Characterization of receptors for endothelins in the perfused arterial and venous mesenteric vasculatures of the rat

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- 1 Endothelin-1 and -3 induced marked arterial and venous constrictions in the perfused mesenteric vasculature of the rat with endothelin-3 being at least 20 times less active than endothelin-1, on both arterial and venous sides of the vasculature.
- 2 Two ET_B selective agonists, BQ-3020 and IRL 1620 (500 pmol), induced weak constrictions of the venous mesenteric vasculature and were inactive in the arterial side at doses up to 1000 pmol.
- 3 In mesenteric vasculatures precontracted with either methoxamine (arterial side) or the thromboxane A₂-mimetic, U46619 (venous side), acetylcholine or bradykinin produced vasodilatations of both arterial and venous vessels, whereas endothelin-3 induced vasodilatations only on the arterial side.
- 4 A selective ET_A receptor antagonist, BQ-123, blocked, in a concentration-dependent and reversible fashion, the vasoconstrictions induced by endothelin-1 on both sides of the mesenteric circulation (IC₅₀; arterial side: 0.013 µM; venous side: 0.032 µM).
- 5 In contrast, the vasodilator responses induced by endothelin-3 on the arterial side of the precontracted mesenteric vasculature were not affected by BQ-123.
- 6 The present study illustrates the presence of ET_A receptors which are responsible for vasoconstriction by endothelins in the arterial and venous mesenteric vasculatures. Furthermore, we suggest that the vasodilatations induced by endothelin-3 in the arterial vasculature uniquely, are ET_B receptor-mediated.

Keywords: Endothelin-1; endothelin-3; vascular effects; antagonist; BQ-123; endothelin receptors; mesenteric vasculature; IRL 1620; BQ-3020

Introduction

The responses of the venous and arterial portions of the mesenteric vasculature of the rat to the potent vasoactive peptides, endothelin-1 and endothelin-3, were recently described (Warner *et al.*, 1989; Warner, 1990). In these two studies, endothelin-1 was shown to be a potent vasoconstrictor of both sides of the rat mesenteric perfused vasculature. In addition, various groups have shown that endothelins have transient depressor properties *in vivo* in conscious and anaesthetized rats as well as in the mesenteric vasculature of the rat *in vitro* (de Nucci *et al.*, 1988; Yanagisawa *et al.*, 1988; Warner *et al.*, 1989; Gardiner *et al.*, 1990; Le Monnier de Gouville *et al.*, 1990; Douglas & Hiley, 1991). The release of vasodilator factors by endothelin-1 in this vascular bed is in accordance with the finding that the vasopressor effects of endothelins are modulated by the release of vasodilator factors, such as endothelium-derived nitric oxide (EDNO) and prostacyclin (de Nucci *et al.*, 1988).

The recent cloning of at least two distinct receptor types, namely ET_A (Arai *et al.*, 1990) and ET_B (Sakurai *et al.*, 1990), has prompted studies to characterize which receptors are involved in the various effects of endothelins. For instance, ET_A receptor activation has been initially associated with the vasoconstrictor effect of endothelins, whereas it was suggested that ET_B receptors were responsible for the release of EDNO (Webb, 1991). However, these concepts have been recently challenged as the vasoconstriction of some venous blood vessels as well as the rabbit pulmonary artery have been shown to be mediated via the activation of ET_B receptors (Ihara *et al.*, 1992b; Moreland *et al.*, 1992). ET_B-mediated vasoconstrictions in the rabbit pulmonary artery were further characterized by the use of the selective ET_B

receptor agonists, IRL 1620 (Suc-[Glu⁹,Ala^{11,15}]-endothelin-1(8-21); Takai *et al.*, 1992) and BQ-3020 ([Ala^{11,15}]-endothelin-1(6-21); Ihara *et al.*, 1992b).

Ihara *et al.* (1992a) have shown that the potent, selective ET_A receptor antagonists, BQ-153 and BQ-123, markedly suppress the pressor effects of endothelin-1 in conscious rats without affecting its initial hypotensive effect. Furthermore, this group suggested that the pressor response to endothelin-1 is due to the activation of ET_A receptors whereas its initial hypotensive effect, which is perhaps due to the release of EDNO (Whittle *et al.*, 1989) and which remained unaffected by the ET_A-receptor antagonists, is mediated by ET_B receptors (Ihara *et al.*, 1992a). These observations were confirmed by Bigaud & Pelton (1992) and Douglas *et al.* (1992). Interestingly, both groups demonstrated that BQ-123 not only reduced the pressor effect but also potentiated the initial depressor response to endothelin-1 in conscious (Bigaud & Pelton, 1992) as well as in anaesthetized rats (Douglas *et al.*, 1992). In addition, the ET_B receptor-mediated vasodilator properties of endothelins have very recently been characterized in rat aortic rings by Karaki *et al.* (1993) using a selective ET_B receptor antagonist, IRL 1038.

In contrast to the ET_B-mediated vasodilator properties of endothelin-1, we have recently shown that the endothelin-induced release of prostacyclin from rat perfused lung is mediated via the activation of ET_A receptors (D'Orléans-Juste *et al.*, 1992). This suggests that even though both ET_A and ET_B receptors are present in pulmonary tissues (D'Orléans-Juste *et al.*, 1992; Nakamichi *et al.*, 1992), the production of endogenous modulators of the vascular effects of endothelin-1 may be triggered by receptors other than ET_B.

As the haemodynamic effects of endothelins are clearly due to a series of interconnected events including the production

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of additional vasorelaxant factors, such as EDNO and prostacyclin, the use of an isolated segmental vasculature, such as the perfused mesenteric bed, is useful in allowing the pharmacological delineation of the constrictor and EDNO-releasing properties of various peptides including the endothelins (Warner, 1990; D'Orléans-Juste *et al.*, 1991; Claing *et al.*, 1992). Hence, this model was chosen to quantify the receptors involved in the responses of the rat mesenteric vasculature to endothelin-1 and endothelin-3.

Methods

The rat mesenteric vascular bed perfused simultaneously through the arterial and venous vasculatures

The rat mesentery was prepared as described previously (Warner, 1990). Male albino Wistar rats (250–350 g) were killed by stunning and exsanguination. The abdomen was opened and the ileocolic and colic branches of the superior mesenteric artery were tied. The portal mesenteric vein was freed of connective and adipose tissues and was then cannulated (Portex tube size 3FG) 1.0–1.3 cm distal to the portal mesenteric junction. The superior mesenteric artery was cannulated as described previously (McGregor, 1965). Warm (37°C), oxygenated (95% O₂: 5% CO₂) Krebs solution (concentration in mM: NaCl 117.5, KCl 4.7, KH₂PO₄ 1.2, CaCl₂ 2.5, MgSO₄ 1.2, NaHCO₃ 25, glucose 5.5) containing heparin (100 u ml⁻¹) was then perfused through the mesenteric artery at a flow of 2 ml min⁻¹ for 5 min. Following this initial perfusion period, the mesentery was separated from the intestine by cutting close to the intestinal border. The venous and arterial vasculatures were subsequently perfused independently at flow rates of 2 ml min⁻¹ with Krebs solution containing indomethacin (5 µM). The pressor responses to the endothelins, angiotensin II and noradrenaline, as well as the vasodilatations induced by endothelins, acetylcholine and bradykinin were measured with pressure transducers (Statham, model P-23A) and recorded on a Grass physiograph (model 7D).

Venous and arterial contractions of the mesenteric vasculature in response to endothelin-1, endothelin-3, angiotensin II and noradrenaline

Following an equilibration period of 45 min, the vasoconstrictor effects of endothelin-1, endothelin-3, BQ-3020, IRL 1620, angiotensin II and noradrenaline were measured on both sides of the mesenteric vasculature. Agonists were administered by bolus injections in volumes of 1 µl. Individual drugs (maximum of four different drugs on each preparation) were administered consecutively at time intervals varying from 10 min (angiotensin II, noradrenaline) to 45 min (endothelin-1, endothelin-3) to avoid tachyphylaxis. After the first administration of the different agonists, BQ-123 (cyclo[D-Asp-L-Pro-D-Val-L-Leu-D-Trp]) (0.01–1 µM) was infused starting from 15 min before the second administration of the agonists. The IC₅₀ of the antagonist was estimated by monitoring the pressor responses of endothelin-1 at the highest dose (arterial: 100 pmol, venous: 25 pmol) in the presence of increasing concentrations of BQ-123 (0.001–1 µM).

Endothelium-dependent vasodilatation

In order to quantify endothelium-dependent relaxations, the perfusion pressures on both sides of the mesenteric circulation were increased by infusing a sympathomimetic, methoxamine (100 µM), on the arterial side and a thromboxane A₂-mimetic, U46619 (0.5 µM), on the venous side (Warner, 1990). The perfusion flow rate was increased to 5 ml min⁻¹ on the arterial side to reduce spontaneous activity (Warner, 1990). When a steady elevation in perfusion pressure was reached on both sides, endothelium-dependent vasodilators

of the arterial and venous mesenteric vasculature of the rat (bradykinin, acetylcholine or endothelin-3: Warner *et al.*, 1989; Warner, 1990; D'Orléans-Juste *et al.*, 1991; Claing *et al.*, 1992) were administered as bolus injections. In some experiments, BQ-123 (0.1 µM) was infused starting from 15 min before the administration of bradykinin, acetylcholine or endothelin-3.

Drugs

Endothelin-1 and endothelin-3 were purchased from Peninsula Laboratories (Belmont, CA, U.S.A.). Bradykinin and angiotensin II were synthesized in the laboratory of Dr D. Regoli (Department of Pharmacology, Université de Sherbrooke, Sherbrooke, Québec, Canada). Acetylcholine, indomethacin, noradrenaline and methoxamine were purchased from Sigma (St. Louis, MO, U.S.A.). U46619 (9,11-dideoxy-9 α ,11 α -methanoepoxy prostaglandin F_{2 α}) was purchased from Cayman Chemical Company (Ann Arbor, U.S.A.). The ET_A receptor antagonist, BQ-123 and the ET_B receptor agonist, BQ-3020, were synthesized at Banyu Pharmaceutical Company (Tsukuba, Japan). The ET_B receptor agonist, IRL 1620, was synthesized at Ciba-Geigy (Takarazuka, Japan).

Endothelin-1, endothelin-3, BQ-3020, IRL 1620 and BQ-123 were dissolved in phosphate-buffered saline (pH: 7.4, Sigma, St. Louis, MO, U.S.A.). All other agents were dissolved in saline (NaCl: 0.9%) or distilled water, except for indomethacin which was dissolved in Trizma base (pH: 7.4, 0.2 M, Sigma).

Statistics

Results are shown as mean values $\pm$ s.e. mean for *n* experiments. Statistical differences between and within groups were compared by analysis of variance (ANOVA; Wallenstein *et al.*, 1980). Statistical significance was assumed when *P* < 0.05.

Results

Effects of BQ-123 on endothelin-1-induced arterial and venous pressor responses

The basal perfusion pressures of the arterial and venous sides of the mesenteric vasculature were 5.7 ± 0.9 mmHg (*n* = 11) and 1.8 ± 0.3 mmHg (*n* = 7), respectively, when perfused at 2 ml min⁻¹. Increasing the perfusion flow to 5 ml min⁻¹ increased the perfusion pressure of the arterial vasculature to 14.3 ± 1.1 mmHg (*n* = 14).

Infusion of BQ-123 had no effect on the basal perfusion pressure of either side of the vasculature while it markedly reduced the increases in perfusion pressures induced by endothelin-1 (25 pmol) (control, arterial side: 3.4 ± 0.3 mmHg, venous side: 10.9 ± 0.8 mmHg; + BQ-123 (0.1 µM), arterial side: 0.7 ± 0.1 mmHg, venous side: 1.9 ± 0.3 mmHg, *n* = 6–8, *P* < 0.001; Figure 1). The response to endothelin-1 was almost restored to control values, 30 min after stopping the infusion of the antagonist (arterial side: 2.6 ± 0.8 mmHg, venous side: 8.0 ± 1.0 mmHg, *n* = 6–8, not significantly different from control).

Characteristically, endothelin-1 was 5–10 times less active in the arterial (Figure 2a) mesenteric vasculature than in the venous vessels (Figure 2b) (e.g. arterial side, 25 pmol: 2.7 ± 0.2 mmHg, 50 pmol: 4.6 ± 0.6 mmHg, *n* = 11; venous side, 5 pmol: 3.0 ± 0.3 mmHg, 10 pmol: 8.0 ± 0.6 mmHg, *n* = 7). BQ-123 (0.01–1 µM) induced a concentration-dependent antagonism ($50 \pm 5\%$ to $100 \pm 1\%$, *n* = 5–8, *P* < 0.001) of the contractions evoked by endothelin-1 (25–100 pmol) on the arterial side of the mesenteric vasculature (Figure 2a). The IC₅₀ value of BQ-123, estimated against the highest dose of endothelin-1 used in the present study (100 pmol), was 0.013 µM. In addition, 30 min after the cessation of the

Figure 1 Typical trace illustrating the pressor effect of endothelin-1 (ET-1: 25 pmol) in the absence and presence of BQ-123 (0.1 μ M, 15 min) in the arterial (a) and venous (b) mesenteric vasculatures of the rat. This trace is representative of 7 other experiments.

Figure 2 The dose-dependent effect of endothelin-1 in absence ($\square$) and presence of BQ-123 ($\blacklozenge$: 0.01 μ M, $\bullet$: 0.1 μ M, $\blacktriangle$: 1 μ M) on the arterial (a) and venous (b) mesenteric vasculatures of the rat. Each point is the mean $\pm$ s.e. mean of 5–8 determinations. * P < 0.001.

infusion of BQ-123 (0.01–0.1 μ M), the responses of arterial mesenteric vasculature were completely recovered for the smaller doses of endothelin-1 (25 pmol: 2.6 ± 0.7 mmHg, 50 pmol: 4.0 ± 1.0 mmHg), but only partially for the highest dose (100 pmol: 15.2 ± 4.1 mmHg). However, at the highest concentration of BQ-123 (1 μ M), the response was only partially restored, 30 min after the cessation of the infusion ($50 \pm 8\%$, $n = 5$ of control values for each dose of endothelin-1 tested, results not shown).

BQ-123 (0.01–1 μ M) induced a concentration-dependent antagonism (35 ± 9 to $90 \pm 2\%$, $n = 5-7$, $P < 0.001$) of the vasoconstrictions induced by endothelin-1 (5–25 pmol) in the venous vasculature (Figure 2b). The IC_{50} value for BQ-123, estimated against the dose of 25 pmol of endothelin-1 was 0.032 μ M. The response to endothelin-1 (5–25 pmol) was totally restored 30 min after the cessation of the infusion of the lower concentrations of the antagonist (0.01–0.1 μ M: 8.0 ± 1.0 mmHg for the dose of 25 pmol of ET-1), but for BQ-123 given at 1 μ M, the response was restored to only $50 \pm 10\%$ of the control value for the three doses of ET-1.

Endothelin-3 increased the perfusion pressures of both sides of the rat mesenteric vasculature (arterial side, 1000 pmol: 3.8 ± 0.6 mmHg, venous side, 200 pmol: 8.0 ± 1.1 mmHg, $n = 5$). However, doses of endothelin-3, 20 times higher than endothelin-1, were required to obtain similar vasoconstrictions (endothelin-1; arterial side, 50 pmol: 4.3 ± 1.1 mmHg, venous side, 10 pmol: 7.9 ± 0.6 mmHg, $n = 5-8$).

BQ-3020 or IRL 1620, caused only small increases in the perfusion pressure on the venous side (BQ-3020, 500 pmol: 1.1 ± 0.1 mmHg; IRL 1620, 500 pmol: 1.8 ± 0.4 mmHg, $n = 4-5$), and were inactive as vasoconstrictors, even at doses of 1000 pmol on the arterial mesenteric vasculature.

Lack of effect of BQ-123 on the pressor responses induced by angiotensin II or noradrenaline

BQ-123 (1 μ M) did not affect the vasoconstrictions induced by angiotensin II (100 pmol: venous, control: 8.4 ± 0.2 mmHg; + BQ-123: 9.4 ± 0.5 mmHg, $n = 4$; Figure 3b) or noradrenaline (1000 pmol: control, arterial side:

Figure 3 Typical trace illustrating the effect of endothelin-1 (ET-1: 25 pmol), angiotensin II (AII: 100 pmol) and noradrenaline (NA: 1000 pmol) in the absence and presence of BQ-123 (1 μM) in the arterial (a) and venous (b) mesenteric vasculatures of the rat. This trace is representative of 3 other experiments.

Figure 4 The dose-dependent effect of endothelin-3 (ET-3: 10–300 pmol) and the vasodilator effects of acetylcholine (ACh: 100 pmol) and bradykinin (BK: 1000 pmol) on the precontracted arterial (methoxamine, 100 μM) and venous (U46619, 0.5 μM) vasculature in the absence (open columns) or presence (solid columns) of BQ-123 (0.1 μM). Columns indicate the mean with s.e. mean of 4–8 determinations. * $P < 0.01$; ** $P < 0.001$.

8.1 ± 1.3 mmHg, venous side: 4.6 ± 1.0 mmHg; + BQ-123, arterial side: 9.5 ± 3.1 mmHg, venous side: 5.1 ± 0.8 mmHg, $n = 4$: Figure 3a and b), while it markedly decreased those induced by endothelin-1 (25 pmol) on both sides of the mesenteric vasculature (Figures 3a and b).

Comparison of the vasoconstrictor and vasodilator effects of endothelin-3 in the perfused mesenteric vascular bed

Infusions of methoxamine (100 μM, arterial side) or U46619 (0.5 μM, venous side) increased the perfusion pressures by 35.1 ± 3.2 mmHg ($n = 14$) and 12.3 ± 0.9 mmHg ($n = 12$), respectively (Figure 4). Endothelin-3 (10–300 pmol) induced dose-dependent vasodilations of the arterial vessels, and vasoconstrictions of the venous side of the mesenteric vasculature. In addition, acetylcholine (100 pmol) and bradykinin (1000 pmol) induced marked vasodilations of the arterial and venous portion of the mesenteric bed, respectively ($20 \pm 3\%$ and $18 \pm 3\%$, $n = 5–6$). BQ-123 (0.1 μM) had no significant effect on the vasodilator response of the arterial vessels to endothelin-3, but significantly reduced its vasoconstrictor effects. The antagonist had no effect on the vasodilation induced by acetylcholine (100 pmol: $19 \pm 3\%$) in the arterial vasculature nor by bradykinin (1000 pmol: $16 \pm 2\%$) in the venous vasculature (Figure 4).

Discussion

Bolus *i.v.* administration of endothelin-1 induces a prolonged pressor response preceded by an initial transient depressor response in conscious and anaesthetized rats (de Nucci *et al.*, 1988; Yanagisawa *et al.*, 1988; Gardiner *et al.*, 1990). The selective ET_A receptor antagonists, BQ-153 and BQ-123, significantly reduce the pressor response to endothelin-1 in conscious rats, yet do not alter its initial hypotensive effect (Ihara *et al.*, 1992a). The latter observation strongly suggests that while the pressor response to endothelin-1 is predominantly mediated by the activation of ET_A receptors, the initial hypotensive response may be due to the activation

of ET_B receptors (Ihara *et al.*, 1992a) and the possible release of EDNO (Whittle *et al.*, 1989).

In order to examine this possibility, we have tested the endothelins in the double perfused mesenteric bed of the rat, in which it is possible to assess separately the EDNO-releasing and vasoconstrictor properties of various peptides and autacoids (Warner, 1990).

The present study shows that the selective ET_A receptor antagonist, BQ-123, markedly reduced in a specific and concentration-dependent fashion, the constrictor effects of endothelin-1 in both the arterial and venous sides of the mesenteric circulation of the rat. This confirms that endothelin-1 vasoconstricts the mesenteric bed via the activation of ET_A receptors. In addition, our results show that BQ-123, even at high concentrations, did not affect the vasodilations induced by endothelin-3. This peptide was used as a vasodilator agent, instead of endothelin-1, since it is a less potent vasoconstrictor agent within this preparation and so more readily permits the characterization of the vasodilator properties of endothelins, which have been reported *in vivo* and in various vascular beds (Warner *et al.*, 1989; Lidbury *et al.*, 1990).

Spinella *et al.* (1991) have reported the antagonist properties of an endothelin analogue ([D-Asp¹]-endothelin-1), which markedly reduced the vasoconstrictive properties of endothelin-1 in the rat perfused lung. In addition, this group has shown that the antagonist was selective for endothelin-1 and did not affect the increase in perfusion pressure induced by endothelin-3 in the same experimental model. In contrast, in the present study, we have shown that BQ-123 also markedly reduced the vasoconstrictions induced by endothelin-3 in the rat perfused mesenteric bed. The recent cloning of two endothelin receptor subtypes, namely ET_A (Arai *et al.*, 1990) and ET_B (Sakurai *et al.*, 1990), has included the observation that the endothelins (endothelin-1, 2 and 3) show different orders of potency on these two receptor subtypes. On the ET_A receptor, endothelin-1 is much more potent than endothelin-3, whereas on the ET_B receptor, both peptides are

equipotent (Arai *et al.*, 1990; Sakurai *et al.*, 1990). As shown in the present study, endothelin-1 was found to be 20 to 40 times more active than endothelin-3 at constricting both sides of the rat mesenteric vasculature, thus suggesting that the vasoconstrictor effects of endothelin-1 in this vasculature are mediated by the activation of ET_A receptors. This hypothesis is confirmed by experiments using BQ-123, which markedly reduced the vasoconstrictor responses to endothelin-1 and -3. The increase in perfusion pressure caused by BQ-3020 or IRL 1620, two highly selective ET_B receptor agonists (Ihara *et al.*, 1992b; Takai *et al.*, 1992) in the venous mesenteric vasculature, illustrates the presence of a relatively small proportion of non-ET_A receptors in the mesenteric vasculature, as initially reported by Randall *et al.* (1989) in the blood perfused mesenteric bed of the rat.

Our data also indicate that endothelins release EDNO via the activation of ET_B receptors in the arterial mesenteric vasculature. This conclusion is reinforced by the observations of Karaki *et al.* (1993) who have reported ET_B-mediated vasodilator properties of endothelin-3 in rat aortic rings. It is also noteworthy that endothelin-3 induced marked vasodilation in the arterial, but not in the venous portion of the mesenteric vasculature, even though the veins responded to bradykinin with endothelium-dependent vasodilations (Warner, 1990).

In conclusion, we have shown that endothelin-1 and endothelin-3 induced arterial and venous constrictions via the activation of ET_A receptors and arterial vasodilation via the activation of BQ-123-insensitive receptors, probably of the ET_B type, in the mesenteric vasculature of the rat.

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