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NOVEL ANTAGONIST OF ENDOTHELIN ET_{B1} AND ET_{B2} RECEPTORS, BQ-788: EFFECTS ON BLOOD VESSEL AND SMALL INTESTINE

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Summary: The effects of a peptide, BQ-788 [*N-cis*-2,6-dimethyl-piperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine], on isolated blood vessel and small intestine were examined. In the rat aorta, BQ-788 antagonized the endothelium-dependent, ET_{B1} receptor-mediated relaxation due to endothelin (ET)-3 with EC_{50} of 3 μM. In the rat aorta without endothelium, 10 μM BQ-788 weakly antagonized the ET_{A1}-mediated contractile effects of ET-1 and ET-3. In the rabbit saphenous vein, it has been shown that ET_{A1}, ET_{A2}, ET_{B1} and ET_{B2} receptors mediate contraction. BQ-788 (10 μM) almost completely inhibited the contractile effect of sarafotoxin S6c (an ET_{B1} and ET_{B2} agonist). BQ-788 also antagonized the contractile effect of ET-3 (an ET_{A1}, ET_{B1} and ET_{B2} agonist) more strongly than desensitization of ET_{B1} and ET_{B2} receptors. However, BQ-788 did not antagonize the effect of ET-1 (agonist of all four receptors). In the guinea pig ileum, 10 μM BQ-788 completely inhibited the relaxation mediated by ET_{B1} and ET_{B2} receptors. These results suggest that BQ-788 is a novel antagonist of ET_{B1} and ET_{B2} receptors with weak antagonistic effect on the ET_{A1} receptor.

The endothelin (ET)(1-3) has dual effects on blood vessels by activating two types of receptor; the ET_A receptor is relatively selectively activated by ET-1 and mediates contraction in the arteries (1,4) whereas the ET_B receptor is activated nonselectively by ET-1 and ET-3 (5) and mediates release of relaxing substances from vascular endothelium, such as nitric oxide and prostacyclin (6-8). BQ-123 (9) and FR139317 (10) are the selective ET_A receptor antagonists whereas IRL 1038 (11-13) and RES-701-1 (14,15) are the selective ET_B receptor antagonists. Using these agonists and antagonists, it has been shown that the ET-induced contractions in rat aorta are mediated by the ET_A receptor (11) whereas the contractions in the vein are mediated by both ET_A and ET_B receptors (16,17). It has also been reported that although the ET_B receptor is desensitized by strong activation, IRL 1038 and RES-701-1 show only partial antagonism, suggesting that the ET_B receptor is classified into the ET_{B1} and ET_{B2} subtypes with their sensitivity to these antagonists (16-18). It has been reported that ET-induced relaxation of the guinea-pig ileum induced is mediated also by ET_{B1} and ET_{B2} receptors (19). Similarly, the ET_A receptor is classified into the ET_{A1} and ET_{A2} subtypes that are respectively sensitive and insensitive to BQ-123 and FR139317 (17). However, there was no antagonist against the ET_{A2} or ET_{B2} receptor. BQ-788 has been shown to bind relatively selectively to the ET_B receptor and inhibits the ET_B-mediated depressor effect without changing the ET_A-mediated pressor effect

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(20). Here we report that BQ-788 antagonizes not only the ET_{B1} receptor but also the ET_{B2} receptor in blood vessels and small intestine.

Materials and Methods

Male Wistar rats (250-300 g) were killed by a sharp blow to the neck and exsanguination. The thoracic aorta was isolated and cut into spiral strips (1-2 mm in width and 5-7 mm in length). Male, New Zealand white rabbits (1.5-2.0 kg) were killed under anesthesia and the saphenous vein was removed and cut into rings of 2-3 mm width. In some experiments, endothelium was removed by gently rubbing the intimal surface with a glass rod moistened with normal physiological salt solution. Male, white guinea pigs (300-400 g) were killed by a sharp blow to the neck and exsanguination. The ileum was removed and the longitudinal muscle layer was isolated. Normal physiological salt solution contained (mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.5, $MgCl_2$ 1.0, $NaHCO_3$ 23.8, ethylenediamine tetraacetic acid (EDTA) 0.01 and glucose 5.5. High K^+ solution was made by substituting NaCl with equimolar KCl. These solutions were saturated with a 95 % O_2 and 5 % CO_2 mixture at 37 °C and pH 7.4.

The force of contraction was recorded isometrically. Muscle preparations were attached to a holder under a resting force of 10 mN and equilibrated for 60-90 min. During this period, high K^+ was repeatedly applied until the peak force was reproducible. The functional integrity of the endothelium was assessed by checking whether 1 μM carbachol (CCh) almost completely (>90%) relaxed the contraction induced by 100 nM norepinephrine (NE)(21). To examine the contractile effects in the aorta and vein, stimulants were added cumulatively. Concentration-response relationship was examined only once in one muscle strip because it was difficult to completely reverse the effect of high concentrations of ET by washing. In some experiments, the ET_{B1} and ET_{B2} receptors were desensitized by the treatment with 300 nM sarafotoxin S6c (STXc) for 30 min followed by wash with normal solution for 30 min (17). The relaxant effects of ET were examined by single application because the effects of ET were transient and cumulative addition did not induce graded responses. ET was added only once in one muscle strip because the second application of ET was ineffective due to desensitization of the ET_B receptor (14,17). Antagonist was added 30 min before the addition of ET.

ET-1, ET-3 and STXc were purchased from the Peptide Institute (Osaka, Japan). BQ-788 was a gift from Dr. T. Okada, Ciba-Geigy Japan. Other chemicals used were NE and CCh (Wako Pure Chemicals, Tokyo, Japan). The logarithmic concentrations of agonists inducing the half maximum response (EC_{50}) were calculated from the concentration-response curves in the absence and presence of antagonist. Results of the experiments are expressed as mean ± S.E.M. Unpaired Student's *t* test was used for the statistical analysis of the results.

Results and Discussion

As shown in Fig. 1, ET-3 induced transient relaxation in the rat aorta with endothelium precontracted with NE. Sequential addition of CCh almost completely inhibited the contraction. BQ-788 (10 μM) changed neither the resting muscle tone nor the contraction induced by NE. However, relaxation induced by ET-3 was inhibited by BQ-788 with the EC_{50} of 3 μM. In contrast, BQ-788 did not inhibit the relaxation induced by CCh. Previously, we have shown that the ET_{B1} antagonists, IRL 1038 and RES-701-1 antagonized the ET-3-induced relaxation with EC_{50} of 0.65 μM and 5 μM, respectively (11,14). These results suggest that BQ-788 antagonizes the ET_{B1} receptor with similar potency to IRL 1038 or RES-701-1.

As shown in Fig. 2 and table 1, cumulative addition of ET-1 or ET-3 induced graded contractions in the rat aorta without endothelium by stimulating the ET_{A1} receptor (17). BQ-788 (3 μM) shifted the concentration-response curves to the right, increasing the EC_{50} by 2 to 3-fold. Since it has been shown that the ET_{A1} antagonists, 3 μM BQ-123 and 3 μM FR139317

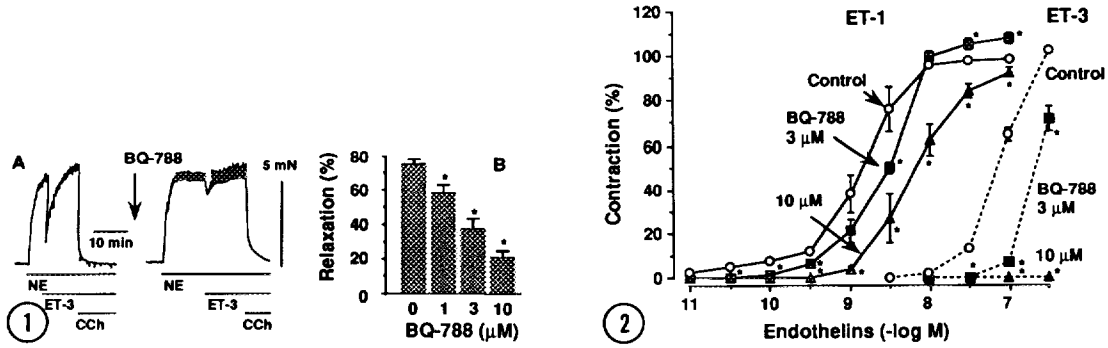

Figure 1. A: Effect of 10 μM BQ-788 on the relaxation induced by 100 nM ET-3 and 1 μM CCh in the rat aorta stimulated by 100 nM NE. B: Concentration-response relationship for BQ-788. The mean and the S.E.M. are shown. *: P<0.05 vs. 0 μM BQ-788. n=6 each.

Figure 2. Effects of ET-1 (left three curves) and ET-3 (right three curves) in the rat aorta. ○: control. ■: 3 μM BQ-788. ▲: 10 μM BQ-788. The mean and the S.E.M. are shown. *: P<0.05 vs. control. n=6 each.

increased the EC_{50} for ET-1 in the swine pulmonary artery by approximately 30-fold (16), BQ-788 seems to be a weaker antagonist against the ET_{A1} receptor than BQ-123 or FR139317.

In the rat aorta with endothelium, ET-1 and ET-3 induced contractions at higher concentrations than in the absence of endothelium (table 1), as reported previously (7). This is because ET activates the smooth muscle ET_{A1} receptor to induce contraction and also the endothelial ET_{B1} receptor to release inhibitory substances. It has been shown that the ET_{B1}-antagonists, IRL 1038 (11) and RES-701-1 (14), augmented the contractile effects of ET. In contrast, BQ-788 antagonized the effects of ET increasing the EC_{50} values (table 1). This result suggests that BQ-788 inhibits the ET_{A1} and ET_{B1} receptors simultaneously.

In the rabbit saphenous vein, cumulative addition of ET-1 (Fig. 3) and ET-3 (Fig. 4) induced graded contraction whereas a selective ET_B agonist, STXc, induced a bell-shaped concentration-response curve (Fig. 5). It was also found that sensitivity of the vein to these stimulants was slightly different from that reported previously (17); the EC_{50} for ET-1 was higher whereas the threshold concentration for STXc was lower in the present experiment. This difference may be due to individual and/or seasonal differences. That the effects of ET-1 (an ET_A/ET_B agonist) and STXc (an ET_B agonist) changed in opposite direction supports the suggestion that the contraction in the vein is mediated by two types of receptors (16,17).

As shown in Fig. 3A and table 1, 10 μM BQ-788 inhibited the contractile effect of only lower concentrations of ET-1 without changing the EC_{50}. As shown in Fig. 3B, desensitization of the ET_B receptor (see Methods) showed similar effect as 10 μM BQ-788. In the ET_B-desensitized vein, 10 μM BQ-788 was ineffective (Fig. 3B). These results suggest that the contractile effect of lower concentrations of ET-1 is mediated by the ET_B receptor. It has been shown that the major portion of the ET-1-induced contraction is mediated by the ET_A receptor that is not sensitive to the conventional ET_A antagonists such as BQ-123 and FR139317 (ET_{A2} subtype)(17). BQ-788 does not seem to antagonize the ET_{A2} subtype.

Table 1. The EC_{50} values for the contractile effects of ET-1, ET-3 and STXc

Conditions	EC_{50} (-log M ± S.E.)	EC_{50} nM	n
Rat aorta (without endothelium)			
ET-1 (control)	8.78 ± 0.14	1.66	8
+ BQ-788 3 μM	8.47 ± 0.03*	3.39	4
+ BQ-788 10 μM	8.19 ± 0.14*	6.46	4
ET-3 (control)	7.14 ± 0.03	73.1	8
+ BQ-788 3 μM	6.66 ± 0.03	221	4
+ BQ-788 10 μM	< 6.5*	>300	4
Rat aorta (with endothelium)			
ET-1 (control)	8.44 ± 0.06	3.63	8
+ BQ-788 3 μM	8.20 ± 0.05*	6.31	4
+ BQ-788 10 μM	7.89 ± 0.05*	12.9	4
ET-3 (control)	6.80 ± 0.01	159	8
+ BQ-788 3 μM	< 6.5*	>300	4
Rabbit saphenous vein			
ET-1 (control)	9.16 ± 0.25	0.692	8
+ BQ-788 10 μM	9.07 ± 0.39	0.851	4
+ STXc[a]	9.10 ± 0.14	0.794	4
+ STXc[a] + BQ-788 10 μM	9.18 ± 0.04	0.661	4
ET-3 (control)	9.13 ± 0.14	0.741	8
+ BQ-788 10 μM	7.74 ± 0.11*	18.2	4
+ STXc[a]	8.21 ± 0.23*	6.17	4
+ STXc[a] + BQ-788 3 μM	7.19 ± 0.09*	64.6	4
+ STXc[a] + BQ-788 10 μM	< 7.0*	>100	4
STXc (control)	10.37 ± 0.35	0.0426	12
+ BQ-788 3 μM	8.34 ± 0.12*	4.57	4
+ BQ-788 10 μM	<7.0*	>100	4

[a] The ET_B receptor was desensitized by the pretreatment with 300 nM STXc (see Methods). *: $P<0.05$ vs. control.

As shown in Fig. 4 and table 1, concentration-response curve for ET-3 was shifted to the right by 10 μM BQ-788, increasing the EC_{50} by 25-fold (Table 1). Desensitization of the ET_B receptor showed weaker effect than BQ-788, increasing the EC_{50} by only 8-fold. In the ET_B-desensitized muscle, 3 and 10 μM BQ-788 showed additional antagonizing effect, increasing the EC_{50} by 87- and >135-fold, respectively. These results support the suggestion that the venous contraction induced by ET-3 is mediated by the ET_{A1}, ET_{B1} and ET_{B2} receptors (17) and that BQ-788 antagonizes not only the ET_B receptor but also the ET_{A1} receptor.

It has been suggested that effect of STXc is mediated by both ET_{B1} and ET_{B2} receptors (17). As shown in Fig. 5 and table 1, BQ-788 (3 and 10 μM) strongly antagonized the effect of STXc increasing the EC_{50} by 107-fold and >2,300-fold, respectively. This result again suggests that BQ-788 antagonizes both of these receptors.

In the ileum stimulated by CCh, 100 nM ET-3 induced biphasic relaxation with a transient phase followed by relatively sustained phase (Fig. 6). STXc (100 nM) showed similar effect (data not shown). It has been shown that the transient relaxation is mediated by the ET_{B1} receptor whereas sustained relaxation is mediated by the ET_{B2} receptor (14,19). BQ-788 (10

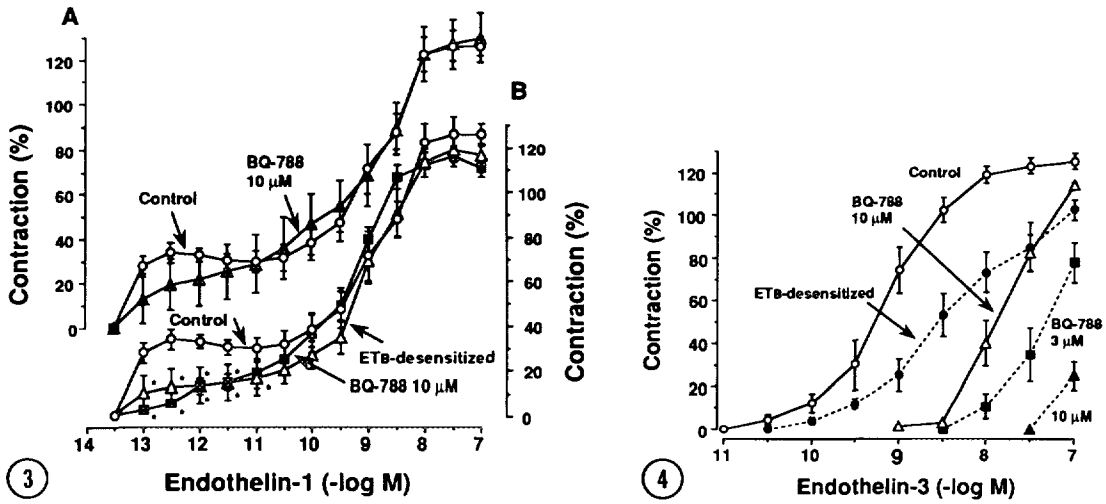

Figure 3. Effects of ET-1 in the rabbit saphenous vein. A: Effect of BQ-788 (left ordinate). ○: control. ▲: 10 μM BQ-788. B: Effect of ET_B receptor-desensitization (right ordinate). ○: control. △: ET_B-desensitization (see Methods). ■: 10 μM BQ-788 and ET_B-desensitization. For further details see Fig. 2. n=4-8.

Fig. 4. Effects of ET-3 in the rabbit saphenous vein. ○: control. △: 10 μM BQ-788. ●: ET_B-desensitization (see Methods). ■: and ▲: 3 and 10 μM BQ-788, respectively, in the ET_B-desensitized preparation. For further details see Fig. 2. n=4-8.

μM) completely inhibited both phases of the relaxation induced by ET-3 (Fig. 6) and STXc (data not shown), again suggesting that BQ-788 antagonizes both of these receptors.

Previously, it has been reported that BQ-788 more strongly inhibited the binding of ET-1 to the ET_B receptor in pCASM cells and human neuroblastoma cell line SK-N-MC cells (EC_{50} = 0.9-1.2 nM) than to the ET_A receptor in porcine cerebellar membranes and hGH cells (EC_{50} = 280-1,300 nM)(20). In the present experiments, we found that BQ-788 weakly antagonized the ET_{A1}-mediated contraction in the rat aorta and rabbit pulmonary vein at the concentrations

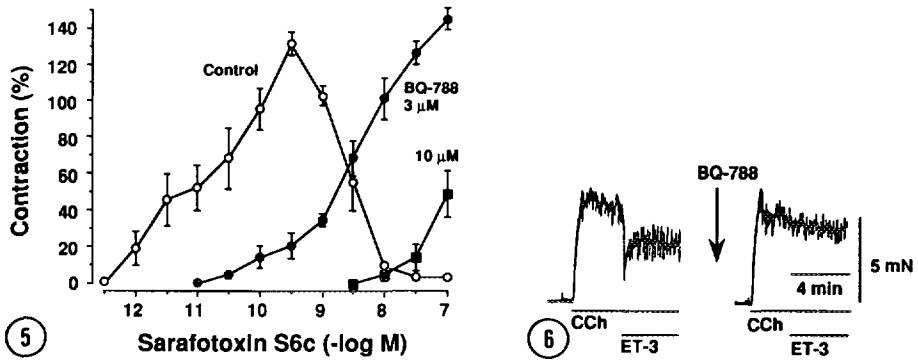

Fig. 5. Effects of STXc in the rabbit saphenous vein. ○: control. ●: 3 μM BQ-788. ■: 10 μM BQ-788. For further details see Fig. 2. n=4-12.

Fig. 6. The inhibitory effect of BQ-788 on the relaxation induced by 100 nM ET-3 in the guinea pig ileum stimulated by 100 nM CCh.

similar to those needed to antagonize the ET_B-mediated effects in the vein, ileum and vascular endothelium. This difference may be due to the tissue difference in the ET receptor subtypes and/or BQ-788 is more easily accessible to the ET_A receptor in the artery and vein than in porcine cerebellar membranes and hGH cells.

From these results, we suggest that BQ-788 is an antagonist against the ET_{B1} and ET_{B2} receptors with weak antagonistic effect on the ET_{A1} receptor and no effect on the ET_{A2} receptor.

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