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Increase in participation of vasoactive intestinal peptide in relaxation of the distal colon of Wistar rats with age

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1 Changes in participation of vasoactive intestinal peptide (VIP) in nonadrenergic noncholinergic (NANC) relaxation of longitudinal muscle of the distal colon with age were studied in 2- to 50-week-old Wistar rats *in vitro*.

2 The extent of the VIP-mediated component of the relaxation induced by electrical field stimulation (EFS) was determined by the effect of VIP_{10-28}, a VIP receptor antagonist. In 2-week-old rats, the extent of the VIP-mediated component of the relaxation was scarce, about 10%, whereas the component gradually increase with age and reached the maximum extent 66% at 50-week-old.

3 Since our previous results suggest that VIP induces NANC relaxation *via* activation of charybdotoxin (ChTx, a blocker of large conductance Ca^{2+}-activated K^+ channel)-sensitive K^+ channels with concomitant slow hyperpolarization in the muscle cells, we next studied whether ChTx-sensitive component and slow hyperpolarization changes with age. Extent of ChTx-sensitive component of the relaxation increased with age, showing a very similar pattern to VIP-mediated one.

4 EFS induced monophasic inhibitory junction potentials (i.j.ps) in longitudinal muscle cells of the distal colon of 2- and 4-week-old. EFS also induced biphasic i.j.ps in many longitudinal muscle cells of 8- and 50-week-old: rapid and subsequent slow hyperpolarization. A VIP receptor antagonist selectively inhibited the slow hyperpolarization.

5 Exogenously added VIP induced no appreciable change in the membrane potential of longitudinal muscle cells of 2-week-old, whereas it induced slight slow hyperpolarization of the cell membrane in 4-week-old and magnitude of the hyperpolarization increased with age. On the other hand, relaxant response of the longitudinal muscle to exogenously added VIP was high in younger rats.

6 The present results suggest that the role of VIP in mediating NANC relaxation of longitudinal muscle of the Wistar rat distal colon is very little at neonatal stage, but it increases with age.
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Keywords: VIP; ageing; NANC relaxation; rat distal colon; charybdotoxin

Abbreviations: ChTx, charybdotoxin; EFS, electrical field stimulation; NANC, nonadrenergic noncholinergic; VIP, vasoactive intestinal peptide

Introduction

There are numerous reports suggesting the role of vasoactive intestinal peptide (VIP) as a mediator of nonadrenergic, noncholinergic (NANC) relaxation of digestive canal smooth muscle: lower oesophageal sphincter of the opossum (Goyal *et al*., 1980) and rabbit (Biancani *et al*., 1984), stomach of the dog (Angel *et al*., 1983), guinea-pig (Grider *et al*., 1985a) and rat (Kamata *et al*., 1988; De Beurme & Lefebvre, 1988), and the taenia coli of the guinea-pig (Grider *et al*., 1985b). We also suggested the role of VIP in the distal colon of the rat (Suthamnatpong *et al*., 1993a; Kishi *et al*., 1996; 2000).

Nitric oxide has been suggested to mediate NANC relaxation of the smooth muscle in various gastrointestinal regions of various kinds of animals (see for review, Moncada *et al*., 1991; Stark & Szurszewski, 1992; Rand & Li, 1995). Recently, we found an interesting change in the participation of nitric oxide in NANC relaxation with age; NANC relaxation of longitudinal muscle in every region studied of the intestine in 2-week-old Wistar rats is almost solely mediated by nitric oxide, and its significance as an inhibitory mediator decreases with age gradually or rapidly, which is dependent on intestinal regions (Takeuchi *et al*., 1998). The present study is planned to study whether extent of the participation of VIP in NANC relaxation of longitudinal muscle of the distal colon changes with age in 2- to 50-week-old Wistar rats to compensate for reduction of nitric oxide participation as a NANC mediator.

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Methods

Two, four-, eight-, twenty- or fifty-week-old Wistar rats obtained from JCL, Inc. (Osaka, Japan) were used (2- and 4-week-old, either sex; 8-, 20- and 50-week-old, male). The strain of rats used which we indicated simply as Wistar in previous

papers (Suthamnatpong *et al*., 1993a,b; Kishi *et al*., 1996) actually was Wistar-ST strain. The strain is included in a subclass of the Wistar strain and supplied from Nippon SLC (Shizuoka, Japan) and widely used in Japan. We used the Wistar strain, instead of the Wistar-ST strain, in the present study. The rats were lightly anaesthetized with diethyl ether and then stunned by a blow on the head and bled *via* the carotid arteries. Segment of the distal colon was removed and placed in Tyrode solution of the following composition: (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 segment were gently flushed out with Tyrode solution. The portion of the colon that is attached by the mesentery to the small intestine was defined as the distal region. After the experiments, the segments were blotted and weighed (Takeuchi *et al*., 1998).

Recording of responses of longitudinal muscle to electrical field stimulation (EFS)

Segments of the distal colon were suspended in an organ bath filled with Tyrode solution aerated with 5% CO_2 in O_2 and maintained at 37°C. Atropine (1 μM) and guanethidine (5 μM) were present throughout the experiment to block cholinergic and adrenergic responses, respectively. Responses of the longitudinal muscle to EFS for 10 s with trains of 1–100 pulses of 0.5-ms width at 30 V, 0.1–10 Hz frequency were recorded isotonically with a 10-min interval between tests. The longitudinal muscle of each segment was subjected to a resting load of 1.0 g. The preparations were equilibrated for at least 30 min before the experiments. 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. Drugs were added to the organ bath in volumes of less than 1.0% of the bathing solution. These volumes of the vehicle of the drugs, redistilled water, did not affect the spontaneous contractile activity, the muscle tone or the NANC response to EFS.

Recording of membrane potentials in longitudinal muscle of distal colon

The segments of the distal colon were mounted in a 1.5 ml organ bath maintained at 30°C and perfused continuously with Tyrode solution at a rate of 3 ml min^{-1}. This temperature allowed stable recording of the membrane potentials, since the spontaneous and evoked mechanical responses were reduced. Atropine (1 μM) and guanethidine (5 μM) were added to the bathing solution throughout the experiment. Membrane potentials were recorded with a conventional glass microelectrode filled with 3 M KCl with a resistance of 50–80 MΩ. Inhibitory junction potentials were elicited by EFS to intramural nerves within the segment with square-wave pulses of 0.5 ms duration at an appropriate intensity (10–30 V). The electrode impalement was made into the longitudinal muscle cells of the superficial layer from the serosal side (Takewaki & Ohashi, 1977). The stimulus pulses were delivered with a pair of Ag-AgCl wire electrodes, one on the serosal surface 1–2 mm away from the impaled glass microelectrode and the other in the solution. The distance between the two electrodes was about 20 mm.

Drugs

N^G-Nitro-L-arginine (L-NOARG), D- and L-arginine were purchased from Sigma (St. Louis, MO, U.S.A.). VIP, VIP_{10-28} and charybdotoxin were from Peptide Institute. Osaka, Japan. (±)-(E)-Methyl-2-[(E)-hydroxyiminol-5-nitro-6-methoxy-3-hexeneamine (NOR1) was from Dojindo laboratories, Kumamoto, Japan. Atropine sulphate and guanethidine were from Wako Pure Chemical, Osaka, Japan. All other chemicals were of analytical grade.

Results

Effect of a VIP antagonist on EFS-induced NANC relaxation of longitudinal muscle of Wistar rat distal colon

Electrical field stimulation (EFS) at 10 Hz for 10 s induced a transient relaxation followed by a large contraction of longitudinal muscle of the distal colon obtained from 2- to 50-week-old Wistar rats in the presence of 1 μM atropine and 5 μM guanethidine. A VIP antagonist, VIP_{10-28} at 3 μM showed the maximal inhibitory effect on the NANC relaxation in Wistar-ST rats (Suthamnatpong *et al*., 1993a) and in 8-week-old Wistar rats in the present study: extent of inhibition by 3 or 10 μM VIP_{10-28} was 36.4±3.4% (n=14) or 31.6±3.6% (n=4), respectively. VIP_{10-28} at 3 μM abolished the relaxations induced by exogenously added 10–30 nM VIP and very significantly inhibited those by 100 nM–1 μM (data not shown).

VIP_{10-28} at 3 μM had no significant effect on the EFS-induced relaxation in 2-week-old rats. However, the antagonist slightly but obviously inhibited the relaxation in 4-week-old rats. The inhibitory effect of the antagonist increased in elder rats and reached the maximum in 20-week-old rats, being about 70% inhibition (Figure 1). Since it was reported that extent of an inhibitory effect of VIP_{10-28} on NANC relaxation was different among the relaxations induced by different frequencies of electrical stimulation in the taenia coli and gastric fundus of the guinea-pig (Grider & Rivier, 1990), and the gastric muscle of the rabbit and rat (Jin *et al*., 1996), effects of VIP_{10-28} on the relaxations induced by 0.1 and 1 Hz were also examined. EFS at 0.1 Hz (a single pulse) or 1 Hz for 10 s also induced NANC relaxations in the segments from 8-week-old rats which was smaller in amplitude and shorter in duration than that induced by 10 Hz stimulation. Both relaxations induced by EFS at 0.1 Hz and 1 Hz were also inhibited by VIP_{10-28} at 3 μM to such an extent that the relaxation by 10 Hz stimulation was inhibited (Figure 2). In 2- and 4-week-old rats, there was no significant difference in the extent of the inhibitory effect of the antagonist on NANC relaxations at different frequencies in each age of the rat (Figure 2).

Next, interrelationship between VIP and nitric oxide as mediators of NANC relaxation was examined. In 8-week-old rats, exogenous VIP (1 μM)-induced relaxation of the longitudinal muscle was not affected by treatment of the preparation with 10 μM N^G-nitro-L-arginine (L-NOARG), an inhibitor of nitric oxide synthesis: 24.7±2.5 or 24.9±2.4% (n=3) relaxation (which was expressed as percentages of the maximum relaxation induced by 30 μM papaverine) induced by VIP without or with L-NOARG, respectively. The relaxation induced by exogenous NOR1 (10 μM), a nitric oxide donor, was not affected by 3 μM VIP_{10-28}: 27.0±3.4 or 25.9±3.4% (n=3) relaxation induced by NORI with or without VIP_{10-28}, respectively. These results suggest that VIP and nitric oxide separately in each other mediate NANC relaxtion in the distal colon. An age-dependent increase in the role of VIP as a mediator of NANC relaxation is summarized in comparison to an age-dependent decrease in the role of nitric oxide in the same tissue as shown previously (Takeuchi *et al*., 1998) (Table 1). Sums of two components of the relaxation are 70–100% in

each age. Therefore, the two mediators must functionally supply the lack of roles of each other.

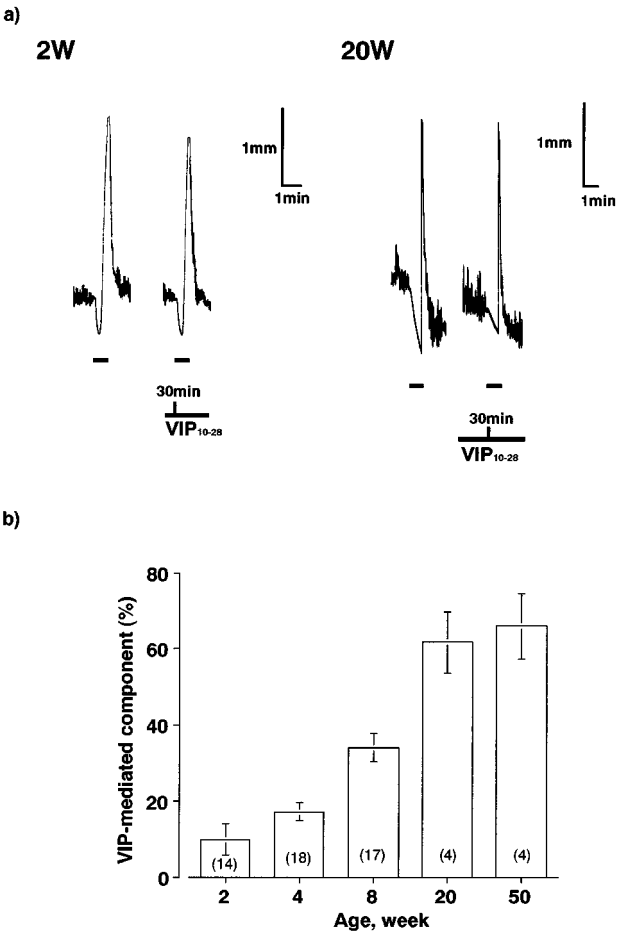

Figure 1 Effect of VIP_{10-28} on EFS-induced relaxation of the distal colonic segments of various-week-old rats. (a) Relaxations of longitudinal muscle of the distal colonic segments prepared from 2- and 20-week-old Wistar rats were induced by EFS (100 train pulses at 10 Hz) in the absence or presence of 3 μM VIP_{10-28}. Atropine (1 μM) and guanethidine (5 μM) were present throughout the experiment. The continuous lines indicate the presence of 3 μM VIP_{10-28}. Times noted on the lines indicate the time after addition of the antagonist. Bold black lines indicate the duration of EFS for 10 s. After recording normal spontaneous movements, the chart was run at a fast speed immediately before the stimulation to make the relaxant response clear. (b) Summary of the effect of VIP_{10-28} on the relaxations in 2-, 4-, 8-, 20- and 50-week-old rats. The component of the relaxation that was inhibited by 3 μM VIP_{10-28} was defined as the VIP-mediated component and expressed as percentages of the relaxation before addition of the antagonist. Either sex of 2- and 4-week-old rats were used (see Methods section). There was no significant difference in the extent of VIP-mediated component of NANC relaxation between male and female rats (data not shown). Values are means$\pm$s.e.mean for the numbers of experiments shown in parentheses.

Effect of charybdotoxin on EFS-induced NANC relaxation of longitudinal muscle of Wistar rat distal colon

Since charybdotoxin (ChTx)-sensitive K^+ channels were suggested to be associated with VIP-mediated NANC relaxation in the Wistar-ST rat distal colon in the previous study (Kishi *et al*., 1996), we next examined the effect of ChTx on the NANC relaxation in Wistar rats. In 2-week-old Wistar rats, ChTx at 100 nM, which exhibited the maximal inhibitory effect on NANC relaxation in Wistar-ST rats (Kishi *et al*., 1996) slightly inhibited the NANC relaxation induced by EFS at 10 Hz. An extent of the inhibitory effect of ChTx increased with age, exhibiting a very similar age-dependent effect to that of VIP_{10-28} (Figure 3).

Although VIP_{10-28} at 3 μM and ChTx at 100 nM slightly or moderately inhibited the NANC relaxation in 4- or 8-week-old rats, respectively, as noted above, simultaneous treatment of the segments with both drugs resulted in inhibition to a similar extent to that induced by each drug alone (Figure 4).

Inhibitory junction potentials induced by EFS in the longitudinal smooth muscle cells of Wistar rat distal colon

Since VIP was shown to be associated with the delayed component of i.j.ps induced by EFS in longitudinal muscle cells of the distal colon of 8-week-old Wistar-ST rats (Kishi

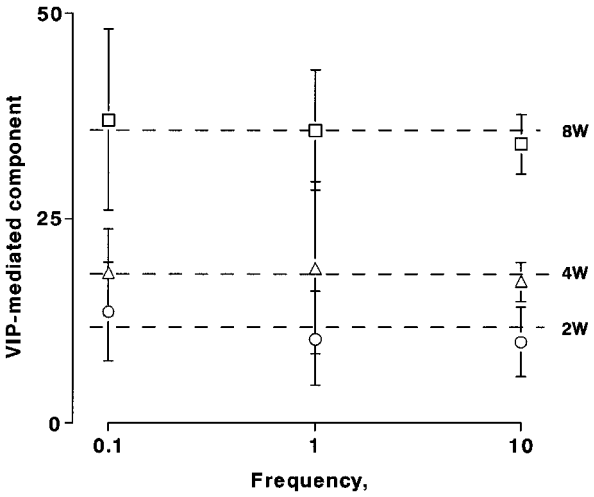

Figure 2 VIP-mediated component in relaxation of longitudinal muscle of the distal colon induced by EFS at various frequencies of trains of pulses. Inhibitory effect of 3 μM VIP_{10-28} on the relaxations induced by EFS at 0.1, 1 or 10 Hz were examined in the distal colonic segments prepared from 2 ○-, 4 △ and 8 □-week-old rats. Values are the mean$\pm$s.e.mean for 3–18 experiments. For further detail, see legend of Figure 1.

Table 1 Changes of VIP- and nitric oxide-mediated component in NANC relaxation of longitudinal muscle of the Wistar rat distal colon with age

	Participation (%)			
Component	*2-week-old*	*4-week-old*	*8-week-old*	*50-week-old*
VIP-mediated	10.0$\pm$4.2 (14)	17.3$\pm$2.4 (18)	34.1$\pm$3.6 (17)	66.0$\pm$8.6 (4)
NO-mediated*	89.3$\pm$3.1 (4)	51.1$\pm$11.1 (5)	37.8$\pm$13.0 (4)	20.6$\pm$11.1 (5)

Relaxations of longitudinal muscle of the distal colon obtained from various-week-old Wistar rats to EFS at 10 Hz were recorded. The component that was inhibited by 3 μM VIP_{10-28} was defined as the VIP-mediated component. Values are the mean$\pm$s.e.mean for the number of experiments shown in parentheses. *Data are from our previous study, Takeuchi *et al.* (1998).

et al., 1996), we next examined the effect of the VIP antagonist on the i.j.ps in 2- to 50-week-old Wistar rats.

In 2-week-old Wistar rats, the resting membrane potential of longitudinal muscle cells of the distal colon was -42.5 ± 2.3 mV ($n=41$). In the presence of atropine (1 μM) and guanethidine (5 μM), EFS with 2–5 pulses at 10 Hz induced monophasic inhibitory junction potentials (i.j.ps) in 15 cells from four animals (Figure 5). But the amplitude of the i.j.ps varied from one cell to another. Even in a certain cell the amplitude was variable, occasionally reached 5 mV or did not occur (Figure 5) in response to EFS. EFS also induced i.j.ps of 15–20 mV in the longitudinal muscle cells of 4-week-old (in 21 cells from four animals; Figure 6a) whose resting membrane potential was -50.8 ± 1.9 mV ($n=33$). VIP_{10-28} at 3 μM did not have any significant effect on the monophasic i.j.ps induced in 2- (not shown) or 4-week-old (Figure 6a) rats. In 8-week-old rats, the resting membrane potential of the cells was -61.2 ± 1.7 mV ($n=115$) and EFS induced two types of i.j.ps of 15–30 mV: monophasic i.j.ps. similar to those in 2- and 4-week-old were induced in 10 of 25 cells (from five animals) and i.j.ps consisting of two phases, rapid and subsequent slow hyperpolarization were induced in other 15 cells. Similar two types of i.j.ps to those in 8-week-old were induced in the cells in 50-week-old rats whose resting membrane potential was -62.0 ± 1.8 mV ($n=47$): monophasic in 10 of 28 cells and biphasic in other 18 cells ($n=28$, from five animals). VIP_{10-28} at 3 μM selectively inhibited the delayed phase, slow hyperpolarization induced by EFS in 8- (not shown) and 50-week-old (Figure 6b).

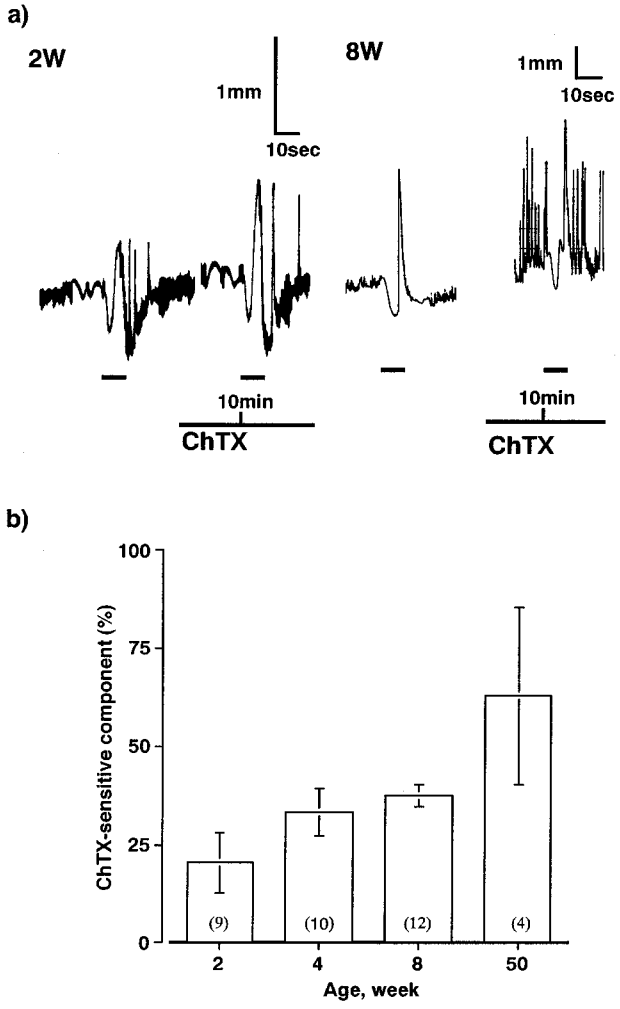

Figure 3 Effect of charybdotoxin (ChTx) on EFS-induced relaxation of the distal colonic segments of various-week-old rats. (a) Relaxations of longitudinal muscle of the distal colonic segments prepared from 2- and 8-week-old Wistar rats were induced by EFS (100 train pulses at 10 Hz) in the absence or presence of 100 nM ChTx. (b) Summary of the effect of ChTx on the relaxations in 2-, 4-, 8- and 50-week-old rats. The component of the relaxation that was inhibited by 100 nM ChTx was expressed as percentages of the relaxation before addition of the antagonist. Values are mean$\pm$s.e. mean for the numbers of experiments shown in parentheses. For further detail, see legend of Figure 1.

Changes in membrane potentials of longitudinal muscle cell and relaxations of longitudinal muscle of the distal colon induced by exogenously added VIP

To confirm an increase in the role of VIP in NANC relaxation of the distal colon with age, we further examined the effects of exogenously added VIP on the resting membrane potentials of longitudinal muscle cells of the distal colon of 2-, 4-, 8- or 50-week-old rats. Bath application of VIP (1 μM) induced no appreciable change in the membrane potential in 2-week-old rats ($n=5$). However, VIP (1 μM) slightly induced slow hyperpolarization in 4-week-old (1.2 ± 0.6 mV, $n=4$), and significantly in 8-week-old (10.4 ± 1.1 mV, $n=7$) and 50-week-old (13.5 ± 1.3 mV, $n=4$) (Figure 7). Thus, the age-dependent hyperpolarization induced by exogenously added VIP is consistent with the age-dependent role of VIP as a mediator of NANC relaxation as shown above.

We next examined the relaxant effect of exogenous VIP on longitudinal muscle of the distal colon in 2- to 20-week-old rats. Exogenous VIP induced relaxation of the longitudinal muscle in a concentration-dependent manner. The magnitude of the relaxant effect was large at 2-week-old and small at 20-week-old, although the relaxation in the concentration-response curve was started from lower concentrations of VIP in the younger age (Figure 8). These results suggest that the relaxant response of the longitudinal muscle of the distal colon to exogenous VIP decreases (not increases) with age.

Discussion

The concentrations of immunoreactive VIP in the duodenum, jejunum, ileum and colon of 3-day-old Sprague Dawley rats are significantly lower than those in the adult. The concentrations continuously increase with age and reach the level approximately equal to adult level by day 28 (Ichihara *et al*., 1983). In the mouse duodenum and colon, the concentration of immunoreactive VIP is maximum in 3-month-old and decreases in 12- and 24-month-old (El-Salhy & Sandstrom, 1999). Immunoreactive nerve fibres which were visible from day 1 in the duodenum of the Wistar rat increased in number in plexi and muscle layers, and the nerve fibres and cell bodies attained the distribution by day 14, described for the adult, within the duodenum and jejunum. The pattern of development observed in these regions was verified in the colon as well (Sikora *et al*., 1984). Significant decrease in number of immunoreactive nerve fibres in the small intestine of senile rats (36-month-old) in comparison to those in young and old (6- and 24-month-old, respectively) rats was shown in immunoelectron microscopic investigation (Fehér & Pénzes, 1987). However, there is little finding on the change with ageing in the role of VIP as a mediator for NANC relaxation in the rat intestine.

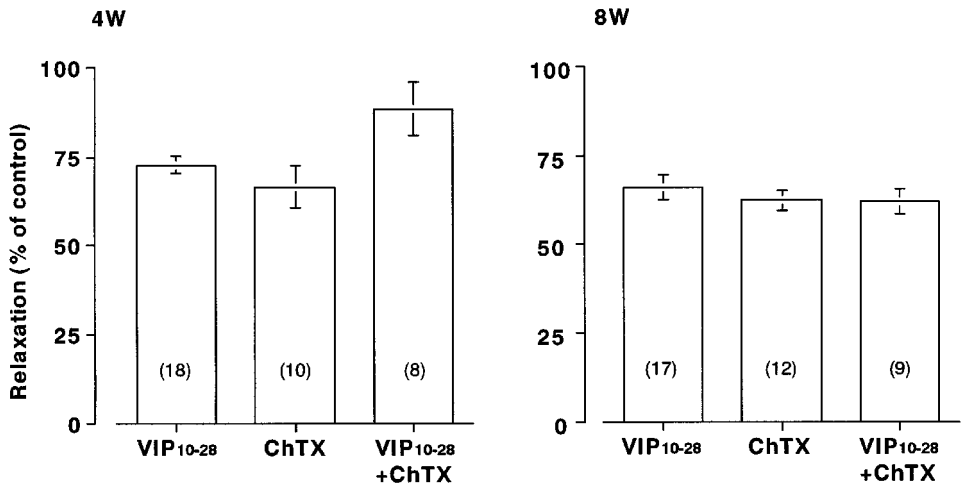

Figure 4 Effects of VIP_{10-28} and charybdotoxin (ChTx) on EFS-induced relaxation of the distal colonic segments. Relaxations of longitudinal muscle of the distal colonic segments prepared from 4- or 8-week-old Wistar rats were induced by EFS at 10 Hz in the absence (control) or presence of 3 μM VIP_{10-28} or 100 nM ChTx, or both drugs. Relaxations are expressed as percentages of control relaxations before addition of the antagonist (control). Values are mean$\pm$s.e.mean for the number of experiments shown in parentheses.

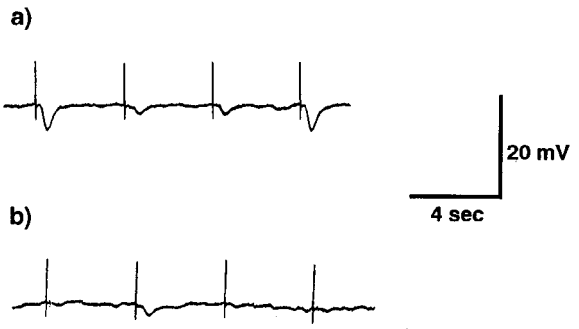

Figure 5 EFS-induced i.j.ps in longitudinal smooth muscle cell of the distal colon of 2-week-old Wistar rats. l.j.ps were induced by two pulses at 10 Hz in longitudinal smooth muscle cell. Records of (a) and (b) were from the different cells.

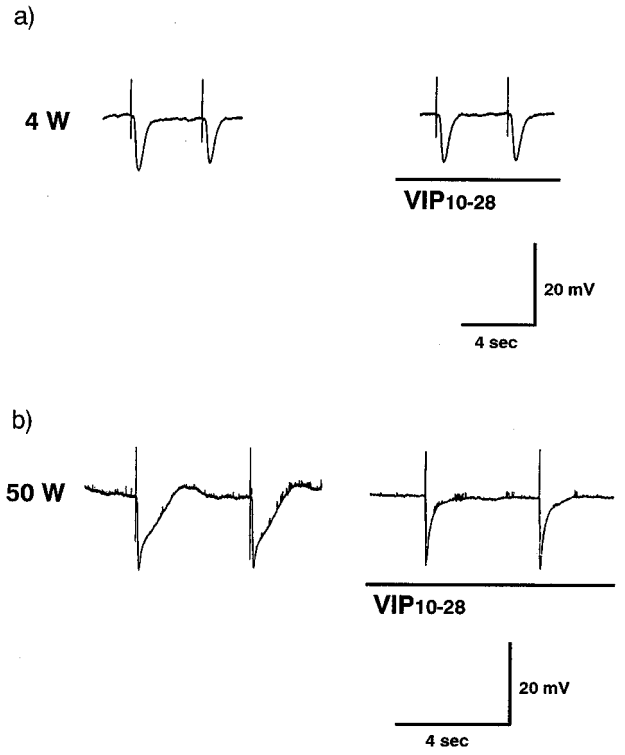

Figure 6 EFS-induced i.j.ps in longitudinal smooth muscle cell of the distal colon of 4-and 50-week-old rats. (a) Monophasic i.j.ps were induced by EFS with five pulses in the absence or presence of 3 μM VIP_{10-28} in 4-week-old rats. VIP_{10-28} was treated for 20 min. (b) I.j.ps were induced by EFS with five pulses in the absence or presence of 3 μM VIP_{10-28} in 50-week-old rats. Note that i.j.ps consist of rapid and slow hyperpolarization and VIP_{10-28} selectively inhibited the latter. Lines indicates the presence of the antagonist. All records in (a) or (b) were from the same longitudinal muscle cell.

In the present study, a maximally effective concentration of VIP receptor antagonist partially inhibited NANC relaxation of longitudinal muscle of the distal colon of Wistar rats. The extent of the inhibition increased with age. Mode of an increase of the inhibition was fairly consistent with that of ChTx-sensitive component (Figures 1 and 3). Inhibitory effects of VIP_{10-28} and ChTx were not additive (Figure 4). VIP_{10-28} selectively inhibited the delayed phase of i.j.ps induced by EFS which was recorded in elder rats (Figure 6). We could not confirm the effect of ChTx on the i.j.ps, since we were unable to record continuously the membrane potentials of the smooth muscle cells due to the stimulatory effect of ChTx on smooth muscle contractility. Since VIP was suggested to mediate NANC relaxation of longitudinal muscle of the distal colon *via* opening of ChTx-sensitive K^+ channels in Wistar-ST rats (Kishi *et al*., 1996), it seems that VIP mediates the NANC relaxation *via* opening of ChTx-sensitive K^+ channels also in the Wistar rat intestine, and that the role of VIP as a mediator of NANC relaxation increases with age. Hyperpolarization of the membrane potential induced by exogenous VIP also seems interesting. Magnitude of the slow hyperpolarization induced by exogenous VIP increased with age (Figure 7). The result suggests that sensitivity of smooth muscle cells to VIP in inducing membrane hyperpolarization is almost absent in the neonatal stage and the sensitivity increases with age. This change in the sensitivity is fairly consistent with an increasing role of VIP with age.

We previously showed that participation of nitric oxide in NANC relaxations in various intestinal regions of the Wistar rats was very significant in 2-week-old and its significance as an inhibitory mediator decreases with age gradually or

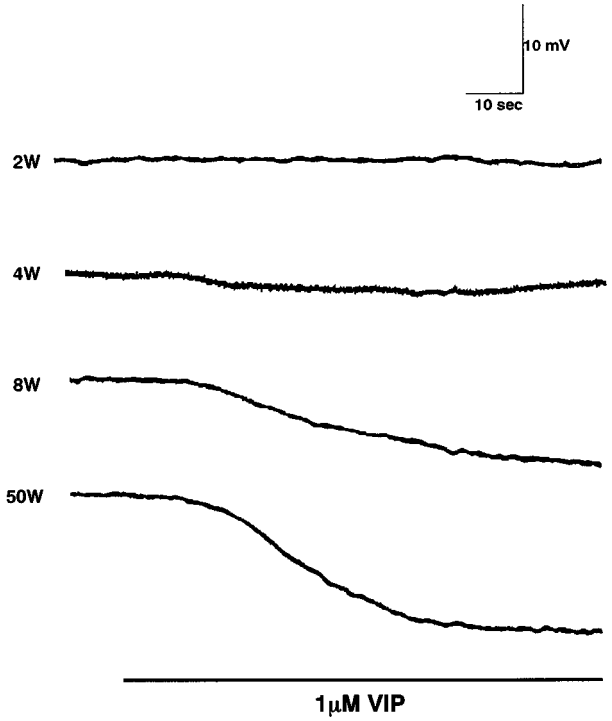

Figure 7 Effects of exogenous VIP on the resting membrane potential of longitudinal muscle cell of the Wistar rat distal colon. Effect of VIP (1 μM) was examined in 2-, 4-, 8- and 50-week-old rats. Note that exogenously added VIP induced age-dependent slow hyperpolarization. Line indicates the presence of VIP.

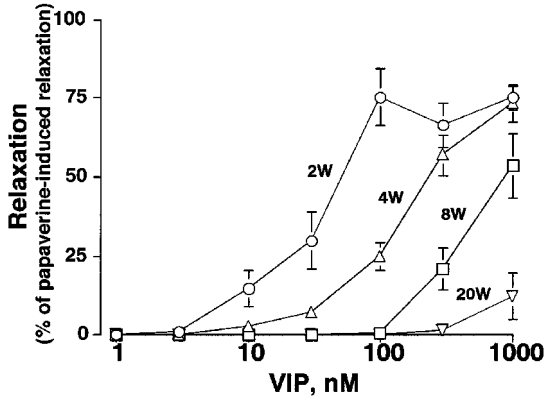

Figure 8 Effects of exogenous VIP on longitudinal muscle of the Wistar rat distal colon. Relaxations were induced by various concentrations of VIP in the segments prepared from 2- to 20-week-old rats. Relaxations are expressed as percentages of the maximum relaxation induced by 30 μM papaverine. Values are mean $\pm$ s.e.mean for 4–11 experiments.

rapidly, differently among the regions (Takeuchi *et al*., 1998). The decrease was remarkable in 50-week-old: nitric oxide-mediated component of the relaxation was absent in the jejunum, ileum and rectum and only slightly present in the proximal and distal colon (Takeuchi *et al*., 1998). Extent of NANC relaxation itself determined by comparing to maximum relaxation induced by 30 μM papaverine in each intestinal segment is similar over the range of age studied (Takeuchi *et al*., 1998). Therefore, mediator(s) other than nitric oxide must compensate for the loss of participation of nitric oxide in inducing NANC relaxation in the elder rat intestine. An increase in participation of VIP in the NANC relaxation in the distal colon with age was first shown in the present study. A rise of VIP-mediated component and a decline of nitric oxide-mediated component with age make a fine contrast (Table 1). If the two mediators work to compensate each other for losses of both roles in mediating NANC relaxation of the distal colon, interrelationship of the two mediators seems the most interesting theme to be solved.

Unexpectedly, relaxant response of longitudinal muscle of the distal colon to exogenous VIP was largest in 2-week-old and gradually decreased with age (Figure 8). However, we can safely say that the significant relaxant effect of exogenous VIP in younger rats has no significant physiological meaning, if any. Namely, exogenous VIP-induced relaxation seems membrane potential independent and EFS-induced relaxation is not inhibited by a VIP receptor antagonist in younger rats, although the relaxation mediated by excitation of myenteric nerves induced by EFS in elder rats is followed by hyperpolarization of the cell membrane and antagonized by the antagonist, as shown in the present study.

Previous studies in the rat intestine showed significant regional differences in the role of nitric oxide as a mediator of NANC relaxation (Suthamnatpong *et al*., 1993a,b; Niioka *et al*., 1997; Takeuchi *et al*., 1998). It has also been shown that the mediators of NANC relaxation in the rat intestine are different among the strains (Hata *et al*., 1998; Okishio *et al*., 2000). Namely, in longitudinal muscle of the colon of 8-week-old Wistar-ST rats, nitric oxide was suggested to mediate the relaxation in the proximal, but not distal region (Suthamnatpong *et al*., 1993a,b), whereas it mediates the relaxation in the distal as well as the proximal region in Wistar rats (Takeuchi *et al*., 1998). Furthermore, VIP was suggested to partially (about 40% of the relaxant response) mediate the relaxation in the distal colon of Wistar and Wistar-ST rats, but not in that of Sprague Dawley rats (Okishio *et al*., 2000). However, present results suggest that these differences and differences among numerous reports which had been published may be due to differences in age and strains of animals used. Thus, when VIP- and nitric oxide-mediated components of the NANC relaxation in the rat intestine are discussed, age and strain of the rat must be carefully considered.

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