Endothelin in a Model of Acute Ischemic Renal Dysfunction: Modulating Action of Atrial Natriuretic Factor

Evan K. Sandok, Amir Lerman, Andrew J. Stingo, Mark A. Perrella, Peter Gloviczki, and John C. Burnett, Jr.

ABSTRACT

This study was undertaken to investigate circulating endothelin (ET) and associated renal hemodynamics in the acute ischemic renal dysfunction associated with suprarenal aortic cross-clamping (ACC) in the presence and absence of prostaglandin inhibition in the anesthetized dog. Second, the modulating action of exogenous atrial natriuretic factor (ANF) was also investigated. In Group I (ACC; N = 6), ACC was performed in the absence of prostaglandin inhibition. No change in mean arterial pressure, GFR, RBF, renal vascular resistance, or ET was noted 2 h after reperfusion when compared with baseline values. In the presence of prostaglandin inhibition with indomethacin (10 mg/kg iv) (Group II, ACC + INDO; N = 10), an increase in plasma ET was first noted to be elevated above baseline ET in Group I as well as during and 2 h after ACC in association with a reduction in GFR, marked renal vasoconstriction, and a sustained increase in arterial pressure. To evaluate the role of the kidney in this increase in ET, another group (Group III, ACC + INDO + NEPH; N = 6) was investigated in the presence of prostaglandin inhibition, and bilateral renal artery clamping was performed 30 min before ACC and maintained throughout the protocol to simulate nephrectomy. In this group, plasma ET concentrations did not increase during ACC. Because ANF may antagonize the renal actions of ET in vivo and may suppress ET release in vitro, the action of ANF upon GFR and plasma ET was evaluated in Group IV (ACC + INDO + ANF; N = 6).

In this group, in the presence of prostaglandin inhibition, plasma ET increased during ACC and returned to baseline after ACC in response to ANF, with a return of GFR, RBF, and renal vascular resistance to baseline. Mean arterial pressure decreased as well to baseline levels. This study demonstrates that ACC results in acute renal dysfunction associated with an increase in circulating ET only in the presence of prostaglandin inhibition, suggesting an important role of prostaglandins in the regulation of circulating ET. This study also suggests that the kidney is involved in the elevation of ET seen in this model. Last, ANF attenuates this increase in ET and maintains GFR and RBF.

Key Words: Endothelin, atrial natriuretic factor, renal failure, renal hypoperfusion

Endothelin (ET) is a potent systemic and renal vasoconstrictor peptide (1,2). Although ET functions as a local autacoid, its circulating concentrations may be increased in pathophysiologic conditions associated with renal hypoperfusion (3-6). Indeed, when ET is exogenously administered to achieve such pathophysiologic concentrations, systemic renal vasoconstriction are observed (7). In rat models of acute ischemic renal failure, increased renal tissue concentrations of ET (8) and reversal of ischemic renal dysfunction with the administration of an anti-ET antibody have been observed (9), supporting an important pathophysiologic role of renal ET in acute renal failure.

Suprarenal aortic cross-clamping (ACC) is used frequently during the reconstruction of the abdominal aorta or the renal arteries, which results in marked renal hypoperfusion despite the maintenance of some collateral circulation. Although repeated studies have demonstrated that the kidney may be resistent to such renal hypoperfusion, the presence of prostaglandin inhibitors such as nonsteroidal anti-inflammatory agents markedly enhances the renal susceptibility to decreases in renal perfusion pressure and increases the risk for the development of acute renal dysfunction (10). In addition to a role for...
prostaglandins in the pathophysiology of acute renal dysfunction, prostaglandin inhibition with indomethacin or aspirin markedly enhances ET-mediated renal vasoconstriction (11–15).

This study was undertaken to investigate circulating ET and associated renal hemodynamics in the acute renal dysfunction associated with ACC in the dog in the presence and absence of prostaglandin inhibition. The first objective was to determine the temporal relationship between circulating ET and renal function during ACC in the presence and absence of prostaglandin inhibition. Our hypothesis was that indomethacin would augment increases in plasma ET and decreases in renal function in response to ACC. The second objective was to determine the role of the kidney in a potential increase in plasma ET with ACC and indomethacin, ACC with indomethacin was performed in the presence of simulated nephrectomy. This was produced by bilateral renal artery clamping to eliminate the low state of RBF that persists during ACC and occurs via aortic collateral flow. Last, because atrial natriuretic factor (ANF) may suppress ET in vitro (16,17) and exogenously administered ANF may be beneficial in acute ischemic renal dysfunction of various causes (18–21), we hypothesized that exogenous ANF would inhibit increases in circulating ET and attenuate the renal responses to ACC.

**MATERIALS AND METHODS**

Acute experiments were conducted in four groups of anesthetized mongrel dogs (18 to 25 kg). In Group I (ACC; N = 6), dogs underwent suprarenal ACC for 1 h, followed by a 2-h period of unclamping in the absence of indomethacin. In Group II (ACC + INDO; N = 10), dogs underwent suprarenal ACC for 1 h with a 2-h period of unclamping but in the presence of indomethacin pretreatment (10 mg/kg, iv bolus). In Group III (ACC + INDO + NEPH; N = 6), dogs underwent bilateral renal artery clamping 30 min before baseline measurements and ACC. Bilateral renal artery clamping remained throughout the remainder of the protocol to eliminate all RBF, thus simulating bilateral nephrectomy. In Group IV (ACC + INDO + ANF; N = 6), dogs underwent 1 h of ACC, with an infusion of ANF (50 ng/kg/min; intra-arterially, proximal to the clamp) beginning just before the release of the aortic clamp and continuing on to the end of the experiment.

**Experimental Design**

Dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv) with additional anesthetic given as needed to maintain a constant level of anesthesia. An endotracheal tube was inserted, and the dogs were ventilated with a Harvard respirator (Harvard Apparatus Co., Inc., South Natick, MA) with room air supplemented with 100% oxygen throughout the experiment. A polyethylene catheter was introduced into the right carotid artery and advanced to the thoracic aorta for measurements of circulating arterial ET before, during, and after ACC. The right femoral artery was cannulated with a polyethylene catheter for monitoring mean arterial pressure (MAP) below the aortic clamp, and the right femoral vein was cannulated for infusions of inulin and saline. Inulin was infused in isotonic saline at a rate of 1 mL/min to establish a plasma level of approximately 50 mg/dL.

The left kidney was exposed through a flank incision, and the left ureter was cannulated for urine collection. A calibrated electromagnetic flow probe was placed around the left renal artery. The flow probe was connected to a flowmeter (Carolina Medical Electronics, King, NC) for the measurement of RBF. A right flank incision was made, and the suprarenal aorta was dissected. Blood flow from the left renal artery and arterial blood pressure were recorded on a Gould Model 2200 strip recorder (Gould, Inc., Cleveland, OH).

After an equilibration period of 1 h during which inulin and saline infusions at 1 mL/min were started, one 30-min baseline control clearance without ET measurements was performed. After this baseline clearance, in Groups II, III, and IV, indomethacin (Sigma Chemical Co., St. Louis, MO) (10 mg/kg iv with NaHCO3 [5 mg/kg] dissolved in 50 mL of distilled water) was slowly infused over a 15-min period to increase the renal susceptibility to renal hypoperfusion as previously reported (22). A second 30-min baseline clearance followed the indomethacin administration in which urine and blood were collected for the subsequent measurement of inulin as well as hemodynamic parameters. After the completion of these baseline clearances, the aorta was cross-clamped above the renal arteries with a Blalock vascular clamp (Mayo Clinic, Rochester, MN) for 1 h. Plasma was sampled to assess circulating plasma ET concentrations at 45 min after the placement of the aortic clamp. After 60 min, the vascular clamp on the aorta was removed. Two hours after unclamping, a final clearance was obtained. In Group I (ACC; N = 6) and Group II (ACC + INDO; N = 10), normal saline infusion (1 mL/min) was begun 5 min before the opening of the clamp and was continued for 2 h. In Group IV (ACC + INDO + ANF; N = 6), ANF (Peninsula Laboratories, Inc., Belmont CA) at a dose of 50 ng/kg/min was infused into the suprarenal aorta beginning 5 min before the opening of the clamp and continuing for 2 h. In Group III (ACC + INDO + NEPH), simulated nephrectomy was performed by bilateral renal artery clamping 30 min before ACC. Plasma ET was determined at baseline after renal artery clamp-


Plasma ET was determined by ET-1,[125I] assay system from Amersham (Amersham, Arlington Heights, IL) as previously reported (5). Blood was drawn from dogs into tubes containing chilled potassium EDTA and was immediately placed on ice until being centrifuged at 4° C. Plasma was separated and frozen at −20° C until analysis. Before the RIA, plasma was acidified with 0.5% trifluoroacetic acid. C8 Bond Elut cartridges (Chromtech, Apple Valley, MN) were washed with 2 mL of normal saline, 4 mL of methanol and 4 mL of water to extract the plasma. After the plasma was applied, cartridges were washed with 2 mL of normal saline and 6 mL of water. ET was eluted from the cartridges with 2 mL of 90% methanol in 1% TFA (trifluoroacetic acid) and was then dried and reconstituted for the RIA. The recovery of the extraction procedure was 81%, as determined by the addition of synthetic ET to plasma, and interassay and intra-assay variations were 9% and 5%, respectively.

Plasma and urinary inulin concentrations were determined by the anthrone method (23). GFR was determined by the clearance of inulin.

For each experimental group, data from the second baseline period and the clamp and postclamp periods are expressed as mean ± SE. All data were analyzed by analysis of variance for repeated measurements and by paired and unpaired t tests. Comparisons within groups were analyzed by analysis of variance for repeated measurement, followed by paired t test when appropriate. Comparisons between groups were performed by unpaired t test. Statistical significance was accepted for P < 0.05.

## RESULTS

Renal hemodynamic and plasma ET responses to ACC in Groups I, II, and IV are reported in Table 1. Plasma ET responses in all four groups are illustrated in Figure 1.

In Group I (ACC), plasma ET remained unchanged during and after aortic clamping (Figure 1) in association with no changes in GFR, RBF, and renal vascular resistance (RVR). Mean arterial pressure (MAP) increased during ACC and returned to baseline after ACC.

In Group II (ACC + INDO), plasma ET markedly increased during ACC and remained increased during the unclamping period (Figure 1). In association with these alterations in plasma ET and in contrast to Group I, GFR and RBF were markedly decreased 2 h after unclamping of the aorta in association with a marked increase in RVR. MAP increased during ACC and remained above baseline after ACC.

In Group III, which underwent ACC in the presence of simulated nephrectomy and indomethacin, plasma

### TABLE 1. Renal hemodynamic actions of ACC

<table>
<thead>
<tr>
<th>Group I (ACC; N = 6)</th>
<th>Baseline</th>
<th>ACC</th>
<th>2 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>141 ± 9</td>
<td>175 ± 8a</td>
<td>126 ± 8</td>
</tr>
<tr>
<td>GFR (mL/min)</td>
<td>24.2 ± 7.5</td>
<td>NDb</td>
<td>26.8 ± 7.8</td>
</tr>
<tr>
<td>RBF (mL/min)</td>
<td>175 ± 3</td>
<td>ND</td>
<td>164 ± 41</td>
</tr>
<tr>
<td>RVR (mm Hg/min/mL)</td>
<td>0.94 ± .20</td>
<td>ND</td>
<td>0.98 ± 20</td>
</tr>
<tr>
<td>ET (pg/mL)</td>
<td>9.5 ± 1.0</td>
<td>12.4 ± 1.7</td>
<td>12.3 ± 1.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group II (ACC + INDO; N = 10)</th>
<th>Baseline</th>
<th>ACC</th>
<th>2 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>125 ± 4</td>
<td>172 ± 5b</td>
<td>141 ± 6a</td>
</tr>
<tr>
<td>GFR (mL/min)</td>
<td>33.2 ± 4.8</td>
<td>ND</td>
<td>7.1 ± 2.4ac</td>
</tr>
<tr>
<td>RBF (mL/min)</td>
<td>142 ± 13</td>
<td>ND</td>
<td>79 ± 13c</td>
</tr>
<tr>
<td>RVR (mm Hg/min/mL)</td>
<td>0.95 ± 0.10</td>
<td>ND</td>
<td>2.5 ± 0.65ac</td>
</tr>
<tr>
<td>ET (pg/mL)</td>
<td>20.7 ± 2.0b</td>
<td>51.7 ± 10.4 b,c</td>
<td>42.3 ± 7.0ac</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group IV (ACC + INDO + ANF; N = 6)</th>
<th>Baseline</th>
<th>ACC</th>
<th>2 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>128 ± 6</td>
<td>168 ± 4c</td>
<td>130 ± 6</td>
</tr>
<tr>
<td>GFR (mL/min)</td>
<td>31.8 ± 4.1</td>
<td>ND</td>
<td>29.8 ± 5.4</td>
</tr>
<tr>
<td>RBF (mL/min)</td>
<td>164 ± 18</td>
<td>ND</td>
<td>139 ± 16</td>
</tr>
<tr>
<td>RVR (mm Hg/min/mL)</td>
<td>0.80 ± .11</td>
<td>ND</td>
<td>1.00 ± 0.10</td>
</tr>
<tr>
<td>ET (pg/mL)</td>
<td>22.0 ± 3.5c</td>
<td>47.4 ± 12.8 b,c</td>
<td>22.9 ± 2.4c</td>
</tr>
</tbody>
</table>

a P < 0.05 versus baseline.
b ND, not determined.
c P < 0.05 versus Group I.
ET did not increase during ACC but modestly increased during the period after unclamping of the aorta but in the presence of continued bilateral renal artery clamping to simulate nephrectomy (Figure 1).

In Group IV (ACC + INDO + ANF), plasma ET increased during ACC before the administration of ANF but decreased to baseline levels during the unclamped period. In association with the normalization of plasma ET, GFR, RBF, and RVR were also not significantly different from baseline. Although MAP increased during ACC, MAP returned to baseline during the postclamp period.

DISCUSSION

This study demonstrates that suprarenal ACC in the dog results in an elevation of circulating ET and an association with marked renal dysfunction when performed in the presence of prostaglandin inhibition. Simulated nephrectomy prevented the observed increase in plasma ET during ACC, suggesting that the kidney either releases ET during ACC or importantly participates by the release of substances, which result in systemic ET release. Last, this investigation demonstrates that the intra-aortic infusion of ANF administered immediately before aortic unclamping resulted in a return of plasma ET and renal function to baseline. These studies support a role for ET in the renal responses associated with renal hypoperfusion produced by suprarenal aortic clamping and a potential therapeutic role for intra-arterial ANF in modulating circulating ET and the renal hemodynamic response to suprarenal ACC in the dog.

In this investigation, renal hypoperfusion produced by the surgical procedure of ACC resulted in no sustained alteration in renal hemodynamic function after aortic unclamping in the absence of prostaglandin inhibition. In contrast, marked vasoconstriction and a reduction in GFR occurred in response to ACC in the presence of indomethacin. Thus, these studies importantly confirm earlier investigations demonstrating that prostaglandins, perhaps prostacyclin or its metabolites, serve to maintain renal hemodynamic function in response to decreases in renal perfusion pressure. Although the mechanism of this action cannot be addressed, the parallel reduction in RBF and GFR supports a modulating action upon the afferent arteriole.

This study also importantly extends previous reports and documents that endogenous prostanooids may participate in the regulation of ET. Indomethacin resulted in an increase in plasma ET before ACC. Although the mechanism of this increase is unclear, these observations are consistent with a possible inhibitory role for the prostaglandins in ET release. Such a concept is supported by the studies of Boulanger and Lüscher, who reported that the endothelium-derived relaxing factor (EDRF), via activation of
extends our previous report of a relationship between study pharmacologic concentrations of ANF reverse ACC. dynamic action of ET. Previous administration of an and ET in an experimental ANF that observed in plasma ET in a rat model of acute ischemic renal failure. The mechanism(s) by which decreased perfusion pressure may further stimulate ET release may be multifactorial and may include the mechanical stimulus of decreased wall stress and/or the activation of chemical stimuli such as angiotensin II and/or hypoxia (25). Because simulated nephrectomy abolished the increase in plasma ET seen in association with ACC in our model, this study importantly extends previous reports and suggests, at least in response to renal hypoperfusion produced by ACC, that the increased circulating ET may be of renal origin and is not due to decreased renal clearance. Alternatively, the kidney may play a key role in the increase in plasma ET during ACC by the release of a factor that stimulates systemic release.

Previous studies support a functional role for an increase in plasma ET immunoreactivity similar to that observed in this study. Previous investigations have reported that pharmacologic concentrations of exogenously administered ET result in marked and preferential renal as compared with systemic vasoconstriction (2, 26). More recently, Lerman et al. have reported that a doubling of circulating concentrations of ET, produced by the infusion of ET-1 in dogs, similar to that observed in this investigation, resulted in both renal and systemic vasoconstriction, consistent with the interpretation that pathophysiologic concentrations of circulating ET are biologically important (7). The observations of Shibouta et al. and Kon et al. that the administration of anti-ET antibodies in a rat model of ischemic renal failure reverses the decrease in GFR again support the concept that ET may play a functionally important role in the renal responses to marked renal hypoperfusion and reperfusion (8,9). However, in the absence of the administration of an ET receptor antagonist, this study provides only circumstantial evidence for a role for ET in the acute renal dysfunction associated with ACC.

Recent studies suggest that the cGMP-linked cardiac peptide, ANF, may also oppose the renal hemodynamic action of ET. Previous reports document that pharmacologic concentrations of ANF reverse ET-mediated renal vasoconstriction (27,28). This study is consistent with such an interaction and extends our previous report of a relationship between ANF and ET in an experimental model of radiocontrast nephropathy (29). In those previous reports, radiocontrast media induced significant increases in plasma ET in dogs with experimental congestive heart failure that are at risk for radiocontrast-induced dysfunction. Pretreatment with ANF, which is protective in this and other models of acute renal dysfunction, increased GFR and maintained GFR at or above baseline levels during and after radiocontrast administration. Despite this protective action of ANF on renal hemodynamics, ANF did not prevent increases in plasma ET after radiocontrast administration. Thus, ANF pretreatment in dogs with congestive heart failure increased GFR above baseline and prevented decreases below baseline during and after radiocontrast without preventing radiocontrast-induced decreases in GFR per se. Thus, in those previous studies, the failure of ANF to completely block radiocontrast-induced decreases in GFR could be related to the intact increases in plasma ET despite ANF pretreatment.

In this study, ANF administration after 1 h of ACC decreased plasma ET concentrations to baseline levels and returned GFR to levels not different from baseline. The difference between the previous study and this one may be multifactorial and reflect different mechanisms responsible for the elevation of ET and renal hemodynamic responses to ANF. Perhaps most important, this study used significantly higher concentrations of ANF than those used in the previous study. Nonetheless, these two studies complement one another and indicate an important functional relationship between ANF and ET in the regulation of renal hemodynamic function in pathophysiologic states.

In this study, ANF administered in aortic arterially to maximize renal actions maintained GFR, RBF, and RVR at baseline levels after ACC. Therefore, ANF emerged as an important modulator of the renal response after ACC when exogenously infused, supported a potential therapeutic action for synthetic ANF during and after ACC. The mechanism through which ANF may mediate its renal hemodynamic actions may include its ability to decrease afferent arteriolar tone, increase the ultrafiltration coefficient (Kf), and/or increase medullary blood flow (30).

During ACC and before the administration of ANF in Group IV, plasma ET increased as in Group II. With ANF infusion and unclamping, plasma ET decreased. Several mechanisms could explain this ANF-mediated decrease. The first explanation is that the ANF-mediated preservation of GFR may have maintained the renal clearance of elevated plasma ET. This mechanism is supported by reports that the kidney may serve as an important site of clearance for ET (31). The second mechanism may be related to the ability of ANF, via the generation of cGMP, to inhibit the release of ET. Kohno et al. recently reported that the release of ET from human umbilical vein endothelial cells in vitro was inhibited by ANF.
This in vitro study would be consistent with the ability of ANF to inhibit the renal release of ET associated with ACC. Alternatively, circulating ET may be a marker for renal cell damage produced by ACC, and to the extent that ANF reduces the degree of renal injury, the decrease in circulating ET could reflect the renal protection produced by ANF (32).

Although this study focused upon the renal adjustments associated with the increase in plasma ET observed with ACC, alterations in MAP during the postclamping period paralleled changes in circulating ET. However, in Group II, only in the presence of a sustained elevation in circulating ET was the hypertensive response to ACC maintained. In the absence of indomethacin (Group I) and in the presence of ANF administration (Group IV), arterial pressure was not elevated after unclamping. In addition, no sustained increase in circulating ET levels were observed in these groups.

In summary, this study supports an important role for endogenous prostaglandins in regulating circulating ET and renal hemodynamic function during and after the cardiovascular surgical procedure of supraprenal ACC. Specifically, endogenous prostaglandins may serve to inhibit ET release both before and after ACC and protect the kidney from the functional consequences of renal hypoperfusion. This investigation also supports a role for the kidney in the elevation of plasma ET during renal hypoperfusion and the ability of intra-arterial ANF after ACC to decrease elevated circulating ET, preserve GFR, and reduce RVR. This investigation thus provides new insight into circulating ET in the pathophysiology of a clinically relevant model of acute ischemic renal dysfunction produced by supraprenal ACC and the therapeutic potential of ANF in modulating these pathophysiologic adaptations to renal hypoperfusion.

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