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Influence of endothelins and sarafotoxin 6c and L-NAME on renal vasoconstriction in the anaesthetized rat

[1]Joanne L. Marshall & *,[1]Edward J. Johns

[1]Department of Physiology, The Medical School, Birmingham B15 2TT

1 An investigation was performed in pentobarbitone anaesthetized rats to compare the renal vasoconstrictor actions of endothelin-1 (ET-1), endothelin-3 (ET-3) and sarafotoxin 6c and their dependency on NO production.

2 Intra-renal arterial infusion of ET-1 and ET-3, from 1–1000 ng had no effect on blood pressure, but reduced renal blood flow maximally by 82 and 81% with EC_{50} values of 510 ± 18 and 1113 ± 17 ng, respectively and correspondingly increased renal vascular resistance and decreased conductance.

3 Direct renal arterial administration of sarafotoxin 6c was without effect on blood pressure but caused a maximum reduction in renal blood flow of 56% at 300 ng and had an EC_{50} of 86 ± 4 ng.

4 Administration of the selective ET_A receptor antagonist FR139317 at 0.3 and 1.0 mg kg^{-1} had no effect on basal levels of blood pressure, renal vascular resistance or renal blood flow. The lower dose of FR139317 had no effect on the ET-1 dose-response curve for renal blood flow while at 1.0 mg kg^{-1}, FR139317 reduced the EC_{50} to 363 ± 32 ng ($P<0.05$).

5 Infusion of L-NAME, 10 μg kg^{-1} min^{-1} increased blood pressure by approximately 15%, increased renal vascular resistance and decreased renal blood flow by some 40%. The EC_{50} values for renal blood flow were reduced to 358 ± 68 ng ($P<0.05$) for ET-1, 638 ± 69 ng ($P<0.05$) for ET-3 and 55 ± 10 ng ($P<0.01$) for sarafotoxin 6c. The maximal reduction in renal blood flow induced by sarafotoxin 6c was raised ($P<0.01$) from 56% to approximately 100% and renal vascular resistance increased when NO production was blocked.

6 These results showed that the vasoconstrictor actions of ET-1 and ET-3 on resistance vessels controlling renal blood flow are mediated *via* ET_B rather than ET_A receptors. Moreover, both ET-1 and ET-3 dependent vasoconstrictions are slightly attenuated by concomitant NO production. By contrast, sarafotoxin 6c appears much more potent at the renal resistance vasculature and is much more powerfully modulated by NO.

Keywords: Endothelins; sarafotoxin 6c; renal blood flow; nitric oxide; L-NAME

Abbreviations: EC_{50}, effective concentration to produce 50% maximal response; ET-1, endothelin 1; ET-2, endothelin 2; ET-3, endothelin 3; ET_A, endothelin receptor subtype A; ET_B, endothelin receptor subtype B; L-NAME, L-nitro-arginine methylester; MAP, mean arterial pressure; NO, nitric oxide; RPF, renal plasma flow

Introduction

The endothelins are a group of peptides produced by the endothelial cells of blood vessels which act in a paracrine and autocrine mode to modulate the tone of vascular smooth muscle cells (Simonson, 1993). There are three endothelin isoforms, endothelin-1 (ET-1) endothelin-2 (ET-2) and endothelin-3 (ET-3) and it is ET-1 which is the most potent and appears to exert most physiological actions of the peptides, with ET-3 also making a significant contribution (Simonson & Dunn, 1993). A related class of compounds are the sarafotoxins, which were originally isolated from the burrowing asp, *Atractaspis engaddensis* and show a high level of peptide sequence homology with the endothelins.

Two major endothelin receptor subtypes have been identified pharmacologically, their genes cloned (Arai *et al*., 1990; Sakurai *et al*., 1990) and have been defined as ET_A and ET_B receptors due to their relative affinities for the endothelins and sarafotoxins, (Sumner *et al*., 1992). The ET_A receptor is preferentially activated by ET-1, and to a lesser extent by ET-3 and sarafotoxin, whereas ET_B receptors have an equal affinity for all endothelin peptides and sarafotoxin 6c (Brooks, 1997). The ET_A receptors are generally found on vascular smooth muscle, and when activated cause a vasoconstriction, while ET_B receptors are found on the endothelial cells themselves, where their activation results in nitric oxide (NO) generation which leads to dilatation of vascular smooth muscle (Hirata *et al*., 1993). However, at the vascular smooth muscle cells, activation of ET_B receptors gives rise to vasoconstriction (Brooks, 1997). It is these relationships which cause complex vascular responses when endothelin production is stimulated or exogenous peptides are applied.

It is generally considered that the majority of endothelin receptors in the systemic vasculature are of the ET_A subtype, but there is increasing evidence that ET_B receptors are located on the smooth muscle cells and mediate vasoconstriction. In the isolated perfused rat kidney, ET-1 and sarafotoxins 6b and 6c produced similar increases in perfusion pressure, which were completely blocked by the mixed ET_A/ET_B antagonist, PD145065 (Williams *et al*., 1991). The ET_A antagonist, BQ-123 partially prevented the fall in renal plasma flow, suggesting that ET_A receptors do have a functional role in the renal vascular bed and indeed our own recent study *in vitro* using rat arcuate arteries confirmed this view (Wu *et al*., 1997). However, the contribution of ET_A receptors in mediating endothelin induced renal vasoconstriction may be less in the rat with a greater contribution of ET_B receptors. Evidence

* Author for correspondence.

from *in vivo* studies (Pollock & Opgenorth, 1993) showed that systemic i.v. infusion of ET-1 significantly increased mean arterial pressure (MAP) and reduced renal plasma flow (RPF) but that administration of the ET_A receptor antagonist BQ123 blocked the increase in MAP, but did not prevent the significant decrease in RPF. This was in contrast to other vessels such as the carotid and iliac arteries where the ET_B agonist (Ala 1, 3, 11, 15) ET-1 elicited vasodilatation as opposed to vasoconstriction. The distribution of endothelin receptor subtypes alters along the renal vascular tree, from predominantly ET_A receptors in the rat renal artery (Clark & Pierre, 1995), to mainly ET_B receptors on post-glomerular vessels (Endlich *et al*., 1996). In the hydronephrotic rat kidney where the exposed vasculature enables detailed study, the ET_A antagonist BQ-123 antagonized ET-1 induced vasoconstriction primarily in pre-glomerular vessels such as arcuate arteries, interlobular arteries and afferent arterioles (Endlich *et al*., 1996). By contrast, the ET_B receptor agonist, IRL1620 constricted all pre-glomerular vessels as well as the efferent arterioles (Endlich *et al*., 1996) with the latter being constricted by almost twice as much compared with the afferent arterioles. ET_B receptors have also been demonstrated in the rat glomerulus, which showed similar affinities for both ET-1 and ET-3 (Takemoto *et al*., 1993).

The role of the ET_A versus the ET_B receptors in mediating renal vasoconstriction still remains to be resolved and also the issue arises as to the contribution of ET_B receptors on endothelial cells which may mediate renal vasodilation *via* NO production. One of the confounding problems at the present time is that many studies have employed systemic infusion of endothelin to study their action on renal haemodynamics. The difficulty is that under these circumstances, the renal vascular responses will be an amalgam of an indirect component due to the raised systemic blood pressure (primarily mediated by ET_A receptors) and a direct one involving both ET_A and ET_B or only the ET_B receptor subtype. In an attempt to address these issues, studies were undertaken in anaesthetized rats prepared for renal haemodynamic measurements, in which localized intra-renal arterial infusions of ET-1, ET-3 and sarafotoxin 6c were given at levels having no systemic effects. Cumulative dose responses were drawn up alone and in the presence of a local infusion of an ET_A receptor antagonist. The contribution of nitric oxide in these responses were investigated by preventing its generation with concomitant L-NAME administration.

Methods

Surgical preparations: All surgical procedures were performed under the provisions of the United Kingdom Government project licence PPL40/1367 and personal investigator licence PIL 40/00371 to E.J. Johns and P1L40/4062 to J.L. Marshall. Male Wistar rats, 270–325 g, were obtained from Charles River (Kent) and after one week acclimatization in the animal holding facility were anaesthetized with sodium pentobarbitone, 60 mg kg^{-1} i.p., and the trachea cannulated to ensure a patent airway. A cannula was placed in the right carotid artery to measure systemic blood pressure (Spectramed Statham, Oxnard CA, U.S.A., transducer linked to a Grass Model 7E polygraph) and a further cannula was inserted into the right femoral vein to allow infusion of pentobarbitone at a rate of 15 mg kg^{-1} h^{-1} (1 ml h^{-1}) to maintain a constant plane of anaesthesia. The right femoral artery was also cannulated with fine tubing (PP10), the end of which was extruded, and this was inserted until the fine tip just entered the left renal artery. It was kept patent by means of a constant infusion of saline (150 mM NaCl) at 5 ml h^{-1} (Braun Perfusor, Melsungen, Germany) and allowed direct intra-renal arterial administration of drugs. The left kidney was exposed *via* a mid-line abdominal incision, the renal artery was carefully isolated and an electromagnetic flow probe placed on it to allow a direct monitoring of renal blood flow (Carolina Medical Electronics Inc., King, NC, U.S.A., EP100 series flow probe linked to a FM 501 flowmeter). The animals were allowed 90–120 min to recover from the surgical procedures. Drugs given intra-renal arterially (i.r.a.) were injected in a volume of 30 μl into the femoral arterial line *via* a self-sealing rubber insert, and then flushed into the kidney by the saline infusion. Once the new stable level of renal blood flow had been achieved (within 5–10 min) readings of flow and blood pressure were taken before the subsequent dose of compound was given. The endothelins have a very long duration of action, extending into hours, consequently cumulative dose-response curves were generated.

Table 1

	Mean blood pressure (mmHg)		*Renal blood flow* (ml/min^{-1}/kg^{-1})		*% Change*	*EC_{50}*	*Renal vascular resistance* (mmHg.ml^{-1} min kg)	
	Before	*After*	*Before*	*After*	*(max)*	(ng)	*Before*	*After*
ET-1 ($n=5$)	88±3	–	25±1	–	82±8	510±18	3.52±0.12	–
ET-1+0.3 mg/kg^{-1} FR139317 ($n=4$)	103±2	101±5	21±1	22±1	94±6	500±36	4.91±0.09	4.59±0.23
ET-1+1.0 mg/mg^{-1} FR139317 ($n=3$)	99±5	93±5	20±4	21±3	99±5	363±32^{+}	4.95±0.25	4.43±0.24
ET-1+L-NAME ($n=3$)	103±4	121±6**††	23±3	15±2	100±0	358±68^{+}	4.48±0.17	8.07±0.40**
ET-3 ($n=4$)	94±2	–	24±4	–	81±7	1113±171^{++}	3.92±0.08	–
ET-3+L-NAME ($n=4$)	115±5	137±6**††	30±4	17±4*	100±0	638±69^{+}	3.83±0.17	8.06±0.35**
Sarafotoxin 6c ($n=4$)	95±8	–	25±5	–	56±5	86±4^{++}	3.80±0.32	–
Sarafotoxin 6c+L-NAME ($n=4$)	100±7	115±7*	24±2	15±2*	92±6**	55±10^{++}	4.17±0.29	7.67±0.46**

This gives mean±s.e.mean of the mean blood pressure, renal blood flow values and renal vascular resistance values before and after either the selective ET_A receptor antagonist FR139317 or L-NAME given at 10 μg kg^{-1} min^{-1}. **Groups of rats then received either endothelin-1 (ET-1), endothelin-3 (ET-3) or sarafotoxin 6c as cumulative doses and the maximum (max) percentage reduction in renal blood flow produced by the highest doses is given along with the EC_{50} calculated from the whole dose-response curve. * = $P<0.05$; ** = $P<0.01$; comparing before and after; † $P<0.05$ and †† $P<0.01$ comparing values obtained in the presence and absence of FR139317 or L-NAME. $^{+}$ = $P<0.05$, $^{++}$ = $P<0.01$ between ET-1, ET-3 or sarafotoxin 6c.

Drugs

Endothelin-1, endothelin-3 and sarafotoxin 6c were obtained from Sigma-Aldrich (Poole, Dorset, U.K.). Stock solutions were made up at 1 mg ml^{-1} and aliquots stored deep frozen until required. Dilutions to the appropriate concentrations were made with saline. Cumulative dose-response curves ranged from 1–1000 ng i.r.a. and in some studies up to 5000 ng i.r.a. The endothelin ET_A-receptor antagonist FR 139317 was obtained from Neosystem Laboratoire (Strasbourg, France) and stored deep frozen at $-20°C$ in aliquots at a concentration of 1 mg ml^{-1}.

L-NAME (Sigma-Aldrich, Poole, Dorset, U.K.) was diluted with saline such that it was delivered systemically *via* the renal arterial cannula at a rate of 10 $\mu g\ kg^{-1}\ min^{-1}$. It was infused for 90 min before experiments were begun as preliminary studies showed that the renal vasodilator effect of i.r.a. administered acetylcholine (1.0 $\mu g\ kg^{-1}$) was blocked by approximately 70% using this approach.

Statistics

All data represent the average values calculated from individual animals and are expressed as means ± s.e.mean. Means were compared between groups using one-way ANOVA and individual points identified using a Scheffe-post-hoc test (SuperAnova, Abacus, Berkley, CA, U.S.A.). Percentage changes given in the tables text and were not used to generate statistical comparisons. Significance was taken when $P<0.05$.

Results

Table 1 contains the basal levels of mean blood pressure, renal vascular resistance and renal blood flow before and following endothelin antagonist and L-NAME administration intra-renal arterially together with the maximum percentage changes in renal blood flow and the EC_{50} values under each condition. Intra-renal arterial infusion of increasing doses of ET-1 had little effect on blood pressure (Figure 1A) over most of the dose range but at 1000 ng caused a significant ($P<0.05$) increase of approximately 5%. Under these conditions, there were dose-related decreases in renal blood flow (Figure 1B) and renal vascular conductance (Figure 1C) reaching a maximum of approximately 82% at a dose of 5000 ng with an EC_{50} of 510 ± 18 ng (Table 1). Administration of ET-3 from 1–1000 ng into the kidney was without effect on blood pressure, showing that it had no systemic action (Figure 1A) but decreased renal blood flow (Figure 1B) and renal vascular conductance (Figure 1C) in a dose-dependent manner, to reach a maximum reduction of some 81% at 5000 ng and with an EC_{50} value of 1113 ± 171 ng. Increasing doses of sarafotoxin 6c given intra-renal arterially up to 1000 ng had no effect on blood pressure (Figure 1A) but caused a progressive reduction in renal blood flow (Figure 1B) and renal vascular conductance (Figure 1C), which reached a maximum at 300 ng but only represented a 55–65% reduction, with an EC_{50} of 86 ± 4 ng. The magnitude and pattern of the reductions in renal blood flow (Figure 1B) and renal vascular conductance (Figure 1C) were markedly different from that observed with both ET-1 and ET-3.

The selective ET_A receptor antagonist, FR139317 was infused at 0.3 and 1.0 mg kg^{-1} intra-renal arterially for 20 min prior to the generation of the endothelin dose-response curves, but it had no effect on basal levels of either blood pressure, renal vascular resistance or renal blood flow (Table 1). Administration of increasing doses of ET-1 concomitantly with the antagonist caused a progressive decrease in renal blood flow reaching a maximum of 94 and 96% reductions with the low and high doses respectively, with EC_{50} values of 500 ± 36 and 363 ± 32 ng (Figure 2A) which was also reflected in the resultant decrease in renal vascular conductance (Figure 2B). It can be seen that there were no significant differences between either the magnitude or the pattern of the ET-1 induced reductions in renal blood flow (Figure 2A) or renal

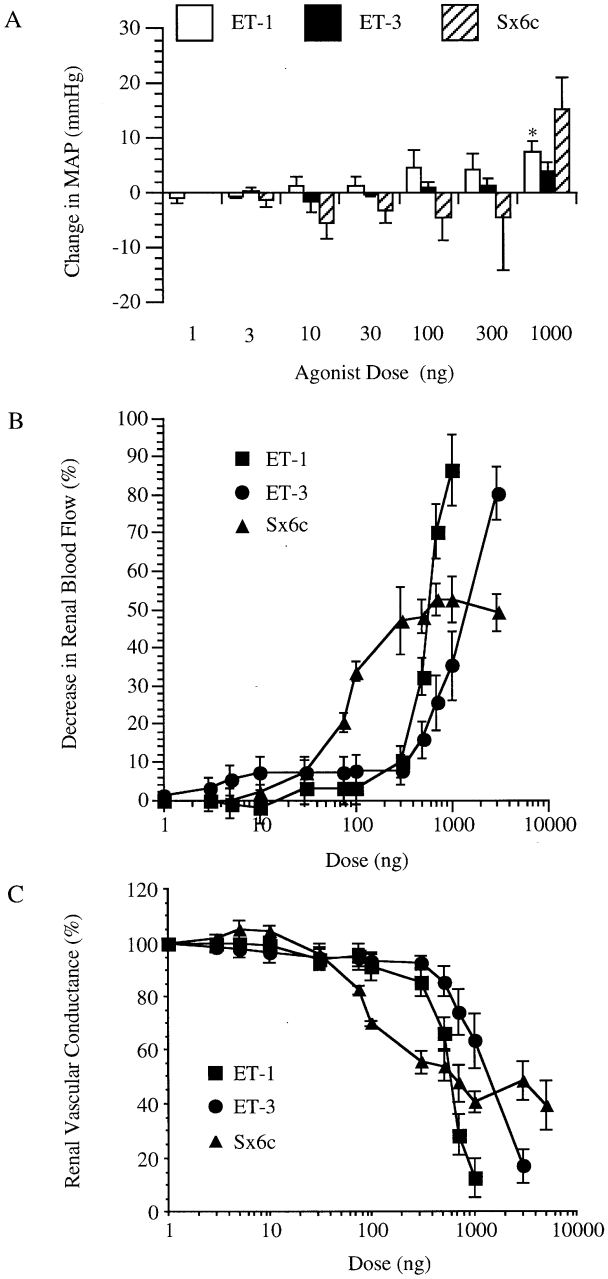

Figure 1 (A) This shows the changes in mean arterial pressure (MAP) caused by the intra-renal arterial infusion of ET-1, ET-3 and sarafotoxin 6c. * = $P<0.05$, ** = $P<0.01$ between the basal state and the level obtained following agonist administration. $n=4$ for all groups except ET-1 where $n=5$. (B) This illustrates the percentage (%) decrease in renal blood flow caused by the intra-renal arterial infusion of ET-1 (■), ET-3 (●) and sarafotoxin 6c (▲) $n=4$ for all groups, except ET-1 where $n=5$. (C) This illustrates the percentage (%) decrease in renal vascular conductance caused the intra-renal arterial infusion of ET-1 (filled squares), ET-3 (●) and sarafotoxin 6c (▲). $n=4$ for all groups, except ET-1 where $n=5$.

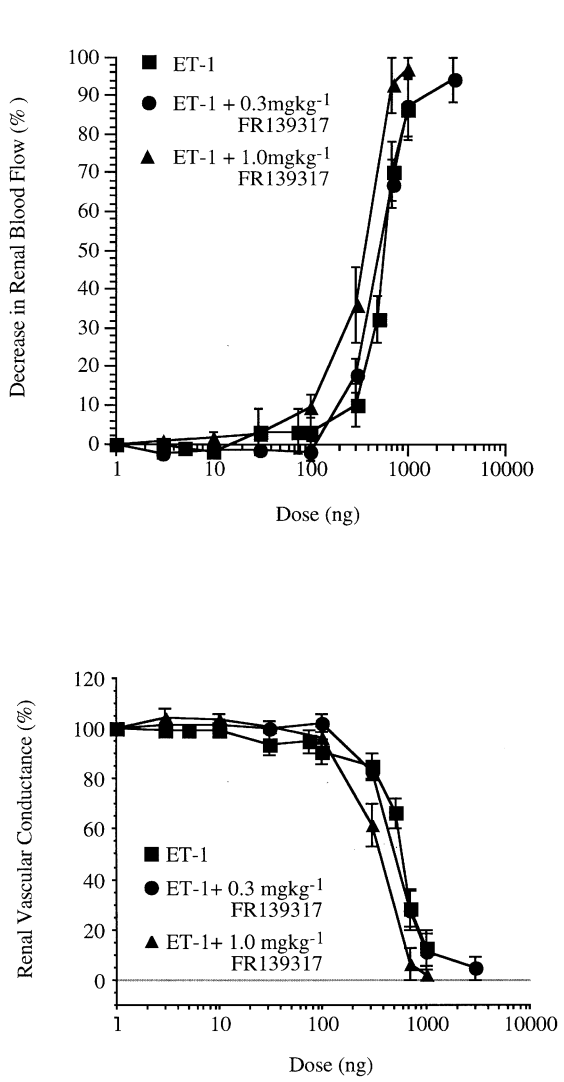

Figure 2 (A) This presents the per cent (%) decrease in renal blood flow in three groups of rats given increasing doses, of ET-1 (■, $n=5$), ET-1 plus 0.3 mg kg^{-1} FR139317 (●, $n=4$) or ET-1 plus 1.0 mg kg^{-1} FR139317 (▲, $n=3$). (B) This illustrates the per cent (%) decrease in renal vascular conductance in three groups of rats given increasing doses of ET-1 (■, $n=5$), ET-1 plus 0.3 mg kg^{-1} FR139317 (●, $n=4$) or ET-1 plus 1.0 mg kg^{-1} FR139317 (▲, $n=3$).

vascular conductance (Figure 2B) in the absence or presence of the antagonist.

The intra-renal arterial infusion of L-NAME at 10 μg kg^{-1} min for 90 min was associated with significant increases ($P<0.05-0.01$) in blood pressure, renal vascular resistance and decreases in renal blood flow in all groups (Table 1). Preliminary studies showed that using this approach, the vasodilator response to a bolus injection of 1.0 μg acetylcholine into the renal arterial cannula was blocked by some 70%, whereas the vasodilator response to 3 μg sodium nitroprusside, a NO donor, was unchanged. Under these conditions, administration of ET-1 intra-arterial reduced renal blood flow (Figure 3A) and decreased renal vascular conductance (Figure 3B) in a dose-related manner reaching almost a total cessation of flow at 1000 ng. In spite of there being a slight enhancement in the ET-1 mediated vasoconstriction at the lower doses, neither the pattern nor magnitude of the renal blood flow or vascular conductance curves were different from those obtained in the absence of L-NAME (Figure 3A,B). Intra-renal infusion of increasing doses of ET-3 in the presence of L-NAME also caused a progressive reduction in renal blood flow, which virtually ceased at a dose of 3000 ng ET-3 (Figure 3C) and in renal vascular conductance (Figure 3D), and although there was a slight leftward shift of the curve over the lower part of the dose range compared with that obtained in the absence of L-NAME was not significant, even though the EC_{50} for renal blood flow was significantly lower at 638 ± 69 ng ($P<0.01$). Figure 3E,F show the dose related reductions in renal blood flow and renal vascular conductance, respectively, caused by sarafotoxin 6c in the absence and presence of L-NAME. It can be seen that in the presence of L-NAME, the dose-response relationships for renal blood flow (Figure 3E) and renal vascular conductance (Figure 3F) were shifted significantly ($P<0.01$) to the left, and in terms of renal blood flow was associated with a lower EC_{50} ($P<0.01$) and a markedly greater maximal reduction of some 92% compared with 56% in the absence of L-NAME ($P<0.01$).

Discussion

At the present time the roles of the different endothelin receptors subtypes mediating vasoconstriction in the rat kidney is unclear. Binding studies have shown a differential pattern of ET_A and ET_B receptors within the renal cortex, medulla and papilla (Gellai *et al*., 1994) which may vary dependent upon the amount of vasculature and tubular tissue at each level. This diversity is supported by functional studies which highlight the complexity of the situation. Clark & Pierre (1995) using the isolated rat renal artery found that the constriction of the conducting artery was primarily mediated by ET_A receptors, although they acknowledged that another endothelin subtype was probably also involved in the contractile response. Indeed, our own work (Wu *et al*., 1997) using the rat arcuate artery *in vitro*, strongly suggested a primary role for ET_A. In other *in vivo* studies in which endothelins were given systemically into the anaesthetized rats, Cristol *et al*. (1993) provided firmer evidence for ET_B receptors mediating the renal vasoconstrictor response which was supported by the work of Pollock & Opgenorth (1993) and Matsuura *et al*. (1997) who in an investigation in the rat indicated that there might be some interaction between ET_A and ET_B receptor subtypes whereby there was a potential mutual inhibition. Thus, one of the major objectives of the present study was to examine the receptor subtypes regulating the renal resistance vasculature more carefully by administering the compounds directly into the kidney thereby avoiding any systemic changes in blood pressure which could indirectly impact on kidney vascular resistance. A second aim was to explore whether the endothelin mediated renal vasoconstriction was in any way dependent upon the production of NO.

It was evident from the initial studies that to a great extent the local administration of the endothelins were successfully confined to the kidney as even at the highest doses used there were no or minor 5–10% increases in blood pressure at a time when there were marked reductions in renal blood flow. The local administration of the ET-1, ET-3 and sarafotoxin 6c all produced graded reductions in renal blood flow and decreases in renal vascular conductances giving good dose-response relationships while at the highest doses of ET-1 and ET-3 there was an almost complete cessation of renal blood flow. Interestingly, the EC_{50} for ET-3 was only twice that of ET-1 which could be taken as indicating that the two agonists were acting at the same receptor, possibly the ET_B subtype. This situation was similar to that described by Panek *et al*. (1992) in

which they used the rabbit pulmonary artery which only contains ET_B receptors, and found the Ki's for ET-1 and ET-3 to be very close at 5.5 ± 0.4 and 4.9 ± 0.5 nM, respectively. In an attempt to provide evidence for a role of ET_A receptors, dose-response curves for ET-1 were drawn up following the administration of the ET_A selective antagonist FR139317, given at doses which caused effective blockade of ET_A receptors when given systemically to rats (Sogabe *et al*., 1993; Gardiner *et al*., 1994). Even though the full dose of the antagonist was delivered *via* the intra-arterial cannula, there was no effect on basal renal blood flow, which could be interpreted as meaning that the basal tone of the renal vasculature was not dependent upon endogenous endothelin release acting upon ET_A receptors. Although the lower dose of FR139317 had no effect on the ET-1 dose-response relationship, as the EC_{50} was unchanged, the higher dose caused a modest, but significant decrease in EC_{50}, suggesting a heightened sensitivity to the peptide. The exact reason for this

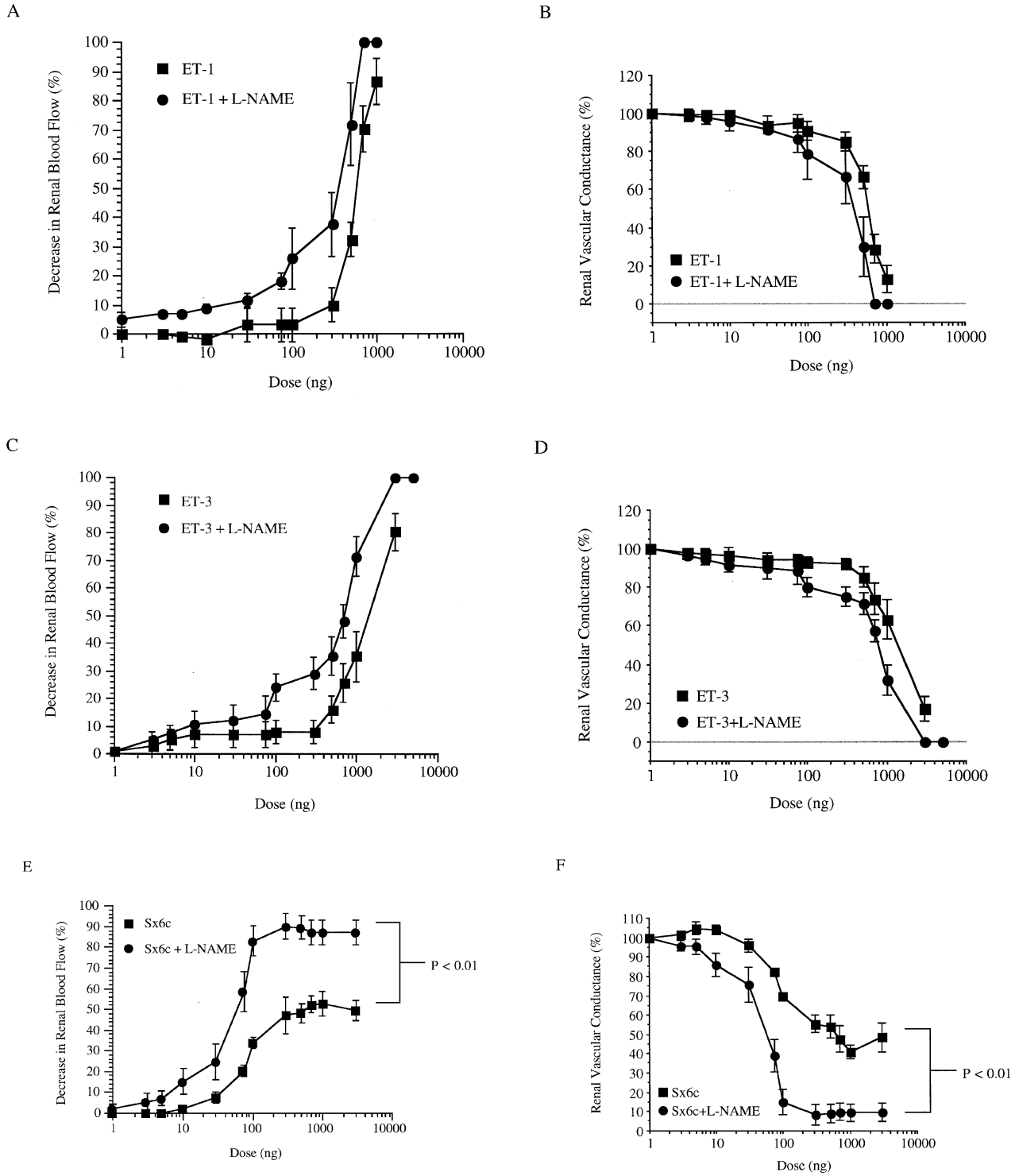

Figure 3 This demonstrates the effect of L-NAME given i.r.a. at 10 μg kg^{-1} min^{-1} for 90 min on the ability of ET-1 to reduce renal blood flow (A) and conductance (B); ET-3 to reduce renal blood flow (C) and conductance (D); and sarafotoxin 6c to reduce renal blood flow (E) and conductance (F). The filled squares are the responses obtained in the absence and the filled circles are those generated in the presence of L-NAME. $P<0.01$ was obtained by comparison of the two curves using one-way ANOVA.

is unclear, but could be partly compatible with the view expressed by Matsuura *et al*. (1997) that there may be an inhibitory interaction between the receptor subtypes.

The dose-response relationship between sarafotoxon 6c, the increase in renal vascular resistance, decrease in vascular conductance and the reduction in renal blood flow was different from that obtained with ET-1 and ET-3 in two very important ways. Firstly, the EC_{50} was much lower, indicating that the renal vasculature was much more sensitive to the sarafotoxin 6c, compared to the endothelins themselves. This finding was of interest in that in our previous studies using isolated rat kidney arcuate arteries (Wu *et al*., 1997), sarafotoxin 6c was without effect at dose levels at which ET-1 was causing a maximal vasoconstrictor response. One explanation could be that the changes in renal blood flow observed in the present study reflected more a change in tone within the major resistance beds, afferent and efferent arterioles, rather than in the larger vessel, the arcuate artery, which provides only a minor component of renal vascular resistance. A second striking difference was that even at the highest doses of sarafotoxin 6c, the maximum degree of reduction achieved was only about 60% of the total possible, i.e. much less than that observed with ET-1 and ET-3. The reason for this difference was not evident initially, but could have been due to a differential action of sarafotoxin 6c on vascular versus endothelial ET_B receptors in which the contribution of NO in the overall response could be different.

These initial observations implicated a role for NO in the renal vascular responses to the endothelins and sarafotoxin 6c and in order to examine this further, a final set of studies were performed in which endogenous production of NO was inhibited. This was done by infusing L-NAME directly into the renal artery at a low dose over a long period of time (90 min) with the aim of causing a gradual inhibition of the NO synthase enzyme. It was evident that the L-NAME caused a modest reduction in basal renal blood flow and increase in renal vascular resistance consistent with NO having a tonic vasodilator action under normal conditions in this tissue. Moreover, it was evident in the preliminary studies that the renal vasodilation caused by a bolus dose of acetylcholine, which mediates its action by stimulating NO generation by the endothelial cells, was largely prevented indicating that endogenous NO generation within the kidney was blocked. There was also a concomitant rise in blood pressure under these conditions which is an effect consistently reported following NO synthase blockade with this compound (Rees *et al*., 1990) and probably reflects spill-over of the L-NAME into the systemic circulation.

Administration of both ET-1 and ET-3 following blockade of NO production resulted in a shift to the left of the renal vascular conductance dose-response curves with significant reductions in the renal blood flow EC_{50} values. This would suggest that the endothelin-mediated renal vasoconstrictions were somewhat buffered by a concomitant production of NO when the peptides exerted their action. The fact that ET_B receptors are present on endothelial cells and when activated to cause NO production (Brooks, 1997) would go part way to explaining the shift in the dose-response curves and reductions in EC_{50}'s observed in this study. The influence of L-NAME on the renal vascular responses to sarafotoxin 6c was perhaps more striking. There was also a leftward shift in the renal vascular resistance dose-response curve and a reduction in the renal blood flow EC_{50}, but more importantly, the maximum reduction in renal blood flow shifted from 56% normally to 100% in the presence of L-NAME. Clearly, the contribution of NO in modulating the renal vasoconstriction responses to sarafotoxin 6c was much greater compared with that for either ET-1 or ET-3.

The current view is that ET-1, ET-3 and sarafotoxin 6c are equi-effective at ET_B receptors on endothelial cells which is considered to result in NO generation (Brooks, 1997), and the fact that sarafotoxin 6c was much less effective on vascular receptors mediating vasoconstriction would support this suggestion. Thus, it is possible to argue that once the NO component of the sarafotoxin 6c mediated action was removed, the full extent of its potency on the vascular ET_B receptors became evident. Importantly, these data do show that sarafotoxin 6c is very potent in causing constriction of vascular smooth muscle in the kidney, being more potent than either ET-1 or ET-3. However, under normal circumstances because of the greater effectiveness of sarafotoxin 6c to cause NO production probably at the endothelial cell, its potency has been underestimated to date. The importance of ET_B mediated NO production in determining basal vascular tone has been recognized by Gellai *et al*. (1996) who observed that administration of a selective ET_B antagonist raised blood pressure and reduced renal blood flow in conscious rats. An alternative possibility which has to be considered is that there may be different subtypes of ET_B receptors present within the kidney which may be differentially sensitive to ET-1 and ET-3 as against sarafotoxin 6c. There is pharmacological evidence to support this view (Sumner *et al*., 1992) in which comparative studies have been undertaken with selective agonists and antagonists. However, as yet at a molecular level only two genes for the endothelin receptors have been cloned (Brooks, 1997).

This study set out to investigate *in vivo* in the rat the way in which ET-1, ET-3 and sarafotoxin 6c mediated vasoconstriction in the kidney. It was apparent that direct intra-renal arterial infusion of ET-1 reduced renal blood flow in a dose-related manner which was relatively insensitive to ET_A receptor blockade with FR139317 suggesting ET_B receptors on the resistance vessels were mediating constriction. The effectiveness of both ET-1 and ET-3 were enhanced following L-NAME administration suggesting that NO buffered endothelin induced vasoconstriction. By contrast, sarafotoxin 6c caused a relatively modest reduction in renal blood flow, with a lower EC_{50} compared to ET-1 and ET-3. However, when NO production was blocked, not only was the EC_{50} decreased, but the maximum reduction in renal blood flow was approximately doubled and became similar to that obtained with ET-1 and ET-3. These findings show that sarafotoxin 6c acts on endothelin receptors within the kidney which generate a much greater level of NO than ET-1 or ET-3 but whether this reflects differing selectivities at vascular or endothelial cell sites, or the presence of further subtypes of ET_B receptors remains to be explored.

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