Role of extrinsic innervation in modulating nitrergic transmission in the canine ileocolonic region

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Role of extrinsic innervation in modulating nitrergic transmission in the canine ileocolonic region. Am J Physiol Gastrointest Liver Physiol 283: G230–G239, 2002. First published March 13, 2002; 10.1152/ajpgi.00468.2001.—The human colon can dilate, often to life-threatening proportions. Our aim was to explore nitrergic mechanisms underlying colonic dilation in conscious dogs with enterically isolated ileocolonic loops either extrinsically innervated (n = 4) or extrinsically denervated (n = 4). We recorded phasic pressures in ileum and ileocolonic sphincter (ICS), colonic tone, compliance, and relaxation during ileal distension. By NADPH-diaphorase histochemistry, we assessed effects of extrinsic denervation and enteric isolation on nitrergic fibers. Extrinsic denervation increased phasic pressures in ileum, ICS, and colon and abolished ICS and colonic relaxation in response to ileal distension. The nitric oxide synthase (NOS) inhibitor Nω-nitro-L-arginine (L-NNA) increased phasic pressures at all sites and ICS tone but did not abolish colonic relaxation during ileal distension in innervated loops. L-NNA reduced compliance and induced colonic high-amplitude propagated contractions in denervated loops. The NOS substrate donor L-arginine reversed effects of L-NNA. The number of NADPH-diaphorase fibers increased in both enterically isolated preparations. Nonnitrergic extrinsic nerve pathways mediate reflex colonic relaxation during ileal distension. Enteric isolation augments the number of NOS fibers, an effect not modified by extrinsic denervation.

The colon has a propensity to relax, often to alarming proportions in pathological states. Although the dilation of toxic megacolon is associated with acute inflammation of the colon, the severe colonic dilation accompanying acute colonic pseudoobstruction (Ogilvie’s syndrome) occurs in the absence of colonic inflammation (4). The dilation of Ogilvie’s syndrome has been attributed to reflexes mediated by an increase in sympathetic inhibition and/or reduced parasympathetic excitation of colonic motor activity (12, 16, 24). This is a plausible hypothesis, because sympathetic interruption increased colonic motility in cats (13). In addition, the vagal nerves provide parasympathetic input to the proximal colon, whereas the distal colon is innervated primarily by the sacral spinal cord. Although extrinsic denervation had relatively modest effects on fasting gastrointestinal motor activity in dogs, frequency and propagation of intestinal migrating motor complexes (MMCs) were altered (17). Similarly, although extrinsic denervation did not markedly affect fasting canine colonic motor activity, contractile response to a meal was reduced significantly (33).

The ileocolonic junction is a high-pressure physiological sphincter that modulates movement of luminal content between the distal ileum and proximal colon (26). Ileal motor events frequently propagate across the ileocolonic sphincter (ICS) into the colon, suggesting that the area functions as a synchronized segment (11). Moreover, the proximal colon relaxes in response to physiological stimuli such as short-chain fatty acids or feeding, facilitating mixing of ileal effluent within the proximal colon (18). We (1) have shown previously that colonic relaxation in response to ileal distension in a canine model can be recorded by a colonic barostat but not by manometric sensors. This reflex resembles long colocolonic or colointestinal inhibitory reflexes. It remains unknown whether extrinsic neural pathways projecting to the prevertebral ganglia participate in this response. Thus the effects of extrinsic denervation on these inhibitory reflex responses at the ICS deserve further study.

Along these lines, nitric oxide (NO) is a major inhibitory neurotransmitter in the canine proximal colon (34). Two studies showed that the extrinsic denervation associated with splanchnic ganglionectomy (23) and with small bowel transplantation (36) increased nitric oxide synthase (NOS) expression in the rat myenteric plexus. These observations support the hypothesis that upregulation of neuronal NOS (nNOS) is an adaptive mechanism that compensates for reduced sympathetic inhibition after extrinsic denervation.

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NITRERGIC COLONIC MECHANISMS

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Our overall goals were to understand the pathophysiology of colonic dilatation and to develop more rational approaches to a clinical condition that can be life threatening. Consequently, we used a previously validated canine model of extrinsic denervation (38) to determine mechanisms that regulate colonic relaxation. By examining in parallel innervated and denervated models, we wished to explore the roles of extrinsic innervation as well as extrinsic denervation in the phenomenon of colonic dilatation. Our goal was to address the following hypotheses: 1) extrinsic denervation will have relatively modest effects on fasting colonic motor activity but abolish colonic relaxation in response to ileal distension? 2) the NOS inhibitor N\textsuperscript{-}nitro-L-arginine (\text{L-NNA}) will increase ileal and colonic motor activity in extrinsically innervated ileocolonic loops; however, 3) the NOS inhibitor \text{L-NNA} will increase ileal and colonic motor activity to a greater extent in extrinsically denervated, compared with extrinsically innervated, ileocolonic loops; and 4) extrinsic denervation will increase NOS fibers in ileocolonic loops.

MATERIALS AND METHODS

The study was approved by the Institutional Animal Care and Use Committee of the Mayo Clinic. Surgical procedures and experiments were performed in accordance with the “Guide for the Care and Use of Laboratory Animals” [DHEW Publication No. (NIH) 85–23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205].

Preparation of Animals

Eight healthy female mongrel dogs weighing 15–20 kg were divided into two groups (n = 4 each). In preparation for construction of an enterically isolated ileocolonic loop, dogs were anesthetized with intravenous methohexital sodium (12.5 mg/kg) and inhaled halothane. Through a 4-cm midventral celiotomy, the mesenteric vascular arcade just proximal to the inferior mesenteric artery in the descending colon was ligated, and the dogs were given a week to recover. Because most of the venous drainage from the distal descending colon drains proximally through the middle colic artery, ligation of this vascular arcade allows collateral vessels to assume the primary venous drainage. This procedure prevents development of venous hypertension in the distal colon at the time of colonic loop construction, which would threaten the anastomosis needed to restore intestinal continuity after construction of the ileocolonic loop.

All dogs were reoperated at least 1 wk later. In one group, the distal ileum was transected 15 cm proximal to the ICS as was the descending colon just proximal to the inferior mesenteric artery of the previous site of the mesenteric vascular arcade ligation. Intestinal continuity was restored by anastomosis of the proximal end of the ileum to the distal end of the colon. Both ends of the ileocolonic loop were exteriorized as a Thiry-Vella loop, thereby creating an enterically isolated but extrinsically innervated ileocolonic segment.

In the other four dogs, a similar enterically isolated ileocolonic loop was constructed, but in addition, all tissue connections to the loop were transected except for the middle colic artery and vein. In brief, this was accomplished by isolating the middle colic artery and vein at the base of the mesocolon, stripping these vessels of investing adventitia using optical magnification for a length of 1 cm, and transecting and ligating all other mesenteric connections to the ileocolonic loop. We have reported and validated a similar preparation in the dog proximal colon (38).

The first group was extrinsically innervated neurologically; they had extrinsically innervated but enterically isolated bowel segments consisting of 15 cm of distal ileum and the first 40 cm of the proximal colon including the ICS (Fig. 1). In the second group, a similar loop was prepared but, in addition, all extrinsic nerves to the colonic loop were transected. In this model, the denervated loop, but not adjacent segments of extrinsically innervated bowel, were found to be devoid of catecholamines, confirming its extrinsic denervation (38). Animals were allowed 2–3 wk to recover from the operation, during which they were trained to stand in a Pavlov sling.

Conduct of Experiments

Before each experiment, dogs were fasted for ≤18 h but were allowed free access to water. On each study day, the ileal catheter assembly, ileal distending balloon, and colonic barostat-manometric assembly (see below) were positioned and secured in position. We then assessed drug effects on fasting motor activity, colonic compliance, and colonic response to ileal balloon distension, in that order (Fig. 2). Four studies on different days were conducted in randomized order in each dog during the intravenous administration of saline (154 mM NaCl), \text{L-NNA}, \text{L-arginine}, or \text{L-NNA} + \text{L-arginine}. \text{L-NNA} (5 mg/kg bolus iv; Sigma, St. Louis, MO) was used to inhibit NOS (20, 21). In the combined (\text{L-NNA} and \text{L-arginine}) studies, \text{L-arginine} (100 mg/kg iv over 5 min followed by an infusion of 100 mg·kg\textsuperscript{-1}·h\textsuperscript{-1} until the end of the study) was administered 30 min after \text{L-NNA} was given (20, 32). Because the effects of \text{L-NNA} on cycling of the duodenojejunal MMC may persist for up to 7 days (32), no experiments were conducted in dogs for 14 days after studies involving the administration of \text{L-NNA}.

Fig. 1. Canine ileocolonic preparation. Separate ileal and colonic stomas were permitted access to the lumen. The extrinsic nerve supply to these loops was preserved in extrinsically innervated loops or completely transected in extrinsically denervated loops. Ileal and colonic phasic activity was recorded by manometric sensors, a sleeve sensor was placed proximate across the ileocolonic sphincter (ICS), and a barostat balloon was passed retrograde to assess colonic tone. Intraluminal distension was performed by distending a Foley catheter with 10 ml of air 2.5 cm proximal to the ileocolonic junction.
motor activity. However, motor events with the appearance and duration of previously characterized phase III activity were identified as phase III activity (26). These MMCs were >3 min of uninterrupted, phasic pressure waves at the maximum frequency for that locus of the small bowel (7–11 cycles/min), or proximal colon (6 cycles/min). To be categorized as propagated, phase III needed to be recorded sequentially in three adjacent channels or from the two most proximal channels. Third, prolonged propagated contractions were single pressure waves, sometimes achieving pressures in excess of 100 mmHg with a duration greater than the ileal slow wave (6–8 s) and often lasting >30 s. They were often seen to propagate rapidly through the distal ileum.

Phasic pressure activity recorded by manometric sensors in the ileum was expressed as the area under the curve (AUC). Tonic pressures at the ICS were measured from the "trough" of phasic contractions to baseline pressure in the sensor judged to be closest to the sphincter segment. Phasic pressure activity recorded by the Dent sleeve was analyzed by customized computerized programs providing measures of mean pressure and tone (2). For calculating ICS tone, the program searched for points in the tracing where the change in slope was small, i.e., an arbitrary value <0.5. These points were subsequently tabulated in ascending order of pressures. In this list of values, observations at low and high pressures corresponded to the baseline pressure and peaks during manometric contractions, respectively. Through an iterative process, we estimated that the initial 25% of values in this table corresponded closely to manual estimates of fluctuations in baseline pressure or tone.

These parameters (AUC for ileal pressure, ICS mean pressure, and ICS tone) were compared for 60 min before and 60 min after the administration of drug or saline. The average AUC recorded by four manometric sensors within the ileum provided a summary measure of ileal motor activity.

Colonic Barostat-Manometric Assembly

Method. A multilumen polyethylene balloon barostat-manometric assembly incorporating two manometric transducers and a 10-cm-long plastic barostat balloon bag was inserted through the colonic stoma retrograde into the proximal colon (Fig. 1). The two water-perfused (0.4 ml/min) manometric sensors were 3 cm oral and 3 cm caudal to the barostat balloon, respectively. The infinitely compliant plastic bag (barostat balloon) had a maximum volume of 250 ml (Hefty Baggies, Mobil Chemical, Pittsford, NY) and was connected to an electronic rigid piston barostat (Mayo rigid barostat; Engineering Department, Mayo Clinic, Rochester, MN). Colonic tone and responses to intraluminal distension were recorded as changes in colonic barostat balloon volume. When the barostat balloon was inflated to a constant pressure of 10 mmHg, contractions and relaxations of the colonic wall were recorded as decreased or increased intraballoon volume, respectively.

Colonic, high-amplitude propagating contractions (HAPCs) were defined as being ≥75 mmHg in amplitude (8, 19) and propagated for ≥15 cm. Colonic phasic pressure activity was averaged for three manometric sensors and expressed as the AUC. Fasting colonic tone and effects of drugs were calculated by separating barostat balloon volume (representing colonic tone) from phasic volume deflections >10 ml from baseline volume. These parameters of colonic motor activity were quantified over 60 min before, and 60 min after the administration of drugs.
Ileal Balloon Distension

Method. A 2.5-cm rubber balloon at the distal end of a Foley catheter was inflated with 10 ml of air for 2 min in the terminal ileum, 2.5 cm proximal to the ileocolonic junction (1).

Data analysis. Pressure recorded by the Dent sleeve within the ICS and the colonic barostat bag volume (see below) were compared for 5 min before and 1 min during ileal balloon distension. Results of ileal balloon distension were expressed as the percent change in ICS pressure during ileal distension compared with before distension.

Assessment of Colonic Pressure-Volume Relationships (Compliance)

Method. Because indexes of colonic tone and compliance are reproducible only after a conditioning distension has been performed (3, 15), intrabarostat balloon pressures were initially increased from 0 to 44 mmHg in 4-mmHg steps at 15-s intervals (3 min total duration of conditioning distension). Thereafter, a second stepwise pressure-volume curve was recorded by inflating the barostat balloon from 0 to 44 mmHg in 4-mmHg steps at 2-min intervals beginning 60 min after the saline or drug infusions were begun (total duration of pressure-volume relationship was 24 min).

Data analysis. Preliminary studies revealed that the colonic barostat balloon volume increased shortly after the intraballoon pressure was increased but stabilized during the last 30 s of each pressure increment. Colonic compliance curves were summarized as the average barostat balloon volume during the last 30 s against the corresponding pressure. Drug effects were assessed as the difference in barostat balloon volume at corresponding pressures during infusion of saline and drugs (i.e., L-NNA, L-arginine, L-NNA + L-arginine). We selected 8, 24, and 44 mmHg to represent low, mid, and high pressures, respectively, during the compliance curve. These differences were compared between extrinsically innervated and extrinsically denervated loops.

NADPH-Diaphorase Histochemistry

Method. These studies were performed in tissues obtained from the ileum and the proximal and distal colon in both groups in the functional studies, i.e., enteric isolated, extrinsically innervated loops (3 dogs), enteric isolated, and extrinsically denervated loops (3 dogs). Tissues from three neurally and enterically intact, nonoperated, naive dogs served as controls. Tissue samples ~1 × 1 cm were snap-frozen after procurement and stored at −70°C until use. The investigator performing the data analysis was blinded to the origin (control vs. extrinsic innervated vs. extrinsic denervated) of the tissues. Serial sections (10–12 μm thick) were cut on a cryostat, placed on glass microscope slides, and air-dried. Sections were fixed for 20 min with 4% paraformaldehyde in 0.1 M PBS, pH 7.4, and rinsed in PBS. Staining for NADPH-diaphorase, an indirect marker for NOS, was performed to identify NOS-containing nerves using standard techniques (10).

Data analysis. NADPH-diaphorase-stained tissue sections were examined with a Zeiss Axioplan 2 microscope equipped with a ×10 objective and AxioCam digital color camera. Selected fields of view were digitized and analyzed using KS400 image-analysis software. NADPH-diaphorase-stained structures were selected and segmented from background based on their blue color. Segmented areas were counted and expressed as number per unit area (mm²) of tissue. Three sections from each tissue, i.e., ileum and proximal colon, were analyzed. From each section, we quantified NADPH-diaphorase fibers in areas measuring 1.5–2 mm².

Statistical Analysis

Baseline or predrug indices of ileal and colonic phasic activity expressed as AUC, ICS (mean pressure, tone), and colonic barostat balloon volume were compared between extrinsically innervated and extrinsically denervated dogs by the two-sample t-test. Treatment effects were analyzed by an analysis of covariance (ANCOVA) fitting terms for groups (extrinsically innervated or denervated) and treatment. In each group, i.e., extrinsically innervated and denervated, three specific comparisons were examined in the overall ANOVA, i.e., saline versus L-NNA, saline versus L-arginine, and L-NNA versus L-NNA + L-arginine. Bonferroni correction was applied to correct for the multiple comparisons, yielding an adjusted P value of 0.017. The average NADPH-diaphorase fiber counts in three sections from each region, i.e., ileum and proximal colon, were compared across groups by an ANOVA, followed by the Tukey Kramer comparison between controls. All values are means ± SE.

RESULTS

As expected, all dogs developed diarrhea during the first 2–4 wk after the operation (38). Therefore, experiments were begun only after a 3-wk recovery period after diarrhea had resolved. Thereafter, the dogs remained healthy throughout the study with good appetites and stable body weights. To prevent stenosis, the stomas were dilated weekly with an inflated balloon (26-F). In one dog, fibrosis of the abdominal fascia around the ileal stoma necessitated a surgical procedure to enlarge the fascial opening.

Effect of Extrinsic Denervation on Baseline Motor Activity

Comparisons of baseline motor activity between innervated and extrinsically denervated dogs were based on qualitative interpretation and quantitative analysis of the 75-min duration before infusion of saline (2 studies in each dog) or drug (i.e., L-NNA, L-arginine, or L-NNA + L-arginine). Predrug ileal phasic contractile activity but not ICS pressure, colonic tone, or phasic activity were (P < 0.04) greater in extrinsically denervated compared with extrinsically innervated dogs (Table 1, Fig. 3). Because we did not synchronize the time of the beginning of each experiment or drug administration to the MMC, we did not analyze the characteristics of MMCs in extrinsically innervated or extrinsically denervated ileal loops.

Effect of L-NNA and L-Arginine on Ileal Motor Activity

L-NNA increased (P ≤ 0.001 vs. saline) ileal motor activity in innervated and extrinsically denervated loops (Table 2, Fig. 3). Ileal phasic contractile activity induced by L-NNA began within a few minutes and lasted for the duration of the study. Whereas L-NNA generally induced uncoordinated ileal phasic pressure
activity, prolonged propagated ileal contractions, followed by simultaneous colonic contractions were observed in one dog from each group. Although L-arginine alone did not affect ileal motor activity, it reversed the effects of L-NNA on ileal phasic activity (Table 2). This effect was greater in denervated, compared with extrinsically innervated, ileocolonic loops ($P = 0.04$ for comparison of L-arginine effects on extrinsically innervated vs. denervated loops after L-NNA).

### Effect of L-NNA and L-Arginine on ICS

L-NNA increased ($P = 0.001$) mean phasic pressures and tone in the ICS to a similar degree in extrinsically innervated and denervated loops (Table 2, Fig. 4). L-Arginine alone did not affect ICS pressure or tone.

#### Table 1. Baseline motor activity in extrinsically innervated and denervated canine ileocolonic loops

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Extrinsically Innervated Loops</th>
<th>Extrinsically Denervated Loops</th>
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<tbody>
<tr>
<td>Ileal phasic motor activity, log AUC</td>
<td>9.7(0.2)</td>
<td>10.2(0.1)*</td>
</tr>
<tr>
<td>Mean ICS pressure, mmHg</td>
<td>30.5(3.0)</td>
<td>39.9(3.2)</td>
</tr>
<tr>
<td>Colonic phasic motor activity, log AUC</td>
<td>8.2(0.6)</td>
<td>8.9(0.3)</td>
</tr>
<tr>
<td>Mean colonic balloon volume, ml</td>
<td>42.6(1.9)</td>
<td>39.4(1.2)</td>
</tr>
</tbody>
</table>

Data are means ± (SE) values for 20 studies, i.e., 5 studies in each dog, including 2 saline studies (on separate days) and studies with L-N^ω^-nitro-L-arginine (L-NNA), L-arginine (L-Arg), and L-NNA followed by L-Arg. *$P = 0.05$, 2-tailed $t$-test. AUC, area under curve; ICS, ileocolonic sphincter.

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**Fig. 3. Effect of L-NNA + L-arginine on fasting motor activity.**

A: extrinsically innervated ileocolonic loop. The arrow points to a migrating motor complex (MMC) in the ileum. L-NNA increased phasic pressure activity in the ileum (IL), ICS, and colon (C). L-NNA also increased ICS baseline pressure and reduced colonic barostat balloon volume, reflecting increased tone at these sites. L-arginine reversed colonic but not ileal motor activity induced by L-NNA. B: extrinsically denervated ileocolonic loop. Compared with A, there was more prominent ileal phasic activity in this tracing even before L-NNA was given. L-NNA increased non-propagated phasic activity, particularly in the colon. L-Arginine reversed the effects of L-NNA in the ileum and colon.
However, L-arginine reversed \( (P < 0.01) \) the effects of L-NNA on ICS pressure and tone and, to a greater extent \( (P \leq 0.03) \), in denervated, compared with extrinsically innervated, loops (Fig. 3).

**Effect of L-NNA and L-Arginine on Colonic Tone and Phasic Motor Activity**

In denervated loops, L-NNA increased nonpropagated colonic phasic pressure activity and reduced colonic barostat balloon volume \( (P < 0.03) \), reflecting increased colonic tone (Table 2, Fig. 3B). Whereas L-NNA increased colonic phasic pressure activity and tone in some dogs with extrinsically innervated loops (Fig. 3A), these effects were not statistically significant. L-NNA \( (P = 0.05) \) increased the number of colonic HAPCs in denervated (mean 8.75; range 2–22 HAPCs/60 min), but not extrinsically innervated (mean 0 HAPCs/60 min) loops (Table 3).

**Effect of Ileal Distension on the ICS and Colonic Tone**

In extrinsically innervated loops, ileal distension was accompanied by a reduction of ICS pressure before = 28 ± 5 mmHg, after = 9 ± 1 ml; \( P = 0.02 \), and an increase in colonic barostat balloon volume from 56 ± 2 to 75 ± 13 ml \( (P < 0.01) \), indicating colonic relaxation (Fig. 5). In contrast, ileal distension did not induce relaxation of the ICS (before = 22 ± 2 ml; after = 19 ± 2 ml) or colon (balloon volume before = 45 ± 2 ml, after = 41 ± 3 ml) in extrinsically denervated loops. Indeed, ileal distension in denervated loops often induced prolonged propagated contractions in the colon. After L-NNA, ileal distension was associated with visible relaxation of the ICS in two of three dogs with extrinsically innervated ileocolonic loops. However, the overall changes in ICS pressure (before = 39 ± 23 mmHg, after = 22 ± 4 mmHg; \( P = 0.3 \)) and colonic tone (balloon volume before = 39 ± 3 ml, after = 45 ± 3 ml; \( P = 0.1 \)) were not statistically significant. The fourth dog became restless and did not permit prolonged ileal distension.

**Effect of L-NNA and L-Arginine on ICS and Colonic Motor Activity During Colonic Distensions**

L-NNA reduced colonic barostat balloon volume during pressure-volume relationships in denervated, but not extrinsically innervated, loops (Fig. 6). However, stepwise colonic distension during assessment of pressure-volume curves was not accompanied by consistent changes in ICS pressure or tone. Neither L-NNA nor L-arginine modified the ICS response to colonic distension.

**Effect of L-NNA and L-Arginine on Hemodynamic Parameters**

The mean blood pressure did not change significantly after L-NNA infusion in dogs either with extrinsically innervated (mean blood pressure before = 122 mmHg, after = 121 mmHg) or extrinsically denervated loops (before = 111 mmHg, after = 122 mmHg).

**Immunohistochemistry**

Distribution of NADPH-diaphorase staining was similar to that reported by others for the canine gastrointestinal tract (9). Cell bodies and their processes in myenteric and submucous plexi and nerve fibers in the ICS were not modified by L-NNA or L-arginine.
the longitudinal and circular muscle layers of the ileum, proximal, and distal colon were labeled with NADPH-diaphorase. Enteric isolation was accompanied by increased NADPH-diaphorase staining in nerve fibers from the circular muscle layer of the ileum ($P < 0.003$) and proximal colon ($P < 0.005$) in both extrinsically innervated and denervated loops compared with controls (Fig. 7, Table 4). However, differences between extrinsically innervated and denervated loops were not statistically significant.

**DISCUSSION**

Acute colonic pseudoobstruction is named after Ogilvie (25), who erroneously attributed colonic dilatation to reduced sympathetic input to the bowel, leaving the parasympathetic action unopposed. Actually, the sympathetic nervous system inhibits colonic motility (5). Thus reflex sympathetic stimulation is a potential pathophysiological mechanism for paralytic ileus and acute colonic pseudo-obstruction. Acute colonic pseudo-obstruction is currently treated by supportive measures, the cholinesterase inhibitor neostigmine, or failing that, colonic decompression (4, 27). Although these measures are generally effective, it may be possible to develop even more effective and safer approaches based on a better understanding of the pathophysiology of the disorder.

This study examined the role of NO in regulating baseline motor activity and reflex responses to ileal distension, the cholinesterase inhibitor neostigmine, or failing that, colonic decompression (4, 27). Although these measures are generally effective, it may be possible to develop even more effective and safer approaches based on a better understanding of the pathophysiology of the disorder.

This study examined the role of NO in regulating baseline motor activity and reflex responses to ileal distension, the cholinesterase inhibitor neostigmine, or failing that, colonic decompression (4, 27). Although these measures are generally effective, it may be possible to develop even more effective and safer approaches based on a better understanding of the pathophysiology of the disorder.

**Table 3. Effect of l-NNA on colonic HAPCs in extrinsically innervated versus denervated loops**

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<th>Innervated</th>
<th>Denervated</th>
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<td>Difference in number of HAPCs</td>
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<tr>
<td>After saline</td>
<td>0.25 ± 0.25 (0-1)</td>
<td>1.25 ± 1.25 (0-5)</td>
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<tr>
<td>After l-NNA</td>
<td>0 (0)</td>
<td>8.8 ± 4.5 (2-22)*</td>
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Values are mean ± SE per 60 min, means ± SE; No. in parentheses is range. Difference in no. of high-amplitude propagating contractions (HAPCs) refers to the difference after or before saline or l-NNA. *$P = 0.05$ vs. effect of l-NNA in innervated loops.

**Fig. 6.** Effect of l-NNA on colonic compliance on individual dogs with extrinsically innervated and denervated loops. Negative values for the denervated preparations indicate lower balloon volume at corresponding pressure after l-NNA, compared with saline, reflecting reduced compliance and a "stiffer" colon. *$P < 0.015$, $#P = 0.05$.
and colonic distension in extrinsically innervated and extrinsically denervated canine ileocolonic loops. Our aims were to determine mechanisms regulating colonic relaxation dependent on and independent of extrinsic innervation to the proximal colon. The NOS inhibitor L-NNA increased fasting ileal and colonic motor activity, and markedly increased ICS alone, confirming that NO inhibits motor activity tonically in the ileum, ICS, and proximal colon (32, 34). L-NNA increased ileal motor activity to a similar degree in both preparations but reduced colonic compliance and induced more HAPCs in extrinsically denervated, compared with innervated, loops. The NADPH-diaphorase stain demonstrated more NOS-containing nerve fibers in the circular muscle layer of the canine ileum and colon after enteric isolation, regardless of extrinsic innervation.

Previous studies support the concept that extrinsic denervation associated with splanchnic ganglionectomy or small bowel transplantation is associated with enhanced nNOS expression in the small intestine (23, 36). Our results suggest that enteric isolation also increases the number of nerve fibers containing NOS, regardless of the state of extrinsic innervation, an observation not previously noted. Without using a universal stain for all nerve fibers, e.g., PGP 9.5, we cannot exclude the possibility that enteric isolation was accompanied by a generalized increase in all nerve fibers, as opposed to a selective effect on NOS-containing fibers. However, intestinal transection with reestablishment of bowel continuity did not affect NOS containing fibers in the rat ileum (36). Although molecular mechanisms regulating changes in expression of nNOS are unclear, disruption of the enteric nervous system by enterostomy may have been important. Thus neuronal disruption by lesions of the ventral root or parasympathetic denervation markedly increased nNOS expression in spinal cord motor neurons (39, 40) and the retina (40). Loss of the luminal milieu in the isolated loops (nutrients and secretions) acting via stimulation of afferent nerves may also have contributed to this manifestation of enteric isolation. However, the increased numbers of NADPH-diaphorase

<table>
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<th>Table 4. NADPH-diaphorase positive nerve fibers in muscle layers of ileocecal loop</th>
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<td>---------------------------------------------</td>
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<td><strong>Ileum</strong></td>
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<td><strong>Colon</strong></td>
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Values are in counts/mm² and are means ± SE, n = 9 (3 sections in each of 3 dogs) for each value. *Significantly different from control (P < 0.05).
fibers in our study may not necessarily indicate an increased release of NO; we did not measure the endogenous release of NO. Nevertheless, the presence of more NADPH-diaphorase-stained nerve fibers in the enterically isolated bowel suggests an increase in NOS activity in these tissues.

Ileal distension by inflation of an intraluminal balloon relaxed the canine ICS and colon in extrinsically innervated ileocolonic loops, confirming previous observations (1) and supporting the concept of motor coordination among the ileum, ICS, and proximal colon (11, 26, 30). This reflex was abolished by extrinsic denervation, but not L-NNA, indicating that ileal distension activated extrinsic pathways mediating intestinoinhibitory reflexes (37) that are not solely dependent on NO. In contrast, reflex relaxation ahead of peristaltic contraction is mediated by intrinsic neural pathways (14). Because colonic relaxation induced by ileal distension was not inhibited by adrenergic, nicotinic, or nitrigenic blockade (1), future studies should assess participation of other candidate nonadrenergic noncholinergic inhibitory neurotransmitters in this response (35).

Whereas extrinsic denervation blocked colonic relaxation during ileal distension, it had relatively modest effects on fasting ileal and colonic motor activity. Baseline fasting phasic activity in the ileum and colon and ICS pressure was greater in extrinsically denervated, compared with extrinsically innervated, loops. Phasic activity recorded by manometric sensors was generally nonpropagated. Although this was sometimes organized into discrete clusters, these usually were continuous, without intervening quiescent periods. We suspect that increased phasic activity after extrinsic denervation was due to diminished tonic-inhibitory sympathetic input and not to enteric isolation per se, which was common to both loops. Indeed, colonic and ileal tissue levels of catecholamines are markedly low or absent in our model of extrinsic denervation (38). Further studies are necessary to ascertain whether pharmacological restoration of α2-tonic-inhibitory sympathetic input by clonidine will restore normal phasic activity, thus confirming our hypothesis.

The NOS inhibitor N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) increased small intestinal motility in humans in a dose-dependent fashion (31). In our study, L-NNA increased ileal motor activity to a comparable extent in extrinsically innervated and denervated loops. L-NNA is 10 times more potent for blocking NOS than is L-NMMA (6). Thus our treatment was probably more potent than the highest dose of L-NMMA used in humans (31). L-Arginine completely reversed the colonic motor effects of L-NNA in extrinsically innervated and denervated loops, confirming that these effects were attributable to NOS inhibition. However, L-arginine, which is converted to NO by NOS, reversed the ileal motor effects of L-NNA to a greater extent when a smaller dose of the inhibitor was administered to human colonic smooth muscle strips (7). Alternatively, the reversal of the ileal motor effects of L-NNA by L-arginine in denervated loops may have been related to greater availability of NOS.

L-NNA often increased nonpropagated phasic pressure activity, but not HAPCs in extrinsically innervated loops, supporting the concept that NOS inhibition prevented relaxation induced by luminal distension, predominantly increased nonpropagated phasic activity, and delayed colonic transit in rats (22). However, the effect of L-NNA on colonic transit in extrinsically denervated loops, wherein it induced HAPCs, is unknown. L-NNA increased colonic stiffness to distension in denervated, but not intact, loops similar to its effects on colonic tone. In contrast to previous studies (26), colonic distension was not followed consistently by contraction of the ICS. The contrasting observations may be a function of differences in the level of colonic distension. Indeed, Quigley et al. (29) showed that the increment in ICS pressure induced by colonic balloon distension was related inversely to distension volume and the average colonic distension volume at our first step of 4 mmHg was greater than the distending volumes, i.e., 5 and 10 ml, which induced ICS contraction in the aforementioned study (29).

In summary, our functional data suggest that extrinsic denervation is associated with increased motor activity in the ileum, ICS, and colon, despite a dramatic increase in the number of NOS staining fibers. Extrinsic nerves mediate reflex relaxation of the ICS and colon during ileal distension, emphasizing the potential importance of sympathetically mediated colonic relaxation in disorders of severe colonic distension such as Ogilvie’s syndrome. Enteric isolation markedly augmented the number of NOS containing fibers in the circular muscle layer of the canine ileum and colon, with or without extrinsic denervation.

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