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Differences in mediator of nonadrenergic, noncholinergic relaxation of the distal colon between Wistar–ST and Sprague–Dawley strains of rats

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Abstract

Participation of nitric oxide and vasoactive intestinal peptide (VIP) in electrical field stimulation-induced nonadrenergic, noncholinergic (NANC) relaxation of longitudinal muscle and in balloon distension-induced descending NANC relaxation of circular muscle were studied in the distal colon of Wistar–ST and Sprague–Dawley rats. The extent of the nitric oxide-mediated component was approximately 50% in longitudinal and circular muscle of Sprague–Dawley rats, whereas this component was absent in both muscles of Wistar–ST rats. The extent of the VIP-mediated component was approximately 40% in longitudinal muscle of Wistar–ST rats and circular muscle of Sprague–Dawley rats, whereas this component was absent in circular muscle of Wistar–ST rats and longitudinal muscle of Sprague–Dawley rats. In circular muscle of Sprague–Dawley rats, in which participation of both nitric oxide and VIP in the relaxation was suggested, inhibition of descending relaxation by N^G-nitro-L-arginine (L-NOARG) together with VIP-(10–28) was similar to that by either of the antagonists, and exogenous VIP-induced relaxation was not affected by L-NOARG, but exogenous nitric oxide-induced relaxation was partly inhibited by VIP-(10–28). These results suggest a linkage of the pathways mediated by nitric oxide and VIP. In the immunohistochemical studies, nitric oxide synthase or VIP immunoreactive neurons were seen in the ganglia, primary internodal strands of the myenteric plexus and in the circular muscle layer. However, the overall appearance of immunoreactive cell bodies in the myenteric plexus and the numbers of immunoreactive fibers in the circular muscle layer appeared to be similar in Wistar–ST and Sprague–Dawley rats. These results suggest that mediators of NANC relaxation in the distal colon are different in different strains of rats, i.e., Wistar–ST and Sprague–Dawley, although no such difference was seen in immunohistochemical studies.

Keywords: NANC (nonadrenergic, noncholinergic) relaxation; Strain difference; Colon, distal; Nitric oxide (NO); VIP (vasoactive intestinal peptide)

1. Introduction

The mediator of nonadrenergic noncholinergic (NANC) relaxation in the gastrointestinal tract has been extensively studied, especially in the last decade. However, several candidates seem to be involved only in restricted gastrointestinal regions of some species. Among the candidates, nitric oxide seems to be the most generally involved NANC mediator in regions such as the lower oesophageal sphincter, stomach, small intestine, large intestine and internal anal sphincter in a number of animal species (see for review, Stark and Szurszewski, 1992; Rand and Li, 1995). Vasoactive intestinal peptide (VIP) was also suggested as the mediator of NANC relaxation in some regions: the lower oesophageal sphincter of opossums (Goyal et al., 1980) and rabbits (Biancani et al., 1984), the stomach of dogs (Angel et al., 1983), guinea pigs (Grider and Rivier, 1990; Grider et al., 1985a) and rats (Kamata et al., 1988), the taenia coli of guinea pigs (Grider and Rivier, 1990; Grider et al., 1985b), and the colon of rats (Grider and Makhlouf, 1986; Suthamnatpong et al., 1993a).

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Interestingly, accumulated evidence indicates that the mediator of inhibitory control is not uniform throughout the gastrointestinal tract, but is quite variable from region to region. In addition to the regional difference, evidence suggests that there is a difference among species; for example, although VIP does not have any role in NANC relaxation in the mouse colon (Fontaine et al., 1986) and in the canine ileocolonic junction (Boeckxstaens et al., 1991), its involvement was suggested in the rat colon, as mentioned above. Furthermore, there is also a significant difference between the results reported by two groups of investigators for the same intestinal region of the same species. Namely, Grider and his collaborators have reported the participation of nitric oxide, in addition to that of VIP, in the descending relaxation in the mid and distal colon of rats (Grider, 1993; Murthy et al., 1996). However, we have never found a role for nitric oxide in the electrically induced relaxation of longitudinal muscle of the distal colon (Suthamnatpong et al., 1993a,b; Kishi et al., 1996) or for VIP in that in the mid colon (Suthamnatpong et al., 1993a). The difference between circular muscle (descending relaxation) and longitudinal muscle had been thought to be a possible reason for the discrepancy. However, we were recently reminded that Grider (1993) and his collaborators generally use Sprague–Dawley rats, while we use Wistar–ST rats. In the present study, we examined whether the difference in strain of rats is important. A marked difference in the mediator of NANC relaxation in the distal colon of rats between the Sprague–Dawley and Wistar–ST strain is described.

2. Materials and methods

Male rats (8 to 9 weeks old) of the Wistar–ST and Sprague–Dawley strains were purchased from Nippon SLC (Shizuoka, Japan). Although we had indicated the strain of rats used as just Wistar in our previous papers (Hata et al., 1990a; Kanada et al., 1992, 1993; Suthamnatpong et al., 1993a,b, 1994; Maehara et al., 1994; Kishi et al., 1996; Takeuchi et al., 1996), Wistar–ST rats belonging to a subclass of the Wistar strain were used in those studies. Wistar–ST strain rats are supplied from Nippon SLC and are widely used in Japan. In the present study, rats of both strains were lightly anaesthetized with ether and then stunned by a blow on the head and bled via the carotid arteries. The colon was removed and placed in Tyrode solution consisting of (in mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.1, NaH_2PO_4 0.42, $NaHCO_3$ 11.9 and glucose 5.6. The contents of the excised segments were gently flushed out with Tyrode solution. The descending colon, which is attached by mesentery to the small intestine, was defined as the distal region.

2.1. Recording of responses of longitudinal muscle of rat distal colon to electrical field stimulation

Entire segments of the distal colon (2.5–3.0 cm in length) were suspended in an organ bath containing 5 ml of Tyrode solution maintained at 37°C and bubbled with 95% O_2: 5% CO_2. One end of the segment was attached to a transducer and the other end was mounted on an electrode placed at the bottom of the bath. The other electrode, a ring type, was placed around the top of the segment. After an equilibration period of 30 min, responses of the longitudinal muscle to electrical field stimulation with trains of 100 pulses of 0.5 ms width at 30 V, 10-Hz frequency, were recorded isotonically with a 10-min inter-

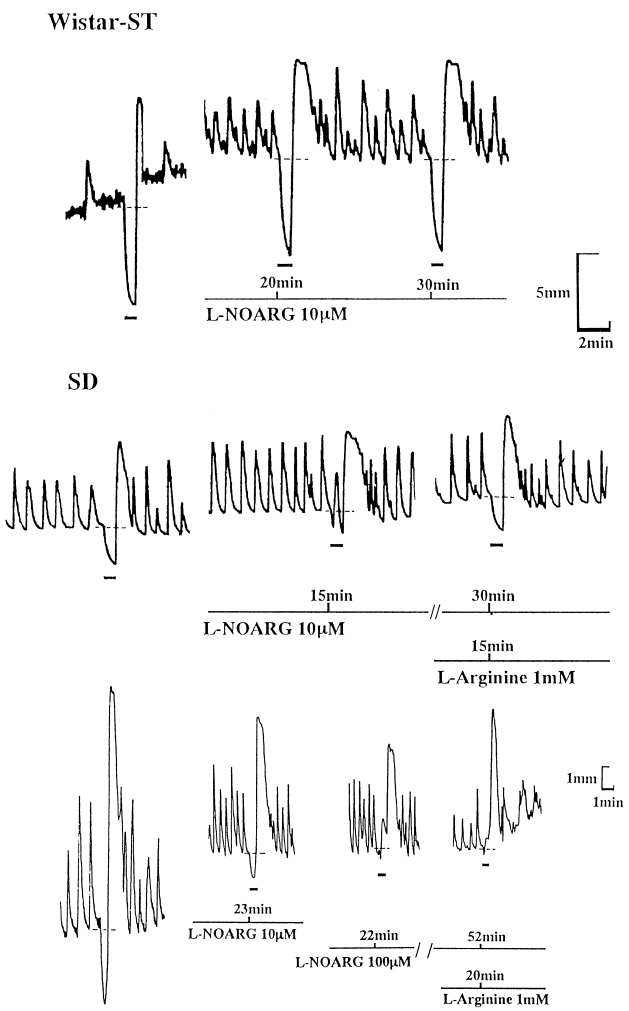

Fig. 1. Effects of L-NOARG and L-arginine on electrical field stimulation-induced relaxation of longitudinal muscle in the distal colon of Wistar–ST and Sprague–Dawley (SD) rats. The lines indicate the presence of L-NOARG (10 or 100 μM) and L-arginine (1 mM) in the bathing fluid. Times noted on the lines indicate the time after addition of the drugs. Bold black lines indicate the duration of electrical field stimulation at 10 Hz for 10 s. After recording of normal spontaneous movements, the chart was run at a fast speed immediately before the stimulation to make the relaxant response clear. The extent of relaxation was expressed as the area under the line of resting tone that was drawn on the bottom of resting spontaneous contractile activity (Dotted lines).

val between tests. The longitudinal muscle was subjected to a resting load of 1.0 g.

2.2. Recording of responses of circular muscle of distal colon to distension stimulus

Colonic segments were held horizontally in a specially designed organ bath described before (Hata et al., 1990b). The middle of the segment was connected by a hook at the joint of the mesentery to anchor it to the bottom of the bath. A rubber balloon connected to a syringe by thin polyethylene tubing was introduced into the lumen, positioned in the middle of the segment and inflated with 0.1–0.15 ml of warm water from the syringe to produce local distension for 30 s. The mechanical response of the circular muscle about 1.0 cm anal to the balloon was recorded by connecting a clip to the wall opposite to the isotonic transducer. This arrangement allowed preferential recording of the response of the circular muscle. The circular muscle was subjected to a resting load of 0.5 g.

2.3. Immunohistochemical study of nitric oxide synthase and VIP

Segments of the distal colon were dissected and immersed in Tyrode solution containing 1 μM nicardipine to relax the longitudinal and circular muscles for 1 min. The segments were then pinned on a plastic sheet, and were immersed in Zamboni fixative (2% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer at pH 7.4) for 72 h. After the mucosa was removed, the segments were rinsed for 24 h at 4°C in 0.1 M phosphate-buffered saline (PBS) containing 30% sucrose. The pattern of the expression and the counting of nitric oxide synthase and VIP immunoreactivity was examined in cryostat sections. Sections (20 μm thick) were cut parallel to the circular muscle layer and put on PLL-coated slides. Sections were incubated in 0.1 M PBS at 4°C for 1 h, and incubated in 0.1 M PBS containing 10% normal goat serum for 1 h before incubation with the first antisera against neuronal isoform of nitric oxide synthase or VIP. Nitric oxide synthase antiserum (1:500) and VIP (1:300) were diluted in 0.1 M PBS containing 0.3% Triton X-100, 1% normal goat serum and 1% bovine serum albumin. After 72 h of incubation with diluted primary antiserum at 4°C, the sections were rinsed in 0.1 M PBS at 4°C for 1 h and incubated at 4°C for 24 h with a second antiserum, goat anti-rabbit immunoglobulin G conjugated with fluorescein isothiocyanate (FITC, Vector Lab., 1:300). Following rinsing as above, the sections were mounted in Vectashield (Vector Lab.). The sections were observed under a fluorescence microscope (Nikon).

2.3.1. Specificity of the antibodies

Antibodies for nitric oxide synthase and VIP were purchased from Chemicon International and Incstar, respectively. The specificity and cross-reactivity of the antisera were evaluated by the supplier. Antibody for nitric oxide synthase was produced in a rabbit treated with synthetic peptide corresponding to amino acid residues 1414–1429 of the rat nitric oxide synthase-1 protein, conjugated to keyhole limpet hemocyanin via a maleimido linkage. This antibody reacts with nitric oxide synthase-containing (and NADPH-diaphorase-positive) neuronal cell bodies throughout the central nervous system of mouse, rat and ferret. Endothelial cells and macrophages within the central nervous system are unstained. The immunostaining is fully abolished by pre-absorption of the antibody with the immunogen peptide. Antibody for VIP was produced in a rabbit, against porcine VIP. The VIP antiserum did not react with 100 pmol amounts of motilin, pancreatic polypeptide, gastric inhibitory polypeptide, peptide histidine isoleucine, insulin, glucagon, somatostatin, gastrin. serotonine, substance P, FMRF-amide, or histamine.

In the present study, the controls were sections incubated without primary antiserum. No specific immunostaining was observed in the myenteric plexus, longitudinal muscle and circular muscle.

The number of nitric oxide synthase immunoreactive neurons with nucleus in 50 ganglions of the myenteric plexus was counted at a 200-fold magnification. For counting the nitric oxide synthase and VIP immunoreactive fibers, photomicrographs of 50 regions in the circular muscle layer were taken at an original magnification of 200 with photographic enlargement of 3 (final magnification: 600). Counting area was 400 μm × 200 μm.

2.4. Drugs

N^G-Nitro-L-arginine (L-NOARG) and L-arginine were purchased from Sigma, St. Louis, USA. Charybdotoxin, VIP and VIP-(10–28) were from the Peptide Institute, Osaka, Japan. Atropine sulfate and guanethidine were from Wako, Osaka, Japan. Drugs were added to the organ bath in a volume of less than 1.0% of the bathing solution. These volumes of the vehicle of the drugs, redistilled water, did not affect either spontaneous contractile activity or muscle tone.

3. Results

3.1. Effects of N^G-nitro-L-arginine (L-NOARG) and L-arginine on NANC relaxation of longitudinal muscle of the distal colon of Wistar–ST and Sprague–Dawley rats

In Wistar–ST rats (see Section 2), according to our earlier report, nitric oxide did not participate in NANC relaxation of longitudinal muscle of the distal colon (Suthamnatpong et al., 1993a; Maehara et al., 1994).

In the present experimtents, segments obtained from the distal colon of Sprague–Dawley rats exhibited moderate spontaneous contractile activity in the presence of 1 μM

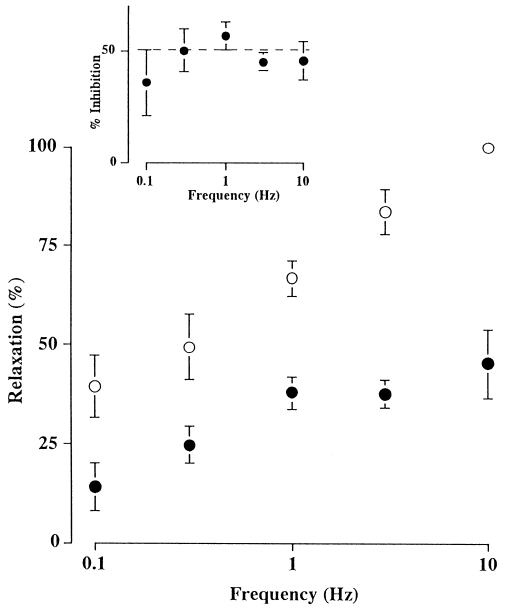

Fig. 2. Inhibitory effects of L-NOARG on relaxations induced by electrical field stimulation at various frequencies in Sprague–Dawley rats. Relaxations were induced by electrical field stimulation at the frequencies indicated for 10 s in the absence (○) or presence (●) of 10 μM L-NOARG. Values are expressed as percentages of relaxation induced at 10 Hz in the absence of L-NOARG. Points are means ± S.E.M. for four experiments. Inset: Inhibitory effects of L-NOARG at various frequencies are expressed as percentages of those obtained in the absence of L-NOARG.

atropine and 5 μM guanethidine. Electrical field stimulation induced contraction without relaxation, but the resting tone of the longitudinal muscle gradually increased during successive trains of electrical field stimulation at 10 Hz for 10 s at 10 min intervals. With the tone increased this way, the segments began to exhibit relaxation followed by a large contraction at the end of electrical field stimulation. When atropine was not added to the bathing solution, the response was always contraction alone. These features in the responses of the segments from Sprague–Dawley rats were similar to those from Wistar–ST rats. In Sprague–Dawley rats, L-NOARG at 10 μM did not have any significant effect on the spontaneous contractile activity or the resting tone of longitudinal muscle of the rat distal colon, but it inhibited the electrical field stimulation-induced relaxation within 20 min of its application. The maximal inhibition was about 40%. Addition of L-arginine (1 mM) to the bathing fluid gradually reversed the inhibitory effect of L-NOARG, causing complete reversal in 20–30 min (Fig. 1). D-Arginine (1 mM) had no significant effect. L-NOARG at 100 μM produced a stronger inhibition than at 10 μM, being 78.9 ± 5.2% ($n = 5$). However, the inhibition was not reversed by addition of L-arginine (Fig. 1). Therefore, the relaxation of longitudinal muscle that was inhibited by 10 μM L-NOARG and completely reversed by a further addition of 1 mM L-arginine was defined as the nitric oxide-mediated component in the present study.

In another series of experiment, we studied the effect of L-NOARG on the relaxations induced by electrical field stimulation at various frequencies. Electrical field stimulation at 0.1 Hz for 10 s (a single pulse) induced a transient relaxation of the longitudinal muscle. With an increase in frequency, greater responses developed. But the response at 100 Hz electrical field stimulation was not reproducible in trials repeated at 10-min intervals. The inhibitory effects of L-NOARG on the relaxations induced by electrical field stimulation at 0.1–10 Hz were similar in magnitude (Fig. 2).

It thus seems that nitric oxide partially participates in NANC relaxation of the longitudinal muscle in the distal colon of the Sprague–Dawley but not of the Wistar–ST strain (Table 1A).

3.2. Effects of L-NOARG on descending relaxation in the distal colon of Wistar–ST and Sprague–Dawley strain of rats

On local distension in the presence of 1 μM atropine and 5 μM guanethidine, the distal segments of both strains showed relaxation of the circular muscle anal to the dis-

Table 1
Differences between Wistar–ST and Sprague–Dawley (SD) rats in the degree of involvement of nitric oxide and VIP in NANC relaxation of the distal colon

(A) The relaxation that was inhibited by L-NOARG (at 10 μM in longitudinal, 100 μM in circular muscle; see for text) and completely reversed by 1 mM L-arginine was defined as the nitric oxide (NO)-mediated component and expressed as a percent of the corresponding control relaxation. (B) The relaxation that was inhibited by 3 μM VIP-(10–28) was defined as VIP-mediated component. Relaxation of longitudinal muscle was induced by electrical field stimulation (EFS) at 10 Hz for 10 s. Values are means ± S.E.M. for the numbers of experiments shown in parentheses. The data for the longitudinal muscle in Wistar–ST rats are from our previous studies.

	A		B	
	NO-mediated component (%)		VIP-mediated component (%)	
	Wistar–ST	SD	Wistar–ST	SD
Longitudinal muscle (EFS-induced relaxation)	3.3 ± 6.3 (9)[a]	38.8 ± 5.6 (3)	44.4 ± 2.4 (6)[b]	1.2 ± 10.7 (3)
Circular muscle (descending relaxation)	2.1 ± 1.1 (3)	50.6 ± 3.6 (6)	3.7 ± 1.1 (3)	42.3 ± 1.7 (5)

[a] Suthamnatpong et al. (1993a).
[b] Kishi et al. (1996).

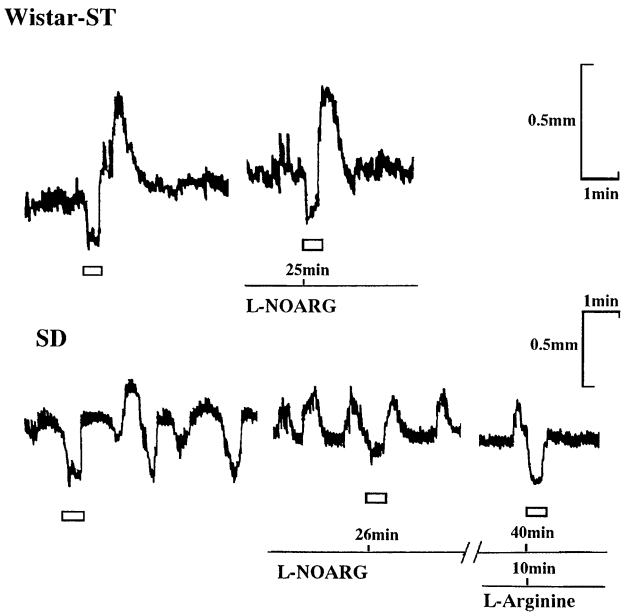

Fig. 3. Effects of L-NOARG and L-arginine on descending relaxation in the distal colon of Wistar–ST and Sprague–Dawley (SD) rats. The lines indicate the presence of L-NOARG (100 μM) and L-arginine (1 mM) in the bathing fluid. Times noted on the lines indicate the time after addition of the drugs. Rectangles indicate 30 s distension.

tended region. This descending relaxation of circular muscle was induced only in the presence of 1 μM atropine.

L-NOARG at concentrations up to 100 μM did not show any appreciable effect on the descending relaxation in the segments from Wistar–ST rats, but significantly inhibited that in segments from Sprague–Dawley rats (Fig. 3). Namely, L-NOARG at 10 μM only slightly inhibited the descending relaxation in the segments from Sprague–Dawley rats, but at 100 μM it inhibited the relaxation by about 50% (Table 1A) within 20–30 min. Addition of L-arginine (1 mM) to the bathing fluid completely reversed the inhibitory effect of L-NOARG within 20–30 min (Fig. 3). D-Arginine (1 mM) had no effect. Thus, in circular muscle, nitric oxide is involved in NANC relaxation in Sprague–Dawley, but not in Wistar–ST rats (Table 1A), indicating a clear difference between Wistar–ST and Sprague–Dawley strain of rats regarding participation of nitric oxide in NANC relaxation of the distal colon.

3.3. Immunohistochemistry of nitric oxide synthase in the distal colon

A few nitric oxide synthase immunoreactive fibers run on the surface of the longitudinal muscle in Wistar–ST and Sprague–Dawley rats. Most of the nitric oxide synthase immunoreactive neurons were seen in the ganglia and primary internodal strands of the myenteric plexus (Fig. 4). The nitric oxide synthase immunoreactive cell

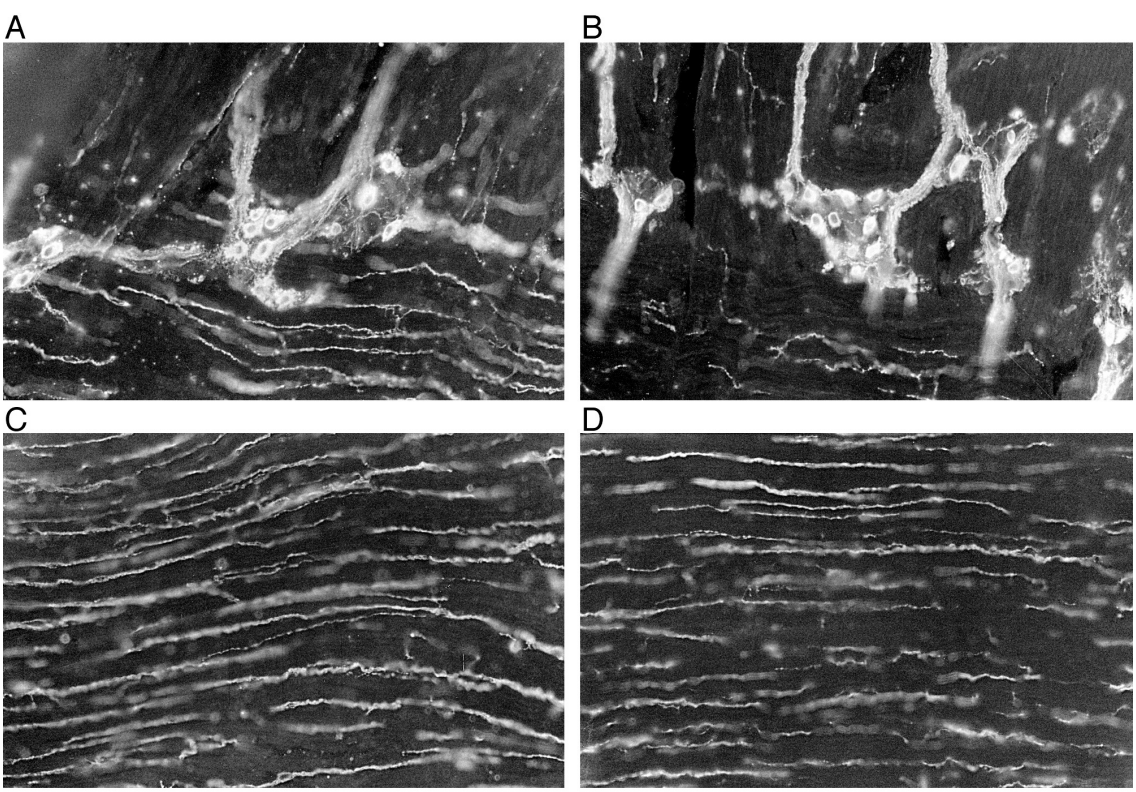

Fig. 4. Fluorescent photomicrographs of nitric oxide synthase immunoreactive neurons in the ganglion of myenteric plexus in Wistar–ST (A) and Sprague–Dawley (B) rats. Fluorescent photomicrographs of nitric oxide synthase immunoreactive fibers in the circular muscle layer in Wistar–ST (C) and Sprague–Dawley (D) rats. Scale bar indicates 100 μm.

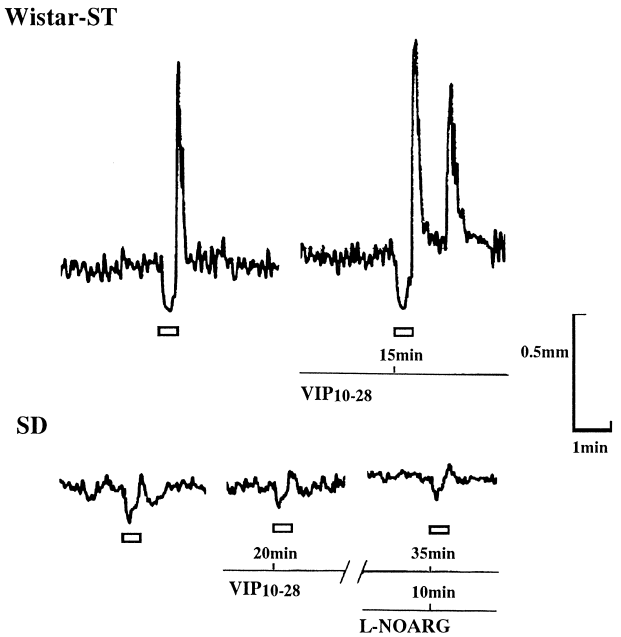

Fig. 5. Effects of VIP-(10–28) and L-NOARG on descending relaxation in the distal colon of Wistar–ST and Sprague–Dawley (SD) rats. The lines indicate the presence of VIP-(10–28) (3 μM) and L-NOARG (100 μM) in the bathing fluid. For further details see legend of Fig. 3.

bodies showed a large variation in size, but all possessed laminalar dendrites and hence are classified as Dogiel type I cells. Most of the nitric oxide synthase immunoreactive cell bodies of the distal colon were found in ganglia of the myenteric plexus (on average 7.6 cell bodies/ganglion in Wistar–ST rats, and on average 8.6 cell bodies/ganglion in Sprague–Dawley rats). The overall appearance of the immunoreactivity in the myenteric plexus in Wistar–ST and Sprague–Dawley rats was essentially similar. Nitric oxide synthase immunoreactive fibers were found in several strata of the circular muscle layer (on average 15.7 fibers/80,000 μm^2 in Wistar–ST, and on average 15.1 fibers/80,000 μm^2 in Sprague–Dawley rats).

3.4. Effects of VIP receptor antagonist and charybdotoxin on NANC relaxation in the distal colon of Wistar–ST and Sprague–Dawley rats

As we have suggested that VIP partially (about 45%) mediates the electrical field stimulation-induced NANC relaxation in the longitudinal muscle of the distal colon of Wistar–ST rats (Kishi et al., 1996), we examined the participation of VIP in the relaxation of the longitudinal muscle in the Sprague–Dawley strain. However, no VIP-mediated component of the relaxation could be shown in the electrical field stimulation-induced NANC relaxation of longitudinal muscle in Sprague–Dawley rats: the VIP receptor antagonist, VIP-(10–28) at 3 μM, which was a sufficient concentration to obtain a maximal inhibitory effect in Wistar–ST rats, did not affect the relaxation induced by electrical field stimulation at 1, 3 (data not shown) or 10 (Table 1B) Hz for 10 s. The antagonist at 3 μM also did not affect the relaxation induced by electrical field stimulation at 10 Hz for a longer duration, 20 s (96.4% of the relaxation before the treatment, $n = 2$), 30 s (100.3%, $n = 2$) or 90 s ($99.4 \pm 7.1\%$, $n = 4$).

The participation of the VIP in the descending relaxation was then also examined. In Wistar–ST rats, VIP-(10–28) at 3 μM did not affect the descending relaxation (Fig. 5), in contrast to its effects in longitudinal muscle. In Sprague–Dawley rats, the antagonist significantly inhibited the descending relaxation by 42% (Fig. 5 and Table 1B). Thus, a clear difference between both strains was also shown for the participation of VIP in NANC relaxation of the distal colon circular muscle. VIP-(10–28) at 3 μM completely inhibited the significant relaxations induced in longitudinal and circular muscle segments by exogenously added VIP at 300 nM (data not shown).

Since an association of the inhibitory effect of VIP with charybdotoxin-sensitive K^+ channels was suggested to exist in the longitudinal muscle of Wistar–ST rats in our previous study (Kishi et al., 1996), we further examined

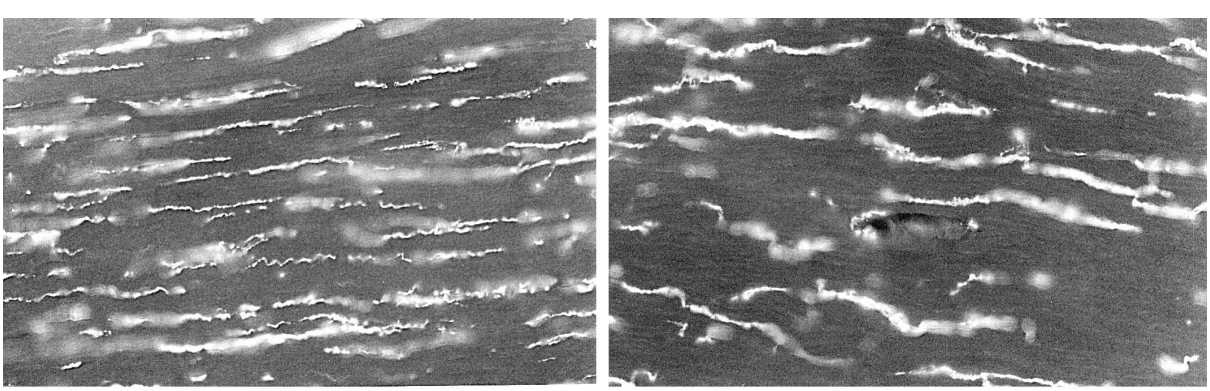

Fig. 6. Fluorescent photomicrographs of VIP immunoreactive fibers in the circular muscle layer in Wistar–ST (A) and Sprague–Dawley (B) rats. Scale bar indicates 100 μm.

whether the effect of VIP on the descending relaxation in Sprague–Dawley rats is associated with charybdotoxin-sensitive K^+ channels. However, such an association was not found: charybdotoxin (100 nM) did not have any significant effect on the descending relaxation (data not shown, $n = 4$) or on the exogenously added VIP-induced relaxation of circular muscle of the distal colon (data not shown, $n = 2$).

3.5. Immunohistochemistry of VIP in the distal colon

A few VIP immunoreactive fibers existed on the surface of the longitudinal muscle in Wistar–ST and Sprague–Dawley rats. Many VIP immunoreactive fibers were seen in the ganglia and primary internodal strands of the myenteric plexus, while few immunoreactive cell bodies of the neuron were observed in the myenteric plexus. The overall appearance of VIP-immunoreactive fibers was essentially similar in the myenteric plexus of Wistar–ST and Sprague–Dawley rats. There were VIP immunoreactive fibers in several strata of the circular muscle layer (on average 16.4 fibers/80,000 μm^2 in Wistar–ST rats, and on average 10.1 fibers/80,000 μm^2 in Sprague–Dawley rats) (Fig. 6).

3.6. Correlation between the nitric oxide- and VIP-mediated components in descending relaxation of the distal colon of Sprague–Dawley rats

Among the relaxant responses of longitudinal and circular muscle of the distal colon of Wistar–ST and Sprague–Dawley rats, only the descending relaxation in Sprague–Dawley rats was suggested to be mediated by both nitric oxide and VIP (Table 1). Therefore, it seemed interesting to study the interaction between both mediators. Exogenously added VIP (10–300 nM) induced a slow gradual relaxation of circular muscle segments from Sprague–Dawley rats and VIP at 300 nM induced a maximal relaxation which corresponded to $151.8 \pm 17.4\%$ ($n = 3$) of the distension-induced relaxation. L-NOARG (100 μM) did not show any significant effect on the VIP-induced relaxation of the circular muscle ($n = 3$). On the other hand, exogenously added nitric oxide (100 nM–1 μM) induced a rapid, transient, relaxation of the circular muscle and nitric oxide at 1 μM induced a maximal relaxation which corresponded to $103.7 \pm 11.7\%$ ($n = 5$) of the distension-induced relaxation. VIP-(10–28) significantly inhibited the nitric oxide (1 μM)-induced relaxation of circular muscle by $40.9 \pm 12.8\%$ ($n = 4$). The inhibition of the descending relaxation induced by L-NOARG together with VIP-(10–28) was similar to that induced by L-NOARG or VIP-(10–28) alone (Fig. 5; $51.1 \pm 3.4\%$ inhibition, $n = 5$). Thus, the results suggest that the nitric oxide- and VIP-mediated components of the descending relaxation are partly associated with each other in the relaxation of the distal colon of Sprague–Dawley rats.

4. Discussion

In comparison to the universal contribution of the neurotransmitter, acetylcholine, to contractile responses throughout the gastrointestinal tract, the mediators of nonadrenergic inhibitory responses vary from region to region. For example, the participation of nitric oxide in NANC relaxation was seen in the proximal, but not in the distal, colon of rats. In contrast, that of VIP was seen in the distal, but not in the proximal, colon (Suthamnatpong et al., 1993a,b). If we carefully examine the many reports on the role of nitric oxide as mediator of inhibitory responses in the gastrointestinal tract, it is seen that the contribution of nitric oxide to the inhibitory response is not uniform. Regions where NANC inhibitory responses seem to be mediated solely by nitric oxide are rather rare, but its participation is partial in nearly all regions (Li and Rand, 1990; Irie et al., 1991; Maggi et al., 1991; Grundy et al., 1993; Postorino et al., 1995; Niioka et al., 1997). In addition to such a regional difference in the participation of nitric oxide in NANC inhibitory responses, differences between rat strains were first shown in the present study. Differences between Wistar–ST and Sprague–Dawley rats were as follows. First, nitric oxide participates partially in the electrical field stimulation-induced relaxation of longitudinal muscle and descending relaxation of circular muscle in Sprague–Dawley rats, but no participation was found in Wistar–ST rats. Second, VIP participates in the relaxation of longitudinal, but not of circular, muscle in Wistar–ST rats and, in contrast, it participates in the relaxation of circular, but not of longitudinal, muscle in Sprague–Dawley rats. Third, a charybdotoxin-sensitive component is associated with the VIP-induced relaxation of the longitudinal muscle in Wistar–ST rats (Kishi et al., 1996), but not with that of circular muscle in Sprague–Dawley rats.

The observations in the immunohistochemical study seem incompatible with the results of the pharmacological study. Especially, a few unexpected observations can not be ignored. Nitric oxide synthase immunoreactive structures were seen to a similar extent in the distal colon of Sprague–Dawley rats and in that of Wistar–ST rats. Nevertheless, there was no participation of nitric oxide in NANC relaxation. Another problem is the absence of nitric oxide synthase immunoreactivity in the longitudinal muscle layer of the distal colon from Sprague–Dawley rats where NANC relaxation was partially (about 40%) mediated by nitric oxide. The population of VIP immunoreactive structures was also similar in the preparations from Sprague–Dawley and Wistar–ST rats. As far as relaxation in the circular muscle of Sprague–Dawley rats is concerned, the dense population of VIP immunoreactive neurons is compatible with the results of the pharmacological study. However, the absence of VIP immunoreactivity in the longitudinal muscle in Wistar–ST rats cannot explain the VIP-mediated NANC relaxation of the longitudinal

muscle. Although there is a possibility that neurons in the myenteric plexus directly affect longitudinal muscle cells, which consist of a thin layer, the differences in the mediators of NANC relaxation between the two rat strains found in the functional experiments were not parallelled by the present immunohistochemical results. Further precise studies on localization of the immunoreactive neurons and on the role of these neurons in smooth muscle relaxation are necessary to solve the discrepancy.

Grider and Makhlouf (1986) first suggested that VIP motor neurons regulate the descending relaxation of the peristaltic reflex in the distal colon of Sprague–Dawley rats. It has also been suggested that nitric oxide produces relaxation in cooperation with VIP in the tissue preparations (Grider, 1993). These results were incompatible with our previous results as mentioned in the Introduction. However, the differences in the mediators of NANC relaxation between rat strains shown in the present study might explain the previous controversy. More recently, this group also suggested a serial pathway in which nitric oxide and VIP released from myenteric neurons relax the colonic smooth muscle, VIP simultaneously producing nitric oxide in the muscle cells and nitric oxide further stimulating VIP release from the neurons (Murthy et al., 1996). In the present study, the inhibitory effect of L-NOARG together with the VIP receptor antagonist on the descending relaxation in Sprague–Dawley rats was similar to that of L-NOARG or the VIP receptor antagonist alone. These results are consistent with a serial link between both mediators. The exogenous nitric oxide-induced relaxation was partly inhibited by the VIP receptor antagonist, whereas the exogenous VIP-induced relaxation was not inhibited by L-NOARG. Therefore, it seems likely that the nitric oxide-mediated mechanism may be present upstream of the VIP-mediated one. However, the present results do not explain how nitric oxide releases VIP from the VIP neurons. Activation of the descending relaxation pathway initiated by balloon inflation may produce and release nitric oxide from the inter-neurons present in the pathway. Then nitiric oxide probably acts presynaptically to enhance the release of VIP, as suggested by the descending relaxation of the rat distal colon (Grider, 1993), and the relaxation of muscle strips obtained from rabbit and rat gastric fundus (Jin et al., 1996) and guinea pig gastric fundus and tenia coli (Grider et al., 1992). The finding that the nitric oxide-induced relaxation was not completely inhibited by the VIP receptor antagonist, which is a blocker of the downstream of nitric oxide-VIP pathway, might seem surprising. However, it is reasonable to postulate that exogenously added nitric oxide diffuses into the whole tissue and relaxes the smooth muscle by a direct action on the smooth muscle cells in addition to the physiological indirect action via VIP release. Thus, the linkage of the nitric oxide- and VIP-mediated pathway in the rat distal colon was reconfirmed in the present study, but the order is the reverse of that suggested by Grider (1993). Evidence for a VIP-nitric oxide linkage whereby VIP induces production of nitric oxide in the smooth muscle cells, was not obtained. The exact reason for the discrepancies between results from the two laboratories is not clear at present. The latter group used 200–400 g Sprague–Dawley rats and prepared a 4- to 6-cm segment of the middle to distal colon (Grider and Makhlouf, 1986), or prepared muscle strips (20 mm long) from the circular muscle of the middle or distal colon (Grider, 1993; strain of the rat used was not noted). We, however reported a regional difference in the mediator of the NANC relaxation in the proximal, middle and distal colon in Wistar–ST rats (Suthamnatpong et al., 1993a). We recently reported that the importance of nitric oxide as the mediator of NANC relaxation in the rat intestine decreases significantly with age (Takeuchi et al., 1998). So, there may be a possibility that an unexpected discrepancy will occur among the results obtained, if the region of the intestine and the age of the rats are not carefully taken into account. Indeed, there are also contradictory reports on the VIP–nitric oxide linkage in the canine colon: the VIP-induced relaxation was reduced (Huizinga et al., 1992) or not changed (Keef et al., 1994) by inhibition of nitric oxide synthesis.

Differences in participation of nitric oxide and VIP in NANC relaxation of the distal colon were now studied. However, mediators other than these two also participate in the relaxation, since the nitric oxide- and VIP-mediated components are only a part of the relaxation in each muscle examined (Table 1). Other candidate NANC transmitters, such as pituitary adenylate cyclase activating peptide, calcitonin gene related peptide and ATP were suggested for the gastrointestinal tract. Therefore, it remains an interesting question whether the participation of other unknown mediators of the relaxation differs among strains of the rats.

In summary, differences in the mediators involved in NANC relaxation in the distal colon between rat strains was shown: participation of nitric oxide in NANC relaxation of longitudinal and circular muscle of the distal colon was shown in Sprague–Dawley but not Wistar–ST rats. Participation of VIP in the NANC relaxation of longitudinal muscle was shown in Wistar–ST but not Sprague–Dawley rats, while the opposite was shown for circular muscle.

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