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Mechanism of neurogenic relaxation and modification of the response by enteric substances in isolated dog colon

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Abstract

The mechanisms of neurogenic relaxation in the longitudinal muscle of the isolated canine colon and its modification by enteric substances were investigated. Relaxations induced by transmural electrical stimulation with electrical pulses, nicotine or K^+ in the muscle strips contracted with bradykinin and treated with atropine were attenuated but not abolished by N^G-nitro-L-arginine (L-NA), and the inhibition was reversed by L-arginine. Oxyhemoglobin and ouabain inhibited the response, whereas K^+ channel inhibitors, such as glibenclamide, tetraethylammonium, apamin and charybdotoxin, were without effect. In L-NA-treated strips, stimulation-induced relaxations were reduced by ouabain but not by oxyhemoglobin. Among substances tested, only norepinephrine, ATP, vasoactive intestinal peptide (VIP) and galanin produced relaxations. However, α- and β-adrenoceptor antagonists and aminophylline did not alter the response to nerve stimulation. In the strips made unresponsive to VIP and galanin, stimulation-induced relaxations were not influenced. Indomethacin, calcitonin gene-related peptide, cholecystokinin, peptide YY, substance P and serotonin did not modulate the neurogenic response. It is concluded that the relaxation associated with nerve stimulation is mediated by nitric oxide (NO) synthesized from L-arginine and also by substance(s) activating the electrogenic Na^+ pump but not that opening K^+ channels. Norepinephrine, ATP, VIP and galanin can be excluded as candidate inhibitory neurotransmitters, and the substances used so far are unlikely to modulate inhibitory nerve function. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Colon; Nitroxidergic nerve; Nicotine; Na^+ pump, electrogenic

1. Introduction

Mechanisms underlying non-adrenergic, non-cholinergic inhibitory neurotransmission in the gastrointestinal tract have been analyzed by electrophysiological, pharmacological and histological techniques with regard to the role of nitric oxide (NO) and neuropeptides (Lundberg, 1996). A contribution of NO to the inhibitory response to nerve stimulation was first defined using NO synthase inhibitors in the canine duodenum (Toda et al., 1990), together with a NO bioassay method for the canine ileocolonic junction (Bult et al., 1990). Accumulated data demonstrate that the involvement of neurogenic NO and peptides in the regulation of gastroenteric motility and tone differs according to location in the digestive tract and also the type of mammals concerned. In the rat stomach, relaxation caused by inhibitory nerve stimulation is associated with NO and vasoactive intestinal peptide (VIP) (Li and Rand, 1990; Lefebvre, 1993), while in the rat ileum, the electrical stimulation-induced contraction is blunted by NO synthase inhibitors, suggesting that NO derived from nerves mediates contraction or changes the release of other neurotransmitters (Bartho et al., 1992). NO is mainly involved in the neurogenic relaxation of longitudinal muscle of the proximal segment of the rat colon, whereas VIP is a likely neurotransmitter in the distal colon (Suthamnatpong et al., 1993). Nitroxidergic nerves may be involved only partially in the stimulation-induced relaxation in the circular muscle of the human ileum (Maggi et al., 1991).

We have reported that neurogenic relaxation of the canine duodenum (Toda et al., 1990, 1991, 1992) and the sphincter of Oddi (Tanobe et al., 1995) is mediated exclusively by NO or its stable analog, such as *S*-nitroso thiol, that possibly activates guanylate cyclase in smooth muscle and increases the production of cyclic GMP (Toda et al., 1992). However, our preliminary study of the isolated canine colon suggested that relaxations induced by nerve

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stimulation are mediated not only by NO but also by other inhibitory factors.

The present study was therefore undertaken to analyze the mechanism of the relaxation induced by nerve stimulation with electrical pulses, nicotine and K^+ in longitudinal muscles of the proximal colon from dogs, in comparison with mechanisms underlying the neurogenic response of the canine duodenum and sphincter of Oddi. We also sought whether enteric substances, such as VIP, cholecystokinin (CCK), calcitonin gene-related peptide (CGRP), peptide YY, galanin, norepinephrine and serotonin, participate in and/or modulate the inhibitory response to nerve stimulation.

2. Materials and methods

2.1. Preparation

Mongrel dogs of either sex, weighing 7 to 14 kg, were anesthetized with intraperitoneal injections of sodium pentobarbital (50 mg/kg) and killed by bleeding from the carotid arteries. Proximal portions of the ascending colon were rapidly removed, and longitudinal muscle strips of approximately 10 mm were prepared. The specimens were fixed vertically between hooks in a muscle bath containing modified Ringer–Locke solution, which was aerated with a mixture of 95% O_2 and 5% CO_2 and maintained at 37 ± 0.3°C. The hook anchoring the upper end of the strips was connected to the lever of a force-displacement transducer. Some of the strips were placed between stimulating electrodes (Toda et al., 1991). Electrical pulses of 0.2 ms duration with a supramaximal intensity at frequencies of 2, 5 and 10 Hz for 10 s were transmurally applied to stimulate intramural nerve terminals. The resting tension was adjusted to 1.5 g, which was optimal for inducing maximal contraction. Constituents of the solution were as follows (mM): NaCl 120, KCl 5.4, $CaCl_2$ 2.2, $MgCl_2$ 1.0, $NaHCO_3$ 25.0, and dextrose 5.6. The pH of the solution was 7.37 to 7.43. Before the start of experiments, all the strips were allowed to equilibrate in the bathing media for 60 to 90 min, during which time the media were replaced every 10 to 15 min.

2.2. Tension recording

Isometric contractions or relaxations were displayed on an ink-writing oscillograph. The contractile response to 5 mM Ba^{2+} was first obtained, then the strips were washed three times with fresh medium and equilibrated for 30 to 40 min. To examine relaxant responses, the strips were precontracted with bradykinin (3×10^{-9} to 10^{-8} M); the contractions were in a range between 50 and 67% of the contraction induced by 5 mM Ba^{2+}. Unless otherwise mentioned, transmural electrical stimulation at 5 Hz, 10^{-4} M nicotine or 10 mM K^+ was applied. At the end of each series of experiment, papaverine (10^{-4} M) was applied to obtain the maximal relaxation; relaxations induced by nerve stimulation, nicotine, K^+, and other relaxing compounds are shown as percentages of those caused by papaverine. Acetylcholine, norepinephrine, ATP and galanin were applied cumulatively to the bathing media to make concentration–response curves. Contractions associated with acetylcholine or transmural stimulation were expressed as values relative to Ba^{2+} (5 mM)-induced contractions. The muscle strips were treated for about 20 min with blocking agents before the response to agonists or nerve stimulation was obtained. Some strips were made unresponsive to VIP or galanin by repeated application of the peptide (10^{-8} M VIP or 10^{-7} M galanin, three to five times).

2.3. Drugs used

The results shown in the text, tables and figures are expressed as mean values ± S.E. Statistical analyses were made using Student's unpaired *t*-test and Tukey's test after one-way analysis of variance. Drugs used were N^G-nitro-L-arginine (L-NA), bradykinin, substance P, VIP, CGRP, peptide YY, CCK, charybdotoxin (Peptide Institute, Minoh, Japan), galanin (Peninsula Lab., San Carlos, CA, USA), L-arginine, yohimbine, tetraethylammonium, nicotine (base) (Nacalai Tesque, Kyoto, Japan), acetylcholine chloride (Daiichi, Tokyo, Japan), atropine sulfate (Tanabe, Tokyo, Japan), timolol maleate (Banyu, Tokyo, Japan), phentolamine mesylate (Novartis-Pharma, Takarazuka, Japan), prazosin hydrochloride (Wako, Osaka, Japan), aminophylline, indomethacin, apamin, glibenclamide, ATP (Sigma, St. Louis, MO, USA), ouabain octahydrate (E. Merck, Darmstadt, Germany), hexamethonium bromide (Yamanouchi, Tokyo, Japan), norepinephrine hydrochloride, tetrodotoxin (Sankyo, Tokyo, Japan), and papaverine hydrochloride (Dainippon, Osaka, Japan). Responses to NO were obtained by adding $NaNO_2$ solution adjusted to pH 2 (Furchgott, 1988); concentrations of acidified $NaNO_2$ solution are given in terms of NO. Oxyhemoglobin was prepared from dog hemoglobin (Sigma) by the method described by Martin et al. (1985).

3. Results

3.1. Effects of transmural electrical stimulation

In longitudinal strips of the canine proximal colon under resting conditions, transmural electrical stimulation at frequencies of 2, 5 and 10 Hz for 10 s produced a frequency-related contraction; mean values relative to contractions induced by 5 mM Ba^{2+} were 3.3 ± 0.7, 8.0 ± 2.5 and 14.7 ± 1.8%, respectively ($n = 5$), which were abolished by 3×10^{-7} M tetrodotoxin. The strips responded to acetylcholine at concentrations of 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M with contractions averaging 0.1 ± 0.1, 24.5 ± 4.5, 60.4 ± 7.3 and 77.2 ± 6.9%, respectively ($n = 7$).

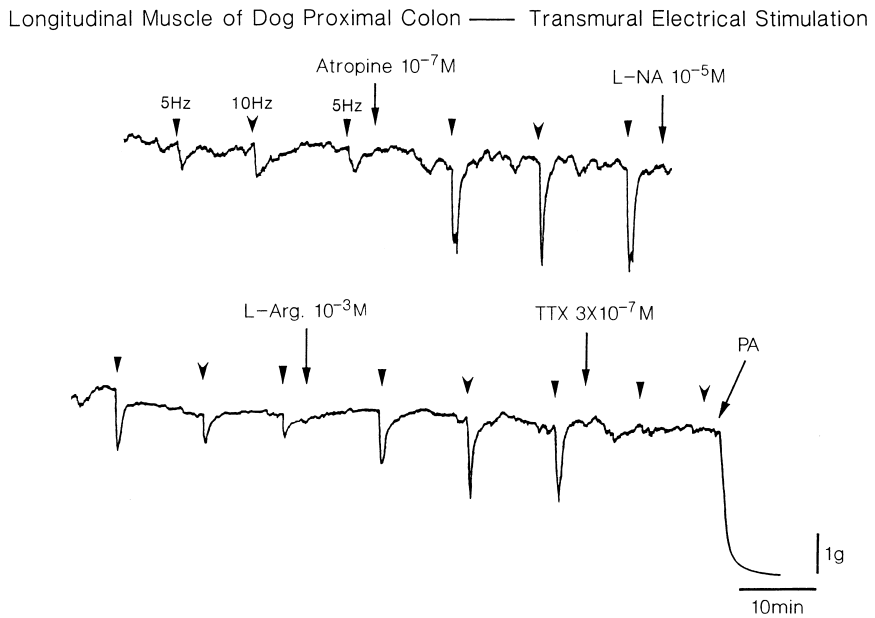

Fig. 1. Typical responses to transmural electrical stimulation (5 and 10 Hz) of a colon strip contracted with bradykinin, as affected by atropine, L-NA, L-arginine (L-Arg.) and tetrodotoxin (TTX). PA represents 10^{-4} M papaverine that produced the maximal relaxation.

When the colon strips were partially contracted with bradykinin (3×10^{-9} to 10^{-8} M), electrical nerve stimulation (2, 5 and 10 Hz) elicited no or slight contractions followed by relaxations of 7.1 ± 2.9, 16.9 ± 2.9 and $29.3 \pm 3.5\%$ ($n = 6$), respectively, relative to those induced by 10^{-4} M papaverine. Treatment with 10^{-7} M atropine abolished the contraction if any and potentiated the relaxant response to nerve stimulation from 13.1 ± 1.2 to $55.1 \pm 2.4\%$ at 5 Hz ($n = 7$, $P < 0.001$, unpaired t-test). Since the relaxation caused by electrical stimulation at 10 Hz was potentiated to a magnitude similar to that obtained at 5 Hz, and since the physiological frequency of neurotransmission has been considered to be less than 10 Hz, mechanisms of relaxation induced by the submaximal frequency of nerve stimulation (5 Hz) were analyzed in strips contracted with bradykinin and treated with atropine in the remainder of this study.

Relaxations induced by transmural nerve stimulation ($51.6 \pm 3.2\%$, $n = 6$) were significantly attenuated by treatment with L-NA (10^{-5} M) ($24.2 \pm 4.3\%$, $P < 0.01$, Tukey's test) and L-arginine (10^{-3} M) reversed the inhibition ($40.5 \pm 4.9\%$). Typical tracings of the response as affected by L-NA and L-arginine are shown in Fig. 1. The stimulation-induced response was not depressed further by increasing the concentration of L-NA to 10^{-4} M ($n = 3$) but was abolished by tetrodotoxin (3×10^{-7} M). Modifications by other pharmacological antagonists or agonists of the response to nerve stimulation are summarized in Table 1. CGRP at 10^{-8} M produced slight relaxations in one out of six experiments, but no significant relaxations in the remaining experiments. The stimulation-induced response was not affected by increasing the concentration of CGRP to 3×10^{-8} M ($n = 3$). CCK, peptide YY, substance P and serotonin at the concentrations used in this study did not produce any relaxations but slight contractions (less than 10%). The stimulation-induced response was not affected by increasing the concentrations of CCK, peptide YY, substance P and serotonin to 3×10^{-8} M ($n = 2$), 10^{-7} M ($n = 2$), 10^{-8} M ($n = 2$) and 10^{-7} M ($n = 2$), respectively. Among the antagonists used, only oxyhemoglobin and ouabain were effective to significantly inhibit the response. The strips responded only to norepinephrine,

Table 1
Modification by antagonists and agonists of the relaxant response to transmural electrical stimulation (5 Hz) of dog colon strips

Treatment	n	Control	Experimental
OxyHb 1.6×10^{-5} M	6	47.4 ± 1.6	25.4 ± 3.9[a]
Phentolamine 10^{-7} M	5	43.4 ± 4.0	41.8 ± 4.2
Timolol 10^{-7} M	6	45.2 ± 1.7	47.2 ± 1.7
Aminophylline 5×10^{-5} M	6	42.5 ± 3.0	42.0 ± 3.4
Indomethacin 10^{-6} M	5	41.6 ± 8.9	40.9 ± 8.7
Ouabain 10^{-6} M	6	49.1 ± 3.4	28.2 ± 4.6[a]
TEA 10^{-3} M	6	42.3 ± 6.6	50.9 ± 6.6
Apamin 5×10^{-7} M	5	43.3 ± 1.7	40.7 ± 2.7
Charybdotoxin 10^{-7} M	3	58.8 ± 4.5	54.0 ± 3.2
Glibenclamide 3×10^{-5} M	6	48.5 ± 5.6	41.0 ± 6.3
Galanin 10^{-7} M (tachyphylaxis)	7	40.8 ± 4.1	42.2 ± 4.0
VIP 10^{-8} M (tachyphylaxis)	6	47.0 ± 2.8	46.9 ± 3.0
CGRP 10^{-8} M	6	49.6 ± 5.4	48.8 ± 5.7
CCK 10^{-8} M	4	50.1 ± 6.8	51.2 ± 7.1
PYY 10^{-8} M	4	40.2 ± 3.6	40.0 ± 5.4
Substance P 10^{-9} M	5	45.7 ± 4.4	47.2 ± 4.6
Serotonin 10^{-8} M	5	47.5 ± 4.5	48.0 ± 3.9

n, number of strips.
Tachyphylaxis strips made unresponsive to the peptide by repeated application.
[a] Significantly different from control, $P < 0.01$ (unpaired t-test).

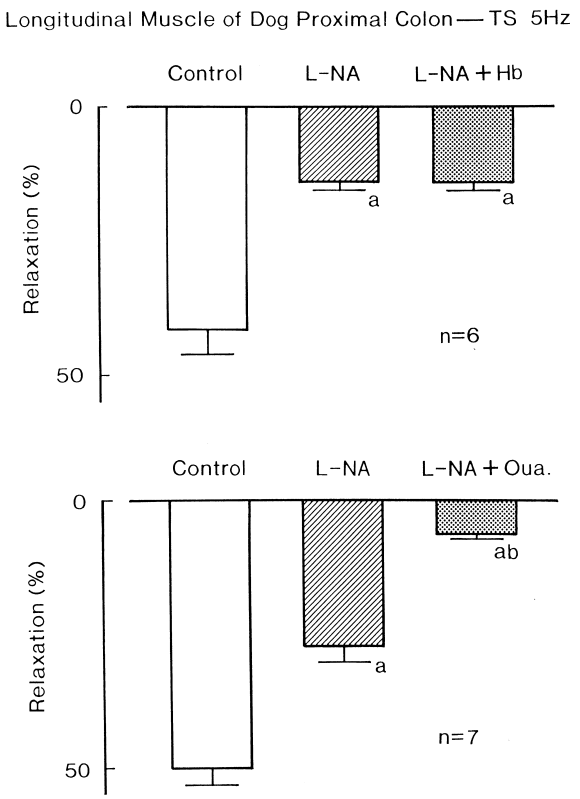

Fig. 2. Modification by L-NA (10^{-5} M) and L-NA + oxyhemoglobin (Hb, 1.6×10^{-5} M) (top panel) and L-NA and L-NA + ouabain (Oua., 10^{-6} M) (bottom) of the response to transmural electrical stimulation (TS, 5 Hz) of the longitudinal muscle of the canine proximal colon contracted with bradykinin and treated with atropine. Relaxations induced by 10^{-4} M papaverine were taken as 100%. Significantly different from control, $^{a}P < 0.01$; significantly different from the value with L-NA, $^{b}P < 0.01$ (Tukey's test). *n* denotes the number of strips from individual dogs. Vertical bars represent S.E.

ATP, galanin and VIP with relaxations. The α- and β-adrenoceptor antagonists and aminophylline were ineffective, and the stimulation-induced relaxation was not reduced in the strips made unresponsive to galanin (10^{-7} M) or VIP (10^{-8} M) by repeated applications (3–5 times) of the peptides.

Despite the significant attenuation by oxyhemoglobin (1.6×10^{-5} M) of the response, this NO-scavenging agent did not produce additional inhibition in L-NA-treated strips (Fig. 2, top panel). On the other hand, ouabain (10^{-6} M) significantly impaired the response in the strips in which a partial inhibition had been induced by L-NA (Fig. 2, bottom). Ouabain at the same concentration did not affect the relaxant responses to ATP, norepinephrine and NO ($n = 4$). In four strips from separate dogs, the stimulation-induced relaxation ($54.7 \pm 3.8\%$) was attenuated by L-NA to $34.7 \pm 3.9\%$ ($P < 0.02$ vs. control, unpaired *t*-test) and then was suppressed by lowering of external K^+ (1/10 of the concentration in control media) to $16.2 \pm 2.9\%$ ($P < 0.01$ vs. the value with L-NA).

3.2. Effects of nicotine and K^+

In bradykinin-contracted colon strips, K^+ (10 mM) and nicotine (10^{-5} M) caused reproducible relaxations. Atropine (10^{-7} M) potentiated the nicotine-induced relaxation from 16.7 ± 2.5 to $50.2 \pm 3.3\%$ ($n = 19$, $P < 0.001$, unpaired *t*-test), but did not alter the response to K^+ (71.4 ± 2.4 vs. $72.1 \pm 2.3\%$, $n = 8$). The subsequent experiments were carried out in strips treated with atropine.

The responses were partially inhibited by L-NA (10^{-5} M) and reversed by L-arginine (10^{-3} M) (Fig. 3). Additional inhibition was not observed on application of 10^{-4} M L-NA ($n = 3$ for K^+ and nicotine), but the responses were abolished by 3×10^{-7} M tetrodotoxin, as shown in Fig. 4. The K^+-induced relaxation was reduced by ouabain (10^{-6} M) from 67.2 ± 4.5 to $30.4 \pm 3.0\%$ ($n = 9$, $P < 0.001$, unpaired *t*-test) and additional treatment with L-NA (10^{-5} M) further depressed the response to $8.8 \pm 3.6\%$ ($P < 0.001$ vs. the value with ouabain). Similar results were obtained with nicotine; the values in control media and those containing ouabain and ouabain plus L-NA were 56.1 ± 4.3, 27.0 ± 6.6 ($n = 10$, $P < 0.01$ vs. control, un-

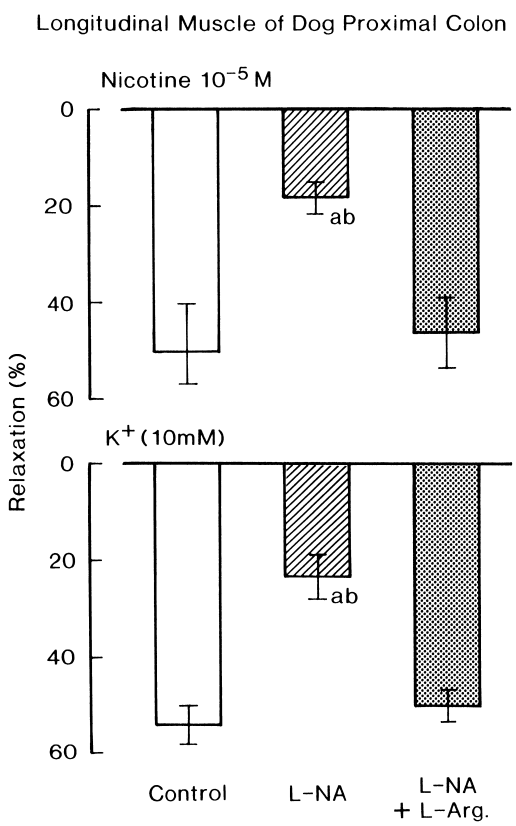

Fig. 3. Modification by L-NA (10^{-5} M) and L-NA + L-arginine (L-Arg., 10^{-3} M) of the response to nicotine (10^{-5} M) (top panel) and K^+ (10 mM) (bottom) of the longitudinal muscle of the canine proximal colon contracted with bradykinin and treated with atropine. Relaxations induced by 10^{-4} M papaverine were taken as 100%. Significantly different from control, $^{a}P < 0.01$; significantly different from the value with L-NA + L-arginine, $^{b}P < 0.01$ (Tukey's test). Number of strips from individual dogs were nine for studies with nicotine and K^+. Vertical bars represent S.E.

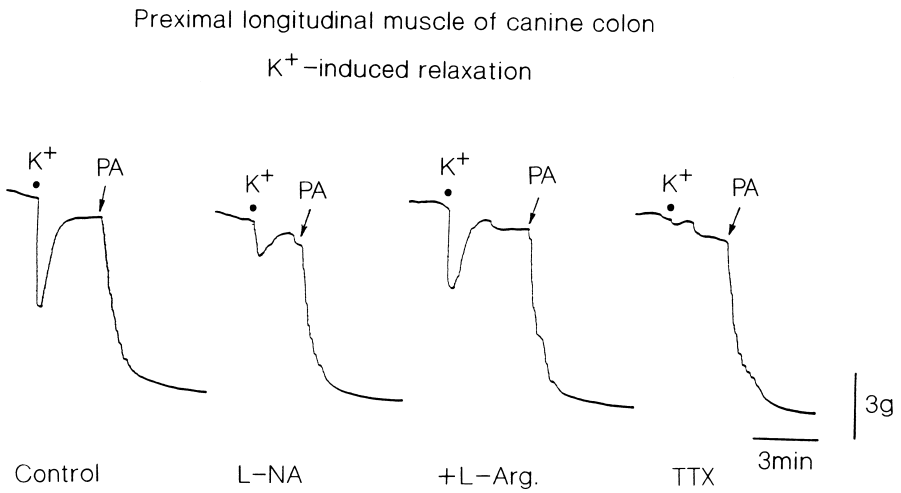

Fig. 4. Tracings of the response to K^+ (10 mM) of a colon strip contracted with bradykinin and treated with atropine, as affected by L-NA (10^{-5} M), L-NA + L-arginine (L-Arg., 10^{-3} M) and tetrodotoxin (TTX, 3×10^{-7} M). PA represents 10^{-4} M papaverine that produced the maximal relaxation.

paired *t*-test) and $8.2 \pm 3.7\%$ ($n = 10$, $P < 0.05$ vs. the value with ouabain), respectively. The nicotine-induced relaxation was abolished by 10^{-5} M hexamethonium ($n = 5$). NO, exogenously applied as acidified $NaNO_2$ solution (10^{-6} to 3×10^{-4} M), induced a concentration-dependent relaxation which was not influenced by treatment with L-NA but was abolished by oxyhemoglobin (Fig. 5).

3.3. Effects of norepinephrine, ATP and galanin

Bradykinin-contracted colon strips responded to norepinephrine (5×10^{-9} to 10^{-5} M) with a concentration-related relaxation (Fig. 6, left panel). This amine, at a concentration (2×10^{-7} M) that induced approximately half-maximal responses, rapidly relaxed the strips and the tone was gradually recovered and stabilized at a level lower than that prior to the addition of norepinephrine. The phasic (A in the Fig. 6, top right panel) and tonic (B) responses were unaffected by L-NA (Fig. 6, bottom right panel). Timolol (10^{-7} M) attenuated the tonic response (40.8 ± 6.2 to $21.8 \pm 5.7\%$, $n = 7$, $P < 0.05$, unpaired *t*-test), whereas prazosin (10^{-7} M) depressed the phasic relaxation (50.0 ± 3.8 to $12.1 \pm 2.7\%$, $n = 6$, $P < 0.001$). Yohimbine (10^{-7} M) was without significant effect ($n = 6$).

ATP (10^{-6} to 10^{-5} M) concentration dependently relaxed the colon strips. The response was not influenced by

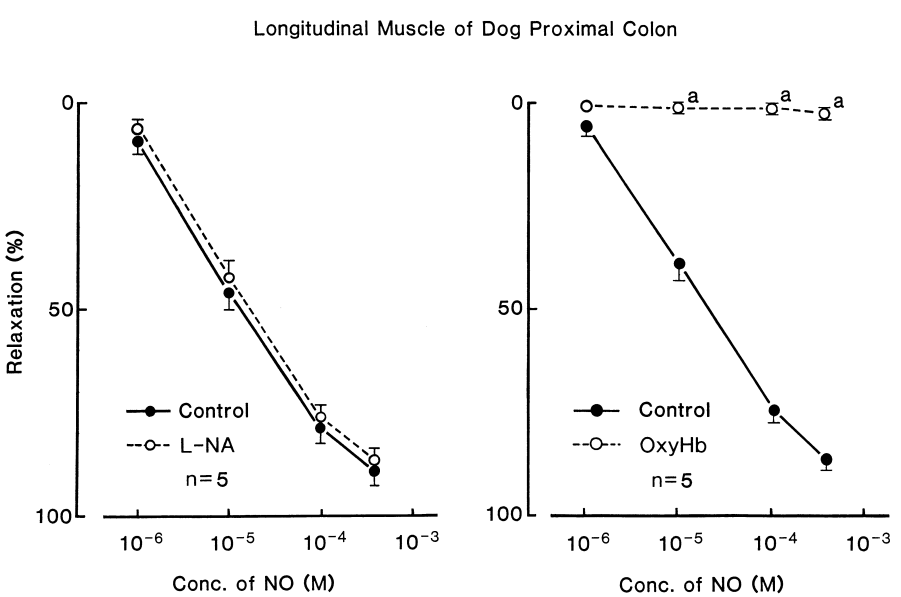

Fig. 5. Modification by L-NA (10^{-5} M) (left panel) and oxyhemoglobin (OxyHb, 1.6×10^{-5} M) (right) of the response to NO of the longitudinal muscle of the canine proximal colon contracted with bradykinin. Relaxations induced by 10^{-4} M papaverine were taken as 100%. Significantly different from control, $^aP < 0.001$ (unpaired *t*-test). *n* denotes the number of strips from individual dogs. Vertical bars represent S.E.

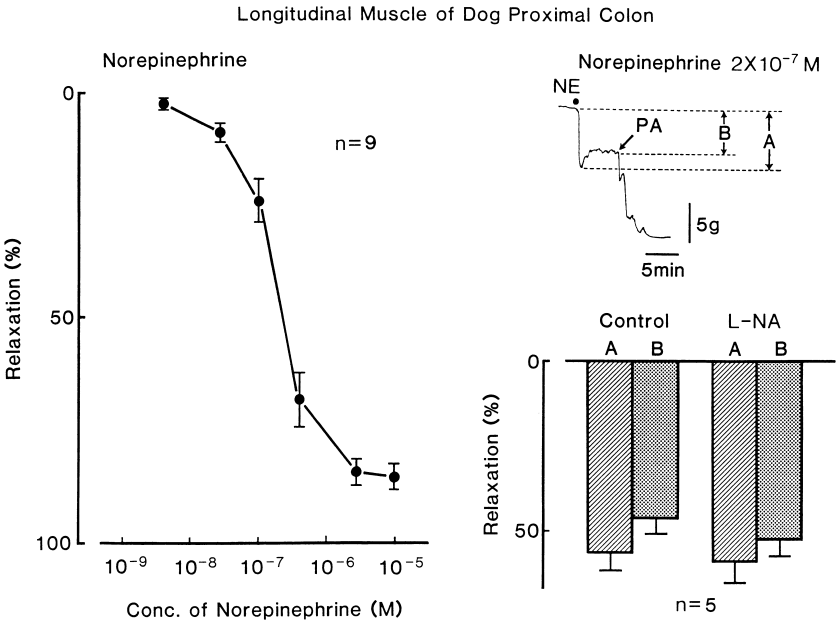

Fig. 6. Dose–response curve for norepinephrine (left panel) and modification by L-NA (10^{-5} M)(bottom right) of the phasic (A in the top right panel) and tonic (B) responses to the amine of longitudinal muscles of the canine proximal colon. The strips were contracted with bradykinin and treated with atropine. Relaxations induced by 10^{-4} M papaverine were taken as 100%. Vertical bars represent S.E.

treatment with L-NA (10^{-5} M, $n = 5$) or apamin (5×10^{-7} M, $n = 4$) but was significantly inhibited by 5×10^{-5} M aminophylline; mean values before and after the treatment were 14.1 ± 1.9 and 0 ($n = 6$, $P < 0.05$, unpaired t-test), respectively, at 10^{-6} M ATP, and those were 41.2 ± 4.3 and $18.4 \pm 2.8\%$ ($n = 6$, $P < 0.01$), respectively, at 10^{-5} M ATP.

Galanin (10^{-9} to 10^{-7} M) relaxed the strips in a concentration-related manner, the maximal relaxation at 10^{-7} M being $69.3 \pm 6.2\%$ ($n = 5$). L-NA did not influence the response ($n = 5$).

4. Discussion

In the longitudinal muscle of the canine proximal colon contracted with bradykinin, relaxations induced by electrical stimulation and nicotine were potentiated by atropine, indicating that the cholinergic excitatory response blunts the neurogenic relaxation. On the other hand, the relaxation elicited by high K^+, possibly due to depolarization of neuronal membranes and the generation of action potentials, is not influenced by atropine. Similar findings were also obtained in canine duodenal longitudinal muscles (Toda et al., 1992). The stimulation of inhibitory, but not excitatory, nerves by high K^+ cannot be explained.

Bradykinin-contracted, atropine-treated colon strips responded to electrical stimulation, nicotine and K^+ with moderate relaxations, which were significantly inhibited but not abolished by L-NA (10^{-5} M), and L-arginine restored the response. The relaxation remaining in the response to electrical stimulation with L-NA treatment was not inhibited further by raising the L-NA concentration to 10^{-4} M or by the addition of oxyhemoglobin, an NO scavenger (Martin et al., 1985), in a concentration sufficient to significantly inhibit the response under control conditions. Inhibitory junction potentials evoked by single pulses of electrical field stimulation in the canine proximal colon are also attenuated but not abolished by 10^{-4} M L-NA methyl ester (Dalziel et al., 1991). Relaxing factor(s) other than NO seem to be involved in the stimulation-induced relaxation. Glibenclamide, an ATP-sensitive K^+ channel inhibitor, and tetraethylammonium, apamin and charybdotoxin, Ca^{2+}-dependent K^+ channel inhibitors, failed to inhibit the neurogenic relaxation. On the other hand, apamin blocks the first, fast phase of the inhibitory junction potential in the circular muscle of the guinea-pig colon in response to electrical field stimulation, and L-NA inhibits the second, late phase of the junction potential (Zagorodnyuk and Maggi, 1994). Electrical stimulation evokes a relaxation of circular muscle strips of the rabbit and human colon that is partially reduced by L-NA; apamin also attenuates the response (Smith and Muir, 1991; Boeckxstaens et al., 1993). According to Thornbury et al. (1991), inhibitory junction potentials due to electrical stimulation of the canine proximal colon and to NO donors are mediated by NO that increases the open probability of Ca^{2+}-activated K^+ channels. In the present study, however, there was no significant attenuation of the neurogenic relaxation by Ca^{2+}-activated K^+ channel inhibitors. Inhibition of mechanical, but not electrical, activity is likely to be associated with cyclic GMP increased by NO (Toda et al., 1992), but not with K^+ channel opening. Norepinephrine, ATP, VIP and galanin relaxed the colon strips

under the experimental conditions used. However, α- and β-adrenoceptor antagonists, aminophylline and indomethacin in concentrations sufficient to depress the relaxant response to the amine and ATP and the production of prostanoids (Miyazaki et al., 1985), respectively, did not impair the neurogenic response, and the response to nerve stimulation in the strips made unresponsive to VIP or galanin was unaltered. The amount of norepinephrine and ATP liberated from adrenergic nerves appears to be insufficient to evoke relaxation of the colon. Boeckxstaens et al. (1993) have reported both that ATP induces relaxations of the circular muscle of the human colon that are inhibited by apamin, and that the inhibitory nerve-mediated relaxation resistant to L-NA is reduced by apamin, suggesting that NO and ATP are involved in the neurogenic response. However, this does not seem to have been the case in the present study, in which apamin did not inhibit the stimulation-induced relaxation, and aminophylline inhibited the ATP-induced relaxation but not the response to nerve stimulation. VIP reportedly acts as a neurotransmitter in the rat gastric fundus (Curro and Preziosi, 1997). According to Keef et al. (1994), VIP and NO are possible co-transmitters in the canine colon; however, evidence for neurogenic VIP acting as an inhibitory neurotransmitter is lacking. Galanin may inhibit neural excitation in the canine pylorus (Allescher et al., 1989). The present study showed that these peptides can be excluded as transmitter candidates.

The relaxation associated with electrical nerve stimulation, nicotine and K^+, resistant to a NO synthase inhibitor and to NO scavengers, was depressed by treatment with ouabain and by lowering of external K^+, both known to inactivate the Na^+,K^+-activated ATPase. Inhibition by ouabain of the neurogenic relaxation is not due to a non-specific mechanism, since relaxations induced by exogenous ATP, norepinephrine and NO were not influenced. It has been demonstrated that hyperpolarization due to endothelium-derived factors released by acetylcholine in isolated canine coronary arteries is suppressed by ouabain, suggesting that the Na^+ pump is involved in the response (Feletou and Vanhoutte, 1988). Substance(s) liberated from inhibitory nerve terminals, although not identified, are expected to activate the electrogenic Na^+ pump and hyperpolarize the membrane, resulting in relaxation of the colon. The possibility remains that ouabain may influence the neural side and stimulate the release of neurotransmitters from the constrictor nerves, but involvement of cholinergic nerve function in the response can be excluded, since cholinergic function was suppressed by treatment with a sufficient concentration of atropine. In addition, neurogenic relaxation caused by nicotine is not affected by ouabain in canine cerebral arteries (Toda, 1975).

Calcitonin gene-related peptide produced relaxation inconsistently, and CCK, peptide YY, substance P and serotonin did not produce relaxations in colon strips, thus being excluded as candidate inhibitory neurotransmitters. In addition, these substances did not significantly modify the response to electrical nerve stimulation. Therefore, as far as the experimental conditions applied in the present study are concerned, the substances are unlikely to act pre- and postjunctionally and regulate either the synthesis and/or release of NO and other relaxing neurotransmitters or their actions on intestinal smooth muscle.

Norepinephrine concentration dependently relaxed the longitudinal muscle of colon strips, and the relaxation induced by the amine in a median effective concentration was separated into two responses, one rapidly developing (phasic) and the other, sustained (tonic). The phasic response was attenuated by timolol, and the tonic response was suppressed by prazosin but not by yohimbine, suggesting that the responses are mediated by β- and α_1-, but not α_2-, adrenoceptors, respectively. Although exogenously applied norepinephrine is a potent relaxant in this tissue, effective concentrations of the amine do not seem to be liberated from adrenergic nerves under the experimental conditions used.

The mechanisms underlying the relaxation induced by nerve stimulation in canine proximal colon, duodenum and sphincter of Oddi differ. It appears that both NO and substance(s) activating the electrogenic Na^+ pump are involved in relaxation of the colon, whereas only NO contributes to the response of the duodenum and sphincter (Toda et al., 1991, 1992; Tanobe et al., 1995). The cholinergically induced contraction is greater in the duodenum (Toda et al., 1991) than in the colon, but no contraction is elicited by cholinergic nerve stimulation in the sphincter of Oddi (Tanobe et al., 1995). Differential regulation by inhibitory and excitatory nerves of the motility and tone of intestinal smooth muscle may contribute to a reasonable regulation of the passage of contents. Intramural humoral factors may act in concert with neural factors for physiological regulation, although the endogenous substances used so far did not modify inhibitory nerve functions.

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