Decrease in Participation of Nitric Oxide in Nonadrenergic, Noncholinergic Relaxation of Rat Intestine With Age

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ABSTRACT—Participation of nitric oxide in the electrical field stimulation-induced nonadrenergic, noncholinergic (NANC) relaxation in various intestinal regions was studied in 2- to 50-week-old Wistar rats. In the jejunum of 2-week-old rats, the extent of the nitric oxide-mediated component of the relaxation of longitudinal muscle was approximately 60-70%, whereas the component was 40-50% in 4-week-old rats and was absent in 8- and 50-week-old rats. Thus, nitric oxide seems to be the most important mediator at young ages but its significance is lost with age. The same tendency as that in the jejunum was also shown in longitudinal muscle of the ileum, proximal and distal colon, and rectum. The tendency was also shown in the circular muscle of the rectum. Sensitivity of the longitudinal muscle of the jejunum and proximal colon to exogenously added nitric oxide was high in younger rats. Immunoreactive structures for nitric oxide synthase were observed in the circular muscle layer of the rectum. The population of the structures was denser in 4-week-old than that in 50-week-old. The results suggest that NANC relaxation in every region of the intestine at 2-week-old is almost solely mediated by nitric oxide, and its significance as an inhibitory mediator gradually or rapidly decreases with age.

Keywords: Nitric oxide, Nonadrenergic, noncholinergic (NANC) relaxation, Aging, Rat intestine, Nitric oxide synthase

Nitric oxide has been suggested to be a possible mediator of the nonadrenergic, noncholinergic (NANC) relaxant response of many smooth muscle tissues. It was recently suggested that participation of nitric oxide in the response changes with age: nitric oxide release from aortic endothelium (1) and from coronary endothelium (2) of rats decreased with age. Decrease in endothelium-dependent coronary microvascular function with age was also suggested in humans (3). There are also a few reports on the influence of age on nitrergic innervation in the gastrointestinal tract. Namely, it was suggested in the rat gastric fundus that importance of the nitrergic innervation increased during development (4) and that the relaxant response to sodium nitroprusside, via changes at the level of the cyclic GMP-dependent protein kinase, decreases with age (5). Slight decrease in contribution of nitrergic innervation to NANC relaxant response with age was also suggested in the rat ileum (6). On the other hand, there are only a few reports on the change in the population of nitric oxide synthase with age. Furthermore, these results are still contradictory. The number of NADPH-diaphorase-positive neurons in the myenteric plexus within the small intestine of rats did not significantly change with age (7). Another report suggested a significant increase in NADPH-diaphorase-positive neurons with age in the myenteric plexus of the proximal colon but not the ileum of rat intestine (8). Recently, the presence of and changes in nitric oxide synthase were studied in fetal and neonatal rat enteric neurons. Since nitric oxide synthase was present at term and the number of immunoreactive neurons gradually increased with age, it was suggested that the neurochemical differentiation is accomplished during the first month of postnatal life (9). Thus, reports were still insufficient to know the influence of aging on the nitric oxide-mediated responses in the gastrointestinal tract.

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Although the role of nitric oxide as a mediator of NANC relaxation was suggested in many regions of the gastrointestinal tract (10, 11), accumulated evidence suggests that the role of nitric oxide is not uniform throughout the intestine. For example, in Wistar-ST rat intestine, an essential role of nitric oxide was suggested in circular (12) and longitudinal (13, 14) muscles of the proximal colon and in the circular muscle of the rectum (15), while only a minor or no role was suggested in the longitudinal muscle of the jejunum (16) or distal colon (13, 17), respectively. In the present study, we carefully examined the influence of aging on the nitric oxide-mediated responses in various intestinal regions of the rat to determine if such a regional difference in the participation of nitric oxide in NANC relaxation is associated with possible changes in the role of nitric oxide with age. It will be shown in this report that an essential role of nitric oxide in NANC relaxation found in every intestinal region of younger rats is lost with age.

MATERIALS AND METHODS

Two-, four-, eight- or fifty-week-old Wistar rats obtained from JCL, Inc. (Osaka) were used (2- and 4-week-old, either sex; 8- and 50-week-old, male). Although we had described the rats used as just Wistar rats in our previous studies (12–14, 18–22), Wistar-ST strain rats had been used. The strain is included in a subclass of the Wistar strain and supplied from Nippon SLC (Shizuoka) and widely used in Japan. We used the Wistar strain, instead of the Wistar-ST strain, in the present study. The rats were lightly anesthetized with diethyl ether and then stunned by a blow on the head and bled via the carotid arteries. Segments of the jejunum, ileum, proximal and distal colon, and the rectum were removed and placed in Tyrode solution of the following composition: 137 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 1.1 mM $MgCl_2$, 0.42 mM NaH_2PO_4, 11.9 mM $NaHCO_3$ and 5.6 mM glucose. The contents of the excised segment were gently flushed out with Tyrode solution. Whole segments of each intestinal region except the ileum were used. Ileal segments, 2.5 cm in length, were excised from the central part of the ileum. The narrow part formed by the sphincter on the ascending colon defined the boundary between the proximal and middle regions. 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. Changes in tissue and body weights during aging are shown in Fig. 1.

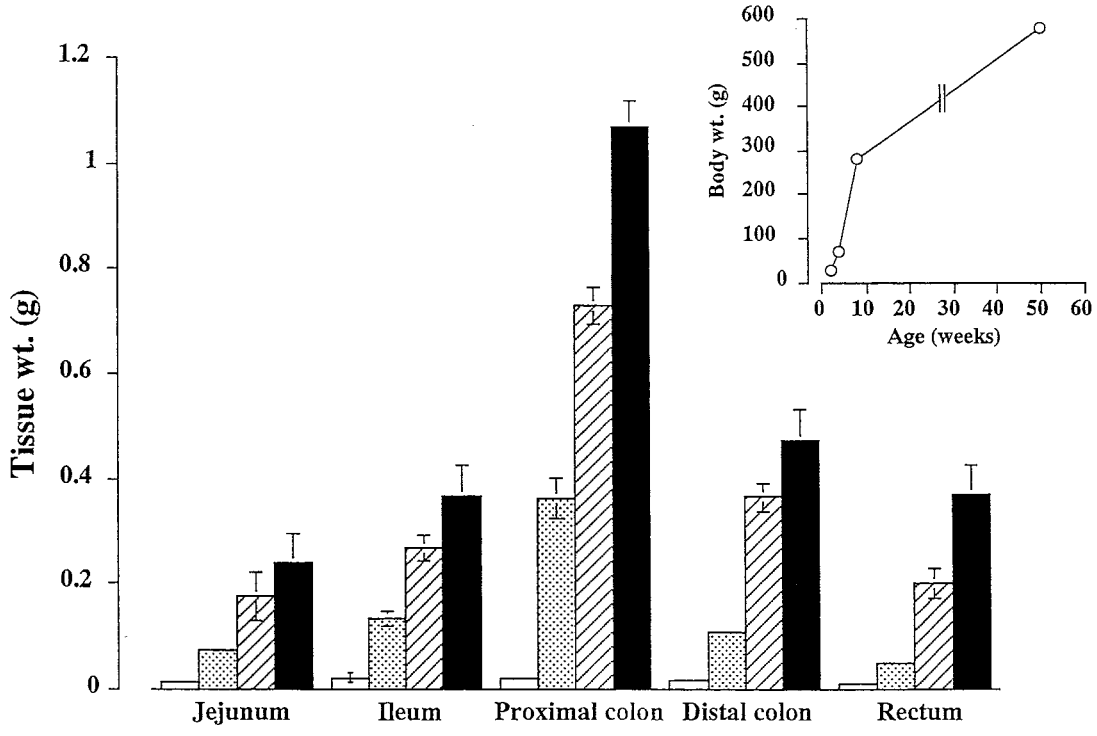

Fig. 1. Changes in body and tissue weights of each intestinal region during increasing age of Wistar rats. Wet weight of segments of each intestinal region prepared from 2 (open columns)-, 4 (dotted)-, 8 (hatched)- and 50 (filled)-week-old rats is shown. Values are each the mean ± S.E.M. for 4–13 preparations. Inset: change in body weight of Wistar rats. Values are the means for 13–16 preparations. For further details, see Materials and Methods.

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

Intestinal segments 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 sec with trains of 1–100 pulses of 0.5-msec 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 suitable resting load of 0.5–1.0 g to obtain the most reproducible responses and stable resting tone. 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 responses of circular muscle of rectum to EFS

Strips were prepared from the rectum by cutting transversely to the longitudinal axis of the tract to record the response of the circular muscle. Responses of the circular muscle to EFS were recorded isotonically in the same way as that in experiments with longitudinal muscle. The circular muscle was subjected to a resting load of 0.5 g.

Immunohistochemical study of nitric oxide synthase

Segments of the rectum were dissected and immersed in Zamboni solution for 72 hr and rinsed for 24 hr at 4°C in 0.1 M phosphate buffer (pH 7.4) containing 30% sucrose. Nitric oxide synthase immunoreactive structures were visualized by the peroxidase anti-peroxidase (PAP) method. Sections (20-μm-thick) were longitudinally cut on a cryostat and were detached on poly-L-lysine-coated slide glasses. The sections were rinsed in 0.1 M phosphate-buffered saline (PBS) and were incubated in 10% normal goat serum (NGS) in 0.1 M PBS for 1 hr before incubation with the first antiserum against nitric oxide synthase. Nitric oxide synthase antiserum was purchased from Sigma (bNOS, Lot No. N2280; St. Louis, MO, USA) and diluted 1:1000 in 0.1 M PBS containing 0.3% Triton X-100, 1% NGS and 1% bovine serum albumin. After 72 hr of incubation with diluted antiserum at 4°C, the sections were rinsed in 0.1 M PBS at 4°C for 1 hr, and then incubated for 24 hr in goat anti-rabbit IgG immunoglobulin (Vector, Burlingame, CA, USA) diluted 1:1000. Following rinsing above, the sections were incubated with PAP complex (dilution 1:1000; Cappel, Durham, NC, USA) at 4°C for 24 hr. Peroxidase reaction products were visualized by incubation at room temperature for 20 min in 0.05 M Tris-HCl buffer containing 3,3-diaminobenzidine (20 mg/100 ml), ammonium nickel (II) sulfate hexahydrate (600 mg/100 ml) and 30% hydrogen peroxide (10 μl/10 ml). The sections were rinsed several times in 0.1 M PBS and dehydrated through a graded series of ethanol solutions.

Drugs

N^G-Nitro-L-arginine (L-NOARG) and L-arginine hydrochloride were purchased from Sigma. All other chemicals were of analytical grade. Gaseous nitric oxide was dissolved in Tyrode solution freshly before experiments as described by Gillespie and Sheng (23). The nitric oxide solution was added to the organ bath in volumes of 0.3–300 μl. These volumes of Tyrode solution alone did not affect the spontaneous contractile activity or the muscle tone.

RESULTS

Relaxation of longitudinal muscle of preparations obtained from various intestinal regions induced by EFS

In the presence of 1 μM atropine and 5 μM guanethidine, EFS with trains of 100 pulses at 10 Hz induced rapid, transient relaxation and then contraction of the longitudinal muscle of segments prepared from the jejunum, ileum, proximal colon and distal colon of 8-week-old Wistar rats. Properties of spontaneous contractile activity and EFS-induced responses in each intestinal region of Wistar rats were similar to those obtained in Wistar-ST rats (see Materials and Methods section) as shown previously: the jejunum (16), ileum (18, 19), proximal colon (13) and distal colon (13, 22).

In the rectum of Wistar rats, longitudinal muscle exhibited small spontaneous contractions at low frequency. In comparison to large spontaneous rhythmic contractions in the proximal colon, their frequency and amplitude were less than 20% of those in the proximal colon. All preparations tested from the rectum showed only contraction in response to EFS with trains of 100 pulses at 10 Hz examined in every 10 min in the initial 2 hr. However, the resting tone of the longitudinal muscle gradually increased during successive trials of EFS. When a higher resting tone was acquired in this way, the preparation began to exhibit a rapid transient relaxation followed by a contraction. When atropine was not added to the bathing fluid, the response was always contraction alone. Tetrodotoxin (1 μM) abolished all the responses to EFS.

Properties of relaxant response to EFS of each region in 2-, 4- and 50-week-old rats were similar to those of the corresponding region in 8-week-old animals as mentioned above. The relaxant responses were expressed as a percentage of the maximum relaxation induced by 30 μM papaverine (Fig. 2). There was not such a significant change in the extent of the relaxation among 2- to 50-week-old rats, although a tendency of a slight decrease with increasing age was seen.

Effects of L-NOARG and L-arginine on EFS-induced relaxation of longitudinal muscle of jejunum prepared from 2-, 4-, 8- and 50-week-old rats

Treatment of the segments of the jejunum of 8-week-old Wistar rats with 10 μM L-NOARG did not affect spontaneous contractile activity, tone of the longitudinal muscle or EFS (100 pulses at 10 Hz)-induced NANC relaxation. Although the treatment also did not affect the EFS-induced relaxation in 50-week-old rats, it resulted in a moderate inhibition of the relaxation in 4-week-old rats and a significant inhibition in 2-week-old rats with the maximum effect within 20–40 min (Fig. 3). Addition of L-arginine (1 mM) to the bathing fluid gradually reversed the inhibitory effect of L-NOARG, causing the complete reversal in 20–30 min (Fig. 3). Thus, the magnitude of the inhibition by L-NOARG was little in elder rats, suggesting a decrease in nitric oxide-mediated component with age (Fig. 3 and Table 1). Concentrations of L-NOARG higher than 10 μM did not show a further inhibitory effect in 2- and 4-week-old rats or any inhibitory effect in 8- and 50-week-old rats. Although the higher concentrations exhibited somewhat stronger inhibition than that at 10 μM in some instances, such inhibition was not reversed by the addition of L-arginine. The effects of L-NOARG on NANC relaxation were also examined on EFS over the range of frequencies tested (0.1–10 Hz): the magnitude of the inhibition at each age was roughly equal at different frequencies (Fig. 4). In subsequent studies, the segments were stimulated at 10 Hz with good reproducibility of response.

Effects of L-NOARG and L-arginine on EFS-induced relaxation of ileum, proximal and distal colon, and rectum

Participation of nitric oxide in the NANC relaxation of longitudinal muscle was also studied in the ileum, proximal and distal colon, and rectum by examining the effects of L-NOARG and L-arginine on the EFS-induced relaxation at 10 Hz. In the ileum, significant participation of nitric oxide was observed in 2-week-old rats and the

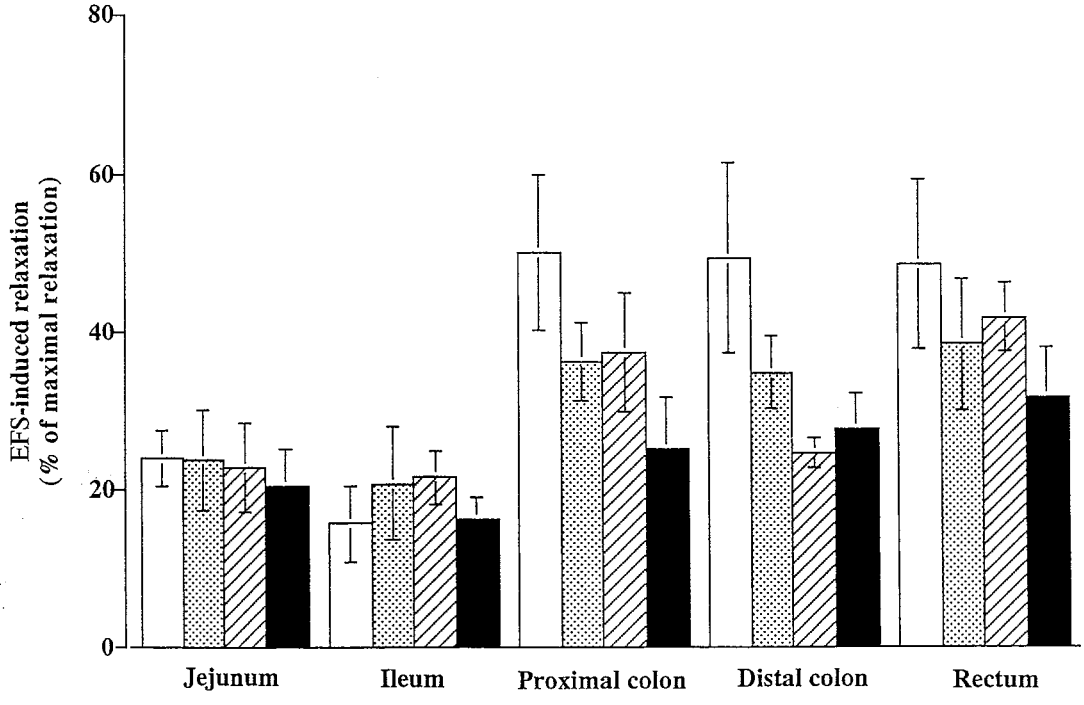

Fig. 2. Magnitude of NANC relaxation induced by EFS of various intestinal regions prepared from 2-, 4-, 8- and 50-week-old Wistar rats. NANC relaxations of longitudinal muscle of various intestinal regions prepared from 2 (open columns)-, 4 (dotted)-, 8 (hatched)- and 50 (filled)-week-old rats were induced by EFS with trains of 100 pulses at 10 Hz. EFS-induced relaxations were expressed as a percentage of the maximal relaxation induced by 30 μM papaverine. Values are each the mean ± S.E.M. for 3–5 experiments.

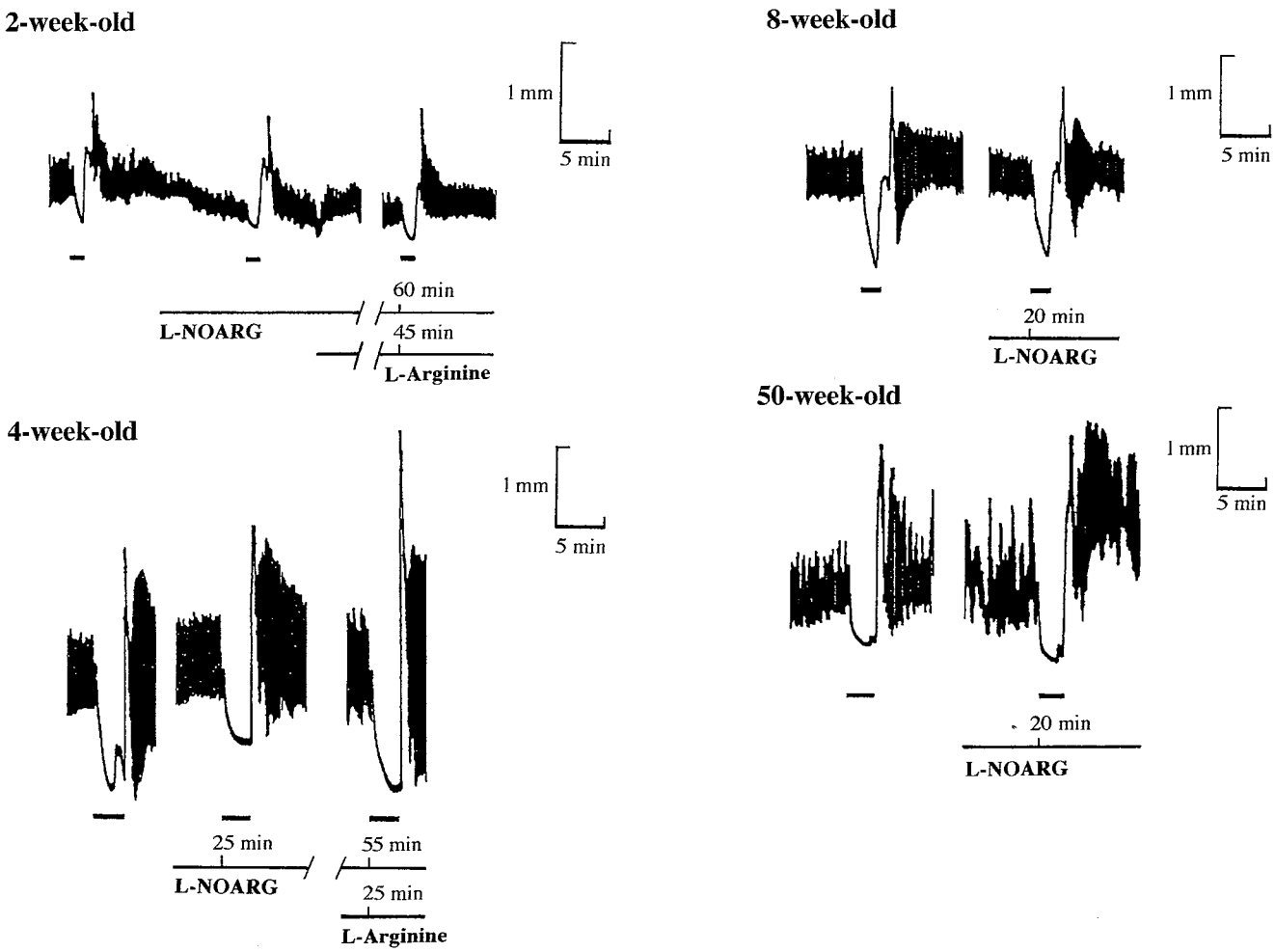

Fig. 3. Effects of L-NOARG and L-arginine on EFS (100 train pulses at 10 Hz)-induced relaxation of longitudinal muscle of the jejunum prepared from 2-, 4-, 8- and 50-week-old rats. The continuous lines indicate the presence of L-NOARG (10 μM) and L-arginine (1 mM). Times noted on the lines indicate the time after addition of the drugs. Bold black lines indicate the duration of EFS for 10 sec. After recording normal spontaneous movements, the chart was run at a fast speed immediately before the stimulation to make the relaxant response clear.

Table 1. Nitric oxide-mediated component in NANC relaxation in 2-, 4-, 8- and 50-week-old rats

	Nitric oxide-mediated component (%)			
	2-week-old	4-week-old	8-week-old	50-week-old
Jejunum	70.2±9.1 (6)	39.6±10.9 (5)	3.5± 3.5 (4)	5.0± 5.0 (3)
Ileum	90.4±4.6 (5)	54.8± 8.2 (5)	43.3±12.5 (3)	6.9± 3.6 (8)
Proximal colon	—	80.8± 8.6 (5)	88.2± 6.8 (4)	11.8± 9.6 (4)
Distal colon	89.3±3.1 (4)	51.1±11.1 (5)	37.5±12.9 (4)	20.6±11.1 (5)
Rectum	96.7±3.3 (3)	2.7± 1.7 (6)	3.7± 3.7 (6)	0 (5)
Rectum (Circular)	—	66.5±11.5 (4)	4.0± 4.0 (3)	0 (2)

Relaxations of longitudinal muscle (circular muscle also in the rectum) of segments obtained from various intestinal regions to EFS at 10 Hz were recorded. The component of the relaxation 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 and expressed as percentages of the relaxation before addition of the drug. Only the segments from the ileum were treated with 100 μM L-NOARG, since the concentration further inhibited the relaxation and 1 mM L-arginine reversed the inhibitory effect in the region. Values are each the mean±S.E.M. for the numbers of experiments shown in parentheses.

participation significantly decreased at 4 weeks and afterwards was still decreasing gradually with age (Table 1).

In the proximal colon, almost full participation of nitric oxide in the NANC relaxation was suggested until 8 weeks and the participation significantly decreased in 50-week-old rats (Table 1).

In the distal colon, significant participation of nitric oxide in the relaxation was also observed at 2-week-old and the most gradual decrease with age among the regions studied was suggested (Table 1).

In the rectum, the most drastic change occurred between 2 and 4 weeks: L-NOARG completely inhibited the relaxation in 2-week-old rats, while it did not show any significant effect on the relaxation in rats older than 4 weeks (Table 1).

As mentioned above, L-NOARG exhibited its maximal inhibitory effect at 10 μM in every preparation with the exception of the ileum in which the maximal effect was exhibited at 100 μM.

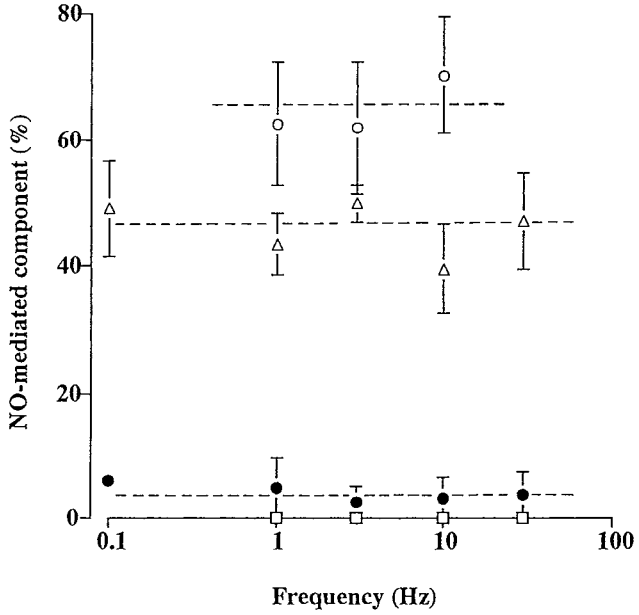

Fig. 4. Nitric oxide (NO)-mediated component in NANC relaxation of longitudinal muscle of the jejunum induced by EFS at various frequencies of trains of pulses. Inhibitory effect of L-NOARG and reversal effect of L-arginine on the NANC relaxations induced by EFS at various frequencies of trains of pulses (0.1, a single pulse, to 30 Hz) were examined in the jejunum prepared from 2 (○)-, 4 (△)-, 8 (□)- and 50 (●)-week-old rats. The component of relaxation 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. The component was expressed as a percentage of the relaxation before addition of L-NOARG. Values are each the mean±S.E.M. for 3–6 experiments.

Examination of participation of nitric oxide in NANC relaxation in circular muscle of rectum

EFS-induced NANC relaxation of the circular muscle of the rectum was not affected by L-NOARG in 8- and 50-week-old rats, but it significantly inhibited the relaxation in 4-week-old rats. The result also suggests the im-

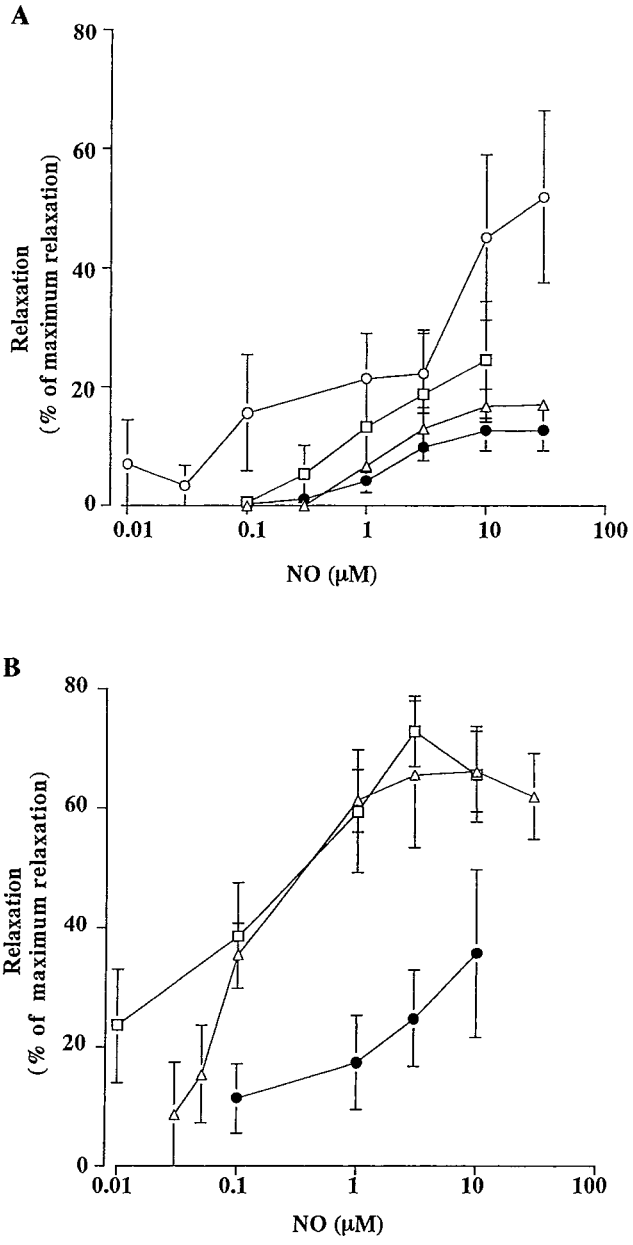

Fig. 5. Effects of exogenous nitric oxide (NO) on segments of jejunum (A) and proximal colon (B). Relaxations were induced by various concentrations of nitric oxide in the segments prepared from 2 (○)-, 4 (△)-, 8 (□)- and 50 (●) (jejunum)-, or 4 (△)-, 8 (□)- and 50 (●) (proximal colon)-week-old rats. Relaxations are expressed as percentages of the maximum relaxation induced by 30 μM papaverine. Values are each the mean±S.E.M. for 3–5 experiments or the mean of 2 experiments.

portance of nitric oxide in the relaxation in the circular muscle during the young age, although its role had already decreased by the time the rats reached 8 weeks of age (Table 1).

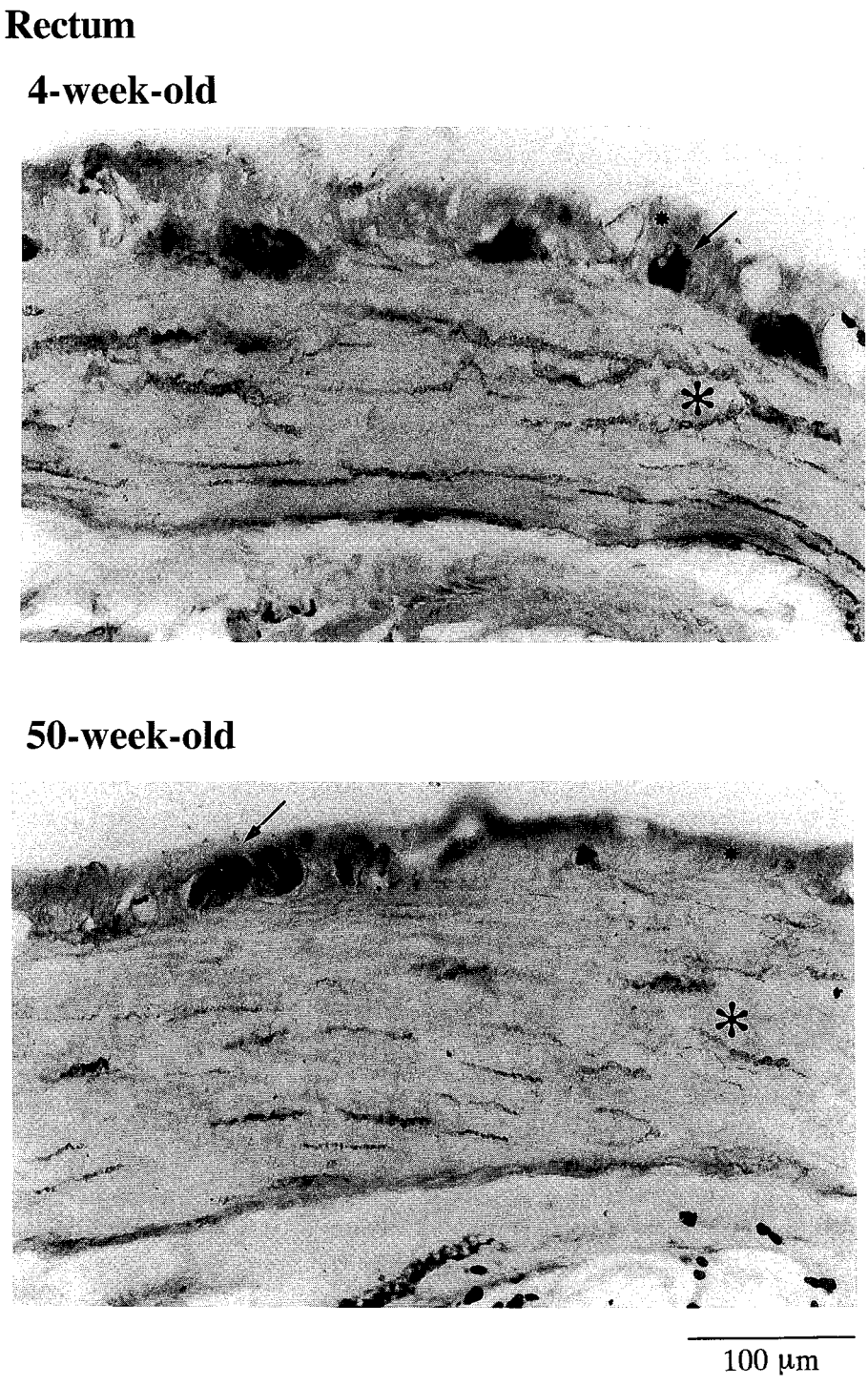

Fig. 6. Nitric oxide synthase immunoreactive structures in rat rectum. Sections were made vertically to the longitudinal axis of the intestine from 4 (upper panel)- or 50 (lower)-week-old rats. Top side of pictures is the serosal side of the tissue. Nitric oxide synthase immunoreactivity is seen in the myenteric plexus (arrows) and circular muscle layer (large asterisks), but not in the longitudinal muscle layer (small asterisks). Note that population of immunoreactive structures is denser at 4-week-old than at 50-week-old.

Relaxant effect of exogenous nitric oxide on longitudinal muscle of jejunum and proximal colon prepared from 2-, 4-, 8- and 50-week-old rats

We next examined the sensitivity of the smooth muscle to exogenous nitric oxide in the preparations of 2- to 50-week-old rats. Exogenous nitric oxide induced relaxation of longitudinal muscle of the jejunum in a concentration-dependent manner. The magnitude of the relaxant effect was large at 2-week-old and small at 50-week-old (Fig. 5A). Also in the proximal colon, magnitude of the relaxation was large in 4- and 8-week-old rats and small in 50-week-old rats, although the relaxation in the concentration-response curve was started from lower concentrations of nitric oxide in the younger age (Fig. 5B). These results suggest that the sensitivity of the smooth muscle of the intestine to nitric oxide decreases with age.

Immunohistochemical examination of nitric oxide synthase in rectum

Since changes in the participation of nitric oxide in NANC relaxation with age were shown in the longitudinal muscle of various regions of the intestine as well as in the circular muscle of the rectum, we further studied the change in the population of nitric oxide synthase in the rectum with age by immunohistochemistry. In the rectum of 4-week-old rats, many nitric oxide synthase immunoreactive structures were observed in the myenteric plexus and circular muscle layer, while there were few immunoreactive structures in the longitudinal muscle layer (Fig. 6). On the other hand, in 50-week-old rats, immunoreactive structures were also observed in the myenteric plexus and circular muscle layer, but the populations were less than those in 4-week-old rats (Fig. 6).

DISCUSSION

Intestinal segments obtained from 2-week-old rats were extremely small and those from 4-week-old rats significantly smaller than those from elder rats examined in the present study. However, there was not such a significant difference in EFS-induced NANC relaxations with respect to their magnitude, which was expressed as a percentage of the papaverine-induced maximum relaxation of the segment. Frequency of spontaneous contractile activity and responses to EFS were also roughly equal among the segments prepared from the same region of different ages, although those were differences among the segments prepared from different regions. Thus, the magnitude of NANC relaxation in each intestinal region does not change appreciably with age. However, the nitric oxide-mediated component was lost with age. It is noteworthy that participation of nitric oxide in NANC relaxation is essential in every region of 2-week-old rats, and that, in contrast, participation of nitric oxide is almost or completely lost in 50-week-old rats. In the longitudinal muscle of the jejunum, ileum, distal colon and rectum in 2-week-old rats and the proximal colon in 4-week-old rats, relaxation is mediated solely by nitric oxide. In other words, no other NANC mediator(s) is important in the relaxation, if indeed any others participate. Participation of nitric oxide in the relaxation gradually decreases with age in the jejunum, ileum and the distal colon or rapidly decreases between 2 and 4 weeks in the rectum. On the other hand, the important role of nitric oxide remained unchanged in the proximal colon of 8-week-old rats. The magnitude of the nitric oxide-mediated component in the relaxation was highest at 2-weeks-old; however, it was not necessarily 100%. Therefore, it is suggested that a rapid developmental change in the participation of nitric oxide in NANC relaxation of rat intestine occurs. Indeed, such developmental changes were suggested in the regulation of vascular resistance in postnatal swine intestine in vivo (24) and in endothelium-dependent responses of ovine pulmonary artery rings in vitro (25). The participation of nitric oxide is almost lost in every region studied in 50-week-old rats. Since similar magnitude of NANC relaxation to that in the younger age was induced at 50-week-old (Fig. 2), other unidentified mediator(s) must mediate the relaxation in elder rats. Thus, the role of nitric oxide as a mediator of NANC relaxation seems to be gradually or rapidly substituted by another mediator in the rat intestine. In other words, time of development of another mediator differs in each intestinal region, although it is still unclear whether the other unknown mediator is identical in every region.

The significant changes in the extent of participation of nitric oxide between 2 and 4 weeks of age is also very interesting, since the weaning period of the rat is 3 weeks after birth. That is, it remains as an interesting question how the luminal content of intestine affects the participation of myenteric nitrergic neurons in NANC relaxation according to whether animals are fed on milk or a standard diet of pellets.

We further examined the change in sensitivity of the tissue to nitric oxide during aging to search for the reason why the role of nitric oxide in the relaxation is lost with age. In two regions examined, the sensitivity tended to be high at 2 weeks of age and low at 50 weeks of age. Since the magnitude of the response to exogenous nitric oxide varied from preparation to preparation and its deviation is significantly larger than that in the mechanical response to EFS, the result of decrease in the sensitivity of the tissue to exogenously added nitric oxide is not necessarily compatible to the result of decrease in the mechanical response. The results, however, suggest that the decrease in the sensitivity is partly, if not fully, associated with the

decrease in the participation of nitric oxide in the relaxation.

Although a decrease with age in the number of nitric oxide synthase-containing nerve fibers was recently reported in the rat penis (26), there are only a few reports on the gastrointestine as mentioned in the Introduction section. Therefore, we tried to detect nitric oxide synthase in the intestine by the immunohistochemical method to determine if its population changes with age. Previously, immunoreactive structures to nitric oxide synthase were found to be mainly present in the myenteric plexus, circular muscle layer and submucus layer in the intestine (27, 28). In the present study, both longitudinal and circular muscle were studied only in the rectum, although mechanical responses in the circular muscle of 2-week-old rats were not studied due to technical reasons (too small to handle similarly to other preparations). Significant participation of nitric oxide in the relaxation of the circular muscle was seen in 4-week-old rats, but it was absent in both muscles of the rectum in 50-week-old rats. Therefore, immunohistochemistry was carried out in the rectum from 4- and 50-week-old rats. Many immunoreactive structures were observed in myenteric plexus and circular muscle layer in the tissue from 4-week-old rats and those from 50-week-old rats were apparently less. Immunoreactivity was scarce in the longitudinal muscle layer. Thus, the difference in population of nitric oxide synthase between the two ages seems fairly consistent with the difference in mechanical responses. However, a certain amount of the immunoreactive structures were still present at 50 weeks of age, although the amount was significantly less than that at 4 weeks of age. Further quantitative examination is necessary to determine the change in nitric oxide synthase with age in relation to the decrease of the nitric oxide-mediated component in the NANC relaxation in rat intestine.

The present study suggests that participation of nitric oxide in NANC relaxation is very important in every intestinal region of the neonatal stage and the participation is lost with age, and that rates of the loss are different in different intestinal regions. Therefore, even if no association of nitric oxide with NANC relaxation was shown in some region of the gastrointestine in some species of animals, the possibility of its role at a younger age of the corresponding region should not be excluded.

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