Biological Role for the Endothelin-A Receptor in Aortic Cross-Clamping

Andrew J. Stingo, Alfredo L. Clavell, Lawrence L. Aarhus, John C. Burnett, Jr

The current study was undertaken to define a biological role for the endothelin-A receptor in a clinically relevant model of altered systemic and renal function produced by suprarenal aortic cross-clamping. This model is associated with profound systemic and renal vasoconstriction, acute renal failure, and a significant increase in circulating endothelin. Studies were performed in three groups of anesthetized mongrel dogs. Group 1 (n=5) underwent aortic cross-clamping for 1 hour; group 2 (n=5) underwent aortic cross-clamping for 1 hour in the presence of BQ-123, a specific antagonist of the endothelin-A receptor; group 3 (n=4) received BQ-123 alone. The marked systemic and renal vasoconstriction associated with aortic cross-clamping in group 1 was markedly attenuated in group 2 in the presence of BQ-123. Unlike the vasoconstrictor response, BQ-123 did not attenuate the decrease in glomerular filtration rate associated with this model. Under unstimulated conditions in group 3, BQ-123 had no actions on systemic or renal hemodynamics. In conclusion, the current study demonstrates that the systemic and renal vasoconstriction associated with aortic cross-clamping are in part mediated through the interaction of endothelin and the endothelin-A receptor. This study demonstrates the functional importance of increased endogenous endothelin in the regulation of vascular tone in this pathophysiological state. (Hypertension 1993;22:62-66)

KEY WORDS • endothelins • receptors, endothelin • vascular resistance • renal failure, acute

Endothelin (ET) is a potent vasoconstrictor peptide of endothelial origin.1 Numerous studies have documented that pathophysiological and pharmacological concentrations of ET result in systemic and renal vasoconstriction.2,3 Such actions may be of clinical relevance because circulating ET is elevated in a number of cardiorenal disease states, including congestive heart failure, hypertension after organ transplantation, and cardiogenic shock.4-6 Recently, Sandok and coworkers7 have documented plasma ET elevation in a clinically relevant animal model of suprarenal aortic cross-clamping (ACC) performed in the presence of prostaglandin inhibition. This model, which is similar to the surgical procedure used during peripheral arterial revascularization and reconstruction, was associated with marked increases in systemic and renal vascular resistances, arterial hypertension, and acute renal failure.

ET is known to exist as three structurally related isoforms termed ET-1, ET-2, and ET-3. The receptors that bind ET have been termed the ETA and ETB receptors and are differentiated on the basis of their relative affinities for the ET isoforms. The ETA receptor, which binds ET-1 preferentially, is highly expressed in vascular smooth muscle and is thought to mediate the potent vasoconstriction associated with ET-1 via increases in intracellular calcium.8 Recent studies have demonstrated that the ETB receptor binds all three isoforms with similar affinity and is localized predominantly to the glomerulus and distal tubules in the kidney as well as lung parenchyma and endothelial cells.9,10 Studies suggest that the ETB receptor may mediate enhanced prostacyclin and endothelium-derived relaxing factor release from endothelial cells.8

BQ-123 is a newly developed five-amino acid peptide that is a specific competitive antagonist of the ETA receptor.11 To date, studies that have addressed antagonism of the actions of ET have relied on functional antagonists such as atrial natriuretic peptide or antibodies to ET.7,12 The availability of a specific receptor antagonist allows us for the first time to address the functional importance of the interaction between the ETA receptor and increased concentrations of ET as well as the significance of this interaction under basal conditions.

Therefore, we undertook the present study to address the pathophysiological role of the ETA receptor and increased endogenous ET in a clinically relevant model of ACC with associated cardiorenal dysfunction by using the specific ETA receptor antagonist BQ-123. Furthermore, the present study was also designed to examine the biological actions of BQ-123 administration in the absence of elevation of circulating ET. Our hypothesis was that BQ-123 would attenuate the increase in systemic and renal vascular resistances associated with ACC, with a maintenance of arterial pressure and glomerular filtration rate (GFR).

Methods

Acute experiments were conducted in three groups of anesthetized mongrel dogs (16 to 22 kg). All studies

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were approved by the institutional animal care and use committee. Group 1 (ACC, n=5) underwent suprarenal ACC for 1 hour followed by a 2-hour period of unclamping. In group 2 (ACC+BQ-123, n=5), dogs underwent suprarenal ACC for 1 hour as in group 1 but in the presence of BQ-123, a specific ETA receptor antagonist. Group 3 (BQ-123, n=4) received BQ-123 infusion at concentrations similar to group 2 without further intervention. 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 respirator (Harvard Apparatus, South Natick, Mass), using room air supplemented with 100% oxygen at 4 L/min throughout the experiment. A polyethylene catheter was introduced into the right external carotid artery and advanced into the thoracic aorta for measurements of circulating arterial ET before and after ACC as well as infusion of either saline in group 1 or BQ-123 in groups 2 and 3. The right external jugular vein was cannulated with a flow-directed thermodilution catheter (Spectramed, Oxnard, Calif) for the measurement of right atrial pressure, pulmonary capillary wedge pressure, and cardiac output (CO). The right femoral artery was cannulated with a polyethylene catheter for monitoring mean arterial pressure (MAP), and the right femoral vein was cannulated for infusion of inulin. Inulin was infused in isotonic saline at a rate of 1 mL/min to maintain a plasma level of approximately 50 mg/dL. The right kidney was exposed through a flank incision, and the right ureter was cannulated for urine collection. A calibrated electromagnetic flow probe was placed around the right renal artery and connected to a flowmeter (Carolina Medical Electronics, King, NC) for measurement of renal blood flow (RBF). The suprarenal aorta was dissected, and a Blalock vascular clamp was placed around the aorta just below the diaphragm in groups 1 and 2 only.

In groups 1 and 2, after an equilibration period of 1 hour during which intravenous inulin and intra-arterial saline infusions at 1 mL/min were started, indomethacin (Sigma Chemical Co, St Louis, Mo) (10 mg/kg IV with 5 mg/kg NaHCO₃ dissolved in 50 mL distilled water) was slowly infused over a 15-minute period to enhance the renal susceptibility to ischemic injury as previously reported for this model. This was followed by a 30-minute baseline clearance period (C1). At the midpoint of this and subsequent clearance periods, hemodynamic parameters including MAP, CO, right atrial pressure, pulmonary capillary wedge pressure, and RBF were measured, and arterial blood was collected in both groups for measurement of plasma ET, sodium, and inulin. Urine was collected during this and subsequent clearance periods and analyzed for sodium and inulin. In group 2, the infusion of BQ-123 (5 μg/kg per minute, Peptides International, Louisville, Ky) was begun into the thoracic aorta 5 minutes before the initiation of ACC. The aorta remained clamped for 1 hour. After 60 minutes, the vascular clamp on the aorta was removed. The renal vascular clamp was followed by a 1-hour postclamp clearances. In group 2, the infusion of BQ-123 continued throughout the first postclamp clearance (C2) and was terminated before the last postclamp or recovery clearance (C3). In group 3, after surgical preparation, 60 minutes was allowed for equilibration during which saline was infused intravenously at 1 mL/min. This was followed by a 30-minute baseline clearance with hemodynamic measurements similar to groups 1 and 2. The baseline clearance was followed by a 15-minute lead-in period during which the infusion of BQ-123 (5 μg/kg per minute) was begun into the thoracic aorta. This was followed by a 30-minute clearance during which the BQ-123 infusion was continued.

Blood for plasma inulin was placed in heparinized tubes, packed in ice, centrifuged at 2500 rpm, and refrigerated along with urine samples pending analysis. Blood for hormone assays was placed in EDTA tubes on ice, and after centrifugation at 2500 rpm, plasma was decanted and stored at −20°C until analysis. Plasma ET was determined by an ET-1 assay system (Amersham, UK) as previously reported. Plasma and urinary inulin concentrations were determined by the anthrone method. GFR was determined by the clearance of inulin.

Data are expressed as mean±SEM. Comparisons within groups were analyzed using analysis of variance for repeated measures followed by Fisher's least significant difference test when appropriate. Absolute changes from baseline between groups were analyzed by unpaired Student's t test and comparisons for repeated measures between groups by factorial analysis of variance. Statistical significance was accepted at a value of P<.05.

Results

The Table reports the systemic hemodynamic and renal responses to ACC in the presence and absence of BQ-123. In group 1, the control group, MAP and systemic vascular resistance (SVR) markedly increased while CO and heart rate decreased. In contrast, in group 2 with BQ-123, MAP did not increase in association with an attenuated increase in SVR and decrease in CO, with no change in heart rate. Right atrial pressure did not change in either group, whereas pulmonary capillary wedge pressure increased in the control group only. The differential response in SVR in the presence and absence of BQ-123 is illustrated in Fig 1, which reports an attenuated increase from baseline in SVR in group 2 versus group 1. The responses of MAP and SVR for individual animals are depicted in Fig 2.

The renal response to ACC in group 1 was characterized by marked decreases in GFR and RBF and increases in renal vascular resistance (RVR). In group 2, in the presence of BQ-123, the changes in GFR were similar to group 1 but with an attenuated decrease in RBF and increase in RVR. This differential response was lost when BQ-123 was terminated in group 2 during the recovery clearance. The differential response in RVR in the presence and absence of BQ-123 is illustrated in Fig 1, which demonstrates the attenuated increase from baseline in RVR in group 2 versus group 1.

Plasma ET after ACC doubled in both groups (19.3±3.9 to 39.4±6.3 pg/mL, baseline to 30 minutes postclamp, P<.05, in group 1; 11.6±2.1 to 22.4±5.3 pg/mL, baseline to 30 minutes postclamp, P<.05, in group 2). The effect of plasma ET for individual animals are illustrated in Fig 3.

In group 3, infusion of BQ-123 alone did not significantly change systemic hemodynamics, including MAP (125±3 versus 123±4 mm Hg), CO (2.75±0.27
### Systemic and Renal Hemodynamics During Aortic Cross-Clamping With and Without BQ-123

<table>
<thead>
<tr>
<th>Hemodynamics</th>
<th>Baseline (C1)</th>
<th>Postclamp (C2)*</th>
<th>Recovery (C3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1: Aortic cross-clamp</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>125±6</td>
<td>156±6†</td>
<td>146±7†</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>126±4</td>
<td>108±4†</td>
<td>104±4†</td>
</tr>
<tr>
<td>RAP (mm Hg)</td>
<td>−2.0±0.5</td>
<td>−1.4±1.1</td>
<td>−1.0±1.0</td>
</tr>
<tr>
<td>PCWP (mm Hg)</td>
<td>2.4±0.9</td>
<td>4.4±2.0†</td>
<td>5.2±1.8†</td>
</tr>
<tr>
<td>CO (L/min)</td>
<td>2.73±0.31</td>
<td>1.60±0.13†</td>
<td>1.31±0.18†</td>
</tr>
<tr>
<td>SVR (mm Hg/L per minute)</td>
<td>48±4</td>
<td>100±7†</td>
<td>119±12†</td>
</tr>
<tr>
<td>GFR (mL/min)</td>
<td>22.9±7.0</td>
<td>7.3±2.1†</td>
<td>7.0±1.4†</td>
</tr>
<tr>
<td>RBF (mL/min)</td>
<td>172±35</td>
<td>108±7†</td>
<td>87±15†</td>
</tr>
<tr>
<td>RVR (mm Hg/mL per minute)</td>
<td>0.84±0.15</td>
<td>1.48±0.10</td>
<td>1.87±0.26†</td>
</tr>
</tbody>
</table>

| **Group 2: Aortic cross-clamp+BQ-123** | | | |
| MAP (mm Hg) | 132±5 | 141±5 | 136±10 |
| HR (bpm) | 139±6 | 144±6† | 147±7† |
| RAP (mm Hg) | 0.4±1.5 | 0.5±1.5 | 0.1±1.3 |
| PCWP (mm Hg) | 2.8±0.4 | 3.0±0.4 | 2.4±0.5 |
| CO (L/min) | 3.77±0.46 | 3.09±0.34‡ ‡ | 2.57±0.41‡ ‡ |
| SVR (mm Hg/L per minute) | 37±4 | 48±4† ‡ | 57±6† ‡ |
| GFR (mL/min) | 21.9±2.0 | 10.5±3.3† | 13.3±3.9† |
| RBF (mL/min) | 194±20 | 145±10† | 96±21† |
| RVR (mm Hg/mL per minute) | 0.70±0.07 | 1.00±0.09‡ | 1.86±0.36† |

MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; RAP, right atrial pressure; PCWP, pulmonary capillary wedge pressure; CO, cardiac output; SVR, systemic vascular resistance; GFR, glomerular filtration rate; RBF, renal blood flow; RVR, renal vascular resistance.

*In group 1, postclamp period (C2) included vehicle; in group 2, postclamp period included BQ-123.

†P<.05 vs baseline.
‡P<.05 vs group 1.

versus 2.58±0.21 L/min), and SVR (46±4 versus 48±2 mm Hg/L per minute). Furthermore, infusion of BQ-123 did not significantly change renal hemodynamics, including RBF (200±32 versus 197±20 mL/min), RVR (0.67±0.11 versus 0.64±0.07 mm Hg/mL per minute), and GFR (17.4±6 versus 18.3±2.6 mL/min). Plasma ET did not significantly change (12.3±2.4 versus 12.5±1.7 pg/mL).

### Discussion

The current study confirms our recent report of the marked systemic and renal vasoconstriction in association with increased circulating ET that characterizes this clinically relevant model of acute hypertension and renal dysfunction associated with the cardiovascular surgical procedure of ACC. This study importantly extends our previous investigation and demonstrates that a specific ETA receptor antagonist, BQ-123, markedly attenuates the systemic vasoconstrictor response associated with ACC with preservation of CO. This receptor antagonism also attenuated the increase in RVR and decrease in RBF associated with this model. In contrast to our previous study, which demonstrated marked attenuation of the renal dysfunction associated with ACC in the presence of atrial natriuretic peptide, ACC-associated decreases in GFR were not attenuated by BQ-123.

The present study provides new insight into the mechanism of the increase in SVR observed in this model of ACC. The blockade of the ETA receptor resulted in significant attenuation of the increase in SVR, suggesting that the peripheral vasoconstriction associated with ACC is in part mediated through endogenous ET. Although there is variability with regard to circulating ET in the experimental groups, the specificity of the interaction between increased ET and the ETA receptor is supported by several factors. These
include the lack of any biological action of BQ-123 under unstimulated conditions and the widespread distribution of the ETA receptor in vascular smooth muscle with its known actions to mediate potent vasoconstriction.7,8 The smaller but significant increase in SVR in the presence of BQ-123 cannot exclude the possibility that other vasoconstrictors in this model of peripheral and renal ischemia and reperfusion may also participate in the vasoconstriction associated with ACC. The increase in SVR in group 1 without BQ-123 was associated with a marked decrease in CO and heart rate, without changes in right atrial pressure and a significant increase in pulmonary capillary wedge pressure. This contrasts with group 2, which in the presence of BQ-123 was associated with a modest decrease in CO, without changes in heart rate or cardiac filling pressures and a significant attenuation in the increase in SVR. This differential response suggests that the observed decrease in CO observed in group 1 may have been mediated through a negative chronotropic action of ET,3 increases in cardiac afterload in association with marked peripheral vasoconstriction, or both.

The modulating action of BQ-123 on renal hemodynamics was also examined in this study. In group 1, without BQ-123, ACC was associated with marked increases in RVR and decreases in RBF, whereas in group 2 the increase in RVR and decrease in RBF were attenuated in the presence of BQ-123. This suggests that the increase in renal vascular tone associated with ACC is in part mediated through the ETA receptor.

This is consistent both with the recent report of Terada et al,9 which demonstrated high expression of ETA receptors in the renal vasculature, and with the known actions of exogenously administered ET on renal function.2,3 Importantly, however, the current model of high endogenous ET is characterized by ischemic renal injury and suggests that the renal vasoconstriction associated with ischemia is in part mediated through ET. This is also consistent with the recent report of Firth and Ratcliffe,14 which demonstrated marked increases in messenger RNA for ET-1 in the kidney in a rat model of acute ischemic renal injury.

Although the renal vasoconstriction was markedly attenuated by BQ-123, the decrease in GFR was not. This suggests that the decrease in GFR is mediated through mechanisms other than ET or that the concentration of BQ-123 used in this study was not sufficient to completely block or competitively antagonize the glomerular actions of ET. Our previous study demonstrated that atrial natriuretic peptide attenuated the decrease in GFR, so one could speculate that the renin-angiotensin-aldosterone system, which is inhibited by atrial natriuretic peptide, may be functionally important in this model. Alternatively, the decrease in GFR may be mediated through the ETb receptor, which is colocalized with the ETA receptor in the glomerulus. Such an alternative explanation is also supported by the study of Kon et al,12 which demonstrated that in a rat model of ischemic renal failure, specific ET antibodies that could prevent ET interactions with both receptor subtypes reversed decreases in GFR.

The current study also documented a differential response in systemic and renal hemodynamics after termination of the BQ-123 infusion during the recovery period. Although the actions of BQ-123 on systemic hemodynamics were maintained for 1 hour after termination of the ETA antagonist, the actions on renal hemodynamics were rapidly lost. This observation has several implications. One could speculate that the renal circulation is more susceptible to the actions of ET or that the peptide antagonist BQ-123 is more rapidly cleared and degraded in the renal as opposed to the peripheral circulation. Alternatively, the concentration of BQ-123 used in this study may represent a threshold dose in terms of renal protection while it is sufficient to potently antagonize the peripheral hemodynamic actions of ET.
In conclusion, the current study documents the biological actions of an ETA receptor antagonist in a clinically relevant model of ACC characterized by acute arterial hypertension with systemic and renal vasoconstriction and elevation of circulating ET. The marked attenuation of systemic and renal vasoconstriction by BQ-123 supports an important functional role for endogenous ET and the ETA receptor in the vascular response associated with ACC. Although increases in systemic and renal vascular tone were attenuated, BQ-123 was unable to attenuate the decrease in GFR in this model, suggesting that mechanisms other than ET or the ETA receptor mediate the acute renal failure associated with ACC. These studies therefore support a role for endogenous ET in the regulation of systemic and renal vascular tone in this pathophysiologic model of altered cardiorenal function.

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References
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