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Mediation via different receptors of the vasoconstrictor effects of endothelins and sarafotoxins in the systemic circulation and renal vasculature of the anaesthetized rat

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1 Using endothelin-1 (ET-1), endothelin-3 (ET-3), sarafotoxin 6b (SX6b) and sarafotoxin 6c (SX6c) as agonists and BQ-123 as a selective ET_A receptor antagonist, we have examined the endothelin receptor subtypes mediating the systemic pressor and renal vasoconstrictor effects of the ET/SX family of peptides.

2 In anaesthetized rats, bolus intravenous injections of ET-1, ET-3, SX6b or SX6c (0.1, 0.25 and 0.50 nmol kg^{-1}) produced initial transient depressor responses followed by sustained and dose-dependent increases in mean arterial pressure (MAP) with the following rank order of potency: SX6b > ET-1 >> SX6c > ET-3. In contrast, in the renal vasculature these peptides caused equipotent dose-dependent falls in renal blood flow (RBF) (ET-1 = ET-3 = SX6b = SX6c).

3 BQ-123 (1 mg kg^{-1}, i.v. bolus) significantly reduced the systemic pressor effects of all the peptides but was largely ineffective against the renal vasoconstrictions.

4 These results indicate that although the systemic pressor effects of the ET/SX peptides are mediated via ET_A receptors, the vasoconstriction in the kidney *in vivo* may be mediated predominantly via ET_B-like receptors. This may be of therapeutic relevance, for an ET_A-receptor-selective antagonist could offer only poor protection of the renal circulation from the deleterious effects of endogenously produced members of this peptide family.

Keywords: Endothelin; sarafotoxin; kidney; BQ-123

Introduction

In the kidney, locally administered endothelin-1 (ET-1) induces vasoconstriction resulting in an increase in renal vascular resistance (RVR) and a decrease in renal blood flow (Badr *et al.*, 1989; King *et al.*, 1989; Kon *et al.*, 1989; Miller *et al.*, 1989). ET-1 also contracts mesangial cells which results in a further decrease in the glomerular filtration rate (Badr *et al.*, 1989; King *et al.*, 1989). Circulating levels of ET-1 have been reported to be increased in both experimental models and clinical studies of acute and chronic renal failure (Firth *et al.*, 1988; Koyama *et al.*, 1989; Warrens *et al.*, 1990; Brooks *et al.*, 1991) and infusion of anti-ET-1 antibodies protects the renal vasculature against ischaemia/reperfusion injury or cyclosporin nephrotoxicity (Kon *et al.*, 1989; 1990). Thus, there is good evidence that endothelins may be involved in renal diseases and that selective ET-receptor antagonists might be useful in the therapy of such diseases.

Two ET-receptor subtypes have been characterized. The ET_A receptor, which has a higher affinity for ET-1, ET-2 and sarafotoxin (SX) 6b than for ET-3 or SX6c (Arai *et al.*, 1990), and the ET_B receptor which is non-isopeptide selective (Sakurai *et al.*, 1990). It has been generally assumed (Masaki *et al.*, 1991) that the ET_A receptor is located on vascular smooth muscle cells, where it mediates the constrictor responses, and the ET_B receptor on the endothelial cell, where it mediates prostacyclin and endothelium-derived relaxing factor (EDRF) release by endothelins (De Nucci *et al.*, 1988; Warner *et al.*, 1989). There may also be a third subtype of ET receptor selective for ET-3 and SX6c (Kloog *et al.*, 1989; Emori *et al.*, 1990; Samson *et al.*, 1990; Harrison *et al.*, 1992).

We have studied which endothelin receptor subtype(s) mediate(s) renal vasoconstriction. For these experiments we used the ET/SX peptides as selective agonists, and the cyclic pentapeptide BQ-123 (cyclo(D-Asp-L-Pro-D-Val-L-Leu-D-trp) (Ihara *et al.*, 1992), an ET_A-receptor-selective antagonist.

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Methods

Surgical procedures

Wistar rats (male, 300–400 g) were anaesthetized with thiopentone sodium (120 mg kg^{-1}, i.p.). The trachea was cannulated to facilitate respiration and body temperature was maintained at 37°C by means of a rectal probe connected to a homeothermic blanket (Bioscience, Sheerness, Kent). The right carotid artery was cannulated and connected to a pressure transducer (Transamerica type 4-422-001) for the measurement of arterial blood pressure which was displayed on a polygraph (Grass 7D, Grass Instruments, Quincy, MA, U.S.A.). Mean arterial blood pressure (MAP) was calculated as diastolic pressure plus one third of the difference between the diastolic and systolic pressures. The left jugular vein was cannulated for the administration of drugs and the right femoral vein for the administration of saline (1.5 ml h^{-1}) to compensate for any fluid loss.

The left kidney was exposed via a mid-line laparotomy and the renal artery was carefully isolated. An ultrasonic flow probe (1RB, internal diameter = 1 mm), embedded in a silicone cuff to provide optimal alignment, was placed around the left renal artery for measurement of total renal blood flow (RBF) using a Transonic T206 Small Animal Flowmeter (Transonic Systems Inc., New York, U.S.A.). A small amount of acoustical couplant (100 mg Nalco 1181, mixed with 10 ml distilled water; Nalco Chemical Co., IL., U.S.A.) was deposited in the acoustic window of the probe adjacent to the artery, in order to displace all air. Renal vascular resistance

(RVR) was calculated by dividing MAP by RBF. Under these conditions in preliminary control experiments MAP, RBF and autoregulation were stable up to 6 h ($n = 3$).

Experimental procedures

After surgery and a stabilization period of at least 30 min, animals ($n = 4$ for each group) were given bolus intravenous injections of 0.1, 0.25, 0.5 and 1 nmol kg^{-1} of ET-1, ET-3, SX6b or SX6c. The time between each injection was 1 h. Five minutes prior to each dose of peptide, animals received either BQ-123 (1 mg kg^{-1}) or vehicle (0.9% w/v sodium chloride containing 10 mM sodium bicarbonate), as i.v. bolus injections of 1 ml kg^{-1}. This dose and time course of BQ-123 administration is effective in antagonizing the pressor effects of the ET/SX peptides (McMurdo *et al.*, 1993).

Changes in MAP and RBF were calculated 3 min after the injection of the ET/SX peptides at which time the initial depressor response was ended, but the renal vasoconstriction was still maintained for all doses of the ET/SX peptides.

Materials

Trapanal (thiopentone sodium) was obtained from Byk Gulden (Konstanz, Germany). ET-1, ET-3 and SX6b were purchased from the Peptide Institute (Osaka, Japan), and SX6c from Peninsula Laboratories Inc. (Belmont, U.S.A.). The peptides were reconstituted in 0.1% v/v acetic acid and then dissolved in 0.9% w/v saline containing 1% w/v bovine serum albumin and 10 mM sodium bicarbonate. We thank Drs A. Doherty and W. Cody of the Chemistry Department of Parke-Davis (Ann Arbor, MI, USA) for providing the BQ-123 which was dissolved in 0.9% w/v saline containing 10 mM sodium bicarbonate.

Statistics

Statistical differences between points were determined by an unpaired two tail Student's t test and $P < 0.05$ was taken to reflect significant difference.

Results

Systemic and renal effects of ET/SX peptides

Basal values for the MAP, RBF and RVR were 120 ± 2 mmHg, 9.4 ± 0.3 ml min^{-1} and 13.2 ± 0.5 mmHg min^{-1} ml^{-1}, respectively ($n = 32$, for each). These values were unaffected by vehicle or BQ-123 at the (1 mg kg^{-1}) dose used in this study.

Injection of all peptides produced similar initial and transient falls in MAP (data not shown). This depressor effect was followed by a sustained, dose-dependent and isopeptide-selective increase in MAP (Figure 1a, Figure 3). The maximum increase in MAP was obtained within 10 min for all peptides except SX6c, in which case the peak occurred at 20 min.

In the kidney, all the peptides produced similar falls in RBF (Figure 2a, Figure 3) associated with a dramatic increase in RVR. The constrictor effect in the renal vasculature reached a maximum within 1 min (Figure 3). At doses greater than 0.25 nmol kg^{-1} the RBF was still affected 1 h after injection although MAP had returned to control levels.

Inhibition by BQ-123 of ET/SX pressor responses

BQ-123 (1 mg kg^{-1}) significantly reduced the systemic pressor effects of SX6b at all doses and of the other ET/SX peptides at doses of 0.25 mmol kg^{-1} and higher (Figure 1b, Figure 3). For instance, 3 min after injection, the increases in MAP induced by ET-1, SX6b, SX6c and ET-3 at 0.5 nmol kg^{-1} were 10 ± 1 mmHg, 4 ± 1 mmHg, 0 ± 4 mmHg and 3 ± 1 mmHg,

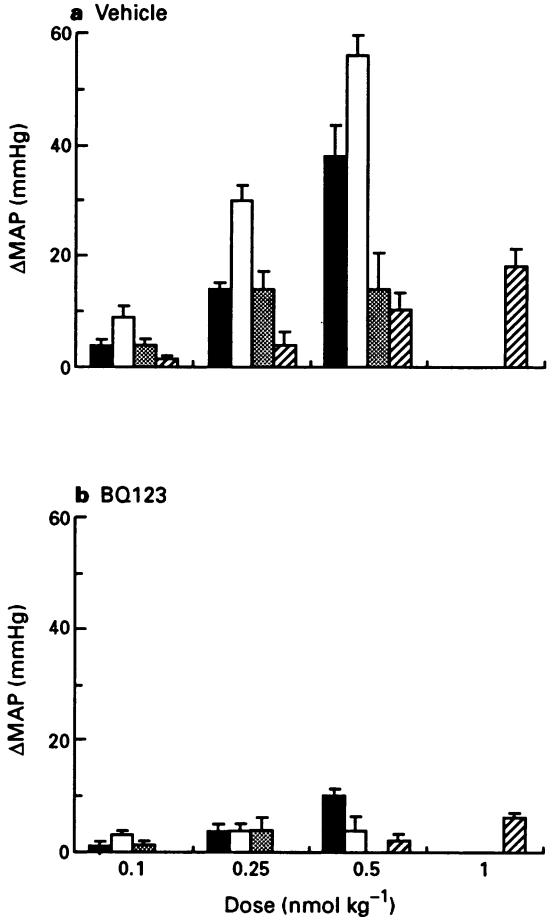

Figure 1 The effect of BQ-123 on the pressor effects of endothelin-1 (ET-1), sarafotoxin 6b (SX6b), SX6c or ET-3 in the anaesthetized rat. (a) The ET/SX peptides (0.1–1 nmol kg^{-1}) produced dose-dependent elevations in blood pressure, 3 min after i.v. administration. Data for 1 nmol kg^{-1} is only shown for ET-3, due to the high mortality in the other groups. (b) Responses to the same doses of the ET/SX peptides in animals pretreated with BQ-123 at 1 mg kg^{-1}, 5 min prior to the peptide. Closed columns, ET-1; open columns, SX6b; stippled columns, SX6c; hatched columns, ET-3. Each column with a vertical bar represents the mean ± s.e.mean from 4 observations.

respectively. In the control experiments the values were 38 ± 6 mmHg, 56 ± 3 mmHg, 14 ± 6 mmHg and 10 ± 4 mmHg ($n = 4$ for each, $P < 0.05$). BQ-123 did not affect the magnitude of the falls in MAP induced by any of the peptides but prolonged their durations (data not shown).

Lack of effect of BQ-123 on renal vasoconstrictions induced by ET/SX peptides

BQ-123 failed to prevent the fall in RBF induced by the ET/SX peptides (Figure 2b, Figure 3). For instance, 3 min after injection, the falls in RBF induced by ET-1, SX6b, SX6c or ET-3 at 0.5 nmol kg^{-1} were 84 ± 5%, 84 ± 9%, 70 ± 9%, 64 ± 5%, respectively. In the control experiments the values were 69 ± 6%, 90 ± 3%, 81 ± 7%, 74 ± 6% ($n = 4$ for each, $P > 0.05$). When RVR values were calculated, BQ-123 significantly decreased the renal vasoconstrictor effects of ET-1 (0.1 nmol kg^{-1}) and SX6b (0.1 and 0.25 nmol kg^{-1}) (Table 1). At higher doses of either ET-1 or SX6b, the very large increases in RVR, due to the great reductions in RBF, were not significantly attenuated by BQ-123. BQ123 did not affect the changes in RVR induced by either ET-3 or SX6c (Table 1). BQ-123 also did not prevent the prolonged effects of the ET/SX peptides at doses greater than 0.25 nmol kg^{-1}.

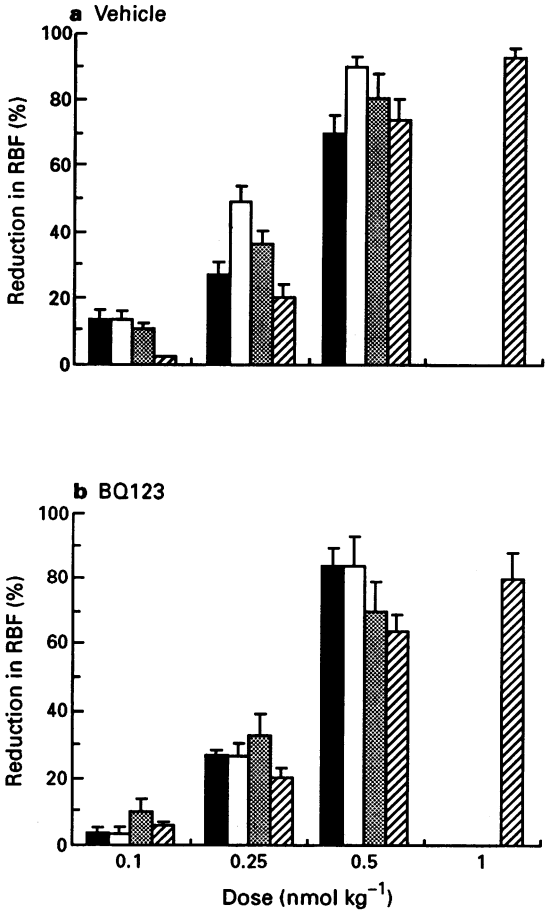

Figure 2 Lack of effect of BQ-123 on the reductions in renal blood flow induced by endothelin-1 (ET-1), sarafotoxin 6b (SX6b), SX6c or ET-3 in the anaesthetized rat. (a) The ET/SX peptides (0.1–1 nmol kg^{-1}) produced dose-dependent falls in renal blood flow 3 min after i.v. administration, expressed as the % reduction in flow from the level immediately prior to peptide injection. Data for 1 nmol kg^{-1} is only shown for ET-3, due to the high mortality in the other groups. (b) Effects of the same doses of the ET/SX peptides in animals pretreated with BQ-123 at 1 mg kg^{-1}, 5 min prior to the peptide. Closed columns, ET-1; open columns, SX6b; stippled columns, SX6c; hatched columns, ET-3. Each column with a vertical bar represents the mean ± s.e.mean from 4 observations.

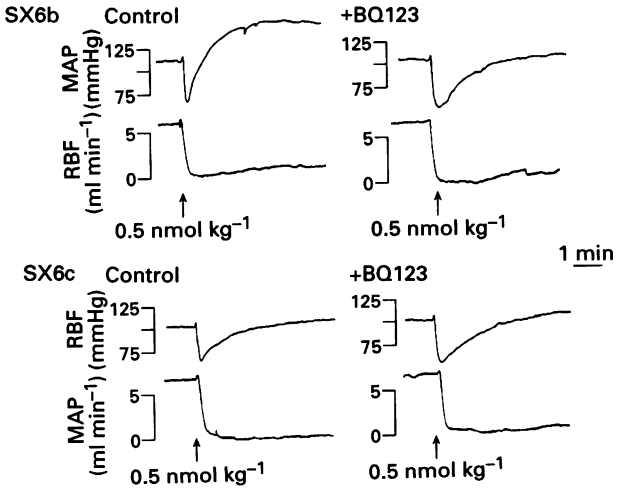

Figure 3 Representative traces of the effect of BQ-123 on the mean arterial blood pressure and renal vasoconstrictor responses to sarafotoxin 6b (SX6b) or SX6c. SX6b or SX6c were administered as i.v. bolus injections (0.5 nmol kg^{-1}). Vehicle or BQ-123 (1 mg kg^{-1}) was administered as an i.v. bolus 5 min prior to the peptide injection. MAP, mean arterial pressure; RBF, renal blood flow.

Table 1 The changes in renal vascular resistances calculated 3 min after bolus intravenous injections of endothelin-1 (ET-1), ET-3, sarafotoxin 6b (SX6b) or SX6c (0.1 or 0.2 nmol kg^{-1})

	0.1 nmol kg^{-1}		*0.25 nmol kg^{-1}*	
	Control	*+ BQ-123*	*Control*	*+ BQ-123*
ET-1	2.1 ± 0.4	0.6 ± 0.2*	7.7 ± 0.3	5.3 ± 1.3
SX6b	3.5 ± 1.0	−0.2 ± 0.1*	23.3 ± 2.3	6.3 ± 0.9*
SX6c	2 ± 0.5	1.9 ± 0.8	11.9 ± 2.2	8.9 ± 2.8
ET-3	0.4 ± 0.2	0.4 ± 0.2	3.6 ± 0.7	2.8 ± 0.5

*Indicates significant differences in the RVR calculated in control versus BQ-123 pretreated (1 mg kg^{-1}, 5 min prior to peptide) animals.

Discussion

This study demonstrates that the vasoconstrictor effects of the ET/SX peptides in the systemic circulation and renal vasculature are mediated by pharmacologically distinct receptor subtypes. The rises in MAP were isopeptide-selective, for ET-1 or SX6b were clearly more potent than ET-3 or SX6c and the elevations in MAP were largely, but not completely, blocked by BQ-123, a selective ET_A antagonist, as described previously (Ihara *et al.*, 1991a; Pollock *et al.*, 1992; McMurdo *et al.*, 1993). This is indicative of a dominant role for the ET_A receptor in these responses. At the same time it is noteworthy that BQ-123 also decreased the pressor effects of SX6c, which has been described as an agonist selective for ET_B receptors (Williams *et al.*, 1991). This may suggest either the presence of another receptor type or that there is an interaction between SX6c and ET_A receptors, or between BQ-123 and ET_B receptors. The vasoconstriction induced by the ET/SX peptides in the renal vascular bed was not isopeptide selective, as the peptides were equipotent, and BQ-123 failed to prevent the ET/SX-induced fall in RBF. At the same time SX6b at a dose of 0.5 nmol kg^{-1} caused a greater increase in RVR than the other ET/SX peptides at the same dose. Taken together with the finding that BQ-123 depressed the reductions in RVR caused by the lowest doses of ET-1 or SX6b, these data are consistent with the premise that the vasoconstriction in the renal vasculature may be mediated via a mixed receptor population. ET_A receptors appear to mediate a proportion of the response to the more selective ET_A agonists whereas the renal vasoconstrictions induced by other ET/SX peptides or to higher doses of ET-1 or SX6b are mediated by another, possibly ET_B, receptor the activation of which can compensate for the blockade of the ET_A-mediated pathway.

Until recently, it was generally thought that the ET_A receptor was the predominant subtype mediating the vasoconstrictor and pressor responses to ET/SX peptides (Ihara *et al.*, 1991a; Masaki *et al.*, 1991; Pollock *et al.*, 1992). The suggestion of non-ET_A-receptors mediating vasoconstrictions first came from *in vitro* studies with selective ET_A receptor antagonists, for in some tissues these antagonists did not fully antagonise endothelin-induced contractions (Ihara *et al.*, 1991b; Fukuroda *et al.*, 1992; Warner *et al.*, 1992). In addition, we have also shown that BQ-123 cannot completely block the pressor effects of ET/SX peptides in the anaesthetized ganglion-blocked rat (McMurdo *et al.*, 1993). The pharmacological characteristics of the responses in most of these reports suggest that this non-ET_A-mediated vasoconstriction is due to ET_B receptor activation (Ihara *et al.*, 1991b; Williams *et al.*, 1991; Fukuroda *et al.*, 1992).

It has been shown previously that ET-1 elicits a strong renal arteriolar vasoconstriction (Badr *et al.*, 1989; King *et al.*, 1989; Miller *et al.*, 1989; Kon *et al.*, 1989). Although the receptor subtype responsible for this effect has not been characterized, [^{125}I]ET-1 binding studies have suggested that binding sites in kidney homogenates are principally of the

ET_B-receptor subtype (Ihara *et al.*, 1991a). These receptors may be located on the vascular smooth muscle of the kidney, or they may be present on another cell type, not necessarily within the kidney, and cause renal vasoconstriction via an indirect mechanism such as activation of the renin-angiotensin system (Miller *et al.*, 1989) or release of a vasoconstrictor autacoid like thromboxane A_2 (Rae *et al.*, 1989). These systems may even be activated by the substantial initial depressor response which would affect autoregulation of kidney blood flow and GFR leading to elevation of angiotensin II, for instance, and so renal vasoconstriction. On the other hand the rapidity of the renal vasoconstriction does argue for a more direct mechanism, at least for the initial phase of the response.

In conclusion, this study demonstrates that in the anaesthetized rat the pressor effects of the ET/SX peptides and the vasoconstrictions they induce in the renal vasculature are predominantly mediated via two different receptor subtypes, respectively ET_A and probably ET_B. This finding attests to the fact that there is a heterogeneity in the distribution of ET/SX receptor subtypes in the circulation. This observation may have therapeutic relevance for an ET_A-receptor selective antagonist might not protect the renal circulation from the deleterious effects of endogenously produced members of this peptide family.

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