Acute Hypoxia and Endogenous Renal Endothelin

Amiram Nir, Alfredo L. Clavell, Denise Heublein, Lawrence L. Aarhus, and John C. Burnett, Jr.

ABSTRACT

Endothelin (ET) is a potent vasoconstrictor peptide of endothelial cell origin. Recent studies have suggested a nonvascular paracrine and/or autocrine role for endothelin in the kidney. This study was designed to elucidate the renal ET response to acute moderate hypoxia, as reflected by urinary ET excretion and renal tissue ET immunoreactivity, and to correlate these responses to the hemodynamic and excretory changes during hypoxia. Experiments were conducted in two groups of anesthetized dogs: a hypoxic group (10% O2 ventilation: P02, 44 mm Hg; N = 7) and a control group (room air ventilation: P02, 114 mm Hg; N = 6). After 60 min of hypoxia or room air ventilation, kidneys were harvested and stained immunohistochemically for ET.

Key Words: Endothelin, hypoxia, urine, kidney

Endothelin (ET) is a potent systemic and renal vasoconstrictor of endothelial cell origin that may function as an autocrine and/or paracrine factor in the regulation of vascular tone. In addition, ET is present in the circulation and may also serve as a circulating hormone in various pathologic states such as in hypoxia (1,2). Although the kidney is a site of ET production and clearance, it is also a target for ET action (3-5). The exogenous administration of ET in pharmacologic concentrations decreases GFR, RBF, urine flow, and renal sodium excretion (6-8). In contrast, exogenous ET, at more physiologic concentrations, may have diuretic and natriuretic actions in the absence of a persistent decrease in GFR (7). A potential tubular action for ET is supported by observations that ET decreases Na+ K+ ATPase activity within proximal tubules (9) and has a concentration-dependent inhibitory effect on fluid absorption in rat isolated proximal nephron (10). Zeidel et al. also reported that ET decreases O2 consumption in inner medullary collecting duct cells (9), which are a site of ET synthesis (5). These studies support a possible nonvascular renal diuretic and natriuretic action of ET. Thus, such a tubular action may represent an important paracrine and/or autocrine role for ET in the regulation of water and sodium homeostasis.

Moderate hypoxia is a state relevant to many pathophysiologic disease states. Reports conflict with regard to the renal hemodynamic and excretory responses to hypoxia (1.1,1.2). These differences may be related to the severity and duration of hypoxia (11). Recent studies have reported increased plasma ET during hypoxia (1.2) and increased ET production by endothelial cells exposed to hypoxia in vitro (13,14). Although Firth and Ratcliff reported increased ET mRNA in rat kidney after acute ischemia (15), Elton et al. recently observed no significant increase in renal ET-1 mRNA in rats exposed to 24 and 48 h of hypoxia (16). This study was therefore designed to further characterize the renal responses to moderate hypoxia and specifically to determine the renal ET response. We therefore determined urinary ET as previous reports have documented urinary ET as a marker of renal ET production and/or release (3). Second, we elucidated ET tissue activity by immunohistochemistry and the contribution of the kidney to circulating ET by determining the plasma gradient of ET across the renal circulation. Last, we correlated these findings to the renal hemodynamic and excretory response.
METHODS

Experiments were conducted in two groups of dogs, a hypoxia group (N = 7) and a control group (N = 6). The dogs were anesthetized with sodium pentobarbital (30 mg/kg) with supplemental doses given as needed. The right femoral artery and vein were cannulated. The left kidney was exposed via a flank incision, and the ureter was cannulated for urine collection. A calibrated electromagnetic flow probe was placed on the renal artery to measure RBF. Saline was infused iv at 1 mL/min throughout the study to maintain volume homeostasis. Inulin was infused iv to achieve plasma concentrations of 40 to 60 mg/dL.

After a 60-min equilibration period with ventilation at room air, a 20-min baseline clearance was performed. During this and subsequent clearances, mean arterial pressure and RBF were measured, blood samples were collected from the aorta, and renal vein and arterial blood was collected in a heparinized syringe for arterial blood gas determination. After the baseline period, 60-min hypoxia (hypoxic group), induced by ventilation with 10% O2 and 90% N2, or room air ventilation (control group) was performed. Two separate clearances were performed during the first and last 20 min of the hypoxia period, and the values were averaged. At the end of the experiment, renal cortical and medullary tissue was collected for immunohistochemistry.

Blood for plasma inulin and electrolyte measurement was placed in heparinized tubes on ice, centrifuged at 2,500 rpm and 4°C, and refrigerated pending analysis. Plasma and urinary inulin concentrations were determined by the anthrone method (17). Blood for hormone analysis was collected in EDTA tubes and immediately placed on ice. Plasma and urine ET were measured by a specific radioimmunoassay as previously described (6). Plasma and urinary sodium concentrations were quantified by use of ion-selective electrodes, Beckman E2A analyzer (Beckman Instruments, Brea, CA).

For immunohistochemical analysis, renal tissue was fixed in 10% buffered formalin and incubated with hydrogen peroxide to block endogenous peroxidase activity and 5% normal goat serum to block nonspecific protein-binding sites. The primary antibody (rabbit anti-ET; Peninsula Laboratories, Belmont, CA) was then applied at a dilution of 1:1,600. Control slides were treated with normal dilute rabbit serum. Sections were then incubated with the secondary antibody–horseradish peroxidase conjugate for visualization.

Statistical Analysis

Values are expressed as mean ± SE. Baseline and hypoxia values were compared by paired t test; values from different groups were compared by unpaired t test. P < 0.05 was considered significant.

RESULTS

Ventilation with 10% O2 resulted in moderate hypoxia (PO2, 43 ± 4 versus 111 ± 4 mm Hg in the control group; P < 0.05). pH increased slightly during hypoxic ventilation (7.37 ± 0.01 to 7.39 ± 0.01) and decreased slightly during the parallel period in the control group (7.36 ± 0.02 to 7.33 ± 0.01; P < 0.05). PCO2 was similar in the two groups.

Urinary ET excretion is shown in Table 1. Excluding outlier values (values > 7 SD from mean), urinary ET excretion increased with hypoxia from 9.8 ± 3.2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Room Air</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>118 ± 3</td>
<td>116 ± 5</td>
</tr>
<tr>
<td>RBF (mL/min)</td>
<td>183 ± 13</td>
<td>166 ± 10^b</td>
</tr>
<tr>
<td>RVR (mm Hg/mL per min)</td>
<td>0.64 ± 0.03</td>
<td>0.70 ± 0.04</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>15.3 ± 1.6</td>
<td>14.8 ± 2.2</td>
</tr>
<tr>
<td>U Flow (mL/min)</td>
<td>0.18 ± 0.03</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>UNaV (mEq/min)</td>
<td>37.3 ± 8.9</td>
<td>34.0 ± 9.1</td>
</tr>
<tr>
<td>FENa (%)</td>
<td>1.6 ± 0.4</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td>UETV (pg/min)</td>
<td>9.5 ± 2.0</td>
<td>8.7 ± 1.3</td>
</tr>
</tbody>
</table>

* Room air and hypoxia, parallel periods in control and hypoxic groups, respectively. MAP, mean arterial pressure; RVR, renal vascular resistance; U flow, urine flow; UNaV, urinary Na excretion; FENa, fractional Na excretion; UETV, urinary endothelin excretion.

^ P < 0.05 versus room air.
^ P < 0.05 versus control.
^ P < 0.05 for change from room air versus control.
to 14.7 ± 2.6 pg/min (P < 0.05) and did not change in the control group (9.5 ± 2.0 to 8.7 ± 1.3 pg/min; P = not significant). The increase was significantly greater in the hypoxic group (Figure 1). Plasma ET tended to increase, but there was no step-up of plasma ET from aortic to renal vein before or during hypoxia in either group.

Immunohistochemical staining for ET was positive in proximal and distal tubules in cortex and medulla in both groups, with augmentation of cortical and medullary staining in the hypoxic group. The glomeruli and blood vessels revealed no staining for ET (Figure 2). No staining was detected with nonimmune rabbit serum in hypoxic or normoxic tissue.

Urine flow, urine sodium excretion, and fractional excretion of sodium increased with acute moderate hypoxia (all P < 0.05) but were not changed with time control (Table 1). The increase in these parameters was greater in the hypoxic group than in the control group (Figure 1). In the hypoxic group, there was positive correlation between the hypoxia-induced increase in urinary ET excretion and the increase in urinary Na excretion (P < 0.05; r² = 0.58). There was no change in GFR or renal vascular resistance in either group (Table 1). In the control group, there was a small, but significant decrease in RBF with time, but there was no difference in RBF between the two groups. There was also no change in mean arterial pressure in either group.

**DISCUSSION**

This study demonstrates for the first time that acute moderate hypoxia in the dog results in a 50% increase in urinary ET excretion and increased ET immunoreactivity in renal tubules. There is no evidence for ET secretion by the kidney to increase circulating concentrations in either group. These alterations in renal ET during hypoxia occurred in association with diuresis and natriuresis.

The predominant tubular immunoreactivity was a consistent finding in all stained samples in these studies. Although immunoreactivity reflects the balance among production, binding, and degradation, Kazutomo et al. found ET production in glomeruli and proximal and distal nephron from normoxic rat kidney, with the highest production in glomeruli and collecting ducts and minimal production in proximal tubules and loop of Henle (18). The increase in urinary ET excretion during hypoxia, as demonstrated in this study, is probably of renal origin, on the basis of elegant studies by Benigni et al. and Abassi et al. (3, 19), who showed minimal urinary detection of infused labeled ET. Thus, one could conclude that hypoxia may be a stimulus for increased renal ET production. This would be consistent with reports that document hypoxia-induced increases in ET production in bovine coronary artery endothelial cells (14) and in human umbilical endothelial cells (13).

ET has been shown to modulate renal tubular cell function by inhibiting Na⁺ K⁺ ATPase activity in proximal tubules and O₂ consumption in inner medullary collecting duct cells (9). Indeed, low-dose ET has diuretic and natriuretic effects independent of renal perfusion pressure (7). The observation presented here is in agreement with previous data and may link the hypoxia-induced diuresis and natriuresis to the diuretic and natriuretic tubular effects of ET, on the basis of the increase in concentrations of ET in proximal and distal tubules, as determined by immunohistochemistry, and the increase in urinary ET. A causal relationship could be further de-
Nir et al.

Figure 2. Representative immunohistochemical staining for ET in kidney of a dog exposed to 1 h of hypoxia or 1 h of room air ventilation. (A) Hypoxic dog medulla. (B) Normoxic dog medulla. (C) Hypoxic dog cortex. (D) Normoxic dog cortex. Brownish-red areas represent the presence of tissue immunoreactive ET.

termined by an assessment of ET receptor blocker effects on hypoxia-induced renal response. However, with regard to the natriuretic actions of ET-1, Clavell et al. reported in preliminary studies that, in the absence of renal vasoconstriction and in the presence of ETA receptor blockade by the ETA receptor antagonist BQ123, ET-1 results in diuresis and natriuresis (20).

Recently, Hoffman et al. found that the ET precursor Big ET (1-39) in the rat is diuretic and natriuretic with pressor effects similar to those of ET in the absence of renal vasoconstriction (8). In this study, we cannot exclude that Big ET participates in these responses.

Acute moderate hypoxia resulted in augmentation of ET immunoreactivity in renal proximal and distal tubular cells. It is unclear whether this increase in ET immunoreactivity in renal tubules represents increased ET production, increased binding of ET to biologically active receptors, or binding to degrading peptidases within renal cells. Further elucidation of this question could be achieved by comparing ET mRNA levels in hypoxic and control kidneys. Nevertheless, these studies demonstrate a clear interaction between endogenous ET and renal tubular cells in hypoxia. One should note that ET immunoreactivity was not detected in endothelial cells. This is in agreement with the concept of the constitutive release of ET from endothelial cells and with preliminary data that revealed little intracellular ET in cultured endothelial cells (21).

In summary, these studies report for the first time the urinary and renal ET responses to moderate hypoxia. Specifically, this study demonstrates increased urinary ET excretion and accumulation of ET in the renal tubular epithelial cells, suggesting a nonvascular role of endogenously produced ET in the regulation of sodium and water excretion. These studies thus continue to support a possible paracrine and/or autocrine role for ET in the control of renal function.

REFERENCES

1. Perrella AM, Edell SE, Krowka JM, Cortese AD, Burnett JC Jr: Endothelium-derived relaxing factor in pulmonary and renal circulation during