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Pre- and postjunctional modulation by endothelin-1 of the adrenergic neurogenic response in canine mesenteric arteries

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Abstract

Transmural electrical stimulation (5–30 Hz) produced a frequency-dependent increase in the perfusion pressure of isolated, perfused dog mesenteric artery segments, which was suppressed by prazosin and abolished by tetrodotoxin. Treatment with endothelin-1 in low concentrations (10^{-10} and 3×10^{-10} M) inhibited the response to electrical nerve stimulation. The effect was not affected by N^G-nitro-L-arginine, indomethacin and removal of the endothelium. The endothelin-1-induced inhibition was antagonized by 10^{-7} M BQ123 [cyclo(D-Trp-D-Asp-L-Pro-D-Val-L-Leu-)sodium], an endothelin ET_A receptor antagonist, but not by 10^{-5} M BQ788 [*N*-cis-2,6-dimethyl-piperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine], an antagonist of endothelin ET_{B1} and ET_{B2} receptors. IRL1620 [Suc-[Glu^9,$Ala^{11,15}$]endothelin-1-(8–21)], a selective endothelin ET_{B1} receptor agonist, did not alter the response to electrical stimulation. However, raising the concentration of endothelin-1 to 10^{-9} M or higher potentiated the response. Similar results were also obtained in mesenteric artery strips in response to electrical stimulation. Endothelin-1 at low concentrations did not alter the contraction caused by exogenous norepinephrine in the artery strips, whereas the peptide at high concentrations potentiated the response. ^{3}H-overflow evoked by transmural electrical stimulation from tissues prelabeled with [^{3}H]norepinephrine was decreased by endothelin-1 (3×10^{-10} M) in the superfused dog mesenteric arteries. It is concluded that endothelin-1 at low concentrations activates prejunctional endothelin ET_A receptors and inhibits adrenergic nerve-mediated contractions by an inhibition of amine release, whereas the peptide at high concentrations potentiates the neurally induced contractions by a postjunctional enhancement, via endothelin ET_A receptors, of the action of norepinephrine. Low concentrations of endothelin-1 appear to act as a vasodilator in adrenergically innervated mesenteric artery.

Keywords: Endothelin-1; Endothelin ET_A receptor; Adrenergic nerve stimulation; Prejunctional inhibition; Postjunctional potentiation; Mesenteric artery

1. Introduction

Endothelin-1 is a potent vasoconstrictor (Yanagisawa et al., 1988; Inoue et al., 1989) and is one of the bioactive peptides secreted by endothelial cells. In addition to direct actions on vascular smooth muscle, endothelin-1 also stimulates the release of vasoactive factors from endothelial cells (Douglas and Hiley, 1990; Rakugi et al., 1989) and affects neurotransmission (Wiklund et al., 1991). The presence of endothelin-like immunoreactive compounds in neural tissue (Yoshizawa et al., 1990) and of specific endothelin-binding sites in the brain, spinal cord, pituitary (Jones et al., 1989; Koseki et al., 1989) and autonomic nerve (Davenport et al., 1989) suggests that these peptides act as neurotransmitters or neuromodulators. There is accumulating evidence that endothelin-1 inhibits responses to autonomic nerve stimulation in guinea pig isolated femoral and pulmonary arteries (Wiklund et al., 1988, 1989) and rat perfused mesenteric artery (Tabuchi et al., 1989). These effects were correlated with decreased output of norepinephrine from adrenergic nerve endings, although the effective concentrations of the peptide differed in the guinea pig and rat arteries. Endothelin-1 potentiates the responses to nerve stimulation through actions on postjunctional sites in guinea pig pulmonary artery (Wiklund et al., 1989) and the vas deferens of the rat (Wiklund et al., 1991, 1990; Warner et al., 1993) and mouse (Rae and Calixto, 1990).

Although three different endothelin receptors have been identified and termed endothelin ET_A, ET_{B1} and ET_{B2} subtypes (Nishiyama et al., 1995; Brooks et al., 1995), the endothelin receptors mediating these effects have not been determined. The endothelin ET_A receptor is more selective for endothelin-1 than for other members of the endothelin family whereas the endothelin ET_B receptor is isopeptide-

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nonselective (Arai et al., 1990; Sakurai et al., 1990; Saeki et al., 1991; Clozel et al., 1992). IRL1620 (Takai et al., 1992; Nishiyama et al., 1995) and BQ788 (Karaki et al., 1994) have been introduced as selective agonist of endothelin ET_{B1} receptors and an antagonist of endothelin ET_{B1} and ET_{B2} receptors, respectively. BQ123 has been shown to be a selective endothelin ET_A receptor antagonist (Ihara et al., 1992).

We designed the present experiments to investigate the effect of endothelin-1 on the response to periarterial nerve stimulation and the contraction evoked by exogenous norepinephrine in isolated dog mesenteric artery segments and strips, to clarify the receptor subtype responsible for the modulation by endothelin-1 of adrenergic neurotransmission using endothelin receptor agonists and antagonists, and to determine the modification by endothelin-1 of the release of [^{3}H]norepinephrine induced by nerve stimulation.

2. Materials and methods

2.1. Studies on mechanical responses

Mongrel dogs of either sex, weighing 8–13 kg, were anesthetized with intravenous injections of sodium thiopental (30 mg/kg) and killed by bleeding from the carotid arteries. Proximal portions of the superior mesenteric artery of 0.9–5.0 mm outside diameter were isolated. The artery segment was placed in 40 ml bathing medium and perfused with modified Ringer-Locke solution maintained at 37 ± 0.3°C and aerated with a mixture of 95% O_2 and 5% CO_2 at a constant rate of 1 ml/min with pressure of a 40–50 mm Hg (Toda et al., 1990). Constituents of the solution were as follows (mM): NaCl 120, KCl 5.5, $CaCl_2$ 2.2, $MgCl_2$ 1.0, $NaHCO_3$ 25.0 and dextrose 5.6. The pH of the solution was 7.36 to 7.43. The perfusion pressure was measured via a pressure transducer (Nihon-Kohden Kogyo Co., Tokyo, Japan) placed upstream to the artery segment. Perfused segments were placed between a pair of stimulating electrodes made of platinum plate. The gap between the segment and the electrodes was wide enough to allow undisturbed contractions and yet sufficiently narrow to permit stimulation of intramural nerve terminals effectively. Under resting conditions, electrical square pulses of supramaximal intensity were applied transmurally at frequencies of 5, 10, 20 and 30 Hz for 40, 20, 10 and 7 s, respectively, every 10–15 min to stimulate perivascular nerves innervating the arterial wall. Transmural electrical stimulation was applied repeatedly until steady responses were obtained, and then the agents, such as endothelin-1, BQ123, indomethacin, N^G-nitro-L-arginine (L-NA), IRL1620 and BQ788, were directly applied to the bathing media. At the end, tetrodotoxin was applied to determine whether the induced response was due to stimulation of perivascular nerves. In preliminary experiments, the stimulation-induced increases in perfusion pressure were observed to be steady over 2 h. When required, the endothelium was removed by rubbing with a cotton-covered polyethylene tube inserted into the lumen of the segment. Successful removal of the endothelium was determined by abolishment of the depressor response induced by 10^{-6} M acetylcholine applied intraluminally.

Some of the mesenteric arteries (0.5–0.8 mm outside diameter) were cut into helical strips approximately 20 mm long to evaluate the effect of endothelin-1 on responses to transmural electrical stimulation and norepinephrine. The endothelium was removed by gently rubbing the intimal surface of the strip with a cotton ball. Removal of the endothelium was verified by abolishment of relaxations caused by 10^{-6} M acetylcholine. The strips were fixed vertically between hooks in a muscle bath of 20 ml capacity containing modified Ringer-Locke solution, which was maintained at 37 ± 0.3°C and aerated with a mixture of 95% O_2 and 5% CO_2. Isometric mechanical responses were recorded on an ink-writing oscillograph. The resting tension was adjusted to 1.5 g, which is optimal for inducing maximum contraction (Toda and Miyazaki, 1978). Perivascular nerve terminals were stimulated transmurally with electrical pulses through polar platinum electrodes at a frequency of 5 Hz for 40 s every 10 min. Norepinephrine (10^{-7} to 10^{-5} M) was applied cumulatively to the bathing media, and the concentration-response curve was obtained.

2.2. ^{3}H-overflow study

Isotope experiments were carried out on helical strips of dog mesenteric arteries as previously described (Toda et al., 1988). The tissue was preincubated for 60 min at 37°C with 0.5 μM [^{3}H]norepinephrine (specific activity of 56.9 Ci/mmol). The tissue was then superfused with the modified Ringer-Locke solution containing cocaine (3×10^{-5} M) and corticosterone (4×10^{-5} M) at a rate of 1 ml/min. The preincubated strips were stimulated electrically 5 times for 3 min at a frequency of 5 Hz. Stimulations were applied after 126 (S_1), 144 (S_2), 162 (S_3), 180 (S_4) and 198 min (S_5) of superfusion. At the end of the superfusion, tritium counts in the superfusate collected each 3 min and in the tissues solubilized by toluene were measured. The stimulation-evoked ^{3}H-overflow was calculated as a percentage of the tissue tritium content at the time of stimulation. Endothelin-1 was added 12 min before S_4. Effects of the drug on stimulation-evoked ^{3}H-overflow were expressed as the ratio between the overflow evoked by S_4 or S_5 and that evoked by S_3. The ratios were compared with those obtained in the absence of treatment with the drug. Endothelin-1 did not affect the spontaneous overflow of tritium.

2.3. Statistics and drugs used

The results shown in the text, table and figures are expressed as mean values ± S.E.M. Statistical analyses

were done by using Student's paired and unpaired *t*-test or Tukey's method after one-way analysis of variance. Drugs used were N^G-nitro-L-arginine (L-NA), endothelin-1 (Peptide Institute, Minoh, Japan), indomethacin, cocaine hydrochloride, corticosterone (Sigma, St. Louis, MO, USA), [^{3}H]norepinephrine (NEN Research Products, DE, USA), tetrodotoxin, norepinephrine hydrochloride (Sankyo Co., Tokyo) and prazosin hydrochloride (Wako Pure Chemical Industries, Osaka, Japan). BQ123 [cyclo(D-Trp-D-Asp-L-Pro-D-Val-L-Leu-)sodium] and BQ788 [*N*-cis-2,6-dimethyl-piperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine] were synthesized in the Tsukuba Research Institute of the Banyu Pharmaceutical Co. (Tsukuba, Japan). IRL1620 [Suc-[Glu^9,Ala^{11,15}]endothelin-1-(8–21)] was synthesized in the International Research Laboratories (Takarazuka, Japan).

3. Results

3.1. Effect on the response to adrenergic nerve stimulation in perfused segments

In dog mesenteric artery segments perfused at a rate of 1 ml/min, transmural electrical stimulation (5, 10, 20 and 30 Hz for 40, 20, 10 and 7 s, respectively) produced a frequency-related increase in the perfusion pressure; the mean values were 4.0 ± 0.7, 24.4 ± 1.9, 45.2 ± 4.1 and 70.8 ± 7.2 mm Hg ($n = 5$), respectively. At a frequency of 20 Hz applied at an interval of 10 min, the pressor responses were reproducible; therefore, the effect of various agents on the response to this frequency of stimulation was evaluated. The pressor response was abolished by treatment with prazosin (10^{-5} M) or tetrodotoxin (3×10^{-7} M), suggesting that norepinephrine released by activation of perivascular adrenergic nerves is involved.

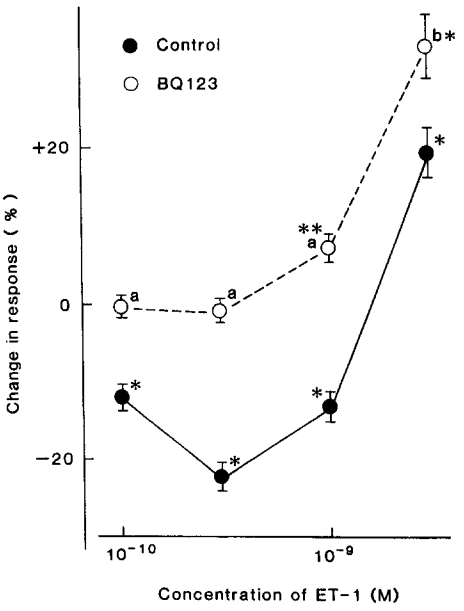

Fig. 1. Effects of endothelin-1 (ET-1, 10^{-10} to 3×10^{-9} M) on the pressor response to transmural electrical stimulation (20 Hz for 10 s) and modification by BQ123 (10^{-7} M) of the inhibitory effect of endothelin-1 on pressor responses induced by transmural stimulation in perfused dog mesenteric artery segments ($n = 5$). Stimulation-induced increases in the perfusion pressure before application of endothelin-1 are expressed as zero; the mean absolute value was 45.4 ± 10.6 mm Hg. Significantly different from zero, * $P < 0.01$, ** $P < 0.05$. Significantly different from control, ^{a} $P < 0.01$; ^{b} $P < 0.05$. Vertical bars represent S.E.M.

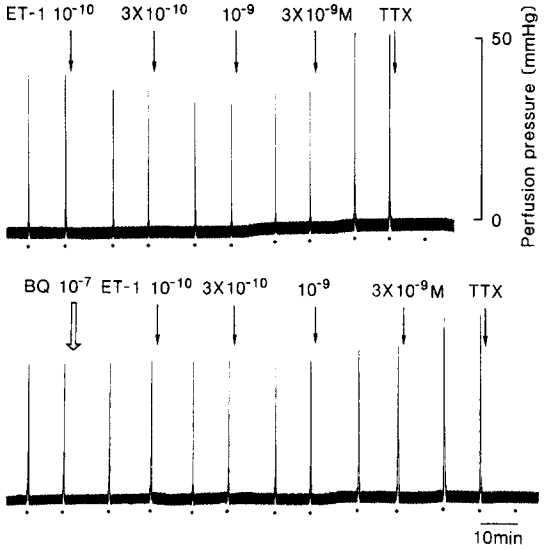

Fig. 2. Typical responses to transmural electrical stimulation (20 Hz for 10 s) of a perfused mesenteric artery segment before and after treatment with endothelin-1 (ET-1, 10^{-10}, 3×10^{-10}, 10^{-9} and 3×10^{-9} M, upper panel), and with BQ123 (BQ, 10^{-7} M) and ET-1 (10^{-10}, 3×10^{-10}, 10^{-9} and 3×10^{-9} M, lower panel). Drugs were cumulatively added directly to the organ bath. Each dot below the tracing represents the application of electrical stimulation. TTX = tetrodotoxin.

Endothelin-1 (10^{-10} and 3×10^{-10} M) did not alter the perfusion pressure, but significantly inhibited the pressor response to transmural electrical stimulation dose dependently in the endothelium-intact segments (Fig. 1). However, raising the concentration of endothelin-1 to 10^{-9} M or higher slightly increased the perfusion pressure and potentiated the stimulation-evoked response from the attenuated level in the presence of the low concentrations of endothelin-1 (Fig. 1). Typical responses are illustrated in Fig. 2. The inhibitory effect of endothelin-1 was not affected by removal of the endothelium. In the endothelium-intact or -denuded segments, treatment with L-NA (10^{-5} M) did not alter the perfusion pressure, but potentiated the response to electrical stimulation. Similar results were also obtained when indomethacin (10^{-6} M) was applied to the segments with and without the endothelium. However, neither L-NA nor indomethacin influenced the inhibition by endothelin-1 of the pressor response to electrical stimulation (Fig. 3). Actual tracings of the responses are shown in Fig. 4.

The endothelin-1-induced inhibition of the pressor response to electrical nerve stimulation was suppressed by

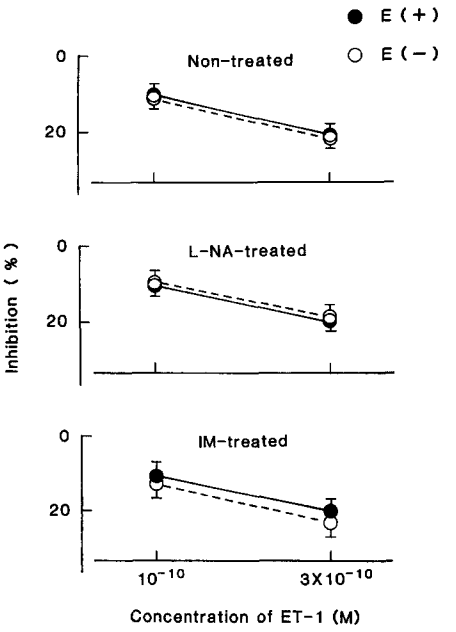

Fig. 3. Modification by N^G-nitro-L-arginine (L-NA) and indomethacin (IM) of the inhibitory effect of endothelin-1 on pressor responses induced by transmural electrical stimulation at 20 Hz of perfused dog mesenteric artery segments ($n = 5$) with and without endothelium (upper panel with non-treated, middle panel with 10^{-5} M L-NA and lower panel with 10^{-6} M IM). Stimulation-induced increases in the perfusion pressure before application of endothelin-1 are expressed as zero; the mean absolute values of non-treated, L-NA-treated and IM-treated group were 45.2 ± 4.1 mm Hg, 45.1 ± 3.3 mm Hg and 48.3 ± 4.6 mm Hg, respectively. Inhibition by endothelin-1 (10^{-10} and 3×10^{-10} M) of the stimulation-induced increases in perfusion pressure was significant ($P < 0.01$). Vertical bars represent S.E.M. E (+) = with endothelium, E (−) = without endothelium.

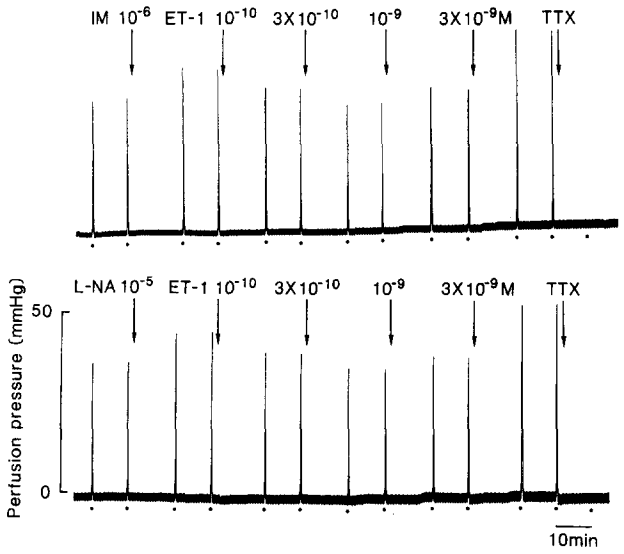

Fig. 4. Typical responses to transmural electrical stimulation (20 Hz for 10 s) of a perfused mesenteric artery segment before and after treatment with indomethacin (IM, 10^{-6} M) and endothelin-1 (ET-1, 10^{-10}, 3×10^{-10}, 10^{-9} and 3×10^{-9} M, upper panel), and with N^G-nitro-L-arginine (L-NA, 10^{-5} M) and endothelin-1 (10^{-10}, 3×10^{-10}, 10^{-9} and 3×10^{-9} M, lower panel). Drugs were cumulatively added directly to the organ bath. Each dot below the tracing represents the application of electrical stimulation. TTX = tetrodotoxin.

treatment with 10^{-7} M BQ123, an endothelin ET_A receptor antagonist, but not by 10^{-5} M BQ788, an antagonist of endothelin ET_{B1} and ET_{B2} receptors (Fig. 5). However, each antagonist alone did not affect the response to electrical nerve stimulation. Mean values of the pressure increase before and after BQ788 were 46.3 ± 7.1 and 45.5 ± 6.1 mm Hg, respectively ($n = 4$, $P > 0.05$), and those of BQ123 were 45.4 ± 10.6 and 45.0 ± 10.8 mm Hg, respectively ($n = 5$, $P > 0.05$). BQ123 at 10^{-7} M did not inhibit the enhancement of the pressor response to periarterial nerve stimulation produced by endothelin-1 of 10^{-9} and 3×10^{-9} M (Fig. 1). IRL1620 (3×10^{-10} to 3×10^{-9} M), a selective endothelin ET_{B1} receptor agonist, did not alter the perfusion pressure nor the response to electrical stimulation (39.8 ± 2.8 vs. 38.8 ± 2.7 mm Hg, at 3×10^{-9} M IRL1620, Fig. 6).

3.2. Effect on the response to adrenergic nerve stimulation in arterial strips and norepinephrine

Transmural electrical stimulation at a frequency of 5 Hz produced a contraction in dog mesenteric artery strips. The responses were abolished by treatment with 3×10^{-7} M tetrodotoxin. The contractile responses to adrenergic nerve stimulation were significantly reduced by treatment with

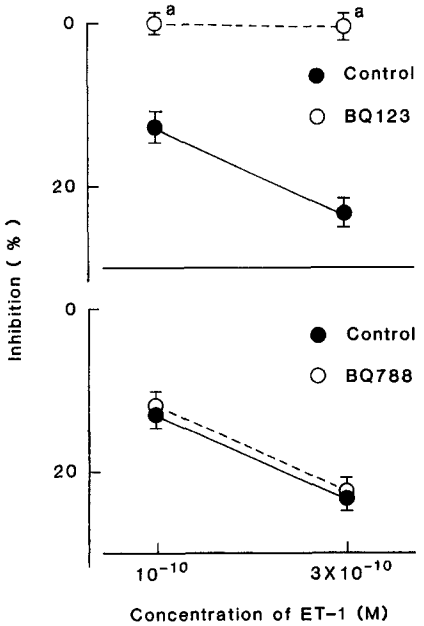

Fig. 5. Modification by BQ123 and BQ788 of the inhibitory effect of endothelin-1 (ET-1) on the pressor responses induced by transmural stimulation at 20 Hz of perfused dog mesenteric artery segments ($n = 5$, upper panel with 10^{-7} M BQ123 and lower panel with 10^{-5} M BQ788). Stimulation-induced increases in perfusion pressure before application of endothelin-1 are expressed as zero; the mean absolute values of BQ123-treated and BQ788-treated group were 45.4 ± 10.6 mm Hg and 46.3 ± 7.1 mm Hg, respectively. Inhibition by endothelin-1 (10^{-10} and 3×10^{-10} M) of the stimulation-induced increase in perfusion pressure was significant ($P < 0.01$). Significantly different from control, [a] $P < 0.01$. Vertical bars represent S.E.M.

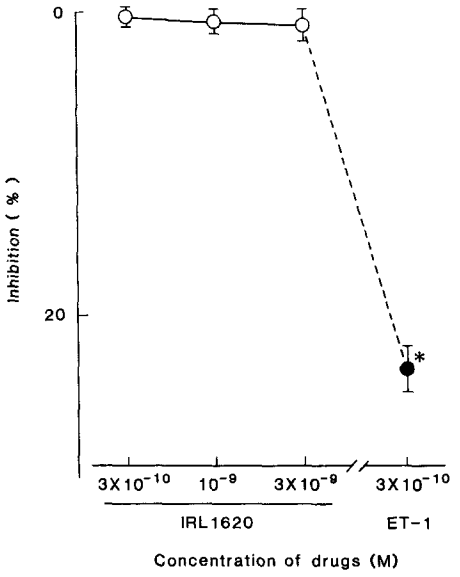

Fig. 6. Effects of IRL1620 (3×10^{-10} to 3×10^{-9} M) and endothelin-1 (ET-1, 3×10^{-10} M) on the pressor responses to transmural stimulation at 20 Hz ($n=5$) of perfused dog mesenteric artery segments ($n=5$). Stimulation-induced increases in perfusion pressure under control conditions are expressed as zero; the mean absolute value was 39.8 ± 2.8 mm Hg. Significantly different from control, * $P<0.01$. Vertical bars represent S.E.M.

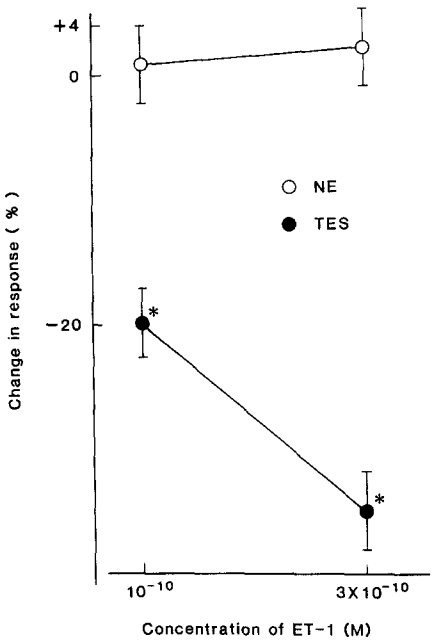

Fig. 7. Modification by endothelin-1 (ET-1) of the contractile responses to transmural electrical stimulation (TES, 5 Hz, $n=9$) and to norepinephrine (NE, 5×10^{-7} M, $n=12$) of dog mesenteric artery strips. Contractions induced by electrical stimulation and norepinephrine before application of endothelin-1 are expressed as zero; the mean absolute values were 175.0 ± 39.4 mg and 272.9 ± 31.5 mg, respectively. Significantly different from the pre-drug values, * $P<0.01$. Vertical bars represent S.E.M.

10^{-10} and 3×10^{-10} M endothelin-1 (Fig. 7), which did not influence the resting tension of the artery strip.

The effects of endothelin-1 on contractions elicited by exogenous norepinephrine (10^{-7} to 10^{-5} M) were studied to determine the postjunctional action of endothelin-1. The contractile responses were not significantly altered by treatment with endothelin-1 (10^{-10} and 3×10^{-10} M), whereas endothelin-1 at 10^{-9} M and 3×10^{-9} M potentiated the response to low concentrations of norepinephrine (10^{-7} and 5×10^{-7} M). Mean values of the potentiation produced by 10^{-9} M endothelin-1 of the response to 10^{-7} and 5×10^{-7} M norepinephrine were $955.6\pm105.9\%$ ($n=5$ $P<0.001$, paired t-test) and $233.7\pm36.8\%$ ($n=5$ $P<0.01$), respectively, and those produced by 3×10^{-9} M endothelin-1 were $1535.0\pm209.8\%$ ($n=5$ $P<0.001$) and $358.6\pm35.3\%$ ($n=5$ $P<0.01$), respectively. Endothelin-1 alone produced a contraction; the mean values of the contraction were $18.5\pm2.4\%$ at 10^{-9} M and $48.1\pm11.3\%$ at 3×10^{-9} M relative to that caused by 30 mM K^+. The potentiating effect of endothelin-1 was not influenced by treatment with BQ123 at 10^{-7} M, whereas in the presence of 10^{-6} M BQ123 the effect on the contractile response to 10^{-7} M norepinephrine was markedly reduced. Potentiations in the absence and presence of 10^{-6} M BQ123 were $955.6\pm105.9\%$ and $376.8\pm70.0\%$ ($n=5$ $P<0.05$, unpaired t-test, for endothelin-1 10^{-9} M), respectively, and $1535.0\pm209.8\%$ and $633.8\pm189.5\%$ ($n=5$ $P<0.01$ for endothelin-1 3×10^{-9} M).

3.3. ^{3}H-overflow from superfused arteries in response to transmural stimulation

In order to determine the prejunctional action of endothelin-1, the modulation by endothelin-1 of the release of [^{3}H]norepinephrine evoked by perivascular nerve stimulation was studied. Transmural electrical stimulation was applied five times at a frequency of 5 Hz for 3 min to mesenteric artery strips exposed for 60 min to the media containing [^{3}H]norepinephrine and superfused for 126 min in the control medium. Endothelin-1 was applied just after the third stimulation (S_3). The ratios of the overflow S_4/S_3 and S_5/S_3 in control series were 1.006 ± 0.002 and 1.004 ± 0.019 ($n=5$), respectively, and were significantly decreased by treatment with 3×10^{-10} M endothelin-1 (Table 1).

Table 1
The ratio of ^{3}H-overflow evoked by transmural electrical stimulation in the absence and presence of endothelin-1

	N	S_4/S_3	S_5/S_3
Control	5	1.006 ± 0.002	1.004 ± 0.019
Endothelin-1 (3×10^{-10} M)	5	0.730 ± 0.031 [a]	0.662 ± 0.051 [a]

N, number of preparations used. Significantly different from controls, [a] $P<0.01$. The strip was treated for 12 min with 3×10^{-10} M endothelin-1 before the application of transmural electrical stimulation (S_4).

4. Discussion

Transmural electrical stimulation at 5, 10, 20 and 30 Hz produced a frequency-related pressor response in isolated perfused dog mesenteric artery segments, a response which was depressed by treatment with prazosin and abolished by tetrodotoxin. Therefore, the observed responses would be elicited mainly by norepinephrine released from electrically stimulated adrenergic nerve endings. The present study showed that endothelin-1 at low concentrations (10^{-10} and 3×10^{-10} M) inhibited the pressor response of the arterial segments to electrical stimulation of perivascular nerves, and decreased the ^{3}H-overflow evoked by nerve stimulation in superfused mesenteric artery strips previously soaked in [^{3}H]norepinephrine-containing media. This observation is in accord with the reports that endothelin-1 inhibits the nerve stimulation-induced release of [^{3}H]-norepinephrine in the isolated guinea pig femoral (Wiklund et al., 1988) and pulmonary arteries (Wiklund et al., 1989) and the perfused rat mesenteric artery (Tabuchi et al., 1989). In addition, endothelin-1 did not inhibit the response to exogenous norepinephrine. Therefore, the inhibitory effect of low concentrations of endothelin-1 on the pressor response to nerve stimulation is expected to be associated with an inhibition of norepinephrine release from nerve endings. In addition, the present study showed that the endothelin-1 at high concentrations (10^{-9} and 3×10^{-9} M) potentiated the pressor response to electrical nerve stimulation. A similar potentiation by endothelin-1 of contractions caused by autonomic nerve stimulation has been described in rat mesenteric circulation (Tabuchi et al., 1990). The potentiating effect of endothelin-1 might be due to postjunctional enhancement, since endothelin-1 increased the contraction of mesenteric artery strips to exogenously applied norepinephrine. Thus, the most likely explanation for these observations is that the inhibition by endothelin-1 at low concentrations is exerted via a prejunctional action, whereas the potentiation is due to its postjunctional action when high concentrations of endothelin-1 are applied.

Binding sites for porcine endothelin-1 have been detected in cultured rat vascular smooth muscle cells (Hirata et al., 1988) and found autoradiographically in organs other than vascular tissue (Davenport et al., 1989). Endothelin prejunctionally inhibits adrenergic nerve function, suggesting the existence of endothelin receptors on nerve terminals. Endothelin receptors have been found in nerve endings of human and porcine coronary arteries (Power et al., 1989). Endothelin-1 has been isolated from porcine spinal cord and its sequence has been determined (Shinmi et al., 1989). Furthermore three different endothelin receptors have also been identified and termed endothelin ET_A, ET_{B1} and ET_{B2} receptor subtypes (Arai et al., 1990; Sakurai et al., 1990; Saeki et al., 1991; Clozel et al., 1992; Brooks et al., 1995; Nishiyama et al., 1995). The present study revealed that the endothelin-1-induced inhibition of the pressor response to adrenergic nerve stimulation was markedly suppressed by treatment with BQ123, an endothelin ET_A receptor antagonist, but not by BQ788, an antagonist of endothelin ET_{B1} and ET_{B2} receptors, and IRL 1620, an endothelin ET_{B1} receptor agonist, did not alter the effect of endothelin-1 on the response to electrical nerve stimulation. These results support the hypothesis that low concentrations of endothelin-1 (10^{-10} and 3×10^{-10} M) activate prejunctional endothelin ET_A receptors, and thereby inhibit the release of norepinephrine, resulting in an attenuation of vascular contraction due to nerve stimulation. Further, the enhancement by endothelin-1 of the norepinephrine-induced contraction was also suppressed by BQ123, suggesting that the postjunctional action of endothelin-1 is mediated by endothelin ET_A receptors. The agonist and the antagonist for endothelin ET_A receptors seem to be more effective at the prejunctional level than at the postjunctional level (about 10^{-10} vs. 10^{-9} M of agonist and 10^{-7} vs. 10^{-6} M of antagonist). We assume that the higher concentration of BQ123 was required to block the postjunctional action induced by the high concentration of the agonist.

Although it has been shown that the endothelium inhibits the response to adrenergic nerve stimulation and the release of norepinephrine from the nerve of the rabbit carotid artery (Tesfamariam et al., 1987; Cohen and Weisbrod, 1988), the present study clearly demonstrated that the inhibitory effect of endothelin-1 on the pressor response to nerve stimulation was not influenced by removal of the endothelium, suggesting that the inhibition of endothelin-1 does not depend on the endothelium. Prostaglandins have been reported to be released by stimulation of adrenoceptors in the isolated perfused superior mesenteric vasculature of the rabbit (Pipili and Poyser, 1981). Prostaglandin I_2, a major metabolite of endoperoxide, is also known to attenuate the response to adrenergic nerve stimulation (Nakajima and Toda, 1986). In the present study, indomethacin (10^{-6} M), a cyclooxygenase inhibitor, was without effect on the inhibition elicited by endothelin-1 of the pressor response to sympathetic nerve stimulation in the mesenteric artery segments. In addition, treatment with L-NA, a NO synthase inhibitor, did not alter the inhibitory effect of endothelin-1. These findings strongly suggest that the metabolites of arachidonic acid and EDRF/NO, even though they are released, are not involved in the mechanism underlying the modulation by endothelin-1 of neurotransmission. However, the possibility that endothelin-1 exerts its effects by affecting the release of other neuromodulatory substances cannot be ruled out.

The potentiation by L-NA of the pressor response to nerve stimulation has been shown to result from a suppression of functions of vasodilator nerves possibly releasing NO as a neurotransmitter (Toda et al., 1991; Zhang et al., 1993). The inability of L-NA to modify the prejunctional action of endothelin-1 may indicate that endothelin-1 does

not alter NO-mediated nerve function in the preparations used. Endothelin-1 prejunctionally inhibits adrenergic nerve function but does not seem to impair the release/synthesis of NO in the vasodilator nerve. Further study is required to clarify whether such a different action is due to distinct mechanisms of transmitter release; that is, the release of norepinephrine in synaptic vesicles by exocytosis in adrenergic nerves and NO release possibly by diffusion immediately after its synthesis by NO synthase from L-arginine.

In conclusion, the present study revealed that endothelin-1, apart from being a potent vasoconstrictor, acts as a vasodilator in adrenergically innervated mesenteric arteries. It appears that endothelin-1 at low concentrations inhibits adrenergic neurogenic contractions by inhibition of neurotransmitter release via activation of prejunctional endothelin ET_A receptors, whereas the peptide at high concentrations potentiates neurally induced contractions due to a postjunctional enhancement of the action of norepinephrine.

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