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Possible Roles of Nitric Oxide and Vasoactive Intestinal Polypeptide on Relaxation Induced by Isoprenaline in Isolated Muscle Strips of the Mouse Gastric Fundus

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The possible role of nitric oxide (NO) and vasoactive intestinal polypeptide on isoprenaline-induced relaxation of the mouse longitudinal gastric fundal strips precontracted with 5.4×10^{-7} M carbachol was investigated. Isoprenaline (5×10^{-7}M, 10^{-6}M and 5×10^{-6}M) produced a concentration-dependent relaxations. N^G-nitro L-arginine (10^{-4}M) partly inhibited isoprenaline-induced relaxation. The inhibitory action of N^G-nitro L-arginine was reversed by 4×10^{-4}M L-arginine but not by 4×10^{-4}M D-arginine. N^G-nitro L-arginine (10^{-4}M) did not affect the relaxation caused by sodium nitroprusside (10^{-6}M). Vasoactive intestinal polypeptide antibody 7913 (1:160 dilution) partly inhibited isoprenaline-induced relaxation. This inhibition was greater on the response to the higher isoprenaline concentration (5×10^{-6}M) than to the lower concentration (10^{-6}M). The combination of vasoactive intestinal polypeptide antibody and N^G-nitro L-arginine significantly enhanced the inhibition on 10^{-6}M isoprenaline action. These results suggest that nitric oxide and vasoactive intestinal polypeptide may partly contribute to the relaxation induced by isoprenaline in the mouse gastric fundus precontracted with carbachol.

Key words: isoprenaline, N^G-nitro L-arginine (L-NOARG), L-arginine (L-ARG), D-arginine (D-ARG), vasoactive intestinal polypeptide (VIP) antibody 7913, isolated mouse gastric fundus

It was shown that isoprenaline-produced concentration-dependent relaxation in the isolated mouse gastric fundus muscle strips precontracted with carbachol (3). In the same tissue (4), the inhibitory action of N^G-nitro L-arginine (L-NOARG) on electrical field stimulation-induced relaxation was observed, suggesting that nitric oxide (NO) may play a role as a mediator. However, the evidence about the contribution of NO to isoprenaline-induced relaxation was obtained from studies on vascular smooth muscle preparations; dependence of isoprenaline-induced relaxation on the endothelium was demonstrated in isolated rat aortic strips (7, 8, 11). In the studies on the same tissue (1), and the rat mesenteric resistance arteries (6), it was suggested that β_1 adrenoceptor activation due to norepinephrine and isoprenaline relaxed the vessels by causing release of endothelium-derived relaxing factor (EDRF) or NO.

According to these findings, it might be interesting to investigate whether NO contributes to relaxation induced by isoprenaline in the longitudinal muscle strips of the mouse gastric fundus. For this purpose, we examined the effect of L-NOARG, an NO synthase inhibitor and its interaction with arginine enantiomers, L-arginine (L-ARG) or D-arginine (D-ARG) on isoprenaline-induced relaxation. Regarding possible stimulation of NO production by vasoactive intestinal polypeptide (VIP) (9), additional experiments were designed using VIP antibody 7913 to determine if isoprenaline causes VIP release.

Materials and Methods

Either sex of mice (*Mus musculus* var. *albino.*) weighing 20-25g were used in the experiments. Mice were fasted for 24h with free access to water. They were killed by a blow on the head and exsanguination via the carotid arteries. The stomach was removed carefully and the fundus was isolated. Strips (about 15mm long and 3mm wide) were prepared by longitudinal incision and were

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mounted under 0.5 g tension in an organ bath filled with Tyrode's solution composed of (in mM): NaCl 136.75, KCl 2.68, $CaCl_2$ 1.8, $MgCl_2 \cdot 6H_2O$ 0.95, $NaH_2PO_4 \cdot 2H_2O$ 0.4, $NaHCO_3$ 11.9 and Glucose 5.5. The bath medium was continuously bubbled with oxygen and the temperature was maintained at 37°C. Changes in the muscle length were recorded via an isotonic lever (×8-10 magnification) on a smoked drum. The tissue was allowed to equilibrate for 60 min, washing with fresh Tyrode's solution every 15 min. After equilibrium was reached, the basal length was recorded for 5 min and then the tissue was treated with Tyrode's solution containing 5.4×10^{-7} M carbachol which was found to be the optimum concentration for inducing reproducible submaximal contraction sustained for at least 50 min without any significant change in contracture length (laboratory observation). The contractile response to carbachol reached a steady state within 10 min. Next, 5×10^{-7} M, 10^{-6} M and 5×10^{-6} M isoprenaline was added to the organ bath and relaxant responses were recorded for 40 min. Then the strip was washed out with fresh Tyrode's solution without carbachol. The same procedure was repeated after allowing the tissue to rest for 60 min. A separate experimental group was used for each isoprenaline concentration. In another series of experiments, after the response to the first isoprenaline administration was recorded, the preparation was washed out with fresh Tyrode's solution and incubated with 10^{-4} M L-NOARG alone or combined with L-ARG or D-ARG (4×10^{-4} M) for 60 min. Ten minutes after the administration of carbachol to the bath, the response of the strip to the second isoprenaline addition was monitored. The inhibitory action of 5×10^{-4} M L-NOARG on isoprenaline induced relaxation was not greater than that of 10^{-4} M L-NOARG. Therefore, the concentration of 10^{-4} M was used in the experiments. The effects of L-NOARG were also examined on sodium nitroprusside (SNP)-induced relaxation in a separate group of similar experiments, using 10^{-6} M SNP instead of isoprenaline. In another series of experiments, VIP antibody 7913 (final dilution 1:160) was added to the bath 60 min before the second carbachol treatment. In a separate group of experiments, the inhibitory action of VIP antibody (1:160 dilution) plus 10^{-4} M L-NOARG were examined on the relaxation produced by 10^{-6} M isoprenaline.

Isoprenaline relaxation was measured in millimeters. Relaxation due to the second isoprenaline addition is expressed as a percentage of the response to the first isoprenaline application, since there was no significant difference between contraction lengths induced by the first and second 5.4×10^{-7} M carbachol additions. Data (mean ± SE) were statistically analyzed by unpaired Student's t test. A probability (P) value of less than 0.05 was considered significant.

The drugs used were isoprenaline hydrochloride, carbachol (carbamylcholine chloride), N^G-nitro L-arginine, L-arginine hydrochloride, D-arginine hydrochloride, SNP dihydrate from Sigma Chemical (St. Louis, MO, USA). Vasoactive intestinal polypeptide (VIP) antibody 7913 was provided by CURE/UCLA/DDC Antibody/RIA Cure, NIH Grant DK41301 as a kindly gift. Isoprenaline stock solution (4×10^{-3} M) was prepared in distilled water (pH 3) containing 5 μM ascorbic acid as an antioxidant. VIP antibody (500 μl) was diluted with Tyrode's solution (final dilution in the bath was 1:160). All other drugs were dissolved in distilled water.

Results

Isoprenaline (5×10^{-7} M, 10^{-6} M and 5×10^{-6} M)

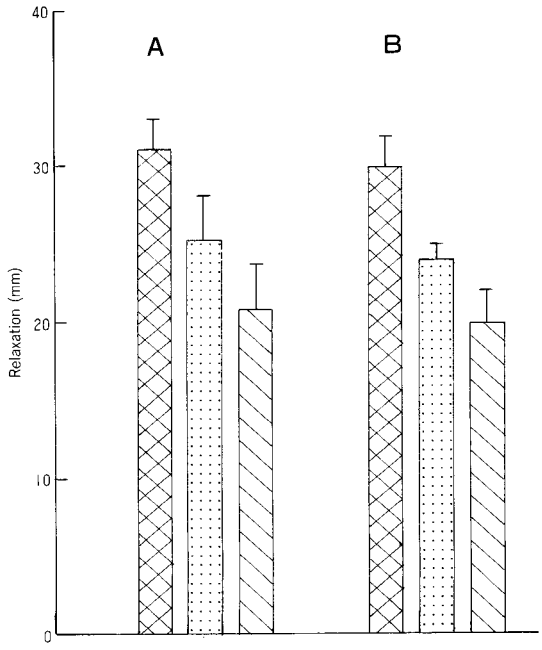


Fig. 1 Relaxations induced by isoprenaline at three different concentrations in the presence of carbachol (5.4×10^{-7} M). Responses of the tissue to the first (**A**), and the second applications (**B**) of the drug are shown. Vertical lines indicate standard errors (S. E.). (▨): 5×10^{-7} M isoprenaline (ISO); (▩): 10^{-6} M ISO; ▧: 5×10^{-6} ISO.

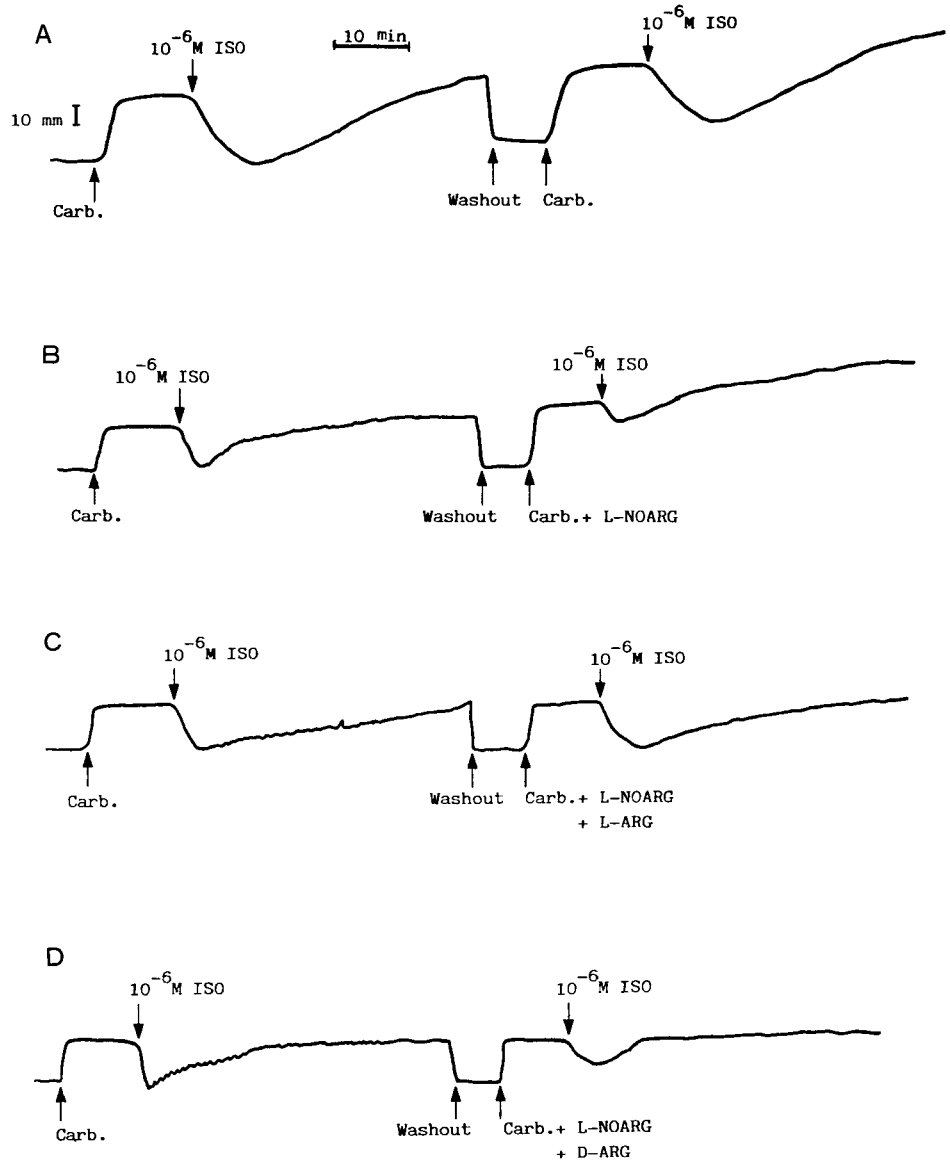
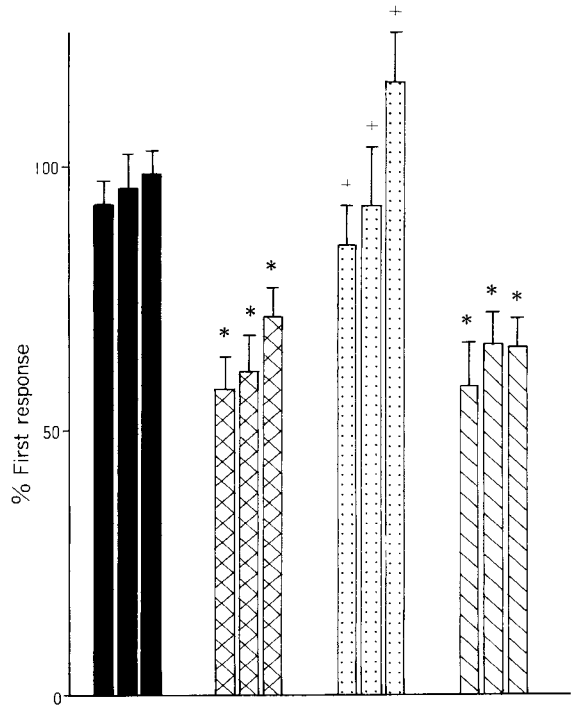


Fig. 2 Relaxation induced by 10^{-6}M isoprenaline (control) (**A**), in the presense of 10^{-4}M L-NOARG (**B**), 10^{-4}M N^{G}-nitro L-arginine (L-NOARG) plus 4×10^{-4}M L-arginine (L-ARG) (**C**), and 10^{-4}M L-NOARG plus 4×10^{-4}M D-arginine (D-ARG) (**D**). ISO: Isoprenaline; Carb: Carbachol (5.4×10^{-7}M).

caused concentration-dependent relaxation in the mouse longitudinal fundal strips precontracted with 5.4×10^{-7}M carbachol (Fig. 1). The response pattern was an initial relaxation followed by a slower recovery phase (Fig. 2A). A slight decrease was observed in the relaxation induced by the second isoprenaline addition. The second response as a percentage of the first isoprenaline relaxation were 92.7 ± 4.6 % (n = 11), 95.8 ± 6.4 % (n = 11) and 98.6 ± 4.3 % (n = 11) from the lower to higher concentration of the drug, respectively. The value for SNP was 96.0 ± 6.5 % (n = 9).

L-NOARG (10^{-4}M) significantly reduced isoprenaline



induced-relaxation but failed to inhibit SNP action. The inhibitory action of L-NOARG seemed to be more considerable on relaxation due to lower isoprenaline concentrations. From lower to higher isoprenaline concentrations, the responses were reduced to $58 \pm 5.8\,\%$ ($P < 0.001$; $n = 9$), $61.1 \pm 6.9\,\%$ ($P < 0.005$; $n = 9$) and $71.4 \pm 5.4\,\%$ ($P < 0.005$; $n = 10$), respectively.

L-ARG (4×10^{-4}M) attenuated the inhibitory action of L-NOARG (10^{-4}M) on the relaxation due to 5×10^{-7} M, 10^{-6}M and 5×10^{-6}M isoprenaline. Values obtained were $84.9 \pm 7.4\,\%$ ($n = 8$), $92.3 \pm 11.2\,\%$ ($n = 8$) and $115.8 \pm 9.1\,\%$ ($n = 8$), respectively. These values were

Fig. 3 (Left) Effects of 10^{-4}M L-NOARG, 10^{-4}M L-NOARG plus 4×10^{-4}M L-ARG and 10^{-4}M L-NOARG plus 4×10^{-4}M D-ARG on the isoprenaline-induced relaxation of the longitudinal muscle of the mouse gastric fundus. In each group of three bars, the isoprenaline concentrations are 5×10^{-7}M, 10^{-6}M and 5×10^{-6}M from left to right. $*P < 0.005$ compared with control. $+P < 0.05$ compared with L-NOARG. L-NOARG; L-ARG; D-ARG: See Fig. 2.
■ Control, ⊠ L-NOARG, ⬚ L-NOARG+L-ARG, ⧅ L-NOARG+D-ARG.

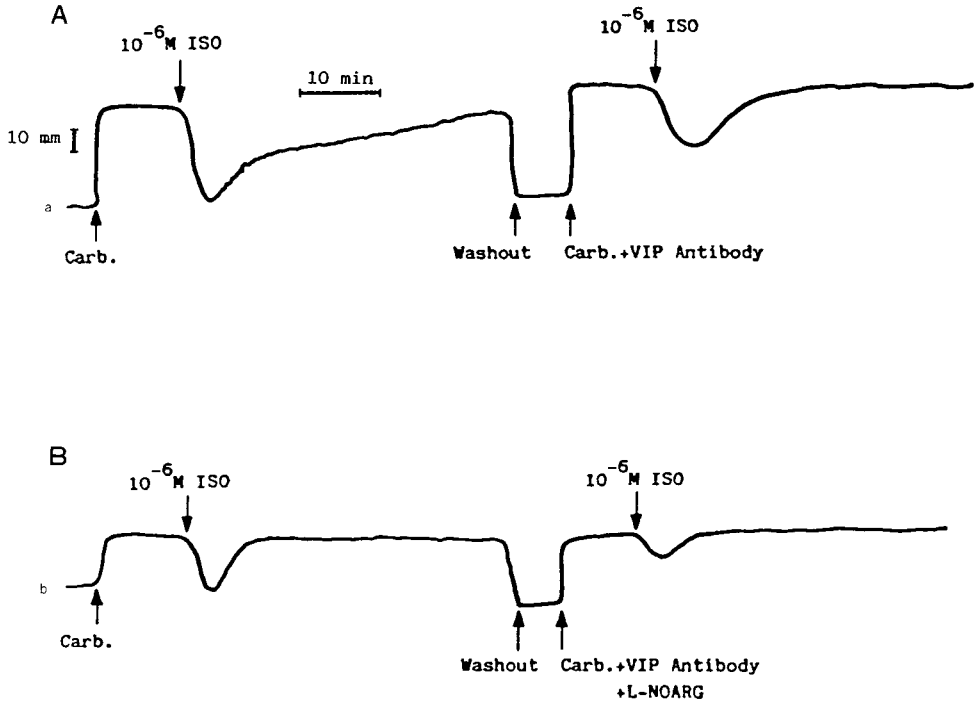


Fig. 4 Relaxation induced by 10^{-6}M isoprenaline in the presense of vasoactive intesinal polypeptide (VIP) antibody 7913 (1 : 160 dilution) (**A**) and VIP antibody 7913 plus 10^{-4}M L-NOARG (**B**). ISO: Isoprenaline; Carb: Carbachol (5.4×10^{-7}M).

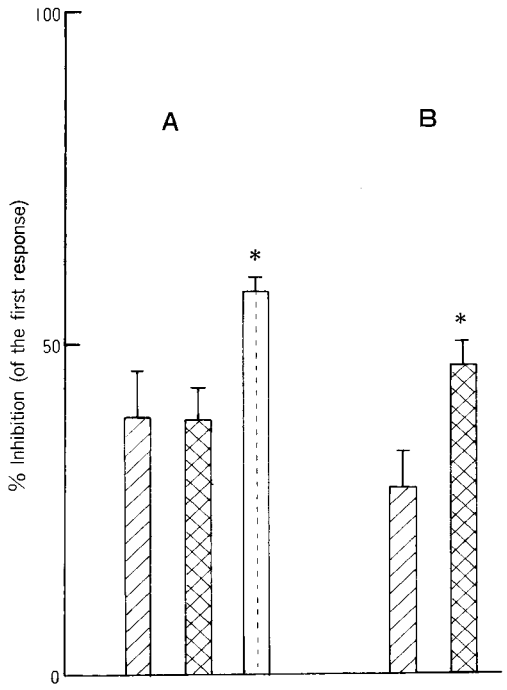


Fig. 5 Percent inhibition caused by L-NOARG (10^{-4}M) and/or VIP antibody 7913 (1:160 dilution) on isoprenaline induced-relaxation in the mouse gastric fundus. **A**; Isoprenaline 10^{-6}M, **B**; Isoprenaline 5×10^{-6}M. (▨): L-NOARG; (▩): Anti-VIP; (▦): Anti-VIP + L-NOARG. $*P < 0.02$ compared with L-NOARG. L-NOARG and VIP: See Figs. 2, 4.

found to be statistically significant ($P < 0.02$, $P < 0.05$ and $P < 0.001$, respectively) compared with that observed in the presence of L-NOARG. On the other hand, 4×10^{-4}M D-ARG did not cause any significant alteration in L-NOARG-produced inhibition. Examples of traces provided from experiments performed under different conditions to test 10^{-6}M isoprenaline action are illustrated in Fig. 2B, C and D. The results of the experiments designed to examine isoprenaline action at three different concentrations in the presence of L-NOARG, L-NOARG plus L-ARG, or D-ARG are shows in Fig. 3.

Incubation of the tissue with VIP antibody 7913 (final dilution 1:160) for 60 min decreased the action of 10^{-6}M isoprenaline (Fig. 4A): relaxation was reduced to 61.4 ± 4.8 % ($P < 0.001$; n = 6). The compound also diminished relaxation due to 5×10^{-6}M isoprenaline to 53.0 ± 3.5 % ($P < 0.001$; n = 6). The percentage inhibition caused by anti-VIP antibody and their comparisons with those of L-NOARG are shown in Fig. 5. Incubation of the preparation with anti-VIP antibody (1:160 dilution) plus L-NOARG (10^{-4}M) for 60 min caused more reduction than that of VIP antibody or L-NOARG alone in the relaxation induced by 10^{-6}M isoprenaline (Figs. 4B, 5).

Discussion

The results of this study suggest that NO and VIP may contribute to the relaxation caused by isoprenaline in the isolated mouse gastric fundus precontracted with carbachol.

L-NOARG partly inhibited isoprenaline relaxation and this inhibition was prevented by L-ARG but not D-ARG, indicating a stereospecific interaction. On the other hand, L-NOARG did not inhibit the relaxation evoked by SNP. This phenomenon is meaningful because it suggests endogenous NO production by isoprenaline. It is possible that a non-adrenergic non-cholinergic (NANC) neuronal mechanism producing NO is present in the mouse gastric fundus (4), and it may contribute to isoprenaline relaxation. Our results show interesting similarity to those of studies performed with endothelium-intact vascular preparations (7, 8, 11). However, observations that L-NOARG and an NO synthase inhibitor, N^G-monomethyl L-arginine (L-NMMA) have no effect on isoprenaline-induced relaxation in human tenia coli (14) and rat gastric fundus (12) seem to be opposite to our results. However, in the latter, isoprenaline was used at a much lower concentration than in the present study.

VIP may also contribute to isoprenaline-induced relaxation, because VIP antibody 7913 did partially inhibit the relaxation. The extent of the anti-VIP antibody-caused inhibition of 5×10^{-6}M isoprenaline-induced relaxation was greater than that of L-NOARG, whereas both agents caused the same degree inhibition on the relaxation produced by 10^{-6}M isoprenaline. This finding implies that as the concentration of isoprenaline increases, so does the contribution of VIP to the relaxation. In the other term, isoprenaline may cause VIP release in a concentration-dependent manner. In a previous study (10), the relaxation induced by VIP was partly inhibited by (R)-p-cAMP, a preferential inhibitor of cAMP-dependent protein kinase or, KT5823, a preferential inhibitor of cGMP-dependent protein kinase, and abolished by a combination of both inhibitors in tenia coli and gastric fundus muscle cells of the guinea pig. The same results were obtained with isoprenaline at a high concentration

(100 M). However, in the same study, contrary to VIP action, isoprenaline did not stimulate NO or cGMP production, suggesting that sympathomimetic drugs may not cause VIP release in the gastric fundus. The reason for these opposite results is not known, but presence of carbachol may facilitate VIP or NO release caused by isoprenaline. The nicotine-like stimulatory action of carbachol on nicotinic receptors is well known. It has been shown that activation of nicotinic receptors led to NO and VIP release in the rat gastric fundus using 10^{-4} M nicotine (13). It is possible that carbachol at the concentration used in the present study facilitates NO and/or VIP release from the mouse gastric fundus. Isoprenaline may have a permissive action which becomes considerable in the presence of carbachol. An observation supporting this hypothesis is that the combination of L-NOARG and trypsin slightly reduced noradrenalin-induced relaxation in the rat gastric fundus (2).

On the other hand, VIP can stimulate NO production (9). Nevertheless, inhibition caused by VIP antibody or L-NOARG on the 10^{-6} M isoprenaline-induced relaxation was significantly increased by combination of both agents. It can not be thought that L-NOARG and VIP antibody concentrations used in the study were insufficient. It has been demonstrated that 2 nM VIP-induced relaxation was completely abolished by VIP antibody 7913 in 1:170 dilution in the rat gastric fundus (12), and maximal amounts of VIP-like immunoreactivity from the same tissue elicited by electrical stimulation for 2 min have been found as to be 709 ± 132 fmol/g wet weight (5). Then, the VIP antibody 7913 concentration used in the present study might be sufficient. Also, L-NOARG was used at its maximal effective concentration. Therefore, the finding suggests that NO production may at least partly be independent of VIP. However, further studies of the exogenous VIP action on the mouse gastric fundus are needed.

In summary, the present findings suggest that NO and VIP may contribute to isoprenaline-induced relaxation in the longitudinal muscles of the mouse gastric fundus precontracted with carbachol.

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