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Role of nitric oxide and neuropeptides in neurogenic vasodilatation of the guinea pig mesenteric artery

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Abstract

Although dense networks of adrenergic nerves are present and noradrenaline causes vasoconstriction, electrical field stimulation failed to elicit any constriction of the isolated ring preparation of the guinea pig mesenteric artery. In the presence of an active tone, a vasodilator response was elicited by electrical field stimulation in endothelium-removed tissues. Nonadrenergic, noncholinergic nerves mediate the electrical field stimulation-induced vasodilator response, since guanethidine and atropine did not affect while tetrodotoxin abolished it. Multiple mediators seem to participate in this vasodilatation. NADPH-diaphorase-positive nerves, calcitonin gene-related peptide (CGRP)- and vasoactive intestinal peptide (VIP)-immunoreactive nerves were present in the mesenteric artery. Nitro-L-arginine but not nitro-D-arginine suppressed the electrical field stimulation-induced vasodilator response with rapid onset and L-arginine restored it. VIP and CGRP relaxed the tissue in a dose-dependent manner. Pretreatment of the animals with capsaicin partly reduced the electrical field stimulation-induced vasodilator response. CGRP-(8–37), a CGRP antagonist, slightly attenuated the vasodilator response induced by both electrical field stimulation and CGRP. Glibenclamide, an inhibitor of ATP-sensitive K^+ channels, decreased the nitro-L-arginine- and capsaicin-insensitive component of the electrical field stimulation-induced vasodilator response. Zinc protoporphyrin IX, an inhibitor of CO formation, did not affect the electrical field stimulation-induced response. In the presence of nitro-L-arginine without an active tone, electrical field stimulation induced a vasoconstrictor response that was sensitive to bunazosin and guanethidine. The results show that the electrical field stimulation-induced vasodilator response of the mesenteric artery of guinea pigs is mediated by nitric oxide (NO), CGRP and some yet unidentified substance(s). Elimination of the vasodilator response unmasked the adrenergic vasoconstrictor response to electrical field stimulation.

Keywords: Vasodilator nerve; Nitric oxide (NO); Mesenteric artery, guinea pig; CGRP (calcitonin gene-related peptide); Capsaicin; Nitro-L-arginine; Zinc protoporphyrin IX

1. Introduction

In addition to adrenergic vasoconstrictor nerves, nonadrenergic, noncholinergic vasodilator nerves are present in cerebral and peripheral vascular tissues (Lee et al., 1978; Toda, 1982; Kawasaki et al., 1988). Electrical field stimulation in vitro results mainly in vasodilatation in cerebral arteries and vasoconstriction in arteries from peripheral tissues of several species (Lee et al., 1976, 1978). In mesenteric arteries of rats and monkeys, vasodilatation is elicited by electrical field stimulation only after blockade of adrenergic transmission (Kawasaki et al., 1988; Toda and Okamura, 1992). Results of pharmacological studies have suggested that endogenous vasodilator substances such as nitric oxide (NO), calcitonin gene-related peptide (CGRP) and vasoactive intestinal peptide (VIP) are vasodilator transmitters (Toda and Okamura, 1990, 1991; Kawasaki et al., 1988; Han et al., 1990; Morris, 1993).

In contrast to the peripheral arteries of other species, electrical field stimulation in guinea pigs causes only a vasodilator response even without blocking adrenergic transmission (Maggi et al., 1990; Morris, 1993). The vasodilator responses upon electrical field stimulation were either capsaicin-sensitive in pulmonary and infe-

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rior mesenteric arteries (Maggi et al., 1990; Meehan et al., 1991) or nitro-L-arginine-sensitive in uterine arteries (Morris, 1993). Morphological studies have shown that the mesenteric artery receives a greater density of innervation than do other systemic arteries in the guinea pig (Dhall et al., 1986; Edvinsson et al., 1989). In the present study we pharmacologically analyzed the neurogenic control of the mesenteric arteries of guinea pigs.

2. Materials and methods

Male guinea pigs (350–800 g) were anesthetized with Na-pentobarbital (50–70 mg/kg). The superior mesenteric arteries were perfused with 3-{(3-cholamidopropyl)dimethylammmonio}-1-propanesulfonate (CHAPS; 0.25%) for 10 s to remove endothelial cells, were isolated and placed in a Krebs-Ringer solution. The composition of the Krebs-Ringer solution was as follows (mM): NaCl, 113; KCl, 4.8; $CaCl_2$, 2.2; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; and glucose, 5.5. The solution was constantly bubbled with a gas mixture of 95% O_2 and 5% CO_2. Ring segments (4 mm long, ca. 0.5 mm in diameter) of the mesenteric arteries were fitted with a pair of wires; a tungsten wire (0.05 mm diameter) and a stainless steel wire (0.1 mm diameter). The tungsten wire was anchored to the bottom of the organ bath containing 20 ml of a Krebs-Ringers solution and the stainless steel wire was connected to a transducer (Nihon Kohden TB 612T). The isometric contraction of the arteries was recorded on a pen-writing recorder. The Krebs-Ringer solution was maintained at 37° C and the resting tension was adjusted to 0.5 g. The organ baths were sterilized to prevent the induction of NO-synthase activity in vascular smooth muscles by bacterial endotoxins (Nakaki et al., 1992).

At the beginning of the experiment, the contractile activity of the artery was tested with noradrenaline (10^{-5} M). When the noradrenaline-induced contraction was under 0.125 g, the tissue was not used for the experiment. The absence of endothelium was confirmed by the lack of vasodilator effect of acetylcholine (10^{-6} M) in tissues precontracted with noradrenaline. Indomethacin (10^{-5} M) was then added to the Krebs-Ringer solution to eliminate the effects of endogenous prostanoids. Electrical field stimulation with biphasic square wave pulses (0.5 ms duration and 180 mA intensity) was applied on tissues through a pair of platinum electrodes that were placed on either side of the artery. For analysis of drug effects, the vasodilator responses upon electrical field stimulation were compared for before and after addition of the drug. Vasodilator activity was expressed as percentage of the contraction. Some animals received two injections of capsaicin (100 mg/kg s.c.), 3 and 4 days before experiments, under anesthesia with ketamine (35 mg/kg i.m.) and xylazine (5 mg/kg i.m.). Dose-response curves were constructed by cumulative addition of CGRP or VIP.

For demonstration of nerve fibers, isolated mesenteric arteries were processed for histochemical experiments in whole mounts. The mesenteric arteries were fixed in paraformaldehyde and picric acid for 72 h, dehydrated with ethanol, cleared with xylene and rehydrated (Saito et al., 1989). Antibodies to CGRP (Cambridge Research Biochemicals, Cambridge, UK) or VIP (CRB) were applied at a concentration of 1 : 1600 dilution for 16–24 h at 4° C. Biotinylated anti-rabbit immunoglobulin G and then fluorescein isothiocyanate-labelled avidin were applied to the tissues. The tissues were mounted in buffered glycerol and examined under a Zeiss fluorescence microscope. For demonstration of noradrenaline-fluorescent nerves, some tissues were treated with glyoxylic acid (20 mg/ml) for 30 min, heated at 105° C, mounted in xylene and examined under a fluorescence microscope (Saito and Goto, 1986). For demonstration of NADPH-diaphorase, the arteries were treated at 37° C in a 0.1 M Tris-HCl buffer (pH 8.0) containing 1 mM NADPH, 0.173 mM tetranitrobluetetrazolium and 0.3% Triton X-100 for 30 min. The tissues were then fixed with 4% paraformaldehyde, dehydrated with ethanol and mounted.

Statistical analysis was made using a *t*-test. A P value less than 0.05 was accepted as showing significance of the difference. Drugs used were acetylcholine chloride (Wako, Osaka Japan), L-arginine HCl (Wako), atropine sulfate (Tanabe, Osaka, Japan), capsaicin (Wako), glibenclamide (Sigma, MO, USA), guanethidine sulfate (Tokyo Kasei Kogyo, Tokyo, Japan), guinea pig VIP (Peninsula, CA, USA; Du et al., 1985), human CGRP (Peptide Institute, Osaka Japan), human CGRP-(8–37) (Peptide Institute), indomethacin (Sigma), noradrenaline bitartrate (Wako), nitro-D-arginine methyl ester hydrochloride (Sigma), $NaNO_2$ (Wako), nitro-L-arginine methyl ester hydrochloride (Sigma), prostaglandin $F_{2\alpha}$ (Funakoshi, Tokyo Japan), tetrodotoxin (Wako), VIP-receptor antagonist ([4Cl-D-Phe^6,Leu^{17}]VIP, Peninsula) and zinc protoporphylin IX (ZnPP-9, Research Biochemicals Institute, MA, USA). $NaNO_2$ (10^{-1} M) and ZnPP-9 (10^{-2} M) were prepared just before use in 0.11 N HCl and dimethyl sulfoxide (DMSO, Kanto Chemical, Tokyo, Japan), respectively. Glibenclamide (10^{-2} M) was prepared in 99.5% ethanol and stored at −20° C.

3. Results

Although noradrenaline (10^{-5} M) constricted the mesenteric artery, electrical field stimulation (10 Hz,

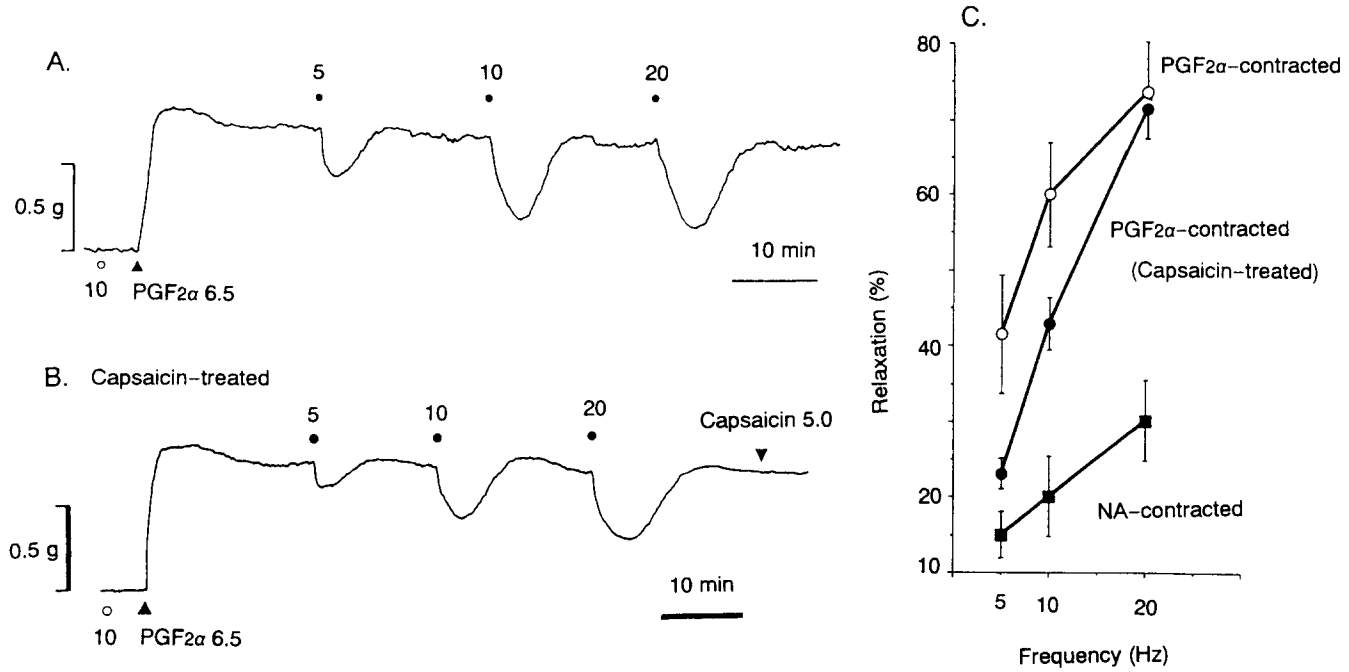

Fig. 1. Typical examples of vasodilator responses of control (A) and capsaicin-treated tissues (B) and the frequency-response curves (C). Electrical field stimulation (10 Hz, 20 s) failed to elicit a contractile response. In the presence of an active tone induced by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 3×10^{-6} M) (○, $n = 12$) and noradrenaline (NA, 10^{-5} M) (■, $n = 5$), electrical field stimulation (5–20 Hz for 10 s) elicited a vasodilator response. Electrical field stimulation also induced a vasodilator response in prostaglandin $F_{2\alpha}$-precontracted arteries from capsaicin-pretreated animals (●, $n = 15$). Numbers in the figure indicate the frequency of electrical field stimulation. Each point and bar represents the mean and the S.E.M. Noradrenaline and prostaglandin $F_{2\alpha}$ increased the tension by $112.5 \pm 6.0\%$ (0.63 ± 0.07 g) and $102.6 \pm 1.3\%$ (0.91 ± 0.09 g) of the first response to noradrenaline (10^{-5} M), respectively.

200 pulses) failed to elicit any constrictor response (Fig. 1A). In tissues precontracted with prostaglandin $F_{2\alpha}$ (3×10^{-6} M) and noradrenaline, electrical field stimulation for 10 s caused a frequency-dependent vasodilator response in every tissue. The electrical field stimulation-induced vasodilator response was larger in tissues contracted with prostaglandin $F_{2\alpha}$ than noradrenaline (Fig. 1C) while the developed tension with prostaglandin $F_{2\alpha}$ and noradrenaline was of a similar magnitude. The relaxation of the tissue started at 7–12 s following initiation of the electrical field stimulation (Fig. 3A) and lasted for 10–15 min. Tetrodotoxin (10^{-6} M) abolished (control $56.7 \pm 4.8\%$ vs. tetrodotoxin 0%, $n = 8$) but neither atropine (10^{-6} M) nor guanethidine

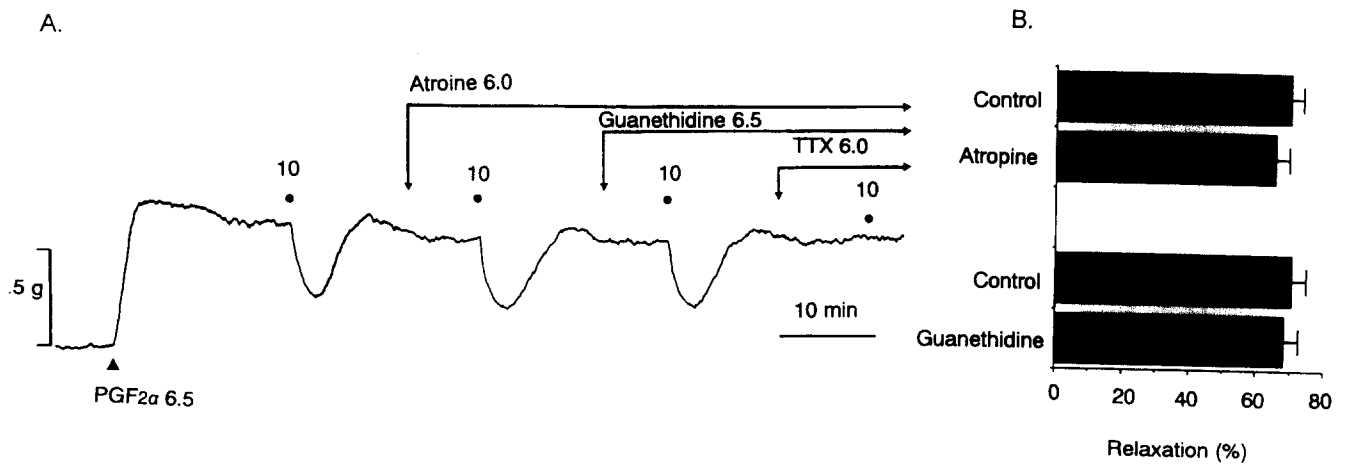

Fig. 2. Typical example of the effect of atropine (10^{-6} M), guanethidine (3×10^{-6} M) and tetrodotoxin (TTX; 10^{-6} M) on the vasodilator response induced by electrical field stimulation (10 Hz for 10 s) (A). While tetrodotoxin abolished the electrical field stimulation-induced response (control $56.7 \pm 4.8\%$ vs. TTX 0%, $n = 8$), atropine and guanethidine did not affect the response (B). Numbers in the figure indicate the drug concentration ($-\log$ M). Each column and bar represents the mean and the S.E.M. for 11 tissues. The drugs were added to the bath 5–10 min before the electrical field stimulation.

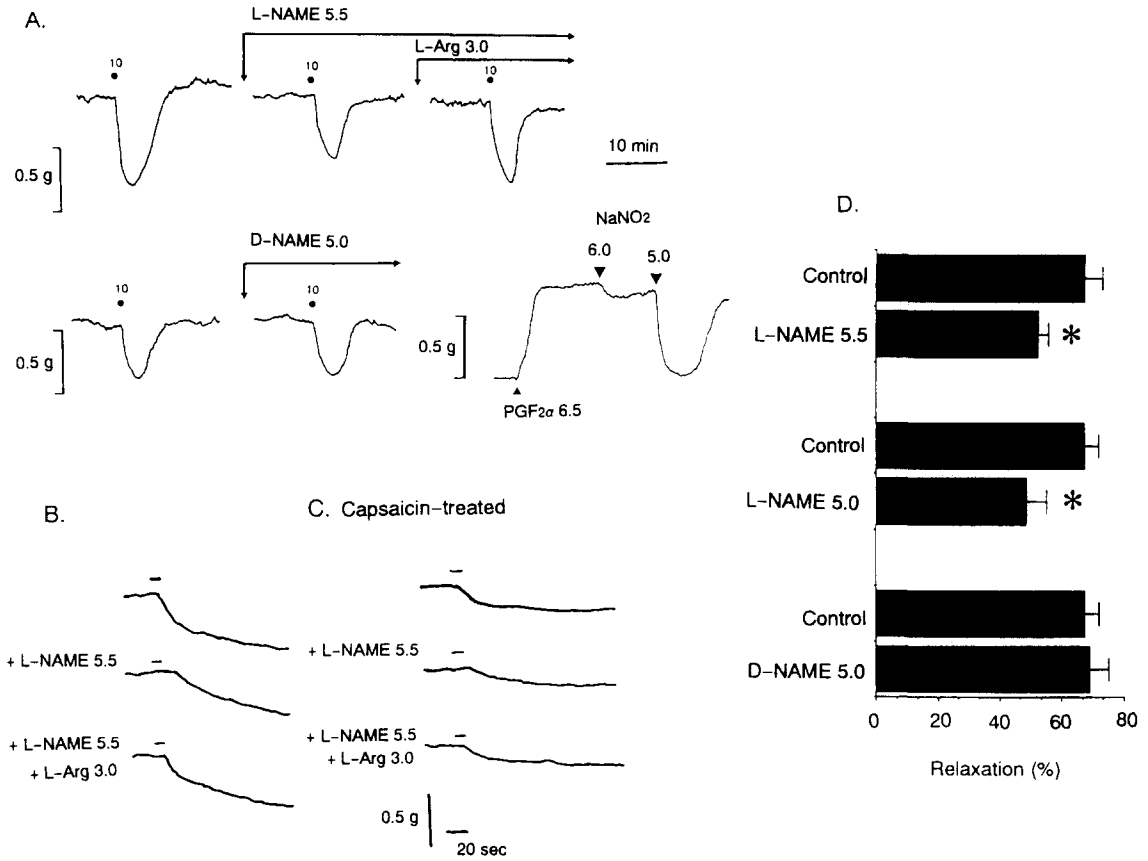

Fig. 3. Typical example of the effect of nitro-L-arginine (L-NAME) and nitro-D-arginine (D-NAME) on the vasodilator response induced by electrical field stimulation and by $NaNO_2$. Nitro-L-arginine but not nitro-D-arginine attenuated the electrical field stimulation-induced response (A). Electrical field stimulation initiated a vasodilator response within 7–9 s in control tissues (B). In the presence of nitro-L-arginine the tissue started relaxation 20–25 s following the initiation of electrical field stimulation. In the presence of L-arginine (10^{-3} M), electrical field stimulation caused vasodilatation within 10 s. Nitro-L-arginine attenuated the electrical field stimulation-induced response with rapid onset and L-arginine restored the response in tissues from capsaicin-treated animals, as well (C). $NaNO_2$ caused a vasodilator response. Numbers in the figure indicate the concentration of the drug ($-\log$ M). In (C), each column and bar represents the mean and the S.E.M. for 7–10 tissues. * Significantly different from control ($P < 0.05$). The drugs were added to the bath 30 min before the electrical field stimulation.

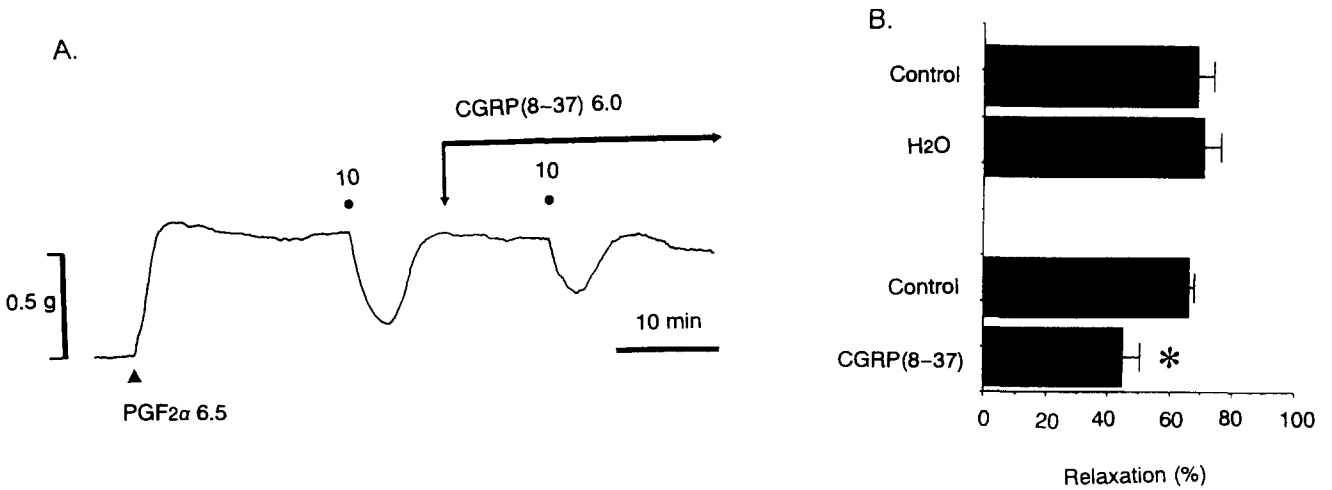

Fig. 4. Typical example (A) and statistical analysis (B) of the effect of CGRP-(8–37) on the electrical field stimulation-induced vasodilator response. CGRP-(8–37) attenuated the response. * Significantly different from control ($P < 0.05$). Each column and bar represents the mean and the S.E.M. from 7 tissues. CGRP-(8–37) was added to the bath 10 min before the electrical field stimulation. H_2O is a solvent for CGRP-(8–37) and served as a control.

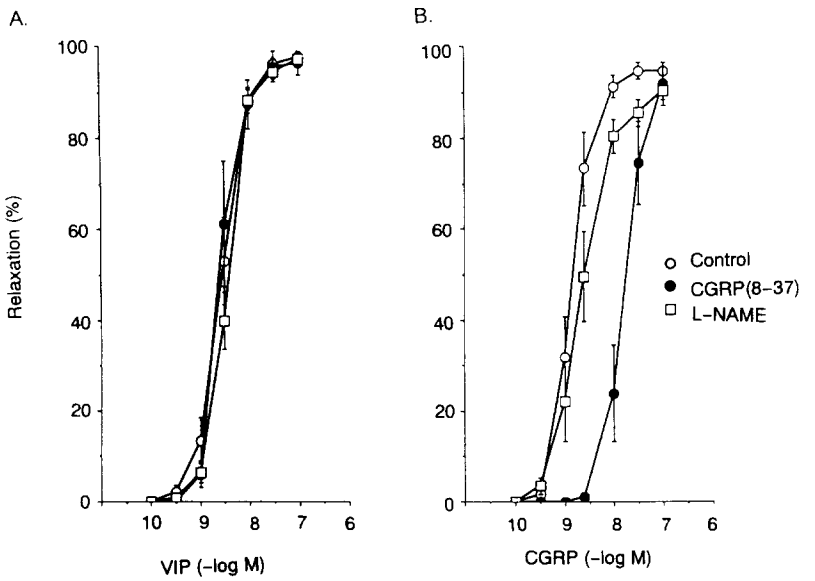

Fig. 5. Vasodilator responses to VIP (A) and CGRP(B). CGRP-(8–37) (●) attenuated the vasodilator response induced by CGRP but not by VIP. Nitro-L-arginine (10^{-5} M) was without effect on both the CGRP- and VIP-induced vasodilator responses (□). Each point and bar represents the mean and the S.E.M. from 4–9 tissues.

(3×10^{-6} M) attenuated the vasodilator responses to electrical field stimulation (Fig. 2A and B). Therefore, nonadrenergic, noncholinergic nerves mediate the electrical field stimulation-induced vasodilator response.

While nitro-L-arginine (10^{-5} M) increased the tone induced by prostaglandin $F_{2\alpha}$ in endothelium-intact preparations (data not shown), it did not cause any change in the tone of endothelium-removed preparations. As shown in Fig. 3A and D, the electrical field stimulation-induced vasodilator response was reduced by nitro-L-arginine (3×10^{-6} M). The tissues started to relax 7–12 s and 20–25 s after the initiation of electri-

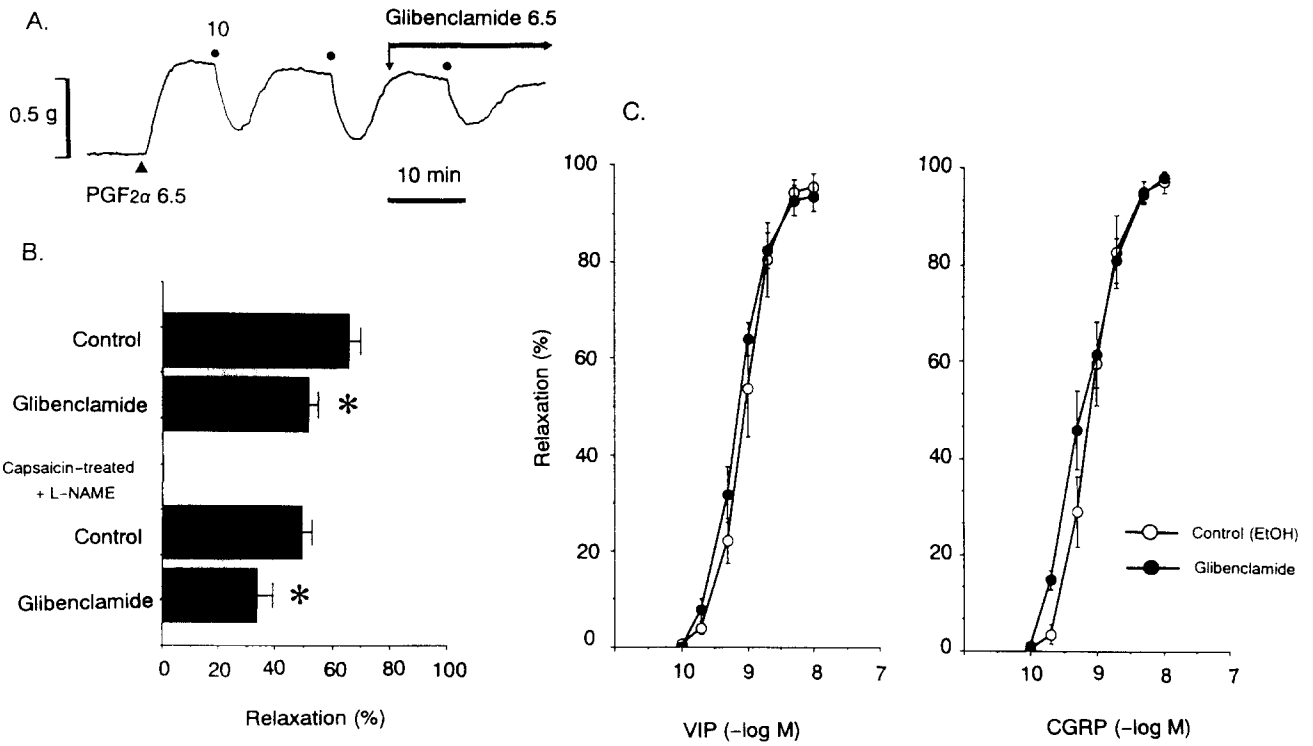

Fig. 6. Typical example (A) and statistical analysis (B) of the effect of glibenclamide on the vasodilator responses. Glibenclamide slightly reduced the electrical field stimulation-induced vasodilator response of control (A) ($n = 8$) and of capsaicin-treated animals in the presence of nitro-L-arginine (10^{-5} M) (B) ($n = 6$). Vasodilator responses to VIP and CGRP were not affected (C). *Significantly different from control ($P < 0.05$). Each point and bar represents the mean and the S.E.M. from 6–9 tissues in (C). Glibenclamide or the vehicle (ethanol) was added to the bath 10 min before the electrical field stimulation.

cal field stimulation in the absence and presence of nitro-L-arginine, respectively (Fig. 3B). Thus the onset of the vasodilator response was delayed by nitro-L-arginine. On the other hand, nitro-D-arginine (10^{-5} M) did not affect the electrical field stimulation-induced response. Addition of L-arginine restored the vasodilatation in the presence of nitro-L-arginine (Fig. 3A and C) (nitro-L-arginine 40.3 ± 3.9% vs. nitro-L-arginine + L-arginine 57.1 ± 3.1%, $n = 5$). Acidified $NaNO_2$ (10^{-5} M), which releases NO (Furchgott, 1988), induced a vasodilator response (Fig. 3A) (85.9 ± 7.8%, $n = 5$).

Capsaicin (10^{-5} M) relaxed the control tissues (data not shown) but not in tissues from capsaicin-treated animals (Fig. 1A). In tissues from capsaicin-treated animals, the amplitude of the electrical field stimulation-induced vasodilator responses at 5 and 10 Hz was smaller than those in intact animals ($P < 0.05$, Fig. 1C). Nitro-L-arginine (10^{-5} M) attenuated the early component of the vasodilator response in these tissues (Fig. 3C). Thus electrical field stimulation still evoked vasodilatation in capsaicin-treated tissues in the presence of nitro-L-arginine.

CGRP and VIP caused vasodilatation in a dose-dependent manner (Fig. 5A and B). Nitro-L-arginine did not affect the vasodilator responses induced by CGRP and VIP. The vasodilator responses to CGRP and electrical field stimulation but not to VIP were reduced by CGRP-(8–37) (10^{-6} M) (Figs. 4A and B, 5A and B). [4Cl-D-Phe^6,Leu^{17}]VIP (3×10^{-7} M) was without effect on vasodilator responses induced by electri-

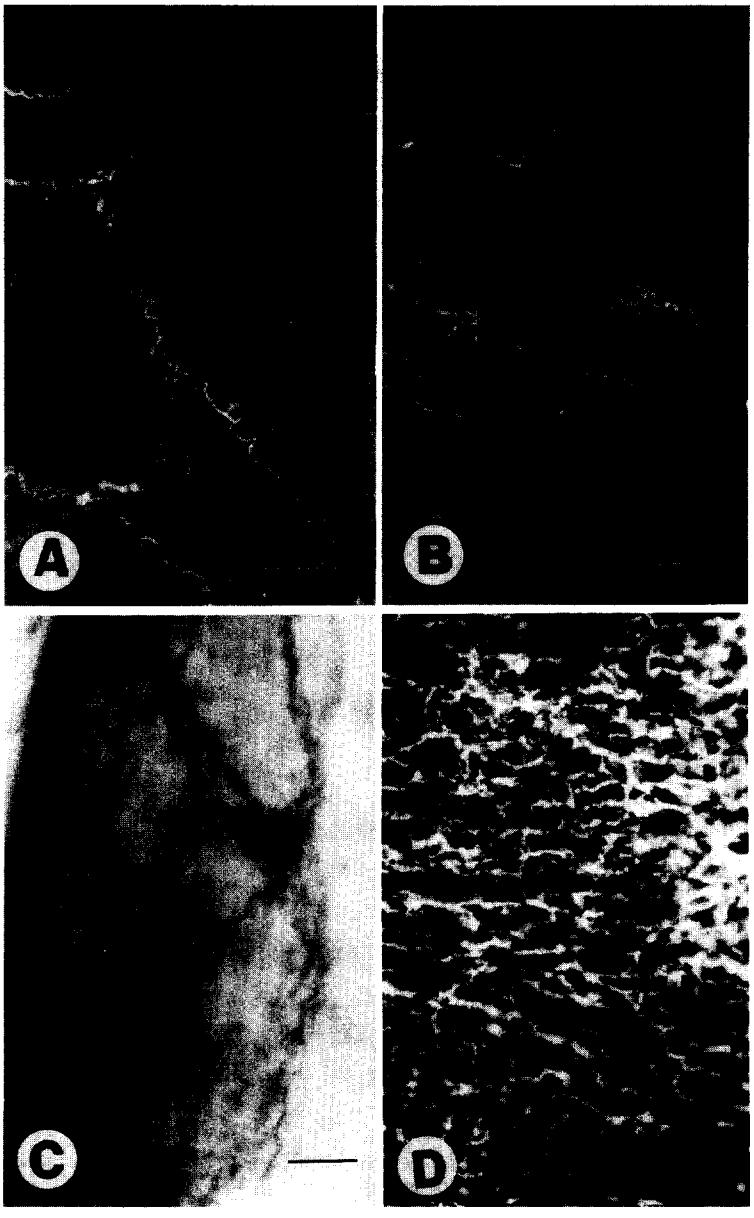

Fig. 7. CGRP-immunoreactive (A), VIP-immunoreactive (B), NADPH-diaphorase-positive (C) and noradrenaline-fluorescence (D) nerves in the mesenteric artery. Bar equals 50 μm.

cal field stimulation (control 68.7 ± 4.5% vs. VIP receptor antagonist 65.7 ± 4.6%, $n = 5$) and VIP ($n = 3$).

Glibenclamide (3×10^{-6} M) slightly yet significantly attenuated the electrical field stimulation-induced response in the presence of nitro-L-arginine (10^{-5} M) in capsaicin-pretreated tissues (Fig. 6A and B). The vasodilator responses to CGRP and VIP were not affected by glibenclamide (Fig. 6C). ZnPP-9, an inhibitor of carbon monoxide (CO) formation, did not affect the electrical field stimulation-induced vasodilator response (control 71.5 ± 3.8% vs. ZnPP-9 67.1 ± 6.8%, $n = 5$).

There is a rich supply of noradrenaline-containing nerve fibers forming a dense network with a spiral or circular orientation along the long axis of the vessel wall of the artery (Fig. 7D). CGRP-like immunoreactive nerve fascicles and fibers appeared to form a less dense plexus (Fig. 7A). A few VIP-like immunoreactive (Fig. 7B) and NADPH-diaphorase-positive nerves (Fig. 7C) were also present. In arteries taken from capsaicin-treated animals, no CGRP-immunoreactive nerves could be demonstrated (data not shown).

In the presence of nitro-L-arginine (10^{-5} M) without an active tone, electrical field stimulation (5–20 Hz, 20 s) caused a vasoconstrictor response (Fig. 8A and C). The tissue contracted in 3–5 s following the initiation of stimulation and the peak of contraction was about 2–5 s after cessation of stimulation (Fig. 8B). Similarly, electrical field stimulation caused a vasoconstrictor response in the presence but not in the absence of nitro-L-arginine in tissues from capsaicin-treated animals. The electrical field stimulation-induced constrictor responses were similar in capsaicin-treated and in control tissues (Fig. 8C). The electrical field stimulation-induced vasoconstriction at 20 Hz was blocked by guanethidine (3×10^{-6} M) in 4 out of 4 tissues and bunazosin (10^{-6} M) in 7 out of 7 tissues (Fig. 8A). Thus electrical field stimulation induced an adrenergic vasoconstriction after the blockade of NO formation.

4. Discussion

In the presence of an active tone induced by prostaglandin $F_{2\alpha}$, electrical field stimulation caused a fre-

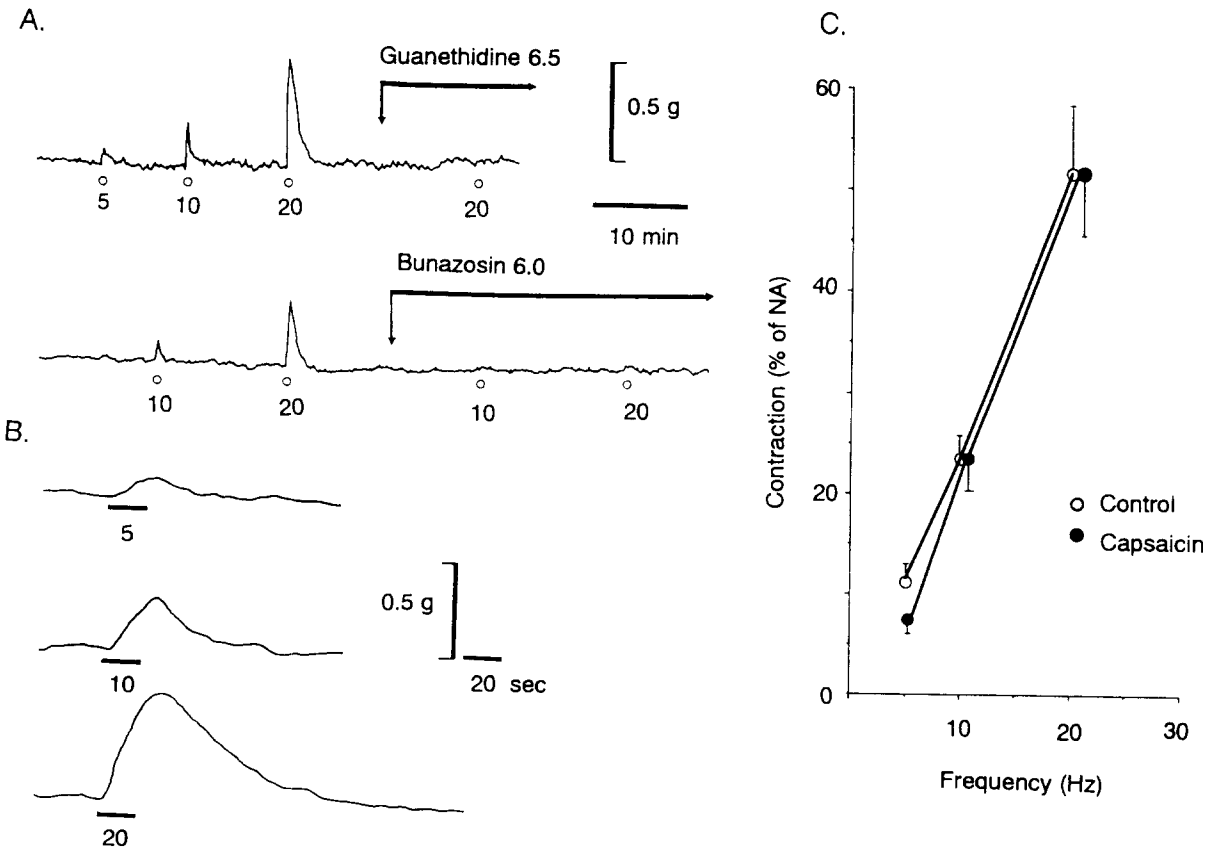

Fig. 8. Typical example of vasoconstriction induced by electrical field stimulation in the mesenteric arteries from control and capsaicin-treated animals. Electrical field stimulation for 20 s at the frequency shown as numbers in the figure induced a frequency-dependent vasoconstriction in the presence of nitro-L-arginine (10^{-5} M) (A, B). The vasoconstrictor response was attenuated by bunazosin (10^{-6} M), and guanethidine (3×10^{-6} M) (A). The amplitude of contraction was similar in control and capsaicin-treated tissues (C). Each point and bar represents the mean and the S.E.M. from 12 tissues.

quency-dependent vasodilator response. Neurogenic vasodilatation induced by electrical field stimulation for 10–40 s has been reported to last for several minutes in cerebral arteries (Toda, 1982; Lee et al., 1978) and more than 10 min in rat mesenteric vascular beds (Kawasaki et al., 1988). The electrical field stimulation-induced vasodilator response in the guinea pig mesenteric artery was long-lasting, similar to the neurogenic vasodilator response of the perfused rat mesenteric vascular bed. Neither atropine nor guanethidine but tetrodotoxin attenuated the vasodilator response induced by electrical field stimulation. Thus nonadrenergic, noncholinergic nerves mediate the electrical field stimulation-induced vasodilator response in the mesenteric artery of guinea pigs. Pharmacological analysis revealed that the electrical field stimulation-induced response consisted of multiple components.

The neurogenic vasodilator response in cerebral arteries of dogs, cats and pigs is rather short-lasting compared to that in the guinea pig mesenteric artery. It has been shown that NO is a vasodilator transmitter in cerebral arteries (Toda and Okamura, 1990, 1991; Chen and Lee, 1993). Nitro-L-arginine delayed the onset of the electrical field stimulation-induced vasodilator response and L-arginine restored it. Thus the early component of the electrical field stimulation-induced response is pharmacologically similar to the neurogenic vasodilator responses in cerebral arteries of dogs, monkeys and pigs and the uterine artery of guinea pigs. A vasodilator response was induced by $NaNO_2$, which releases NO in physiological salt solution (Furchgott, 1988). NO-synthase is the same enzyme as NADPH-diaphorase (Dawson et al., 1991). NADPH-diaphorase-positive nerves were present in the mesenteric artery. NO is a labile substance that may be degraded promptly in oxygenated solution. Thus, it is suggested that NO plays a role as an early component of the electrical field stimulation-induced vasodilator response.

CO was recently proposed as a neuronal messenger (Verma et al., 1993). ZnPP-9, which is an inhibitor of heme oxygenase and prevents the formation of CO, blocks the induction of long-term potentiation in hippocampal slices (Zhuo et al., 1993) and slightly inhibits the neurogenic relaxation of anal sphincter muscles (Rattan and Chakder, 1993). It has been shown that CO dilates arterial preparations by increasing intracellular cGMP (Furchgott and Jothianandan, 1991). However, the lack of effect of ZnPP-9 suggests that CO is not involved in the electrical field stimulation-induced response in the mesenteric artery of guinea pigs.

CGRP is contained in capsaicin-sensitive nerves and causes vasodilatation. Neurogenic vasodilatation in rat mesenteric resistance vessels disappears following treatment of the tissue with capsaicin (Kawasaki et al., 1988) and by CGRP-(8–37), an antagonist of CGRP receptors (Chiba et al., 1989; Han et al., 1990). Activation of capsaicin-sensitive nerves produces inhibitory junction potentials in the guinea pig inferior mesenteric arteries (Meehan et al., 1991). Pretreatment of guinea pigs with capsaicin depletes endogenous CGRP from cardiovascular tissues (Saito and Goto, 1986; Wharton et al., 1986). CGRP-containing nerves were present in the guinea pig mesenteric arteries and the electrical field stimulation-induced vasodilator response was reduced in tissues from capsaicin-pretreated animals. Furthermore, CGRP-(8–37) attenuated the vasodilatation induced by electrical field stimulation as well as CGRP. These results suggest that CGRP mediates the electrical field stimulation-induced vasodilator response.

Although both NO and CGRP are likely to mediate the vasodilatation, the two vasodilators seem to act independently. Inhibition of NO formation by nitro-L-arginine did not affect the vasodilator response induced by CGRP. The nitro-L-arginine-sensitive component of the vasodilatation is induced by electrical field stimulation even in CGRP-depleted tissues. Neurogenic vasodilatation in guinea pigs is reported to be totally sensitive to capsaicin in pulmonary and inferior mesenteric arteries (Maggi et al., 1990; Meehan et al., 1991). Thus there is regional diversity in the control mechanism of vascular tone. Even after blockade of NO formation in CGRP-depleted tissues, electrical field stimulation still evoked a vasodilator response. VIP is a putative vasodilator transmitter in cerebral and uterine arteries (Lee et al., 1984; Morris, 1993). VIP-immunoreactive nerves were present and VIP caused full relaxation of the mesenteric arteries. In the present study, [4Cl-D-Phe^6,Leu^{17}]VIP, which is reported to antagonize VIP activity in pancreatic cells (Pandol et al., 1986), did not affect the vasodilatation induced by electrical field stimulation. However, this does not rule out a possible role of VIP since the antagonist did not affect the VIP-induced vasodilatation. It is still to be determined whether VIP is involved in the electrical field stimulation-induced vasodilatation.

Glibenclamide, an inhibitor of ATP-sensitive K^+ channels, attenuated the nitro-L-arginine- and capsaicin-insensitive component of the electrical field stimulation-induced vasodilatation. Thus some factor that activates ATP-sensitive K^+ channels may partly participate in the electrical field stimulation-induced vasodilator response. Hyperpolarization of the smooth muscle membrane through activation of ATP-sensitive K^+ channels is reported to be responsible for the vasodilatation caused by endothelium-derived hyperpolarizing factor (EDHF), CGRP and VIP in some vascular tissues (Nelson et al., 1990; Standen et al., 1989; Brayden et al., 1991; Hong et al., 1994) although con-

tradictory results are also reported (Parsons et al., 1991; Kageyama et al., 1993; Hattori et al., 1992). Neither VIP- nor CGRP-induced vasodilatation was affected by glibenclamide. In the present study, EDHF of endothelium origin is not responsible for the vasodilator response since the vascular endothelium was removed. However, release of a similar substance from nerves cannot be ruled out, as NO is released from both nerves and endothelium.

The mesenteric arteries of guinea pigs receive dense networks of noradrenaline-containing nerves and noradrenaline is a vasoconstrictor. However, electrical field stimulation did not constrict the mesenteric arteries, unless the early component of vasodilatation was suppressed by nitro-L-arginine methyl ester. Even in tissues from capsaicin-treated animals, vasoconstriction was not induced without preventing NO formation. Electrical field stimulation initiated the vasoconstriction within several seconds and thus the time course of the electrical field stimulation-induced vasoconstriction seems to overlap that of the nitro-L-arginine-sensitive vasodilatation. It is, therefore, likely that the NO-induced vasodilatation masked the vasoconstriction following electrical field stimulation. The abolishment by blockade of α-adrenoceptors shows the involvement of adrenergic nerves in the electrical field stimulation-induced vasoconstriction.

In summary, the present study demonstrated that electrical field stimulation induced a nonadrenergic, noncholinergic vasodilator response that was mediated by NO, CGRP and some unidentified substance(s) in the guinea pig mesenteric artery. ATP-sensitive K^+ channels seem to be responsible for some portion of the response. The profound neurogenic vasodilatation masks the adrenergic vasoconstriction induced by electrical field stimulation.

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