Rat gastroduodenal motility in vivo: interaction of GABA and VIP in control of spontaneous relaxations

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Krantis, Anthony, Kamal Mattar, and Ian Glasgow. Rat gastroduodenal motility in vivo: interaction of GABA and VIP in control of spontaneous relaxations. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G897–G903, 1998.—Spontaneous relaxations occurring within motor activity in the rat gastroduodenum in vivo can be distinguished by their dependence on either nitric oxide (NO) or ATP. We examined the interaction of γ-aminobutyric acid (GABA) and vasoactive intestinal peptide (VIP) within pathways controlling this activity in the antrum (S) and duodenum (D) of anesthetized Sprague-Dawley rats, using miniaturized extraluminal foil strain gauges oriented perpendicular to (S1, D1) or in the axis of (S2) the circular smooth muscle. The NO synthase inhibitor N\textsuperscript{\text{-}}-nitro-L-arginine methyl ester (L-NAME; 10 mg/kg iv) attenuated (P < 0.05) antral relaxations and, in the duodenum, nonpropagating “intergroup” relaxations. The GABA\textsubscript{A} receptor antagonist bicuculline (350 µg/kg sc) had similar effects. The GABA\textsubscript{A} agonist 3-amino-1-propanesulfonic acid stimulated L-NAME-sensitive relaxations at S1 and D1. Propagating “grouped” responses were unchanged. VIP (6 µg/kg iv) always induced a relaxation of the duodenum, which was attenuated by bicuculline and L-NAME. VIP caused simultaneous responses at S1 and S2; however, the antrum displayed either contraction or relaxation in response to VIP. All antral relaxations in response to VIP were attenuated (P < 0.05) by L-NAME; however, only VIP-induced relaxations at S1 were nonpropagated. GABA\textsubscript{A} receptors mediate the pathway(s) controlling NO-related spontaneous relaxations of the antrum and duodenal circular muscle. All VIP-induced relaxations are mediated by NO. Spontaneous relaxations of the rat gastroduodenum include responses that involve a GABA\textsubscript{A}-NANC NO-related pathway, which is targeted by VIP. In addition, VIP can target NO relaxations of the antrum via other pathways.

γ-aminobutyric acid; vasoactive intestinal peptide; nitric oxide

SPONTANEOUS MOTOR ACTIVITIES of the rat gastroduodenum in vivo can be differentiated by their pattern and dependency on either nitric oxide (NO) or ATP (8a). NO is responsible for antral and duodenal (“intergroup”) nonpropagating relaxant activity, whereas propagating duodenal (“grouped”) relaxant activity is mediated by ATP, via P\textsubscript{2x} purinoceptors. These spontaneous motor activities are independent, indicative of their control through distinct neural pathways.

It has previously been shown that intravenously administered γ-aminobutyric acid (GABA) and its selective GABA\textsubscript{A} receptor agonist, homotaurine, induce dose-dependent transient relaxations of the duodenum in anesthetized rats (22). These GABA-induced relaxations were sensitive to treatment with the GABA\textsubscript{A} receptor antagonist bicuculline and the nerve toxin TTX, indicative of the involvement of neurogenic mechanisms. This is consistent with the now well-established pharmacology of GABA actions in isolated intestine of a number of species in which GABA, via GABA\textsubscript{A} receptors, stimulates nonadrenergic, noncholinergic (NANC) relaxations. In the rat duodenum in vitro, desensitization with ATP blocks GABA neurogenic-induced relaxations (23). However, NANC relaxations induced by GABA in the rat isolated duodenum were insensitive to desensitization with ATP but blocked by NO synthase inhibition (12, 16). In addition, in the canine terminal ileum and ileocolonic junction (2), ATP desensitization does not affect relaxations induced by GABA. Rather, NO has been shown to be the mediator of these GABA-induced relaxations in vitro. In addition to targeting both ATP- and NO-mediated relaxations, experiments with rat colon muscle strips have shown that GABA-induced relaxations are accompanied by release of vasoactive intestinal peptide (VIP), and the relaxations are inhibited by the VIP receptor antagonist VIP-(10—28) (10). VIP has been proposed to be the primary relaxant transmitter in the stomach and taeenia coli (19). In vivo studies have shown that infusion of VIP causes gastric relaxation in the dog, rat, and cat (13, 21, 23), and preadministered VIP antiserum attenuated VIP-induced gastric relaxations in the rat (3). Indeed, VIP is localized and released by neurons within the gut wall (6, 8), and VIP can directly relax the smooth muscle layers within all regions of the gut (11, 12).

The targeting of enteric inhibitory and/or excitatory motoneurons by GABA appears to be important in reflex control of gut motility (10, 18, 29). At least three distinct functional GABAAergic innervations of the mammalian gut neurons occur: GABA\textsubscript{A}-related (A-GABAAergic) innervation of cholinergic motoneurons, A-GABAAergic innervation of NANC motoneurons, and GABA\textsubscript{B}-related (B-GABAAergic) prejunctional innervation of cholinergic motoneurons. The nomenclature A-GABAAergic and B-GABAAergic has been adopted from the work of Krantis et al. (19). This correlates with the now well-characterized GABA nerve cell and fiber innervation of the mammalian gut wall (14, 20, 28). Anatomic studies of the rat large intestine, performed in our laboratory, show that 50% of myenteric ganglion cells are immunopositive for the GABA\textsubscript{A} receptors (19). A subpopulation (30—35%) of GABA\textsubscript{A}-immunoreactive ganglion cells was also NO synthase positive. Indeed, GABA\textsubscript{A} receptors were found to be present on 90% of all NO synthase-positive neurons. Preliminary studies (unpublished) by our laboratory on the rat duodenum, jejunum, and ileum have shown that myenteric neu-
rons and submucosal ganglion cells also localize GABA<sub>A</sub> receptors.

A-GABAergic innervation of NANC inhibitory motoneurons in the intestine has been established; therefore, in this study we sought to test whether any of the relaxant motor activities in the rat gastroduodenum in vivo involve an A-GABAergic component. We hypothesized that GABA<sub>A</sub> receptors are functionally involved in the pathways controlling spontaneous nitrergic gastric and duodenal relaxant activity. In addition, we sought to examine whether there is an interaction of VIP within these pathways, as might be predicted from in vitro studies.

**METHODS**

Male Sprague-Dawley rats (250–350 g) were fasted for 24 h with free access to water before surgery. Rats were then anesthetized with halothane (4%) and then transferred to a scavenging table electrically heated to maintain the rat at 37°C. The level of anesthesia was reduced to 2% for the surgical procedure; a midline incision was made in the neck region of the animal to allow access to the right jugular vein and the right carotid artery. The right jugular vein was cannulated for the purpose of intravenous drug infusion. Blood pressure was monitored continuously by a pressure transducer connected to a cannula in the right carotid artery. For experiments that required close intra-arterial administration of drugs, the femoral artery was exposed (after a 2- to 3-cm incision of the skin in the area of the right hindlimb), and a cannula (PE-10 polyethylene tubing) was introduced and fed retrogradely 6 cm into the lower part of the thoracic aorta to position the tip close to the opening of the superior mesenteric artery, therefore allowing drug delivery directly into the blood supply of the antrum. To ensure proper tip position, a small injection of VIP (<3 µg/kg) was given via the cannula to validate the injection site. With the patency of the close intra-arterial cannulation (characterized by a hemodynamic effect), the hindlimb incision was closed with wound clips. At the conclusion of the experiment and after the animal was killed, the position of the intra-arterial cannula was verified. Animals that did not respond to the test VIP injection or that did not have a correctly positioned cannula were not included for data analysis. Drugs injected systemically were always given in 0.5-ml volumes and flushed with 0.9% saline.

After the cannulation of blood vessels, a median laparotomy (3–4 cm) exposed the gastrointestinal regions of interest. With the use of cotton-tipped applicators moistened with saline, gut segments were sequentially positioned to facilitate the implantation of foil strain gauges. The first gauge (S<sub>1</sub>) was glued, using Vetbond, onto the serosa of the gastric antrum oriented parallel to the axis of the longitudinal muscle, 2 cm proximal to the pyloric sphincter. Another foil strain gauge (D<sub>1</sub>) was glued to the serosa 1–2 cm distal to the gastroduodenal junction, on the antimesenteric border. This foil strain gauge was also oriented such that the longitudinal axis of the gauge ran parallel to the longitudinal muscle layer. In the duodenum, this orientation affords the most sensitive setting for recording circumferential motor activity (19). In some experiments, a second foil strain gauge (S<sub>2</sub>) was glued onto the antrum, juxtaposed to the S<sub>1</sub> foil strain gauge but oriented to the axis of the circular muscle, as described by Sarna (30). The abdominal incision was then closed, the animal was carefully rotated to the prone position, and the exteriorized strain gauge leads were attached to an IBM-based data acquisition system via an interface box. The animal was then covered with a blanket to help maintain normal optimal body temperature, and the concentration of halothane was reduced to 1% and maintained at that level for the remainder of the experiment. The animal was monitored for vital signs, and a blood pressure between 70 and 100 mmHg was deemed acceptable. After surgery, the animal was allowed a 1-h period of stabilization before the motility recordings began. Experiments consisted of a 60-min control period of recording, at which point drug treatment was administered, followed by motility recordings for up to 4 h.

Motility recording and analysis. The method for recording and analyzing motor activity using foil strain gauges has previously been described in detail (17). Briefly, with the use of an IBM data acquisition system, the motility data from up to three recording points were acquired, digitized, and stored. Data were stored as events, related to a change in voltage due to the bending of the foil gauge in either direction, corresponding to a contraction (upward deflection) or relaxation (downward deflection) response. Files (motility recordings) for each channel (gauge) were simultaneously analyzed for contractions and relaxations, using a proprietary gastrointestinal analysis software program.

Statistical analysis. We prepared data for statistical analysis using spreadsheet software (Quattro Pro). Data from each channel were first grouped according to the event (contraction or relaxation) and then further grouped into control or treatment period and then according to the parameter being analyzed. We examined 1) the amplitude (grams tension) and 2) the frequency (min<sup>-1</sup>) of the events. All tabulated data are expressed as means ± SE of these parameters. For graphed data, the treatment period means are expressed as a percentage of the control mean, and the control mean was represented by 100%. An ANOVA with Tukey’s multiple-comparison test (Statsgraphics Plus, version 5.2) was used to determine significance between the treatment and control periods. P < 0.05 was considered statistically significant.

Chemicals. 3-Amino-1-propanesulfonic acid (3-APS), bicuculine methiodide, N<sup>6</sup>-nitro-l-arginine methyl ester (l-NAME), and VIP were obtained from Sigma (Toronto, ON, Canada). VIP antiserum AC115 was obtained from Cambridge Biochemicals (Wilmington, DE).

**RESULTS**

In the antrum (S<sub>1</sub>), typical rhythmic activity (predominantly relaxations) was evident during the predrug control recording period, similar to that reported in our companion study (8a). In the proximal duodenum (D<sub>1</sub>), control motor activity consisted of periods of intense propagating motor activity (grouped) alternating with periods of less intense nonpropagating intergroup activity. Similar to the results described in our companion study (8a), relaxations in the antrum and within the intergroup periods in the duodenum were specifically attenuated (P < 0.05) by the NO synthase inhibitor l-NAME (10 mg/kg iv, n = 6), and this could be reversed by l-arginine (300 mg/kg iv).

Effects of GABA<sub>A</sub> agonist and antagonist drugs. Within 1 min of 3-APS administration, antral motor activity was altered, and within 30 min spontaneous antral relaxations showed a significant (P < 0.05, n = 4) increase in amplitude (Fig. 1). These effects of 3-APS on antral activity persisted for up to 70 min. In the proximal duodenum (Fig. 2) only the relaxant events in the intergroup period were affected by 3-APS. Both
amplitude and frequency were significantly enhanced (Fig. 1). The increased intergroup relaxant activity was maximal within the first 30 min postinjection; however, by 90 min there was a disappearance of intergroup activity (not shown) indicative of desensitization.

The GABA<sub>A</sub> receptor antagonist bicuculline (350 µg/kg sc) administered after 60 min of control recording significantly attenuated spontaneous antral relaxations (Fig. 1). Duodenal grouped motor activity was unaffected by bicuculline. However, intergroup relaxations were decreased significantly. The specificity of bicuculline actions in the duodenum is graphically represented in the sample recording in Fig. 2, in which bicuculline reversed the effects of 3-APS-evoked responses.

**Effects of systemically administered VIP or VIP antiserum.** VIP (6 µg/kg) administered either intravenously or injected intra-arterially directly into the blood supply of the antrum after 60 min of control recording caused either a single contraction or a single relaxation of the gastric antrum (S<sub>1</sub>); examples of this are presented in Fig. 3. The VIP-induced response was evident within 1 min of injection and was maximal 1 min after the initiation of the response. However, the duration of the evoked response was long, sometimes taking up to 40 min before a recovery to baseline tone. We found no quantitative or qualitative difference in the action of VIP applied intravenously or intra-arterially, and the likelihood of a VIP-induced relaxation vs. contraction was equal. In a separate group of rats (n = 8), we repeated our experiments, but with two antral foil strain gauges, S<sub>1</sub> (oriented to record circular muscle activity, as before) and S<sub>2</sub> (oriented to record longitudinal muscle activity). The responses to applied VIP always occurred simultaneously at both the S<sub>1</sub> and S<sub>2</sub> recording points (Fig. 3).

In contrast to the antrum, in the proximal duodenum systemically administered VIP (6 µg/kg) always caused an immediate but transient (duration ≤2 min) relaxation (Fig. 4). A consistent feature of VIP injection (n = 8) was the attenuation (P < 0.05) of intergroup relaxations. This effect on intergroup relaxations was also evident after treatment with VIP antiserum (100 µl/kg iv).

**Effects of L-NAME on VIP-evoked responses.** After 60 min of control recording, animals (n = 8) were injected with VIP (6 µg/kg iv) to establish a control VIP-induced relaxation at the recording sites. Motor activity was allowed to recover for at least 40 min, at which time L-NAME (10 mg/kg iv) was administered, and 30 min...
later VIP (6 µg/kg iv) was retested. In this way, the mitigating effects of tachyphylaxis were avoided. VIP-induced contractions were not affected by l-NAME treatment (not shown). However, all VIP-induced antral relaxations at S1 and S2 (Fig. 5) and VIP-induced duodenal relaxations (Fig. 6) were significantly reduced by l-NAME treatment.

Effects of bicuculline on VIP-evoked responses. The experimental protocol used in the l-NAME-VIP experiments was repeated (n = 4) using the GABA<sub>A</sub> antagonist bicuculline (350 µg/kg sc). VIP-induced relaxations at S1 but not S2 (Fig. 7) and at D1 (Figs. 7 and 8) were significantly attenuated by bicuculline administration.

**DISCUSSION**

As shown here and in our companion study (8a), spontaneous relaxant activity of the gastroduodenum in the anesthetized rat occurs within distinct patterns,
which can be distinguished by their dependence on either NO or ATP. All spontaneous relaxant activity in the gastric antrum and in the duodenum within intergroup periods is dependent on NO. Grouped relaxations are propagatory and dependent on ATP. Our pharmaco-logical examination of these spontaneous motor activities demonstrates that only spontaneous antral (circular muscle) relaxations and duodenal intergroup relaxations are significantly reduced by treatment with the GABA<sub>A</sub> receptor antagonist bicuculline. Conversely, application of the GABA<sub>A</sub> receptor agonist 3-APS evokes relaxations of circular muscle in the antrum; in the duodenum relaxations are evoked only during the intergroup period. These actions of 3-APS were reversed by bicuculline. Taken together, these results suggest that the A-GABAergic system functionally targets the NO-mediated relaxation responses occurring spontaneously in the rat gastroduodenum.

In addition to NO modulating purinergic motor innervation of the duodenum, the results of our companion study show that NO-mediated relaxations of the duodenum can be stimulated by the P2Y-purinoceptor agonist 2-methylthio-ATP. P2Y-purinoceptors may exist either directly on duodenal nitrergic inhibitory motoneurons or on some unknown interneuron that is part of an excitatory innervation of the nitrergic inhibitory motoneurons. The fact that duodenal NO-mediated relaxations are targeted by A-GABAergics suggests that the related GABAergic interneurons may well be stimulated by ATP via P2Y-receptors. Whether ATP or GABA is involved in the same or separate excitatory pathways controlling NO-inhibitory motoneurons in the proximal duodenum is the focus of our ongoing in vivo motility studies.

We found grouped relaxant activity to be unaffected by A-GABAergic agonist and antagonist drugs. The interaction of GABAergic and purinergic elements in duodenal relaxation is controversial: GABA-induced neurogenic relaxations of the rat duodenum could be blocked by ATP desensitization (23). Furthermore, relaxations induced by ATP, GABA, and electrical stimulation were all antagonized by apamin, which has been shown to block ATP-mediated relaxations. However, other in vitro studies of the rat duodenum (12, 16) report that NO and not ATP mediates GABA-induced relaxations.

The sensitivity of NO-related responses in the duodenum to treatment with bicuculline indicates that pathways controlling NO (NANC) neurons contain GABA<sub>A</sub> receptors. Studies show that NO neurons are widespread in the mammalian gut wall (25, 26) and that NO colocalizes with other neurotransmitters in the myenteric plexus of various species. Studies in our laboratory show that in the human colon NO synthase-positive neurons colocalize neuropeptide Y (NPY) (27). In another study of the human colon, Nichols et al. (28) presented evidence for a subpopulation of GABAergic myenteric neurons to be NO synthase positive (28). In the guinea pig colon the interplay of ATP, VIP, and NO in neurogenic relaxations has been shown (4), and in the guinea pig small intestine and rat gut, VIP coexists with NO synthase in a subpopulation of neurons (1, 5). Thus the nitrergic components identified in the present

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**Fig. 5.** Effects of N<sup>ω</sup>-nitro-L-arginine methyl ester (L-NAME) on amplitude of VIP (6 µg/kg iv)-evoked relaxations in antrum (n = 6) recorded from circular (S<sub>1</sub>) and longitudinal (S<sub>2</sub>) muscles and proximal duodenum (D<sub>1</sub>). Data are presented as % (± SE) of relaxation in response to VIP (control) preinjection of L-NAME (10 mg/kg iv). *Statistically significant difference (P < 0.05) compared with control.

**Fig. 6.** Typical recording of motor activity in proximal duodenum showing effect of L-NAME treatment on spontaneous motor patterns and on VIP-evoked relaxation. VIP caused a large relaxation followed by a reduction in intergroup relaxations. L-NAME injection also caused augmentation of grouped motor activity and blocked the relaxation response to a second challenge with VIP.

**Fig. 7.** Effects of bicuculline on amplitude of VIP (6 µg/kg iv)-evoked relaxations of antral (n = 4) circular (S<sub>1</sub>) and longitudinal (S<sub>2</sub>) muscle and proximal duodenum (D<sub>1</sub>). *Statistically significant difference (P < 0.05) compared with control.
study may well represent one or another of these NO neuronal subpopulations.

Our results show that VIP applied either systemically or close intra-arterially can evoke relaxations of the rat antrum and duodenum and subsequently inhibit duodenal intergroup relaxations. Treatment with VIP antiserum also attenuated these relaxations. VIP-evoked antral relaxant responses (recorded from either strain gauge orientation) were attenuated by pretreatment with L-NAME. In light of our observation that duodenal intergroup relaxations and VIP-induced duodenal relaxations were also attenuated by L-NAME, it would therefore appear that all VIP-induced gastroduodenal relaxations in the rat in vivo are mediated by NO.

In the stomach, it has been shown that VIP receptors exist on smooth muscle, via which VIP appears to evoke NO-dependant relaxations (3). This targeting of NO myogenic components also occurs in the large intestine (9). In the present study, we could not determine whether NO in smooth muscle cells was involved in VIP actions. However, we were able to characterize VIP actions based on the sensitivity of VIP-evoked relaxations to bicuculline. In the duodenum, all VIP-evoked relaxations were attenuated by bicuculline. However, in the antrum, only relaxations to VIP recorded at the antral circular muscle were antagonized by bicuculline. Therefore, in the rat gastric antrum in vivo, VIP must induce relaxation of the circular and longitudinal muscle layers via different NO-related pathways. The nitrergic neurons innervating the antral circular muscle layer and duodenal smooth muscle are stimulated via an A-GABAergic mechanism, and this pathway is targeted by VIP. We also propose that VIP is targeting a separate NO-mediated relaxation of the antral longitudinal muscle that does not have an A-GABAergic component. This may represent a separate NO innervation. Alternatively, NO might be synthesized by longitudinal smooth muscle cells stimulated directly by VIP, as has previously been proposed (24).

VIP could also induce contractions of the rat gastric antrum. There is limited evidence that VIP is an excitatory agent at enteric smooth muscle. For instance, in guinea pig intestine, VIP evokes a TTX-sensitive contraction in vitro (15). Moreover, electrophysiological studies show that VIP can excite about 45% of the nerve cells in the myenteric plexus of the ileum (7). Therefore, the findings of the present in vivo study may represent functional evidence for VIP to induce antral contractions via cholinergic and/or non-cholinergic excitatory motoneurons.

In conclusion, the findings of the in vivo studies described here and in our companion study provide evidence that all three putative NANC inhibitory neurotransmitters, NO, ATP, and VIP, are involved in spontaneous relaxant motor activity of the rat gastro-duodenum. Each candidate neurotransmitter is capable of evoking relaxations of the gastric antrum and the proximal duodenum. However, in the gastric antrum, all spontaneous relaxant activity was NO mediated. In the duodenum, relaxations within the distinct patterns of spontaneous motor activity involved different transmitters: intergroup relaxations were mediated by NO, whereas grouped relaxations were shown to be mediated by ATP via P2x-purinoceptors. Furthermore, there are functionally active GABA_a neural sites within the pathways controlling NO-mediated spontaneous relaxations of the rat antrum and duodenum. VIP also appears to be functionally important within spontaneous relaxant motor activity. In the antrum, VIP relaxed circular muscle via an A-GABAergic-NO mechanism. VIP-induced relaxations of antral longitudinal muscle were also mediated by NO but insensitive to A-GABAergic antagonism. In the duodenum, a VIP-GABA-NO pathway is also evident, since spontaneous intergroup relaxations were sensitive to antagonism of each of these components. We propose that this VIP action represents a site for integration of the A-GABAergic-NO pathway with other enteric reflex control mechanisms.

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REFERENCES


