Characterization of endothelin receptors in rat renal artery *in vitro*

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1 The aim of this study was to investigate the function and characteristics of endothelin receptors in rat main branch renal artery *in vitro*.

2 Endothelin(ET)-1 (mean EC₅₀ = 9.8 nM) was approximately 12 fold more potent than ET-3 (mean EC₅₀ = 120 nM) as a contractile agonist and produced a greater maximum response. In contrast, neither of the ET_B receptor-selective agonists, alanine^[1,3,11,15]ET-1 nor sarafotoxin S6c, (0.1 nM–1 µM), induced any contractile effect, or any relaxant effect in endothelium-intact preparations pre-contracted with the thromboxane A₂ mimetic, U-46619. Sarafotoxin S6c (30 nM) also failed to induce any further contraction in tissues pre-contracted with an EC₅₀ concentration of ET-1.

3 The ET_A receptor-selective antagonist, BQ123, behaved as a weak and variable antagonist of the contractile effects of ET-1 (mean pA₂ estimates in the range 5.8–6.3). In contrast, BQ123 antagonized ET-3 with a potency (mean pA₂ = 7.6) consistent with its affinity for ET_A receptors. Co-incubation of BQ123 (3 µM) with the putative ET_B receptor-selective antagonist, IRL1038 (10 µM), produced no greater antagonism of ET-1 responses than was induced by BQ123 (3 µM) alone.

4 In conclusion, ET_B receptors do not appear to be present in rat main branch renal artery. The contractile effects of ET-3 in this tissue seem to be mediated by ET_A receptors. While ET_A receptors partly mediate the contractile effects of ET-1, these data raise the possibility that a population of novel BQ123-insensitive endothelin receptors may also contribute to this response.

Keywords: Rat renal artery *in vitro*; endothelin; sarafotoxin S6c; BQ123; ET_A receptor; ET_B receptor; receptor subtypes

Introduction

The endothelins represent a class of recently discovered potent vasoactive 21 amino acid peptides (Yanigasawa *et al.*, 1988). There is evidence to support the existence of discrete pathways for the production of three closely related peptides, endothelin-1 (ET-1), endothelin-2 (ET-2) and endothelin-3 (ET-3).

Administered intravenously, the most prevalent effect of the endothelins is vasoconstriction, although a vasodilator component often precedes the vasoconstriction (Wright & Fozard, 1988; Lippton *et al.*, 1988). Current knowledge supports the existence of two main classes of endothelin receptors, termed ET_A and ET_B, which have been successfully cloned and expressed (Arai *et al.*, 1990; Sakurai *et al.*, 1990; Elshourbagy *et al.*, 1993). It was initially thought that ET_A receptors mediate the vasoconstrictor effects of the endothelins, while ET_B receptors mediate their vasodilator effects. This view has recently been challenged following the demonstration of ET_B receptor-mediated vasoconstrictor responses (Sumner *et al.*, 1992). Whether the same subtype of ET_B receptor mediates both constrictor and dilator responses remains to be clarified. Moreover, although only two subtypes of mammalian endothelin receptor have as yet been cloned, many groups have observed atypical agonist and antagonist potency profiles in radioligand binding and isolated tissue studies, raising the possibility that further subtypes of endothelin receptors occur (Sokolovsky *et al.*, 1993; Bodelsson & Stjernquist, 1993; Bax *et al.*, 1993a,b; Eglezos *et al.*, 1993; Warner *et al.*, 1993).

Although much studied, the physiological and pathophysiological significance of endothelin remains largely undetermined. Interestingly, the renal vasculature appears particularly sensitive to the vasoconstrictor effects of endothelin (Pernow *et al.*, 1988; Lanese *et al.*, 1992), and ET-1 can be produced in several renal cell types (Kon &

Badr, 1991; Perico & Remuzzi, 1993). Since several forms of acute renal failure involve intense renal vasoconstriction, it is not surprising that endothelin has been postulated as a potential mediator. Indeed, studies in rat models support some potential involvement of endothelin in both ischaemia and cyclosporine-induced renal failure (Kon & Badr, 1991). In addition, there is some evidence to support a role for endothelin in animal models of chronic renal disease, possibly as a consequence of its mitogenic effects and through interactions with other mediators of inflammation (Benigni *et al.*, 1993; Perico & Remuzzi, 1993).

The possible pathophysiological involvement of endothelin in the kidney has provoked excitement in the possibility that endothelin receptor antagonists may have efficacy in the treatment of renal disease. However, much remains to be learned regarding the pharmacology of renal endothelin receptors. In terms of the renal vasculature, there is evidence from studies in the rat to suggest that both ET_A and ET_B receptors contribute to vasoconstrictor responses *in vivo* (Wellings *et al.*, 1993; Pollock & Opgenorth, 1993). While perfused kidney preparations or studies *in vivo* are invaluable in allowing microvascular responses to endothelin to be studied, they make it difficult to control drug concentrations and hence quantitatively characterize receptors. The aim of the present study was to use an *in vitro* test system, where conditions could be closely controlled, in order to characterize functional endothelin receptors in rat main branch renal artery. A preliminary account of these findings has been presented to the British Pharmacology Society (Pierre & Clark, 1994).

Methods

General

Male Sprague-Dawley rats (250–350 g) were killed by cervical dislocation. The main branch renal artery to the left

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kidney was carefully removed along with a portion of the abdominal aorta so that handling of the vessel was minimized. The artery was placed into ice cold gassed (95% O₂, 5% CO₂) Krebs-Henseleit solution, pH 7.4. Indomethacin (30 µM) was also present in the solution to prevent the formation of cyclo-oxygenase products. The vessel was cut into rings, approximately 1 mm in length and 400 µm in diameter. Where required, the endothelium was removed by gently rolling the rings on a steel wire 100 µm in diameter. The arterial rings were suspended between stainless steel wires, 100 µm in diameter, in 10 ml glass tissue baths containing gassed Krebs-Henseleit solution at 37°C. Changes in isometric tension were measured with isometric force transducers (Gould-Statham UC3) and displayed on an eight channel chart recorder (Electromed, MT8).

Arterial rings were put under an initial tension of 0.4 g and left to equilibrate for 20 min. KCl (80 mM) was then added to test the contractile viability of the preparations. Once any contractile response had plateaued, the tissues were washed, allowed to relax and the tension was readjusted to 0.4 g. The peptidase inhibitors, phosphoramidon (1 µM) and bestatin (10 µM), were added 45 min before the construction of agonist concentration-response curves in an attempt to prevent any peptide degradation. Where required, the resting tension was readjusted to 0.4 g, 15 min prior to construction of agonist concentration-response curves.

Effects of agonists: relaxant responses

A 10 fold cumulative contractile concentration-response curve to the thromboxane A₂ mimetic, U-46619 (0.1 nM–10 µM), was constructed and the EC₈₀ concentration determined (concentration required to produce 80% of the maximum response). The tissues were then washed four-times, at 5 min intervals and the tension re-adjusted to 0.4 g. After 15 min, the tissues were contracted with an EC₈₀ concentration of U-46619. After the contraction had stabilized, acetylcholine (1 µM) was added. A marked relaxant response (at least 80% relaxation of the induced tone) to acetylcholine was taken as evidence of a functional endothelium (Furchgott & Zawadzki, 1980) and preparations were discarded if this was not achieved.

The tissues were then washed four times, at 5 min intervals. After a further 45 min, the tissues were again contracted with an EC₈₀ concentration of U-46619. Once the contraction had stabilized, a 10 fold cumulative concentration-response curve to either of the ET_B receptor-selective agonists, sarafotoxin S6c (S6c) or alanine^[1,3,11,15]ET-1 ([Ala⁴]ET-1) (both 0.1 nM–1 µM) or vehicle was constructed.

Effects of agonists: contractile responses

Endothelium-denuded preparations were contracted with an approximate EC₈₀ concentration (0.1 µM) of U-46619 and tissues which showed greater than 10% relaxation to a subsequent acetylcholine challenge (1 µM) were discarded. Tissues were then washed four times at 5 min intervals; 45 min later, cumulative three fold concentration-response curves to an endothelin isopeptide were constructed. In some tissues, after a maximum response to ET-3 had been attained, a single maximal concentration (1 µM) of ET-1 was added to determine whether any additional contractile response could be achieved.

Agonist interaction studies

Endothelium-denuded preparations were contracted with an approximate EC₅₀ concentration of ET-1 (concentration required to evoke 50% of the maximum response). When the contraction had stabilized, the ET_B receptor-selective agonist, S6c (30 nM) was added and any response observed was compared to that which occurred in a time-matched vehicle control tissue.

Antagonist studies

Initial attempts to wash out ET-1 following the construction of a concentration-response curve were unsuccessful, so consecutive reproducible concentration-response curves could not be obtained. Therefore, for a given endothelium-denuded preparation, contractile responses to ET-1 could not be studied in the absence and subsequent presence of an antagonist. Hence, antagonists were added 45 min (or in some cases 15 min) prior to the construction of a 3 fold cumulative curve to either ET-1 or ET-3 and responses compared with those observed in time-matched vehicle-treated control preparations.

Expression of results

Agonist studies Agonist-induced contractile responses were expressed as a percentage of the response to the initial U-46619 challenge and concentration-response curves were plotted. Where appropriate, geometric mean EC₅₀ values with 95% confidence intervals were determined for each agonist.

Antagonist studies Agonist concentration-ratios in the absence and presence of antagonist were calculated at the EC₅₀ level. Antagonist potencies were expressed as estimated pA₂ values. These were derived from single concentrations of antagonist according to Schild (1957), using the following equation and assuming a slope (–n) of unity:

$$\log (x-1) = \log K - n \text{ pA}_x$$

where, x is the concentration-ratio, and K is the antagonist affinity constant. Estimated mean pA₂ values are presented as arithmetic mean ± s.e.mean.

Where required, differences between groups of data were tested for statistical significance by Student's *t* test for unpaired data.

Drugs

ET-1, ET-3 and sarafotoxin S6c were purchased from Peptide and Protein Research Consultants (England) or Peptide Institute Inc (Japan). Acetylcholine, phosphoramidon and bestatin were purchased from Sigma Chemical Company Ltd. IRL1038 ([Cys¹¹-Cys¹⁵]-endothelin 1(11-21)) was obtained from Cambridge Research Biochemicals (England). U-46619 (15S)-hydroxy-11α, 9α-(epoxymethano) prosta -5z, 13E-dienoic acid, [Ala⁴]ET-1 and BQ123 (cyclo(-D-Val-Leu-D-Trp-D-Asp-Pro)) were synthesized by Glaxo Research and Development Ltd. All chemicals for the Krebs-Henseleit solution were of Analar grade and obtained from BDH Ltd. (UK). Drugs were dissolved in distilled water with the following exceptions. ET-1, S6c and [Ala⁴]ET-1 were dissolved in dimethylsulphoxide, whilst ET-3 and IRL1038 were dissolved in 0.1 M ammonium acetate. U-46619 was dissolved in 0.1 M NaHCO₃. All drugs were stored as frozen aliquots (1 mM) and defrosted for use as required. Dilutions of drugs to working concentrations were made with distilled water.

Results

Agonist profile

Cumulative addition of ET-1 (0.1 nM–1 µM) and ET-3 (0.1 nM–3 µM) produced concentration-dependent contractions of endothelium-denuded preparations. ET-3 was less potent than ET-1 (approx. 12 fold) and evoked a significantly smaller maximum response (Figure 1; Table 1). In eight tissues, after constructing a concentration-response curve to ET-3 (0.3–3 µM) and obtaining a maximum response, addition of ET-1 (1 µM) evoked a further significant increase in tension (from 98.9 ± 7.7 to 130.4 ± 8.2% of the initial U-46619-induced contraction).

In contrast to ET-1 and ET-3, the selective ET_B receptor agonists, S6c (Williams *et al.*, 1991) and [Ala⁴]ET-1 (Saeki *et al.*, 1991) (0.1 nM–3 µM), had no contractile effect (Figure 1). S6c (30 nM) also failed to evoke any contractile response when added to endothelium-denuded preparations (*n* = 5) pre-contracted with an approximate EC₅₀ concentration (10 nM) of ET-1 (data not shown).

In endothelium-intact preparations pre-contracted with an EC₈₀ concentration of the thromboxane A₂-mimetic, U-46619, the selective ET_B receptor agonists, S6c and [Ala⁴]ET-1 (0.1 nM–1 µM), failed to elicit any relaxant or contractile response (Figure 2).

Antagonist studies

Effects of antagonists on ET-1-induced contractile responses Incubation with the ET_A receptor-selective antagonist, BQ123 (Ihara *et al.*, 1991), at 0.3, 1 and 3 µM caused a parallel rightward displacement of the concentration-response curves to ET-1, with no significant change in the maximum response (Figure 3). However, Figure 3 also illustrates that BQ123 was a rather weak and inconsistent antagonist of the effects of ET-1, and consequently the antagonism appeared poorly concentration-related. Estimates of the pA₂ were made at each concentration of antagonist and were in the range 5.8 to 6.3 (Table 2). When the incubation period with BQ123 was reduced from

Figure 1 Contractile effects of endothelin-1 (●), endothelin-3 (○), [Ala⁴]ET-1 (▲) and sarafotoxin S6c (■) on rat endothelium-denuded renal artery rings. Values are arithmetic mean ± s.e.mean (*n* = 5–6).

Figure 2 Example trace showing the effects of the selective ET_B receptor agonists, [Ala^{1,3,11,15}]ET-1 and sarafotoxin S6c, in rat endothelium-intact renal artery rings pre-contracted with U-46619 (0.1 µM).

45 to 15 min (*n* = 4), the same profile of antagonism was observed and the potency of BQ123 was not altered (data not shown).

The putative ET_B receptor antagonists, IRL1038 (Urade *et al.*, 1992), at a concentration of 10 µM, caused no rightward displacement of the ET-1 concentration-response curve and did not affect the maximum response observed (*n* = 4; data not shown). Furthermore, a combination of IRL1038 (10 µM) with BQ123 (3 µM) resulted in no greater antagonism than that exerted by BQ123 (3 µM) alone (Figure 4). The mean EC₅₀ for ET-1 in the presence of IRL1038 and BQ123 (12.7, 5.6–29 nM) was not significantly different from that observed in the presence of BQ123 alone (43, 12.3–152 nM).

Effects of BQ123 on ET-3-induced contractile responses At a single concentration of 0.3 µM, BQ123 caused a marked rightward displacement of the ET-3 concentration-response curve without reducing the maximum response (Figure 5), and a mean pA₂ estimate of 7.6 ± 0.2 (*n* = 5) was calculated. Thus, BQ123 was approximately 20–60 fold more potent as an antagonist of ET-3 in comparison with ET-1-induced contractions.

Table 1 Contractile potencies of endothelins and their analogues in endothelium-denuded rat renal artery rings (*n* = 5–6)

Compound	EC ₅₀ (nM)	95% confidence limits	Maximum response (% U-46619)	s.e.mean
ET-1	9.8	7.6–12.5	143	8.4
ET-3	120*	56.4–251	107*	7.8
[Ala ⁴]ET-1	Inactive up to 1 µM			
S6c	Inactive up to 1 µM			

**P* < 0.05 ET-1 vs ET-3, Student's unpaired *t* test.

Table 2 Mean concentration-ratios (CR) and estimated pA₂ values for the ET_A receptor-selective antagonist, BQ123, versus endothelin-1 in endothelium-denuded rat renal artery rings

	BQ123 (µM)		
	0.3	1.0	3.0
CR	1.5 ± 0.6	3.8 ± 0.9	4.9 ± 1.4
Estimated pA ₂	< 6.5	6.3 ± 0.2	5.8 ± 0.3

Values are mean ± s.e.mean; *n* = 4–6.

Figure 3 Effects of the ET_A receptor-selective antagonist, BQ123, (control, □; BQ123 0.3 µM ○, 1 µM ■ and 3 µM ●) on contractile responses to endothelin-1 in rat endothelium-denuded renal artery rings. Values are arithmetic means ± s.e.mean (*n* = 4–6).

Figure 4 Effects of the ET_A receptor-selective antagonist, BQ123 ($3 \mu M$), and a combination of BQ123 ($3 \mu M$) and the ET_B receptor selective antagonist, IRL1038 ($10 \mu M$), on contractile responses to endothelin-1 in rat endothelium-denuded renal artery rings: (□) control; (■) BQ123 alone; (●) BQ123 & IRL1038. Values are arithmetic mean $\pm$ s.e.mean ($n = 5$).

Discussion

There is growing evidence to suggest that endothelin may contribute to the pathology of certain forms of acute and chronic renal disease (Kon & Badr, 1991; Perico & Remuzzi, 1993; Benigini *et al.*, 1993). Consequently, it is possible that endothelin receptor antagonists may find a role in the treatment of these conditions. However, in order to identify appropriate antagonists, it is essential to have an understanding of the location and function of renal endothelin receptors. The aim of this paper was to study the function and characteristics of endothelin receptors in rat main branch renal artery *in vitro*; a test system which should allow for quantitative receptor characterization.

The initial approach taken was to compare the potencies of various endothelin receptor agonists. The results indicate that ET-1 and ET-3 both contract rat renal artery, although ET-1 is more potent (~ 12 fold) than ET-3 and induces a slightly but significantly greater maximal response. This profile of agonist action is consistent with that seen in other ET_A receptor containing tissues, such as rabbit carotid artery and rat thoracic aorta (Moreland *et al.*, 1992; Sumner *et al.*, 1992). In contrast to these two agonists, neither of the ET_B receptor-selective agonists, sarafotoxin S6c nor [Ala⁴]ET-1, induced any contractile effect at concentrations up to $30 \mu M$, nor did they induce relaxation of pre-contracted endothelium-intact renal arteries. Thus, it appeared that neither ET_B relaxant nor contractile receptors were present. The agonist profile, therefore, suggested that rat renal artery contains exclusively ET_A receptors. However, classification of receptors solely on the basis of rank orders of agonist potencies can be misleading. Consequently, we went on to test our preliminary hypothesis using the ET_A receptor-selective antagonist, BQ123 (Ihara *et al.*, 1991).

It has previously been demonstrated by several groups (Ihara *et al.*, 1991; Sumner *et al.*, 1992; Moreland *et al.*, 1992) that in ET_A receptor containing tissues (e.g. rabbit carotid artery, pig coronary artery, rat thoracic aorta) BQ123 competitively antagonizes the contractile effects of ET-1, with pA_2 values in the range 6.9–7.5. However, in the present study BQ123 was a relatively weak and inconsistent antagonist of ET-1; pA_2 estimates were in the range 5.8–6.3. It seems unlikely that the weak antagonism exerted by BQ123 was due to breakdown or uptake of the cyclic peptide antagonist since (a) all experiments were carried out in the presence of the peptidase inhibitors, phosphoramidon and bestatin, and (b) BQ123 still exerted weak and inconsistent

Figure 5 Effects of the ET_A receptor-selective antagonist, BQ123, (control, □; BQ123 $0.3 \mu M$, ●) on contractile responses to endothelin-3 in rat endothelium-denuded renal artery rings. Values are arithmetic means $\pm$ s.e.mean ($n = 5$).

antagonism when the contact time was reduced from 45 to 15 min.

Previous workers (LaDouceur *et al.*, 1993) reported that BQ123 was a weak antagonist of ET-1-induced contractions of rabbit pulmonary artery and demonstrated that this was a result of the tissue containing a mixed population of ET_A and ET_B receptors. Thus, an alternative hypothesis to account for the low potency of BQ123 in the present study could be that a small population of contractile ET_B receptors are present in rat renal artery. Although the agonist profile suggested that ET_B receptors were not in this tissue (since the selective ET_B receptor agonists tested failed to evoke a contractile or relaxant response), we questioned whether it was possible that ET_B receptors were present, but unable to elicit a response under basal conditions. Hence, we postulated that when ET_A receptors were stimulated and the tissue partially contracted, ET_B receptor stimulation may also trigger a contractile response. This scenario could explain the low potency of BQ123, since even when ET_A receptors were blocked, ET_B receptors could continue to mediate the contractile effects of ET-1. To test this possibility, we examined whether co-incubation with BQ123 and the putative ET_B receptor antagonists, IRL1038 (Urade *et al.*, 1992), resulted in greater antagonism of the effects of ET-1 than that observed in the presence of BQ123 alone. This was not the case, although it is worth noting that Urade and co-workers (1994) have recently raised concerns regarding variation between experimental batches in the affinity of IRL1038 for ET_B receptors. Nevertheless, further evidence that ET_B receptors are absent from rat renal artery was derived from the observation that when tissues were pre-contracted with an approximate EC_{50} concentration of ET-1, the ET_B receptor-selective agonist, S6c, failed to elicit any further contractile response. Thus, we feel it is unlikely that the weak antagonism exerted by BQ123 in this tissue can be explained by the argument that a small population of ET_B contractile receptors are present.

Given these data, we begin to consider the speculative but intriguing possibility that rat renal artery contains both ET_A receptors and a novel subtype of contractile endothelin receptor which is BQ123-insensitive. To investigate this possibility further, we went on to study the ability of BQ123 to antagonize another endothelin receptor agonist, ET-3. Interestingly, BQ123 was more potent (approximately 20–60 fold) as an antagonist of ET-3 and the pA_2 value (7.6) obtained was consistent with an action at ET_A receptors. The increased potency of BQ123 as an antagonist of ET-3 relative to ET-1 supports the possibility of receptor heterogeneity in this tissue since in a single receptor system the potency of an antagonist should be independent of the agonist studied. Thus, the data can be explained by the hypothesis that ET-3 contracts rat renal artery solely via an action on ET_A recep-

tors, while ET-1 may stimulate both ET_A receptors and a novel endothelin receptor subtype which has low affinity for BQ123. Consistent with this view is the observation that ET-1 can evoke an additional contractile response when added on top of a maximal ET-3-induced contraction. Moreover, although carried out using a modified Krebs solution, we have also found (unpublished observations) that while ET-1 causes cross desensitization in rat renal artery, ET-3 desensitizes responses to itself, but does not alter sensitivity to ET-1. These data support the hypothesis that ET-1 recognises a subtype of endothelin receptor not bound by ET-3.

Alternatively, it could be argued that the weak antagonism of ET-1 by BQ123 may be due to the kinetics of the interaction of ET-1 with ET_A receptors. Radioligand binding studies indicate that ET-1 binds almost irreversibly to ET_A receptors in vascular smooth muscle cells (Hirata *et al.*, 1988). Thus, the weak antagonism by BQ123 of ET-1-induced contractions in rat renal artery could reflect an inability of the antagonist to displace bound ET-1 from rat vascular ET_A receptors. However, this seems unlikely since studies on rat aorta (Sumner *et al.*, 1992), under almost identical conditions to the present work, show that BQ123 competitively antagonizes ET-1-induced contractions with a pA₂ of 6.9. Thus, we feel that the present data raise the possibility that rat renal artery contains a novel subtype of BQ123-resistant endothelin receptor. Since the concentration-response curve to ET-1 in the presence of BQ123 was not biphasic, this suggests that the putative population of BQ123-resistant receptors must be able to mediate a full contractile response to ET-1. A question of interest is whether a similar receptor subtype may occur in the human renal vasculature. Present evidence suggests this is unlikely. Maggi and co-workers (1990) reported that human renal artery showed an agonist selectivity profile consistent with it containing ET_A receptors while a very recent study by Maguire *et al.* (1994) found no evidence to suggest that human renal artery contains a novel BQ123-insensitive endothelin receptor.

The possible existence of a novel contractile endothelin receptor subtype in rat renal artery is supported by reports that a similar BQ123-resistant receptor subtype may occur in other vascular and non-vascular tissues including rat vas deferens (Eglezos *et al.*, 1993), guinea-pig iliac artery (Schoeffter *et al.*, 1993) and rabbit renal artery (Moreland *et*

al., 1994). Moreover, recent evidence suggests that a novel BQ123-resistant receptor may occur in human vascular smooth muscle preparations including saphenous vein (Bax *et al.*, 1993a), coronary artery (Bax *et al.*, 1993b; Godfraind *et al.*, 1993) and umbilical artery (Bodelsson & Stjernquist, 1993). Assessment in these tissues of the antagonist potencies of recently reported non-peptide ET_A receptor antagonists, such as Ro 46-2005 (Clozel *et al.*, 1993) and BMS182,874 (Stein *et al.*, 1994), may help define whether they truly possess a novel endothelin receptor subtype. Similarly, investigations at the molecular level should help determine whether an additional contractile endothelin receptor subtype is present in smooth muscle.

While studies using isolated conductance arteries *in vitro* allow for quantitative characterization of receptors it cannot be assumed that the same subtypes of receptor occur in the microvasculature. Interestingly, the ineffective antagonism by BQ123 or ET-1-induced contractions in rat main branch renal artery has also been observed in the rat renal microvasculature by workers using perfused kidneys or anaesthetized rat preparations (Wellings *et al.*, 1993; Cristol *et al.*, 1993; Pollock & Oppenorth, 1993). However, in contrast to the present results from main branch renal artery, these workers (Wellings *et al.*, 1993; Cristol *et al.*, 1993) found that ET_B receptors play an important role in mediating the contractile effects of ET-1 in renal microvasculature. If confirmatory evidence can be found for a BQ123-insensitive endothelin receptor subtype, then it will be important to determine whether it is present in renal microvessels and plays a role in regulating glomerular blood flow.

In conclusion, we have shown that rat main branch renal artery does not contain ET_B receptors mediating either relaxation or contraction. The contractile effects in this tissue of ET-3, and to some extent ET-1, appear to be mediated by ET_A receptors. However, the weak antagonism exerted by the ET_A receptor-selective antagonist, BQ123, against the contractile effects of ET-1 raises the possibility that in rat renal artery, ET-1 may stimulate a novel subtype of BQ123-insensitive endothelin receptor.

The authors would like to acknowledge the advice and constructive criticism of Dr Ian Watts, Dr David White, Dr Mike Sumner, Dr Mike Drew & Dr Pete Strong. The technical assistance of Miss Hazel Garratt is also gratefully acknowledged

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(Received June 13, 1994

Revised September 22, 1994

Accepted October 24, 1994)