Renal Protection by a Dual ET_A/ET_B Endothelin Antagonist, L-754,142, after Aortic Cross-Clamping in the Dog

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Abstract. Renal insufficiency is a significant complication that occurs after surgical procedures, requiring cross-clamping of the aorta. The mechanism for this renal dysfunction is currently not known, but studies suggest a potential role of endothelin in mediating the insufficiency. Accordingly, the role of endothelin was assessed using the nonpeptidyl, dual ET_A/ET_B endothelin antagonist L-754,142 in a model of renal insufficiency in the anesthetized dog induced by cross-clamping the suprarenal aorta for 60 min, followed by 2 h of reperfusion. In vehicle-treated animals (saline, n = 8) after 2 h of reperfusion, plasma [ET-1] increased 66% and renal blood flow (RBF) was reduced by 38% compared with baseline. This decline was associated with an 84% increase in renal vascular resistance and a 54% reduction in GFR (baseline, 46 ± 5 ml/min; 21 ± 3 ml/min at 2 h; P < 0.01) and sodium reabsorption (baseline, 6.7 ± 0.7 μEq/min; 3.0 ± 0.5 μEq/min at 2 h, P < 0.01). After baseline measurements, pretreatment with L-754,142 at 0.3 mg/kg bolus + 0.1 mg/kg per h continuous infusion (low dose; n = 8) or 3.0 mg/kg bolus + 1 mg/kg per h infusion (high dose; n = 8) initiated 45 min before aortic cross-clamp led to a dose-dependent normalization of RBF and renal vascular resistance within 2 h of cross-clamp removal. GFR was also improved and returned to within 75% of baseline (P < 0.01 versus vehicle) by 2 h of reperfusion with L-754,142 (baseline, 55 ± 5 ml/min; 42 ± 5 ml/min at 2 h with the high dose). The improvement of GFR with L-754,142 treatment was associated with a preservation of sodium reabsorption compared with vehicle-treated animals. This study supports a role of endothelin in the pathogenesis of renal insufficiency after aortic cross-clamping and demonstrates that pretreatment with the dual ET_A/ET_B endothelin antagonist L-754,142 preserves RBF and sodium reabsorption, leading to a significant improvement in GFR. (J Am Soc Nephrol 8: 1061–1071, 1997)
addition, Sandok et al. (10) and Stingo et al. (7) reported that 60 min of suprarenal ACC in the dog was associated with a greater than twofold increase in plasma ET-1 levels. Stingo et al. (7) went on to demonstrate that treatment with the peptidic ET_A antagonist BQ-123 demonstrated partial protection of RBF but not GFR after ACC. These data suggest that the mechanism of GFR reduction after acute renal ischemia may be mediated via either a non-ET-1 mechanism or may involve both ET_A and ET_B receptors. Support for the latter explanation comes from the studies of Kon et al. (15) and López-Farré et al. (16), who were able to reverse the decline in GFR after acute renal ischemia in the rat using an antienothelin antibody, which would interfere with endothelin interacting with either receptor subtype. These reports, combined with the observation by Brooks et al. (17) that activation of ET_B receptors in the dog reduces reabsorption of sodium while ET_A receptor activation appears to mediate vasoconstriction, suggest that a dual ET_A/ET_B receptor antagonist may be beneficial in acute renal ischemia. On the basis of these observations, we examined the potential benefits of a new, potent, dual ET_A/ET_B receptor antagonist, L-754,142 (18), to preserve RBF and kidney function after acute renal ischemia in a canine model of suprarenal ACC.

Materials and Methods

All studies were conducted under the guiding principles of the American Physiological Society and the Guide for the Care and Use of Laboratory Animals published by National Institutes of Health (publication no. 85-23, revised 1985). The methods and protocols used were a modification of those described by Sandok et al. (10). Mixed-breed dogs of either sex (9 to 14 kg) were anesthetized with sodium pentobarbital (30 mg/kg, intravenously), intubated, and allowed to breathe room air. The right carotid artery was cannulated for the measurement of systemic blood pressure. The right jugular vein was cannulated for administration of creatinine (25 mg/kg prime + 0.5 mg/kg per min). The left femoral artery and vein were cannulated for measuring arterial pressure below the cross-clamp and for the administration of anesthesia and a saline drip, respectively. The right femoral vein was cannulated for the administration of L-754,142 or the saline vehicle. A midline incision was made in the lower abdomen, and bilateral ureteral catheters were inserted just proximal to the bladder for urine collection. A lateral incision was made in the right flank, and the right kidney was exposed by careful dissection, allowing the peritoneum to remain intact. The abdominal aorta was dissected free above the right renal artery, and umbilical tape was placed around it. The right renal artery was dissected free, and an electromagnetic (Carolina Medical, Inc., King, NC) or ultrasonic (Transonic, Ithaca, NY) flow probe was placed around it for assessing RBF. Arterial blood pressure, RBF, and the electrocardiogram were continuously monitored and a Gould R53800 physiograph. The output was also digitized and stored on disk using an M100 data acquisition system (Modular Instruments, Inc., Malvern, PA).

The animals that underwent 60 min of ACC were divided into three groups: those that received L-754,142 before cross-clamping at 0.3 mg/kg bolus + 0.1 mg/kg per h continuous infusion (low dose, n = 8) or 3 mg/kg bolus + 1 mg/kg per h continuous infusion (high dose, n = 9) and animals that received normal saline (vehicle, n = 9). All treatments were initiated 45 min before ACC and were infused at a rate of 15 ml/h. A separate group of five animals received a 3 mg/kg bolus + 1 mg/kg per h continuous infusion of L-754,142, but were not subjected to ACC and served as a time-matched control group (L-754,142, control). An additional control group of three animals received 10 mg/kg indomethacin and were not subjected to ACC.

Each animal was allowed to stabilize for 45 to 60 min, during which time an infusion of creatinine was initiated. A priming dose of 25 mg/kg creatinine was followed by a continuous infusion of 0.5 mg/kg per min creatinine in isotonic saline at a rate of 15 ml/h. This dosing regimen maintained plasma levels of creatinine at 6 to 12 mg/dl. After stabilization, a 30-min baseline clearance was determined. At the midpoint of this and each subsequent clearance period, a blood sample was taken for determining plasma levels of ET-1, creatinine, Na^+, K^+, and Cl^-1. Hemodynamic measurements were reported at the end of each clearance period. Urine was collected during the entire 30-min period, the volume and flow rate (V) was determined, and an analysis for creatinine, Na^+, K^+, and Cl^- were conducted. This baseline period before treatment represented the function to which all subsequent measurements were compared.

At the end of this first baseline clearance period, indomethacin (Sigma Chemical Co., St. Louis, MO) (10 mg/kg dissolved with 5 mg/kg NaHCO_3 in a total volume of 50 ml of distilled water) was administered as an intravenous infusion over 15 min to render the kidney more susceptible to ischemic injury (12,13). Indomethacin was used because ET-1 has been shown to release prostacyclin (19), which antagonizes the ET-1-induced vasoconstriction (12) and could compromise our ability to assess the role of ET-1 in acute renal failure. In addition, the use of nonsteroidal anti-inflammatory agents in the elderly has been noted to be a potential complicating factor in patients at risk of acute renal failure after surgery (5). Saline vehicle or L-754,142 administration was also initiated concomitantly with the administration of the indomethacin at a rate of 15 ml/h.

After completion of the indomethacin infusion (15 min), a second, 30-min clearance was determined (pre-cross-clamp). At the end of the second clearance period, the suprarenal aorta was cross-clamped above the right renal artery for 60 min using an atrumatic Cooley-Derra pediatric anastomosis clamp to completely occlude the abdominal aorta. After 60 min, the clamp was removed and reperfusion was followed for 2 h. Renal clearance rates were determined at 60 and 120 min of reperfusion. At the end of the experiment, each dog in the L-754,142-treated group was administered a single intravenous dose of 0.3 nmol/kg Big ET-1 to confirm blockade of endothelin receptors by L-754,142.

In the case of the time-matched control animals, L-754,142 was continuously infused over 4 h, with renal clearances taken at time points that were comparable to the time points taken in the ACC groups. Renal clearances were taken at similar times in the indomethacin control group.

The dosing regimen for L-754,142 was determined by evaluating the ability of the antagonist to inhibit the systemic pressor response to an intravenous dose of 0.3 nmol/kg Big ET-1 (Peptides International, Louisville, KY) by greater than 90% (Figure 1). Big ET-1 was used rather than ET-1 to avoid the potential compromising vasodilatory effects of ET-1, which occur before the prolonged vasoconstriction.

Exclusion Criterion

The dogs in this study were maintained on a normal diet and received water ad libitum. If renal function values deviated more than 2 SD from the mean at baseline, the values for these animals were excluded from the final analysis. As a result, two animals were eliminated from the study due to high GFR: one from the vehicle control group and one from the high-dose L-754,142 group.
Materials and Analyses

L-754,142 (18) was synthesized by the Department of Medicinal Chemistry (Merck Research Laboratories, Rahway, NJ). Blood samples for plasma analysis were drawn into heparinized tubes and immediately centrifuged at 3000 rpm. The plasma was then decanted and stored at 4°C along with the urine samples, pending analysis. Plasma samples for ET-1 determination were frozen at -70°C until analyzed. Plasma and urine concentrations of creatinine were determined using the GEM Profiler® (Electro-Nucleonics, Inc., Fairfield, NJ), using an automated methodology developed in our laboratory. GFR was estimated by the clearance of creatinine. Urine and plasma electrolyte concentrations were determined using an ion-selective electrode-type analyzer (ELISE®, Beckman Instruments, Irvine, CA). The fractional excretion of Na (\(FE_{Na}\)) was calculated by:

\[
\% FE_{Na} = \frac{\left( U_{Na} \times V \right)}{\left( P_{Na} \times GFR \right)} \times 100.
\]

Absolute urinary sodium excretion \(U_{Na}V\) was calculated by:

\[
U_{Na}V = U_{Na} \times V.
\]

The fractional excretion of potassium \(FE_{K}\) and the absolute potassium excretion were calculated by similar equations. The net reabsorption of sodium \(T_{Na}\) was calculated by:

\[
T_{Na} = \left( P_{Na} \times GFR \right) - \left( U_{Na} \times V \right) \times 20.
\]

RVR was calculated by dividing femoral mean arterial pressure by RBF.

ET-1 Determination

The plasma samples were thawed, and 1-ml aliquots were removed to 12×75 mm polypropylene test tubes. Quality control samples of control dog plasma “spiked” with 2, 5, and 10 fmol/ml unlabeled ET-1 (Peptides International) were included with the unknowns. To all 1-ml sample aliquots, 1 ml of 20% acetic acid was added. The acidified samples were vortexed and centrifuged for 1 min at 2800 × g (3200 rpm, Beckman J6, JS 4.0 rotor \(r_{max} = 22.6 \text{ cm} \)). Samples were applied to Si-C18 Sep-pak cartridges (500 mg of C18 in a 3-cm syringe; Waters part no. 20805; Millipore, Milford, MA) that had been conditioned previously with 3 ml of methanol, 3 ml of water, and 3 ml of 10% acetic acid. Columns were washed with 3 ml of 10% acetic acid and 6 ml of ethyl acetate. Columns were eluted with 3 ml of 80% methanol/20% 0.05 M ammonium bicarbonate. Eluted samples were dried overnight in a Savant Speed-Vac centrifugal evaporator (Farmingdale, NY). Dried samples were assayed the next day for immunoreactive ET-1.

The plasma samples were vortexed and centrifuged for 1 min at 2600 × g (3200 rpm, Beckman J6, JS 4.0 rotor \(r_{max} = 22.6 \text{ cm} \)). Samples were applied to Si-C18 Sep-pak cartridges (500 mg of C18 in a 3-cm syringe; Waters part no. 20805; Millipore, Milford, MA) that had been conditioned previously with 3 ml of methanol, 3 ml of water, and 3 ml of 10% acetic acid. Columns were washed with 3 ml of 10% acetic acid and 6 ml of ethyl acetate. Columns were eluted with 3 ml of 80% methanol/20% 0.05 M ammonium bicarbonate. Eluted samples were dried overnight in a Savant Speed-Vac centrifugal evaporator (Farmingdale, NY). Dried samples were assayed the next day for immunoreactive ET-1.

An effective dose of L-754,142 was established by the antagonism of the pressor response to Big ET-1. In a separate group of animals \((n = 4)\), dogs were repeatedly challenged with 0.3 nmol/kg Big ET-1 administered intravenously. This dose could be repeated without the development of tachyphylaxis for more than 4 h and gave a reproducible increase in systemic diastolic pressure of 37 ± 2 mmHg. Pretreatment of two animals with the 3 mg/kg bolus + 1 mg/kg per h infusion of L-754,142, followed by repetitive Big ET-1 challenges, led to a 90 ± 2% antagonism of the baseline Big ET-1 pressor response (5.7 ± 1 mmHg increase). An additional verification of the efficacy of the dose, animals treated with L-754,142 were challenged with an intravenous bolus dose of 0.3 nmol/kg Big ET-1 at the end of the experimental protocol (4 h of L-754,142 treatment). Figure 1 shows the effects of Big ET-1 dosing at the end of the experimental protocol in animals receiving the low and high dose of L-754,142 compared with control animals. The pressor response to 0.3 mg/kg Big ET-1 was reduced by >90% with both L-754,142 dosing regimens, thus confirming the efficacy of the doses.

The hemodynamic consequences of ACC are shown in Table 1. Occlusion of the aorta proximal to the right renal artery results in an immediate and significant decrease in femoral

Statistical Analyses

All data were presented as the mean ± SEM. A multivariate ANOVA consisting of repeated-measures analysis within the treatment group and a single-factor repeated analysis of variance between the active treatment group and the vehicle group was used to detect significant effects of L-754,142 treatment within each procedure group. Statistical significance was determined within each group over time using a repeated-measures ANOVA followed by a Dunnett’s test, with comparisons made to the baseline period. When a significant difference between treatment groups was indicated by two-way repeated-measures ANOVA, multiple comparisons were conducted using a Student-Newman-Keuls method to evaluate the difference from baseline between the two groups at each time point. Data were considered statistically significant if the \(P\) value was <0.05. All statistical tests were performed using SigmaStat for Windows (Jandel Scientific, San Rafael, CA).

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artrial pressure and a significant increase in carotid arterial pressure in all groups that was sustained for the entire 60-min cross-clamp period. Data represent 45 min of cross-clamping compared with baseline. The magnitude of this change was similar in all groups of animals. Removal of the aortic clamp resulted in a prompt return of both carotid and femoral arterial pressure to baseline values in both vehicle and high-dose L-754,142 treatment groups. In the low-dose L-754,142 group, RBF was significantly reduced at 1 h of reperfusion. Femoral arterial pressure was also observed to progressively decline below baseline in the vehicle-treated group by 2 h of reperfusion. Because there is no significant difference in arterial blood pressure among groups at any of the time points (P = 0.32), we conclude that L-754,142 had no effect on systemic blood pressures during or after ACC. This was confirmed in the time-matched control study, in which the high dose of L-754,142, administered in the absence of ACC, was shown to have no significant time-dependent effect on systemic arterial pressure.

Table 1 also presents the effects of ACC on heart rate for all treatment groups. Despite an increase in heart rate during ACC, this was found to be statistically significant only in the low-dose L-754,142 group. There were no other time-dependent changes in heart rate in any of the other groups.

The time-dependent changes in RBF before and after ACC are shown in Figure 2. Baseline RBF was similar among groups (P = 0.23). During cross-clamping, RBF was reduced significantly to <10 ml/min. After reperfusion, there was an immediate and transient hyperemic response in RBF in all animals. In the vehicle-treated animals, this was followed by a rapid decline in RBF, which remained significantly reduced by 38 to 41% for the entire reperfusion period compared with the baseline RBF values (P < 0.01). In contrast, with L-754,142 treatment, RBF was improved during reperfusion. In the low-dose group, RBF was significantly reduced at 1 h of reperfusion (P < 0.05), but returned to baseline by 2 h of reperfusion. With the high dose, RBF returned to baseline more rapidly within the first hour and remained at this level (P = 0.34 versus baseline). Thus, after aortic cross-clamp, animals treated with L-754,142 demonstrated a return to baseline RBF within 2 h of reperfusion compared with the vehicle-treated animals. The time-matched L-754,142 control group demonstrated no significant changes in RBF from baseline for the duration of the protocol.

The dramatic effect of ET-1 blockade on RBF during the first 5 min of reperfusion is illustrated in Figure 3 by a representative tracing from a saline vehicle dog and a dog that received the high dose of L-754,142. In contrast to the saline vehicle animal in which RBF was markedly reduced after reperfusion, treatment at the high dose of L-754,142 led to an immediate return of RBF to baseline that was sustained.

With the release of the cross-clamp and a return of systemic arterial pressure in all animals to baseline, the sustained reduc-

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**Table 1. Effect of L-754,142 on systemic hemodynamic parameters in anesthetized dogs after suprarenal aortic cross-clamping (ACC)**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Pre-ACC</th>
<th>45 min of ACC</th>
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<th>2 h Reflow</th>
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<td>vehicle</td>
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<td>139 ± 4</td>
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<td>vehicle</td>
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<td>153 ± 6</td>
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<td>140 ± 3</td>
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<tr>
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<td>162 ± 11</td>
<td>173 ± 9</td>
<td>167 ± 14</td>
<td>168 ± 17</td>
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</table>

* Hemodynamic measurements were reported at the end of the baseline clearance period, before ACC (after L-754,142 or vehicle and indomethacin treatment), at 45 min of ACC, and at the end of 1 and 2 h of reperfusion. After the baseline clearance period, L-754,142 was administered with indomethacin as an intravenous bolus of 0.3 mg/kg followed by a continuous infusion at 0.1 mg/kg per h in the low-dose group and 3.0 mg/kg bolus followed by 1 mg/kg per h infusion in the high-dose group. Vehicle animals received normal saline. Data are expressed as the mean ± SEM with n = 8 for each group except the control L-754,142 group, in which n = 5.

<sup>b</sup> P ≤ 0.01 versus baseline.
<sup>c</sup> P ≤ 0.05 versus baseline.
<sup>d</sup> Control animals were administered L-754,142 at the high dose in the absence of ACC with data reported at similar time points as the ACC animals for comparison.
The effect of aortic cross-clamping (ACC) on renal blood flow (RBF) for animals pretreated with either saline vehicle (n = 8) or the endothelin antagonist L-754,142 at either 3 mg/kg bolus + 1 mg/kg per h (high dose; n = 8) or 0.3 mg/kg bolus + 0.1 mg/kg per h (low dose; n = 8) and for high-dose L-754,142 time-matched controls (control, n = 5). Bars represent function at baseline, before ACC (after indomethacin and either L-754,142 or saline), 45 min into ACC, and at 1 and 2 h of reperfusion. For the L-754,142 controls, the bars represent data reported at similar time points as the ACC groups. Statistical significance versus baseline function within groups is represented by **P < 0.01.

Figure 2. The effect of aortic cross-clamping (ACC) on renal blood flow (RBF) for animals pretreated with either saline vehicle (n = 8) or the endothelin antagonist L-754,142 at either 3 mg/kg bolus + 1 mg/kg per h (high dose; n = 8) or 0.3 mg/kg bolus + 0.1 mg/kg per h (low dose; n = 8) and for high-dose L-754,142 time-matched controls (control, n = 5). Bars represent function at baseline, before ACC (after indomethacin and either L-754,142 or saline), 45 min into ACC, and at 1 and 2 h of reperfusion. For the L-754,142 controls, the bars represent data reported at similar time points as the ACC groups. Statistical significance versus baseline function within groups is represented by **P < 0.01.

Reperfusion after 60 min of ACC is associated with reductions in renal function, which are summarized in Figure 5 and Table 2. The effects of L-754,142 or saline vehicle on GFR after ACC, estimated by the clearance of exogenously infused creatinine, are presented in Figure 5. At baseline, there was no significant difference in GFR among groups (P = 0.24). Although GFR was significantly reduced in the vehicle group after saline and indomethacin administration, this was assumed to be a transient response because the indomethacin control animals demonstrated a return to baseline function 3 and 4 h after administration (similar time points as the 1- and 2-h reperfusion points). Baseline GFR was 32 ± 7 ml/min compared with 28 ± 2 and 33 ± 2 ml/min at 3 and 4 h, respectively. Treatment with L-754,142 and indomethacin also led to an initial reduction of GFR in all groups, but this reduction did not achieve statistical significance.

After the release of the cross-clamp, reperfusion was shown to significantly reduce GFR in all groups of animals compared with baseline. The mean decrease in vehicle-treated dogs was 70 ± 6% and 54 ± 6% from baseline at 1 and 2 h, respectively. In contrast, at both 1 and 2 h of reperfusion, treatment with L-754,142 was shown to blunt the decline in GFR compared with vehicle treatment. After 2 h of reperfusion, GFR had returned to within 25 ± 5% of baseline in the low-dose L-754,142 treatment group and 26 ± 5% in the high-dose L-754,142 group, which was significantly greater (P < 0.01) in both treatment groups compared with the vehicle group after 2 h of reperfusion. In time-matched controls, GFR was not significantly different from baseline during the entire infusion despite a slight drop after indomethacin infusion (P = 0.08).

The effects of 60 min of ACC on the FE_{Na}, T_{Na}, U_{Na,V}, FE_{K}, urinary potassium excretion (U_{K,V}), and urine flow are pre-
Figure 3. A representative tracing from two dogs showing the effects of ACC on RBF (ml/min) before, during, and after the release of the cross-clamp for a vehicle control dog and one pretreated with the high dose of L-754,142. Recording speeds were 25 mm/s for the expanded tracings and 0.25 m/mls for the compressed tracings.

presented in Table 2. Reperfusion was found to have little effect on the urine flow in the vehicle and low-dose L-754,142 group of animals compared with baseline. In contrast, both of the high-dose L-754,142 treatment groups exhibited a significant reduction in urine flow from baseline during the entire infusion period that did not vary after the initial decline. An explanation for this decline in urine flow appears to be associated with a decrease in \( U_{Na} \), although not changed from baseline in the vehicle and low-dose L-754,142 groups, was significantly reduced from baseline in both high-dose L-754,142 groups. This was also reflected in the decline in \( FENa \); however, this was only statistically significant in the high-dose L-754,142 ACC group. After reperfusion, \( FENa \) tended to be lower in the L-754,142-treated animals, whereas there was a trend toward an increase in \( FENa \) in the saline vehicle group; however, this did not achieve statistical significance.

To further assess this tendency for a decrease in sodium excretion with L-754,142 treatment and an increase in sodium excretion in the vehicle group, \( T_{Na} \) was calculated as the difference between filtered and excreted sodium. These data indicated that \( T_{Na} \) was improved by L-754,142 treatment after release of the cross-clamp. In vehicle-treated animals after release of the ACC, \( T_{Na} \) was significantly reduced by 70 and 54% at both 1 and 2 h of reperfusion, respectively, compared with baseline \( (P < 0.001) \). With low-dose L-754,142, \( T_{Na} \) was slightly, but not significantly, reduced from baseline after reperfusion. In the high-dose L-754,142 group, \( T_{Na} \) returned to within 22% of baseline by 2 h of reperfusion and was significantly greater than saline vehicle-treated animals at both 1 and 2 h of reperfusion. In the absence of ACC, L-754,142 administration was shown to have no effect on \( T_{Na} \) over the 4-h infusion period \( (P = 0.19) \).

\( U_K \) did not significantly differ from baseline at any time point, although at 1 h of reperfusion \( U_K \) was significantly lower than the L-754,142-treated animals. With L-754,142 treatment, \( U_K \) also was not different from baseline at any time point. These results are minimized in the calculation of \( FE_K \). After 1 h of reperfusion, \( FE_K \) was significantly increased in the vehicle-treated animals compared with baseline but returned to baseline by 2 h of reperfusion, whereas L-754,142 treatment preserved \( FE_K \) at all time points.

The improvement in vascular function with an endothelin antagonist suggests that plasma ET-1 levels should be increased by 60 min of ACC. Evaluation of plasma samples from the ACC dogs in this study confirms this. Figure 6 presents the plasma ET-1 concentrations in all animals subjected to 60 min of ACC. Baseline plasma ET-1 concentrations were not significantly different among groups \( (P = 0.52) \). Addition of indomethacin led to a small but nonsignificant rise in plasma ET-1. However, in the high-dose L-754,142 group, administration of the compound and indomethacin led to a significant increase in plasma ET-1. Sixty minutes of ACC followed by 2 h of reperfusion further increased the plasma levels of ET-1 compared with baseline. Multivariate analysis demonstrated that the increase in plasma ET-1 was significantly greater in the high-dose L-754,142 treatment group compared with the vehicle group at all time points except baseline.
Figure 4. The effect of ACC on renal vascular resistance for animals pretreated with either saline vehicle (n = 8) or the endothelin antagonist L-754,142 at either 3 mg/kg bolus + 1 mg/kg per h (high dose; n = 8) or 0.3 mg/kg bolus + 0.1 mg/kg per h (low dose; n = 8) and for high-dose L-754,142 time-matched controls (control, n = 5). Bars represent function at baseline, before ACC (after indomethacin and either L-754,142 or saline), 45 min into the ACC, and at 1 and 2 h of reperfusion. For the L-754,142 controls, the bars represent data reported at similar time points as the ACC groups. Statistical significance versus baseline function within groups is represented by *P < 0.05 and **P < 0.01 and between groups versus vehicle by †P < 0.05.

Discussion

The results of the study presented here confirm previous reports (7,10) linking endothelin to postischemic increases in RVR and decreases in renal function in a model of acute renal failure that used 60 min of aortic cross-clamp. This model has been suggested (7,10) to represent a clinically relevant model of postsurgical acute renal failure in which kidney hypoperfusion, as opposed to cessation of flow during cross-clamp, is an important etiological factor (21). This study extends these observations further by demonstrating that use of a dual (ETA/ETB) endothelin receptor antagonist such as L-754,142 (18) can completely protect RBF and RVR and also significantly improve GFR. These results are in contrast to a previous study, in which the ETA-specific antagonist BQ-123 was shown to only partially protect renal hemodynamics and afford no protection of GFR (7). Therefore, our study supports and advances the hypothesis that endothelin contributes to renal dysfunction after ACC in the dog via both ETA and ETB receptors.

The role of ET-1 in renal injury is supported by the twofold rise in circulating [ET-1] during the occlusion period measured above the cross-clamp, which remained elevated during reperfusion observed in this and other studies (7,10). The source of ET-1 may either be the kidney in response to ischemia or the crushing injury of the aortic endothelium. Using a similar ACC model with a simulated bilateral nephrectomy before ACC, Sandok et al. (10) found no increase in ET-1 during ACC and only a slight increase after reperfusion. They concluded that the kidney releases endothelin or releases substances that lead to systemic release of ET-1. Exposure of the kidney to a large increase in ET-1 leads to marked vasoconstriction and a reduction in GFR. However, this increase in [ET-1] was not sufficient to produce an increase in systemic arterial pressure, demonstrating that the kidney is more sensitive to ET-1 than the systemic vasculature consistent with reports from other laboratories (14).

The effects of ET-1 on the kidney are complex. ET-1 mediates its response via two different receptor subtypes in mammals, ETA and ETB. In both the rat and the dog, ET-1 elicits vasoconstriction of the systemic vasculature via both receptors (17,22–25). Although unclear in the renal vasculature, vasoconstriction appears to be mediated predominantly by the ETA receptor in the dog (17) and predominantly by the ETB receptor in the rat (23,26), despite an approximately equal distribution of receptor types (26). BQ-123, an ETA-selective antagonist, has been shown to effectively increase both RBF and GFR in a rat model of renal ischemia (27). However, in the dog it was shown to improve, but not normalize, both RBF and RVR. In contrast, pretreatment with L-754,142, a dual ETA/ETB antagonist, restored RBF and RVR after reperfusion, confirming both the role of ET-1 in renal vascular constriction (7) and the
Figure 5. The effect of ACC on glomerular filtration rate as determined by clearance of exogenously administered creatinine for animals pretreated with either saline vehicle (n = 8) or the endothelin antagonist L-754,142 at either 3 mg/kg bolus + 1 mg/kg per h (high dose; n = 8) or 0.3 mg/kg bolus + 0.1 mg/kg per h (low dose; n = 8) and for high-dose L-754,142 time-matched controls (control, n = 5). Bars represent function at baseline, before ACC (after indomethacin and either L-754,142 or saline), and at 1 and 2 h of reperfusion. For the L-754,142 controls, the bars represent data reported at similar time points as the ACC groups. Each bar represents the mean ± SEM. Statistical significance versus baseline function is represented by *P < 0.05 and **P < 0.01 and between groups versus vehicle by tP < 0.05.

participation of the ET_B receptor in renal vasoconstriction in the dog.

Endothelin receptors also participate in the regulation of renal function. In the dog, the ET_B receptors have been localized mainly within the proximal tubules and glomeruli (17,22,26) and inhibit sodium reabsorption when stimulated (17). In the rat, however, inhibition of sodium reabsorption has been reported to be mediated by the ET_A receptor (26). Therefore, the role of the ET_A and ET_B receptors in renal function is not completely understood and may vary among species. Consequently, it may be beneficial to block both types of receptors to obtain the greatest protection of renal function. This may explain why BQ-123, an ET_A-selective antagonist, was found to have no benefit on GFR after 60 min of ACC (7), whereas L-754,142 was shown to significantly improve GFR. The inability of BQ-123 to protect GFR was suggested to be due to either too low a dose or a component of the damage associated with the ET_B receptor (7). This latter explanation is supported by our study. Although the reduction in GFR in our study was not as great as reported by Stingo et al. (7) in their vehicle group (54% versus 70%, respectively), this may be explained by the fact that we collected urine flow from both ureters rather than only one. Of note, the effects of ET-1 antagonism on GFR were not as immediate or dramatic as the improvement in RBF, demonstrating that there may be a time delay in beneficial effects or that other potential mediators may also contribute to the decrease in renal function.

The observation in the present study that using a dual ET_A/ET_B receptor antagonist significantly attenuates the reduction in GFR caused by ACC supports the notion that the ET_B receptor is involved with factors that influence GFR, such as the reabsorption of sodium (17). Brooks et al. (17) demonstrated that stimulation of ET_B receptors leads to a decrease in sodium reabsorption in the dog. Thus, blocking the ET_B receptor should maintain sodium reabsorption and GFR after ACC given the significant rise in plasma ET-1 levels (7,10). Our results support this hypothesis because T_Na was shown to be preserved in the L-754,142-treated animals, but was significantly reduced after reperfusion in the vehicle group. Thus, blockade of both the ET_A and ET_B receptors is probably the principle mechanism by which protection of renal function was achieved. Blockade of the ET_B receptor is also the most likely explanation for the significantly greater increase in plasma ET-1 in the L-754,142-treated animals compared with vehicles. Previous studies have suggested that clearance of ET-1 from the plasma is mediated by the ET_B receptors (28,29). Under normal physiological conditions, the vasoconstricting effects of ET-1 appear to be balanced by prostacyclin (12,19).
Table 2. Effect of L-754,142 on renal function in anesthetized dogs after suprarenal ACC

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Pre-ACC</th>
<th>1 h Reflow</th>
<th>2 h Reflow</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urine flow (ml/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vehicle</td>
<td>0.27 ± 0.09</td>
<td>0.13 ± 0.04</td>
<td>0.13 ± 0.04</td>
<td>0.20 ± 0.06</td>
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<tr>
<td>high dose 142</td>
<td>0.52 ± 0.18</td>
<td>0.14 ± 0.03</td>
<td>0.16 ± 0.03</td>
<td>0.21 ± 0.03</td>
</tr>
<tr>
<td>low dose 142</td>
<td>0.63 ± 0.24</td>
<td>0.91 ± 0.47</td>
<td>0.19 ± 0.03</td>
<td>0.26 ± 0.06</td>
</tr>
<tr>
<td>control 142(^d)</td>
<td>0.36 ± 0.04</td>
<td>0.19 ± 0.04</td>
<td>0.22 ± 0.04</td>
<td>0.22 ± 0.04</td>
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<tr>
<td><strong>Urinary sodium excretion (μEq/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vehicle</td>
<td>31.5 ± 11.5</td>
<td>30.3 ± 11.0</td>
<td>17.7 ± 5.6</td>
<td>21.2 ± 8.1</td>
</tr>
<tr>
<td>high dose 142</td>
<td>37.8 ± 13.2</td>
<td>13.5 ± 4.9</td>
<td>12.1 ± 2.3</td>
<td>14.3 ± 3.1</td>
</tr>
<tr>
<td>low dose 142</td>
<td>45.9 ± 16.8</td>
<td>43.2 ± 16.9</td>
<td>21.3 ± 5.3</td>
<td>23.3 ± 6.5</td>
</tr>
<tr>
<td>control 142(^d)</td>
<td>63.5 ± 4.6</td>
<td>27.5 ± 5.6</td>
<td>27.8 ± 8.0</td>
<td>33.7 ± 9.5</td>
</tr>
<tr>
<td><strong>Sodium reabsorption (μEq/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>vehicle</td>
<td>6.7 ± 0.7</td>
<td>3.9 ± 0.8</td>
<td>2.1 ± 0.5</td>
<td>3.0 ± 0.5</td>
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<tr>
<td>high dose 142</td>
<td>8.1 ± 0.7</td>
<td>7.6 ± 0.4</td>
<td>4.2 ± 0.7</td>
<td>6.3 ± 0.8</td>
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<tr>
<td>low dose 142</td>
<td>6.1 ± 0.8</td>
<td>4.9 ± 0.9</td>
<td>4.7 ± 0.3</td>
<td>4.5 ± 0.6</td>
</tr>
<tr>
<td>control 142(^d)</td>
<td>7.3 ± 1.2</td>
<td>5.2 ± 0.4</td>
<td>6.0 ± 1.0</td>
<td>6.8 ± 1.1</td>
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<td><strong>FENa</strong></td>
<td></td>
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<td></td>
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<tr>
<td>vehicle</td>
<td>0.52 ± 0.18</td>
<td>0.84 ± 0.29</td>
<td>0.86 ± 0.17</td>
<td>0.75 ± 0.3</td>
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<tr>
<td>high dose 142</td>
<td>1.06 ± 0.35</td>
<td>0.39 ± 0.16</td>
<td>0.40 ± 0.14</td>
<td>0.22 ± 0.05</td>
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<tr>
<td>low dose 142</td>
<td>0.73 ± 0.31</td>
<td>1.47 ± 0.76</td>
<td>0.46 ± 0.10</td>
<td>0.58 ± 0.18</td>
</tr>
<tr>
<td>control 142(^d)</td>
<td>0.91 ± 0.13</td>
<td>0.55 ± 0.11</td>
<td>0.60 ± 0.27</td>
<td>0.60 ± 0.23</td>
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<tr>
<td><strong>UKV (μEq/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vehicle</td>
<td>30.6 ± 3.6</td>
<td>20.9 ± 4.4</td>
<td>22.9 ± 6.0</td>
<td>24.5 ± 3.6</td>
</tr>
<tr>
<td>high dose 142</td>
<td>41.6 ± 5.0</td>
<td>34.1 ± 4.2</td>
<td>38.5 ± 9.1</td>
<td>53.3 ± 6.6</td>
</tr>
<tr>
<td>low dose 142</td>
<td>44.0 ± 4.9</td>
<td>36.5 ± 4.4</td>
<td>46.6 ± 6.5</td>
<td>39.1 ± 7.9</td>
</tr>
<tr>
<td>control 142(^d)</td>
<td>33.7 ± 5.7</td>
<td>28.3 ± 6.7</td>
<td>35.4 ± 7.6</td>
<td>43.7 ± 11.4</td>
</tr>
<tr>
<td><strong>FEK</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vehicle</td>
<td>19.4 ± 2.4</td>
<td>21.5 ± 2.2</td>
<td>44 ± 6.7</td>
<td>30.9 ± 3.6</td>
</tr>
<tr>
<td>high dose 142</td>
<td>21.3 ± 2.0</td>
<td>16.5 ± 2.0</td>
<td>26.3 ± 4.6</td>
<td>30.4 ± 3.8</td>
</tr>
<tr>
<td>low dose 142</td>
<td>30.2 ± 3.5</td>
<td>25.4 ± 3.6</td>
<td>38.0 ± 5.2</td>
<td>34.3 ± 4.7</td>
</tr>
<tr>
<td>control 142(^d)</td>
<td>21.3 ± 3.8</td>
<td>25.1 ± 4.7</td>
<td>27.0 ± 2.6</td>
<td>27.8 ± 3.5</td>
</tr>
</tbody>
</table>

\(^a\) The treatment groups and times are similar to those reported in Table 1. \(F_{\text{ENa}}\), fractional excretion of sodium; \(U_{\text{K}}V\), urinary potassium excretion; \(F_{\text{EK}}\), fractional excretion of potassium.

\(^b\) \(P < 0.05\), L-754,142 treated versus vehicle.

\(^c\) \(P < 0.05\) versus baseline.

\(^d\) For the controls, the data were reported at similar time points as the cross-clamped animals for comparison.

\(^e\) \(P < 0.01\) versus baseline.

\(^f\) \(P < 0.01\), L-754,142 treated versus vehicle.

Thus, inhibition of prostacyclin synthesis by indomethacin could unmask the detrimental effects of ET-1 on renal function (10,12). This may explain why indomethacin treatment led to an initial reduction in GFR in some of the vehicle group animals. This effect, however, does not persist, because a control study demonstrated no persistent effects on GFR. Although the use of indomethacin in this model of young healthy animals is apparently required in combination with ACC to achieve renal dysfunction (10), indomethacin itself does not result in prolonged renal dysfunction. In addition, the increase in susceptibility to renal injury with indomethacin may be reflective of what happens in unhealthy kidneys in which endothelial dysfunction due to atherosclerosis, underlying renal disease, or aging may lead to a reduction in prostacyclin release (5,30-32).

The significant improvement in renal function observed in our model of acute renal failure using ACC is in sharp contrast to the results observed by Brooks et al. (33) using a 60-min renal artery occlusion (RAO) model in the dog. Interestingly, the use of either BQ-123 (an ETA-selective antagonist) or SB209,670 (an ET antagonist) in the RAO model provided only modest protection of renal function despite a prompt return of RBF to baseline. With SB209,670, after 60 min of reperfusion, GFR was still only 30% of baseline. Although at first glance both of these models may appear similar, the RAO model will cause a cessation of flow, whereas the ACC model...
The effect of ACC on plasma [ET-1] for animals pretreated with either saline vehicle (n = 8) or the endothelin antagonist L-754,142 at either 3 mg/kg bolus + 1 mg/kg per h (high dose; n = 8) or 0.3 mg/kg bolus + 0.1 mg/kg per h (low dose; n = 8) and for high-dose L-754,142 time-matched controls (control, n = 5). Bars represent function at baseline, before ACC (after indomethacin and either L-754,142 or saline), 45 min into the ACC, and at 1 and 2 h of reperfusion. For the L-754,142 controls, the bars represent data reported at similar time points as the ACC groups. Statistical significance versus baseline function within groups is represented by *P < 0.05 and **P < 0.01 and between groups versus vehicle by †P < 0.05.

still allows hypoperfusion of the kidney. The difference between these models is readily apparent when one compares the effects of reperfusion on RBF. In studies that involve ACC as opposed to RAO, we and others (6,7,10,34) have shown that removal of the cross-clamp is associated with a profound decrease in RBF, which is maintained for several hours. This sustained reduction in RBF after reperfusion in the ACC model is in sharp contrast to and represents an important difference from studies that involve direct RAO (33). A similar observation was made by Pass et al. (9). The reason for this difference in RBF after reperfusion is not readily apparent, but may be related to the sustained hypoperfusion of the kidney in the ACC model, leading to ET-1 release (10), which is absent in the RAO model. Thus, there are important distinctions between the two models that are reflected in the results.

The only other pharmacologic agent shown to protect both RBF and renal function in the dog after ACC has been atrial natriuretic factor (ANF) (10). It is important to note that in addition to improving RBF and GFR in this model, ANF was also shown to reduce the plasma level of ET-1 to baseline. Therefore, it is possible that the protection afforded by ANF may be partially mediated either through a reduction in ET-1 release or an increase in the clearance of ET-1.

In conclusion, the results of the present study suggest that the decline in RBF after 60 min of suprarenal aortic occlusion in the dog is primarily due to an increase in RVR associated with an increase in circulating [ET-1], leading to a significant reduction in GFR. The significant increase in plasma [ET-1] is also associated with a decrease in sodium reabsorption, which may also contribute to the reduction in GFR. The dual ET/ET antagonist L-754,142, when administered as a bolus followed by a constant infusion 45 min before ACC, was shown to prevent the renal vasoconstriction observed after reperfusion, thereby increasing RBF, which contributed to a significant improvement of GFR and sodium reabsorption. In light of previous studies in this canine model of ACC that have shown that few agents preserve RBF (6–10), the findings of the study presented here are important because this is one of the few instances in which a pharmacological agent has been shown to protect RBF and attenuate the reduction in GFR after ACC. These results suggest that ET-1 plays an important role in the vasoconstriction after ACC and that the use of a dual endothelin antagonist can preserve RBF and significantly improve GFR and sodium reabsorption. These results provide useful insight that may lead to a breakthrough in an area in which there is as yet limited therapy.

References
2. Paul MD, Mazer CD, Byrick RJ, Rose DK, Goldstein MB:


