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# Pharmacology of two novel mixed $ET_A/ET_B$ receptor antagonists, BQ-928 and 238, in the carotid and pulmonary arteries and the perfused kidney of the rabbit

Marie-Claude Maurice, Jean-Philippe Gratton & [1]Pedro D'Orléans-Juste

Department of Pharmacology, Medical School, Université de Sherbrooke, Sherbrooke (Québec) J1H 5N4, Canada

**1** In the present study, we have pharmacologically characterized two novel mixed endothelin $ET_A/ET_B$ receptor antagonists, namely BQ-928 and BQ-238, in $ET_A$ and $ET_B$ preparations, the rabbit carotid artery (RbCA) and the rabbit pulmonary artery (RbPA), respectively. These two antagonists were compared to established $ET_A$ (BQ-123 and BMS 182874), $ET_B$ (BQ-788) and mixed $ET_A/ET_B$ (SB 209670) receptor antagonists.

**2** In the RbCA, the $ET_A$ monoreceptor preparation, BQ-238 and BQ-928 had apparent affinities ($pA_2$) of $7.42 \pm 0.22$ and $7.22 \pm 0.18$, respectively, BQ-788 being inactive in this preparation. In the $ET_B$ monoreceptor preparation, the RbPA (when IRL-1620 was used as an $ET_B$ receptor agonist), the $pA_2$ for BQ-238 was $7.05 \pm 0.14$ and for BQ-928 was $8.43 \pm 0.04$. BQ-123 and BMS 182874 were inactive in this preparation. Similar to SB 209670, BQ-238 but not BQ-928 had a higher affinity for the $ET_A$ than the $ET_B$ receptor.

**3** All of the antagonists were tested for their ability to block and reverse endothelin-1-induced vasoconstrictions in the rabbit perfused kidney. In this preparation endothelin-1-induced increases in vascular resistance have been shown to be mediated solely by $ET_A$ receptors. All compounds (except BQ-788) blocked the pressor effects of endothelin within the kidney; the calculated $IC_{50}$ values for BQ-123, BMS 182874, SB 209670, BQ-928 and BQ-238 were 0.4 $\mu$M, 2 $\mu$M, 0.01 $\mu$M, 0.4 $\mu$M and 0.09 $\mu$M, respectively.

**4** In all experiments in the rabbit perfused kidney, endothelin-1 was readministered for a third time, 60 min following cessation of infusion of the above-mentioned antagonists. The response to the third infusion of endothelin-1 following cessation of infusion of BQ-123, BMS 182874 and SB 209670 was not significantly different from that to the third infusion of endothelin in control conditions. However, the response to endothelin-1 was significantly higher than control in tissues pre-infused with BQ-788 or BQ-928 ($56 \pm 9$ and $41.6 \pm 15\%$, respectively, $n = 8$ each, $P < 0.05$).

**5** Our results suggest that in a system where $ET_A$ receptor activation is responsible for vasoconstriction and $ET_B$-receptor activation for vasodilatation, $ET_A$ receptor selective antagonists or mixed $ET_A/ET_B$ receptor antagonists which possess high affinity for $ET_A$ receptors do not induce hyperresponsiveness to endothelin-1. In contrast, $ET_B$ selective antagonists or mixed antagonists possessing a high affinity for $ET_B$ receptors (such as BQ-928) interfere with the $ET_B$-receptor-dependent physiological antagonism of endothelin-1-induced pressor responses in these same tissues.

**Keywords:** $ET_A$ receptors; $ET_B$ receptors; isolated blood vessels; rabbit kidney; BQ-238; BQ-928

## Introduction

A recent study by Allcock *et al*. (1995) showed that in contrast to a selective $ET_A$ receptor antagonist, the mixed $ET_A/ET_B$ receptor antagonist PD-145065 (Doherty *et al*., 1993) failed to prevent the reductions in blood flow in the kidney, splanchnic area, lung and skin induced by administration of endothelin-1 (ET-1) to the anaesthetized rat. From these results, the authors suggested that the predominant role of the $ET_B$ receptors within blood vessels of the rat is to limit the pressor effects of endothelin-1. It had been previously suggested that the systemic pressor effect of endothelin-1 in the rat is partly mediated by $ET_B$ receptors located on the smooth muscle of resistance vessels (McMurdo *et al*., 1993). The observations of Allcock *et al*. (1995) confirm our results obtained in a selective $ET_A$-receptor dependent constrictive organ, the rabbit perfused kidney, in which the $ET_B$ receptor antagonist, BQ-788, sharply potentiates the vasoconstrictive effect of endothelin-1 (D'Orléans-Juste *et al*., 1994).

In a more recent study, it was further shown that following interruption of the application of a mixture of $ET_A$ and $ET_B$ antagonists (BQ-123 plus BQ-788) (D'Orléans-Juste *et al*., 1995), a renal hyperresponsiveness to endothelin-1 (i.e. enhanced vasoconstriction) was found in the rabbit perfused kidney. In that same study, we proposed that a reduction of the minimal effective plasma concentration of a mixture of $ET_A/ET_B$ antagonists may lead to an exaggerated constriction to exogenous endothelin due to the non-reversible impairment of the $ET_B$-dependent release of endothelium-derived relaxing factor (EDRF).

We were therefore interested, in the present study, to examine whether mixed $ET_A/ET_B$ receptor antagonists would induce the same type of hyperresponsiveness as found in our previous experiments (D'Orléans-Juste *et al*., 1995). For that purpose, we have used a highly potent mixed $ET_A/ET_B$ receptor antagonist, SB 209670 (Ohlstein *et al*., 1994), together with BQ-123 and another selective $ET_A$ receptor antagonist, BMS 182874 (Stein *et al*., 1994). In addition, a recent study by Fukami *et al*. (1995, 1996) disclosed two new mixed $ET_A/ET_B$ receptor antagonists, namely BQ-928 and BQ-238. These antagonists have, as interesting characteristics, inverse affinities for the two receptor populations. Namely, BQ-928 has a higher affinity for $ET_B$ than $ET_A$ receptors, whereas BQ-238 possesses a higher affinity for $ET_A$ than $ET_B$ receptors, similar to SB 209670.

Therefore, we initally characterized pharmacologically on $ET_A$ and $ET_B$ receptor containing vascular preparations, the pharmacodynamic characteristics of all these antagonists. Secondly, we compared their efficiencies as blockers of en-

[1] Author for correspondence.

dothelin-1-induced vasoconstrictions in the rabbit kidney, where vasoconstrictions are mediated soley by $ET_A$ receptors (Télémaque *et al*., 1993). Thirdly, we evaluated the capacities of all of these antagonists to induce a significant hyperresponsiveness of the kidney to endothelin-1 following interruption of their application.

## Methods

All experiments were performed on tissues or organs removed from New-Zealand White rabbits (1.7–2.5 kg) (Réjean Brisebois, Fleurimont, Canada) of either sex, anaesthetized with ketamine/xylazine (40/10 mg $kg^{-1}$, i.m.) and then killed by spinal transection.

### *Rabbit carotid and pulmonary artery experiments*

The vessels were removed and mechanically denuded of their endothelium. The rabbit carotid arteries were cut into 4–6 mm wide rings, while rabbit pulmonary arteries were cut into spiral strips about 15–20 mm long and 3 mm wide. The tissues were immediately suspended in 10 ml baths containing warmed (37°C) and oxygenated (95% $O_2$, 5% $CO_2$) Krebs solution with the addition of indomethacin (1 $\mu$M). The tissues were stretched for an equilibration period of 60 min with initial resting tensions of 2 g and 1 g for the carotid and pulmonary arteries, respectively, before initiation of experimental procedures. The incubation medium was changed every 15 min. During the 60 min equilibration period, the tension of tissues was constantly reapplied in all blood vessels. Tissues were linked to transducers (Grass Model FT03C) which were connected to a physiograph (Grass Model 7) to measure isometric contractile force. Before each experiment, the absence of endothelium was confirmed by the inability of the tissue, precontracted with angiotensin II (10 nM), to relax in response to acetylcholine (1 $\mu$M). Concentration-response curves to agonists were constructed 15 min after application of the antagonist. The antagonist effects were evaluated in terms of displacement to the right of the agonist-induced concentration-response curves and $pA_2$ values were obtained by Schild plot analysis (Arunlakshana & Schild, 1959). Control tissues, treated with vehicle, were always run in parallel. $ED_{50}$ values for the agonists were calculated from the linear regression performed on the dose-response curve between 20% and 80% of the maximal effect for each agonist. $EC_{50}$ values were then transformed into $pD_2$ values (negative logarithm expression of the $EC_{50}$).

### *Rabbit perfused kidney experiments*

In another series of experiments, a polyethylene catheter (Portex, tube size 6FG) was placed in the right renal artery from the freshly killed animals (as previously described) and the kidney was excised (within 1 min after death) while being continuously perfused (5 ml $min^{-1}$) with heparinized (100 u $ml^{-1}$) Krebs solution. The kidney was then rapidly placed in a heated chamber (37°C) and perfused (5 ml $min^{-1}$) with oxygenated (95% $O_2$, 5% $CO_2$) protein-free Krebs solution. After an equilibration period of 60 min, endothelin-1 (10 nM) was infused intraarterially (i.a.) for 5 min, twice at 60 min time intervals and the perfusion pressure was measured with a pressure transducer (Statham P23AC) and recorded on a Grass physiograph (Grass model 7D). The response to a median concentration of endothelin-1 (10 nM; D'Orléans-Juste *et al*., 1995) was monitored in the absence or presence of increasing amounts of all antagonists (1 nM to 10 $\mu$M). Each kidney was treated with one antagonist at a single concentration. Intrapolated $IC_{50}$ values were subsequently calculated from linear regression analysis of the inhibition curves for each antagonist (D'Orléans-Juste *et al*., 1993; Claing *et al*., 1994).

In further experiments, antagonists at concentrations which reduced the response to endothelin (10 nM) by at least 60% were infused from 15 min before the second administration of the agonist. Administration of antagonists was interrupted 5 min after the maximal response to endothelin-1 had been attained. Sixty minutes after treatment, endothelin-1 (10 nM) was infused a third time to measure reversibility.

To monitor oedema formation, the kidneys were weighed before being placed in the heated chamber and at the end of each experiment, which lasted for a maximum time period of 180 min. The mean weight of the kidneys was $8.4 \pm 0.2$ g before and $11.1 \pm 0.3$ g at the end of the experiments ($n = 51$) ($P < 0.01$).

### *Drugs and solutions*

Synthetic endothelin-1 was purchased from American Peptide (Sunnyvale, CA, U.S.A.). BQ-123 (cyclo[D-Trp-D-Asp-Pro-D-Val-Lev]) (Ihara *et al*., 1992), BQ-788 (N-cis-2,6-dimethylpiperidinocarbonyl-L-Y-Me-Leu-D-Trp (COO Me)-DNle. Sodium salt) (Ishikawa *et al*., 1994), BQ-928 (N-cis-2, 6-dimethylpiperidinocarbonyl-L-2-cyclopropylglycyl-D-2-bromotryptophanyl-D-norleucine) and BQ-238 (cyclo(-D-2-bromotryptophanyl-D-aspartyl-L-prolyl-D-2-cyclopentylglycyl-L-2-cyclopropylglycyl) were synthesized at Banyu Pharmaceutical (Tsukuba, Japan). IRL-1620 (Suc-[$Glu^9$, $Ala^{11,15}$]-endothelin-1 (8–21)) was purchased from Bachem California (Torrance, CA, U.S.A.). Angiotensin II was synthesized in the laboratory of Dr D. Regoli (Sherbrooke, Canada). Heparin, trizma base and phosphate-buffered saline (pH 7.4) were purchased from Sigma (St-Louis, MO, U.S.A.). BMS 182874 (5-(dimethylamino)-N-(3,4-dimethyl-5-isoxazolyl)-1-naphtalene sulfonamide) and SB 209670 ((+)-(1S, 2R, 3S)-3-(2-carboxymethoxy-4-methoxyphenyl)-1-(3,4-methylenedioxyphenyl)-5-(prop-l-yloxy) indane-2-carboxylic acid) were generously supplied by Dr S. Moreland (Bristol-Mayers Squibb, Princeton, NJ, U.S.A.) and Dr E.H. Ohlstein (SmithKline Beecham, King of Prussia, PA, U.S.A.), respectively. All agents were dissolved either in phosphate-buffered saline (pH, 7.4) or distilled water, except for indomethacin which was dissolved in trizma base (0.2 M; pH 7.4).

### *Statistics*

Data used in the text and figures are expressed as mean ± s.e.mean of $n$ observations. Statistical comparisons between groups were performed by paired or unpaired Student's $t$ test. ANOVA followed by Dunnett multiple comparisons test was used for comparison of the response to one agonist varying with time. Values of $P < 0.05$ were considered statistically significant.

### *Ethics*

The care of animals and all research protocols conformed to the guiding principles for animal experimentation as enunciated by the Canadian Council on Animal Care and approved by the Ethical Committee on Animal Research of the Université de Sherbrooke Medical School.

## Results

### *Effect of selective and non-selective endothelin receptor antagonists in the rabbit carotid and pulmonary arteries*

Endothelin-1 and IRL-1620 induced concentration-dependent contractions of the rabbit carotid artery (RbCA) and pulmonary artery (RbPA), respectively, with apparent affinities ($pD_2$) of $9.36 \pm 0.14$ (maximal tension of $2.2 \pm 0.12$ g) and $8.82 \pm 0.10$ (maximal tension of $1.15 \pm 0.10$ g), respectively ($n = 21$ for each preparation).

On the rabbit carotid artery, BQ-123, BQ-928, BQ-238, BMS 182874 and SB 209670 induced concentration-dependent rightward shifts of the concentration-response curves to endothelin-1, with Schild plot-extrapolated apparent affinities

($pA_2$) of 6.41±0.24 (Calo *et al.*, 1996), 7.22±0.18, 7.42±0.22, 6.20±0.21 and 9.22±0.21, respectively (Table 1). In contrast, BQ-788 did not effect endothelin-1 induced contractions of the rabbit carotid artery at concentrations up to 1 μM (Calo *et al.*, 1996).

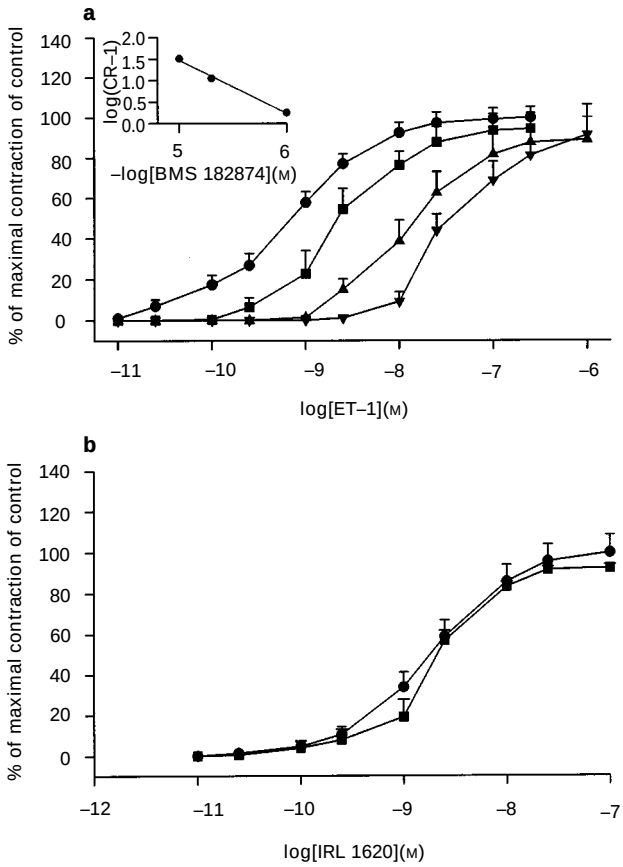


**Figure 1** (a) Effect of the $ET_A$ receptor antagonist BMS 182874 on constrictions of the rabbit carotoid artery induced by endothelin-1 (ET-1). The tissues were incubated for 15 min with either vehicle (●) or BMS 182874 1 μM (■), 5 μM (▲) and 10 μM (▼) before exposure to endothelin-1. Each point represents the mean of at least 5 experiments; vertical lines show s.e.mean. Inset: Schild plot of BMS 182874 against endothelin-1 induced contractions. The slope of regression did not differ significantly from unity (slope 1.22±0.22, correlation 0.99). The $pA_2$ estimate was 6.20±0.21. (b) Lack of effect of BMS 182874 on constrictions of the rabbit pulmonary artery induced by IRL-1620. The tissues were incubated for 15 min with either vehicle (●) or BMS 182874 10 μM (■) before exposure to IRL-1620. Each point represents the mean of at least 5 experiments; vertical lines show s.e.mean.

In another series of experiments, BQ-788, BQ-928, BQ-238 and SB 209670 induced rightward shifts of the concentration-response curve to IRL-1620 in the rabbit pulmonary artery, with Schild plot extrapolated apparent affinities ($pA_2$) of 9.01±0.16 (Calo *et al.*, 1996), 8.43±0.04, 7.05±0.14 and 8.09±0.37, respectively (Table 1). BQ-123 and BMS 182874 were without effect. In either preparation, all of the active antagonists competitively antagonized responses to the agonist with a Schild plot slope value not significantly different from unity (Figures 1 to 4).

In a last series of control experiments, all these agonists were found to be inactive against contractions induced by angiotensin II (results not shown).

### *Effect of the selective or mixed $ET_A/ET_B$ receptor antagonists on endothelin-1-induced constrictions of the rabbit perfused kidney*

Before the various pharmacological agents were tested, the basal pefusion pressure of the kidney was 40.2±1.9 mmHg ($n=51$).

In an initial series of control experiments the vasocontrictor responses of the kidneys to three consecutive infusions of endothelin-1 (10 nM) were established to be 86±6, 100±10 and 117±5 mmHg ($n=8$) (Figure 5a). The second and third administration of the peptide exhibited a 17.7±4.2% (non-significant) and 39.1±7.8% ($P<0.05$), increased response respectively, when compared to the effect of the first infusion. In contrast, angiotensin II (1 μM, 5 min infusion) induced similar increases in renal perfusion pressure following three consecutive administrations (27.2±3.0; 23.0±3.3 and 27.5±5.2 mmHg, respectively, $n=6$).

All the antagonists, except BQ-788, reduced in a concentration-dependent manner the vasoconstrictor response of the rabbit perfused kidney to the second endothelin-1 infusion (10 nM) (Table 2). The response to a second challenge with endothelin-1 was monitored in each kidney treated with a single concentration of one of the six antagonists.

The intrapolated $IC_{50}$ values for antagonists tested against the renal response to endothelin-1 are shown in Table 2. The $IC_{50}$ value for BQ-123 against the response of the kidney to endothelin-1 has previously been established at 0.4 μM (Télémaque *et al.*, 1993). The rank order of potency of the antagonists was: SB 209670 > BQ-238 > BQ-928 = BQ-123 > BMS 182874 >>> BQ-788.

### *Reversibility of the antagonistic effects of selective or mixed $ET_A/ET_B$ receptor antagonists in the rabbit perfused kidney*

The response of the rabbit perfused kidney to a third infusion of endothelin-1 was not significantly different in BQ-123, BQ-238 and SB 209670 treated organs compared to untreated

**Table 1** Apparent affinities ($pD_2$ or $pA_2$ values) of endothelin receptor agonists and antagonists in the rabbit carotid and pulmonary arteries

| | *Rabbit carotid artery* | | *Rabbit pulmonary artery* | |
|---|---|---|---|---|
| | *$pD_2$* | *Max response* (g) | *$pD_2$* | *Max response* (g) |
| *Agonist* | | | | |
| ET-1 | 9.36±0.14 | 2.22±0.12 | – | – |
| IRL 1620 | – | – | 8.82±0.10 | 1.15±0.10 |
| *Antagonist* | *$pA_2$* | | *$pA_2$* | |
| BQ-238 | 7.42±0.22 | | 7.05±0.14 | |
| BQ-928 | 7.22±0.18 | | 8.43±0.04 | |
| BQ-788 | Inactive* | | 9.01±0.16* | |
| BQ-123 | 6.41±0.24* | | Inactive* | |
| BMS 182874 | 6.20±0.21 | | Inactive | |
| SB 209670 | 9.22±0.21 | | 8.09±0.37 | |

Each value represents the mean±s.e.mean of at least 6 experiments. $pD_2$: the negative logarithm of the molar concentration of agonist producing 50% of the maximal response. $pA_2$: the negative logarithm of the molar concentration of antagonist that is able to reduce the effect of a double concentration of agonist to that of a single concentration. *Taken from Calo *et al.* (1996).

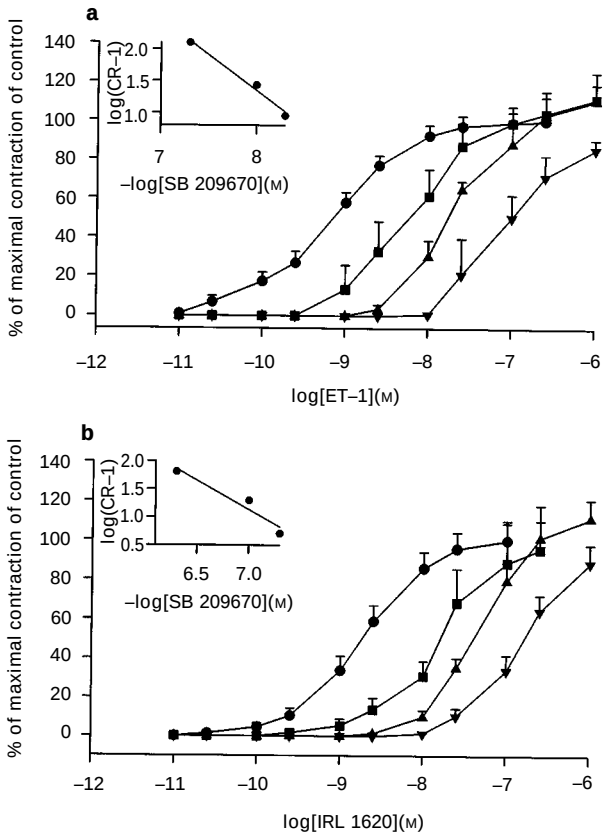


**Figure 2** (a) Effect of the mixed $ET_A/ET_B$ receptor antagonist SB 209670 on constrictions of the rabbit carotid artery induced by endothelin-1 (ET-1). The tissues were incubated for 15 min with either vehicle (●) or SB 209670 5 nM (■), 10 nM (▲) and 50 nM (▼) before exposure to endothelin-1. Each point represents the mean of at least 5 experiments; vertical lines show s.e. mean. Inset: Schild plot of SB 209670 against endothelin-1 induced contractions. The slope of regression did not differ significantly from unity (slope $1.11 \pm 0.15$, correlation 0.98). The $pA_2$ estimate was $9.22 \pm 0.21$. (b) Effect of SB 209670 on constrictions of the rabbit pulmonary artery induced by IRL-1620. The tissues were incubated for 15 min with either vehicle (●) or SB 209670 50 nM (■), 100 nM (▲) and 500 nM (▼) before exposure to IRL-1620. Each point represents the mean of at least 5 experiments; vertical lines show s.e. mean. Inset: Schild plot of SB 209670 against IRL-1620-induced contractions. The slope of regression did not differ significantly from unity (slope $1.04 \pm 0.29$, correlation 0.93). The $pA_2$ estimate was $8.09 \pm 0.37$.

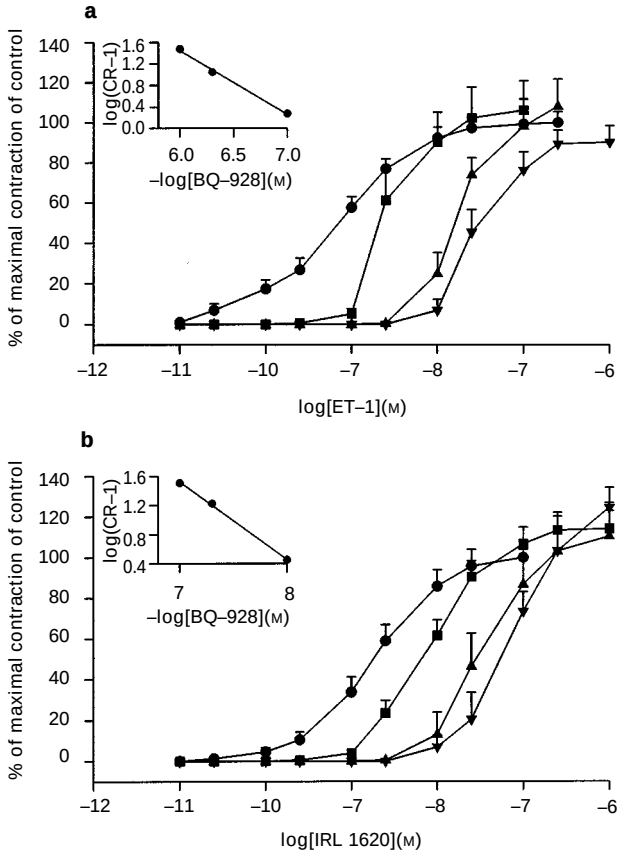


**Figure 3** (a) Effect of the mixed $ET_A/ET_B$ receptor antagonist BQ-928 on constrictions of the rabbit carotid artery induced by endothelin-1 (ET-1). The tissues wre incubated for 15 min with either vehicle (●) or BQ-928 0.1 $\mu$M (■), 0.5 $\mu$M (▲) and 1 $\mu$M (▼) before exposure to endothelin-1. Each point represents the mean of at least 5 experiments; vertical lines show s.e.mean. Inset: Schild plot of BQ-928 against endothelin-1 induced contractions. The slope of regression did not differ significantly from unity (slope $1.19 \pm 0.19$, correlation 0.99). The $pA_2$ estimate was $7.22 \pm 0.18$. (b) Effect of BQ-928 on constrictions of the rabbit pulmonary artery induced by IRL-1620. The tissues were incubated for 15 min with either vehicle (●) or BQ-928 10 nM (■), 50 nM (▲) and 100 nM (▼) before exposure to IRL-1620. Each point represents the mean of at least 5 experiments; vertical lines show s.e.mean. Inset: Schild plot of BQ-928 against IRL-1620-induced contractions. The slope of regression did not differ significantly from unity (slope $1.07 \pm 0.08$, correlation 0.99). The $pA_2$ estimate was $8.43 \pm 0.04$.

controls. Furthermore, the response to BMS 182874 was found to be less ($-22.7 \pm 8.3\%$) ($P<0.05$, $n=7$) than in untreated controls. In contrast, the response to endothelin-1 was found to be higher than in controls in reversibility experiments of kidneys treated with either BQ-788 ($56.0 \pm 9.0\%$) ($P<0.05$, $n=8$) or BQ-928 ($41.6 \pm 15.0\%$) ($P<0.05$, $n=6$).

In another series of experiments, the responses of rabbit perfused kidney to angiotensin-II (1 $\mu$M) were shown to be unaffected by any of the antagonists used in the present study (results not shown).

## Discussion

One of the main purposes of the present study was firstly to establish the pharmacological characteristics of the recently developed mixed $ET_A/ET_B$ antagonists, BQ-238 and BQ-928, in comparison to other well-established antagonists, such as SB 209670 (Ohlstein *et al*., 1994). It was found in our study that BQ-238, but not BQ-928, possesses a higher affinity for $ET_A$ than for $ET_B$ receptors. These results have been confirmed in two highly selective preparations, the rabbit carotid artery and pulmonary artery (with IRL-1620 as a selective $ET_B$ agonist). The higher affinity of BQ-928 or BQ-238 for the $ET_A$ or $ET_B$ receptors confirmed the observations of Fukami *et al*. (1996); they showed, using the natural agonist endothelin-1, the same receptor selectivity for these particular antagonists in porcine aortic smooth muscle or cerebellum membrane selective for the $ET_A$ or $ET_B$ receptors, respectively. With these two preparations, we have also confirmed the affinity and selectivity of BMS 182874 for $ET_A$ receptors, as initially demonstrated by Stein *et al*. (1994).

In a recent study, we have shown that a mixture of BQ-123 (1 $\mu$M) and BQ-788 (10 nM) although efficiently blocking the vasoconstriction to endothelin-1 in the rabbit perfused kidney, induced a marked hyperresponsiveness to a third infusion of endothelin-1 given 1 h after cessation of the antagonist treatment (D'Orléans-Juste *et al*., 1995). In the present study, the mixed $ET_A/ET_B$ receptor antagonist BQ-928 also induced the same hyperresponsiveness to endothelin-1. Interestingly, this

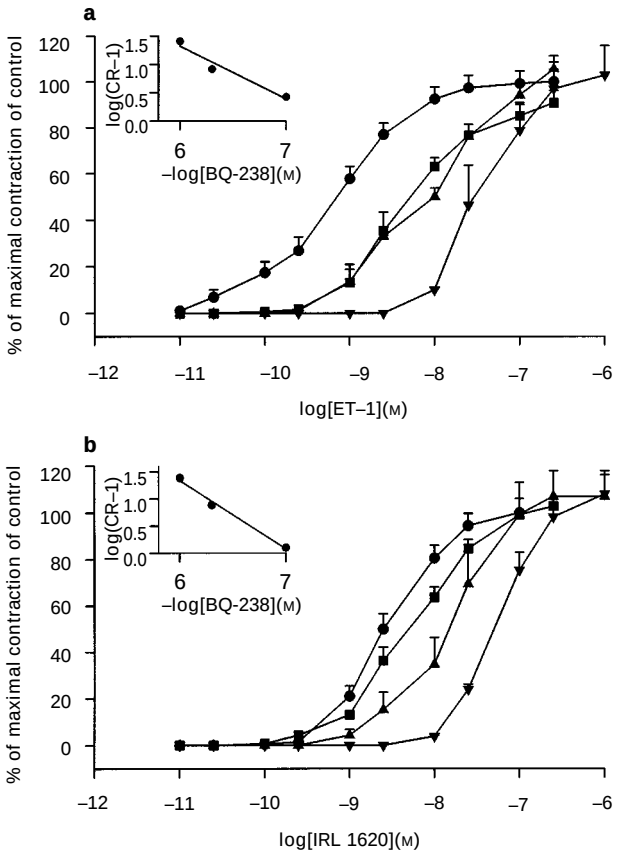


**Figure 4** (a) Effect of the mixed $ET_A/ET_B$ receptor antagonist BQ-238 on constrictions of the rabbit carotid artery induced by endothelin-1 (ET-1). The tissues were incubated for 15 min with either vehicle (●) or BQ-238 0.1 $\mu$M (■), 0.5 $\mu$M (▲) and 1 $\mu$M (▼) before exposure to endothelin-1. Each point represents the mean of at least 5 experiments; vertical lines show s.e. mean; Inset: Schild plot of BQ-238 against endothelin-1 contractions. The slope of regression did not differ significantly from unity (slope $0.93 \pm 0.22$, correlation 0.95). The $pA_2$ estimate was $7.42 \pm 0.22$. (b) Effect of BQ-238 on constrictions of the rabbit pulmonary artery induced by IRL-1620. The tissues were incubated for 15 min with either vehicle (●) or BQ-238 0.1 $\mu$M (■), 0.5 $\mu$M (▲) and 1 $\mu$M (▼) before exposure to IRL-1620. Each point represens the mean of at least 5 experiments; vertical lines show s.e.mean. Inset: Schild plot of BQ-238 against IRL-1620-induced contractions. The slope of regression did not differ significantly from unity (slope $1.22 \pm 0.22$, correlation 0.97). The $pA_2$ estimate was $7.05 \pm 0.14$.

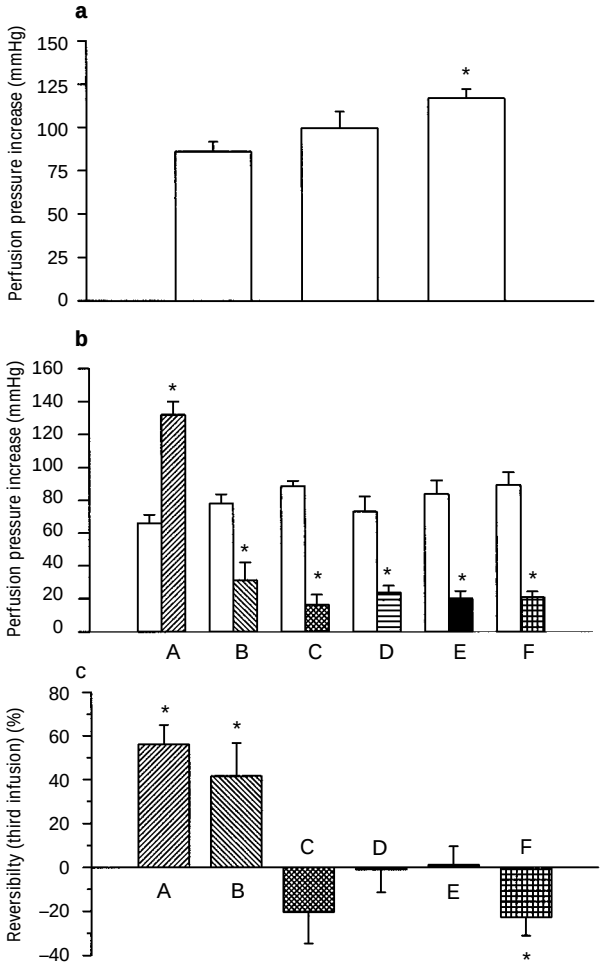


**Figure 5** (a) Changes in perfusion pressure induced by three successive infusions of endothelin-1 (ET-1, 10 nM for 5 min) (60-min intervals) in the rabbit perfused kidney. $^*P<0.05$ compared to first infusion ($n=8$). (b) Changes in perfusion pressure induced by ET-1 (10 nM) in the absence (open columns) or presence of BQ-788 (0.01 $\mu$M) (column A), BQ-928 (0.5 $\mu$M) (B), SB 209670 (0.05 $\mu$M), (C), BQ-238 (0.1 $\mu$M) (D), BQ-123 (1 $\mu$M) (E) or BMS 182874 (5 $\mu$M) (F). $^*P<0.05$ compared to control ($n=5-8$). (c) Responses of the rabbit kidney to a third administration of endothelin-1 (10 nM), 60 min after termination of the infusion of either BQ-788 (0.01 $\mu$M) (column A), BQ-928 (0.5 $\mu$M) (B), SB 209670 (0.05 $\mu$M) (C), BQ-238 (0.1 $\mu$M) (D), BQ-123 (1 $\mu$M) (E) or BMS 182874 (5 $\mu$M) (F). $^*P<0.05$ compared to the third control infusion of endothelin-1 (10 nM) ($n=5-8$).

type of hyperreactivity was not seen following interruption of the infusion of SB 209670 or BQ-238. We would suggest that this lack of hyperresponsiveness is due to the fact that both compounds have much higher affinities for $ET_A$ than $ET_B$ receptors. The highly tachyphylactic nature of the endothelial $ET_B$ receptor-mediated response (Le Monnier De Gouville *et al*., 1990) may explain the significant increase in responsiveness of the kidney to a third control infusion of endothelin-1, a phenomenon not seen with angiotensin II. Nonetheless, treatment of the kidney with a mixture of BQ-123 and BQ-788 (D'Orléans-Juste *et al*., 1995) or with BQ-928 still triggered a significant increase in the response to a third infusion of endothelin-1 when compared to the third control infusion.

On the other hand, it has been established that the $ET_B$ receptor found on the endothelium is pharmacologically and biochemically indistinguishable from the $ET_B$ receptors found on the smooth muscle (Clozel & Gray, 1995). Moreover, Warner (1993) demonstrated the presence of $ET_B$ receptor subtypes ($ET_{B1}$ and $ET_{B2}$) in rat arterial mesenteric vasculature and stomach strips. Nonetheless, results obtained from the $ET_B$-receptor selective, and contractile, preparation of the rabbit pulmonary artery (with IRL-1620 as an agonist) may be extrapolated to the function of the same receptor type on the endothelium of the kidney, as previously suggested (D'Orléans-Juste *et al*., 1994). As we have previously shown in the rabbit that BQ-788 potentiates the pressor and renal constrictor effects of endothelin-1 (D'Orléans-Juste *et al*., 1994; 1995), the results of the present study support the preferential use of selective $ET_A$ antagonists in the human renal circulation in pathological situations where raised levels of endogenous endothelin-1 may be important. This hypothesis is based on the assumption that small diameter vessels will respond to endothelin-1, principally via $ET_A$ receptors, as already demonstrated in larger human renal vessels (Maguire *et al*., 1994).

The above-mentioned concept is further supported by the recent observation of Allcock *et al*. (1995) that in the anaesthetized rat mixed $ET_A/ET_B$ receptor antagonists such as PD-145065 are incapable, unlike BQ-123, of reversing the reductions in blood flow in different tissues induced by endothelin-1, due to their ability to block the $ET_B$ receptor-dependent release of endothelium-derived relaxing factor. Interestingly, the

**Table 2** Maximal pressor responses induced by a 5-min infusion of endothelin-1 (10 nM) via the renal artery of rabbit perfused kidneys, in the absence and presence of either BQ-123, BMS 182874, SB 209670, BQ-238, BQ928 or BQ-788

| *Antagonist* | *Concentration* ($\mu$M) | *% of antagonism* | *$IC_{50}$* ($\mu$M) |
|---|---|---|---|
| BQ-123 | 0.1 | 0.7±15.1 | 0.4 |
| ($ET_A$) | 0.5 | 56±8.2 | |
| | 1 | 82±4.4 | |
| BMS 182874 | 0.5 | 0 | 2 |
| ($ET_A$) | 1 | 38.4±13.6 | |
| | 5 | 76.4±3.2 | |
| | 10 | 97.4±1.8 | |
| SB 209670 | 0.001 | 0 | 0.01 |
| ($ET_A > ET_B$) | 0.005 | 32.9±13.9 | |
| | 0.01 | 54.1±8.3 | |
| | 0.05 | 81.4±5.9 | |
| | 0.1 | 100 | |
| BQ-238 | 0.01 | 0 | 0.09 |
| ($ET_A > ET_B$) | 0.05 | 25.6±13.8 | |
| | 0.1 | 67.5±3.2 | |
| | 0.5 | 93.1±2.3 | |
| | 1 | 100 | |
| BQ-928 | 0.1 | 0.7±9.0 | 0.4 |
| ($ET_A < ET_B$) | 0.5 | 60.5±12.7 | |
| | 1 | 94.2±1.9 | |
| BQ-788 | 0.01 | 0 | – |
| ($ET_B$) | | | |

Each value represents the mean of at least 5 experiments. Preferential affinity for either receptor type is shown in parentheses.

mixed $ET_A/ET_B$ receptor antagonist, BQ-928, similar to PD-145065, possesses a higher affinity for $ET_B$ than for $ET_A$ receptors.

Although BQ-238 and BQ-928 are almost equipotent as antagonists of contractions of the RbCA induced by endothelin-1, the latter is much more potent in blocking the constriction induced by IRL-1620 in the RbPA. This difference in affinity for both $ET_A$ and $ET_B$ receptor types may explain the lower potency of BQ-928 compared to BQ-238 as a blocker of the renal constriction to endothelin-1 and the hyperresponsiveness that follows the interruption of its infusion into the rabbit kidney.

The non-peptidic antagonist, BMS 182874, which is selective for $ET_A$ receptors, was significantly less reversible as a blocker of ET-1-induced renal constriction than were BQ-123 or the mixed $ET_A/ET_B$ receptor antagonists. Indeed, although SB 209670 and BMS 182874 are metabolically stable compounds, only the latter $ET_A$ receptor selective blocker retained a significant antagonist effect for more than 60 min following its interruption.

Bosentan, which possesses a higher affinity for $ET_A$ than for $ET_B$ receptors, has been shown to improve cardiopulmonary vascular performance in a porcine endotoxic model (Weitzberg *et al*., 1996). In contrast, Ruetten *et al*. (1996) have recently demonstrated that endothelin-1 may be important in the maintenance of vascular tone in conditions of septic shock induced by LPS. In this latter study, the authors demonstrated that SB 209670 exacerbates the damage induced by septic shock in the anaesthetized rat. It is noteworthy that both $ET_A$ and $ET_B$ receptors mediate increases in vascular resistance induced by administration of exogenous endothelin-1 in anaesthetized rats (McMurdo *et al*., 1993). However, the pathological states seen in various vasculatures during septic shock cannot be entirely explained by reductions in the blood supply to these organs, caused by a drop in mean arterial pressure. A particular example of this is the marked increase in renal resistance encountered in endotoxin-treated rats (Mulder *et al*., 1993). Antagonists such as SB 209670, which interfere with both $ET_A$ and $ET_B$ receptor-mediated vasoconstriction in the rat as well as $ET_B$-receptor dependent release of EDRF (Ohlstein *et al*., 1994), will interfere not only with the increase in vascular resistance induced by endothelin, but will also affect the $ET_B$-receptor-dependent increase in blood flow, which is L-NAME-sensitive, at least in the renal circulation of the rabbit (D'Orléans-Juste *et al*., 1994). On the other hand, treatment with bosentan in the endotoxic pig model (Weitzberg *et al*., 1996) was significantly less lethal than treatment with SB 209670 in the rat model (over 60% of the animals died; Ruetten *et al*., 1996). The lesser toxicity of bosentan, when compared to SB 209670, may be due to the predominant role of $ET_A$ receptors as mediators of the pressor effects of ET-1 in the pig (Cirino *et al*., 1992) as opposed to the rat where both $ET_A$ and $ET_B$ receptors are involved in the endothelin-1-induced increase of mean arterial pressure (McMurdo *et al*., 1993) and reduction of renal blood flow (Wellings *et al*., 1993). Interestingly, Clozel and Breu (1996) have recently demonstrated a predominant role of endothelial 'vasorelaxant' $ET_B$ receptors in deoxycorticosterone acetate (DOCA) and spontaneously hypertensive rats but not in normal rats, with the orally-available $ET_B$ receptor antagonist Ro 46-8443.

A recent study by Haynes *et al*. (1996) has shown that the mixed $ET_A/ET_B$ receptor antagonist TAK-044 increases blood flow by 22% in the forearm arteries of healty subjects whereas BQ-123 increases it by 75% (Haynes & Webb, 1994). The weaker effect of TAK-044 in increasing forearm blood flow may well be due to its ability to interfere with $ET_B$ receptor-dependent vasodilation induced by endogeneous endothelin-1.

Overall, our results show that mixed $ET_A/ET_B$ receptor antagonists which possess a higher affinity for $ET_B$ than for $ET_A$ receptors induce hyperresponsiveness following interruption of their application to organs in which $ET_A$ receptors largely mediate endothelin-1 resistance vasoconstrictions, such as the rabbit kidney (Télémaque *et al*., 1993). In order to avoid these deleterious effects, which may be dependent on the *in vivo* pharmacokinetic characteristics of these compounds, we would advocate the use of either highly selective $ET_A$ receptor antagonists or mixed $ET_A/ET_B$ receptor antagonists which possess much higher affinities for $ET_A$ than $ET_B$ receptors.

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