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BQ-153, a novel endothelin (ET)$_A$ antagonist, attenuates the renal vascular effects of endothelin-1

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Abstract—Endothelin (ET)-1, leukotriene D_4 and the thromboxane analogue, U-44069, were all shown to produce dose-dependent reductions in renal blood flow after direct injection into the renal artery of anaesthetized pigs. The effects of ET-1 differed from the other two mediators in that ET-1 caused a transient vasodilator followed by a prolonged vasoconstrictor response. The pressor response was not mediated by the secondary release of either leukotriene D_4 or thromboxane A_2 as evidenced by the lack of effect of appropriate receptor antagonist MK571 (3-{-2(7-chloro-2 quinolinyl) ethenyl}phenyl{3-(dimethylamino-3-oxopropyl)thio}methyl thio propionic acid) and L-670,596 respectively. This response, however, could be inhibited in a dose-dependent fashion by the selective ET_A antagonist, BQ-153 (cyclo-D-sulphalanine-L-Pro-D-Val-L-Leu-D-Trp-). Following blockade by BQ-153 the vasodilator response was unaffected and a residual pressor response remained, suggesting that either or both of these effects were mediated either through an ET_B or a novel, as yet undefined, endothelin receptor.

Endothelin (ET)-1 is a potent, 21 amino acid, vasoconstrictor peptide first isolated from medium conditioned by cultured endothelial cells (Yanagisawa et al 1988). Subsequent studies have revealed the presence of two additional, closely related peptides (ET-2 and ET-3), all three peptides being coded for by three separate genes (Inoue et al 1989). Further research has shown the presence of two distinct endothelin receptors, which have been termed ET_A (selective for ET-1) and ET_B (nonselective for ET isopeptides) (Arai et al 1990; Sakurai et al 1990). These peptides and receptors are widely distributed in a number of tissues and mediate a number of biological responses (Lerman et al 1990).

There has been a considerable interest in the role of endothelin peptides in renal pathology (Simonson & Dunn 1991). Evidence for a role for ET-1 in post-ischaemic acute renal failure and cyclosporine-induced glomerular dysfunction has been obtained through the use of anti-endothelin antibodies (Kon et al 1989, 1990; Shibouta et al 1990). The ET-1 gene is expressed not only in vascular endothelial cells, but also in mesangial cells where its expression can be upregulated by various inflammatory mediators (Sakamoto et al 1990; Zoja et al 1991). ET-1-induced reductions in renal blood flow and function can be mediated through both efferent and afferent arteriolar constriction (Kon et al 1989), as well as through effects on ET_A receptors on mesangial cells (Simonson & Dunn 1990). The vasoconstriction mediated by ET-1 may be counteracted in the kidney by the secondary release of vasodilators, such as prostaglandin I_2 (Chou et al 1990). Because ET-1 can induce eicosanoid synthesis, it is possible that the renal vasoconstrictor effects could be mediated, in part, by the release of pressor arachidonic acid metabolites, such as thromboxane A_2 and leukotriene D_4. This has been investigated in the present work through the use of specific receptor antagonists. Recently a highly selective, but relatively weak, ET_A antagonist, BE-18257B, a novel cyclic pentapeptide, has been isolated from *Streptomyces misakiensis* (Ihara et al 1991). Analogues of this have been synthesized and potent, selective ET_A antagonists, such as BQ-153 (cyclo-D-sulphalanine-L-Pro-D-Val-L-Leu-D-Trp-), have been described (Ihara et al 1992). The effects of BQ-153 on renal vasoconstriction in the pig have been investigated in the present work.

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Materials and methods

Materials. Leukotriene D_4, L-670,596 and MK-571 (3-{-2(7-chloro-2 quinolinyl) ethenyl}phenyl{3-(dimethylamino-3-oxo-propyl)thio}methyl thio propionic acid), synthesized at Merck Frosst; BQ-153 (cyclo-D-sulphalanine-L-Pro-D-Val-L-Leu-D-Trp-) synthesized at Banyu Central Research Laboratories; U-44069, Upjohn; ET-1, Peninsula labs; Azaperone, Pitman-Moore Ltd; sodium pentobarbitone, MTC Pharmaceuticals.

In-vivo procedures. Yorkshire domestic pigs, 2 months old, with a mean weight of $15{\cdot}0 \pm 2$ kg were sedated with azaperone (2–4 mg kg^{-1}, i.m.) and anaesthetized with sodium pentobarbitone (20 mg kg^{-1}, i.v.) Surgical anaesthesia was maintained with a continuous infusion of the sodium pentobarbitone (5–10 mg kg^{-1} h^{-1}, i.v.) from which the animals were not permitted to recover. Body temperature was maintained at 37·5°C with a homeothermic blanket (Harvard Instruments).

An endotracheal tube was inserted through a tracheostomy and the animals were ventilated with room air (Harvard Instruments respirator). Systemic arterial pressure was monitored with a catheter-tip pressure transducer (Millar Instruments) inserted into the right femoral artery. A port located 5 mm from the tip of the catheter permitted the withdrawal of arterial blood (0·3 mL) for blood gas (pO_2, pCO_2) and pH determinations (Instrumentation Laboratories blood gas analyser, model 1312). The respiratory volume (150–200 mL/stroke) and frequency (10–20 breaths min^{-1}) were adjusted to maintain pO_2 at 80–100 mmHg, pCO_2 at 35–45 mmHg, and pH at 7·35–

7·45. The heart rate was obtained for either the systemic blood pressure signal (Buxco Electronics cardiovascular analyser) or from the electrocardiogram (ECG) signal that triggered a cardiotachometer. A lead-II ECG was recorded and continuously displayed (Marquette Electronics defribrillator monitor). A flexible double lumen (Swan-Ganz flow-directed thermodilution) catheter was inserted into the jugular vein and the tip advanced into the main pulmonary artery for measurement of pulmonary arterial pressure.

The left renal artery was exposed through a subcostal incision and carefully freed from its connective tissue. A 25-gauge needle connected to polyethylene tubing (PE-50) was inserted into the artery at its juncture with the abdominal aorta for injection of either ET-1, U44069, leukotriene D_4 or 0·9% NaCl (saline). U44069, the stable endoperoxide analogue, was used in these studies in place of TxA_2 which is unstable and difficult to synthesize. Renal blood flow (RBF) was measured with a noncannulating electromagnetic flow probe (Biotronex Laboratories, model BL-6025-H43) applied to the renal artery and connected to a flowmeter (Biotronex Laboratories, model 613). Transducers of 2·0–4·0 mm circumference were used to obtain a fit on the artery that produced an estimated 10–20% reduction in vessel diameter.

The output signals from the transducers and flowmeter were monitored and recorded with a Beckman dynograph. Analogue signals from the recorder were fed to a microprocessor interface (Buxco Electronics cardiovascular analyser) which digitized the signals from an IBM PC. The physiological parameters included mean arterial pressure, heart rate, pulmonary arterial pressure and RBF. After surgery, the animals were allowed to stabilize until consistent readings were obtained in all parameters measured, after which baseline pressure and RBF values were established. All experiments were carried out under the guidelines of the Canadian Council of Animal Care.

Experimental protocol. After the stabilization period and when the baseline was established, ET-1, U44069 or leukotriene D_4 was injected into the renal artery at increasing doses until a maximum response was obtained. Sufficient time was allowed between doses to permit complete return to baseline values before injection of the subsequent dose. The dose that produced the maximal response was repeated to confirm the reproducibility of the ET-1, U44069 or leukotriene D_4 effect on RBF. The doses were plotted on a log scale (x-axis) with the corresponding response on the y-axis. A straight line which best fitted the data points was drawn and the dose which produced 50% of the maximal response (ED50) was determined from the graph for each agonist. Once the maximum response was determined separate groups of animals were pretreated either 2 min before the intrarenal arterial injection of ET-1 with the ET_A antagonist, BQ-153 (i.v.) or 5 min before with the thromboxane receptor antagonist, L-670,596 (2·0 mg kg^{-1}, i.v.), the leukotriene antagonist, MK-571 (5·0 mg kg^{-1}, i.v.) or the drug vehicles. U44069 and leukotriene D_4 were injected into the renal artery 30 min after treatment with either L-670,596 or MK-571, respectively. In a single pig, U44069 and leukotriene D_4 were injected into the renal artery 5 min after the administration of BQ-153 (0·1 mg kg^{-1}). L-670,596, MK-571, U44069 and leuotriene D_4 were dissolved in saline, ET-1 in phosphate-buffered saline and BQ-153 in distilled water. The data are expressed as mean ± s.e.m. Statistical analysis was carried out using the Student's *t*-test.

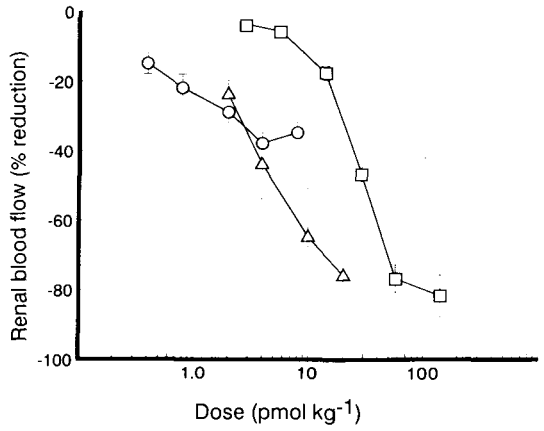

FIG. 2. Log–dose response curves for ET-1 (○), leukotriene D_4 (△) and U44069 (□) showing the percent reductions in renal blood flow produced by each peptide. Each point represents the mean ± s.e.m. of n = 8 for ET-1, n = 3 for leukotriene D_4, n = 10 for U44069.

Results

Following injection of low doses of ET-1 into the renal artery of the anaesthetized pig, biphasic changes in RBF were observed

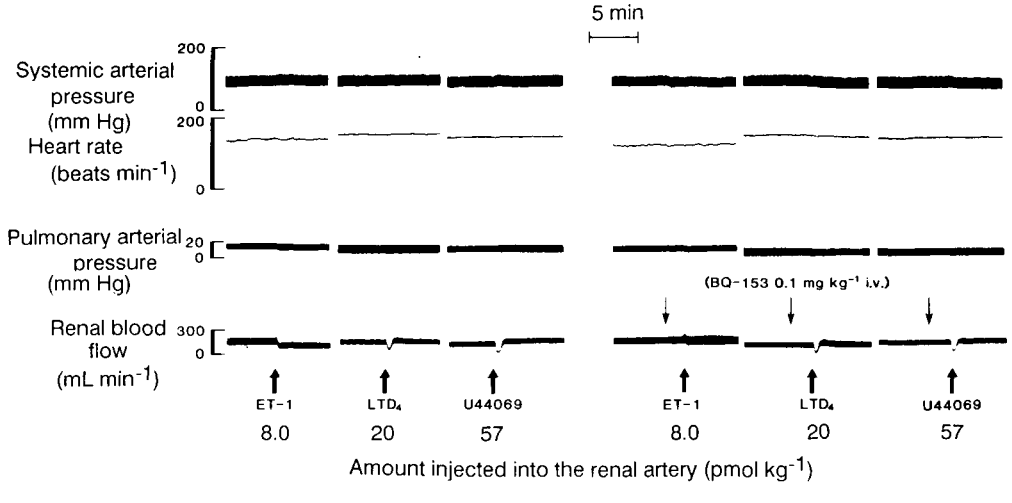

FIG. 1. A typical tracing showing the effects of ET-1, leukotriene D_4 and U44069 on changes in renal blood flow, pulmonary arterial pressure, heart rate and systemic arterial pressure in the presence and absence of BQ-153.

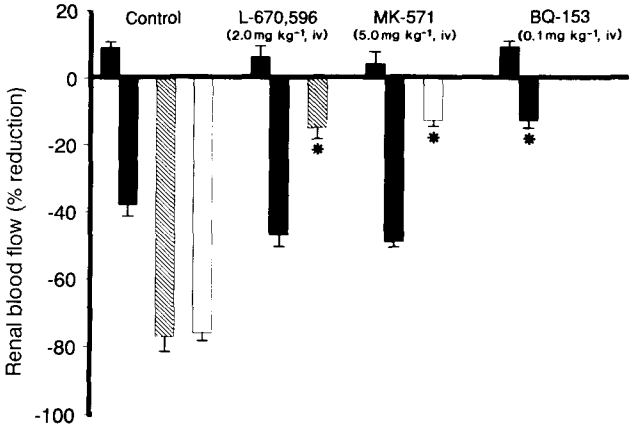

FIG. 3. The effects of L-670,596, MK-571 and BQ-153 on ET-1-induced reductions in renal blod flow. Results are shown as means ± s.e.m. (n = 8, 10, 3, respectively) for control responses, n = 3 for drug-treated groups). ■ ET-1 (4 pmol kg^{-1}, i.a.); ▩ U44069 (57 pmol kg^{-1}, i.a.); □ LTD_4 (20 pmol kg^{-1}, i.a.). * $P < 0.05$.

(Fig. 1). Thus, within 12 s following the injection of ET-1 there was a transient elevation in RBF followed by a sustained dose-related reduction in flow. RBF increased 3 ± 1% after the 1·0 ng kg^{-1} dose; the lowest amount of ET-1 injected into the renal artery. The maximum dose of ET-1 administered was 10 ng kg^{-1} which resulted in a 9 ± 1% rise in RBF. The increases in flow lasted 6·0 s and were not seen when U44069, leukotriene D_4 or phosphate-buffered saline was injected. The transient increases in RBF were followed by sustained dose-related reductions in flow which lasted considerably longer than the responses to either U44069 or leukotriene D_4. The duration of the ET-1-induced reduction in RBF was 24 ± 3 min, whereas the diminished flow produced by either U44069 or leukotriene D_4 lasted only 1·0 ± 0·1 min and 1·6 ± 0·2 min, respectively. The maximal reduction in flow with ET-1 was 38 ± 3% and was achieved at a dose of $4{\cdot}0 \times 10^{-12}$ mol kg^{-1} (10 ng kg^{-1}). Higher doses of ET-1 produced no further reductions in RBF. These decreases in RBF were produced without any effects on systemic arterial pressure, heart rate or pulmonary arterial pressure (Fig. 1).

U44069 and leukotriene D_4 also decreased RBF in a dose-dependent manner but the responses were transient. The U44069-induced reduction in flow, but not the leukotriene D_4 effect, was followed by a reactive hyperaemia which lasted 4·6 ± 0·9 min. At a dose of 10 ng kg^{-1}, leukotriene D_4 produced a maximal decrease in RBF of 76 ± 2%, whereas twice this dose was required to reduce flow by 77 ± 4% with U44069. Log dose response curves for the effects of ET-1, U44069 and leukotriene D_4 on RBF are shown in Fig. 2 The half-maximal dose (ED50) for leukotriene D_4 was achieved at 3·6 pmol kg^{-1} and for U44069 at 26 pmol kg^{-1}. ET-1 produced a maximal decrease in RBF of 38 ± 3% achieved at a dose of 10 ng kg^{-1}. The ED50 for ET-1 was determined to be 0·6 pmol kg^{-1} (Fig. 2).

The effects of various blockers on the reductions in renal blood flow induced by ET-1 are shown in Fig. 3. The thromboxane receptor antagonist, L-670,596 (Ford-Hutchinson et al 1989), and the leukotriene D_4 receptor antagonist, MK-571 (Jones et al 1989), failed to modify the response induced by the injection of 4 pmol kg^{-1} of ET-1 directly into the renal artery when administered intravenously 5 min before ET-1. These drugs, however, were administered at appropriate doses because 30 min later in the same animals they produced significant attenuation of the changes in RBF induced by the appropriate agonist. The constrictor, but not the dilator, response to ET-1 could be significantly blocked by administration of BQ-153 as shown in Fig. 3. The selectivity of BQ-153 was shown in a single experiment in which administration of bolus doses of BQ-153 (0·1 mg kg^{-1}) 5 min before the injection of either leukotriene D_4 or U44069 failed to modify the constrictor response to these agents (Fig. 1).

In a separate series of experiments the effects of various doses of BQ-153 against a standardized dose of ET-1 were tested. The maximal reduction in RBF induced by ET-1 (4 pmol kg^{-1}) was 38 ± 2% (n = 8). Following 5 min pretreatment with BQ-153 at doses of 0·01, 0·02, 0·05 and 0·1 mg kg^{-1}, the reductions in RBF induced by ET-1 were 31 ± 1 (n = 3), 22 ± 1 (n = 3, $P < 0.05$), 17 ± 1 (n = 3, $P < 0.05$) and 13 ± 2 (n = 3, $P < 0.05$) (all results shown as percent means ± s.e.m.). Higher doses of BQ-153, from 0·2 to 1·0 mg kg^{-1}, failed to attenuate further the decreases in RBF induced by ET-1. The residual reduction in blood flow following maximal inhibition by BQ-153 was 19 ± 6 mL min^{-1} (n = 4), or 13 ± 2% of the initial flow.

Discussion

As described previously in the pig and other species, ET-1 is a potent renal vasoconstrictor (Lippton et al 1988; Pernow et al 1989a; Chou et al 1990). When compared with the thromboxane analogue, U44069 and leukotriene D_4, the effects of ET-1 were observed at lower doses and were considerably prolonged, probably reflecting slow dissociation of ET-1 from its receptor (Hirata et al 1988). Previous studies have indicated that the pressor actions of endothelin are limited by the release of vasodilators, in particular prostaglandin I_2 and endothelium-derived relaxing factor (DeNucci et al 1988; Chou et al 1990). This is consistent with the present observations where a transient increase in blood flow and a limited (as compared with leukotriene D_4 and U44069) decrease in renal blood flow were observed. The role of prostaglandins may be less important in the porcine renal vasculature as, in a single animal, pretreatment with indomethacin (5 mg kg^{-1} i.v.) failed to modify the pressor response to ET-1.

The reductions in RBF were dose-dependent and caused no systemic cardiovascular changes after the intrarenal arterial injection of ET-1. This suggests a direct action of the peptide on the renal vasculature. The concentrations of ET-1 used in the present study were below the threshold needed to cause a rise in systemic arterial blood pressure (Nakamoto et al 1989). The lack of systemic effects at the higher doses of ET-1 used in this study may indicate that the peptide was completely metabolized or eliminated after one passage through the kidney. Indeed, Pernow et al (1989b) demonstrated that the kidney was a major site for the elimination of ET-1.

As ET-1 is known to stimulate the synthesis of arachidonic acid metabolites, one objective of the present work was to investigate whether the reductions in RBF could be mediated through the release of either leukotriene D_4 or thromboxane A_2, potent constrictors of the renal vasculature. As shown in the present work, the time course and profile of the ET-1 response was different from that observed with U44069 and leukotriene D_4. Injection of either eicosanoid resulted in an immediate and transient (< 1·0 min) reduction in RBF which was distinctly different from the biphasic, transient depressor followed by the prolonged pressor response of ET-1.

Further evidence that neither thromboxane A_2 nor leukotriene D_4 receptors are involved in the ET-induced vasoconstriction came from the studies with L-670,596, a potent, competitive and selective TxA_2 receptor antagonist (Ford-Hutchinson et al 1989) and MK-571, a selective leukotriene D_4 receptor antagonist (Jones et al 1989) which failed to attenuate the response to ET-1. This indicates that despite the fact that ET-1 activates eicosanoid pathways, the vasoconstrictor actions associated

with some members of this pathway are not involved in the renal vasoconstriction produced by ET-1 in the pig.

The present work, however, clearly demonstrates that the pressor actions of ET-1 in the kidney can be attenuated by the ET_A antagonist, BQ-153. BQ-153 has been shown to be a potent antagonist of ET-1 binding to ET_A receptors in porcine aortic smooth muscle cells, a very weak antagonist of ET-1 binding to ET_B receptors in porcine cerebellum, a competitive antagonist of ET-1-induced contractions of the porcine coronary artery and an inhibitor of ET-1-induced sustained pressor actions in the conscious rat (Ihara et al 1992). A residual component of the pressor action of ET-1 and all the depressor response were unaffected by BQ-153. This is consistent with studies in conscious rats where BQ-153 affected only the pressor response and had no effect on the depressor responses and in-vitro studies in the porcine coronary artery where a component of the contraction was unaffected by BQ-153 (Ihara et al 1992). These residual responses have been postulated to be mediated through either ET_B receptors or an additional, as yet undefined receptor. Thus the present work would indicate that both ET_A and ET_B receptors are functionally active in the porcine kidney, but that ET_A receptor activation is the predominant event in the pressor responses. These results suggest that BQ-153 will be an appropriate agent to investigate the role of ET_A receptor activation in animal models of renal failure and that this may provide important information as to the potential role of endothelin as a mediator of human renal disease.

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