ET_A receptor-mediated responses to endothelin-1 and big endothelin-1 in the rat kidney

¹David M. Pollock & Terry J. Opgenorth

Pharmaceutical Discovery, Abbott Laboratories, D-47V AP9, Abbott Park, IL 60064, U.S.A.

1 Renal clearance experiments were conducted in anaesthetized Sprague-Dawley rats to determine the effect of the ET_A receptor antagonist, BQ-123, on the renal haemodynamic response to endothelin-1 (ET-1) and its precursor, big endothelin-1 (big ET-1) at doses that produce an equivalent degree of renal vasoconstriction.

2 Infusion of either big ET-1 at 100 pmol kg⁻¹ min⁻¹ or ET-1 at 12 pmol kg⁻¹ min⁻¹ for 60 min produced almost identical decreases in renal blood flow (RBF) and glomerular filtration rate (GFR). Big ET-1 produced an increase in mean arterial pressure (MAP) that was significantly larger than the increase produced by ET-1.

3 Co-infusion with BQ-123 (0.1 mg kg⁻¹ min⁻¹) prevented the rise in MAP produced by big ET-1 and completely blocked the renal response. Similarly, BQ-123 inhibited both the increase in MAP and the decrease in RPF and GFR produced by ET-1.

4 Big ET-1 but not ET-1, produced a significant increase in water and sodium excretion. BQ-123 had no effect on the diuretic and natriuretic response to big ET-1 consistent with possible ET_B-mediated inhibition of tubular reabsorption.

5 We have previously shown that at higher doses of ET-1, BQ-123 was unable to inhibit the renal vasoconstrictor response despite blockade of the pressor response. Taken together, these results indicate that ET-1 activates primarily ET_A receptors at moderately low doses to produce renal vasoconstriction while higher doses also involve non-ET_A receptors.

6 Since ET-1 has a similar binding affinity for both ET_A and ET_B receptors, we suggest that there may be a third type of ET-1 receptor in the rat kidney with lower affinity for ET-1 that is coupled to a vasoconstrictor mechanism.

Keywords: Endothelin-1 (ET-1); big endothelin-1 (big ET-1); endothelin receptors; endothelium; renal haemodynamics

Introduction

Endothelin-1 (ET-1) produces contraction of vascular smooth muscle by activation of ET_A receptors and, in some tissues, via ET_B receptors (Clozel *et al.*, 1992; Cristol *et al.*, 1992; Pollock & Opgenorth, 1993). The rat kidney contains a predominance of ET_B receptors (Nambi *et al.*, 1992; Terada *et al.*, 1992; Karet *et al.*, 1993) and several lines of evidence have suggested that ET-1 activates primarily ET_B receptors to produce decreases in renal blood flow (RBF) and glomerular filtration rate (GFR). First, the receptor-selective ET_B agonist, sarafotoxin 6c, has been reported to produce renal vasoconstriction in this species (Clozel *et al.*, 1992; Cristol *et al.*, 1992). We and others have also reported that the ET_A receptor antagonist, BQ-123, effectively blocks the systemic pressor response to a high dose of exogenously administered ET-1 but has no effect on decreases in RBF or GFR (Cristol *et al.*, 1992; Pollock & Opgenorth, 1993). Furthermore, a non-selective receptor antagonist, PD145065, can block the effects of ET-1 in the kidney (Wellings *et al.*, 1993). These findings bring into question the physiological role of ET_A receptors in the rat kidney. In addition to haemodynamic effects, ET_B receptors may also play a role in fluid-electrolyte balance. Evidence suggests that ET-1 inhibits the actions of vasopressin in the kidney through ET_B receptor activation (Ohta *et al.*, 1990; Terada *et al.*, 1992; Migas *et al.*, 1993).

The ET-1 precursor, referred to as big ET-1, is thought to produce effects similar to ET-1 as a result of proteolytic cleavage by an endothelin converting enzyme (Opgenorth *et al.*, 1992). Big ET-1 is a less potent renal vasoconstrictor than ET-1 despite being capable of producing much larger increases in systemic arterial pressure (Hoffman *et al.*, 1990; Pollock & Opgenorth, 1991). Due to the apparent autocrine

and/or paracrine nature of endothelin, a common hypothesis is that the site of conversion of big ET-1 to ET-1 represents a location where the endothelin system plays a physiological and pathophysiological role. Thus, infusion of big ET-1 may provide insight into the relevant actions of ET-1.

The purpose of the current study was first to compare the haemodynamic and renal excretory effects of i.v. administered ET-1 and big ET-1 at doses that produce similar degrees of renal vasoconstriction in the anaesthetized rat, and secondly, to elucidate the role of ET_A receptors in these responses by determining the effect of the ET_A receptor antagonist, BQ-123, on the response to ET-1 and big ET-1.

Methods

Experiments were conducted on male Sprague-Dawley rats (200–300 g; Charles River, Cambridge, MA, U.S.A.) anaesthetized with Inactin (100 mg kg⁻¹, i.p.; 5-sec-butyl-5-ethyl-2-thiobarbituric acid; synthesized in-house) and placed on a servo-controlled heating table to maintain rectal temperature at 37°C. A tracheostomy was performed to allow for unobstructed breathing. Two catheters (PE-50) were placed in the jugular vein for infusion of endothelin peptides (0.9% NaCl vehicle was infused during the control periods at 10 µl min⁻¹) and BQ-123 (0.25 M NaHCO₃ vehicle was infused during the control periods and in the control groups at 10 µl min⁻¹). A catheter was placed in the femoral artery (PE-50) for measurement of mean arterial pressure (MAP; Modular Instruments Inc., Southeastern, PA, U.S.A.) and blood sampling. [³H]-inulin (0.75 µCi kg⁻¹ min⁻¹; Amersham, Arlington Heights, IL) and [¹⁴C]-*p*-aminohippurate (PAH; 2 µCi kg⁻¹ min⁻¹; New England Nuclear, Boston, MA,

¹ Author for correspondence.

U.S.A.) were infused via a catheter (PE-50) placed in the femoral vein ($10 \mu\text{l min}^{-1}$ in 0.9% NaCl). Urine was collected via a bladder catheter (PE-90).

After surgery, a 90 min equilibration period was allowed before starting a 30 min control clearance period. Animals then received one of four infusions regimens: ET-1 alone ($12 \text{ pmol kg}^{-1} \text{ min}^{-1}$), ET-1 in combination with BQ-123 ($0.1 \text{ mg kg}^{-1} \text{ min}^{-1}$), big ET-1 alone ($100 \text{ pmol kg}^{-1} \text{ min}^{-1}$), and big ET-1 in combination with BQ-123 ($n = 6$ in each group). BQ-123 infusion was initiated 2–5 min prior to ET-1 or big ET-1. Thirty min after starting the infusions, a 30 min experimental clearance period was obtained. An arterial blood sample ($40\text{--}50 \mu\text{l}$) was collected into a heparinized capillary tube at the midpoint of each clearance period for determination of haematocrit and plasma radioactivity. At the end of the clearance protocol, a terminal blood sample (1.5 ml) was collected into a heparinized centrifuge tube for determination of plasma electrolyte concentrations. Kidneys were then removed, decapsulated and weighed.

Urine and plasma sodium and potassium concentrations were measured with ion-selective electrodes (EL-ISE, Beckman, Instruments, Brea, CA, U.S.A.). Activity of [^3H]-inulin and [^{14}C]-PAH in urine and plasma was determined by liquid scintillation counting (Beckman Instruments, Fullerton, CA, U.S.A.).

Human endothelin-1-(1-21) and human big endothelin-1-(1-38) were purchased from American Peptide, Santa Clara, CA, U.S.A.). BQ-123 (cyclo(-D-Trp-D-Asp-Pro-D-Val-Leu)) was synthesized in-house.

Renal blood flow (RBF) was calculated as the clearance of [^{14}C]-PAH/(1-haematocrit). Using 5 mmHg as an estimate of renal venous pressure, renal vascular resistance was calculated as $(\text{MAP}-5)/\text{RBF}$. Glomerular filtration rate (GFR) was determined from the clearance of inulin. Fractional excretion rates were calculated as the absolute excretion rate as a percentage of the filtered load.

Student's *t* test for paired data was used to determine significant differences between control vs. experimental periods and for unpaired data to determine the effect of BQ-123 (Statview II, Abacus Concepts, Berkeley, CA, U.S.A.). $P < 0.05$ was considered significant. All values are reported as mean $\pm$ s.e.mean.

Results

By design, i.v. infusion of ET-1 or big ET-1 at 12 and $100 \text{ pmol kg}^{-1} \text{ min}^{-1}$ produced nearly identical decreases in RBF and GFR, approximately 60% and 50%, respectively, in both groups (Figure 1). Renal vascular resistance values were increased to a similar level at these doses. MAP and haematocrit were also significantly increased although the changes produced by big ET-1 were more than 2 fold larger. MAP was increased by $22 \pm 3\%$ and $49 \pm 7\%$ by ET-1 and big ET-1, respectively ($P < 0.05$), while haematocrit was increased by $11 \pm 2\%$ and $28 \pm 1\%$ by ET-1 and big ET-1, respectively ($P < 0.05$).

Simultaneous infusion of BQ-123 in rats receiving either ET-1 or big ET-1 significantly inhibited the systemic and renal haemodynamic responses (Figure 1). Infusion of BQ-123 and ET-1 resulted in no significant change in MAP, HCT, renal vascular resistance, or RBF. However, a small ($16 \pm 5\%$), but significant decrease in GFR was observed. BQ-123 completely prevented all of the responses to big ET-1.

The effect of ET-1 and big ET-1 with or without co-infusion of BQ-123 on water and sodium excretion is depicted in Figure 2. Big ET-1, but not ET-1, produced a significant increase in both urine flow rate and sodium excretion as well as fractional excretion of water and sodium. Water and sodium excretion rates were also unchanged during co-administration of BQ-123 and ET-1. During infusion of both big ET-1 and BQ-123, a significant diuresis and

Figure 1 Haemodynamic responses to endothelin-1 (ET-1) and big ET-1 in anaesthetized rats during infusion of BQ-123 or vehicle (0.25 N NaHCO_3). ET-1 was infused at $12 \text{ pmol kg}^{-1} \text{ min}^{-1}$ with (○) or without (●) simultaneous infusion of BQ-123 at $0.1 \text{ mg kg}^{-1} \text{ min}^{-1}$. Big ET-1 was infused at $100 \text{ pmol kg}^{-1} \text{ min}^{-1}$ with (□) or without (■) simultaneous infusion of BQ-123. Values are mean $\pm$ s.e.mean. $n = 6$ in all four groups of rats. * $P < 0.05$ vs. control; † $P < 0.05$ vs. ET-1 or big ET-1 alone. MAP, mean arterial pressure; HCT, haematocrit; RVR, renal vascular resistance; RBF, renal blood flow; GFR, glomerular filtration rate.

natriuresis was still observed despite blocking the increase in MAP and the decreases in RBF and GFR. The increases in urine flow rate and sodium excretion were actually larger when big ET-1 was infused along with BQ-123 than with big ET-1 alone although the difference in sodium excretion did not achieve statistical significance ($0.1 < P < 0.05$).

Discussion

Our study provides new information regarding the role of ET_A receptors in the rat kidney. Since the ET_A -selective antagonist, BQ-123, was capable of blocking the haemodynamic responses to ET-1 and big ET-1, we have provided evidence that ET_A receptor activation is capable of producing a significant degree of renal vasoconstriction in the rat. Furthermore, we provide evidence to suggest that big ET-1 infusion activates non- ET_A receptors, presumably ET_B , to inhibit tubular sodium and water reabsorption.

We have previously reported that the renal haemodynamic effects of a relatively high dose of ET-1, $25 \text{ pmol kg}^{-1} \text{ min}^{-1}$,

Figure 2 Renal excretory responses to endothelin-1 (ET-1) and big ET-1 in anaesthetized rats during infusion of BQ-123 or vehicle (0.25 N NaHCO₃). ET-1 was infused at 12 pmol kg⁻¹ min⁻¹ with (○) or without (●) simultaneous infusion of BQ-123 at 0.1 mg kg⁻¹ min⁻¹. Big ET-1 was infused at 100 pmol kg⁻¹ min⁻¹ with (□) or without (■) simultaneous infusion of BQ-123. Values are mean ± s.e.mean. *n* = 6 in all four groups of rats. **P* < 0.05 vs. control; †*P* < 0.05 vs. ET-1 or big ET-1 alone. UV, urine flow rate; FE H₂O, fractional excretion of water; UNaV, sodium excretion; FE Na, fractional excretion of sodium.

cannot be inhibited by BQ-123. Since the renal haemodynamic effects of a lower (yet probably still pharmacological) dose of ET-1 can be inhibited by ET_A receptor antagonism, it is possible that a non-ET_A receptor with a lower affinity for ET-1 may exist in the kidney that is insensitive to BQ-123. Furthermore, Cirino *et al.* (1992) reported that ET_A receptor blockade could not completely prevent the vasoconstrictor actions of ET-1 in the pig kidney. ET_A receptor blockade has been shown to inhibit completely the renal vasoconstrictor response to ET-1 in the dog and rabbit (Brooks *et al.*, 1994; Télamaque *et al.*, 1993).

One of the goals of the current study was to determine the role of ET_A receptors in the renal response to big ET-1. Our finding that big ET-1-induced renal vasoconstriction is mediated by ET_A receptors agrees with a preliminary report of Slee *et al.* (1993) who observed that BQ-123 inhibits the increase in renal vascular conductance produced by big ET-1 in anaesthetized rats. Big ET-1 is a less potent renal vasoconstrictor than ET-1 despite being capable of producing a higher degree of systemic hypertension (Pollock & Opgenorth, 1991). In order to make the comparison between big ET-1 and ET-1 more meaningful for the study of renal haemodynamics and excretion, we utilized a lower dose of ET-1 that produced a similar degree of renal vasoconstriction to a high dose of big ET-1. Under these conditions, we observed that the renal vasoconstriction produced by both peptides is mediated by ET_A receptors. The difference in potency between big ET-1 and ET-1 to produce haemodynamic changes in the kidney as compared to other vascular beds has been previously reported (Hoffman *et al.*, 1990; Pollock & Opgenorth, 1991). Although the reason for this observation has not been elucidated, it can be postulated that conversion of big ET-1 by the endothelin converting enzyme may occur at a lower rate in the kidney.

Our results confirm the findings of Hoffman and colleagues (1990) that big ET-1, but not ET-1, is a diuretic agent. However, their results were somewhat complicated by the use of doses of ET-1 that produced a much larger decrease in GFR compared to big ET-1. In our experiments, the divergent effects of big ET-1 and ET-1 on sodium and water excretion were somewhat unexpected given our experimental design that the doses be chosen based on equivalent degrees of renal vasoconstriction. We observed that ET-1 had no natriuretic activity at the dose used and that ET_A receptor antagonism prevented the haemodynamic but not the renal excretory response to big ET-1. Several possible mechanisms could explain these findings. First, since it is known that ET-1 can be metabolized by brush-border endopeptidases (Abassi *et al.*, 1992; Asaad *et al.*, 1993), it is possible that the additional 11 amino acids on big ET-1 could protect it from proteolytic degradation thus facilitating access of mature ET-1 to tubular endothelin receptors. Also, there are a few studies that suggest a direct effect of big ET-1, producing certain haemodynamic effects independent of conversion to ET-1 (Auguet *et al.*, 1992; Salvati *et al.*, 1992). Since the ET_B receptor has been linked to endothelium-dependent vasodilatation (Warner *et al.*, 1989), we can hypothesize that big ET-1 may produce ET_B receptor mediated increases in medullary blood flow as a mechanism for producing diuresis and natriuresis. However, there is no direct evidence to support this idea at present. Other possibilities are that big ET-1 may directly activate a non-ET_A receptor or that big ET-1 is converted by an endothelin converting enzyme that allows it to gain access to a site not accessible to exogenous ET-1.

Our results are consistent with the idea that the diuretic and natriuretic effects of big ET-1 are mediated by non-ET_A receptors since BQ-123 did not prevent these effects. In fact, the potentiation of the diuretic and natriuretic responses by BQ-123 suggest that the intense renal vasoconstriction decreases the filtered load thus attenuating the increases in water and sodium excretion. Although the mechanism of inhibiting reabsorption is not clear, it does not appear to be a pressure-dependent phenomenon since BQ-123 blocked the pressor response to big ET-1 yet the changes in urine flow rate and sodium excretion remained intact. Zeidel *et al.* (1989) have reported that ET-1 can inhibit tubular Na⁺-K⁺-ATPase activity as a means of inhibiting tubular sodium reabsorption. There is also increasing evidence that ET_B receptors located on inner medullary collecting duct cells may be responsible for inhibiting vasopressin-stimulated increases in water reabsorption (Terada *et al.*, 1992; Migas *et al.*, 1993).

While ET-1 was roughly 10 fold more potent than big ET-1 in terms of renal vasoconstriction, this difference was not evident with respect to changes in mean arterial pressure and haematocrit. We have now observed that ET-1 can activate multiple receptor subtypes in the kidney, yet increases in mean arterial pressure and haematocrit appear to be mediated exclusively by ET_A receptors. Big ET-1 also increases these variables via ET_A receptor activation.

In conclusion, we have provided evidence that ET-1 can activate ET_A receptors to produce vasoconstriction in the rat kidney. Since ET-1 has been proposed as a mediator of detrimental haemodynamic effects in various forms of renal failure, ET_A receptor antagonists may provide useful pharmacological tools for elucidating the physiological and pathophysiological role of ET-1 in the kidney (Benigni *et al.*, 1993; Mino *et al.*, 1993). This does not preclude an important role for non-ET_A receptors in the renal vasculature. Our study also demonstrated that big ET-1 inhibits tubular reabsorption of sodium and water by a non-ET_A receptor and non-pressure-dependent mechanism. Therefore, the role of ET_B receptors in the control of sodium and water excretion warrants further investigation.

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