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Esophageal-gastric relaxation reflex in rat: dual control of peripheral nitrergic and cholinergic transmission

Gerlinda E. Hermann, R. Alberto Travagli, and Richard C. Rogers

Department of Neuroscience, Pennington Biomedical Research Center-Louisiana State University System, Baton Rouge, Louisiana

Abstract

It has long been known that the esophageal distension produced by swallowing elicits a powerful proximal gastric relaxation. Gastroinhibitory control by the esophagus involves neural pathways from esophageal distension-sensitive neurons in the nucleus tractus solitarius centralis (cNTS) with connections to virtually all levels of the dorsal motor nucleus of the vagus (DMV). We have shown recently that cNTS responses are excitatory and primarily involve tyrosine hydroxylase-immunoreactive cells, whereas the DMV response involves both an α_1 excitatory and an α_2 inhibitory response. In the present study, using an esophageal balloon distension to evoke gastric relaxation (esophageal-gastric reflex, EGR), we investigated the peripheral pharmacological basis responsible for this reflex. Systemic administration of atropine methyl nitrate reduced the amplitude of the gastric relaxation to $52.0 \pm 4.4\%$ of the original EGR, whereas N^G -nitro-L-arginine methyl ester (L-NAME) reduced it to $26.3 \pm 7.2\%$ of the original EGR. Concomitant administration of atropine methyl nitrate and L-NAME reduced the amplitude of the gastric relaxation to $4.0 \pm 2.5\%$ of control. This reduction in the amplitude of induced EGR is quite comparable ($4.3 \pm 2.6\%$) to that seen when the animal was pretreated with the nicotinic ganglionic blocker hexamethonium. In the presence of bethanechol, the amplitude of the esophageal distension-induced gastric relaxation was increased to $177.0 \pm 10.0\%$ of control; administration of L-NAME reduced this amplitude to $19.9 \pm 9.5\%$. Our data provide a clear demonstration that the gastroinhibitory control by the esophagus is mediated via a dual vagal innervation consisting of inhibitory nitrergic and excitatory cholinergic transmission.

Keywords

receptive relaxation reflex; nonadrenergic; noncholinergic; vagovagal reflex

The general hypothesis of a vagovagal reflex connecting vagal afferent input, brain stem interneurons, and vagal efferent projections to the stomach was formulated some years ago (18,20,22,35,36,42,53,54). Although early models are certainly an oversimplification, they still explain many of the salient features of brain stem reflex control of the stomach: reflex action is initiated by stimulation of sensory vagal afferent pathways that enter the brain stem via the solitary tract and terminate primarily in the medial divisions of the nucleus of the solitary tract (NTS). These NTS neurons make excitatory and/or inhibitory synaptic connections with efferent cholinergic preganglionic neurons of the dorsal motor nucleus of the vagus (DMV). DMV neurons then project to excitatory cholinergic or to inhibitory nonadrenergic, noncholinergic (NANC) postganglionic neurons of the stomach and complete the loop.

Although the general scheme of vagovagal reflex control of the stomach is accepted, there are only a few examples in which the connectional and neuropharmacological details of a specific circuit approach completeness. The esophageal distension-gastric relaxation reflex (EGR), or “receptive relaxation reflex” first described by Cannon and Leib (10), is a possible exception. Vagal distension-sensitive afferents in the esophagus of the rat (19,45) project to a highly circumscribed region of the NTS, the subnucleus centralis (cNTS) (2,7,8,23,29,30,39,41). The cNTS is composed of neurons with two clearly defined neurochemical phenotypes: a dense “core” with nitric oxide synthase-immunoreactive (NOS-IR) neurons and an outer thin “shell” of neurons that contain tyrosine hydroxylase (TH-IR; i.e., catecholaminergic neurons) (41). We showed recently that repetitive distension of the esophagus primarily activates the TH-IR neurons in the cNTS, whereas NOS-IR-positive neurons are unresponsive (41).

Using in vivo electrophysiological recordings, we showed (39) that esophageal distension produced activation of cNTS neurons exclusively (e.g., Fig. 1); that is, there were no inhibitory responses. These NTS neurons project to virtually all parts of the DMV, the nucleus ambiguus, and other subdivisions of the NTS, as well as subjacent areas involved in control of the oral cavity and pharynx such as the reticular formation and the area postrema (55,56). Our neurophysiological studies also showed that esophageal distension tends to activate those DMV neurons in areas containing presumptive preganglionic efferent projections to NANC neurons, whereas DMV neurons located in areas likely to control cholinergic excitation of the stomach were inhibited by esophageal distension (39). We also showed that the EGR is partially blocked by either an α_1 - or an α_2 -adrenoceptor antagonist application onto the floor of the fourth ventricle (i.e., onto the area postrema and solitary nucleus) and is nearly eliminated by their combination. In contrast, antagonism of NOS, GABA, or β -adrenoceptor actions did not affect the reflex (39,41). In vitro neurophysiological studies (17,31) on gastric DMV neurons have shown that these neurons may be either activated or inhibited by norepinephrine via α_1 - or α_2 -adrenoceptors, respectively. Parallel studies (16,37) show that brain stem reflex regulation of gastric function may be dependent on noradrenergic neurons in the NTS.

Together, the data available in the literature suggest that esophageal distension activates catecholaminergic neurons of cNTS that, in turn, activate, via α_1 -adrenoceptors, preganglionic DMV neurons projecting to postganglionic NANC cells and inhibit, via α_2 -adrenoceptors, preganglionic DMV cells that control postganglionic cholinergic neurons.

Although the general central nervous system (CNS) organization of vagal reflex control of the stomach is accepted (see Fig. 1), there is still considerable debate concerning the relative importance of either activation of the NANC vs. withdrawal of excitatory cholinergic vagal pathways in the expression of EGR. For example, observations from in vivo neurophysiological studies (33) estimate that ~90% of DMV neurons are inhibited by afferent signals that would normally cause gastric inhibition, e.g., gastric/duodenal distension. This finding supports the view that the dominant gastroinhibitory mechanism involves reflex withdrawal of excitatory cholinergic input to the stomach. In contrast, pharmacological studies of the same reflex mechanisms have shown that reflex activation of inhibitory vagal NANC pathways are dominant (50,51). This obvious disparity in attributing relative roles of these pathways in generating this reflex is probably due to the technical weaknesses inherent in either approach. Neurophysiological approaches may overestimate the importance of cholinergic withdrawal due to sampling errors (38). Pharmacological approaches can easily detect the presence of an active inhibitory process but have difficulty in detecting the reduction in a tonic excitation (38). We recently devised a method for studying gastric reflex mechanisms that eliminates some of these biases. By combining highly sensitive strain gauge recording methods with mild gastric fluid preloading, we are

able to use relatively simple pharmacological techniques to parse the relative significance of the two vagal control pathways in the control of gastroinhibition. The aims of present study were to 1) test the hypothesis that the receptive relaxation reflex is mediated by simultaneous inhibition of excitatory cholinergic and activation of inhibitory NANC inputs to the stomach and 2) assess the relative contribution of these pathways to the overall reflex.

METHODS

All experimental protocols were performed according to the guidelines set forth by the National Institutes of Health and were approved by the Institutional Animal Care and Use Committees at the Pennington Biomedical Research Center.

Drugs and chemicals

Animals were anesthetized with thiobutabarbital (Inactin; Sigma, St Louis, MO; 150–200 mg/kg ip). This long-term anesthetic has been shown not to interfere with brain stem autonomic reflexes (9). The following drugs were used to suppress the expression of the EGR: the nonselective muscarinic receptor blocker atropine methyl nitrate, the NOS inhibitor *N*^G-nitro-L-arginine methyl ester (L-NAME), the nicotinic receptor blocker hexamethonium, and the muscarinic receptor agonist bethanechol (used to maximally clamp the cholinergic drive of smooth muscle). These agonists and antagonists were purchased from Sigma.

Surgical preparation

Male Long-Evans rats (250- to 500-g body weight; Charles River Laboratories, Wilmington, MA; *n* = 35) were food deprived overnight (16 h) and anesthetized with thiobutabarbital. An adequate depth of anesthesia was assessed by the absence of the foot pinch withdrawal reflex. Body temperature was monitored with a rectal thermometer and maintained at $37 \pm 1^\circ\text{C}$ with a heating pad. Once anesthetized, rats were fitted with a jugular cannula for the systemic delivery of drugs and a tracheal catheter to aid in the maintenance of a clear airway. A midline abdominal incision was then made exposing the stomach and proximal duodenum. A 1-mm inner diameter silicon catheter was inserted through the pylorus and into the lumen of the stomach. The catheter was placed via a small incision in the duodenum 1–2 cm distal to the pylorus and secured in place by a ligature around the catheter.

A miniature strain gauge (RB, Madison, WI) was then aligned with circular smooth muscle fibers and sutured to the fundus. Strain gauge leads and the catheter exited through the midline incision, which was then closed with a suture. Strain gauge leads were then connected to a bridge amplifier and, in turn, to a polygraph and IBM personal computer-based data logger (Run Technologies Data Pac 2000) for the recording of changes in fundic tone. Note that the recorded magnitude of the esophageal-induced fundic relaxation can be influenced by such factors as the size of the animal, size of the stomach, size of the strain gauge, and placement of the strain gauge on the fundus. There can be slight variations in the induced EGR between individual animals; therefore, each animal served as its own control for purposes of comparing the effects of peripheral pharmacological manipulations (i.e., basal vs. drug treatment EGR).

An esophageal distension balloon was constructed from a 1.5-cm length of 1-mm outer diameter, 0.5-mm inner diameter silicone tubing (AM Systems, Seattle, WA) that was stretched to increase its compliance. This distension balloon was attached to noncompliant polyethylene tubing (PE-50). The tubing was filled with water and attached to a 1-ml syringe, also filled with water. The balloon was placed orally in the esophagus such that the tip was located 1 cm above the esophageal hiatus at the level of the thoracic esophagus.

Injecting water to a final expanded volume of 160 μ l modestly distends the esophageal balloon. Our previous studies (39,41) showed that this distension produced a transmural pressure increase of ~14 mmHg. This degree of pressure activates vagal mechanosensors but not spinal nociceptors (45,46). Surgically prepared anesthetized rats were mounted in the stereotaxic frame.

Experimental design

Gastric motility was monitored throughout duration of the experiment. After a 1-h postsurgical recovery period, we gradually infused 1 ml of PBS into the stomach to provide a mild (~0.5-g strain) preload on the fundus. After 5 min of acclimation to gastric preload, the esophageal distension balloon was filled to a volume of 160 μ l and held for 1 min; fundic tone was monitored continuously. Five minutes after esophageal stimulation, the gastric preload was relieved. This first elicitation of the EGR served as a baseline for subsequent comparisons.

One hour later, rats received one of the following intravenous drug treatments: 1) PBS (isotonic PBS, 1 ml/kg; served as injection volume and time control; $n = 5$), 2) L-NAME [10 mg/kg (50), NOS inhibitor; $n = 7$], 3) atropine methyl nitrate [50 μ g/kg iv vagolytic dose (34,50); nonselective muscarinic antagonist; $n = 4$], 4) atropine methyl nitrate plus L-NAME (antagonized both the cholinergic and nitrergic peripheral pathways; $n = 5$), 5) hexamethonium [20 mg/kg iv (50); nicotinic ganglionic blocker; $n = 4$], 6) bethanechol [50 μ g/kg iv (11); nonselective muscarinic agonist; $n = 5$], or 7) bethanechol plus L-NAME ($n = 5$). In each instance, the intravenous drug treatment preceded the second gastric preload by 10 min; preload and esophageal distension were then repeated as described above. In most cases, intravenous drug treatment had a transient effect on baseline fundic tone. Before the EGR was evoked, the gastric preload (1 ml of PBS injected into the stomach) was applied and the stomach was allowed to acclimate to this load (5 min). By the time the EGR was elicited, baseline gastric tone was not significantly different from that under the basal (i.e., predrug) condition. The only exception to this scenario was with intravenous bethanechol. This cholinergic agonist noticeably increased gastric tone, and this maximal peripheral cholinergic stimulation was maintained. Indeed, this increase in tone provoked by bethanechol provided an even greater background against which to elicit the EGR. Thus, in this case, the continued demonstration of the EGR truly speaks to the contribution of the nitrergic pathway in maintaining this reflex.

Data analysis

The change in fundic tone elicited by the esophageal distension was evaluated by measuring the difference in tone before and during esophageal distension. Esophageal distension-induced reductions in fundic tone and the duration of the reflex effect were compared between the first (basal) and second (experimental) periods, i.e., after intravenous drug treatment. Changes in the magnitude of the EGR-induced reduction in tone were normalized for each animal by dividing the magnitude of the induced response in the experimental condition by the magnitude of the induced response in the basal condition and multiplied by 100. This number represents the effects of peripheral interference on the elicited EGR; that is, a fundic relaxation index of “100%” is indicative of no change to the control reflex due to the drug, whereas an index of “50%” indicates a loss in the relaxation reflex by 50%. Thus each animal served as its own control, and the percentage of change (basal vs. experimental responses) was compared across each of the seven treatment groups.

Percent differences between the basal and experimental condition reflexes elicited in each group were evaluated using one-way analysis of variance (ANOVA) across all treatment groups followed by Dunnett's post hoc test (i.e., intravenous PBS time and injection

control). To evaluate the relative contributions of the cholinergic versus the nitrergic efferent pathways, we performed a Bonferroni post hoc test for these two treatment groups. Data are expressed as means $\pm$ SE. *P* values <0.05 were considered statistically significant.

RESULTS

In the food-deprived animal, mild distension of the esophagus (i.e., fictive swallowing of a food bolus) induced a decrease in fundic tone, i.e., a receptive relaxation of the fundic portion of the stomach (5,6,10,23,36,39,41). This reflex can be elicited repeatedly without significant variations in the amplitude of the fundic relaxation (see “PBS” in Fig. 2.)

The magnitude of the fundic relaxation (EGR) following intravenous injection of PBS was $100.6 \pm 12.5\%$ ($n = 5$) of the basal EGR elicited in these animals (Figs. 2 and 3). Percent differences between the magnitudes of the basal vs. experimental condition reflexes elicited in each group were evaluated using one-way ANOVA across all treatment groups [$F_{(6,28)} = 58.71$; $P < 0.0001$]. Dunnett's post hoc tests (i.e., control group = intravenous PBS) indicated that the reflexes evoked after each treatment were significantly different from those generated after PBS intravenous pretreatment (Fig. 3; $*P < 0.001$). Systemic administration of atropine methyl nitrate reduced the amplitude of the gastric relaxation to $52.0 \pm 4.4\%$ ($n = 4$) of the original EGR, whereas L-NAME reduced it to $26.3 \pm 7.2\%$ ($n = 7$) of the original EGR. Concomitant administration of atropine methyl nitrate and L-NAME ($n = 5$) reduced the amplitude of the gastric relaxation to $4.0 \pm 2.5\%$ of control. This reduction in amplitude of induced EGR was quite comparable ($4.3 \pm 2.6\%$) to that seen when the animal was pretreated with the nicotinic ganglionic blocker hexamethonium. Thus withdrawal of cholinergic input to the stomach, via atropine methyl nitrate pretreatment, causes ~48% reduction in reflex size. Eliminating the nitrergic path with L-NAME causes ~74% reduction in EGR size (Fig. 3; $t = 2.22$, $P = <0.05$; Bonferroni post hoc test). The disproportional contribution of these two pathways is even more pronounced when one considers that the cholinergic portion makes up more than 90% of the efferent fibers of the DMV, whereas the NANC comprises ~5% of this efferent pathway.

In the presence of the muscarinic agonist bethanechol, the evoked EGR was significantly larger than the original EGR ($177.0 \pm 10.0\%$); administration of L-NAME reduced the magnitude of the gastric relaxation to $19.9 \pm 9.5\%$ ($n = 5$) of the original EGR. Data are summarized in Fig. 3.

DISCUSSION

Our previous studies (39,41) have established that the EGR is entirely vagally mediated in that central vagotomy eliminates this relaxation reflex. The purpose of this study was to resolve the relative contributions of the efferent vagal pathways involved in the EGR. The present study clearly demonstrates that the gastroinhibitory control by the esophagus is mediated via a dual vagal innervation consisting of inhibitory NANC-nitrergic and excitatory cholinergic transmission. Our data suggest that the inhibitory NANC component of the EGR accounts for ~60–70% of the fundic relaxation, whereas the remaining portion is attributable to withdrawal of the excitatory cholinergic tone.

Our conclusions are based on the following experimental results. Esophageal distension in rats pretreated with the non-selective muscarinic antagonist atropine methyl nitrate induced ~40% gastroinhibition of the reflex that could be elicited under control conditions. These data suggest that one portion of the EGR is attributable to the removal of an excitatory cholinergic input to the fundus and that an additional, noncholinergic (likely NANC), component also plays a relevant role in the EGR. Indeed, esophageal distension in rats

pretreated with the NOS inhibitor L-NAME induced a much reduced EGR, only ~25% of that evoked under control conditions. These data support the view that a large portion of the gastroinhibition/relaxation is attributable to activation of nitrergic inputs to the proximal stomach (51). Concomitant administration of atropine methyl nitrate and L-NAME essentially abolished the gastroinhibition induced by esophageal distension to the same degree as pretreatment with the selective ganglionic nicotinic antagonist hexamethonium. These data indicate that the site of action of the effects of atropine methyl nitrate and L-NAME are most likely at the postganglionic level and that the EGR is a purely parasympathetic/vagal reflex (39,44,51,57).

Despite the fact that the agonists and antagonists in this study were administered systemically, the possibility exists that their responses are elicited via centrally mediated effects. However, previous work has shown (21,39,41,49) that the central neuropharmacological components of the EGR circuit do not utilize nitrergic or cholinergic transmission. Therefore, we are confident in our interpretation that the site of action of L-NAME (as well as atropine methyl nitrate) is peripheral.

Reports from recent studies on vasodilation and sweating (12,26) have suggested that there may be an interaction between L-NAME and muscarinic receptors such that L-NAME may act as a cholinergic antagonist. However, in our studies, we used an intravenous dose of atropine methyl nitrate that is slightly higher than the frequently cited intravenous vagolytic dose of 40 $\mu\text{g/kg}$ (34,50,51). Thus any further suppressive effect of L-NAME that we observed in addition to atropine methyl nitrate was probably an effect to block nitrergic effects of the EGR.

The involvement of the nitrergic, NANC pathway is further supported by the experiments conducted in the presence of the nonselective muscarinic agonist bethanechol. Systemic administration of bethanechol (50 $\mu\text{g/kg}$) maximally activates muscarinic receptors in gastric smooth muscle. Under these conditions, a reflex-induced withdrawal of cholinergic input to gastric smooth muscle will have no effect, because muscarinic receptors are maximally occupied and active. Therefore, any reflex-induced relaxation observed under these circumstances must, by definition, be due to an active, NANC-induced inhibition. Clearly, the use of the NOS inhibitor L-NAME verified this hypothesis by nearly eliminating the evoked EGR. In addition, the evoked EGR was significantly increased with the bethanechol treatment [i.e., muscarinic (cholinergic) receptors were maximally occupied]. These data demonstrate that another pathway other than cholinergic withdrawal plays a critical role in the EGR (28).

The present results combined with earlier observations allow us to construct an outline of the circuitry and function of the EGR. Low-threshold vagal afferent tension receptors in the esophagus are activated by mild esophageal distension (45). These afferent fibers, in turn, activate a subset of noradrenergic neurons in the cNTS (16,41). cNTS neurons project broadly throughout the DMV (39). Esophageal distension generally inhibits neurons in the medial DMV but activates those in the lateral and extreme caudal division of the DMV (39). The medial DMV is presumed to contain the neurons of origin for the excitatory projection to the stomach, whereas the lateral and caudal divisions have been suggested as the site of origin for the inhibitory projections (25,39). In vitro neurophysiological studies have shown that gastric-projecting DMV neurons are inhibited by norepinephrine via α_2 -adrenoceptors, whereas α_1 -adrenoceptors activate DMV cells (17,31). In vivo experiments have shown that the gastroinhibition induced by esophageal distension is antagonized partially by fourth ventricle administration of either α_1 - or α_2 -adrenoceptor antagonists, whereas concomitant administration of these agents blocks, almost completely, the EGR (41). Because it has long been acknowledged that essentially all vagal efferent preganglionic input to the gastric

enteric plexus is conveyed by acetylcholine release onto nicotinic receptors (44), the only possible scenario that explains the experimental data shown so far is that α_1 -adrenoceptor activation excites preganglionic DMV cells that are part of the NANC circuit and α_2 -adrenoceptor activation inhibits preganglionic DMV cells that are part of the cholinergic pathway. Indeed, the data presented herein provide the first functional demonstration in an in vivo model that the gastroinhibition occurring during the EGR is mediated by both pathways via the coordinated activation of the vagal gastroinhibitory NANC pathway and the inhibition of the excitatory cholinergic vagal path to the stomach.

Despite the overwhelming evidence that two pathways are involved in this reflex, i.e., an activation of the NANC pathway and withdrawal of the cholinergic pathway, a recent article by Ferreira et al. (15) from Gillis's laboratory states that the EGR is determined exclusively by the withdrawal of cholinergic tone induced by α_2 -adrenoceptor-mediated activation of DMV neurons and that the noncholinergic contribution to EGR is limited to activation of a nitrergic interneuron. Although these authors claim that "the specific purpose of the present study (Ferreira et al.) was to employ the same reflex-stimulating technique as Rogers et al. (41)...," comparison of the methods used in these two papers reveals that Ferreira et al. (15) did not use the same reflex-stimulating techniques as we did, nor did they investigate the esophageal-gastric reflex but, probably, a gastrogastic reflex. Thus the motility traces reported in Fig. 2 of the report by Ferreira et al. shows a transient increase in antral tone and motility during balloon distension. This type of response was first described by Andrews and colleagues (3,4) in the early 1980s in response to a proximal gastric distension. This reflex is the only known example of an excitatory gastrogastic reflex.

There are several other differences in methods and instrumentation used in the study by Ferreira et al. (15) vs. our study. First, Ferreira et al. used an esophageal balloon distension measuring 10 mm in diameter; our stimulation was an esophageal balloon with a distension diameter of ~2 mm. Second, Ferreira et al. used a stimulation volume of 700 μ l, whereas ours was 160 μ l. Note that according to work by Dong et al. (14), the physiological range of vagal afferents sensitive to distension-mediated esophageal stimuli is 100–300 μ l in volume. The upper end of this range is near noxious.

Third, Ferreira et al. (15) monitored antral gastric tone using an intragastric balloon inserted via the fundus. Intragastric balloons are poor choices for measuring gastric tone for two reasons. First, a balloon placed in the antrum activates contact sensors in the antrum (47), and this elicits an inhibitory gastric vagovagal reflex [i.e., gastrogastic reflex (1)]. Second, because the inflated balloon is almost always less compliant than the gastric wall, it will be difficult to observe relaxation of the gastric wall. Better systems to measure intragastric pressure without a balloon include direct insertion of a microtip pressure transducer as described by Takahashi and Owyang (50), use of a gastric barostat as described by Krowicki et al. (24), or use of a gastric wall strain gauge as we have described. Finally, use of chloral compounds (e.g., chloral hydrate or α -chloralose as used by Gillis's laboratory group) as an adjunct to urethane anesthesia is known to induce adynamic ileus (43,48). Thus the combined effects of α -chloralose anesthesia, balloon distension, and insensitive instrumentation all combine to provoke a background of profound gastroinhibition against which further reductions may be difficult to observe.

In contrast, we monitored the change in fundic (not antral) tone with the use of an extraluminal strain gauge secured to the circular musculature. These strain gauges are sensitive to changes in wall tensions between ~0.1 and 5.0 g. This corresponds to a range of intragastric pressures of 0 to 8 mmHg. Clearly, Gillis's laboratory is not studying the same reflexes or responses that we are.

Dual vagal innervation to the stomach is not a novel concept. In fact, studies from the last century to the present have shown that section of the vagus can have conflicting effects on the behavior of the stomach (5,42,45,46,50,51); for reviews, see also Refs. 38 and 52. The dichotomous effects of vagotomy (e.g., increased fundic tone and reduced antral motility) suggested to Pavlov that the vagus controlled gastric motility through both inhibitory and excitatory pathways. This differential function of vagal input to the stomach is reinforced by results obtained from vagal stimulation experiments. In the antrum, stimulation of the vagus leads to an increase in tone and contractility, whereas in the fundus, the result is often a profound relaxation (42). These results provided the foundation for the notion that vagal reflex control of the stomach, in general, and the EGR, in particular, involves the activation of a vagal inhibitory path simultaneous with the inhibition of a tonically active vagal excitatory path. A large array of anatomic and physiological studies suggest that a substantial portion of the NANC pathway's action to inhibit fundic tone is due to the release of NO onto gastric smooth muscle (6,13,27,32,50,51). Therefore, it comes as no surprise that we find that the gastroinhibition that occurs during the EGR of the food-deprived animal is mediated by both pathways via the coordinated activation of the vagal gastroinhibitory NANC pathway and the inhibition of the excitatory cholinergic vagal path to the stomach.

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References

1. Abrahamsson H. Studies on the inhibitory nervous control of gastric motility. *Acta Physiol Scand Suppl* 1973;390:1–38. [PubMed: 4521069]
2. Altschuler SM, Bao XM, Bieger D, Hopkins DA, Miselis RR. Viscerotopic representation of the upper alimentary tract in the rat: sensory ganglia and nuclei of the solitary and spinal trigeminal tracts. *J Comp Neurol* 1989;283:248–268. [PubMed: 2738198]
3. Andrews PL, Grundy D, Scratcherd T. Reflex excitation of antral motility induced by gastric distension in the ferret. *J Physiol* 1980;298:79–84. [PubMed: 7359444]
4. Andrews PL, Grundy D, Scratcherd T. Vagal afferent discharge from mechanoreceptors in different regions of the ferret stomach. *J Physiol* 1980;298:513–524. [PubMed: 7359436]
5. Behrns KE, Sarr MG. Diagnosis and management of gastric emptying disorders. *Adv Surg* 1994;27:233–255. [PubMed: 8140975]
6. Berthoud HR. Anatomical demonstration of vagal input to nicotinamide acetamide dinucleotide phosphate diaphorase-positive (nitroergic) neurons in rat fundic stomach. *J Comp Neurol* 1995;358:428–439. [PubMed: 7560296]
7. Bieger D. The brainstem esophagomotor network pattern generator: a rodent model. *Dysphagia* 1993;8:203–208. [PubMed: 8102966]
8. Bieger D. Rhombencephalic pathways and neurotransmitters controlling deglutition. *Am J Med* 2001;111(Suppl 8A):85S–89S. [PubMed: 11749931]
9. Buelke-Sam J, Holson JF, Bazare JJ, Young JF. Comparative stability of physiological parameters during sustained anesthesia in rats. *Lab Anim Sci* 1978;28:157–162. [PubMed: 642434]
10. Cannon WB, Leib CW. The receptive relaxation of the stomach. *Am J Physiol* 1911;29:267–273.
11. Cho CH, Qiu BS, Ballard HJ, Ogle CW. Adenosine and cholinergic-induced gastric contraction in rats. *Digestion* 1993;54:98–104. [PubMed: 8100543]

12. Dambrova M, Chlopicki S, Liepinsh E, Kirjanova O, Gorshkova O, Kozlovski VI, Uhlen S, Liepina I, Petrovska R, Kalvinsh I. The methylester of γ -butyrobetaine, but not γ -butyrobetaine itself, induces muscarinic receptor-dependent vasodilatation. *Naunyn Schmiedeberg Arch Pharmacol* 2004;369:533–539. [PubMed: 15060760]
13. Desai KM, Sessa WC, Vane JR. Involvement of nitric oxide in the reflex relaxation of the stomach to accommodate food or fluid. *Nature* 1991;351:477–479. [PubMed: 1675430]
14. Dong H, Loomis CW, Bieger D. Vagal afferent input determines the volume dependence of rat esophageal motility patterns. *Am J Physiol Gastrointest Liver Physiol* 2001;281:G44–G53. [PubMed: 11408254]
15. Ferreira M Jr, Sahibzada N, Shi M, Niedringhaus M, Wester MR, Jones AR, Verbalis JG, Gillis RA. Hindbrain chemical mediators of reflex-induced inhibition of gastric tone produced by esophageal distension and intravenous nicotine. *Am J Physiol Regul Integr Comp Physiol* 2005;289:R1482–R1495. [PubMed: 16051723]
16. Ferreira M Jr, Sahibzada N, Shi M, Panico W, Niedringhaus M, Wasserman A, Kellar KJ, Verbalis J, Gillis RA. CNS site of action and brainstem circuitry responsible for the intravenous effects of nicotine on gastric tone. *J Neurosci* 2002;22:2764–2779. [PubMed: 11923442]
17. Fukuda A, Minami T, Nabekura J, Oomura Y. The effects of noradrenaline on neurones in the rat dorsal motor nucleus of the vagus, in vitro. *J Physiol* 1987;393:213–231. [PubMed: 2895810]
18. Gillis, RA.; Quest, JA.; Pagani, FD.; Norman, WP. *Handbook of Physiology. The Gastrointestinal System. Motility and Circulation. Vol. I.* Bethesda, MD: Am Physiol Soc; 1989. Control centers in the central nervous system for regulating gastrointestinal motility; p. 621–683.sect. 6
19. Goyal RK, Padmanabhan R, Sang Q. Neural circuits in swallowing and abdominal vagal afferent-mediated lower esophageal sphincter relaxation. *Am J Med* 2001;111:95S–105S. [PubMed: 11749933]
20. Grundy, D.; Schemann, M. Motor control of the stomach. In: Brookes, S.; Costa, M., editors. *Innervation of the Gastrointestinal Tract.* London: Taylor and Francis; 2002. p. 57–102.
21. Hornby PJ. Receptors and transmission in the brain-gut axis. II. Excitatory amino acid receptors in the brain-gut axis. *Am J Physiol Gastrointest Liver Physiol* 2001;280:G1055–G1060. [PubMed: 11352796]
22. Jansson G. Vagovagal reflex relaxation of the stomach in the cat. *Acta Physiol Scand* 1969;75:245–252. [PubMed: 5785146]
23. Jean A. Brain stem control of swallowing: neuronal network and cellular mechanisms. *Physiol Rev* 2001;81:929–969. [PubMed: 11274347]
24. Krowicki ZK, Nathan NA, Hornby PJ. Gastric motor and cardiovascular effects of insulin in dorsal vagal complex of the rat. *Am J Physiol Gastrointest Liver Physiol* 1998;275:G964–G972.
25. Krowicki ZK, Sharkey KA, Serron SC, Nathan NA, Hornby PJ. Distribution of nitric oxide synthase in rat dorsal vagal complex and effects of microinjection of nitric oxide compounds upon gastric motor function. *J Comp Neurol* 1997;377:49–69. [PubMed: 8986872]
26. Lee K, Mack GW. Role of nitric oxide in methacholine-induced sweating and vasodilation in human skin. *J Appl Physiol.* October 20;2005 10.1152/japplphysiol.00122.2005
27. Lefebvre RA, Baert E, Barbier AJ. Influence of N^G -nitro-L-arginine on non-adrenergic non-cholinergic relaxation in the guinea-pig gastric fundus. *Br J Pharmacol* 1992;106:173–179. [PubMed: 1504726]
28. Lewis MW, Hermann GE, Rogers RC, Travagli RA. In vitro and in vivo analysis of the effects of corticotropin releasing factor on rat dorsal vagal complex. *J Physiol* 2002;543:135–146. [PubMed: 12181286]
29. Lu WY, Bieger D. Vagal afferent transmission in the NTS mediating reflex responses of the rat esophagus. *Am J Physiol Regul Integr Comp Physiol* 1998;274:R1436–R1445.
30. Lu WY, Bieger D. Vagovagal reflex motility patterns of the rat esophagus. *Am J Physiol Regul Integr Comp Physiol* 1998;274:R1425–R1435.
31. Martinez-Pena y Valenzuela I, Rogers RC, Hermann GE, Travagli RA. Norepinephrine effects on identified neurons of the rat dorsal motor nucleus of the vagus. *Am J Physiol Gastrointest Liver Physiol* 2004;286:G333–G339. [PubMed: 12936913]

32. Mashimo H, Kjellin A, Goyal RK. Gastric stasis in neuronal nitric oxide synthase-deficient knockout mice. *Gastroenterology* 2000;119:766–773. [PubMed: 10982771]
33. McCann MJ, Rogers RC. Impact of antral mechanoreceptor activation on the vagovagal reflex in the rat: functional zonation of responses. *J Physiol* 1992;453:401–411. [PubMed: 1464835]
34. Millard, P. Anesthetic procedures. In: Aspinall, V., editor. *Clinical Procedures in Veterinary Nursing*. 2. London: Elsevier; 2003. p. 121-155.
35. Miolan JP, Roman C. Discharge of efferent vagal fibers supplying gastric antrum: indirect study by nerve suture technique. *Am J Physiol Endocrinol Metab Gastrointest Physiol* 1978;235:E366–E373.
36. Miolan JP, Roman C. The role of oesophageal and intestinal receptors in the control of gastric motility. *J Auton Nerv Syst* 1984;10:235–241. [PubMed: 6481090]
37. Rinaman L. Hindbrain noradrenergic lesions attenuate anorexia and alter central cFos expression in rats after gastric viscerosensory stimulation. *J Neurosci* 2003;23:10084–10092. [PubMed: 14602823]
38. Rogers, RC.; Hermann, GE.; Travagli, RA. Brainstem control of gastric function. In: Johnson, LR., editor. *Physiology of the Gastrointestinal Tract*. 4. New York: Elsevier; 2005.
39. Rogers RC, Hermann GE, Travagli RA. Brainstem pathways responsible for oesophageal control of gastric motility and tone in the rat. *J Physiol* 1999;514:369–383. [PubMed: 9852320]
40. Rogers RC, Travagli RA, Hermann GE. Brainstem catecholaminergic neurons are activated during the esophageal-gastric receptive relaxation reflex (Abstract). *FASEB J* 2003;17:605.3.
41. Rogers RC, Travagli RA, Hermann GE. Noradrenergic neurons in the rat solitary nucleus participate in the esophageal-gastric relaxation reflex. *Am J Physiol Regul Integr Comp Physiol* 2003;285:R479–R489. [PubMed: 12714355]
42. Roman, C.; Gonella, J. Extrinsic control of digestive tract motility. In: Johnson, LR., editor. *Physiology of the Gastrointestinal Tract*. 2. New York: Raven; 1987. p. 507-553.
43. Sababi M, Nylander O. Comparative study of the effects of nitric oxide synthase and cyclo-oxygenase inhibition on duodenal functions in rats anaesthetized with Inactin, urethane or α -chloralose. *Acta Physiol Scand* 1996;158:45–52. [PubMed: 8876747]
44. Schemann M, Grundy D. Electrophysiological identification of vagally innervated enteric neurons in guinea pig stomach. *Am J Physiol Gastrointest Liver Physiol* 1992;263:G709–G718.
45. Sengupta JN. An overview of esophageal sensory receptors. *Am J Med* 2000;108(Suppl 4a):87S–89S. [PubMed: 10718458]
46. Sengupta JN, Kauvar D, Goyal RK. Characteristics of vagal esophageal tension-sensitive afferent fibers in the opossum. *J Neurophysiol* 1989;61:1001–1010. [PubMed: 2723726]
47. Sengupta JN, Petersen J, Peles S, Shaker R. Response properties of antral mechanosensitive afferent fibers and effects of ionotropic glutamate receptor antagonists. *Neuroscience* 2004;125:711–723. [PubMed: 15099685]
48. Silverman J, Muir WW III. A review of laboratory animal anesthesia with chloral hydrate and chloralose. *Lab Anim Sci* 1993;43:210–216. [PubMed: 8355479]
49. Smith BN, Dou P, Barber WD, Dudek FE. Vagally evoked synaptic currents in the immature rat nucleus tractus solitarius in an intact in vitro preparation. *J Physiol* 1998;512:149–162. [PubMed: 9729625]
50. Takahashi T, Owyang C. Characterization of vagal pathways mediating gastric accommodation reflex in rats. *J Physiol* 1997;504:479–488. [PubMed: 9365919]
51. Takahashi T, Owyang C. Vagal control of nitric oxide and vasoactive intestinal polypeptide release in the regulation of gastric relaxation in rat. *J Physiol* 1995;484:481–492. [PubMed: 7602539]
52. Travagli RA, Hermann GE, Browning KN, Rogers RC. Brainstem circuits regulating gastric function. *Annu Rev Physiol* 2005;68:279–305. [PubMed: 16460274]
53. Travagli RA, Hermann GE, Browning KN, Rogers RC. Musings on the wanderer: what's new in our understanding of vagovagal reflexes? III. Activity-dependent plasticity in vagovagal reflexes controlling the stomach. *Am J Physiol Gastrointest Liver Physiol* 2003;284:G180–G187. [PubMed: 12529266]

54. Travagli RA, Rogers RC. Receptors and transmission in the brain-gut axis: potential for novel therapies. V. Fast and slow extrinsic modulation of dorsal vagal complex circuits. *Am J Physiol Gastrointest Liver Physiol* 2001;281:G595–G601. [PubMed: 11518671]
55. Travers JB, Dinardo LA, Karimnamazi H. Motor and premotor mechanisms of licking. *Neurosci Biobehav Rev* 1997;21:631–647. [PubMed: 9353796]
56. Travers SP, Norgren R. Organization of orosensory responses in the nucleus of the solitary tract of rat. *J Neurophysiol* 1995;73:2144–2162. [PubMed: 7666129]
57. Zheng ZL, Rogers RC, Travagli RA. Selective gastric projections of nitric oxide synthase containing vagal brainstem neurones. *Neuroscience* 1999;90:685–694. [PubMed: 10215170]

Fig. 1.

Schematic drawing illustrating proposed neurocircuitry of vagovagal reflex responsible for the esophageal-gastric relaxation reflex (EGR). Afferent activation (e.g., esophageal distension as during swallowing of bolus of food) causes second-order noradrenergic neurons of the nucleus tractus solitarii (NTS) to inhibit cholinergic tone of some neurons of the dorsal motor nucleus of the vagus (DMV) (via α_2 -adrenergic receptors) as well as activate other DMV neurons (via α_1 -adrenergic receptors) (40). DMV neurons inhibited by NTS input are tonically active and control cholinergic inputs (striped cells) to gastric smooth muscle. DMV neurons excite nonadrenergic, noncholinergic (NANC) neurons (solid cells), causing a direct inhibition of gastric smooth muscle through release of NO, VIP, and/or adenosine. This dual withdrawal of cholinergic tone and activation of an inhibitory pathway (NANC) results in relaxation of the fundic stomach in preparation for receiving incoming foodstuff. NE, norepinephrine. [Neurophysiological data of the NTS and DMV neurons responding to esophageal distension are modified from Rogers et al. (39).]

Fig. 2.

Raw polygraph records of fundic motility and tone immediately before, during, and after esophageal stimulation. Records at *left* represent basal EGR elicited from each individual animal. Records at *right* represent experimental EGR elicited after systemic administration of agonist or antagonists indicated. IV, intravenous; L-NAME, N^G -nitro-L-arginine methyl ester. See bar graphs in Fig. 3 for quantified percent changes in EGRs following experimental treatments.

Fig. 3.

Summary of effects of systemic agonists or antagonists on esophageal distension-evoked gastric relaxation. Each animal served as its own control: amplitude of evoked EGR after experimental treatment was compared with the amplitude of the original EGR and expressed as a percentage of the original EGR. The evoked gastric relaxation reflex can be repetitively elicited. The magnitude of the EGR after intravenous PBS was the same as the original EGR in this group ($100.6 \pm 12.5\%$; $n = 5$). Percent differences between the magnitudes of the basal vs. experimental condition reflexes elicited in each group were evaluated using 1-way analysis of variance across all treatment groups [ANOVA $F_{(6, 28)} = 58.71$; $P < 0.0001$]. Dunnett's post hoc tests (i.e., control group = PBS IV) indicated that the reflexes evoked after each treatment were significantly different from that generated after PBS IV pretreatment ($*P < 0.001$). Systemic administration of atropine methyl nitrate reduced the amplitude of the gastric relaxation to $52.0 \pm 4.4\%$ ($n = 4$) of the original EGR, whereas L-NAME reduced it to $26.3 \pm 7.2\%$ ($n = 7$) of the original EGR. Concomitant administration of atropine methyl nitrate and L-NAME ($n = 5$) reduced the amplitude of the gastric relaxation to $4.0 \pm 2.5\%$ of control. This reduction in amplitude of induced EGR was quite comparable to that seen when the animal was pretreated with the nicotinic ganglionic blocker hexamethonium ($4.3 \pm 2.6\%$; $n = 4$). Thus withdrawal of cholinergic input to the stomach, via atropine methyl nitrate pretreatment, causes ~48% reduction in reflex size. Eliminating the nitrergic path with L-NAME causes ~74% reduction in EGR size. This difference is statistically significant (Bonferroni post hoc test on these two groups: $t = 2.22$, $\#P < 0.05$). In the presence of the muscarinic agonist bethanechol, the evoked EGR was significantly larger than the original EGR ($177.0 \pm 10.0\%$); administration of L-NAME under this condition reduced the magnitude of the gastric relaxation to $19.9 \pm 9.5\%$ ($n = 5$) of that elicited under bethanechol treatment (refer to *bottom trace* of Fig. 2).