ELSEVIER

European Journal of Pharmacology 384 (1999) 163–167

ejp

www.elsevier.nl/locate/ejphar

Basal inhibitory action of endogenous endothelin on the sympathetic contraction in the isolated rat tail artery

Angel Luis García-Villalón *, Luis Monge, Nuria Fernández, Miguel Angel Sánchez, María Angeles Martínez, Bernardino Gómez, Godofredo Diéguez

Departamento de Fisiología, Facultad de Medicina, Universidad Autónoma, Arzobispo Morcillo 2, 28029 Madrid, Spain

Received 1 July 1999; received in revised form 23 September 1999; accepted 28 September 1999

Abstract

In order to test whether endogenous endothelin modulates the sympathetic vasoconstriction, arterial segments, 2 mm long, from rat tail artery were mounted in organ baths for isometric tension recording. Electrical field stimulation (2–8 Hz, 0.2 ms, 70 V during 1 s) produced frequency-dependent arterial contraction (maximal contraction 770 ± 49 mg) that was nearly abolished (over 95% reduction) by tetrodotoxin (10^{-6} M) or phentolamine (10^{-6} M). This contraction was increased by pretreatment with the antagonist of endothelin ET_B receptors *N*-(*N*-(*N*-(2,6-dimethyl-1-piperidinyl)carbonyl)-4-methyl-L-leucyl)-1-(methoxycarbonyl)-D-tryptophyl)D-norleucine (BQ-788, 10^{-7}–3×10^{-6} M), and was not modified either by the antagonist of endothelin ET_A receptors cyclo(D-α-aspartyl-L-prolyl-D-valyl-L-leucyl-D-tryptophyl) (BQ-123, 10^{-7}–3×10^{-6} M) or the agonist of endothelin ET_B receptors endothelin-1 (8–21), *N*-Suc-(Glu^9, $Ala^{11,15}$) (IRL-1620, 10^{-8}–10^{-7} M). The potentiating effect of BQ-788 was not modified in arterial segments without endothelium or pretreated with the inhibitor of nitric oxide synthesis N^W-nitro-L-arginine (L-NA, 10^{-4} M) or with the inhibitor of endothelin converting enzyme *N*-(α-rhamnopyranosyloxy-hydroxyphosphinyl)-leu-trp (phosphoramidon, 10^{-4} M). Exogenous noradrenaline (10^{-9}–10^{-4} M) produced concentration-dependent arterial contractions that were not modified by BQ-788 (3×10^{-6} M), BQ-123 (3×10^{-6} M) or IRL-1620 (10^{-7} M). Therefore, an inhibitory action of endogenous endothelin on sympathetic vasoconstriction may be present under basal conditions. This inhibition could be produced by endothelin through activation of prejunctional endothelin ET_B receptors, which may inhibit noradrenaline release from perivascular sympathetic nerves.

Keywords: Endothelin ET_B receptor; Tail artery, rat; BQ-788; Electrical stimulation

1. Introduction

Experimental observations suggest that endothelin-1 is produced by endothelial cells and has marked effects on the cardiovascular system (Levin, 1995), but as its plasma concentrations in the basal conditions are very low, the participation of this peptide in the vascular regulation under normal conditions remains controversial (Rubanyi and Polokoff, 1994). There exists the possibility that endothelin-1, by reaching higher concentrations locally in the vascular wall than in plasma, may act as a paracrine substance and have a functional significance (Levin, 1995). Although exogenous endothelin-1 at relatively high concentrations produces a powerful vasoconstriction, mainly by activating endothelin ET_A receptors located on vascular smooth muscle cells, at low concentrations it may produce vasodilatation mediated by endothelin ET_B receptors located on endothelial cells (Karaki et al., 1993), or it may reduce the vasoconstriction to sympathetic stimulation by reducing the release of noradrenaline from perivascular nerve terminals (Tabuchi et al., 1989). It has been thus proposed that the vasodilator effect of endothelin-1 may be of greater physiological importance than the vasoconstrictor effect for the vascular regulation in normal conditions (Gellai et al., 1996).

The present study was performed to test the hypothesis that endogenous endothelin modulates the vasoconstriction in response to sympathetic stimulation. The experiments were performed in segments from the rat tail artery, which were mounted in organ baths for isometric tension recording and exposed to electrical field stimulation and exoge-

* Corresponding author. Tel.: +34-91-3975412; fax: +34-91-3975324.
E-mail address: angeluis.villalon@uam.es (A.L. García-Villalón)

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00687-1

nous noradrenaline under control and different experimental conditions.

2. Methods

Seventeen male Sprague–Dawley rats, weighing 200–300 g, were killed by pentobarbital overdose, and the ventral caudal (tail) artery was dissected free and cut into cylindrical segments 2 mm in length. Each segment was prepared for isometric tension recording in a 4-ml organ bath at 37°C, containing modified Krebs–Henseleit solution with the following composition (millimolar): NaCl, 115; KCl, 4.6; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; glucose, 11.1. The solution was equilibrated with 95% oxygen and 5% carbon dioxide to give a pH of 7.3–7.4. Briefly, the method consists of passing two fine, stainless steel pins, 100 μm in diameter, through the lumen of the vascular segment. One pin is fixed to the organ bath wall, while the other is connected to a strain gauge for isometric tension recording, thus permitting the application of passive tension in a plane perpendicular to the long axis of the vascular cylinder. The recording system included a Universal Transducing Cell UC3 (Statham Instruments), a Statham Microscale Accessory UL5 (Statham Instruments) and a Beckman Type RS Recorder (model R-411, Beckman Instruments). A previously determined resting passive tension of 750 mg was applied to the vascular segments, and then they were allowed to equilibrate for 60–90 min.

Electrical field stimulation (2, 4 and 8 Hz, 0.2 ms pulse duration, at a supramaximal voltage of 70 V, for 1 s) was applied to the arteries via two platinum electrodes placed either side of the artery and connected to a CS-14 stimulator (Cibertec). An interval of at least 10 min was imposed between stimulation periods to allow recovery of the response, and the stimulation was repeated in every case until the responses were reproducible during over 40 min under control conditions. The vascular response to electrical stimulation was recorded in the presence of tetrodotoxin (10^{-6} M) or phentolamine (10^{-6} M), to test whether the response is mediated by stimulation of perivascular nerve terminals, and activation of α-adrenoceptors, respectively.

The effects of the antagonist of endothelin ET_B receptors BQ-788 (10^{-7}–3×10^{-6} M), the antagonist of endothelin ET_A receptors BQ-123 (10^{-7}–3×10^{-6} M) and the agonist of endothelin ET_B receptors IRL-1620 (10^{-8}–10^{-7} M) on the arterial response to electrical field stimulation were analysed. The effects of BQ-788 were studied on three stimulation frequencies (2, 4 and 8 Hz), so that each of these three frequencies was tested in one different group of arteries before or after adding the different concentrations of BQ-788 cumulatively. As the maximal effect of BQ-788 was observed with electrical stimulation at 4 Hz, this frequency was selected for studying the effects of BQ-123 and IRL-1620. Each of these substances was added cumulatively to the organ bath, and electrical stimulation was applied before (control) and 10 min after adding each concentration of the substance. In each experiment, a vascular segment which received electrical stimulation but was not treated with any substance was used as a time control.

The effect of BQ-788 on the response to electrical stimulation (4 Hz) was also studied in arteries after inhibition of nitric oxide synthesis with N^W-nitro-L-arginine (L-NA, 10^{-4} M) and in arteries without endothelium. After obtaining a reproducible response to electrical stimulation, L-NA was added to the organ bath, and after 20-min incubation, electrical stimulation was applied; then BQ-788 was also added and electrical stimulation was again applied to the arteries in the presence of both L-NA and BQ-788. Endothelium removal was accomplished by gently rubbing of the vascular lumen with a steel rod, and functionally tested by abolition of the relaxation to acetylcholine (10^{-5} M) after precontraction with 5-hydroxytryptamine (10^{-7} M) at the end of the experiment.

The effect of BQ-788 on the response to electrical stimulation (4 Hz) was also studied in the presence of the inhibitor of the endothelin converting enzyme phosphoramidon (10^{-4} M), to block the possible production of endothelin-1 in the arteries once placed in the organ bath. In this case, phosphoramidon was added immediately after mounting the vascular segment, and it was present in the organ bath throughout the duration of the experiment.

To examine the site of action, prejunctional or postjunctional, of the agonists and antagonists of endothelin receptors on the arterial response to electrical stimulation, cumulative concentration–response curves to noradrenaline (10^{-9}–10^{-4} M) were performed in arteries treated with BQ-788 (3×10^{-6} M), BQ-123 (3×10^{-6} M) or IRL-1620 (10^{-7} M), these substances being incubated during 10 min before testing noradrenaline. The response to noradrenaline was also recorded in a non-treated vascular segment from the same artery to be taken as control. EC_{50} values for noradrenaline were calculated as the concentration producing 50% of the maximal effect by geometric interpolation.

The values of the contraction are shown in absolute values for noradrenaline and as absolute increments over the control response for the effects of endothelin receptor agonists and antagonists on electrical stimulation, and expressed as means ± S.E.M. Data were evaluated by a analysis of variance applied to each group of data, followed by a Dunnet's test to compare each experimental condition with its control. $P < 0.05$ was considered significant.

Drugs used were: (−)-arterenol, bitartrate salt (noradrenaline); L-NA; phentolamine hydrochloride; *N*-(α-rhamnopyranosyloxy-hydroxyphosphinyl)-leu-trp, sodium salt (phosphoramidon); and tetrodotoxin (Fugu poison), all from Sigma; cyclo(D-α-aspartyl-L-prolyl-D-valyl-L-leucyl-D-tryptophyl), peptide free base (BQ-123); *N*-(*N*-(*N*-(2,6-dimethyl-1-piperidinyl)carbonyl)-4-methyl-L-leucyl)-1-(me-

thoxycarbonyl)-D-tryptophyl)D-norleucine monosodium (BQ-788), and endothelin-1 (8–21), *N*-Suc-(Glu^{9}, Ala^{11,15}), peptide free base (IRL-1620) from Research Biochemicals International. All drugs were dissolved in distilled water and further diluted in isotonic NaCl.

3. Results

3.1. Response to electrical field stimulation

Electrical stimulation produced frequency-dependent contraction (219 ± 31; 394 ± 69 and 770 ± 49 mg for 2, 4 and 8 Hz, respectively), which in time control experiments did not change significantly during the period of the experimental procedure. This response was nearly abolished (over 95% reduction, $P < 0.01$), similarly by tetrodotoxin or phentolamine.

The contraction to electrical stimulation was increased in a concentration-dependent way in the presence of BQ-788 ($P < 0.01$). This increment induced by BQ-788 was higher when the stimulation frequency was 4 Hz, and it was lower with 2 and 8 Hz (Fig. 1A). The absolute increments produced by BQ-788 at 10^{-7}; 3×10^{-7} and 10^{-6} M, of the response to electrical stimulation, were significantly ($P < 0.01$) higher for 4 Hz than for 8 or 2 Hz, although for the highest BQ-788 concentration (3×10^{-6} M), the increments of the response were not significantly different between 2, 4 and 8 Hz. Therefore, the rest of the studies were performed using a stimulation frequency of 4 Hz.

The vascular contraction to electrical stimulation (4 Hz) was not modified by BQ-123 or IRL-1620 (not shown). Pretreatment with L-NA increased the response to electrical stimulation (4 Hz; $51 \pm 14\%$, $P < 0.01$), and the increment of the contraction to electrical stimulation produced by BQ-788 was similar in the absence and in the presence of L-NA ($P > 0.05$) (Fig. 1B). The contraction to electrical stimulation (4 Hz) in the arteries without endothelium (405 ± 65 mg) was not different from control arteries ($P > 0.05$), and the BQ-788-induced potentiation on electrical stimulation in these endothelium-deprived arteries was not significantly different to that in control arteries ($P > 0.05$) (Fig. 1B). In the arteries treated with phosphoramidon, both the response to electrical stimulation (4 Hz) and the potentiation of this response after BQ-788 were similar to that in control arteries (Fig. 1B).

3.2. Response to noradrenaline

Noradrenaline produced concentration-dependent contraction of every arterial segment (maximal contraction = 3535 ± 118 mg; $EC_{50} = 2 \times 10^{-7}$ M, 95% confidence interval = 1.1×10^{-7} M–3.5×10^{-7} M). This response to noradrenaline was not modified by pretreatment with BQ-788 (3×10^{-6} M), BQ-123 (3×10^{-6} M) or IRL-1620 (10^{-7} M) (Fig. 1C).

4. Discussion

The response to electrical stimulation found in our experiments is adrenergic in nature, probably mediated by noradrenaline released from perivascular sympathetic nerves, because it was nearly abolished by phentolamine

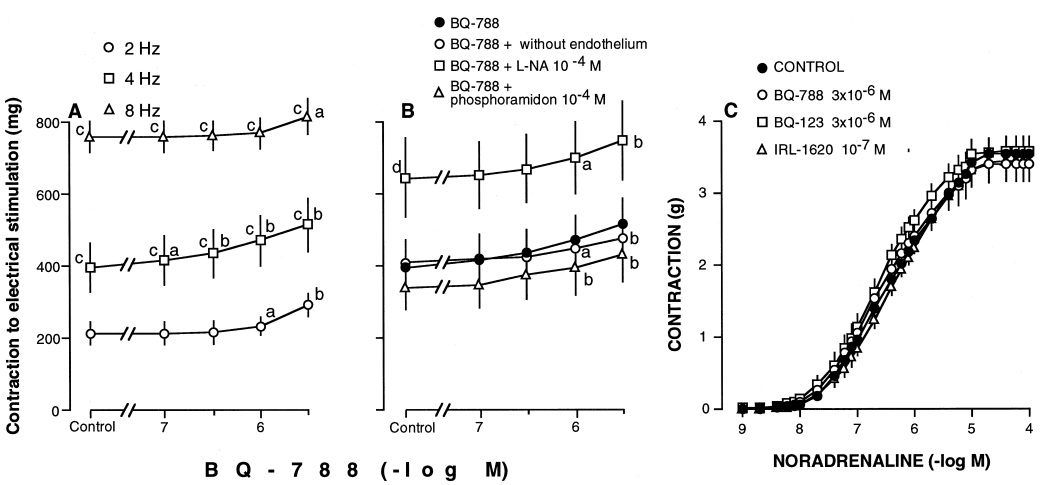

Fig. 1. (A) Potentiation of the contraction to electrical field stimulation (2, 4 and 8 Hz, 0.2 ms, supramaximal voltage, during 1 s) in rat tail arteries by BQ-788. (B) Potentiation of the contraction to electrical field stimulation (4 Hz) in rat tail arteries by BQ-788, in intact arteries and in arteries without endothelium, in the presence of L-NA (10^{-4} M) or in the presence of phosphoramidon (10^{-4} M). (C) Contraction of rat tail arteries to noradrenaline in the absence (control) and in the presence of BQ-788 (3×10^{-6} M), BQ-123 (3×10^{-6} M) or IRL-1620 (10^{-7} M).
Points are means ± S.E.M. In each case, data are average from 6–7 animals. a, b: Significant difference (a, $P < 0.05$; b, $P < 0.01$) in the presence of BQ-788 compared with control. c: Significant difference ($P < 0.01$) compared with 2 Hz. d: Significant difference ($P < 0.05$) compared with control in the absence of L-NA.

and tetrodotoxin. The present results suggest that under basal conditions there may be an inhibitory action on sympathetic contraction produced by endogenous endothelin through activation of endothelin ET_B receptors. This is based on the observation that the arterial contraction to electrical stimulation was increased by the antagonist of endothelin ET_B receptors BQ-788, and was not affected by the antagonist of ET_A receptors BQ-123. As the agonist of endothelin ET_B receptors IRL-1620 did not affect the sympathetic contraction, it is also suggested that this type of receptor may be already saturated by endogenous endothelin under basal conditions, and it cannot be stimulated further by an exogenous agonist.

The inhibitory effect of endothelin ET_B receptors on the sympathetic response may be due to these receptors are located prejunctionally and reduce noradrenaline release from sympathetic nerve endings, or they may be located postjunctionally and reduce the response of the smooth muscle to released noradrenaline. Although measurement of noradrenaline release should be accomplished to clarify this issue, our results may suggest that postjunctional mechanisms are not involved, because the arterial contraction to exogenous noradrenaline was not affected after inhibition of endothelin receptors of ET_A and ET_B subtypes, as well as after activation of endothelin ET_B receptors. Although the maximal contraction to exogenous noradrenaline was much higher than the response to electrical stimulation, low noradrenaline concentrations (10^{-9}–10^{-7} M) produced contractions that were comparable to those produced by electrical stimulation, and were not modified by endothelin ET_A and ET_B receptor agonists or antagonists. Therefore, a reasonable interpretation of our results is that the basal inhibitory action of endothelin on the sympathetic contraction may be due to endogenous endothelin reduces noradrenaline release from perivascular nerve endings, probably by activation of prejunctional endothelin ET_B receptors. This agrees with previous findings suggesting that exogenous endothelin inhibits the release of noradrenaline from sympathetic nerve endings (Tabuchi et al., 1989), and this effect may be mediated by prejunctional endothelin receptors of the ET_B subtype in arteries of dog kidney (Matsumura et al., 1996) or rat tail artery (Mutafova-Yambolieva and Westfall, 1998). To our knowledge, there are no previous data on whether these prejunctional endothelin ET_B receptors are active in physiological conditions. Although vasodilator endothelin ET_B receptors may play a role in the maintenance of vascular tone (Gellai et al., 1996), it has not been determined whether these receptors are pre- or postjunctional.

The potentiation by BQ-788 of the contraction to electrical stimulation was dependent on the stimulation frequency, being greater for 4 Hz than for 2 and 8 Hz. We hypothesize that low frequencies (2 Hz) may produce in nerve endings weak stimulation, which may be also weakly potentiated by inhibition of endothelin ET_B receptors. However, 8 Hz may already produce near maximal noradrenaline release, which may not be increased further by blockade of endothelin ET_B receptors.

It is known that endothelin may stimulate the release of nitric oxide by activation of endothelial endothelin ET_B receptors (Hirata et al., 1993), and that endothelin ET_B receptor-mediated inhibitory action on noradrenaline release from renal sympathetic nerves is dependent on nitric oxide (Matsuo et al., 1997). This mechanism may not be involved in the inhibition of sympathetic vasoconstriction by endothelin ET_B receptors observed in the present study, because the potentiating effect of BQ-788 on this contraction was not modified by inhibition of nitric oxide production with L-NA. Endothelium removal tended to reduce the enhancing effect of BQ-788, although this reduction was not statistically significant, therefore the involvement of the endothelium through the release of other factors (prostacyclin, hyperpolaryzing factor) in the effects of BQ-788 cannot be excluded with the present results.

In our preparation, the suggested inhibitory action of endogenous endothelin on sympathetic contraction is not probably produced by endothelin synthesised by the arterial segment after its placement in the organ bath because the effects of sympathetic stimulation were not modified by phosphoramidon, in spite of this blocker of endothelin synthesis was present in the organ bath throughout all the experiments. As endothelin-1 may bind strongly to its receptors, and this binding may be resistant to washing and last for a long time (Hilal-Dandan et al., 1997), it is suggested that endothelin previously synthesised may bind to endothelin ET_B receptors of the rat tail artery in vivo, and may remain bound to these receptors after the artery is removed from the animal and placed in the organ bath.

In conclusion, we suggest that endogenous endothelin activates prejunctional endothelin ET_B receptors, which may reduce the release of noradrenaline from perivascular sympathetic nerves, thus causing an inhibitory action on the sympathetic vascular contraction. Therefore, endogenous endothelin might play a modulatory role in vascular sympathetic regulation in physiological situations.

Acknowledgements

The authors are grateful to Mrs. M.E. Martínez and H. Fernández-Lomana for technical assistance. This work was supported, in part, by MEC (No. PM 95-0032), FIS (No. 96/0474) and CAM (No. AE 00236/95).

References

Gellai, M., Fletcher, T., Pullen, M., Nambi, P., 1996. Evidence for the existence of endothelin-B receptor subtypes and their physiological roles in the rat. Am. J. Physiol. 271, R254–R261.

Hilal-Dandan, R., Villegas, S., Gonzalez, A., Brunton, L.L., 1997. The quasi-irreversible nature of endothelin binding and G protein-linked signaling in cardiac myocytes. J. Pharmacol. Exp. Ther. 281, 267–273.

Hirata, Y., Emori, T., Eguchi, S., Kanno, K., Imai, T., Ohta, K., Marumo, F., 1993. Endothelin receptor subtype B mediates synthesis of nitric oxide in cultured bovine endothelial cells. J. Clin. Invest. 91, 1367–1373.

Karaki, H., Sudjarwo, S.A., Hori, M., Sakata, K., Urade, Y., Takai, M., Okada, T., 1993. ET_B receptor antagonist, Irl 1038, selectively inhibits the endothelin induced endothelium dependent vascular relaxation. Eur. J. Pharmacol. 231, 371–374.

Levin, E.R., 1995. Endothelins. N. Engl. J. Med. 333, 356–363.

Matsumura, Y., Matsuo, G., Egi, Y., Tadano, K., Morimoto, S., 1996. Inhibitory effects of endothelin-3 on antidiuresis and norepinephrine overflow induced by stimulation of renal nerves in anesthetized dogs. J. Cardiovasc. Pharmacol. 28, 278–284.

Matsuo, G., Matsumura, Y., Tadano, K., Hashimoto, T., Morimoto, S., 1997. Involvement of nitric oxide in endothelin ET_B receptor-mediated inhibitory actions on antidiuresis and norepinephrine overflow induced by stimulation of renal nerves in anesthetized dogs. J. Cardiovasc. Pharmacol. 30, 325–331.

Mutafova-Yambolieva, V.N., Westfall, D.P., 1998. Inhibitory and facilitatory presynaptic effects of endothelin on sympathetic cotransmission in the rat isolated tail artery. Br. J. Pharmacol. 123, 136–142.

Rubanyi, G.M., Polokoff, M.A., 1994. Endothelins: molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. Pharmacol. Rev. 46, 325–415.

Tabuchi, Y., Nakamaru, M., Rakugi, H., Nagano, M., Mikami, H., Ogihara, T., 1989. Endothelin inhibits presynaptic adrenergic neurotransmission in rat mesenteric artery. Biochem. Biophys. Res. Commun. 161, 803–808.