British Journal of Pharmacology (1996) 117, 1204–1208

Nitric oxide-mediated relaxation without concomitant changes in cyclic GMP content of rat proximal colon

[1,*],†Tadayoshi Takeuchi, *Masami Kishi, *,†Toshiaki Ishii, *Hideaki Nishio & *,†Fumiaki Hata

*Department of Veterinary Pharmacology, College of Agriculture, †Department of Molecular Physiology and Biochemistry, Research Institute for Advanced Science and Technology, Osaka Prefecture University, Sakai 593, Japan

1 We studied the relation of nitric oxide-mediated relaxation of longitudinal muscle to changes in cyclic GMP content of the tissue in the proximal colon of rats.

2 Dimethylphenylpiperazinium (DMPP) and electrical field stimulation (EFS) induced nitric oxide-mediated relaxation of the segments with a concomitant increase in cyclic GMP content.

3 LY 83583 and methylene blue, soluble guanylyl cyclase inhibitors, significantly inhibited the stimulatory effects of DMPP and EFS on the cyclic GMP content, but did not affect the relaxant responses of the segments to DMPP and EFS.

4 Rp-8 bromo cyclic GMPS, an inhibitor of cyclic GMP-dependent protein kinase had no effect on DMPP- and EFS-induced relaxation.

5 These data strongly suggested that nitric oxide-mediated relaxation of the rat proximal colon is not associated with change in cyclic GMP content of the tissue.

Keywords: Rat proximal colon; nitric oxide; nonadrenergic noncholinergic relaxation; cyclic GMP; LY 83583

Introduction

Several lines of evidence suggest that nitric oxide or a nitric oxide-related compound is a mediator of nonadrenergic, noncholinergic (NANC) inhibitory response in the gastrointestinal tract (Stark & Szurszewski, 1992). We have reported that electrical field stimulation (EFS) induced NANC-induced relaxation of longitudinal muscle of the proximal colon of rats. N^G-nitro-L-arginine (L-NOARG), an inhibitor of nitric oxide synthase, inhibited the EFS-induced relaxation and L-arginine reversed this inhibition, while exogenous nitric oxide induced relaxation (Suthamnatpong *et al.*, 1993a). EFS of the preparations significantly increased the guanosine 3':5'-cyclic monophosphate (cyclic GMP) content and L-NOARG inhibited while L-arginine reversed this inhibition. Exogenous nitric oxide increased the cyclic GMP content of the preparations (Suthamnatpong *et al.*, 1993b). We also showed a relaxant effect of cyclic GMP in 'skinned smooth muscle' preparations obtained from the longitudinal muscle layer of rat proximal colon (Maehara *et al.*, 1994). From these findings, we suggested that the mechanism of NANC inhibition in rat proximal colon involves a nitric oxide and cyclic GMP generation system. However, there has been no direct evidence for coupling of nitric oxide-cyclic GMP-relaxation in rat proximal colon. To study this coupling, we examined the effects of inhibitors of soluble guanylyl cyclase on the increase in cyclic GMP content and NANC inhibitory responses induced by EFS in longitudinal muscle of the proximal colon of rats.

We have reported participation of cholinergic interneurones in the descending neural pathway in rat proximal colon (Hata *et al.*, 1990) and ileum (Kanada *et al.*, 1993). Irie *et al.* (1991) first suggested that activation of nicotinic acetylcholine (ACh) receptors within the myenteric plexus resulted in nitric oxide-mediated NANC relaxation in the rat duodenum. In the present study, we examined whether activation of nicotinic ACh receptors by dimethylphenylpiperazinium (DMPP) results in NANC relaxation in rat proximal colon and found that DMPP induced NANC relaxation mediated by nitric oxide and cyclic GMP elevation. Therefore, we further examined the effects of inhibitors of soluble guanylyl cyclase on the increase in cyclic GMP content and NANC inhibitory responses induced by DMPP.

[1] Author for correspondence.

Methods

Male Wistar rats (250–350 g) were lightly anaesthetized and then stunned by a blow on the head. The colon was removed and placed in Tyrode solution consisting of (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 narrow part formed by a sphincter in the ascending colon defined the boundary between proximal and middle regions. The contents of the excised segment were gently flushed out with Tyrode solution.

Recording of responses of longitudinal muscle of the proximal colon to EFS or DMPP

Colonic 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 noradrenergic responses, respectively. Responses of the longitudinal muscle of the proximal colon to DMPP or EFS with trains of 100 pulses of 0.5 ms width and supramaximal voltage (30 V) at a frequency of 10 Hz were recorded isotonically with a 10 min interval between tests. The longitudinal muscle was subjected to a resting load of 1.0 g. The preparations were equilibrated for at least 30 min before experiments. Drugs were added to the organ bath in volumes of less than 1.0% of the bathing solution. These volumes of vehicle of the drugs, distilled water, did not affect the spontaneous contractile activity or the muscle tone. When effects of LY83583 (1–10 μM) or methylene blue (30 μM) on DMPP- or EFS-induced relaxation were examined, three trials at least were examined after addition of the drug. The extent of the relaxation was measured as the area under the line for the resting tone immediately before addition of drugs. Relaxations after treatment with drugs were expressed as percentages of those before treatment.

Measurement of cyclic GMP content of longitudinal muscle myenteric plexus preparations

Longitudinal muscle preparations with adhering myenteric plexus were prepared from the proximal colon as described by Paton & Zar (1968). The preparations (10–20 mg wet weight) were equilibrated for 20 min with Tyrode solution at 37°C without or with LY83583 (1–10 μM) or methylene blue (30 μM) and aerated with 95% O_2 and 5% CO_2. The preparations were then incubated with various concentrations of DMPP (1–100 μM) for 30 s or stimulated electrically for 10 s. After the incubation or stimulation period, preparations were quickly frozen on dry ice. Frozen preparations were then homogenized in 2 ml 6.0 N trichloroacetic acid (TCA) solution. After removal of TCA by mixing with ether, the cyclic GMP contents were determined with a cyclic GMP assay kit (Amersham Japan, Tokyo). Every determination of cyclic GMP was carried out in the absence of phosphodiesterase inhibitor.

Drugs

Dimethylphenylpiperazinium iodide was purchased from Aldrich, U.S.A. N^G-nitro-L-arginine (N^5-nitroamidino-L-2,5-diaminopentanoic acid, L-NOARG), L-arginine hydrochloride, D-arginine hydrochloride and 8-bromo cyclic GMP were from Sigma Chemical Co., St. Louis, U.S.A. 8-Bromoguanosine-3′, 5′-cyclic monophosphate, **Rp**-isomer (**Rp**-8 bromo cyclic GMPS) was from BIOLOG Life Sci, Inst., Bremen, Germany. 6-(Phenylamino)-5, 8-quinolinedione (LY83583) was from Res. Biochem. Inc., MA, U.S.A. Tetrodotoxin was from Wako Pure Chemical, Osaka, Japan. Nicotine was from Nakarai Tesque, Kyoto, Japan. All other chemicals were of analytical grade.

Statistical analysis

Results were analysed by Student's t test and a P value of < 0.05 regarded as significant.

Results

Relaxant responses of longitudinal muscle of rat proximal colon to DMPP and EFS

DMPP induced relaxation of longitudinal muscle of the proximal colon in a concentration-dependent manner in the absence or presence of atropine (1 μM) and guanethidine (5 μM; Figure 1). Tetrodotoxin (TTX; 1 μM) or hexamethonium (500 μM) inhibited the DMPP (1–10 μM)-induced relaxation completely. Since the relaxation was clearer when resting tone of the preparations was increased by treatment with prostaglandin (PG) $F_{2\alpha}$, the proximal segments were treated with 1 μM $PGF_{2\alpha}$ in the following experiments. Nicotine (30 μM) also relaxed the segments (Figure 1), though its effect could not be examined repeatedly due to prompt desensitization. N^G-nitro-L-arginine (N^5-nitroamidino-L-2,5-diaminopentanoic acid; 100 μM) markedly inhibited the DMPP-induced relaxation, causing significant inhibition within 20–40 min (Table 1). Addition of L-arginine (1 mM) to the bathing fluid reversed the effect of L-NOARG, causing complete reversal in 20–30 min. D-Arginine had no effect (Table 1).

As we have already shown, EFS also induced nitric oxide-mediated relaxation of the smooth muscle (Suthamnatpong *et al.*, 1993a; Table 1).

Effect of Rp-8 bromo cyclic GMPS on the DMPP- or EFS-induced relaxation of longitudinal muscle of rat proximal colon

8-Bromo cyclic GMP, a membrane permeable analogue of cyclic GMP, inhibited the spontaneous contractile activity of

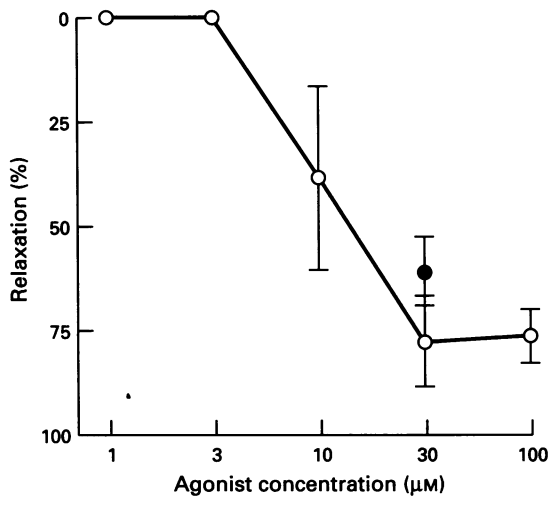

Figure 1 Relaxations of longitudinal muscle of rat proximal colon in response to DMPP and nicotine. The proximal colonic segments were first contracted with 1 μM $PGF_{2\alpha}$. When the contraction reached a constant level, various concentrations of DMPP (○; $n=5$) or 30 μM nicotine (●; $n=5$) were applied. Relaxations were expressed as percentages of amplitude of precontraction with $PGF_{2\alpha}$. Values are means with standard errors.

Table 1 Effects of N^G-nitro-L-arginine (L-NOARG) and inhibitors of soluble guanylate cyclase or PKG on DMPP- or EFS-induced relaxation

	Treatment	*% relaxation*
DMPP-induced relaxation	L-NOARG (100 μM)	27.9±15.9* (5)
	+L-Arginine (1 mM)	100.9±8.5** (5)
	+D-Arginine (1 mM)	39.6±16.0 (3)
	LY83582 (5 μM)	123.5±19.4 (3)
	Methylene blue (100 μM)	102.7±15.4 (3)
	Rp-8 bromo cGMPS (30 μM)	113.0±23.4 (3)
EFS-induced relaxation	L-NOARG (10 μM)	12.7±6.7*# (11)
	+L-Arginine (1 mM)	92.6±4.3**# (8)
	LY83583 (5 μM)	122.9±11.2 (5)
	Methylene blue (100 μM)	103.6±6.1 (4)
	Rp-8-bromo cGMPS (30 μM)	114.4±15.9 (3)

The proximal segments of rats were treated with drugs indicated for 20–40 min. Relaxations induced by 100 μM DMPP or EFS after the treatments are expressed as percentages of those before treatments (control). Values are mean±s.e.mean for the numbers of experiments shown in parentheses. Significantly different from the value of corresponding control (*) and from the value with L-NOARG (**) by Student's t test at $P<0.01$. #These data are from the reference, Suthamnatpong *et al.* (1993a).

the segments at concentrations over 10 μM. At 30 μM it also induced significant sustained relaxation of the longitudinal muscle precontracted by $PGF_{2\alpha}$. Rp-8 bromo cyclic GMPS, an inhibitor of cyclic GMP-dependent protein kinase (PKG), at 30 μM significantly inhibited the relaxant effect of 8-bromo cyclic GMP (Figure 2). However, the same concentration of the inhibitor did not affect the DMPP- and EFS-induced relaxations (Table 1).

Effects of DMPP and EFS on cyclic GMP content of longitudinal muscle of the proximal colon of rats

Although whole segments of rat proximal colon were used to record spontaneous contractile activity and relaxation of longitudinal muscle, longitudinal muscle myenteric plexus preparations were obtained from rat proximal colon for cyclic GMP measurement to avoid contamination by circular muscle cells. DMPP increased the cyclic GMP content of the preparations concentration-dependently (Figure 3). Nicotine (1–10 μg ml^{-1}) also increased the cyclic GMP content of the preparations, though nicotine was less effective than DMPP (Figure 3). TXX (1 μM) completely inhibited the stimulatory effect of 100 μM DMPP on the cyclic GMP content. Treatment of the muscle preparations with 10 μM L-NOARG inhibited

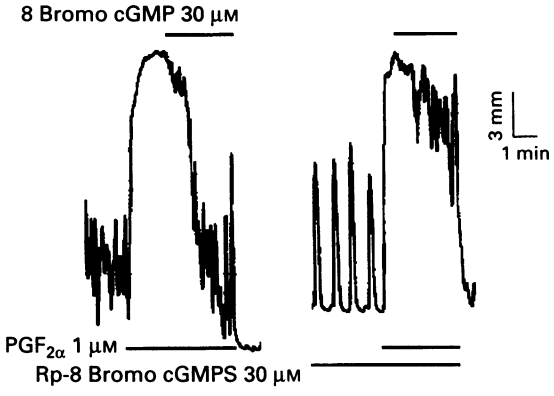

Figure 2 Effect of Rp-8 bromo cyclic GMPS on 8-bromo cyclic GMP-induced relaxation of the rat proximal colon. When the contraction induced by 1 μM $PGF_{2\alpha}$ reached a constant level, 30 μM 8-bromo cyclic GMP was applied in the absence (left side) or presence (right side) or 30 μM Rp-8 bromo cyclic GMPS. Lines indicate the presence of drugs. The recording is typical of three preparations.

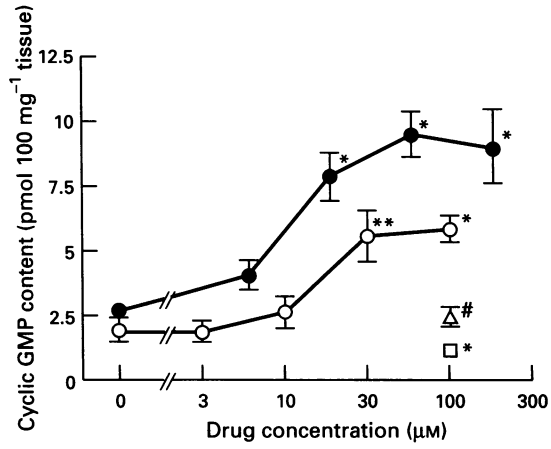

Figure 3 Effects of DMPP and nicotine on cyclic GMP content of longitudinal muscle myenteric plexus preparations of rat proximal colon. Longitudinal muscle myenteric plexus preparations were incubated with indicated concentrations of DMPP (○) or nicotine (●) for 30 s at 37°C. Effects of 100 μM DMPP in the presence of 1 μM TTX (△) or 10 μM L-NOARG (□) were also examined. Values are means with standard errors for 3–6 experiments. Significance of difference from the value in the absence of agonist: $*P<0.01$, $**P<0.02$. Significance of difference from the value of DMPP alone: $^{\#}P<0.01$. For further details, see Methods.

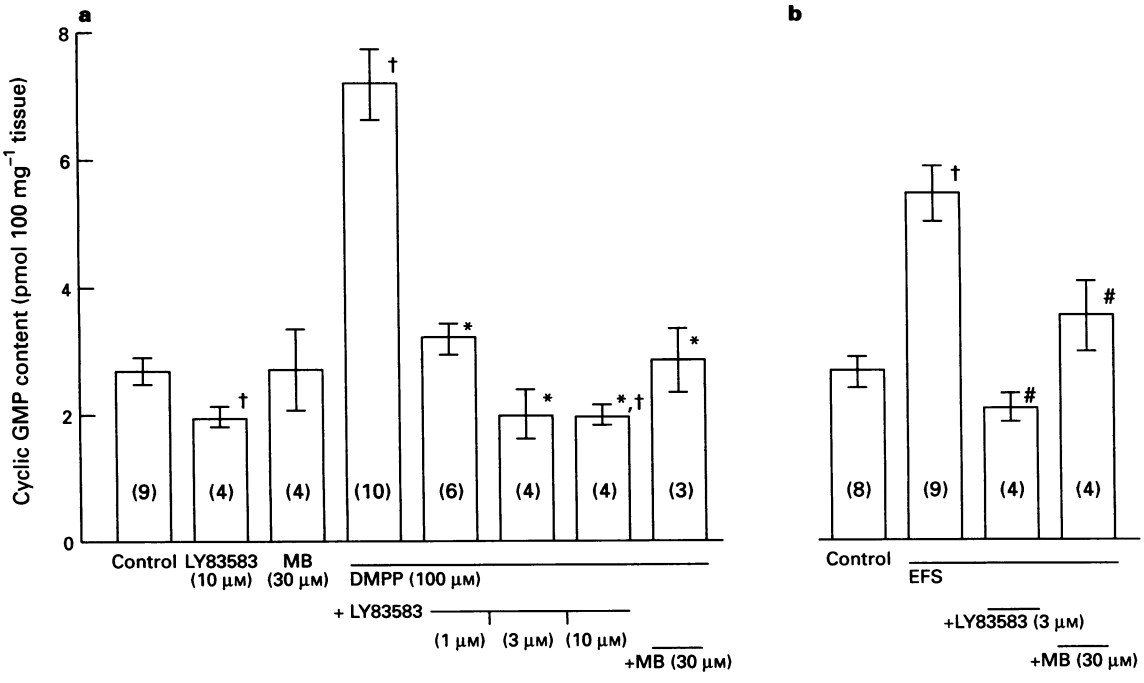

Figure 4 Effects of DMPP and EFS in the absence or presence of LY 83583 or methylene blue on cyclic GMP contents of longitudinal muscle myenteric plexus preparations of rat proximal colon. Longitudinal muscle myenteric plexus preparations were incubated with 100 μM DMPP for 30 s at 37°C, or were stimulated electrically for 10 s in the absence or presence of indicated concentrations of LY 83583 or 30 μM methylene blue (MB). Columns and bars represent means with standard errors from three to ten experiments. Values significantly different at $P<0.01$ from the value of DMPP alone (*) and at $P<0.05$ from the value of control (†). Values significantly different at $P<0.01$ from the value of EFS (#).

not only the stimulatory effect of DMPP on the cyclic GMP content and decreased it below the resting level (Figure 3).

As we have already shown, EFS also increased the cyclic GMP content of the preparations (Suthamnatpong *et al.*, 1993b; Figure 4).

Effects of LY83583 and methylene blue on the DMPP- or EFS-induced increase in cyclic GMP content and relaxation of longitudinal muscle of rat proximal colon

LY83583, an inhibitor of soluble guanylyl cyclase, significantly inhibited the stimulatory effects of DMPP and EFS on cyclic GMP content of the longitudinal muscle myenteric plexus preparations. At 10 μM LY83583 not only inhibited the stimulatory effect of DMPP, but also decreased the cyclic GMP content below the resting level (Figure 4). Methylene blue, another inhibitor of soluble guanylyl cyclase, at 30 μM also inhibited the increase in cyclic GMP content elicited by DMPP and EFS (Figure 4).

However LY83583 (5 μM) and methylene blue (100 μM) did not have any appreciable effect on the basal tone and the relaxations induced by DMPP and EFS (Table 1).

Discussion

Katsuki *et al.* (1977) and Schultz & Schultz (1977) reported activation of guanylyl cyclase by nitrosocompounds and suggested that cyclic GMP played a role in relaxation of smooth muscle. There have been many studies which report an association of increases in cyclic GMP with NANC relaxation of gastrointestinal smooth muscle, such as canine internal anal sphincter (Grous *et al.*, 1991), and opossum (Barnette *et al.*, 1989) and human (Barnette *et al.*, 1991) lower esophageal sphincter. Moreover, there is recent evidence for an association of the cyclic GMP level with nitric oxide-mediated relaxation in preparations of gastrointestinal tract, such as the lower esophageal sphincter of dogs (Shikano *et al.*, 1988), the gastric fundus of guinea-pigs (Jin *et al.*, 1993), ileum (Kanada *et al.*, 1992; 1993) and proximal colon (Suthamnatpong *et al.*, 1993b) of rats, the taenia coli of rabbits (Shikano *et al.*, 1988) and guinea-pigs (Shikano *et al.*, 1988; Jin *et al.*, 1993), and dispersed gastric muscle cells (Murthy *et al.*, 1993; Murthy & Makhlouf, 1995). These findings strongly suggest that a nitric oxide-cyclic GMP generating system is responsible for the NANC relaxation in the gastrointestinal tract.

In the present study, relaxation of the segments and increase in cyclic GMP content of longitudinal muscle preparations of rat proximal colon induced by DMPP and EFS were inhibited by L-NOARG and the inhibition was reversed by a high concentration of L-arginine, indicating an essential role of nitric oxide in these responses. LY83583 has been reported to destroy nitric oxide and inhibit soluble guanylyl cyclase (Mulsch *et al.*, 1988). Methylene blue has also been reported to inhibit soluble guanylate cyclase (Gruetter *et al.*, 1981; Martin *et al.*, 1985; Ignarro *et al.*, 1986). Although LY83583 and methylene blue are not very specific, their inhibitory effects on soluble guanylyl cyclase were also confirmed in longitudinal muscle strips from rat proximal colon in the present study. LY83583 at 10 μM was especially potent: the cyclic GMP levels decreased below the resting level. Under these conditions, surprisingly, the relaxant response induced by EFS or DMPP treatment remained unchanged, indicating a dissociation between EFS or DMPP effect and cyclic GMP-relaxation coupling. The result that a PKG inhibitor, Rp-8 bromo cyclic GMPS (Butt *et al.*, 1990; Bäumner & Nawrath, 1995) did not affect the relaxant response to DMPP and EFS was consistent with this finding. Nevertheless, cyclic GMP did relax skinned muscle preparations obtained from rat proximal colon (Maehara *et al.*, 1994) and a high concentration of 8-bromo cyclic GMP induced gradual sustained relaxation in the present study. It seems that cyclic GMP could induce relaxation of the muscle strips under some experimental conditions, probably at higher concentrations, but the nitric oxide-mediated relaxation induced by treatment with DMPP or EFS is cyclic GMP-independent. We previously showed that nitric oxide-mediated relaxation of rat proximal colon is not associated with the inhibitory junction potentials of the cell membrane (Suthamnatpong *et al.*, 1994). Thus, it is likely that nitric oxide mediates relaxation of rat proximal colon by an unknown mechanism which is not associated with changes in cyclic GMP content and membrane potential of the smooth muscle cells.

This work was supported in part by a Grant-in-Aid for Scientific Research from Ministry of Education, Science, Sports and Culture of Japan.

References

BARNETTE, M., BARONE, F.C., FOWLER, P.J., GROUS, M., PRICE, W.J. & ORMSBEE, H.S. (1991). Human lower oesophageal sphincter relaxation is associated with raised cyclic nucleotide content. *Gut*, **32**, 3–9.

BARNETTE, M., TORPHY, T.J., GROUS, M., FINE, C. & ORMSBEE, H.S. III. (1989). Cyclic GMP: a potential mediator of neurally- and drug-induced relaxation of opossum lower oesophageal sphincter. *J. Pharmacol. Exp. Ther.*, **249**, 524–528.

BÄUMNER, D. & NAWRATH, H. (1995). Effects of inhibitors of cGMP-dependent protein kinase in atrial heart and aortic smooth muscle from rats. *Eur. J. Pharmacol.*, **273**, 295–298.

BUTT, E., VAN BEMMELEN, M., FISCHER, L., WALTER, U. & JASTORFF, B. (1990). Inhibition of cGMP-dependent protein kinase by Rp-guanosine 3',5'-monophosphorothioates. *FEBS Lett.*, **263**, 47–50.

GROUS, M., JOSLYN, A.F., THOMPSON, W. & BARNETTE, M.S. (1991). Change in intracellular cyclic nucleotide content accompanies relaxation of the isolated canine internal anal sphincter. *J. Gastrointest. Motil.*, **3**, 46–52.

GRUETTER, C.A., GRUETTER, D.Y., LYON, J.E., KADOWITZ, P.J. & IGNARRO, L.J. (1981). Relation between cyclic guanosine 3',5'-monophosphate formation and relaxation of coronary arterial smooth muscle by glycerine trinitrate, nitroprusside, nitrite and methemoglobin. *J. Pharmacol. Exp. Ther.*, **219**, 181–186.

HATA, F., KATAOKA, T., TKEUCHI, T., YAGASAKI, O. & YAMANO, N. (1990). Differences in control of descending inhibition in the proximal and distal regions of rat colon. *Br. J. Pharmacol.*, **101**, 1011–1015.

IGNARRO, L.J., HARBISON, R.G., WOOD, K.S. & KADOWITZ, P.J. (1986). Dissimilarities between methylene blue and cyanide on relaxation and cyclic GMP formation in endothelium-intact intrapulmonary artery caused by nitrogen oxide-containing vasodilators and acetylcholine. *J. Pharmacol. Exp. Ther.*, **236**, 30–36.

IRIE, K., MURAKI, T., FURUKAWA, K. & NEMOTO. T. (1991). L-N^G-nitro-arginine inhibits nicotine-induced relaxation of isolated rat duodenum. *Eur. J. Pharmacol.*, **202**, 285–288.

JIN, J.-G., MURTHY, K.S., GRIDER, J.R. & MAKHLOUF, G.M. (1993). Activation of distinct cAMP- and cGMP-dependent pathways by relaxant agents in isolated gastric muscle cells. *Am. J. Physiol.*, **264**, G470–G477.

KANADA, A., HATA, F., SUTHAMNATPONG, N., MAEHARA, T., ISHII, T., TAKEUCHI, T. & YAGASAKI, O. (1992). Key roles of nitric oxide and cyclic GMP in nonadrenergic and noncholinergic inhibition in rat ileum. *Eur. J. Pharmacol.*, **216**, 287–292.

KANADA, A., HOSOKAWA, M., SUTHAMNATPONG, N., MAEHARA, T., TAKEUCHI, T. & HATA, F. (1993). Neuronal pathway involved in nitric oxide-mediated descending relaxation in rat ileum. *Eur. J. Pharmacol.*, **250,** 59–66.

KATSUKI, S., ARNOLD, W., MITTAL, C. & MURAD, F. (1977). Stimulation of guanylate cyclase by sodium nitroprusside, nitroglycerin and nitric oxide in various tissue preparations and comparison to the effects of sodium azide and hydroxylamine. *J. Cyc. Nuc. Res.*, **3,** 23–35.

MAEHARA, T., FUJITA, A., SUTHAMNATPONG, N., TAKEUCHI, T. & HATA, F. (1994). Differences in relaxant effects of cyclic GMP on skinned muscle preparations from the proximal and distal colon of rats. *Eur. J. Pharmacol.*, **261,** 163–170.

MARTIN, W., VILLANI, G.M., JOTHIANANDAN, D. & FURCHGOTT, R.F. (1985). Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. *J. Pharmacol. Exp. Ther.*, **232,** 708–716.

MULSCH, A., BUSSE, R., LIEBAU, S. & FORSTERMANN, U. (1988). LY 83583 interfers with the release of endothelium-derived relaxing factor and inhibits soluble guanylate cyclase. *J. Pharmacol. Exp. Ther.*, **247,** 283–288.

MURTHY, K.S. & MAKHLOUF, G.M. (1995). Interaction of cA-kinase and cG-kinase in mediating relaxation of dispersed smooth muscle cells. *Am. J. Physiol.*, **268,** C171–C180.

MURTHY, K.S., ZHANG, K.M., JIN, J.-G., GRIDER, J.R. & MAKHLOUF, G.M. (1993). VIP-mediated G protein-coupled Ca^{2+} influx activates a constitutive NOS in dispersed gastric muscle cells. *Am. J. Physiol.*, **265,** G660–G671.

PATON, W.D.M. & ZAR, M.A. (1968). The origin of acetylcholine released from guinea-pig intestine and longitudinal muscle strips. *J. Physiol.*, **194,** 13–33.

SCHULTZ, Z. & SCHULTZ, K. (1977). Sodium nitroprusside and other smooth muscle relaxants increase cGMP levels in rat ductus deferens. *Nature* **265,** 750–752.

SHIKANO, K., LONG, C.J., OHLSTEIN, E.H. & BERKOWITZ, B.A. (1988). Comparative pharmacology of endothelium-derived relaxing factor and nitric oxide. *J. Pharmacol. Exp. Ther.*, **247,** 873–881.

STARK, M.E. & SZURSZEWSKI, J.H. (1992). Role of nitric oxide in gastrointestinal and hepatic function and disease. *Gastroenterology*, **103,** 1928–1949.

SUTHAMNATPONG, N., HATA, F., KANADA, A., TAKEUCHI, T. & YAGASAKI, O. (1993a). Mediators of nonadrenergic, noncholinergic inhibition in the proximal, middle and distal regions of rat colon. *Br. J. Pharmacol.*, **108,** 348–355.

SUTHAMNATPONG, N., HOSOKAWA, M., TAKEUCHI, T., HATA, F. & TAKEWAKI, T. (1994). Nitric oxide-mediated inhibitory response of rat proximal colon: independence from changes in membrane potential. *Br. J. Pharmacol.*, **112,** 676–682.

SUTHAMNATPONG, N., MAEHARA, T., KANADA, A., TAKEUCHI, T. & HATA, F. (1993b). Dissociation of cyclic GMP level from relaxation of the distal, but not the proximal colon of rats. *Jpn. J. Pharmacol.*, **62,** 387–393.

(Received August 15, 1995
Revised November 6, 1995
Accepted November 24, 1995)