Nitrergic regulation of colonic transit in rats

YOHEI MIZUTA, TOKU TAKAHASHI, AND CHUNG OYWANG

Division of Gastroenterology, Department of Internal Medicine, University of Michigan Health System, Ann Arbor, Michigan 48109

Mizuta, Yohei, Toku Takahashi, and Chung Owyang. Nitrergic regulation of colonic transit in rats. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G275–G279, 1999.—Nitric oxide has been shown to be an inhibitory neurotransmitter in the mammalian colon, although its role in colonic transit remains unclear. We investigated the effect of the nitric oxide biosynthesis inhibitor N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME) on colonic transit in conscious rats. Colonic transit was determined by calculating the geometric center of the distribution of radiochromium instilled into the proximal colon. We also studied the effect of L-NAME on colonic motility in vivo and on descending relaxation in vitro. L-NAME (10 mg/kg) significantly delayed colonic transit compared with saline. The inhibitory effect of L-NAME was prevented by L-arginine (100 mg/kg) but not by D-arginine (100 mg/kg). L-NAME (10 mg/kg) induced random and uncoordinated phasic contractions throughout the rat colon in vivo. Luminal distension evoked descending relaxation in the proximal and distal rat colon in vitro. L-NAME (10 \textsuperscript{–4} M) significantly inhibited this relaxation. It is suggested, therefore, that nitric oxide enhances transit in the rat colon by mediating descending relaxation, which, in turn, facilitates propulsion of the colonic contents.

descending relaxation: geometric center; giant contraction; phasic contraction; N\textsuperscript{G}-nitro-L-arginine methyl ester

NITRIC OXIDE (NO) plays an important role in the mediation of nonadrenergic noncholinergic (NANC) relaxation of smooth muscle and in regulation of gastrointestinal motility. It has been suggested that NO is also involved in the regulation of gastric emptying (1, 23, 24) and in small intestinal transit (17). In colonic transit, however, the understanding of the physiological role of NO remains unclear.

The mid- and distal colons expel fecal bolus and display a pattern of intense peristaltic contractions and mass action associated with propulsion of dehydrated feces. Colonic transit is mediated by the migrating motor complex, a contractile pattern that propels colonic contents as it propagates aborally through the long segment of the mid- and distal colon (25). With the use of isolated mouse colon, it has been demonstrated that NO is involved in mediating colonic motor complexes (7). Furthermore, Ohta and colleagues (22) reported that inhibition of nitric oxide synthase (NOS) in the brain suppressed colonic motor activity in dogs.

Colonic transit is also regulated by colonic peristalsis in the mid- and distal colon, which is characterized by ascending contraction and descending relaxation. Descending relaxation allows rapid propulsion of a large bolus by a giant motor contraction and prevents the development of tone in the distal receiving segment, allowing the segment to accommodate the colonic contents propelled by ascending giant motor contractions in the mid- and distal colon (6, 25). It has been demonstrated that NANC inhibitory neurons, including NO, mediate descending relaxation (9, 11, 13).

The aim of this study is to clarify the role of NO in colonic motor activity and colonic transit in rats, with particular emphasis on descending relaxation in the colon.

MATERIALS AND METHODS

Colonic transit. Male Sprague-Dawley rats (220–270 g) were fasted overnight and anesthetized with an intramuscular injection of xylazine and ketamine (13 and 87 mg/kg body wt, respectively). An indwelling Silastic cannula was inserted into the cecum (1 cm proximal to the cecocolic junction) and positioned to enter the proximal colon (1 cm distal to the cecocolic junction), as previously described (2). This catheter was brought through the abdominal wall and led subcutaneously into the midscapular region. Three days after surgery, a nonabsorbable radioactive marker, radiochromium (0.2 ml, 0.5 \textmu Ci; Na\textsuperscript{51}CrO\textsubscript{4} in saline) (26), was instilled into the proximal colon through the indwelling Silastic catheter. The catheter was flushed with isotonic saline (0.3 ml). After 90 min, the entire colon was surgically removed under xylazine and ketamine anesthesia and divided into 10 equal segments. Each segment was placed into a vial, and the radioactivity in the stool was counted by a gamma counter for 1 min. The radioactivity in the stool was counted and referred to as segment 11. The geometric center of the distribution of \textsuperscript{51}Cr within the colon is the center of gravity for the distribution of radiochromium, and it is calculated using the following equation:

\[
\text{Geometric center} = \Sigma (\text{fraction of } \text{51}^{\text{Cr}} \text{ per segment} \times \text{segment number})
\]

The rats were divided into the following six groups according to the agents administered: 1) saline (0.1 ml), 2) N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME; 10 mg/kg), 3) N\textsuperscript{G}-nitro-L-arginine (L-NNA; 10 mg/kg), 4) L-NAME (10 mg/kg) + L-arginine (100 mg/kg), 5) L-NAME (10 mg/kg) + D-arginine (100 mg/kg), and 6) L-arginine (100 mg/kg). These doses were selected based on the previous studies in vivo (20, 30, 31). It has been shown that administration of L-NAME at 10 mg/kg to rats resulted in consistent inhibition of NO biosynthesis. No additional inhibition was observed with doses >20 mg/kg (14). L-NAME (10 mg/kg) has been shown to inhibit gastric emptying in rats (24). L-NNA (5 mg/kg) has also been shown to inhibit the transpyloric flow in conscious dogs (1). These agents were administered subcutaneously, followed by instillation of Na\textsuperscript{51}CrO\textsubscript{4} into the colon. It has been well demonstrated that Na\textsuperscript{51}CrO\textsubscript{4} is a nonabsorbable radioactive marker that is widely used for the measurement of gastric emptying, small intestinal transit, and colonic transit (2, 26).

Our preliminary studies showed that no \textsuperscript{51}Cr radioactivity was detectable in blood samples obtained from the portal vein. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
in either control or L-NAME-treated rats. Hence, it is unlikely that L-NAME affects Na\(^{51}\)CrO\(_4\) absorption, resulting in misinterpretation of transit data. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Every effort was made to minimize animal suffering and reduce the number of animals used.

In vivo recording of colonic motility. Rats were fasted overnight and anesthetized with an intramuscular injection of xylazine and ketamine. Extraluminal force transducers (6 × 3 mm) were implanted on the serosal surface of the proximal, mid-, and distal colon to monitor circular muscle contraction as previously described (27). The first transducer was sutured to the proximal colon, 1 cm from the border of the cecum and proximal colon. The second was sutured to the midcolon and the third to the distal colon, 2 cm from the pelvic brim. Body temperature was kept at 37°C with a heating pad. After 2 h of basal recording, L-NAME (10 mg/kg) was administered into the jugular vein.

The area under the curve was calculated using a computer-assisted system (Mac Lab; ADInstruments, Castle Hill, Australia) and expressed as the motility index. The area under the curve was evaluated for 10 min before and after the administration of L-NAME.

In vitro study of descending relaxation. Rats were fasted overnight. A xylazine and ketamine anesthetic was administered, and the entire colon was surgically removed. As previously described by Hata and colleagues (13), segments (3–4 cm) of the proximal and distal colon were held horizontally with a hook at the junction of the mesentery to an anchor fixed on the bottom of an organ bath containing Krebs-Henseleit buffer composed of the following (in mM): 118 NaCl, 4.8 KCl, 2.5 CaCl\(_2\), 25 NaHCO\(_3\), 1.2 KH\(_2\)PO\(_4\), 1.2 MgSO\(_4\), and 11 glucose. A rubber balloon connected to a syringe by a thin polyethylene tube was introduced into the lumen and positioned on the oral side of the segment. The balloon was inflated with warmed saline (0.1–0.3 ml) at 5-min intervals to produce local distension for 15 s. An isometric transducer recorded the mechanical response of the circular muscle, 1.0 cm anal to the center of the balloon. The colonic segments were maintained at a resting load of 1.5 g and equilibrated for 1 h. Descending relaxation in response to balloon distension was studied in the presence and absence of various antagonists.

Materials. The following materials were purchased: atropine sulfate, guanethidine, L-arginine, D-arginine, and TTX were purchased from Sigma (St. Louis, MO); L-NAME and L-NNA were from Research Biochemicals International (Natick, MA); and sodium chromate in sodium chloride solution (Na\(^{51}\)CrO\(_4\); 1 mCi/ml) was from Amersham (Arlington Heights, IL).

Statistical analysis. Results are expressed as means ± SE. Statistical analysis was performed by Student’s t-test or ANOVA with Tukey-Kramer multiple comparisons test. P values <0.05 were considered significant.

RESULTS

Effects of NO inhibitor on colonic transit in conscious rats. The geometric center of the distribution of \(51\)Cr in the saline-treated group was 5.4 ± 0.1 (n = 7). The geometric center of \(51\)Cr distribution in the L-NAME (10 mg/kg)-treated group was significantly less [3.6 ± 0.4 (n = 4, P < 0.01, ANOVA); Fig. 1]. The administration of L-NNA (10 mg/kg) also significantly inhibited the colonic transit, resulting in a lower geometric center (3.8 ± 0.1; n = 4, P < 0.01, ANOVA). The inhibitory effect of L-NAME on colonic transit was prevented by the simultaneous administration of L-arginine (100 mg/kg, geometric center = 5.4 ± 0.3; n = 4). In contrast, the simultaneous administration of L-arginine (100 mg/kg) failed to prevent the inhibitory effects of L-NAME on colonic transit (geometric center = 3.9 ± 0.3; n = 4, P < 0.01, ANOVA; Fig. 1). The administration of L-arginine alone did not significantly affect colonic transit (geometric center = 5.9 ± 0.4; n = 3).

Effects of NO inhibitor on colonic motility in anesthetized rats. Under basal conditions, there were high-frequency phasic contractions (5.6 ± 0.6 cycles/min) in the proximal colon of anesthetized rats. Giant contractions in the mid- and distal colon were characterized by the typical features of long duration (>30 s) and large amplitude (>10 g). The frequency of the giant contractions was 0.43 ± 0.09 cycles/min in the midcolon and 0.35 ± 0.04 cycles/min in the distal colon (Fig. 2). The motility index under basal conditions was 2,792 ± 420, 1,324 ± 334, and 1,632 ± 410 g·s in the proximal, mid-, and distal colon, respectively (means ± SE, n = 5).

Intravenous administration of L-NAME (10 mg/kg, bolus) significantly increased the basal tone and enhanced phasic contractions in the proximal colon (Fig. 2). In the mid- and distal rat colon, L-NAME increased the basal tone and evoked a change in the colonic motility pattern to one of phasic contractions (Fig. 2). L-NAME significantly increased the motility index throughout the entire colon: 378 ± 133% increase in the proximal colon, 266 ± 67% increase in the midcolon, and 259 ± 28% increase in the distal colon.

Effects of NO inhibitor on descending relaxation in vitro. In the proximal and distal colonic segments, local
distension induced muscle relaxation on the anal side. Of the 15 segments tested, descending relaxation was observed in 13 proximal colonic specimens and in 12 distal colonic specimens. In control experiments, increasing the inflation of the balloon (0.1–0.3 ml) caused descending relaxation in a volume-dependent manner. Atropine (10^−6 M) and guanethidine (10^−6 M) did not affect descending relaxation in response to balloon inflation. TTX (10^−6 M) completely abolished descending relaxation in response to a balloon inflation of 0.1–0.2 ml. In subsequent experiments, the effects of various antagonists on descending relaxation were investigated with the use of a 0.2-ml balloon inflation. L-NAME (10^−4 M) significantly inhibited descending relaxation by 74.2 ± 10.2% in the proximal colon (P < 0.01, n = 9) and by 59.9 ± 12.5% in the distal colon (P < 0.05, n = 9). L-NNA (10^−4 M) showed similar inhibitory effects on descending relaxation (data not shown). The addition of L-arginine (10^−3 M) to the bath prevented the inhibitory effects of L-NAME in the proximal and distal colon (Fig. 3).

**DISCUSSION**

It has been demonstrated that administration of the NO biosynthesis inhibitor, L-NAME, delays gastric emptying in dogs (1, 23) and rats (24). L-NAME also has an inhibitory effect on small intestinal transit in rats (17). The effect of L-NAME on colonic transit, however, remains unknown.

The geometric center calculated after ^51^Cr instillation provides a reliable measurement of colonic transit (2, 19, 21). Using this method, we investigated the effect of L-NAME on colonic transit in conscious rats. Colonic transit calculated as a geometric center was significantly delayed by L-NAME. The concomitant administration of L-arginine, but not D-arginine, prevented the inhibitory effect of L-NAME. Although it is thought that L-NAME may act as muscarinic antagonist (3), our present study showed that both L-NAME and L-NNA inhibited colonic transit to a similar degree. It has been demonstrated that L-arginine, but not D-arginine, reverses morphine-induced constipation (4). Pretreatment with L-NAME inhibits castor oil-induced diarrhea in rats (18). Foxx-Orenstein and Grider (8) showed that the velocity of pellet propulsion was inhibited in a concentration-dependent manner by L-NNA in the guinea pig colon in vitro. These observations suggest that NO mediates colonic motility. Our studies clearly indicate that NO plays an important role in the regulation of colonic transit, and our results offer an explanation for the inhibition by L-NAME of castor oil-induced diarrhea (18). It is important to acknowledge that the inhibitory effect of L-NAME on diarrhea may also be mediated by modulation of fluid secretion because NO stimulates intestinal electrolyte secretion in some pathophysiological states (15). We cannot rule out the possibility that altered intestinal secretion evoked by L-NAME may affect colonic transit.
The involvement of NO in the regulation of the colonic motor complex has been demonstrated recently (7, 22). Ohta and associates (22) have shown that intravenous infusion of l-NAME significantly enhanced colonic motor activity and increased the frequency of the colonic motor complex in conscious dogs. Fida and colleagues (7) also demonstrated that l-NAME increased the frequency of the colonic motor complex in mice in vitro. However, these studies do not explain the delayed colonic transit observed in our studies.

To further investigate the effects of l-NAME on colonic motility, we recorded contractions of the entire rat colon in vivo. The primary role of the proximal colon is to mix colonic contents and absorb fluid, whereas the mid- and distal colon propels and eliminates feces (25, 28). Phasic to-and-fro movements are dominant in the proximal colon, and propulsive movements and giant contractions are often observed in the mid- and distal colon. In anesthetized rats, the frequency of giant contractions in the midcolon is 0.3–0.4 cycles/min, and 25 to 30% of these contractions migrate to the distal colon (29). We have shown that l-NAME significantly changed this spontaneous pattern of motility in the mid- and distal colon, inducing a pattern of random phasic contractions. Therefore, it is quite likely that the impairment of coordinated motility evoked by l-NAME delays colonic transit.

It has been demonstrated that the peristaltic reflex, which is characterized by ascending contraction and descending relaxation, determines the propulsive velocity of intestinal contents (8). Therefore, it is likely that the inhibition of descending relaxation by l-NAME may contribute to the delayed colonic transit. Descending relaxation allows rapid propulsion of a large bolus by ascending giant motor contractions. This relaxation may also prevent the development of tone in the distal receiving segment so that it may accommodate the colonic contents propelled by ascending giant motor contractions (6, 25). Descending relaxation has been shown to be mediated by NANC inhibitory neurons. It has been demonstrated that ATP (5), vasoactive intestinal polypeptide (VIP) (9), pituitary adenylate cyclase activating peptide (PACAP) (10, 16), and NO (9, 13) released from NANC nerves mediate the descending relaxation in the colon. NO has also been reported to be produced from the smooth muscle cells in response to VIP and PACAP (9, 12, 16). Our in vitro study demonstrated that l-NAME inhibited descending relaxation in response to luminal distension in the rat colon. We confirmed that descending relaxation is mediated by NANC inhibitory neurons and showed that NO plays an important role in mediating descending relaxation in the rat colon. This suggests that the inhibitory effects of NO biosynthesis inhibitor on colonic transit can be explained, at least in part, by its inhibitory effects on the descending relaxation.

In conclusion, the NO biosynthesis inhibitor, l-NAME, significantly delayed colonic transit in rats. The present study strongly suggests that NO plays an important role in the coordination of propulsive motility and peristalsis in the rat colon.

This study was supported in part by a fund from Daiichi Pharmaceutical Co. Ltd., Tokyo, Japan (T. Takahashi).

Address for reprint requests and other correspondence: T. Takahashi, 6520 MSRB1, Univ. of Michigan Health System, Ann Arbor, MI 48109-0682 (E-mail: ttakahas@umich.edu).

Received 8 December 1998; accepted in final form 31 March 1999.

REFERENCES