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Endothelin-1-induced potentiation of adrenergic responses in the rabbit pulmonary artery: role of thromboxane A_2

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Abstract

To examine whether low concentrations of endothelin-1 potentiate the vasocontrictor response to adrenergic stimulation, we recorded the isometric response of rings of rabbit pulmonary artery to electrical stimulation and noradrenaline. Endothelin-1 (10^{-10} M) potentiated the contractions induced by electrical stimulation and noradrenaline. The endothelin ET_B receptor antagonist (2,6-dimethylpiperidine-carbonyl-γ-methyl-Leu-N_{in}-[Methoxycarbonyl]-D-Trp-D-Nle) (BQ-788, 10^{-6} M), but not the endothelin ET_A receptor antagonist cyclo(D-Asp-Pro-D-Val-Leu-D-TRP) (BQ-123, 10^{-6} M), inhibited the potentiating effects of endothelin-1. Pretreatment with the cyclooxygenase inhibitor indomethacin, the thromboxane synthase inhibitor furegrelate and the thromboxane receptor antagonist [1*S*-[1α,2α(*Z*),3α,4α]]-7-[3-[[[[(1-oxoheptyl)amino]acetyl]amino] methyl]-7-oxabicyclo-[2.2.1]hept-2-yl]-5-heptenoic acid (SQ-30741) (all at 10^{-5} M) prevented the potentiation induced by endothelin-1 on adrenergic stimulation. The Ca^{2+} channel antagonist nifedipine (10^{-6} M) did not affect the potentiation induced by endothelin-1. The results indicate that endothelin-1 potentiates the responses to electrical stimulation and noradrenaline by activating endothelin ET_B receptors. This potentiation depends on the production of cyclooxygenase-generated factors, probably thromboxane A_2.

Keywords: Endothelin-1; Electrical stimulation; Noradrenaline; Thromboxane A_2; Synergism

1. Introduction

The major isoform of endothelin in the cardiovascular system, endothelin-1, contracts isolated pulmonary vessels (Ryan et al., 1989; Hay et al., 1993; Cases et al., 1996) and increases pulmonary vascular resistance (Lippton et al., 1989; Horgan et al., 1991). The action of endothelin-1 is mediated by two pharmacologically distinct receptors, endothelin ET_A receptor and endothelin ET_B receptor (Arai et al., 1990; Sakurai et al., 1990). The endothelin ET_A receptor is localized in the vascular smooth muscle and its stimulation causes constriction. Stimulation of endothelin ET_B receptors, present on endothelial cells and on smooth muscle, causes dilatation or constriction (De Nucci et al., 1988; Tsukahara et al., 1994; Haynes et al., 1995; Verhaar et al., 1998). Endothelin-1 may also modulate the effects of other vasoactive substances that are found in plasma or released from perivascular nerve endings. Endothelin-1 has been shown to potentiate vascular contractions to noradrenaline and serotonin (Yang et al., 1990; Henrion and Laher, 1993; Kita et al., 1998). In adition, endothelin-1 enhances the contractile response to nerve stimulation in some vascular beds (Wiklund et al., 1989; Wong-Dusting and Rand, 1991; Reid, 1993; Mutafova-Yambolieva and Radomirov, 1994) or it may reduce sympathetic constriction by reducing the release of noradrenaline from perivascular nerve-endings (Tabuchi et al., 1989). In other instances, the interaction between endothelin-1 and sympathetic response is modulated by changes in temperature (Padilla et al., 1997; García-Villalón et al., 1997). Because endothelin-1 may release thromboxane A_2 from vascular preparations (Takayasu-Okishio et al., 1990; Reynolds and Mok, 1990; Horgan et al., 1991; Zaugg et al., 1996) and thromboxane A_2, in turn, may affect neurotransmission and the vascular response to vasoconstrictors (Timimi et

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al., 1978; Makita, 1983), the possibility exists that thromboxane A_2 may be involved in the effects of endothelin-1 on sympathetic responses. In the present study, we examined the effects of subpressor concentrations of endothelin-1 on the response of the rabbit pulmonary artery to sympathetic stimulation and noradrenaline. To analyze the contribution of endothelin-1-induced release of thromboxane A_2 to the vascular responses, the effects of endothelin-1 were measured after inhibition of cyclooxygenase activity, thromboxane A_2 synthase inactivation or blockade of thromboxane A_2 receptor.

2. Materials and methods

2.1. Pulmonary artery preparations

New Zealand white male rabbits (2.5–3 kg) were anesthetized with ketamine (50 mg/kg i.m.) and exanguinated. The lungs were removed and the pulmonary arteries (secondary segments) were isolated, cleaned of connective tissue, and cut into 4 mm rings for isometric recording of tension. The outside diameter of the rings was measured with an ocular micrometer within a Wild M8 zoom microscope (Heerbrugg, Switzerland) and ranged from 0.5 to 1 mm. Ring preparations were suspended between two L-shaped stainless steel pins. One pin was fixed to the organ bath wall while the other was connected to a strain gauge (model Grass FT03). Changes in isometric force were recorded on a Macintosh computer by use of Chart v 3.4/s software and a MacLab/8e data acquisition system (ADInstruments). Each preparation was set up in a 4-ml bath containing modified Krebs–Henseleit solution of the following millimolar composition: NaCl, 115; KCl, 4.6; $MgCl_2 \cdot 6H_2O$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; glucose, 11.1; and disodium EDTA, 0.01. The solution was equilibrated with 95% oxygen and 5% carbon dioxide to give a pH of 7.3–7.4. The temperature was held at 37°C.

To establish the resting tension for maximal force development, a series of preliminary experiments was performed on artery rings of similar length and outer diameter which were exposed repeatedly to 60 mM KCl. Basal tension was increased gradually until contractions were maximal. The optimal resting tension was $1g$. The artery rings were allowed to attain a steady level of tension during a 2–3-h equilibration period before testing. In some arterial rings, the endothelium was removed mechanically by inserting a roughened stainless steel wire into the lumen and gently rubbing the ring on a wet filter paper. This procedure has been shown morphologically to result in essentially complete removal of the endothelium (Martínez et al., 1994, 1995). Functional integrity of the endothelium was confirmed routinely by the presence of relaxation induced by acetylcholine (10^{-7}–10^{-6} M) during contraction obtained with noradrenaline (10^{-7}–3×10^{-7} M).

2.2. Experimental protocols

Following the equilibration period, concentration–response curves for endothelin-1 (10^{-11}–10^{-7} M) were obtained in paired rings under resting tension in the absence and in the presence of the endothelin ET_A receptor antagonist BQ-123, the endothelin ET_B receptor antagonist BQ-788 (10^{-6}–10^{-5} M), or both the endothelin ET_A receptor antagonist BQ-123 plus the endothelin ET_B receptor antagonist BQ-788 (10^{-6} M). Electrical field stimulation was provided by a Grass S88 stimulator (Grass Instruments, Quincy, MA, USA) via two platinum electrodes positioned on each side and parallel to the axis of the artery ring. To asses the nature of the contractile responses and avoid direct stimulation of smooth muscle, frequency–response relationships were determined on a group of arteries in the presence and absence of 10^{-6} M tetrodotoxin, following a procedure previously described (Aldasoro et al., 1993; Martínez et al., 1994). In summary, the protocol was designed to find the optimal stimulation parameters (15 V, 0.2 ms duration) causing a contractile response that was completely eliminated by 10^{-6} M tetrodotoxin. Frequency–response relationships were determined using 30 s trains of pulses at 1, 2 and 4 Hz. A period of 10 min was allowed between stimulations.

To study the effects of experimental substances on electrical field stimulation-induced contractions, we performed the following protocol: after an initial set of stimulations (1, 2, and 4 Hz) at 10-min intervals, another set of stimulations was given in the presence or absence of experimental substances. The drugs tested included endothelin-1 (10^{-12} to 10^{-10} M), the selective endothelin ET_B receptor agonist sarafotoxin S6c (3×10^{-11} M), the endothelin ET_A receptor antagonist BQ-123 (10^{-5} M), the endothelin ET_B receptor antagonist BQ-788 (10^{-5} M), the cyclooxygenase inhibitor indomethacin (10^{-5} M), the thromboxane A_2 receptor antagonist SQ-30741 (10^{-5} M), and the thromboxane synthase inhibitor furegrelate (10^{-5} M). Concentration–response curves to endothelin-1 and noradrenaline were also constructed in the presence of indomethacin, SQ-30741 and furegrelate (10^{-5} M). Antagonists were added to organ bath chambers 15 min before the initiation of frequency– or concentration–response relationships.

Concentration–response curves for noradrenaline were determined in a cumulative manner. Control (in the absence of endothelin-1) and experimental (in the presence of endothelin-1) data were obtained from separate vascular preparations. Another group of arterial rings was incubated with the endothelin ET_B receptor antagonist BQ-788 or the endothelin ET_A receptor antagonist BQ-123 before exposure to noradrenaline or noradrenaline plus endothelin-1 10^{-10} M.

To study the participation of dihydropyridine Ca^{2+} channels on the endothelin-1 induced potentiation of adrenergic responses, arterial rings were preincubated with

the Ca^{2+} channel antagonist nifedipine (10^{-6} M) for 20 min prior to make the frequency–response or concentration–response curves in the absence and in the presence of endothelin-1 (10^{-10} M).

2.3. Drugs

The following drugs were used: tetrodotoxin, prazosin hydrochloride, noradrenaline hydrochloride, acetylcholine chloride, endothelin-1, sarafotoxin S6c, guanethidine sulphate, furegrelate sodium salt, nifedipine, indomethacin, (2,6-dimethylpiperidine-carbonyl-γ-methyl-Leu-N_{in}-[Methoxycarbonyl]-D-Trp-D-Nle) sodium salt (BQ-788), cyclo(D-Asp-Pro-D-Val-Leu-D-TRP) (BQ-123) (Sigma, St. Louis, MO, USA), and [1*S*-[1α,2α(*Z*),3α,4α]]-7-[3-[[[[(1-oxoheptyl)amino]acetyl]amino]methyl]-7-oxabicyclo-[2.2.1]hept-2-yl]-5-heptenoic acid (SQ-30741, Bristol-Myers Squibb, Princeton, USA).

All drugs were dissolved in Krebs solution except indomethacin, which was dissolved initially in ethanol and further diluted in Krebs solution to the proper final concentration. Drugs were added to the organ bath in volumes of less than 70 μl. Stock solutions of the drugs were freshly prepared every day, and kept on ice throughout the experiment.

2.4. Analysis of data

The data are expressed as means ± S.E.M. pD_2 (negative logarithm of the molar concentration at which half-maximum contraction occurs) was determined from individual concentration–response curves by nonlinear regression analysis. The pA_2 values for endothelin receptor antagonist were determined from a Schild plot (Arunlakshana and Schild, 1959). The concentration-ratios (CR) were calculated as the ratio between the EC_{50} value for endothelin-1 in the presence and absence of different concentrations of the endothelin ET_B receptor antagonist BQ-788. A Schild plot was constructed with the CRs: log (CR-1) (ordinate scale) was plotted against log (antagonist concentration) (abscissa scale) and pA_2 was estimated as the intercept of the regression line with the abscissa scale (Arunlakshana and Schild, 1959). In each experimental group *n* indicates the number of animals. Differences between agonist and antagonist-treated groups were assessed by one-way analysis of variance (ANOVA). Differences between groups were identified by *t* test. Statistical significance was accepted at $P < 0.05$.

3. Results

3.1. Effects of endothelin-1

Endothelin-1 (10^{-11}–10^{-7} M) caused concentration-dependent contractions with a pD_2 of 8.8 ± 0.2. These contractions were endothelium-independent (Fig. 1A). The presence of the endothelin ET_B receptor antagonist BQ-788 (10^{-6}–10^{-5} M) in the organ bath induced significant shifts ($P < 0.05$) of the control curve to the right in a concentration-dependent manner, with no change in the maximum response (Fig. 1A). Schild analysis of these data yielded a pA_2 value of 7.16 with a slope of 1.05 ± 0.11 indicating competitive antagonism. The endothelin ET_A receptor antagonist BQ-123 (10^{-5} M) did not affect the contractile responses to endothelin-1 (Fig. 1B). Combination of both endothelin ET_A and ET_B receptor antagonists (10^{-6} M) caused a similar rightward shift on concentration–response curve to endothelin-1 (Fig. 1A) to that produced by the endothelin ET_B receptor antagonist (10^{-6} M) given alone. The α_1-adrenoceptor antagonist prazosin (10^{-6} M) did not change the concentration response curve to endothelin-1 (Fig. 1B). There was not significant difference in the contraction of rings with and without endothelium to the addition of 60 mM KCl (3080 ± 125 mg vs. 2945 ± 160 mg, $n = 8$, $P > 0.05$).

3.2. Effects of endothelin-1 on electrical stimulation-induced contractions

Electrical stimulation induced frequency-dependent increases in tension which were abolished by tetrodotoxin (10^{-6} M), guanethidine (10^{-6} M) and prazosin (10^{-6} M), thus suggesting that the effect was due to the release of noradrenaline from adrenergic nerves acting on α_1-adrenoceptors. Endothelin-1 (10^{-11}–10^{-10} M) caused concentration-dependent potentiation of the electrically evoked

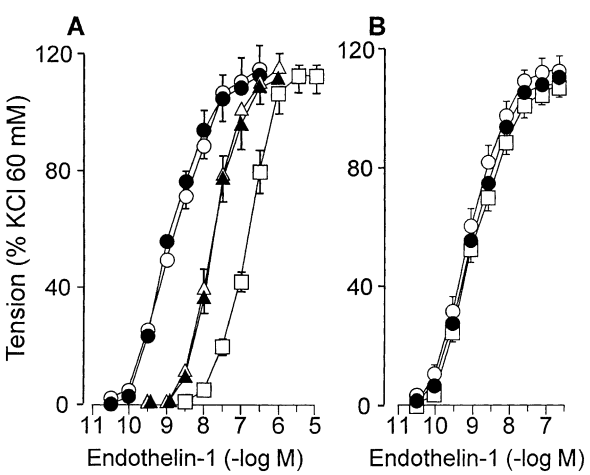

Fig. 1. (A) Concentration–response curves for endothelin-1 determined in artery rings with (●, $n = 8$) and without (○, $n = 8$) endothelium, in the presence of endothelin ET_B receptor antagonist BQ-788 (▲ 10^{-6} M, $n = 5$; □ 10^{-5} M, $n = 5$) and in the presence of both endothelin ET_B receptor antagonist BQ-788 plus endothelin ET_A receptor antagonist BQ-123 (△ 10^{-6} M, $n = 4$). (B) Concentration–response curves to endothelin-1 in the absence (●, $n = 8$) and in the presence of the endothelin ET_A receptor antagonist BQ-123 (□ 10^{-5} M, $n = 5$) and in the presence of the α_1-adrenoceptor antagonist prazosin (○ 10^{-6} M, $n = 3$). Values are mean ± S.E.M.

responses (Fig. 2A). The endothelin ET_B receptor antagonist BQ-788 (10^{-5} M), but not the endothelin ET_A receptor antagonist (10^{-5} M), prevented the amplifying effect of endothelin-1 (10^{-10} M) (Fig. 2B). The selective endothelin ET_B receptor agonist sarafotoxin S6c (3×10^{-11} M) induced potentiation of electrical stimulation which was also inhibited in the presence of the endothelin ET_B receptor antagonist BQ-788 (10^{-5} M) (Fig. 2C).

3.3. Effect of endothelin-1 on noradrenaline-induced contractions

Endothelin-1 potentiated noradrenaline-induced contractions in a concentration-dependent manner (Fig. 3). The noradrenaline pD_2 values and maximal response in the presence and absence of endothelin-1 are shown in Table 1. The endothelin ET_B receptor antagonist BQ-788 (10^{-5} M), but not the endothelin ET_A receptor antagonist BQ-123 (10^{-5} M), produced a parallel, rightward shift of the potentiating effects of 10^{-10} M endothelin-1 on the noradrenaline concentration–response curve (Fig. 3).

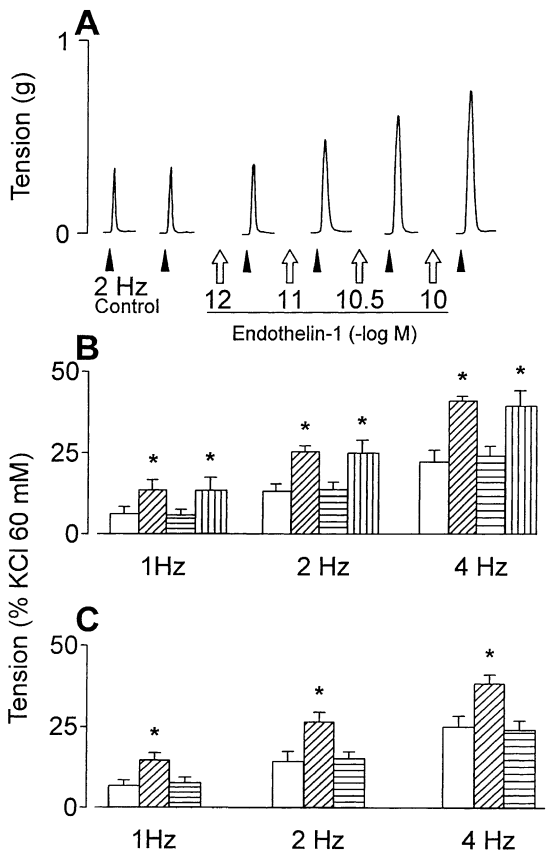

Fig. 2. (A) Original tracings of contractile responses to electrical stimulation (2 Hz) of rabbit pulmonary artery under control conditions and after incubation with various concentrations of endothelin-1 (10^{-12} to 10^{-10} M). (B) Contractile responses to electrical stimulation in the absence, control (□, $n=10$) and in the presence of endothelin-1 10^{-10} M (▨, $n=10$), endothelin-1 together with the endothelin ET_B receptor antagonist BQ-788 10^{-5} M (▤, $n=6$), or endothelin-1 together the endothelin ET_A receptor antagonist BQ-123 10^{-5} M (▥, $n=6$). (C) Contractile responses to electrical stimulation in the absence, control (□, $n=5$) and in the presence of the selective endothelin ET_B receptor agonist sarafotoxin S6c 3×10^{-11} M (▨, $n=5$), and sarafotoxin S6c plus the endothelin ET_B receptor antagonist BQ-788 10^{-5} M (▤, $n=5$). Values are mean ± S.E.M. * Significant difference from control value, $P < 0.05$.

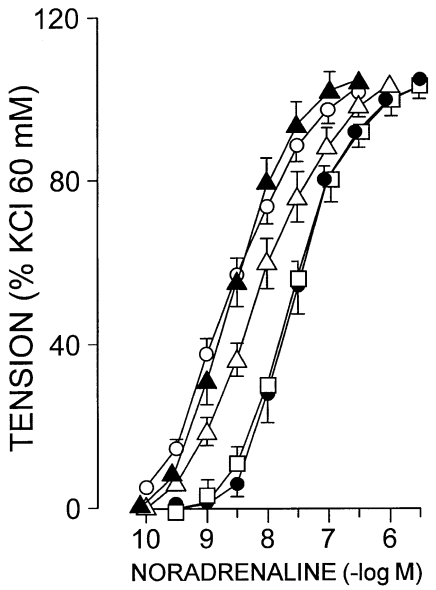

Fig. 3. Concentration–response curve to noradrenaline in the absence (●, $n=8$) and in the presence of endothelin-1 (△, 10^{-11} M, $n=6$) (○, 10^{-10} M, $n=6$), and endothelin-1 (10^{-10} M) together with the endothelin ET_B receptor antagonist BQ-788 (□, 10^{-5} M, $n=6$) or the endothelin ET_A receptor antagonist BQ-123 (▲, 10^{-5} M, $n=6$). Values are mean ± S.E.M.

Table 1
pD_2 values and maximal contractions elicited by noradrenaline alone (control), in the presence of either endothelin-1 or the endothelin ET_B receptor antagonists BQ-788, and the endothelin ET_A receptor antagonist BQ-123 together with endothelin-1

Noradrenaline	pD_2 (M) (95% confidence interval)	Maximal responses (percentage of KCl 60 mM ± S.E:M.)
Control ($n=9$)	7.53 ± 0.25	104 ± 5
With endothelin-1		
10^{-12} M ($n=9$)	7.50 ± 0.34	100 ± 3
10^{-11} M ($n=9$)	8.36 ± 0.09[a]	107 ± 6
10^{-10} M ($n=9$)	8.76 ± 0.07[a]	105 ± 2
With endothelin-1 (10^{-10} M) + BQ-788		
10^{-6} M ($n=9$)	8.28 ± 0.05[a]	105 ± 6
10^{-5} M ($n=9$)	7.76 ± 0.14	102 ± 3
With endothelin-1 (10^{-10} M) + BQ-123		
10^{-6} M ($n=9$)	8.57 ± 0.07[a]	111 ± 3
10^{-5} M ($n=9$)	8.62 ± 0.10[a]	105 ± 4

Values are means ± S.E.M.
n, number of rabbits.
[a] $P < 0.05$, vs. control rings.

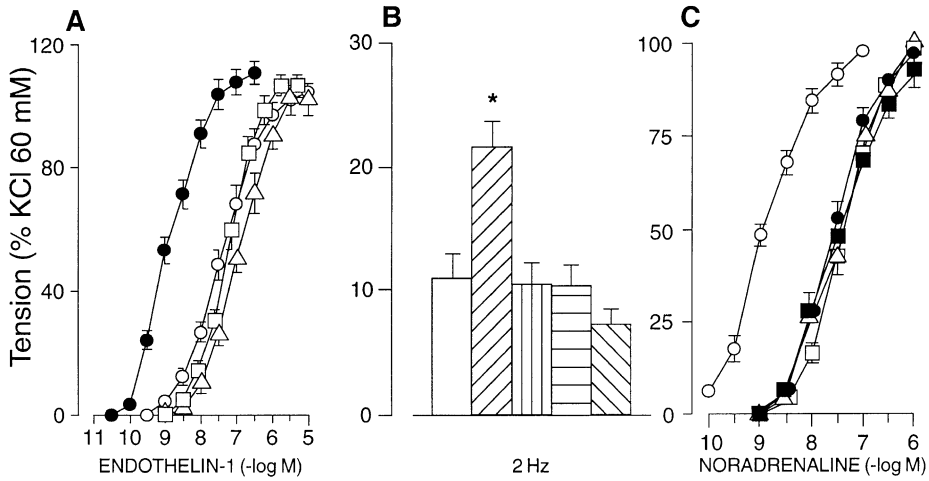

Fig. 4. (A) Concentration–response curves to endothelin-1 in the absence (●, $n = 7$) and in the presence of indomethacin (○, 10^{-5} M, $n = 4$), SQ-30741 (△, 10^{-5} M, $n = 4$), or furegrelate (□, 10^{-5} M, $n = 4$). (B) Contractile responses to electrical stimulation (2 Hz) under control conditions (□, $n = 10$), and in the presence of 10^{-10} M of endothelin-1 (▨, $n = 10$), endothelin-1 plus 10^{-5} M indomethacin (▥, $n = 6$), endothelin-1 plus 10^{-5} M SQ-30741 (▤, $n = 6$), or endothelin-1 plus 10^{-5} M furegrelate (▧, $n = 6$). (C) Concentration–response curves to noradrenaline in the absence (●, $n = 7$) and in the presence of either endothelin-1 10^{-10} M (○, 10^{-5} M, $n = 5$), endothelin-1 plus indomethacin, (△, 10^{-5} M, $n = 4$), endothelin-1 plus SQ-30741 (□, 10^{-5} M, $n = 4$), or endothelin-1 plus furegrelate (■, 10^{-5} M, $n = 4$). Values are mean ± S.E.M. * Significant difference from control value, $P < 0.05$.

3.4. Role of cyclooxygenase product release and thromboxane A_2

If thromboxane A_2 mediates part of endothelin-1-induced contraction, then inhibition of cyclooxygenase, inhibition of thromboxane A_2 synthase, and thromboxane A_2 receptor blockade should have an inhibitory effect on endothelin-1-induced contraction and should also inhibit the endothelin-1-induced potentiation of neurogenic and noradrenaline contractions. Fig. 4A shows that indomethacin, furegrelate and SQ-30741 (all at 10^{-5} M) displaced to the right the concentration–response curve to endothelin-1 and prevented the amplifying effects of endothelin-1 on neurogenic (Fig. 4B) and noradrenaline (Fig. 4C) contractions. Preincubation with indomethacin, furegrelate or SQ-30741 (all at 10^{-5} M) had no significant effects on the concentration–response curves to noradrenaline, indicating that these antagonists did not have a non-specific inhibitory effect on agonist-induced contraction (results not shown).

3.5. Endothelin-1 and Ca^{2+}

The dihydropyridine Ca^{2+} channel antagonist nifedipine (10^{-6} M) did not modify the concentration–response curves to noradrenaline and endothelin-1 nor the frequency–response curves to electrical stimulation (data not shown). In the presence of nifedipine (10^{-6} M), the enhancement by endothelin-1 (10^{-10} M) of the contractile responses to noradrenaline and electrical stimulation was identical to that observed in the absence of nifedipine (Fig. 5A and B).

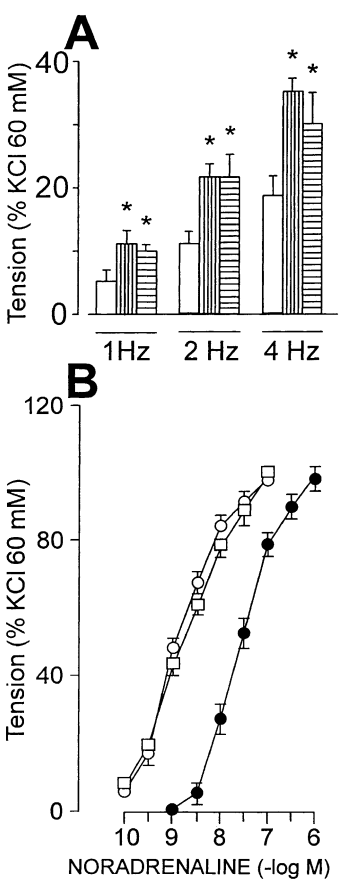

Fig. 5. (A) Frequency–response relationship under control conditions (□, $n = 6$) and in the presence of either 10^{-10} M endothelin-1 (▥, $n = 6$) or 10^{-6} M nifedipine plus 10^{-10} M endothelin-1 (▤, $n = 6$). (B) Concentration–response curves to noradrenaline in the absence (●, $n = 6$) and in the presence of either 10^{-10} M endothelin-1 (○, $n = 6$) or 10^{-6} M nifedipine plus endothelin-1 (□, $n = 6$). Values are mean ± S.E.M. * Significant difference from control value, $P < 0.05$.

4. Discussion

This study confirms previous findings showing that endothelin-1 is primarily a constrictor of the rabbit pulmonary artery due to endothelin ET_B receptor stimulation (Fukuroda et al., 1994; Maurice et al., 1997). BQ-123 did not affect the endothelin-1-induced contraction, thus suggesting that endothelin ET_A receptors do not contribute to this effect. Previous studies have shown that neither BQ-123 alone nor BQ-788 alone inhibited the endothelin-induced contraction of rabbit pulmonary artery, whereas the combination of both BQ-123 and BQ-788 completely inhibited the endothelin-induced contraction (Fukuroda et al., 1994). It was concluded that both endothelin ET_A and endothelin ET_B receptors contribute to endothelin-induced responses in rabbit pulmonary artery. Similar results have been reported in rat mesenteric arteries (Palea et al., 1998). However, in our results, the endothelin ET_A receptor antagonist BQ-123 did not affect the endothelin-induced contraction but the use of the endothelin ET_B receptor antagonist BQ-788 shifted to the right the concentration–response curves for endothelin-1 in a parallel manner. Schild analysis showing unitary slopes and antagonist pA_2 values obtained from these data indicate a competitive antagonism at the endothelin ET_B receptor. In addition, combined treatment with BQ-123 and BQ-788 did not modify the antagonism induced by BQ-788 given alone. Therefore, our results exclude a significant role for endothelin ET_A receptors in the contractile response of the rabbit pulmonary artery to endothelin-1 and they are consistent with the hypothesis that stimulation of endothelin ET_B receptors is responsible for the endothelin-induced contractions in rabbit pulmonary artery.

A main finding of our study is that subpressor concentrations of endothelin-1 enhance the vascular contractions induced by sympathetic stimulation and noradrenaline. The endothelin ET_B receptor antagonist BQ-788, but not the endothelin ET_A receptor antagonist BQ-123, inhibited the potentiating effects of endothelin-1. In addition, the selective endothelin ET_B agonist sarafotoxin S6c (3×10^{-11} M) induced potentiating effects on electrical stimulation-induced contractions similar to those observed in the presence of 10^{-10} M of endothelin-1. It is unlikely that the endothelin ET_B agonist sarafotoxin S6c could have produced nonspecific potentiation of neurogenic-induced contractions, because its potentiating effect was completely blocked by the endothelin ET_B receptor antagonist BQ-788. These results are consistent with the idea that endothelin ET_B receptor stimulation by endothelin-1 is followed by enhancement of responses to both endogenous and exogenous noradrenaline. The effects of endothelin-1 on neurogenic contractions could involve an effect on adrenergic nerves leading to release of noradrenaline or, alternatively, endothelin-1 could act with noradrenaline at postjunctional receptor sites. Because noradrenaline release was not measured in this study, a contribution of presynaptic facilitating effects cannot be excluded. The fact that the concentration–response curve to endothelin-1 was not modified by prazosin, an α_1-adrenoceptor antagonist, suggests that the action of this peptide does not involve release of noradrenaline.

Endothelin-1 is known to activate phospholipase A_2 in vascular smooth muscle cells (Resink et al., 1989; Reynolds et al., 1989), leading to the release of arachidonic acid metabolites that can be transformed by cyclooxygenase into the endoperoxide prostaglandin H_2, which then serves as a common precursor for a diverse group of prostanoids including thromboxane A_2 (Reynolds and Mok, 1990; Zaugg et al., 1996). Thromboxane A_2 potentiates the response to vasoconstrictors (Timimi et al., 1978; Trachte and Stein, 1988; Stein and Trachte, 1989). If thromboxane A_2 participates in the endothelin-1-induced potentiation of vascular responses, cyclooxygenase inhibition, TP receptor blockade or thromboxane A_2 synthase inhibition should attenuate or inhibit the potentiation. In our experiments, the cyclooxygenase inhibitor indomethacin caused complete inhibition of the potentiating effects of endothelin-1. The TP receptor antagonist SQ-30741 abolished the potentiation of sympathetic stimulation and noradrenaline-induced contractions. These results indicate that the augmentation of vascular responses by endothelin-1 is due to synthesis of a cyclooxygenase product that activates the TP receptor. Incubation of pulmonary artery rings with furegrelate, a selective inhibitor of thromboxane A_2 synthase (Johnston et al., 1986), the enzyme that converts prostaglandin H_2 to thromboxane A_2, inhibited the potentiating effects of endothelin-1, indicating that thromboxane A_2 is the endothelin-stimulated cyclooxygenase product that activates the TP receptor and mediates the potentiation on symphatetic nerve stimulation and noradrenaline. Our results support previous observations in the guinea pig perfused lung suggesting that the bronchoconstriction of endothelin-1 is mediated by release of thromboxane A_2 following activation of endothelin ET_B receptors (D'Orleans-Juste et al., 1994; Noguchi et al., 1996).

The role of Ca^{2+} in the interaction between endothelin-1 and adrenergic stimulation may vary considerably among different vascular beds and species. In our experiments, nifedipine, a dihydropyridine Ca^{2+} channel antagonist, did not affect the direct effect of endothelin-1 or prevent the potentiating action of endothelin-1 on noradrenaline and electrical stimulation-induced contractions. This indicates that the influx of extracellular Ca^{2+} through dihydropyridine-sensitive Ca^{2+} channels does not contribute to the direct contractile effects of endothelin-1 nor participates in the potentiating effects of the peptide on adrenergic contractions. Thus, the increase in cytosolic Ca^{2+} necessary to produce the potentiation may be released from internal storage. However, other mechanisms may be involved in the potentiating effect. For instance, Henrion and Laher (1993), using rings of rabbit aorta, suggested that endothelin-1 could potentiate noradrenaline-induced contractions

by increasing the sensitivity of the smooth muscle to Ca^{2+} by activating protein kinase C-dependent mechanism. In contrast, in porcine coronary artery (Kasuya et al., 1992) and rat trachea (Henry, 1993), contractions mediated by the endothelin ET_B receptor are due to Ca^{2+} influx via an inositoltriphosphate-independent pathway.

In conclusion, the results of the present study indicate that endothelin-1, in addition to its vasocontrictor effect, strongly potentiates the responses to electrical stimulation and noradrenaline. This probably takes place by the binding of endothelin-1 to the endothelin ET_B receptor, since the potentiating effect was reversed by BQ-788. The potentiating effects of endothelin-1 is due to stimulation of the production of cyclooxygenase-generated vasoconstrictor factors, probably thromboxane A_2, which in turn may contract vascular smooth muscle through TP receptors stimulation.

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