

Calcitonin Gene-Related Peptide Mediated Neurogenic Vasorelaxation in the Isolated Canine Lingual Artery

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ABSTRACT—The nature of neurogenic relaxation was investigated in ring preparations of canine lingual artery. In all experiments, the preparations were previously treated with guanethidine (5×10^{-6} M) to block neurogenic constrictor responses. In the presence of norepinephrine (10^{-5} M) to induce tone, electrical stimulation (10 V, 4 to 16 Hz, for 45 sec) produced relaxation of the rings in an endothelium-independent fashion. The relaxant response in endothelium-denuded rings was not changed by propranolol (10^{-5} M), and atropine (10^{-5} M) did not affect the relaxation elicited by electrical stimulation in endothelium-intact rings. *N*^G-monomethyl-L-arginine (10^{-4} M) or *N*^G-nitro-L-arginine methyl ester (10^{-4} M), a nitric oxide (NO) synthase inhibitor, had no effect on the electrical stimulation-induced relaxation of endothelium-denuded rings. Human calcitonin gene-related peptide (CGRP)-(8–37) (2×10^{-8} M), a CGRP₁-receptor antagonist, inhibited neurogenic relaxation of endothelium-denuded rings; substance P (10^{-6} M) failed to mimic the observed effect of electrical stimulation. The demonstrated effect of electrical stimulation was inhibited by glibenclamide (10^{-5} M), but not tetraethylammonium (2×10^{-4} M); glibenclamide abolished the relaxation in response to exogenous CGRP or the ATP-sensitive K⁺ channel opener cromakalim (10^{-6} M) in endothelium-denuded rings. Moreover, tetrodotoxin (3.13×10^{-6} M) inhibited the relaxation of endothelium-denuded rings induced by electrical stimulation. The relaxation was selectively inhibited when endogenous CGRP had been depleted from perivascular nerves by capsaicin (10^{-6} M). These results suggest that CGRP, but not NO, released from non-adrenergic non-cholinergic nerves by electrical stimulation produces relaxation of canine lingual artery that is mediated by activation of CGRP₁ receptors.

Keywords: Lingual artery, Calcitonin gene-related peptide, Glibenclamide, Adenosine 5'-triphosphate-sensitive potassium channel, Non-adrenergic non-cholinergic nerve

The inhibitory mechanical responses of the rabbit lingual artery consisted of two components; one is atropine-sensitive but the other is a non-cholinergic component (1). This type of mixed dilator response is not unusual; it has been noted in a number of different tissues including the cat salivary gland (2), nasal mucosa (3), tongue (4) and the dog hind limb (5). The identity of the non-cholinergic inhibitory transmitter in these tissues is not known. Calcitonin gene-related peptide (CGRP), a 37-amino acid peptide, is synthesized via the alternative processing of the primary RNA-transcript of the calcitonin gene (6, 7). CGRP has been shown to be widely distributed in the central and peripheral nervous system (8), and it also exists in nerve fibers throughout the cardiovascular system (9). CGRP is one of the most potent vasodila-

tors known (10). The first goal of this study is to determine whether CGRP is involved in the neurogenic relaxation in canine lingual artery.

Recently, Nelson et al. (11) reported that CGRP produces relaxation by activation of ATP-sensitive K⁺ channels in the rabbit mesenteric artery. In contrast, activation of ATP-sensitive K⁺ channels does not appear to mediate relaxation in other arteries (12, 13) in response to CGRP *in vitro*. The second goal of this study was to test the hypothesis that relaxation of the lingual artery is mediated by activation of ATP-sensitive K⁺ channels.

Finally, we examined the effect of nitric oxide (NO) synthase inhibitors, *N*^G-monomethyl-L-arginine (L-NMMA) and *N*^G-nitro-L-arginine methyl ester (L-NAME), on the neurogenic relaxation in the lingual artery. There has been much attention focused on NO, as well as CGRP, as a neurotransmitter released from non-adrenergic non-

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cholinergic (NANC) nerves (14, 15).

MATERIALS AND METHODS

Animals

Mongrel dogs of either sex (10–15 kg) were used for all studies.

Vessel preparation, isometric tension recording and electrical stimulation

In accordance with our institutional Animal Care Committee guidelines, the animals were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and then sacrificed by rapid exsanguination. The lingual artery, which is the largest collateral branch of the external carotid artery, was carefully isolated and dissected into several ring segments, without disturbing the intimal layer, after immersion in ice-cold modified Krebs-Ringer solution (118.3 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl_2 , 1.2 mM MgSO_4 , 1.2 mM KH_2PO_4 , 25.0 mM NaHCO_3 , 0.026 mM calcium disodium ethylenediaminetetraacetic acid and 11.1 mM glucose, aerated with 95% O_2 – 5% CO_2 ; pH 7.2–7.3). The endothelium was removed from most rings by gently rubbing the luminal surface with a wooden implement.

The rings were suspended in a 20-ml water-jacketed tissue bath (37°C) with one end tied to a fixed point and the other, to a force transducer (TB-612T; Nihon Kohden, Tokyo); changes in isometric force were recorded with an amplifier (AP-601T, Nihon Kohden) attached to a recorder (RM-6000, Nihon Kohden). Before the start of the experiment, the rings were allowed to equilibrate for 90 min in Krebs-Ringer solution. During this time, the rings were stretched to a passive tension of 15.3 millinewton (mN) (1.5 g).

The ring preparations were placed between a pair of rectangular platinum electrodes. The gap between the preparation and the electrodes was wide enough to allow undisturbed contractions and yet sufficiently narrow to permit effective stimulation of intramural nerve terminals. The ring preparations were stimulated for 45 sec by trains of 2 msec square pulses of supramaximal intensity at 4, 8 or 16 Hz, 10 V, provided by a direct current power supply and a switching transistor triggered by a stimulator (SEN-3201, Nihon Kohden).

In all experiments, the adrenergic neuron blocker guanethidine (5×10^{-6} M) was applied to block neurogenic constrictor responses; the rings were serially washed, and then after contraction with norepinephrine (10^{-5} M), the functional integrity of the endothelium was checked by the presence of relaxation induced by acetylcholine (10^{-5} M). The concentration (5×10^{-6} M) of guanethidine is larger than normally used to block transmitter release from sympathetic nerves. However, with lower con-

centrations of guanethidine (10^{-6} M), nerve stimulation produced small contractions that produced artifacts with electrical recording. The effects of guanethidine (5×10^{-6} M) on electrical stimulation in the absence (abolition of contraction) or presence (relaxation instead of the abolition of contraction) of active tone induced by norepinephrine were routinely confirmed in the endothelium-intact and denuded ring preparations used in the present study. Preliminary experiments demonstrated that relaxation produced by electrical stimulation of previously exposed ring preparations to guanethidine in the presence of active tone induced by norepinephrine was stable for 5 hr.

Further details are given in the Results section.

Immunohistochemistry

The intact and capsaicin-exposed (10^{-6} M for 30 min) endothelium-denuded ring preparations were fixed in 4% paraformaldehyde and picric acid for 24–72 hr at 4°C (16). The tissues were dehydrated with ethanol, embedded in paraffin and sectioned at 5 μm . Anti-CGRP polyclonal antibody (RPN 1842; Amersham, Buckinghamshire, England) was applied at 1:300 dilution for 16–24 hr at 4°C, and sections were stained by the immunoperoxidase technique using a streptavidin-biotin kit (Nichirei, Tokyo); then they were counter stained lightly with Mayer's hematoxylin. Negative control staining was also carried out by the substitution of the normal rabbit serum for the primary antiserum.

Drugs

Sources of drugs were as follows: acetylcholine chloride, norepinephrine hydrochloride, α, β -methylene ATP, substance P acetate, capsaicin, tetrodotoxin, guanethidine monosulfate, glibenclamide, N^G -monomethyl-L-arginine acetate salt, N^G -nitro-L-arginine methyl ester (Sigma Chemical Co., St. Louis, MO, USA); atropine sulfate (Tanabe Seiyaku Co., Ltd., Osaka); propranolol hydrochloride (Sumitomo Pharmaceuticals Co., Ltd., Osaka); *l*-isoproterenol hydrochloride (Nichiken Chemical Co., Tokyo); papaverine hydrochloride, adenosine, dimethyl sulfoxide, acetic acid (Wako Chemicals, Tokyo), cromakalim (Pola Cosmetics, Yokohama); calcitonin gene-related peptide, and h-8–37 calcitonin gene-related peptide (Peptide Institute, Inc., Osaka). All agents except for capsaicin, tetrodotoxin, glibenclamide and cromakalim were dissolved in distilled water. The tetrodotoxin stock solution was prepared by dissolving it in 1.0% acetic acid. Capsaicin, glibenclamide or cromakalim was dissolved in dimethyl sulfoxide and diluted in Krebs-Ringer solution before being added to the tissue bath. All other reagents were of analytical grade.

Statistical analyses

Means \pm S.E.M. are given throughout. Statistical analyses were performed by Student's *t*-test for paired and unpaired observations. Differences were considered to be statistically significant when *P* was < 0.05 .

RESULTS

Responses to electrical stimulation

The endothelium-denuded canine lingual artery contracted during the electrical stimulation (4 to 16 Hz), and this response was abolished when guanethidine (5×10^{-6} M) was added 20 min before the initiation of the electrical

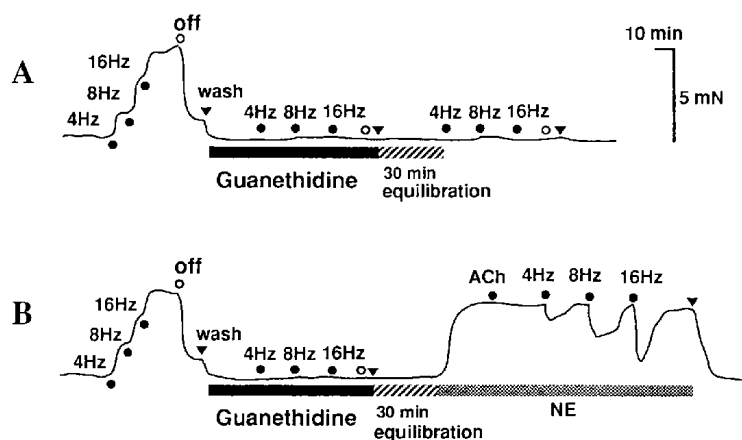


Fig. 1. Responses of endothelium-denuded canine lingual artery rings in the absence (A) or presence (B) of active tone induced by NE (10^{-6} M) to electrical stimulation (10 V, 4 to 16 Hz, for 45 sec). The rings confirmed to have an ability to respond (contraction) to electrical stimulation were exposed to guanethidine (5×10^{-5} M), and then after 20 min, neural elements in the arteries were stimulated electrically. The rings were serially washed and reequilibrated for 30 min, and then the effect of electrical stimulation was assessed in the absence (A) or presence (B) of active tone induced by NE; the functional integrity of the endothelium was checked by the addition of ACh (10^{-5} M) before the assessment (B). In the absence of guanethidine (30 min after washing), electrical stimulation produced no response (A); therefore, lingual artery rings previously treated with guanethidine were used in the following experiments in the absence of guanethidine.

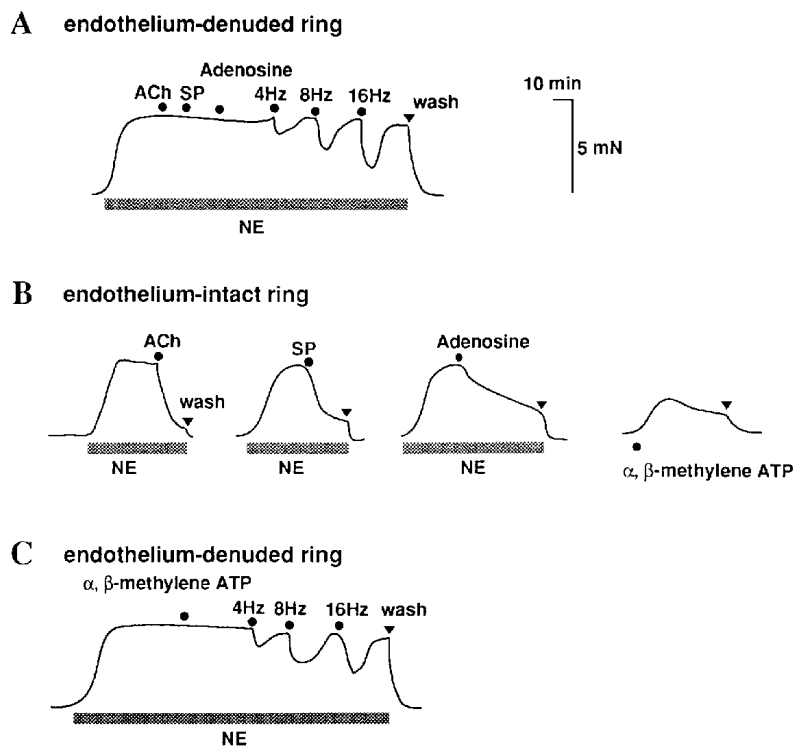


Fig. 2. Responses of endothelium-denuded (A and C) and intact (B) canine lingual artery rings to SP (10^{-6} M), adenosine (10^{-6} M) and α , β -methylene ATP (10^{-6} M). The effect of guanethidine (5×10^{-6} M) on electrical stimulation in the absence (abolition of contraction) or presence (relaxation instead of the abolition of contraction) of active tone induced by NE (10^{-6} M) (see Fig. 1 for protocol) were confirmed in all ring preparations before the start of the experiments.

stimulation; even in the absence of guanethidine (30 min after washing), electrical stimulation elicited no response (Fig. 1A). In the presence of active tone induced by the addition of norepinephrine (NE, 10^{-5} M), the endothelium-denuded preparations confirmed to have no ability to respond to electrical stimulation (in the absence of NE) relaxed in a frequency-dependent fashion when neural elements in the arteries were stimulated electrically (Fig. 1B).

In addition to the classical transmitters NE and acetylcholine (ACh), co-transmitters have been identified in perivascular nerves, such as the purinergic component in sympathetic transmission (17), and vasoactive neuropeptides such as substance P (SP) and CGRP have been shown to be present in some blood vessels (18, 19). In our experimental system (in guanethidine-treated ring preparations), exogenously added SP (10^{-6} M); adenosine ($2 \times$

10^{-6} M), a purine (P_1)-receptor agonist; and α,β -methylene ATP (10^{-6} M), a purine (P_2)-receptor agonist, did not mimic the observed effect of electrical stimulation (Fig. 2, A and C); SP and adenosine produced endothelium-dependent relaxation (Fig. 2, A and B), and endothelium-intact rings contracted in response to the addition of α,β -methylene ATP (10^{-6} M) (Fig. 2B).

Effects of atropine, propranolol, L-NMMA and L-NAME

At the steady-state (equilibrium) contraction induced by NE, electrical stimulation (4 to 16 Hz) produced an atropine (10^{-5} M)-resistant and a propranolol (10^{-5} M)-resistant relaxation in endothelium-intact (Fig. 3A) and endothelium-denuded ring preparations (Fig. 3B), respectively (see summarized data in Table 1). Furthermore, the

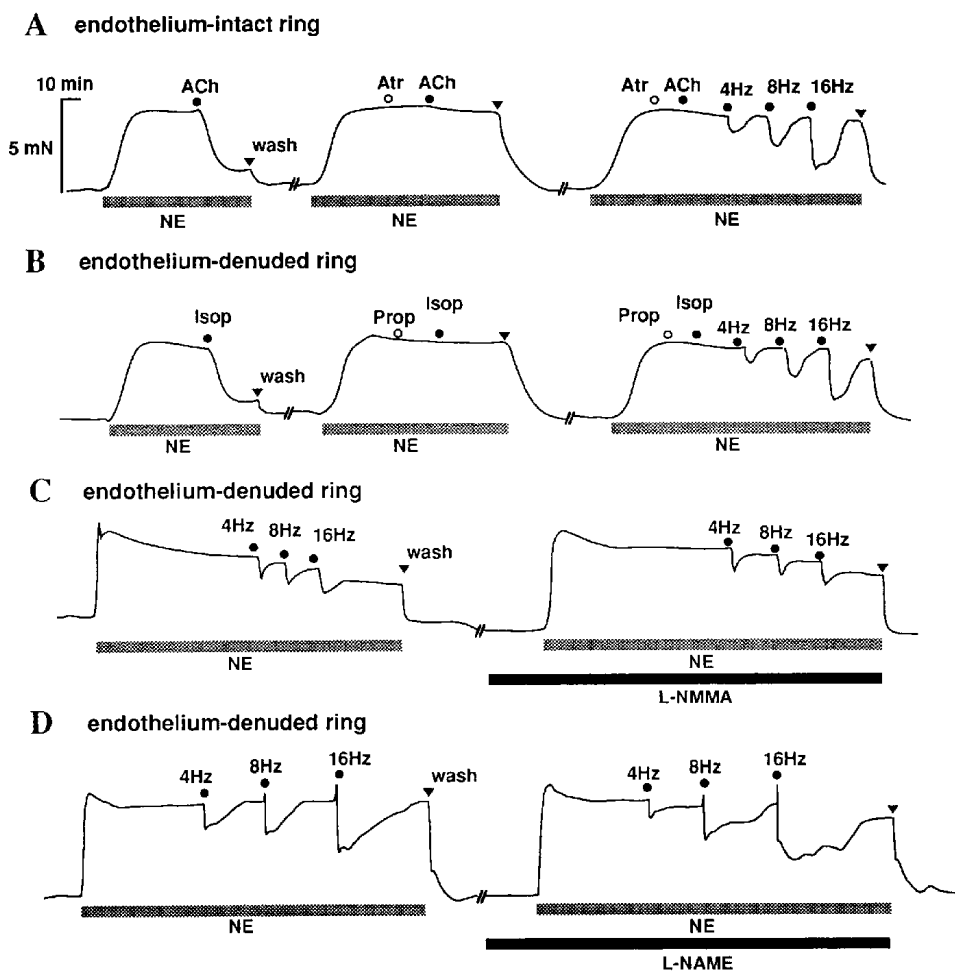


Fig. 3. Effects of atropine (A), propranolol (B), L-NMMA (C) and L-NAME (D) on the relaxant response to electrical stimulation of endothelium-intact (A) and -denuded (B, C and D) canine lingual artery rings. The rings used were previously treated with guanethidine (5×10^{-6} M), and the effect was confirmed in all ring preparations before the start of the experiments (see Fig. 1 for protocol). When added, doses were the following: 10^{-5} M ACh, 10^{-5} M atropine (Atr), 10^{-6} M isoproterenol (Isop), 10^{-5} M propranolol (Prop), 10^{-4} M L-NMMA and 10^{-4} M L-NAME. L-NMMA or L-NAME was added 30 min before the addition of NE (10^{-6} M) to the bathing media.

Table 1. Electrical stimulation-induced relaxation (%) in the presence of active tone induced by NE (10^{-6} M) and effects of tetrodotoxin, capsaicin, atropine and propranolol in endothelium-intact and -denuded canine lingual artery rings

Treatment with:	Endothelium-intact rings		Endothelium-denuded rings			
	None	Atropine	None	Tetrodotoxin	Capsaicin	Propranolol
16 Hz	69.0±3.3	69.8±5.5	70.1±6.0	32.7±19.2*	16.7±8.7*	70.0±3.5
8 Hz	55.7±7.3	56.5±3.3	56.7±5.6	12.4±12.3*	16.6±5.5*	55.5±4.5
4 Hz	44.4±5.3	44.5±7.1	45.7±6.5	4.1±5.3*	8.9±2.4*	43.2±5.5

The rings used were previously treated with guanethidine (5×10^{-6} M), and the effect was confirmed in all rings before the start of the experiments (see Fig. 1 for protocol). The preparation was treated with tetrodotoxin (3.13×10^{-6} M) or capsaicin (10^{-6} M) for 15 min or 30 min, respectively; the rings were serially washed and reequilibrated, and then the relaxation response to electrical stimulation was determined in the rings contracted to a stable plateau tension by the addition of NE. Atropine (10^{-5} M) or propranolol (10^{-5} M) treatment for 10 min was performed before the initiation of the electrical stimulation in the rings contracted to a stable plateau tension by the addition of NE. The relaxant response of the rings is expressed as a percentage of the maximum relaxation induced by 2×10^{-4} M papaverine applied at the end of the experiments. Data are means±S.E.M. ($n=5$). *Significantly ($P<0.01$) different from the corresponding value for no drug (none).

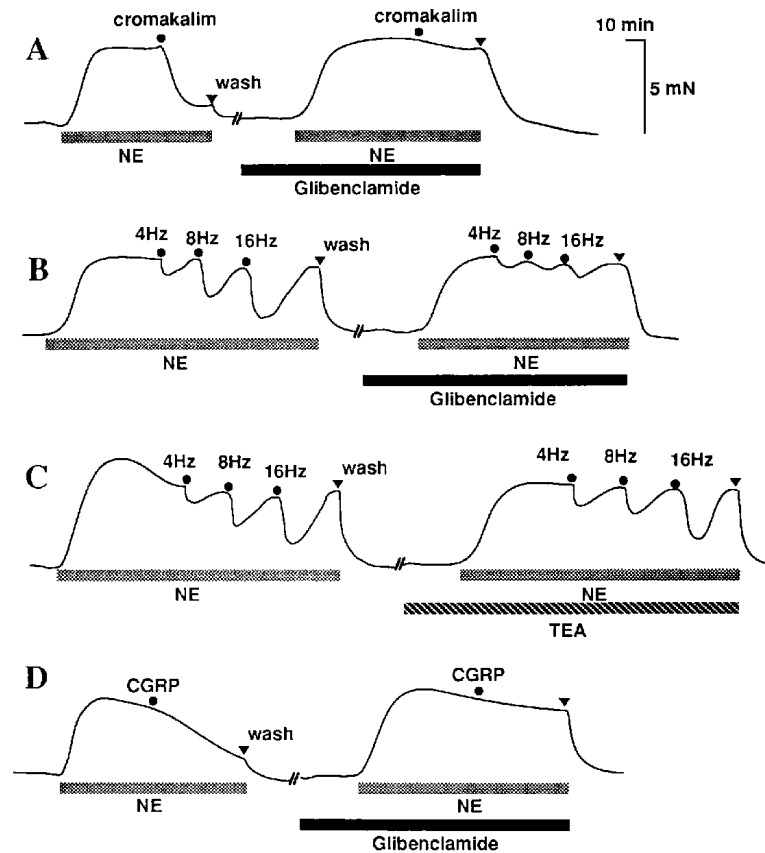


Fig. 4. Effects of glibenclamide (on the relaxant responses to cromakalim, A; electrical stimulation, B; and to exogenous CGRP, D) and tetraethylammonium (TEA, on the relaxant response to electrical stimulation, C) in the rings precontracted with NE. The endothelium-denuded rings were previously treated with guanethidine (5×10^{-6} M), and the effect was confirmed in all rings before the start of the experiments (see Fig. 1 for protocol). When added, doses were the following: 10^{-6} M cromakalim, 10^{-5} M glibenclamide, 2×10^{-4} M TEA and 10^{-8} M CGRP. Glibenclamide or TEA was added 30 min before the addition of NE (10^{-6} M) to the bathing media.

relaxation induced by electrical stimulation was endothelium-independent (Table 1). L-NMMA (10^{-4} M) or L-NAME (10^{-4} M) had no effect on electrical stimulation-induced relaxation in endothelium-denuded rings (Fig. 3, C and D). L-NMMA or L-NAME at the concentration used

abolished the relaxation elicited by ACh (10^{-5} M) in rings confirmed to have the ability of endothelium-dependent relaxation (data not shown). Thus, the relaxation in response to electrical stimulation is mediated by NANC nerves, but not by NO.

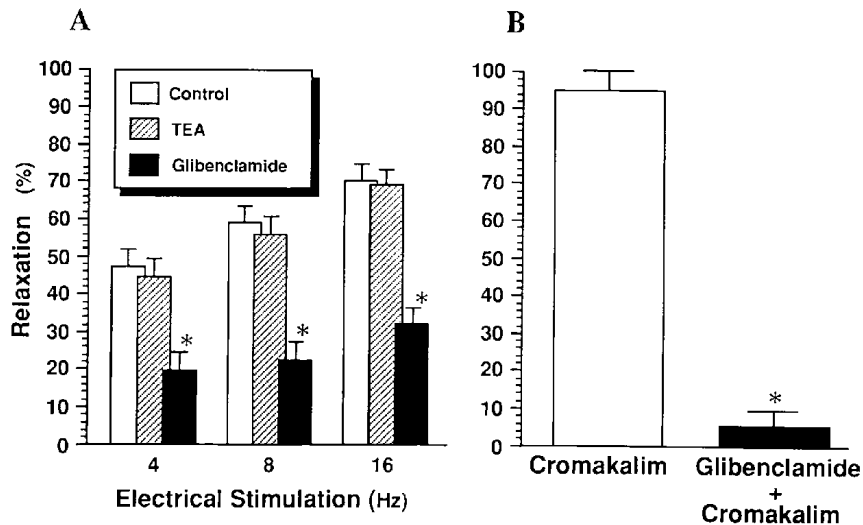


Fig. 5. Effects of glibenclamide and tetraethylammonium (TEA) on the relaxant response of endothelium-denuded canine lingual artery rings to electrical stimulation (A) and inhibition afforded by glibenclamide on cromakalim-induced relaxation of the rings (B). The experiments were performed under the same experimental conditions as those of Fig. 4 (B to D). The relaxant response of the rings precontracted with NE (10^{-6} M) is expressed as a percentage of the maximum relaxation induced by 2×10^{-4} M papaverine applied at the end of the experiments. Mean values \pm S.E.M. ($n=5$) are shown. *Significantly ($P < 0.01$) different from the corresponding value without the channel blockers used (control in panel A or cromakalim alone in panel B).

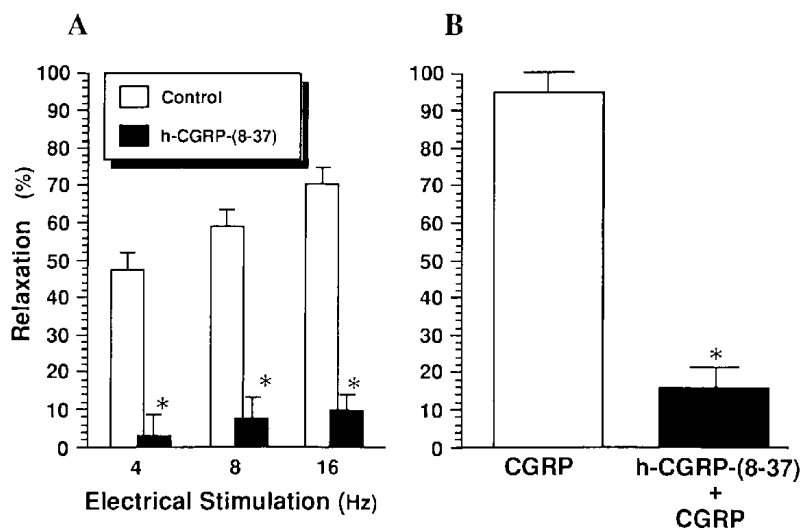


Fig. 6. Effect of human CGRP-(8-37) on the relaxant response in the presence of active tone induced by NE (10^{-6} M) of endothelium-denuded canine lingual artery rings to electrical stimulation (A) and exogenously added CGRP (B). The rings used were previously treated with guanethidine (5×10^{-6} M), and the effect was confirmed in all ring preparations before the start of the experiments (see Fig. 1 for protocol). Human CGRP-(8-37) (2×10^{-8} M) was added 30 min before the addition of NE; the relaxation response to electrical stimulation or to exogenous CGRP (10^{-8} M) was determined in the rings contracted to a stable plateau tension by the addition of NE. The relaxant response of the rings is expressed as a percentage of the maximum relaxation induced by 2×10^{-4} M papaverine applied at the end of the experiments. Mean values \pm S.E.M. ($n=5$) are shown. *Significantly ($P < 0.01$) different from the corresponding value without human CGRP-(8-37) (control in panel A or CGRP alone in panel B).

Effects of tetrodotoxin and capsaicin

Tetrodotoxin (3.13×10^{-6} M, treatment for 15 min before NE precontraction) potently depressed the frequency-response curve for electrical stimulation (4 to 16 Hz) (Table 1), consistent with the responses being neurogenic. To identify possible neurotransmitters involved in the relaxation mediated by NANC nerves in the lingual artery, we used capsaicin. When the ring preparations were previously treated with capsaicin (10^{-6} M) for 30 min, the relaxation caused by electrical stimulation was greatly

reduced (Table 1). These results clearly indicate that the NANC relaxant response is mediated by the capsaicin-sensitive nerves.

Effects of glibenclamide and tetraethylammonium

Glibenclamide (10^{-5} M), a blocker of ATP-sensitive K^+ channels, but not the Ca^{2+} -sensitive K^+ channel blocker tetraethylammonium (TEA, 2×10^{-4} M, Fig. 4C), at a concentration that abolished the relaxant response to 10^{-6} M cromakalim (Figs. 4A and 5B), markedly attenu-

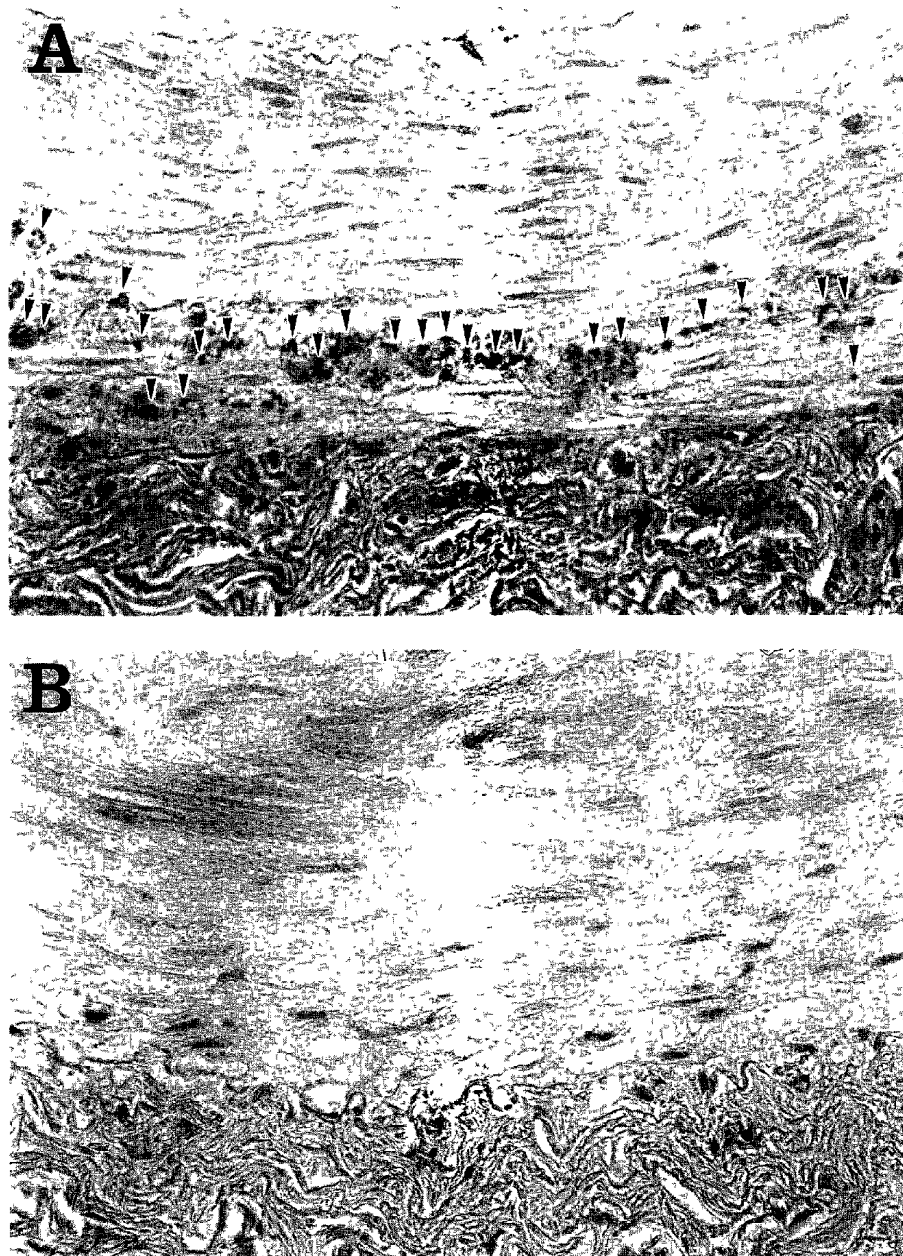


Fig. 7. CGRP-like immunoreactivity in canine lingual artery. There were many immunoreactive fibers (arrowheads) in the control tissue (A), immunoreactive material was observed in the adventitial layer. After 30 min of exposure to capsaicin (10^{-6} M), the intensity of CGRP-like immunoreactivity was reduced nearly fully (B).

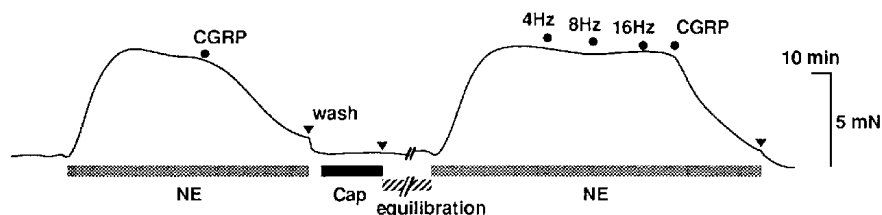


Fig. 8. Effect of capsaicin on the relaxant response in the presence of active tone induced by NE (10^{-6} M) of endothelium-denuded canine lingual artery ring to exogenous CGRP (10^{-8} M). The ring was previously treated with guanethidine (5×10^{-6} M), and the effect was confirmed before the start of the experiment (see Fig. 1 for protocol). Capsaicin (Cap, 10^{-6} M) treatment was performed for 30 min.

ated the demonstrated effect of electrical stimulation in endothelium-denuded rings precontracted with NE (Figs. 4B and 5A).

Nelson et al. (11) have proposed that CGRP activates ATP-sensitive K^{+} channels, the action which is involved in a substantial part of its relaxant effect in rabbit mesenteric arteries. They demonstrated that glibenclamide attenuates the relaxation and hyperpolarization of the arteries induced by CGRP. Indeed, in our experiments, glibenclamide (10^{-5} M) attenuated the relaxation produced by exogenously added CGRP (10^{-8} M) (Fig. 4D).

Effect of human CGRP-(8–37)

If the relaxant response to electrical stimulation is due solely to released CGRP from NANC vasodilator nerves in the lingual artery, the CGRP receptor antagonist human CGRP-(8–37) (20) should be able to inhibit the effect elicited by electrical stimulation. The results of the experiments are shown in Fig. 6; human CGRP-(8–37) (2×10^{-8} M) inhibited not only the neurogenic relaxation induced by electrical stimulation but also the relaxant response to exogenously added CGRP (10^{-8} M) in the endothelium-denuded ring preparations. Relaxation produced by isoproterenol (10^{-6} M) in endothelium-denuded rings was not affected by human CGRP-(8–37) (data not shown), suggesting the selective effect of human CGRP-(8–37). Taken together, these results have led to the suggestion that CGRP might be involved in the relaxant response to electrical stimulation.

Capsaicin and histochemical study

The role of CGRP as a possible transmitter for relaxation of canine lingual artery was examined further by immunohistochemistry in endothelium-denuded ring preparations. Numerous CGRP-like immunoreactive fibers were found to be present in the adventitia; the CGRP-like immunoreactive fibers showed typical varicose profiles (Fig. 7A). After the ring preparation was treated with capsaicin (10^{-6} M), the CGRP-like immunoreactivity was markedly diminished (Fig. 7B). Capsaicin did not inhibit the relaxation induced by exogenous CGRP (10^{-8} M)

(Fig. 8), suggesting that the relaxant response to electrical stimulation disappeared when endogenous CGRP (localized prejunctionally) was depleted by capsaicin.

DISCUSSION

There are three major new findings in the present study. First, CGRP (released from NANC nerves in response to electrical stimulation) produced marked relaxation of canine lingual artery that is mediated by activation of CGRP₁ receptors. Second, although it has been suggested that NO or a NO-related compound has an important role as a primary messenger of transmitting information from nerves to vascular smooth muscles (14), the major mechanism of relaxation appears to be activation of CGRP₁ receptors on the vascular muscle. Third, a mechanism of CGRP-induced relaxation of the lingual artery may be due, in part, to activation of ATP-sensitive K^{+} channels. These postulates are inferred from the following significant observations: 1) In the presence of NE to induce tone, electrical stimulation produced relaxation of guanethidine (to block contractile responses)-treated endothelium-denuded ring preparations (Fig. 1B); 2) This relaxation was endothelium-independent (Table 1) and not changed by propranolol, a β -adrenoceptor antagonist (Fig. 3B and Table 1), and atropine, a muscarinic cholinergic antagonist, at the concentration that abolished the relaxant response to ACh, but did not affect the relaxation exhibited by electrical stimulation in endothelium-intact ring preparations (Fig. 3A and Table 1); 3) L-NMMA or L-NAME, a NO synthase inhibitor, at the concentration that abolished the relaxation afforded by ACh in endothelium-intact rings, had no effect on the electrical stimulation-induced relaxation in endothelium-denuded rings (Fig. 3, C and D); 4) Exogenously added SP failed to mimic the effect of electrical stimulation (Fig. 2A); 5) Tetrodotoxin depressed electrical stimulation-induced relaxation in endothelium-denuded rings (Table 1); 6) Human CGRP-(8–37), at the concentration that nearly completely abolished the effect of exogenous CGRP, inhibited neurogenic relaxation induced by electri-

cal stimulation of endothelium-denuded rings (Fig. 6), and this relaxation was also inhibited by glibenclamide, at the concentration that abolished the effects of exogenous CGRP and cromakalim, a ATP-sensitive K^+ channel opener (Fig. 4, A, B and D); and 7) The neurogenic relaxation was selectively inhibited by depleting endogenous CGRP from perivascular nerves (Figs. 7 and 8 and Table 1).

The relaxation elicited by electrical stimulation was not completely, but partially, inhibited by tetrodotoxin in our system (Table 1), while Kawasaki et al. (21) have reported that periarterial nerve stimulation in precontracted rat mesenteric resistance vessels produces a tetrodotoxin-abolishable neurogenic vasodilation that is mediated by NANC nerves. Therefore, it is possible that there are segmental or regional differences or species differences in the effect of tetrodotoxin in responses of blood vessels to electrical stimulation, and the partial refractoriness to tetrodotoxin of the electrical stimulation-induced relaxation may point to a nonneurogenic and consequently postsynaptic origin for the relaxations. However, there appears to be no obvious answer, and no consideration has been given to this problem as far as we can tell from the current literature.

A COOH-terminal fragment of human CGRP, CGRP-(8–37), was introduced as a putative CGRP-receptor antagonist (20). CGRP-(8–37) inhibited CGRP-induced contractile responses of guinea pig atrium (22) and relaxation of the mesenteric artery (23) and guinea pig basilar artery (24). In contrast, CGRP-(8–37) is a weak antagonist of responses of the vas deferens to CGRP (22). The existence of at least two classes of CGRP receptors has been suggested, one being sensitive to CGRP-(8–37) (CGRP₁ receptor) and the other being insensitive (CGRP₂ receptor) (22). In the present study, CGRP-(8–37) (2×10^{-8} M) almost completely inhibited relaxation of lingual artery in response to electrical stimulation of neural elements in the artery (Fig. 6A) or to exogenous CGRP (Fig. 6B). Thus responses of the canine lingual artery to the electrical stimulation and CGRP are mediated by activation of CGRP₁ receptors. The residual responses after inhibition by CGRP-(8–37) may reflect a very small population of CGRP₂ receptors that are not inhibited by CGRP-(8–37) or simply may reflect <100% inhibition of CGRP₁ receptors by CGRP-(8–37) at the concentration used.

Recent evidence suggests that activation of ATP-sensitive K^+ channels is an important mechanism of vasorelaxation (11, 25, 26). Furthermore, many investigators have shown that the relaxant effects of K^+ channel openers, such as cromakalim and pinacidil, are counteracted by an increase in extracellular K^+ concentration (20, 27, 28). CGRP is reported to stimulate adenylate cyclase and in-

crease the intracellular content of cyclic AMP in rat aortic smooth muscle cells (29). In support of the possibility that a cyclic AMP-dependent mechanism may be involved in activation of ATP-sensitive K^+ channels in smooth muscle cells is the recent finding that cyclic AMP activates ATP-sensitive K^+ channels in myocytes (30). Thus we anticipated that CGRP-induced relaxation of the lingual artery may also involve activation of ATP-sensitive K^+ channels. Glibenclamide produced marked inhibition of relaxant responses of the lingual artery to electrical stimulation (Fig. 4B) and exogenous CGRP (Fig. 4D). The relaxation to electrical stimulation was unaltered by tetraethylammonium, a Ca^{2+} -sensitive K^+ channel blocker (Fig. 4C). The findings suggest that activation of ATP-sensitive K^+ channels is a major mechanism that mediates relaxation of canine lingual artery in response to CGRP. The remaining relaxation may be mediated by other cyclic AMP-dependent mechanisms, such as Ca^{2+} desensitization of contractile elements in the smooth muscle (31, 32), or cyclic GMP-dependent mechanisms, including NO release (33). Wei et al. (33) suggested that a cyclic GMP-dependent mechanism is involved in CGRP-induced dilatation of feline cerebral arterioles. However, electrical stimulation-induced relaxation was endothelium-independent (Table 1), and L-NMMA or L-NAME had no effect on the neurogenic relaxation of the lingual artery (Fig. 3, C and D) in our system; therefore, the neurogenic relaxation is apparently independent of NO production linked to generation of cyclic GMP.

Inasmuch as the relaxation of lingual artery caused by electrical stimulation was greatly attenuated by capsaicin, a sensory neurotoxin, which depletes SP and CGRP but not vasoactive intestinal polypeptide from nerves (16, 34), CGRP is probably contained in the sensory nerves of the lingual artery. The addition of SP did not mimic the effect of electrical stimulation (Fig. 2A), suggesting that SP-like substances are not involved in the neurogenic relaxation. The immunohistochemistry showing that CGRP-like immunoreactivity was markedly diminished after treatment with capsaicin (Fig. 7) provides more evidence that the vasodilator substance released by electrical stimulation is most likely CGRP in the canine lingual artery. The activation of peripheral terminals of sensory nerves has been shown to cause antidromic vasodilation through the local axon reflex (35). Since the tongue is blood-supplied via the lingual artery, its vascular reactivity for active substances including peripheral neurotransmitters of sensory nerves might be important in the regulation of tongue circulation. However, identification of the substance mediating the NANC responses through a local reflex mechanism in the canine lingual artery awaits further study.

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