Rat gastroduodenal motility in vivo: involvement of NO and ATP in spontaneous motor activity

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Glasgow, Ian, Kamal Mattar, and Anthony Krantis. Rat gastroduodenal motility in vivo: involvement of NO and ATP in spontaneous motor activity. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G889–G896, 1998.—Our studies of fasted anesthetized rats have shown that all spontaneous relaxations of the antrum are nitric oxide (NO) dependent. Duodenal motility is patterned into propagating “grouped” motor activity interposed with “intergroup” periods of nonpropagating motor activity; in the duodenum, only intergroup relaxations are NO dependent. We examined the involvement of NO and ATP in spontaneous motor activities of the gastroduodenum in vivo; contractions and relaxations were recorded and analyzed simultaneously from the antrum (S1) and proximal duodenum (D1) of anesthetized Sprague-Dawley rats (n = 10/group), using extraluminal foil strain gauges. Treatment with the NO synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME; 10 mg/kg iv) attenuated (P < 0.05) antral and intergroup relaxations, whereas grouped relaxations were enhanced (P < 0.05). These effects were reversed with L-arginine (300 mg/kg iv), L-NAME also increased (P < 0.05) the amplitude of duodenal contractions. ATP (8 mg·kg⁻¹·min⁻¹ iv) stimulated relaxations at S1 and D1 that were blocked by the P2y-purinoceptor antagonist suramin (60 mg/kg iv). This treatment did not affect spontaneous antral relaxations; however, duodenal grouped relaxations were attenuated. Desensitization to the P2y-purinoceptor agonist α,β-methylene ATP (300 µg/kg iv) gave results similar to suramin. In contrast, the P2x-purinoceptor agonist 2-methylthio-ATP (2-MeS-ATP; 360 µg/kg iv) evoked duodenal relaxations that were attenuated by L-NAME, and desensitization to 2-MeS-ATP attenuated intergroup relaxations. Spontaneous relaxations of the rat antrum and duodenal intergroup relaxations are NO dependent. Both gut regions relax in response to systemically administered ATP; this response is sensitive to suramin. Grouped duodenal relaxations display functional sensitivity to suramin and P2x-purinoceptor desensitization, indicative of the involvement of ATP and P2x purinoceptors. P2y purinoceptors must also be present; however, these occur on elements releasing NO. Although NO does not mediate grouped relaxations or duodenal contractions, the sensitivity of these responses to L-NAME indicates that the pathways controlling these responses is modulated by NO.

nitric oxide; α,β-methylene-ATP; 2-methylthio-ATP; gastroduodenum; relaxations

SINCE THE FIRST PROPOSAL that all relaxations of mammalian gastrointestinal smooth muscle are mediated by extrinsic adrenergic nerves, overwhelming pharmacological and physiological evidence has accumulated to show that the mammalian gut wall contains a distinct class of intrinsic inhibitory motoneurons, the so-called nonadrenergic, noncholinergic (NANC) neurons (3, 8, 13). These neurons mediate functional relaxations of the gut. However, the identity of the neurotransmitter of this NANC inhibitory motor innervation is controversial, with three main candidates: 1) a purinergic neurotransmitter, involving ATP, 2) a nitrergic neurotransmitter, acting via release of nitric oxide (NO), and 3) a peptidergic neurotransmitter, such as vasoactive intestinal peptide (VIP).

Both neural stimulation and exogenous application of ATP have been shown to produce relaxations in isolated preparations of enteric smooth muscle from the rat duodenum and fundus and from the guinea pig fundus, small intestine, and taenia coli (16–19, 25). NO is a potent inhibitory neurotransmitter (9), and NO-synthesizing neurons are distributed extensively in the myenteric and submucosal nerve networks of guinea pigs, rats, and humans (1, 20–22). Functional in vitro and in vivo studies provide convincing evidence in support of the notion that NO is released by NANC inhibitory motoneurons mediating relaxations of the mammalian gut (2, 5, 7, 23).

Taken together, this evidence suggests that no single neurotransmitter is responsible for mediating all NANC relaxations in the mammalian gut. These putative transmitters may represent distinct inhibitory nerve types or may be colocalized and as such may represent a single inhibitory nerve type with a variety of transmitters released under differing stimulus conditions (6). In addition to their direct actions on enteric smooth muscle, evidence exists that some of these putative NANC neurotransmitters may be present in other types of intrinsic neurons, including interneurons (5, 10). As transmitters of interneurons, these agents could mediate stimulation of inhibitory or excitatory motoneurons, as well as modulate motoneurons.

Because there is compelling evidence that more than one population of intrinsic inhibitory innervation exists, it is important to identify 1) how these innervations and their transmitters are involved in spontaneous gut motor activity and 2) whether these neurotransmitters can be differentiated on the basis of their involvement within specific patterns of motor activity. This study characterizes relaxant activity in the rat gastroduodenum in vivo and investigates the involvement of nitrergic and purinergic mechanisms in the pathways controlling this motor activity. Follow-up studies of the involvement of VIP and the putative transmitter of gut interneurons, γ-aminobutyric acid (GABA), are presented by Krantis et al. (9a) in a companion study.

METHODS

The methodology used was developed in our laboratory for recording motor activity from the gastroduodenum of Sprague-Dawley rats (13). A brief description of the recording and analysis techniques is presented in Data recording and analysis.
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 (3–4%) and transferred to a scavenging table maintained 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. Mean arterial pressure (MAP) was monitored continuously by means of a pressure transducer (P23 ID; Gould Statham) connected to a cannula inserted into the right carotid artery. The blood pressure transducer was subsequently attached to an IBM PC data acquisition system via an interface box.

After midline laparotomy, the stomach and ~6–8 cm of the proximal duodenum were exteriorized onto saline-soaked gauze pads. Two foil strain gauges (attached to 30-cm-long fine 32-gauge wires) were then glued to the serosal surface of the stomach and the proximal duodenum, using Vetbond glue (tissue adhesive no. 1469; 3M). The first strain gauge (S₁) was glued onto the gastric antrum, ~2 cm proximal to the pyloric sphincter, and the second gauge (D₁) was glued onto the antimesenteric border of the duodenum, 2 cm distal to the sphincter. Both strain gauges were oriented parallel to the longitudinal muscle. In the antrum, this orientation preferably detects circular muscle activity. In the duodenum, the tubular structure confines the gauge (irrespective of the axis of orientation) to detect only circumferential muscle force, and alignment in the longitudinal axis affords the greatest sensitivity (13). The fine wire leads attached to the strain gauges were advanced caudally to the distal end of the midline laparotomy, where they were exteriorized. The abdominal incision was then closed, and the animal was carefully rotated to a prone position to allow the exteriorized wires to be attached to the IBM-based data acquisition system via an interface box (channel 1: S₁; channel 2: D₁). The animal was then covered with a blanket to help maintain normal body temperature, and anesthesia was reduced to 1% halothane and maintained at that level for the remainder of the experiment. The animal was monitored carefully 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. For all drugs except suramin, the treatment period started at the time of drug injection (t = 0). Suramin is a slowly equilibrating competitive antagonist at P₂x purinoceptors with maximum effect in vitro evident within 90 min (14). On this basis, the treatment period for animals injected with suramin in this study started at 120 min after suramin.

Ex vivo experiments. Ex vivo experiments were conducted to allow comparison of the effects of drugs previously tested intravenously. Sprague-Dawley rats were surgically prepared as for the in vivo studies described above, except the animals were stabilized for 60 min in a supine position. After the stabilization period, the abdominal cavity was carefully re-opened and exposed using two hemostat clamps. With the use of saline-soaked cotton-tipped applicators, the stomach and a segment of proximal duodenum were very gently exteriorized onto saline-soaked gauze pads. The gut maintained on the gauze was kept moist with saline (37°C). Drugs were applied serosally close to the strain gauge of interest. Fresh gauze pads and cotton-tip applicators were used to absorb excess drug, so as to prevent the applied drug from entering the abdominal cavity.

Data recording and analysis. Measurement and analysis of long-term recordings was based on the method of Krantis et al. (13). During the experiment, an IBM data acquisition system (acquisition software provided by Dr. Frank Johnson, Institute of Medical Engineering, University of Ottawa) was used to acquire, digitize, and store the motility data for both channels 1 (S₁) and 2 (D₁). Data are recorded as events and long-term recordings was based on the method of Krantis et al. (13). During the experiment, an IBM data acquisition system (acquisition software provided by Dr. Frank Johnson, Institute of Medical Engineering, University of Ottawa) was used to acquire, digitize, and store the motility data for both channels 1 (S₁) and 2 (D₁). Data are recorded as events and

![Figure 1. Typical recording of rat antral (S₁) spontaneous motor activity before and after injection of N°-nitro-L-arginine methyl ester (L-NAME; 10 mg/kg iv). The 3 panels represent a recording from t = 40 min to t = 90 min. L-NAME was injected as a single bolus at t = 60 min. Control activity consisted predominantly of relaxations and a few contractions. After L-NAME injection, relaxations were inhibited within 10 min. Tension scale shown on y-axis can be used to compare alteration in tone and amplitude of responses.](http://ajpgi.physiology.org/DownloadedFrom/10.22032/ajpgi.2017-03-30)
increased accuracy of recording motor activity. Typical recordings presented in the results always show the recording scale (grams tension) in the ordinate and allow assessment of relative change in tone only. For each animal, the ability of the gauges to detect relaxations and contractions was tested using papaverine (10 mM) and carbachol (1 mM), respectively, applied directly ex vivo onto the gut serosa (13). Only animals in which relaxations and contractions were recorded were subsequently used for our studies.

Statistical analysis. Experimental data for each channel were first grouped according to the event being analyzed (contractions or relaxations) and then further grouped into control or treatment period and then according to the parameter being analyzed. For this study, we examined 1) the amplitude (grams tension) of the event and 2) the frequency of the events. All tabulated results are expressed as means \( \pm \) SE of either amplitude or frequency. Each animal served as its own control, and for pooled data the treatment period means are expressed as a percentage of the control mean, where the control mean was represented by 100%. An ANOVA with a Tukey multiple comparison test (Statsgraphics Plus, version 5.2) was used to determine significance between the raw data from the treatment period and the control period. \( P < 0.05 \) was considered statistically significant.

Chemicals. ATP, \( \alpha,\beta \)-methyleno-ATP (\( \alpha,\beta \)-Me-ATP), L-arginine, and \( N^G \)-nitro-L-arginine methyl ester (L-NAME) were obtained from Sigma (Toronto, ON, Canada). Suramin was obtained from CB Chemicals (Woodbury, CT). 2-Methyl-thio-ATP (2-MeS-ATP) was obtained from Research Biochemicals International (Natick, MA).

RESULTS

Control motor activity. Spontaneous patterns of gastroduodenal motility recorded under control conditions in anesthetized rats \( (n = 6) \) included nonpropagating single and propagating “grouped” motor events. In the gastric antrum \( (S_1) \), motor activity consisted primarily of periodic relaxations (Fig. 1). These relaxations were similar in amplitude \( (0.07 \pm 0.02 \text{ g}) \), with a frequency of \( \sim 5 \) per minute, and were often oscillatory in appearance. Contractions of \( 0.04 \pm 0.01 \text{ g} \) were also observed, but these were typically less frequent \( (\sim 3 \text{ per minute}) \) and their occurrence was random.

In the proximal duodenum \( (D_1) \), motor activity was clearly different, consisting of two obvious patterns that we have termed grouped and “intergroup” motor events. In the gastric antrum \( (S_1) \), motor activity consisted primarily of periodic relaxations (Fig. 1). These relaxations were similar in amplitude \( (0.07 \pm 0.02 \text{ g}) \), with a frequency of \( \sim 5 \) per minute, and were often oscillatory in appearance. Contractions of \( 0.04 \pm 0.01 \text{ g} \) were also observed, but these were typically less frequent \( (\sim 3 \text{ per minute}) \) and their occurrence was random.

In the proximal duodenum \( (D_1) \), motor activity was clearly different, consisting of two obvious patterns that we have termed grouped and “intergroup” activity (13), as shown in Fig. 2. Grouped activity was characterized by distinct groups of large-amplitude, high-frequency relaxations and/or contractions. Each grouped activity period lasted 0.5–3.0 min and was periodic, occurring approximately every \( 6.6 \pm 0.4 \text{ min} \) (minimum \( 4.1 \text{ min} \), maximum \( 10.5 \text{ min} \)). Within this period of grouped activity, responses appeared to build in
in anesthetized Sprague-Dawley rats

The interdigestive motor pattern of the duodenum

Table 1. Characteristics of the spontaneous interdigestive motor pattern of the duodenum in anesthetized Sprague-Dawley rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>Intergroup</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude, g tension</td>
<td>Control</td>
<td>L-NAME</td>
</tr>
<tr>
<td>Contraction</td>
<td>0.08 ± 0.01</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Relaxation</td>
<td>0.19 ± 0.02</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>Frequency, min⁻¹</td>
<td>Control</td>
<td>L-NAME</td>
</tr>
<tr>
<td>Contraction</td>
<td>9.2 ± 1.4</td>
<td>2.7 ± 1.0</td>
</tr>
<tr>
<td>Relaxation</td>
<td>11.1 ± 0.6</td>
<td>4.1 ± 0.7</td>
</tr>
<tr>
<td>Duration, s</td>
<td>Control</td>
<td>L-NAME</td>
</tr>
<tr>
<td>Contraction</td>
<td>3.0 ± 0.1</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>Relaxation</td>
<td>2.9 ± 0.1</td>
<td>3.0 ± 0.1</td>
</tr>
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</table>

Values are means ± SE of data obtained from 8 rats.

intensity reminiscent of migrating myoelectric complexes (MMCs), and these coincided with motor activity that could be seen to propagate caudally down the duodenum, as can be seen in the recordings obtained from multiple foil strain gauges in the proximal duodenum (Fig. 3). Recorded motor activity occurring between the episodes of grouped activity, herein termed intergroup, consisted of both contractions and relaxations. Compared with relaxations within the grouped period at the most distal recording site.

grouped relaxation amplitude. Quantitative parameters of the duodenal motor activities are presented in Table 1. Injection of saline vehicle (0.9% iv) or direct application of saline onto the serosa in the ex vivo protocol (not shown here) had no effect on gastroduodenal motor activity.

Effects of treatment with the NO synthase inhibitor L-NAME. L-NAME is widely used as an inhibitor of NO synthase activity both in vitro and in vivo (5, 7). A previous in vivo study of anesthetized rats (15) has determined that L-NAME (10 mg·kg⁻¹·ml⁻¹ iv) is effective in inhibiting gastric fundus relaxations, with only a transient effect on MAP. On this basis, we chose to test L-NAME (10 mg·kg⁻¹·ml⁻¹ iv) for its effects on motor activity of the rat gastroduodenum in vivo under conditions of anesthesia.

L-NAME always caused a transient (up to 10 min) 10 mmHg increase in MAP. This served as an indicator of effective injection of the drug. Antral contractions showed no significant (P > 0.05) change in either amplitude or frequency after L-NAME injection (Table 2). However, within 10–15 min of L-NAME injection, spontaneous antral relaxations were significantly (P < 0.05) reduced in both amplitude and frequency (Fig. 4A). The effects of L-NAME lasted for the duration of the experiment (up to 3 h) with no recovery of motility patterns to those observed in the control recording period.

In the proximal duodenum, the two types of motor activity patterns, grouped and intergroup, were affected differently by L-NAME (Fig. 2). Grouped relaxations were enhanced by L-NAME, characterized by a significant (P < 0.05) increase in amplitude and frequency (Fig. 4B). In contrast, intergroup relaxations were either reduced or abolished on L-NAME treatment. Although antral contractions were not affected by L-NAME treatment, in the duodenum, the amplitude of grouped contractions was significantly increased (Table 2). Comparable results were obtained with L-NAME (3.7 mM, 100 µl) administered onto the serosa of the antrum or duodenum (data not shown).

When injected 60 min after L-NAME (10 mg/kg iv) administration, L-arginine (300 mg/kg iv) transiently reversed (for up to 10 min) the effects of L-NAME in both the antrum and duodenum (Fig. 4). Injection of L-arginine alone (300 mg/kg iv) generally caused effects opposite to those of L-NAME treatment. In the antrum, L-arginine significantly increased the amplitude and frequency of spontaneous relaxations (Fig. 4A). In contrast to the antrum, duodenal grouped relaxation amplitude.

Table 2. Effects of L-NAME on spontaneous gastroduodenal contractions

<table>
<thead>
<tr>
<th>Region</th>
<th>Amplitude, g tension</th>
<th>Frequency, events/min</th>
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<tbody>
<tr>
<td>S1</td>
<td>Control: 0.07 ± 0.02</td>
<td>L-NAME: 0.06 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>L-NAME: 0.14 ± 0.03</td>
<td>Control: 0.8 ± 0.4</td>
</tr>
<tr>
<td>D1 grouped</td>
<td>Control: 0.05 ± 0.01</td>
<td>L-NAME: 0.02 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>L-NAME: 0.3 ± 0.1</td>
<td>Control: 3 ± 1*</td>
</tr>
<tr>
<td>D1 intergroup</td>
<td>Control: 0.05 ± 0.02</td>
<td>L-NAME: 0.05 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>L-NAME: 0.9 ± 0.5</td>
<td>Control: 4 ± 1*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 3. L-NAME, N⁶-nitro-L-arginine methyl ester (10 mg/kg iv). *Significant (P < 0.05) compared with control.
amplitudes were significantly reduced (Fig. 4B). The action of L-arginine in the duodenum was confined to the grouped relaxations, and all of the effects of L-arginine were transient, lasting 4–10 min. L-Arginine did not affect gastroduodenal spontaneous motor contractions.

Effects of ATP, suramin, α,β-Me-ATP, and 2-MeS-ATP. ATP (8 mg·kg\(^{-1}\)·min\(^{-1}\) iv, for 1 min) caused a transient (up to 10 min) decrease (Δ20 mmHg) in MAP. In the gastric antrum, ATP infusion always evoked large-amplitude (215% of control) relaxations (Fig. 5). These were easily distinguishable from control spontaneous relaxations and lasted for ~10–15 min before a return to control patterns. Similarly, in the duodenum, ATP always evoked relaxations. However, in contrast to the antrum, the duodenum usually only responded with a single, transient relaxation within 1 min of ATP injection. In addition, this response occurred (or was visible) only if ATP was injected during the intergroup activity. This relaxation could easily be distinguished from the random spontaneous relaxations during intergroup activity by its long duration and large amplitude. ATP did not affect the spontaneous activity of either the grouped or intergroup periods.

To further elucidate the involvement of ATP in gastroduodenal motor activity, parallel experiments were conducted using the P\(_{2\times}\)-purinoceptor antagonist suramin. To our knowledge, this antagonist, which has been used to investigate ATP sites of action in the gut in vitro, has not been used previously for in vivo motility studies. Preliminary in vivo experiments in this laboratory, using a dose of 30 mg/kg [which is slightly higher than the dosage effective against parasites in humans (19)], found the effects of suramin on spontaneous motor activity to be inconsistent. However, suramin at a dose of 60 mg/kg iv was consistently effective without altering vital signs in the test animals (n = 6). Suramin (60 mg/kg) administered as a single intravenous bolus injection attenuated the amplitude of the ATP-induced antral relaxation by 49 ± 6% compared with control. In the duodenum, ATP-induced relaxations were reduced by 57 ± 4%. Spontaneous motor activity of the antrum and duodenal intergroup activity were not affected by suramin treatment. However, suramin caused changes in grouped motor activity evident from 90 min and maximal at 120 min after drug injection. This long equilibration time for suramin action is comparable to that observed in other studies (14). Suramin actions were characterized by an increase in the average duration of grouped activity periods (5–7 min) and inhibition (P < 0.05) of grouped relaxation amplitude and frequency (Fig. 6). Grouped contractile activity was not affected.

The P\(_{2\times}\)-purinoceptor agonist α,β-Me-ATP (300 µg/kg iv) caused a transient increase in the amplitude of grouped relaxations (Fig. 7). This effect lasted up to 2 min and was followed by a dramatic reduction in both the amplitude and frequency of grouped relaxations (Figs. 6 and 7). α,β-Me-ATP-induced inhibition lasted until the end of the experiment, indicative of a persistent desensitization. At a lower dosage (150 µg/kg iv),
α,β-Me-ATP exerted similar effects, but these were not sustained, and in some cases there was recovery as early as 30 min after injection. There were no significant changes to the spontaneous antral activity or duodenal contractile activity.

On injection, 2-MeS-ATP (360 µg/kg iv) evoked a single large relaxation (~30 s duration) easily distinguishable from control spontaneous activity. 2-MeS-ATP-induced relaxations were almost abolished by L-NAME treatment (Table 3). Within 3 min of injection, 2-MeS-ATP significantly reduced intergroup relaxation amplitude and frequency (Fig. 6); grouped relaxations were not affected. In addition, there were no significant changes to the spontaneous motor activity of the antrum or contractile duodenal activity.

**DISCUSSION**

The results obtained in this study show intravenous injection or direct serosal application of the NO synthase inhibitor L-NAME to differentially alter spontaneous relaxant motor activity in the rat gastric antrum and proximal duodenum in vivo. Spontaneous antral relaxations were either significantly attenuated or abolished. However, in the duodenum, only the relaxations of the intergroup period were attenuated. These intergroup periods of motor activity comprise sparsely distributed small-amplitude responses and are easily distinguished from the regular periods of intense propagating motor activity, referred to herein as grouped activity. The profile and duration of this grouped activity in the duodenum are similar to those described previously by researchers in this laboratory (13). Grouped activity consisted of both relaxations and contractions, and often one type of response predominated. This may be due to the intrinsic tissue tone, since it is well established that localized regions of the gut are under a preset tone. Together, the alternating periods of grouped vs. intergroup activity accounted for all of the spontaneous duodenal motor activity in the anesthetized rat. Whereas intergroup relaxations were attenuated or abolished by L-NAME, grouped relaxations were enhanced. All of the L-NAME effects were reversed by the NO synthase substrate L-arginine.

These results indicate that NO is not the primary transmitter of the pathway controlling relaxations occurring within grouped activity. However, our results

![Fig. 6. Effects of ATP agonist and antagonist drugs on spontaneous duodenal relaxations. Treatment groups (n = 6-10/group) are shown on x-axis. Collected data are expressed as percent change ± SE compared with control. Amplitude and frequency data are presented separately. 2-MeS-ATP, 2-methylthio-ATP. *P < 0.05 compared with control.](http://apjpphysiology.org/

![Fig. 7. Effects of α,β-methylene-ATP on duodenal motor activity. Grouped and intergroup periods are shown, before and after treatment with α,β-methylene-ATP (300 µg·kg⁻¹·min⁻¹ iv). In this example, grouped periods occurred relatively close together just before drug injection. These can be used to compare the effects of α,β-methylene-ATP, which transiently augmented and then reduced grouped motor activity.](http://apjpphysiology.org/)

**Table 3. Effects of L-NAME on 2-MeS-ATP-induced relaxation of the duodenum**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>After L-NAME</th>
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<tbody>
<tr>
<td>2-MeS-ATP-induced relaxation, g</td>
<td>1.49 ± 0.01</td>
<td>0.02 ± 0.01*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6. 2-MeS-ATP, 2-methylthio-ATP (360 µg/kg iv); L-NAME, 10 mg/kg iv. *Significant (P < 0.05) compared with control.
clearly show that NO has a primary role in relaxations of the antrum and within the duodenal intergroup activity.

The involvement of NO in relaxations of the stomach has been previously reported (15) in studies in which L-NAME either abolished or greatly reduced vagally stimulated relaxations of the gastric fundus in anesthetized rats. In addition, L-NAME treatment enhanced vagally stimulated gastric contractions. However, we found no significant change in spontaneous antral contractions with L-NAME treatment. It is likely that the contractions evoked by vagal stimulation involve pathways distinct from those mediating the spontaneous contractile activity described in this study and that vagally stimulated contractions are in some way modulated by NO.

The effect of L-NAME on grouped relaxations was unexpected and contrary to the notion that NO functions only as a transmitter of NANC inhibitory motoneurons or as a relaxant factor within smooth muscle cells. In an in vivo study of the rat jejunum, Calignano et al. (5) reported an increase in motility in vivo after administration of a NO synthase inhibitor and proposed that NO modulates the release of certain mediators from local neuronal or intestinal cellular sources. Likewise, studies of anesthetized rats show that inhibition of NO synthesis causes an increase in phasic activity of the small intestine (11), indicative of a neuromodulatory role for NO in these regions. We believe that NO is not only a transmitter of NANC motoneurons in the gastrointestinal but is also released by inhibitory interneurons targeting neurons in the pathway(s) controlling propagating motor activity.

NO-synthesizing varicose fibers occur within almost all intestinal ganglia of the rat, guinea pig, and human myenteric and submucosal nerve layers (20, 21), which strongly supports the notion that NO may also be a transmitter of enteric interneurons. Functional proof of this has been provided by Young et al. (26), who showed that NO stimulates cGMP production within both myenteric and submucosal ganglion cells. This notion is further strengthened by the results of a recent anatomic study in this laboratory in which a subpopulation of neuronal GABAergic ganglion cells, and therefore by definition enteric interneurons (22), was found to display the capacity to constitutively synthesize NO.

Direct serosal application of L-NAME onto the antrum in the anesthetized rat caused effects identical to those of intravenous L-NAME. This strongly suggests the involvement of local NO in the generation of these responses. However, NO-related elements may not be the final component in the pathway mediating antral relaxations. NO could be stimulating the release of ATP, since ATP injection also relaxed the gastric antrum and ATP is known to have direct actions on gastrointestinal smooth muscle (8, 16, 18). In our study, ATP caused relaxations in the antrum without affecting spontaneous antral relaxations. In addition, treatment with suramin at a dose that inhibited relaxations to systemically injected ATP had no effect on spontaneous antral relaxations. Furthermore, development of tachyphylaxis to α,β-Me-ATP or 2-MeS-ATP also had no effect in the antrum. It would appear that NO and not ATP is the transmitter of the inhibitory (presumably NANC) motor nerves mediating spontaneous relaxation of the rodent stomach. This is further supported by D’Amato et al. (7), who showed that the NO synthase inhibitor N G-nitro-L-arginine had no effect on ATP-evoked relaxations of rat gastric fundus muscle strips.

For the rat small intestine, at least two different inhibitory motor systems have been proposed to occur, with ATP being a major NANC inhibitory transmitter in the duodenum (16). Our findings suggest that the inhibitory transmitter responsible for grouped relaxations is not NO. Rather, ATP mediates these relaxations, since treatment with suramin attenuated grouped relaxations. We propose that grouped relaxations are mediated (in part or in whole) by ATP and involve P 2 purinoceptors.

Although P 2y purinoceptors are considered to be the sites by which ATP mediates NANC relaxation of the intestine (24), there is also evidence for ATP to induce relaxations via P 2x purinoceptors (17, 25). Identification of the P 2-purinoceptor subtype involved in ATP-related responses of the gut in vivo is made difficult by the lack of specific antagonists. Suramin may be suitable for antagonizing P 2 purinoceptors but cannot distinguish between the subtypes. An alternative approach is to differentiate these subtypes by their rank order of potency for ATP analogs (4). α,β-Me-ATP exhibits greater affinity for the P 2x-receptor subtype, which readily desensitizes with continuous exposure to this ATP analog. Fortunately, 2-MeS-ATP is more potent at the P 2y purinoceptor. Our results show that α,β-Me-ATP specifically blocked duodenal grouped relaxations after an initial but transient augmentation of grouped relaxation amplitude. Unexpectedly, 2-MeS-ATP induced a large relaxation in the proximal duodenum that was found to be sensitive to L-NAME. Like α,β-Me-ATP, prolonged exposure to 2-MeS-ATP also caused a reduction in motor activity; however, this was apparent only for the intergroup relaxations.

In conclusion, the results presented here provide compelling evidence that NO is the primary inhibitory neurotransmitter of the pathway(s) mediating spontaneous relaxant motor activity in the rat gastric antrum. There is more than one type of inhibitory motor innervation in the proximal duodenum. These can be functionally separated with respect to the distinct patterns of spontaneous motor activity within the duodenum: intergroup relaxations, like antral relaxations, are mediated by NO, whereas grouped relaxations are mediated by ATP. Since the grouped motor activity is reminiscent of MMCs, we propose that ATP, through P 2x purinoceptors, is the primary inhibitory transmitter involved in mediating propagatory motor activity in the duodenum of fasted rats. In addition, ATP, via P 2y purinoceptors, appears to be targeting neurons in the pathway controlling NO-mediated intergroup relaxations. The involvement of ATP within both types of duodenal motor activity is also true of NO. NO appears to be more than just an inhibitory motor neurotransmit-
ter within the rat gastroduodenum, since treatment with L-NAME enhanced grouped relaxations. We propose that NO exerts a tonic neuromodulatory control over ATP-mediated relaxations within grouped motor activity. Moreover, this tonic neuromodulatory action of NO extends to the excitatory motor innervation of the duodenum.

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