for 6.64 and 9.20%, respectively, of that obtained by EFS and showing that nAChRs stimulate excitatory EMN much less efficiently than EFS. Two additional observations further confirmed the low sensitivity of excitatory esophageal EMN to nAChRs stimulation. First, we found, as others have (23), a strong direct stimulation of excitatory EMN by EFS and no responses to nicotine in patients with achalasia and fully impaired inhibitory neurotransmission (not shown). Second, we found that atropine inhibits nicotine-LES relaxation (the opposite effect than on EFS relaxation) in our study, an effect very similar to that caused by atropine during vagal efferent stimulation (15). The effect caused by atropine during LES stimulation by nAChRs could be due to a direct pharmacological antagonism (50) or be exerted on an interneuronal synapse, which would further indicate a strong stimulation of a polysynaptic inhibitory motor pathway by nAChRs, overriding a weak stimulation of the excitatory one. In addition, the differences between the responsiveness of excitatory EMN to EFS and to nicotine are also clearly observed in our experiments with longitudinal strips. Because few inhibitory EMN were found in the outer longitudinal smooth muscle layer of human esophagi in morphological studies (37), EFS induced an on contraction in our basal studies (43). Stimulation of nAChRs in these conditions did not induce any contraction nor were any responses obtained by nicotine following simultaneous blockade by L-NNa and apamin in longitudinal studies. Thus the low responsiveness of esophageal excitatory EMN to stimulation through nAChRs was observed in our study of circular LES and EB circular strips as well as in the longitudinal EB layer.

Classic in vivo studies by Goyal and Rattan (15, 32) on the opossum suggested that the vagi do not mediate any influence on excitatory LES EMN and the vagal influence on the sphincter is entirely inhibitory. These authors also found that nicotine only stimulated inhibitory EMN (33). Recent studies (49) on the same animal also found that vagal stimulation did not evoke measurable excitatory responses even following complete blockade of inhibitory LES pathways by L-NNa and apamin. These results also agreed with those of our study but did not show how cholinergic EMN in LES and EB were stimulated during peristalsis. In vivo studies (17) found HEX-sensitive transmission to excitatory EMN in rat LES, contrasting with in vitro studies (6) also in the LES of the same animal showing the absence of direct nicotinic inputs to excitatory EMN and suggesting a polysynaptic pathway. In addition, studies in mice found separate sets of preganglionic neurons located in different parts of the dorsal motor nucleus of the vagus projecting onto nitrergic or cholinergic LES EMN (14), providing an anatomic base for the physiological differences observed. Otherwise, synaptic transmission in the ganglia of the autonomic nervous system is classically described as me-
diated by ACh acting on nAChRs; however, ganglionic neurotransmission in the esophagus can include important non-cholinergic and also noncholinergic synaptic responses. On the one hand, classic studies on the opossum LES found that muscarinic (M1) and serotoninergic (5-HT3) receptors also mediate vagal inputs to inhibitory EMN (12, 26). On the other hand, recent studies (10, 11) on animal models found that most fast excitatory postsynaptic potentials in the MP are mediated by a slight contribution of ACh acting on nAChRs and a strong contribution of ATP acting on purine P2X receptors or 5-HT acting on 5-HT3 receptors. The relevance of each of these alternative mechanisms of ganglionic neurotransmission can vary along the gastrointestinal tract, and their role within neurotransmission to excitatory esophageal EMN needs further investigation.

Our results suggest that whereas vagal fibers or interneurons could easily and efficiently stimulate inhibitory EMN through nAChRs in human LES and EB, full stimulation of intrinsic excitatory EMN requires other neurotransmitters or other circuits. Little is known about the anatomy and physiology of the neuronal circuitry that directly controls the smooth muscle of the esophagus. Understanding these circuits could improve our current pharmacological approach to transient LES relaxations and gastroesophageal reflux disease and also our current treatments for primary and secondary esophageal motor disorders. Further studies are needed to describe these neuronal circuits and to assess their relevance in health and disease.

ACKNOWLEDGMENTS

The authors thank Drs. Sans, Martí-Ragó, Moreno, and Llatjos (Hospital de Bellvitge), Dr. Rius (Hospital de Sant Pau), Dr. Fernández-Llamazares (Hospital Can Ruti), Drs. Sánchez-Ortega and Navarro (Hospital del Mar), Drs. Bianchi and Sutò (Hospital de Mataró), and Drs. Rodríguez-Santiago and Martí-Gallostra (Hospital Mutua de Tarrasa) for providing human tissue; and the Fundación Vila-romà (Hospital Can Ruti), Drs. Sans, Martí-Ragó, Moreno, and Llatjos (Hospital de Bellvitge), and Drs. Sañez and Jane Lewis for reviewing the manuscript. This study was presented, in part, at the 2000 Annual meeting of the American Gastroenterological Association in San Diego, CA and at the VIII Little-Brain Big-Brain Meeting in S´Agaró, Girona, Spain, 1–5 October, 2003.

GRANTS

This work was supported, in part, by Grants 98/0794 and 2002-P1020662 from the Fondo de Investigaciones Sanitarias of Spain, by Grant 2001-SGR 00214 from the Direcció General de Recerca of the Generalitat de Catalunya, by a grant from the Fundació de Gastroenterologia Dr Francisco Villarreal, by the Fundació Jaume Esperalba i Terrades del Consorci Sanitari del Maresme, and by the Fundació Institut Gottmann.

REFERENCES

G306

IN VITRO MOTOR RESPONSES TO NICOTINE IN THE HUMAN ESOPHAGUS


31. Preiksaitis HG, Tremblay L, and Diamant NE. *G306 IN VITRO MOTOR RESPONSES TO NICOTINE IN THE HUMAN ESOPHAGUS* Preiksaitis HG, Tremblay L, and Diamant NE.

32. Rattan S and Goyal RK. *G306 IN VITRO MOTOR RESPONSES TO NICOTINE IN THE HUMAN ESOPHAGUS* Rattan S and Goyal RK.


34. Schneider DA, Perrone M, and Galligan JJ. *G306 IN VITRO MOTOR RESPONSES TO NICOTINE IN THE HUMAN ESOPHAGUS* Schneider DA, Perrone M, and Galligan JJ.


