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Incomplete inhibition of the pressor effects of endothelin-1 and related peptides in the anaesthetized rat with BQ-123 provides evidence for more than one vasoconstrictor receptor

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1 The effects of the ET_A receptor antagonist, BQ-123 on blood pressure changes induced by various members of the endothelin (ET)/sarafotoxin (SX) peptide superfamily were investigated in the anaesthetized rat.

2 ET-1 (1 nmol kg^{-1}, i.v. bolus) induced a sustained increase in mean arterial pressure (MAP, maximum increase 44 ± 3 mmHg). Intravenous injection of BQ-123 at 0.2, 1.0 or 5.0 mg kg^{-1} 5 min before ET-1 inhibited the pressor response by 18, 50 and 61%, respectively. The ET-1 pressor response was inhibited by 75% when the peptide was given 60 min after the start of a 120 min i.v. infusion of BQ-123 (0.2 mg kg^{-1} min^{-1}).

3 In addition to ET-1, BQ-123 (1 mg kg^{-1}, i.v. bolus) attenuated the pressor responses to big ET-1 (1 nmol kg^{-1}, i.v., bolus, maximum increase in MAP: 68 ± 7 mmHg), ET-3 (3 nmol kg^{-1}, i.v., bolus, maximum response: 30 ± 3 mmHg), SX6b (1 nmol kg^{-1}, i.v., bolus, maximum response: 41 ± 5 mmHg) and SX6c (1 nmol kg^{-1}, i.v., bolus, maximum response: 24 ± 4 mmHg) by 65, 60, 88 and 50%, respectively.

4 With the exception of big ET-1, all the peptides used in this study induced an initial transient depressor response (−32 ± 3 mmHg, $n = 18$). Although BQ-123 (1 mg kg^{-1}, i.v., bolus) did not affect the absolute magnitude of the fall in MAP, the ET_A receptor antagonist significantly prolonged the depressor responses induced by ET-3 and SX6b.

5 Thus, BQ-123 attenuates the pressor, but not the depressor effects of ET-1, big ET-1, ET-3, SX6b and SX6c. Complete inhibition of the pressor responses could not be achieved, suggesting that a component of the pressor response is not mediated via the ET_A receptor.

Keywords: Endothelin; sarafotoxin; ET_A receptor; ET_B receptor; BQ-123

Introduction

The endothelins (ETs) and sarafotoxins (SXs) constitute a family of structurally homologous peptides composed of 21 amino acids, of which there are now considered to be at least 8 members. ET-1, ET-2 and ET-3 are found in mammalian tissues (see Simonson & Dunn, 1990), and the sarafotoxins SX6a, SX6b, SX6c, and SX6d are constituents of the venom of the Israelia burrowing asp, *Atractaspis engaddensis* (Kloog *et al.*, 1988; Bdolah *et al.*, 1989). Murine vasoactive intestinal contractor (VIC) is an additional member of this peptide family (Saida *et al.*, 1989). A number of studies have demonstrated a widespread tissue distribution of specific ET receptors (see Randall, 1991).

At present the cDNAs encoding two ET receptors, classified as ET_A and ET_B have been cloned and expressed. Each receptor contains 7 trans-membrane domains and shows remarkable similarity to the rhodopsin receptor and other G protein coupled receptors (see Webb, 1991). The ET_A receptor is highly selective for ET-1 and is characterized by the rank order of binding affinities: ET-1 > SX6b > ET-3 (Arai *et al.*, 1990), while the ET_B receptor is non-isopeptide selective (ET-1 = ET-3) (Sakurai *et al.*, 1990). The existence of a third ET receptor has been proposed, present on bovine endothelial cells (Emori *et al.*, 1990) and probably mediating the release of nitric oxide (NO) (Emori *et al.*, 1991). It may be similar to the receptor present in porcine isolated pulmonary and coronary vessels (Fukuroda *et al.*, 1992).

Elevated levels of circulating ET-1 have been reported in a variety of cardiovascular diseases, including hypertension, renal failure, myocardial ischaemia and subarachnoid haemorrhage (see Thiemermann, 1991). However, it is not yet clear whether ET-1 is involved in the pathogenesis of these disorders. Clarification will only be obtained with the use of potent and selective ET receptor antagonists for assessing the contribution of the ETs to physiology and pathophysiology. BQ-123 (cyclo[D-Asp-L-Pro-D-Val-L-Leu-D-Trp]) antagonizes ET-1-induced constriction of porcine isolated coronary artery strips and inhibits ET-1 binding to porcine aortic smooth muscle cells (Ihara *et al.*, 1992) and is therefore a putative ET_A receptor antagonist.

Here, we have investigated the effects of BQ-123 on blood pressure changes induced by ET-1, big ET-1, ET-3, SX6b and SX6c in the anaesthetized rat.

Methods

Surgical procedure

Male Wistar rats (250–400 g) were anaesthetized with Trapanal (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 (Harvard, Edenbridge, Kent). The right carotid artery was cannulated and connected to a pressure transducer (Transamerica type 4-422-0001) for the measurement of systemic blood pressure, from which mean arterial blood pressure (MAP) and heart rate were derived and recorded on a Grass 7D polygraph (Grass Instruments, Quincy, Mass., U.S.A.). The left jugular and right femoral veins were cannulated for the administration of drugs.

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Experimental design

After surgery, all animals were allowed to stabilize for 20 min before receiving a 5 min infusion of hexamethonium (10 mg kg^{-1}, i.v.). Twenty min later, animals were given either BQ-123 (0.2, 1.0 or 5.0 mg kg^{-1}, i.v., bolus; approximately 0.28, 1.4 and 7 μmol kg^{-1}) or vehicle (0.9% w/v saline containing 10 mM sodium bicarbonate, 1 ml kg^{-1}). Five minutes later, animals ($n = 4-6$ for each group) were given either ET-1 (1 nmol kg^{-1}), ET-3 (3 nmol kg^{-1}), big ET-1 (1 nmol kg^{-1}), SX6b (1 nmol kg^{-1}) or SX6c (1 nmol kg^{-1}), all as i.v. bolus injections. In a separate series of experiments BQ-123 was administered as an infusion of 0.2 mg kg^{-1} min^{-1} (preceded by a loading dose of 1 mg kg^{-1}) for 60 min prior to and continued for a further 60 min following an i.v. bolus injection of ET-1 (1 nmol kg^{-1}; $n = 6$).

Calculations of changes in mean arterial pressure (MAP)

Changes in MAP were calculated as the peak increase or decrease of MAP (mmHg) from control levels. In some experiments, the changes in MAP were calculated as the area under the curve, expressed in mm^2, to permit comparisons between the durations of depressor responses induced by the various peptides.

Materials

Sodium-thiopentone (Trapanal) was obtained from Byk Gulden (Konstanz, Germany). Hexamethonium bromide, phenylephrine and angiotensin II were purchased from Sigma Chemical Co. (Poole, Dorset) and were dissolved in 0.9% w/v saline. ET-1, ET-3, big ET-1 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% acetic acid and then diluted in 0.9% w/v saline containing 1% w/v bovine serum albumin and 0.06% sodium bicarbonate. BQ-123 was synthesized by the Parke-Davis Pharmaceutical Research Division of Warner-Lambert Co. (Ann Arbor, U.S.A.) and was dissolved in 0.9% w/v saline containing 10 mM sodium bicarbonate. Aliquots of the peptides and BQ-123 were stored frozen (−20°C) until use.

Statistical comparisons

All values in the figures and text are expressed as mean ± s.e.mean of n observations. Statistical evaluation of the data was by Student's t test for unpaired determinations or by ANOVA. A P value of <0.05 was considered significant.

Results

Mean resting values for mean arterial blood pressure (MAP) were 109 ± 2 mmHg ($n = 74$) and for heart rate were 377 ± 5 beats min^{-1} ($n = 74$). Treatment with hexamethonium (2 mg kg^{-1} min^{-1} for 5 min) caused MAP to fall to 83 ± 1 mmHg and heart rate to 330 ± 4 beats min^{-1}. These values were unaffected by vehicle or BQ-123 at any of the doses used in this study.

Duration of action of BQ-123

BQ-123 (1 mg kg^{-1}) was given as an i.v. bolus injection 1, 5, 15 or 30 min before ET-1 (1 nmol kg^{-1}, i.v., bolus; $n = 4-6$) or as an infusion 60 min before and continuing for 60 min after ET-1 ($n = 6$). BQ-123 was ineffective when given 30 or 15 min prior to ET-1. Furthermore, BQ-123 was no more effective when given 1 min rather than 5 min before ET-1 (data not shown). A pretreatment time of 5 min (for BQ-123 bolus injections) was, therefore, used in this study.

BQ-123 inhibits ET/SX induced pressor responses

ET-1 (1 nmol kg^{-1}) produced a sustained increase in arterial blood pressure which reached a maximum (44 ± 3 mmHg) within 5 min and returned to control levels within 60 min (Figure 1a). The peak effect was decreased significantly to 22 ± 3 mmHg ($n = 6$) and 17 ± 3 mmHg ($n = 6$) by 1 and 5 mg kg^{-1} BQ-123 (i.v., bolus), respectively (Figure 2b). BQ-123 at 5 mg kg^{-1} did not cause a significantly greater reduction in either the maximum response or the duration of the ET-1 induced pressor effect than 1 mg kg^{-1} (Figure 2a and 2b). In comparison to BQ-123 at 1 and 5 mg kg^{-1}, 0.2 mg kg^{-1} was clearly a threshold dose for inhibition of the ET-1 pressor response (Figure 2a and 2b). BQ-123 at 10 mg kg^{-1} (i.v., bolus) was not more effective than at lower doses ($n = 2$, data not shown). BQ-123 (1 mg kg^{-1}, i.v., bolus) was, therefore, selected for use in subsequent experiments with the other ET/SX agonists.

SX6c or SX6b (1 nmol kg^{-1}) or big ET-1 (1 nmol kg^{-1}) caused peak increases in MAP of 24 ± 4 mmHg ($n = 4$), 41 ± 5 mmHg ($n = 4$) and 67 ± 7 mmHg ($n = 6$), respectively. ET-3 at 3 nmol kg^{-1} increased MAP by 30 ± 3 mmHg ($n = 4$). SX6c at 3 nmol kg^{-1} caused rapid death (2 out of 2 experiments). The maximum elevations of MAP were seen at 5–10 min after peptide injection except with SX6c where the peak occurred at 20 min (Figure 3a–3d). The peak pressor effects of SX6b, big ET-1, ET-3, SX6c and ET-1 were decreased in the presence of BQ-123 to 12, 35, 40, 50 and 50% of control, respectively. The ET-1-induced increase in MAP was reduced by 75% when the peptide was given at 60 min after the start of a 120 min infusion of BQ-123 (0.2 mg kg^{-1} min^{-1}) (Figure 1c).

BQ-123 did not inhibit the pressor actions of phenylephrine or angiotensin II. Phenylephrine (0.1 μmol kg^{-1}, i.v., bolus; $n = 4$) increased MAP by 62 ± 6 mmHg in the absence and 66 ± 5 mmHg in the presence of BQ-123. Angiotensin II (1 nmol kg^{-1}, i.v., bolus; $n = 4$) increased MAP by 76 ± 4

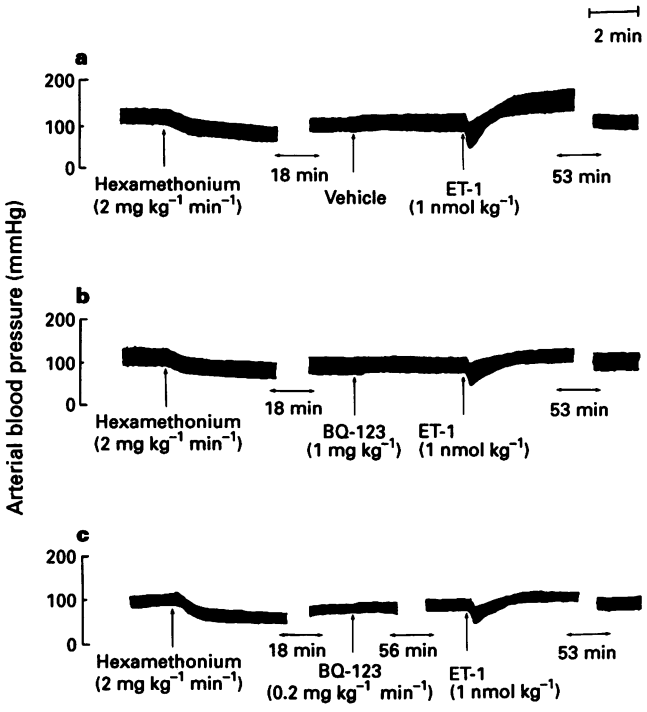

Figure 1 The figure shows three representative, original traces comparing endothelin-1 (ET-1, 1 nmol kg^{-1}, i.v., bolus)-induced pressor responses in (a) vehicle-treated rats; (b) rats given BQ-123 (1 mg kg^{-1}, i.v., bolus) 5 min prior to ET-1 injection and (c) rats given ET-1 at 60 min after the start of an infusion of BQ-123 (loading dose of 1.0 mg kg^{-1} then 0.2 mg kg^{-1} min^{-1} for 120 min, i.v.).

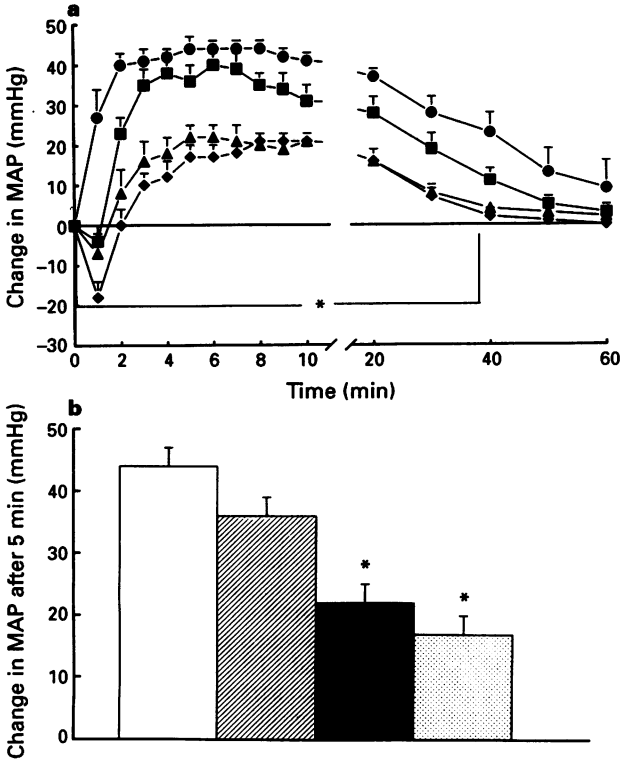

Figure 2 BQ-123 inhibits endothelin-1 (ET-1) pressor responses in a dose-dependent manner. (a) Time course of the effect of ET-1 (1 nmol kg^{-1}, i.v., bolus given at time = 0 min) on mean arterial pressure (MAP). Five minutes prior to ET-1 injection, different animals received either vehicle (●, $n = 6$), BQ-123 at 0.2 mg kg^{-1} (■, $n = 5$), BQ-123 at 1.0 mg kg^{-1} (▲, $n = 6$) or BQ-123 at 5.0 mg kg^{-1} (◆, $n = 6$), all as i.v. bolus injections. (b) BQ-123 inhibits the ET-1-induced rise in MAP (at 5 min) in a dose-dependent fashion. For example, 0.2 mg kg^{-1} (hatched column; $n = 6$) caused 18% inhibition and 1.0 mg kg^{-1} (solid column; $n = 6$) caused 50% inhibition. While, 5.0 mg kg^{-1} (stippled column; $n = 6$) produced 61% inhibition. Data are expressed as mean ± s.e. mean (vertical bars) of n observations. *$P < 0.05$ when compared to vehicle control.

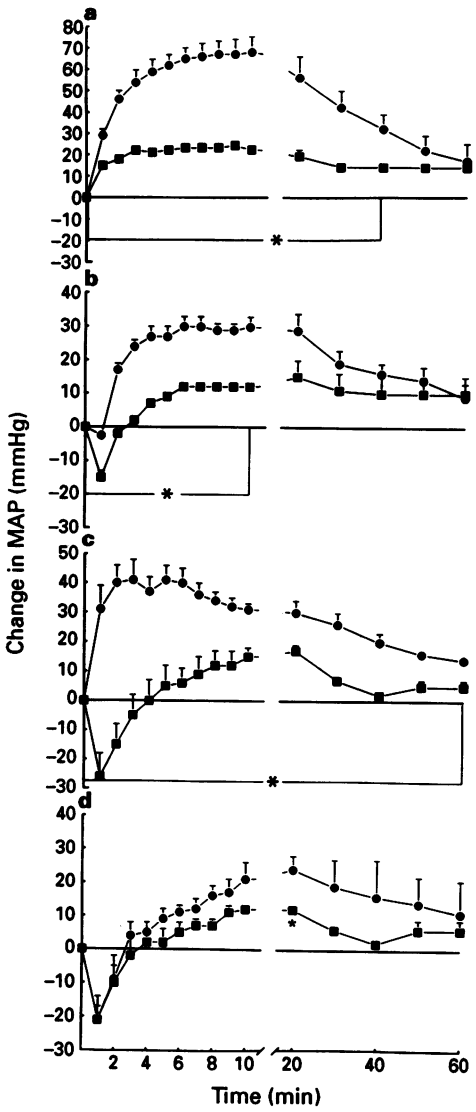

Figure 3 BQ-123 inhibits the pressor responses elicited by big endothelin 1 (ET-1), ET-3, sarafotoxin 6b (SX6b) or SX6c in the anaesthetized rat. Different groups of animals received (a) big ET-1 (1 nmol kg^{-1}; $n = 4$); (b) ET-3 (3 nmol kg^{-1}; $n = 4$); (c) SX6b (1 nmol kg^{-1}; $n = 4$) or (d) SX6c (1 nmol kg^{-1}; $n = 4$), all as i.v. bolus injections at time 0 min in the absence (●, vehicle-treatment) or presence (■) of BQ-123 (1 mg kg^{-1}, i.v. bolus). Data are mean ± s.e. mean (vertical bars) of n observations.
*$P < 0.05$ when compared to vehicle control.

mmHg in the absence and 79 ± 4 mmHg in the presence of BQ-123.

BQ-123 prolongs the duration of the ET/SX induced depressor responses

Injection of all peptides except big ET-1 produced an initial depressor response. ET-1 (1 nmol kg^{-1}), ET-3 (3 nmol kg^{-1}), SX6b (1 nmol kg^{-1}) and SX6c (1 nmol kg^{-1}) caused similar falls in MAP (ranging from −25 ± 5 mmHg to −36 ± 3 mmHg; data not shown). However, the SX6c-induced depressor response was longer than the other peptides (Figure 3) as confirmed by measurement of area under the curve (Table 1).

Although, BQ-123 (1 mg kg^{-1}) had no effect on the fall in MAP induced by any of the peptides (range: −30 ± 3 mmHg to −40 ± 5 mmHg), it prolonged the depressor actions of ET-1, ET-3 and SX6b. The duration of the SX6c-induced fall in MAP was unaffected by BQ-123 (Table 1).

Discussion

Our results show that the ET_A receptor antagonist, BQ-123, attenuates the pressor but not the depressor responses to ET-1, big ET-1, ET-3, SX6b and SX6c in the anaesthetized rat. BQ-123 was developed from another ET_A receptor antagonist (BE-18257B) which was isolated from the fermentation products of *Streptomyces misakiensis* and found to antagonize ET-1 pressor responses in conscious rats (Ihara *et al.*, 1991). Moreover, BQ-123 is similar to the ET_A receptor antagonist, BQ-153, which also antagonizes ET-1 pressor effects *in vivo* (Ihara *et al.*, 1992).

When ET-1 is injected into an anaesthetized rat there is an initial transient depressor response followed by a sustained pressor response. These changes in arterial blood pressure are mediated via at least two different ET receptors. The receptor responsible for the initial fall in blood pressure appears to be non-isopeptide selective (Inoue *et al.*, 1989). This ET_B receptor may be located on the endothelium and mediate vasodilatation via the release of prostacyclin (Lidbury *et al.*, 1990) or NO (Warner *et al.*, 1989; Whittle *et al.*, 1989). The 'vasoconstrictor' receptor (ET_A) has greater selectivity for ET-1 than ET-3 and is located on the vascular smooth muscle (see Sakurai *et al.*, 1992). Therefore, BQ-123 would be expected to inhibit the ET_A-mediated pressor, but not the

Table 1 Depressor effects of endothelin-1 (ET-1), endothelin-3 (ET-3), sarafotoxin S6b (SX6b) and sarafotoxin S6c (SX6c) in the presence or absence of BQ-123

	Area under the curve (mm^2)	
Peptide	*Control*	*+BQ-123*
ET-1	1.9 ± 0.3	3.4 ± 0.9
ET-3	2.0 ± 0.2	3.4 ± 0.4*
SX6b	1.1 ± 0.2	8.3 ± 2*
SX6c	4.3 ± 0.7	5.0 ± 2

This table shows the depressor responses induced by ET-1 (1 nmol kg^{-1}; $n = 6$), ET-3 (3 nmol kg^{-1}; $n = 4$), SX6b (1 nmol kg^{-1}; $n = 4$) or SX6c (1 nmol kg^{-1}; $n = 4$), all as i.v. bolus injections, in vehicle or BQ-123 (1 mg kg^{-1}, i.v., bolus)-treated rats. Data are mean ± s.e. mean of n observations. *$P < 0.05$ when compared to control.

ET_B-induced depressor responses.

On injection into the anaesthetized rat, the rank order of pressor potency of the peptides we used was: big ET-1 > ET-1 = SX6b > ET-3 ≥ SX6c. These data show that big ET-1 was significantly more potent than ET-1 at the same dose. The reason for this is unclear. The ET-1 pressor response was not completely abolished by any of the doses of BQ-123 used in this study. Even an infusion of BQ-123 (0.2 mg kg^{-1} min^{-1} for 60 min) only reduced the pressor response to 25% of control. Thus, there is a component of the ET-1-induced vasoconstrictor response which cannot be inhibited by BQ-123 and, therefore, may not be mediated via the ET_A receptor. The hypothesis that the ET-1-induced rise in blood pressure is not entirely due to activation of the ET_A receptor is supported by the finding that BQ-123 at concentrations up to 10 mg kg^{-1} does not completely inhibit the pressor action of ET-1 in conscious rats (Ihara *et al.*, 1992). BQ-123 failed to abolish the pressor responses to the other members of the ET/SX superfamily of vasoconstrictor peptides. Although SX6b was equipotent to ET-1 in this study, BQ-123 inhibited the SX6b pressor activity by 88% compared to a reduction of only 50% for ET-1. It is possible that the pressor activity produced by SX6b is mediated almost entirely via the ET_A receptor, while ET-1 may also be acting on another receptor or activating a mechanism which is not blocked by BQ-123. Alternatively, SX6b may have a greater intrinsic activity than ET-1 on the ET_B receptor. This would result in a greater vasodilator response which, when unmasked after inhibition of the ET_A receptor, would contribute to a more pronounced attenuation of the pressor response in the presence of BQ-123.

With the exception of big ET-1 all of the peptides used induced similar vasodepressor effects. Significantly, SX6c produced a longer lasting depressor response than the other peptides and this was unaffected by BQ-123. BQ-123 did not affect the depth of the fall in MAP, but significantly prolonged the depressor action probably by suppressing agonist-induced vasoconstrictor activity. This was best seen in the SX6b-treated animals in which BQ-123 was most effective at inhibiting the pressor response, allowing the depressor response to persist unchallenged by vasoconstriction. This prolonged fall in blood pressure in the presence of BQ-123 may indicate that the ET/SX peptides cause sustained activation of endothelial cells producing NO or prostacyclin. This would give rise to a tonic release of these vasodilators, thereby ameliorating the pressor effects of the peptides. The development of specific ET_B receptor antagonists will help further to characterize this depressor response.

It has been reported that SX6c (in doses up to 0.3 nmol kg^{-1}) is equipotent to SX6b as a pressor agent in the pithed rat and that this rise in blood pressure is an ET_B-mediated effect (Williams *et al.*, 1991). In contrast, in the present study in the ganglion-blocked rat, SX6c caused a smaller and more slowly developing increase in blood pressure when compared to SX6b or any of the other peptides. It has been suggested that there are two distinct phases of ET pressor response: an early phase immediately following the depressor response (3–10 min), followed by a later pressor effect which develops 10–20 min after peptide injection and lasts for more than an hour (Inoue *et al.*, 1989). These phases may be mediated via different receptors or mechanisms and SX6c may elicit only the latter of the phases. In our study the SX6c induced maximum increase in MAP was inhibited (by 50%) by BQ-123, indicating that the SX6c-induced pressor response is not wholly mediated via the ET_B receptor as suggested previously (Williams *et al.*, 1991). However, in non-ganglion blocked rats Clozel and co-authors (1992) have demonstrated that SX6c (0.8 nmol kg^{-1}) produces an increase in MAP which is not affected by BQ-123 (3 mg kg^{-1}). Although the different effects of BQ-123 reported in our study and by Clozel *et al.* may be due to the different preparations used, this seems unlikely for Cristol *et al.* (1992) have recently demonstrated that the dose-dependent increases in MAP obtained with 0.1, 0.25 and 0.5 nmol kg^{-1} SX6c are significantly attenuated by BQ-123 (1 mg kg^{-1}) in the anaesthetized rat. In our study, SX6c at 3 nmol kg^{-1} was lethal. The SXs probably cause death by inducing coronary constriction accompanied by ST-segment elevation, a slow positive inotropic effect and atrio-ventricular block (Wollberg *et al.*, 1987).

This work extends a preliminary report on the ET_A receptor antagonist, BQ-123 (Pollock *et al.*, 1992). We have shown that complete inhibition of the ET- or SX-induced pressor responses could not be achieved using BQ-123. It could be that ET/SX peptides induce the release of other agents which will increase blood pressure such as renin, aldosterone or catecholamines (Goetz *et al.*, 1988; Miller *et al.*, 1989). Contrary to this suggestion, the pressor effects of SX6c do not appear to be mediated via the central nervous system, by catecholamines from the adrenal medulla, by prostanoids or by ET-1 (Clozel *et al.*, 1992). Alternatively, the ET_B receptor may mediate a pressor action, as already indicated (Williams *et al.*, 1991). There is increasing evidence pointing to the existence of multiple ET receptor subtypes (Emori *et al.*, 1990; Samson *et al.*, 1990; Fukuroda *et al.*, 1992; Harrison *et al.*, 1992). Supporting the contention that ET/SX-induced smooth muscle contractions are not all mediated via the ET_A receptor is the finding that BQ-123 (10^{-5} M) did not affect the EC_{50} threshold concentration or maximum response to ET-1 in rings of guinea-pig bronchi, rabbit pulmonary artery or rat stomach strip. In contrast, this dose of BQ-123 shifted the EC_{50} for ET-1 in the rat thoracic aorta from 3×10^{-10} M to 1×10^{-7} M and the threshold concentration for contraction from 10^{-10} M to 3×10^{-8} M (Warner *et al.*, 1992). In a similar study Hay (1992) demonstrated that BQ-123 antagonizes ET-1-induced contractions of guinea-pig aorta but is without effect on ET-1-induced contractions of guinea-pig bronchus. Furthermore, BQ-123 does not inhibit ET- or SX-induced renal vasoconstriction *in vivo* (Cristol *et al.*, 1992).

In summary, the present study supports the hypothesis that the pressor response to members of the ET/SX family of vasoconstrictor peptides is not mediated entirely via activation of the ET_A receptor.

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