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

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

González, Asensio A., Ricardo Farré, and Pere Clave. 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 motor neurons (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 N'G-nitro-L-arghine (l-NNA; 100 μM), and after 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 ± 3.19 and 9.20 ± 5.51% of that induced by EFS. EFS elicited a contraction in longitudinal strips; after 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 small number of 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 motor neurons 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...
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 Clínic 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 distal to the line 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 using isometric force transducers and recorded on a chart recorder (model 03 force transducer and model 7 series polygraph; Grass Instruments, Quincy, MA). In each experiment, up to six strips from the same specimen were simultaneously studied. After an equilibration period of 1 h, strips were stretched to 150% of their initial length and placed between two parallel platinum wire electrodes 10 mm apart (41, 43). Thereafter, the tension of most strips taken from the GEJ progressively increased over the following 1–2 h. This increase in tension was defined as the active resting tone (43).

Experimental design. The influence of EMN on active LES resting tone was studied by measuring the changes in tension of LES strips after exposure to TTX (5 μM; Sigma, St. Louis, MO), to hexamethonium (HEX; 100 μM, Sigma), to N⁶-nitro-L-arginine (l-NNA; 100 μM, Sigma), to atropine (3 μM; Braun Medical, Barcelona, Spain). The myogenic contribution was evaluated after 10 min of exposure to a Krebs calcium-free buffer (14). The concentrations of TTX and l-NNA had previously been shown to abolish maximal EFS-induced relaxation (12–14). The effect of these antagonists was evaluated 30 min after their being added to the tissue bath. Responses of circular LES and circular and longitudinal EB strips to EFS and nicotine were assessed (1) at basal conditions, (2) during nitric blockade by l-NNA (100 μM, and 3) during simultaneous blockade by l-NNA and apamin (1 μM; Sigma). The concentration of apamin was the same as that which abolished the relaxation induced by exogenously ATP (10 mM) or pituitary adenylate cyclase-activating peptide (PACAP; 10 μM) in porcine LES in our previous study (8).

EFS. EFS was applied by means of an electrical stimulator (model S88; Grass Instruments) and a power booster (Stimu-Splitter II; Med-Lab Instruments, Loveland, CO) to obtain four identical and undistorted signals. Pulses of 0.4-ms duration and 0.3- to 70-Hz frequency were delivered in 5-s trains using supramaximally effective voltage previously set at 26 V (5, 41). LES and EB responses to EFS were characterized using TTX (5 μM), HEX (100 μM), or atropine (3 μM). The concentration of atropine was the same as that which had abolished the response of LES strips to carbachol (10 μM; Sigma) in our previous studies on human LES (13).

Stimulation of nAChRs. The effect of nicotine (Sigma) was evaluated by exposing the strips to the drug (2–100 μM) for 2 min. After the strips were washed with 100 ml of fresh buffer, there was a 30-min period before the next exposure. Repetitive additions of nicotine (20 μM) did not result in desensitization of nAChRs (not shown). LES and EB responses to nicotine (100 μM) were characterized by HEX, TTX (10 μM), or atropine. The effect of nicotine was studied on circular LES and EB strips at rest, on circular EB strips precontracted with carbachol (2 μM), and on longitudinal EB strips.

Data analysis. The effect of EFS or pharmacological agents was determined in terms of changes in tone. Relaxation was expressed in grams and/or in the percentage of active LES resting tone or carbachol-induced tone in EB strips. Contraction was expressed in grams.

The numbers of experiments were represented as either number of strips (n) or number of specimens (N). Values were expressed as means ± SE. Student’s t-test was selected for comparisons, using the paired mode when appropriate. Statistical significance was accepted if P < 0.05.

RESULTS

Control of LES resting tone and effect of esfs and nicotine on LES strips. Seventy-five strips, taken from the GEJ of 17 specimens that developed active tone and relaxed on EFS, were considered as pertaining to the LES. Mean resting tone was 3.6 ± 0.36 g. Both TTX (5 μM) and l-NNA (100 μM) significantly increased (P < 0.01) LES resting tone by 13.43 ± 2.47 (n = 11, N = 6) and 21.54 ± 2.9% (n = 20, N = 9), respectively. Neither HEX (100 μM; n = 6, N = 4) nor atropine (3 μM; n = 5, N = 4) significantly affected resting tone. Atropine also failed to significantly modify LES resting tone following TTX (−2.20 ± 0.35%; n = 4, N = 2) or l-NNA, (−0.76 ± 0.23%; n = 4, N = 2). Exposure of the LES strips to a Krebs calcium-free buffer for 10 min significantly decreased resting tone by 47.5 ± 4.8% (n = 8, N = 4).

At basal conditions, EFS induced an isolated “on” relaxation during stimulus in 35 of the 75 LES strips and an on relaxation followed by an “off” contraction at the end of the stimulus in 40 (Fig. 1A). When strips from the same specimen were compared, LES strips that showed biphasic responses to EFS had always been obtained from higher parts of the LES than the strips that showed only an isolated on relaxation (n = 16, N = 8). The amplitude of both on and off responses was frequency dependent. Maximal relaxation in lower 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 apamin, EFS induced a strong cholinoergic on contraction (6.28 ± 1.65 g at 10 Hz, n =
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. 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).

Fig. 3. Effect of the neurotoxin TTX, the ganglionic blocker hexamethonium (HEX), and ATR on EFS (5 Hz) or NIC-induced LES relaxation (#, **, *P < 0.05 vs. control; #blockade, #inhibition, #increase). HEX did not affect EFS relaxation (n = 5, N = 3) and fully blocked NIC-induced relaxation (n = 3, N = 2). TTX fully blocked EFS-induced relaxation (n = 5, N = 3); in contrast, relaxation induced by NIC was significantly reduced but not abolished by TTX (n = 4, N = 2), suggesting the presence of somatodendritic as well as nerve terminal nicotinic acetylcholine receptors (nAChRs) in nitric enteric motoneurons (EMN). Atropine significantly enhanced EFS-induced relaxation (10 Hz, n = 5, N = 3) and, in contrast, significantly inhibited NIC-induced relaxation (n = 5, N = 3).

38 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
Longitudinal EB strips did not develop active resting tone. EFS induced a frequency-dependent contraction on longitudinal EB strips (Fig. 5D) reaching 14.03 ± 2.52 g at 70 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 showing intense stimulation of excitatory EMN, and in the same experimental conditions, stimulation of nAChRs still induced a slight relaxation. B: during simultaneous blockade with L-NNA and APA, EFS also induced a strong contraction and NIC induced a slight 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 esophag-
contractions or preganglionic during stimulus without direct activation of muscle (5, 35, 41, rather than at peripheral sites. induced fall in LES pressure in humans occurs in the CNS Altogether, these cross the blood-brain barrier, did not alter LES pressure (22). hyoscine butylbromide, a muscarinic antagonist that does not atropine reduced LES pressure in healthy humans (7, 24), and intrinsic cholinergic contribution to LES tone in vitro, agreeing with previous in vitro studies on the human LES (30). In vivo, EMN continuously inhibits the myogenic LES resting tone. The lack of effect of atropine suggests the absence of an apamin-sensitive neurotransmitter in the human esophagus. The human LES is constituted by the sling muscle on the angle of Hiss 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 mainly 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 intrasphinchnic 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

Fig. 6. Frequency-response curves of EB strips. Circular EB strips: A: EFS-induced off contractions (n = 24, N = 9) were of higher amplitude than on contractions (n = 19, N = 9). On contractions were fully cholinergic (n = 5, N = 4), whereas off contractions were only partly inhibited by ATR (n = 5, N = 4). B: EFS induced on contractions alone in the presence of L-NNA that were fully blocked by ATR (n = 6, N = 4). Longitudinal EB strips: C: EFS-induced on contractions that were antagonized but not blocked by ATR (n = 6, N = 3).

Fig. 7. Quantitative effects of stimulation of EB EMN by EFS or N. At basal conditions (A and B, left), EFS caused an off contraction; in contrast, NIC did not induce any effect. A: after nitricergic blockade, EFS induced a strong cholinergic on contraction showing intense stimulation of excitatory EMN, and in the same experimental conditions, stimulation of nAChRs did not induce any effect. B: during simultaneous blockade with L-NNA and APA, EFS also induced a strong contraction and stimulation of nAChRs, a weak cholinergic contraction amounting to 9.20% of that induced by EFS and suggesting a less efficient stimulation of excitatory EB EMN. C: EFS also induced an on contraction in longitudinal EB strips at rest and also in those pretreated by L-NNA and APA, NIC did not induce any effect on longitudinal strips in both experimental settings.
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 cuneoneurotransmitter, 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 (30) 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-
iated by ACh acting on nAChRs; however, ganglionic neurotransmission in the esophagus can include important noncholinergic 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 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-Ràgul, 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 Sotlo (Hospital de Mataro), and Drs. Rodríguez-Santiago and Martí-Gallostra (Hospital Mutua de Terrassa) for providing human tissue; and Drs. López-Nadiv and Caballero (Hospital de Sant Pau) and Drs. Manyalich and Net (Hospital Clínic i Provincial) for coordination during organ donor procedures. The authors also thank Emma Martínez for technical support and Prof. Marcel Jiménez and Jane Lewis for reviewing the manuscript.

This study was presented, in part, at the 2000 Annual meeting of the American Gastrointestinal 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 de Spain, by Grant 2001SGR 00214 from the Direcció General de Recerca of the Generalitat of Catalonia, by a grant from the Fundación de Gastroenterología Dr Francisco Villardell, by the Fundación Jaume Esperalba i Terrades del Consorci Sanitari del Maresme, and by the Fundació Institut Guttmann.

REFERENCES


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

32. Rattan S and Goyal RK. *32. Rattan S and Goyal RK.*

33. Rattan S and Goyal RK. *33. Rattan S and Goyal RK.*


