Renal and Adrenal Responses to Hypoxemia during Angiotensin-Converting Enzyme Inhibition in Lambs

Douglas N. Weismann, James E. Herrig, Oliva J. McWeeny, Nancy A. Ayres, and Jean E. Robillard

From the Department of Pediatrics and the Cardiovascular Research Center, College of Medicine, University of Iowa, Iowa City

SUMMARY. Chronically catheterized lambs (4–37 days postnatal age) (n = 35) were studied to test the hypothesis that the products of angiotensin-converting enzyme activity are involved in renal and adrenal responses to normocapnic hypoxemia in immature lambs. Arterial angiotensin II (from 111.0 ± 38.8 to 71.0 ± 38.8 pg/ml, P < 0.01) and aldosterone (from 128.0 ± 98.0 to 62.1 ± 27.9 pg/ml, P < 0.01) concentrations were significantly decreased and vasopressor responses to angiotensin I were greater than 90% inhibited by continuous intravenous infusion of angiotensin-converting enzyme inhibitor (captopril, 2.5 μg/kg/min, n = 16). Baseline mean arterial pressure (64 ± 14 vs. 78 ± 9 mmHg) and urinary sodium excretion rate (UN/V 3.04 ± 2.83 vs. 15.00 ± 20.00 μEq/min) were significantly (P < 0.05) decreased in captopril-treated lambs vs. control lambs. Baseline arterial plasma renin activity was significantly (P < 0.01) increased in captopril-treated vs. control lambs (8.6 ± 9.0 vs. 100.0 ± 64.0 ng/ml per hr). Normocapnic hypoxemia (P02 38 ± 6 torr for 30 minutes) during captopril infusion was associated with no significant (P > 0.05) changes in renal hemodynamics and function, including glomerular filtration rate (from 0.34 ± 0.24 to 0.35 ± 0.25 ml/min per g). Urinary prostaglandin E excretion rate (from 0.655 ± 0.703 to 1.310 ± 1.020 mg/ml per g) and adrenal blood flow (from 2.67 ± 1.69 to 6.24 ± 3.73 ml/min per g) increased significantly (P < 0.05) under these conditions. Arterial epinephrine (from 0.11 ± 0.07 to 1.1 ± 1.8 ng/ml), norepinephrine (0.48 ± 0.38 to 3.2 ± 5.4 ng/ml), and arginine vasopressin (from 5.11 ± 2.20 to 10.70 ± 8.61 μU/ml) also increased significantly (P < 0.05) in response to hypoxemia during angiotensin-converting enzyme inhibition with captopril. None of these responses to hypoxemia were significantly different from that of uninhibited (control) lambs (n = 19). In contrast, cortisol response to hypoxemia was significantly (P < 0.05) less in captopril-treated lambs (captopril vs. control, —1.00 ± 1.90 vs. 3.40 ± 3.30 μg/dl). These data suggest that the products of angiotensin-converting enzyme activity are not important regulators of renal responses to hypoxemia, but may be involved in cortisol responses to normocapnic hypoxemia in immature lambs. (Circ Res 52: 179–187, 1983)
Surgical Procedures

Surgical procedures in these lambs have been described previously (Weismann and Clarke, 1981). Briefly, lambs were anesthetized with halothane, followed by placement of chronic indwelling catheters in femoral vessels with catheter tip placement in the left ventricular cardiac chamber, distal abdominal aorta, and distal inferior vena cava, respectively. A suprapubic cystostomy tube also was placed. Catheter tip placements were verified by direct observation when the lambs were killed. Vascular catheters were tunneled subcutaneously to exit the skin at the left flank, and the bladder catheter was tunneled a short distance to exit the skin of the abdomen anteriorly. All incisions were closed with chromic sutures. The lambs were administered glucose-saline solution intravenously during the postoperative period until they were able to stand and feed by mouth, which was usually within 4 hours of the operative procedures. Thereafter, the lamb nursed from the ewe ad libitum. The lambs received ampicillin, 200 mg/kg, intravenously, every 12 hours until studied. The lambs were allowed 64-72 hours for recovery from the operative procedures prior to participation in the experimental protocol.

Experimental Protocol

Lambs were studied over a range of postnatal age (experimental group, 4-37 days; mean age, 15 ± 9 days. Control group, 4-30 days; mean age, 11 ± 8 days) similar to that of the previous study (Weismann and Clarke, 1981). Studies were performed while the lambs were supported in a standing posture by a specially designed canvas harness. Arterial blood pressure (MAP) was recorded continuously on a Beckman R611 Dynograph using a pressure transducer (Statham) connected to the femoral artery catheter. In each lamb, \( ^{3}H \)-inulin (New England Nuclear) in a 5% dextrose-in-water solution was administered as a constant intravenous infusion at a rate of 0.4 ml/min (0.1 \( \mu \)Ci/min) by a Harvard infusion/withdrawal pump. The experimental group (n=16) received treatment with the ACE inhibitor, captopril (\( \text{d-mercapto-2-methyl propanoyl-L-proline: SQ-14225} \); Squibb and Sons, generously supplied by Dr. Z. P. Horovitz). Arterial blood was sampled for pre-inhibitor levels of angiotensin II (AlI) and aldosterone (Aldo) concentrations. Baseline arterial pressure responses to an intravenous bolus of saline, angiotensin I (AI, 1 \( \mu \)g/kg), and II (0.5 \( \mu \)g/kg) also were determined. Captopril was solubilized in 0.9% saline solution and immediately given as a constant intravenous infusion at a rate of 2.5 \( \mu \)g/kg per min (40 \( \mu \)l/min). Lambs not treated with captopril (control group, n=19) were given 0.9% saline alone and subjected to an identical protocol.

After a 60-minute equilibration period, adequacy of ACE inhibition was documented as greater than 90% suppression of the arterial pressure response to an iv bolus of AI (1 \( \mu \)g/kg). Intact arterial pressure response to an iv bolus of All (0.5 \( \mu \)g/kg) was also confirmed in each lamb. Thereafter, three clearance periods of 20 minutes each were performed during continuous captopril infusion. Urine was collected quantitatively. At the midpoint of each period, arterial blood was sampled for determinations of \( ^{3}H \)-inulin content, sodium concentration (Na), hematocrit, oxyhemoglobin saturation, pH, Pco\(_2\), and P\(_O_2\). During the third clearance period, arterial blood was also sampled for All, Aldo, plasma renin activity (PRA), cortisol concentration (Cort), vasopressin concentration (AVP), epinephrine concentration (E), and norepinephrine concentration (NE). Withdrawn blood was volumetrically replaced with plasma protein fraction (Plasmanate, Cutter Laboratories). After completion of the third clearance period during captopril infusion, approximately 1.5 \( \times \) 10\(^5\) microspheres (3M Company), 15 ± 3 \( \mu \)m in diameter and labeled with a single radioactive isotope (\( ^{41} \text{Ce}, ^{85} \text{Sr}, ^{46} \text{Sc}, \text{or} \ ^{95} \text{Nb} \)), were suspended in 3 ml of 0.9% saline. The microspheres then were agitated thoroughly and injected into the left ventricular catheter over 20-30 seconds, then flushed immediately with 5 ml of 0.9% saline solution. An independent lower body reference sample was obtained by withdrawal of blood (Harvard infusion/withdrawal pump) through the left femoral arterial catheter at a rate of 1.94 ml/min for a period of 3 minutes starting approximately 20 seconds before the microspheres injection (Makowsky et al., 1968).

Subsequently, systemic hypoxemia was produced during continuous captopril infusion by directing a 12% oxygen in nitrogen gas mixture into a clear plastic bag which was placed over the lamb’s head and secured with a drawstring at the neck. Partial rebreathing of expired gases containing CO\(_2\) within the bag encircling the head occurred under these circumstances, so that hyperpernia produced by the hypoxemia would not induce hypocarbia. During this initial equilibration to the oxygen-deficient inspired gas mixture, arterial blood pH and gases were monitored frequently for regulation of gas flow into the bag encircling the lamb’s head. From these measurements and adjustments, the desired degree of hypoxemia could be induced while maintaining pH and PCO\(_2\) in the normal range. Approximately 20 minutes after initiation of the oxygen-deficient inspired gas mixture, appropriate pH, PCO\(_2\), and P\(_O_2\) values were confirmed by arterial blood gas analysis, and a 10-minute clearance period was performed. Urine was again, collected quantitatively. At the midpoint of the clearance period, arterial blood was sampled for determinations, as in the third baseline period. Sampled blood was again replaced volumetrically with plasma protein fraction. At the end of the 10-minute clearance period during hypoxemia and continuous captopril infusion, an injection of radioactive microspheres labeled with a different isotope was performed as previously described.

Following the microsphere injection during hypoxemia, normoxemia was restored by allowing the lamb to breath room air with continued captopril infusion. After 20 minutes of equilibration to normoxemia, two 20-minute clearance periods were performed, as previously, followed by a third injection of microspheres, as described previously. Continued suppression of ACE activity was again documented by greater than 90% suppression of the vasopressor response to an iv bolus of AI (1 \( \mu \)g/kg). Non-suppression of All responsiveness was also documented, as previously. The lamb was then killed by intravenous bolus administration of pentobarbital sodium (Somlethal, med-tech, inc.). The adrenals and kidneys were immediately harvested, weighed, cut into sagittal sections of approximately 1 g, and placed-in counting vials.

Lambs given vehicle only were studied in an identical fashion for determination of responses to hypoxemia in an uninhibited state.

Analytical Procedures

The concentrations of sodium in blood and urine were measured with a flame photometer (Corning). Arterial blood pH, P\(_O_2\), and Pco\(_2\) were determined by a Radiometer pH/blood gas analyzer. Oxyhemoglobin saturation was determined by an IL Cooximeter. \( ^{3}H \)-Inulin in blood and urine was determined by scintillation counting (Beckman LS-330). Radiolabeled microsphere (\( ^{85} \text{Sr}, ^{41} \text{Ce}, ^{46} \text{Sc}, ^{95} \text{Nb} \)) content in blood and tissue was determined by counting in a \( \gamma \)-
spectrophotometer (Beckman 300) with separation of isotopes by standard methods (Heymann et al., 1977). Hematocrit was determined by standard micro-methodology. Samples of blood for determination of plasma renin activity (PRA) were collected in chilled tubes containing EDTA, placed on ice, and centrifuged at 4°C within 20 minutes. Plasma renin activity was determined by radioimmunoassay, using the method of Haber et al. (1969) as modified by Oparil (1975). Blood samples for plasma aldosterone determinations were collected in heparinized syringes, placed on ice, and centrifuged at 4°C. Aldosterone was determined by radioimmunoassay by the method of Ito et al. (1972). This assay system has been fully characterized previously (Robillard et al., 1980).

Blood samples for plasma All determinations were collected in chilled tubes containing 0.3 mM EDTA and 0.025 M O-phenanthroline. Precipitation of cells and proteins was performed immediately with 65% acetonitrile, and the supernatant was dried under air for subsequent chromatographic isolation of All on SP sephadex in sodium acetate buffer. All was measured by radioimmunoassay (Cain et al., 1972) which has been fully characterized in our laboratory (Robillard et al., 1982). Cross-reactivity of the All antiserum based on All as 100% reactive is 131% for angiotensin III and less than 3% for angiotensin I. Cross-reactivity was also evident for All metabolites (156% cross-reactivity with the hexapeptide, 130% cross-reactivity with the heptapeptide and 103% cross-reactivity with the pentapeptide). The intra-assay coefficient of variation was 5.7% and the interassay coefficient of variation was 8.8%.

Plasma vasopressin was extracted, using the benzotropine extraction, and measured by radioimmunoassay procedures of Skowsky et al. (1974). Urine samples for prostaglandin determinations were collected in chilled tubes and immediately frozen at −70°C. Urine samples underwent extraction, ethylacetate and separation into prostaglandin classes by silicic acid chromatography. Urinary PGE and PGF levels were determined by radioimmunoassay with specific antisera (Van Orden et al., 1973, 1977). Arterial blood epinephrine and norepinephrine content were determined by radioenzymatic assay (Cat-a-Kit, Upjohn Co.) as described by Peuler and Johnson (1977). Arterial cortisol concentration was determined by radioimmunoassay (Farmer and Pierce, 1974) using a commercially available kit (Premix, Diagnostic Products).

**Calculations**

Glomerular filtration rate was determined as the calculated renal clearance of $^{3}H$-inulin. Renal and adrenal blood flow were calculated as: total kidney or adrenal counts X femoral artery reference flow rate/total femoral blood counts.

**Data Analysis**

Comparisons were performed by Wilcoxon’s signed rank and rank sum tests. Correlation coefficients and linear regressions were computed by least squares formulas (Steel and Torrie, 1960). A two-sided significance limit $P$ value of 0.05 or less was required for a difference or a correlation to be declared significant. Experimental data are expressed as mean ± SD.

**Results**

Administration of captopril by constant infusion to these chronically catheterized lambs produced marked inhibition of converting enzyme activity, as verified by at least 90% inhibition of the vasopressor response (25 ± 8 mm Hg pre-captopril vs. 2 ± 2 mm Hg post-captopril) to intravenously administered bolus of angiotensin I (Al, 1 μg/kg) without altering vasopressor response (26 ± 8 mm Hg) to iv bolus administration of angiotensin II (All, 0.5 μg/kg). Furthermore, arterial All (from 111.0 ± 38.8 to 71.0 ± 38.8 pg/ml, $P < 0.01$ by Wilcoxon signed rank test) and aldosterone (Aldo, from 128.0 ± 98.0 to 62.1 ± 27.8 pg/ml, $P < 0.01$ by Wilcoxon signed rank test) were significantly decreased during captopril infusion. Captopril infusion (Table 1) was associated with relatively large but nonsignificant ($P > 0.05$ by Wilcoxon rank sum test) differences in urinary flow rate (V) and fractional sodium excretion rate in comparison with control lambs (Table 2). Baseline glomerular filtration rate (GFR), urinary prostaglandin E excretion rate ($U_{PGE}$), urinary prostaglandin F excretion rate ($U_{PGF}$), adrenal blood flow (ABF), and renal blood flow (RBF) were not significantly different in captopril-treated (Table 1) relative to control lambs (Table 2).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Renal Hemodynamic and Function Values during Converting Enzyme Inhibition (Captopril-Treated Group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Baseline (mEq/min)</td>
</tr>
<tr>
<td>V (ml/min)</td>
<td>0.28 ± 0.19</td>
</tr>
<tr>
<td>GFR (m/l/min)</td>
<td>0.34 ± 0.24</td>
</tr>
<tr>
<td>$U_{Na}$ (mEq/min)</td>
<td>3.04 ± 2.63</td>
</tr>
<tr>
<td>FE$Na$ (%)</td>
<td>0.20 ± 0.24</td>
</tr>
<tr>
<td>$U_{NaV}$ (ng/min)</td>
<td>0.655 ± 0.703</td>
</tr>
<tr>
<td>$U_{NaV}$ (ng/min)</td>
<td>1.410 ± 0.791</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>64 ± 14</td>
</tr>
<tr>
<td>ABF (m/l/min)</td>
<td>2.67 ± 1.69</td>
</tr>
<tr>
<td>RBF (m/l/min)</td>
<td>8.90 ± 5.33</td>
</tr>
</tbody>
</table>

Values are mean ± SD. $V$ = urinary flow rate; $GFR$ = glomerular filtration rate; $U_{NaV}$ = urinary sodium excretion rate; $FE_{Na}$ = fractional sodium excretion rate; $U_{PGE}$ = urinary prostaglandin E excretion rate; $U_{PGF}$ = urinary prostaglandin F excretion rate; MAP = mean arterial pressure; $ABF$ = adrenal blood flow; $RBF$ = renal blood flow.

$*$ Value is significantly different from baseline, $P < 0.05$ by Wilcoxon signed rank test.
2). Baseline mean arterial pressure (MAP, 64 ± 14 vs. 78 ± 9 mm Hg, captopril vs. control, P < 0.01 by Wilcoxon rank sum test) and urinary sodium excretion rate (UNaV, 3.04 ± 2.83 vs. 15.00 ± 20.00 μEq/min, captopril vs. control, P < 0.05 by Wilcoxon rank sum test) were significantly decreased in captopril-treated lambs. Baseline epinephrine (E), norepinephrine (NE), vasopressin (AVP), cortisol (Cort), aldosterone (Aldo), and angiotensin II (All) were not significantly different (P > 0.05 by Wilcoxon rank sum test) in captopril-treated lambs relative to controls. Baseline plasma renin activity (PRA), on the other hand, was significantly higher in captopril-treated vs. control lambs (100.0 ± 64.0 vs. 8.6 ± 9.0 ng/ml per hr, P < 0.01). Baseline values in captopril-treated lambs (Table 3) vs. control lambs (Table 4) for P02 (95 ± 10 vs. 88 ± 8 torr), oxyhemoglobin (91.9 ± 2.3 vs. 95.6 ± 1.3% saturation), Pco2 (32 ± 4 vs. 35 ± 3 torr), pH (7.45 ± 0.04 vs. 7.40 ± 0.06) were slightly, but significantly (P > 0.05 by Wilcoxon rank sum test) different. Baseline hematocrit (Hct) values were not significantly (P > 0.05) different.

Administration of oxygen-deficient inhaled gas mixture to lambs during continuous angiotensin-converting enzyme inhibition with captopril produced significant declines in arterial P02 and oxyhemoglobin saturation without significant changes in arterial pH and Pco2 (Table 1). There was a very small but significant increase in hematocrit in response to hypoxemia (Table 3). Similar responses were seen in control lambs, although Pco2 slightly, but significantly, decreased in response to hypoxemia in the control group (Table 4). Comparison of the change (Δ) in Pco2 with hypoxemia (ΔPco2, captopril vs. control, −0.10 ± 7.0 vs. −5.1 ± 6.7 torr) and Pco2 during hypoxemia (captopril vs. control, 33 ± 6 vs. 31 ± 7 torr) revealed no significant differences (P < 0.05 by Wilcoxon rank sum test). The change in P02, oxyhemoglobin, hematocrit, and pH in response to hypoxemia were not significantly different (P > 0.05 by Wilcoxon rank sum test) in comparisons of captopril to control groups. Linear regression analysis of changes in arterial pH (r = 0.0005, P > 0.5), Pco2 (r = 0.10, P > 0.5), P02 (r = 0.21, P > 0.4), oxyhemoglobin (r = 0.14, P > 0.5), or hematocrit (r = 0.13, P > 0.5) in response to hypoxemia during converting enzyme inhibition demonstrated no significant correlation of these variables with postnatal age. Similar results were noted with linear regression analysis of changes in arterial pH (r = −0.29, P > 0.2), Pco2 (r = 0.25, P > 0.3), P02 (r = 0.34, P > 0.1), oxyhemoglobin (r = −0.28, P > 0.5), or hematocrit (r = 0.45, P > 0.5) in control lambs. These relationships also were similar when the variables were expressed as percent change, except for hematocrit, which became statistically significant (AHct expressed as percent change, r = 0.47, P < 0.05). Recovery values were not significantly different from baseline values, except for hematocrit (Tables 3 and 4).

Hypoxemia during converting enzyme inhibition was associated with a small but significant increase in arterial plasma renin activity (PRA), but no change in

### Table 3
Arterial Blood Values during Converting Enzyme Inhibition (Captopril-Treated Group)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Hypoxemia</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>V (ml/min)</td>
<td>14</td>
<td>0.46 ± 0.38</td>
<td>0.46 ± 0.51</td>
</tr>
<tr>
<td>GFR (ml/min per g)</td>
<td>14</td>
<td>0.43 ± 0.12</td>
<td>0.35 ± 0.13</td>
</tr>
<tr>
<td>UNaV (μEq/min)</td>
<td>14</td>
<td>15.00 ± 20.00</td>
<td>18.00 ± 23.00</td>
</tr>
<tr>
<td>FEna (%)</td>
<td>14</td>
<td>0.44 ± 0.57</td>
<td>0.70 ± 0.81</td>
</tr>
<tr>
<td>Uo2/Cr (ng/min)</td>
<td>8</td>
<td>0.658 ± 0.301</td>
<td>1.230 ± 1.270</td>
</tr>
<tr>
<td>Uo2/creatinine (ng/g)</td>
<td>8</td>
<td>1.610 ± 1.890</td>
<td>0.991 ± 1.240</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>19</td>
<td>78 ± 9</td>
<td>79 ± 12</td>
</tr>
<tr>
<td>ABF (ml/min per g)</td>
<td>16</td>
<td>3.18 ± 1.61</td>
<td>5.56 ± 2.73*</td>
</tr>
<tr>
<td>RBF (ml/min per g)</td>
<td>16</td>
<td>6.91 ± 2.33</td>
<td>6.34 ± 3.45</td>
</tr>
</tbody>
</table>

Values are mean ± sd. * Value is significantly different from baseline, P < 0.05 by Wilcoxon signed rank test.

### Table 4
Arterial Blood Values in Lambs Not Administered Captopril (Control Group)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Hypoxemia</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>P02 (torr)</td>
<td>95 ± 10</td>
<td>95 ± 10</td>
<td>95 ± 10</td>
</tr>
<tr>
<td>Oxyhemoglobin (% sat)</td>
<td>91.2 ± 2.3</td>
<td>91.8 ± 2.3</td>
<td>91.8 ± 2.3</td>
</tr>
<tr>
<td>Pco2 (torr)</td>
<td>32 ± 4</td>
<td>32 ± 3</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>pH</td>
<td>7.46 ± 0.04</td>
<td>7.44 ± 0.09</td>
<td>7.44 ± 0.05</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>30 ± 5</td>
<td>31 ± 6*</td>
<td>28 ± 5*</td>
</tr>
</tbody>
</table>

Values are mean ± sd; n = 16. * Value is significantly different from baseline, P < 0.05 by Wilcoxon signed rank test.
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FIGURE 1. Baseline, hypoxemia and recovery values of arterial plasma renin activity (PRA), angiotensin II (All) and aldosterone (Aldo) concentration during continuous angiotensin-converting enzyme inhibition with captopril (Panel a) and in control lambs (Panel b). Values are mean ± SD. * Value is significantly greater than baseline value, P < 0.05 by Wilcoxon signed rank test.

All or Aldo (Fig. 1a). Recovery values were not significantly different from baseline values during captopril infusion. Linear regression analysis of changes in PRA (r = 0.15, P > 0.5), Aldo (r = 0.35, P > 0.1) and All (r = 0.001, P > 0.5) in response to these conditions demonstrated no significant correlations with postnatal age. Arterial vasopressin (AVP), epinephrine (E), and norepinephrine (NE) concentrations, on the other hand, increased significantly under these conditions (Fig. 2a). Arterial AVP remained significantly elevated in the recovery period as well. In contrast, arterial cortisol concentration (Cort) did not change significantly in response to hypoxemia during inhibition of converting enzyme (Fig. 2). The changes (Δ) in these variables with hypoxemia did not correlate significantly with postnatal age (ΔAVP r = 0.20, P > 0.4; ΔE r = -0.01, P > 0.5; ΔNE r = 0.01, P > 0.5; ΔCort r = 0.02, P > 0.5) by linear regression analysis.

Hypoxemia in control lambs was associated with a significant increase in PRA and Aldo (Fig. 1b). The increase in All in response to hypoxemia (Fig. 1b) did not reach statistical significance. Linear regression analysis of changes in PRA (r = -0.07, P > 0.5) and All (r = 0.14, P > 0.5) with hypoxemia in control lambs demonstrated no significant correlations with postnatal age. The change in arterial aldosterone concentration with hypoxemia correlated significantly with postnatal age (r = 0.75, P < 0.02). However, when expressed as percent change, the correlation was not significant (r = 0.42, P > 0.2). Arterial AVP, E, NE, and cortisol also increased significantly to these conditions in control lambs (Fig. 2b). Linear regression analysis of changes in AVP (r = 0.19, P > 0.5), E (r = -0.06, P > 0.5), NE (r = -0.09, P > 0.5), and Cort (r = 0.29, P > 0.3) with hypoxemia in control lambs revealed no significant correlation of these variables with postnatal age. Comparisons (Wilcoxon rank sum test) of the change in these variables with hypoxemia in captopril-treated vs. control lambs (ΔPRA 17.00 ± 27.00 vs. 6.72 ± 7.82 ng/ml per hr, P > 0.1; ΔAll 1.2 ± 30.0 vs. 29.0 ± 30.0 pg/ml, P > 0.05; ΔAVP 6.10 ± 7.60 vs. 29.0 ± 54.00 μU/ml, P > 0.1; ΔE 0.64 ± 1.30 vs. 4.80 ± 1.36 ng/ml, P > 0.05; ΔNE 1.2 ± 2.1 vs. 3.9 ± 9.0 ng/ml, P > 0.05) revealed significant differences only for ΔCort (-1.00 ± 1.90 vs. 3.40 ± 3.30 pg/dl, P < 0.01) and ΔAldo (-2.4 ± 29.0 vs. 35.0 ± 37.0 pg/ml, P < 0.02).

Hypoxemia during converting enzyme inhibition with captopril did not significantly change hemodynamic and renal functional values, with the exception of adrenal blood flow and urinary prostaglandin E excretion rate (Table 1). Adrenal blood flow (ABF) and urinary prostaglandin E excretion rate (U_{PGE2}) increased significantly in response to hypoxemia during captopril infusion, and U_{PGF2α} remained elevated during the recovery period. Changes in glomerular filtration rate (ΔGFR r = 0.45, P < 0.05), urinary sodium excretion rate (ΔUNa r = 0.07, P > 0.5), fractional sodium excretion rate (ΔFE_{Na} r = -0.19, P > 0.4), mean arterial pressure (ΔMAP r = 0.28, P > 0.2), renal blood flow (ΔRBF r = -0.41, P > 0.05), renal vascular resistance (ΔRVR r = -0.09, P < 0.5), adrenal blood flow (ΔABF r = -0.20, P > 0.2), and U_{PGF2α} (ΔU_{PGF2α}, P > 0.5) in response to hypoxemia during converting enzyme inhibition were not significantly related to postnatal age. Although change in U_{PGF2α} in response to hypoxemia (ΔU_{PGF2α}) during ACE inhibition correlated significantly with postnatal age (r = -0.60, P < 0.05), when values were corrected for kidney weight no significant correlation was noted (r = -0.17, P > 0.5). Similar nonsignificant relationships were noted when these variables were expressed as percent change.

Similar responses to hypoxemia were evident in control lambs (Table 2). Comparisons of the change in these variables with hypoxemia in captopril-treated vs. control lambs revealed no significant differences (P > 0.05 by Wilcoxon rank sum test). These results included lack of significant difference in ΔABF (captopril vs. control, 3.90 ± 4.00 vs. 3.00 ± 2.90 ml/min per g, P > 0.5) and ΔU_{PGF2α} (captopril vs. control, 0.660 ± 0.687 vs. 0.780 ± 1.560 ng/ml, P > 0.5). Changes in these variables with hypoxemia in control
Captopril treatment inhibited the vasopressor response to intravenously administered angiotensin I in the lambs of the current study, demonstrating inhibition of the conversion of angiotensin I to angiotensin II which is mediated by angiotensin-converting enzyme (Skeggs et al., 1976). Furthermore, baseline plasma renin activity was markedly increased in captopril-treated lambs, probably due to loss of the negative-feedback effect of angiotensin II on renin release (Schiffrin et al., 1981). The decline in serum angiotensin II levels in response to captopril therapy was compatible with that of previous studies (Vinci et al., 1979; Morton et al., 1980; Swartz et al., 1980; Atkinson et al., 1981; Moore et al., 1981). The relatively small change in angiotensin II levels induced by captopril may be due to a combination of factors: First, angiotensin II metabolites may persist despite blockade of angiotensin II production. A proportion of these angiotensin II metabolites will be detected as angiotensin II due to considerable cross-over of angiotensin II antibody to various angiotensin II metabolites. Second, accumulation of plasma angiotensin I will occur as an immediate precursor at the site of enzymatic blockade. Very large accumulations of angiotensin I may artifactually increase angiotensin II concentration measurements (Morton et al., 1980), even with very low cross-over reactivity of angiotensin II antibody to various angiotensin II metabolites. Third, vascular response to intravenously administered angiotensin I may be regulated by vascular tissue-converting enzyme activity which may not be
reflected in circulating plasma angiotensin II levels (Horovitz, 1980).

Previous studies of chronically catheterized lambs of similar postnatal age as those of the current study (Weismann and Clarke, 1981), as well as human infants (Torrado et al., 1974; Guignard et al., 1976; Broberger and Aperia, 1978; Müller et al., 1980) and newborn piglets (Alward et al., 1978), have demonstrated a significant decline in glomerular filtration rate (GFR) in response to hypoxemia. This decrease in renal function has been accompanied by significantly increased plasma renin activity, aldosterone concentration (Weismann and Clarke, 1981), and plasma renin concentration (Alward et al., 1978). The increase in plasma renin appears to be due to increased renal renin production, which has been documented in response to hypoxemia in rats (Mattioli et al., 1975) and lambs (Weismann and Williamson, 1981). It is not known whether this increase in renin secretion rate in response to hypoxemia is due to increased sympathetic activity (Fray, 1980) or decreased negative feedback due to decreased angiotensin II formation secondary to decreased angiotensin-converting enzyme (ACE) activity, as shown in adult dogs (Leuenberger et al., 1978; Stalcup et al., 1979). However, studies in hypoxic newborn infants have shown elevated serum ACE levels and lung ACE activity under these conditions (Mattioli et al., 1975). These findings suggested that increased activity of the renin-angiotensin-aldosterone system may be involved in the glomerular filtration rate response to hypoxemia in newborns through increased production of the potent vasoconstrictor, angiotensin II. In contrast, the current study demonstrated that arterial angiotensin II levels were not affected by hypoxemia. Furthermore, hypoxemia induced a 19 ± 32% decrease in glomerular filtration rate in uninhibited lambs of the current study, similar to the 23 ± 33% decline seen in our previous study (Weismann and Clarke, 1981), and was unaltered by captopril treatment. Thus, increased angiotensin II production appears not to be a mediator of this response.

Captopril infusion decreased baseline arterial pressure in the lambs of the current study. The decreased baseline urinary sodium excretion rate during captopril infusion was probably a reflection of this decreased perfusion pressure. This vasodepressor response to captopril is compatible with the results of previous studies (Heel et al., 1980). Hatton et al. (1981) suggested that the vasodepressor effect of angiotensin-converting enzyme inhibition may be due to blockade of angiotensin II formation and consequent removal of angiotensin II involvement in homeostatic baroreceptor reflex activity, as well as decreased direct vasoconstrictor effects. Depressor responses to captopril have also been strongly correlated with increased vasodilator prostaglandin production (Swartz et al., 1980; Moore et al., 1981) and prolonged kinin effect (Williams and Hollenberg, 1977; Vinci et al., 1979). Definite conclusions concerning the mechanism of this vasodepressor response to captopril cannot be determined from the current data, due to the role of angiotensin-converting enzyme in catabolism of bradykinin as well as formation of angiotensin II (Skeggs et al., 1976). Furthermore, the extensive interaction of these systems with the prostaglandin system and their role in baroreceptor function were not specifically studied.

The responses to hypoxemia and the relationships of the change in these variables with hypoxemia to postnatal age in control lambs of the current study were similar to those noted previously in lambs (Weismann and Clarke, 1981). Exceptions were the lack of significant change in fractional sodium excretion rate and lack of significant correlation of the change in urinary flow rate and sodium excretion rate to postnatal age. However, when the change in fractional sodium excretion rate with hypoxemia was expressed as percent change, the increase in value in the current study (60 ± 70%) was similar to the results of our previous study (120 ± 240%). There are too few animals in the current study to determine adequately the relationship of the responses to hypoxemia to postnatal age in control and captopril-treated lambs.

Hypoxemia is a potent stimulus to catecholamine release in adult (Toyooka and Blake, 1961; Harrison and Seaton, 1965; Sylvestor et al., 1979) and fetal (Comline and Silver, 1961; Comline et al., 1965; Jones and Robinson, 1976; Robillard et al., 1981) experimental animals. The present study demonstrates that an increase in circulating catecholamines occurs in response to hypoxemia in uninhibited immature lambs as well. Responses of the adrenergic system are facilitated by angiotensin II in many experimental systems (Zimmerman, 1981). However, the present study suggests that angiotensin-converting enzyme activity is not required for the increase in circulating catecholamines in response to hypoxemia in the maturing lamb. Likewise, the urinary prostaglandins E and F, arterial AVP, and hematocrit responses to hypoxemia were similar to those of a previous study of maturing lambs (Weismann and Clarke, 1981), and do not appear to be significantly altered by converting enzyme inhibition with captopril.

Cortisol release in response to hypoxemia in uninhibited lambs of the current study is compatible with previous studies of anesthetized adult experimental animals (Marotta et al., 1963, 1965; Hirai et al., 1963; Marotta, 1972; Raff et al., 1981), and appears to be controlled by multiple mechanisms (Marotta, 1972; Raff et al., 1981). The mechanism by which captopril changes this cortisol response to hypoxemia is unknown. The differential response does not appear to be modulated by adrenal blood flow, as blood flow to the adrenal is increased in response to hypoxemia in immature lambs in a similar fashion, with or without captopril therapy. Captopril does not readily cross the blood brain barrier (Heel et al., 1980); thus, it is unlikely that the drug exerts a direct effect on hypothalamic function. Although alteration of baroreceptor reflexes has been reported with captopril (Hatton et al., 1981), the baroreceptor-mediated increase in vasopressin in response to hypoxemia (Anderson et
al., 1978) was intact in these lambs. It is also possible that captopril altered chemoreceptor responsiveness to hypoxemia, which may be a mechanism for glomerular filtration rate (Korner, 1963) as well as cortisol (Marotta, 1972) responses to hypoxemia. Alternatively, captopril may have decreased adrenocorticotropic hormone response to hypoxemia by preventing its stimulation by angiotensin II (Gann, 1979). Also, decreased adrenal responsiveness to adrenocorticotropic hormone or direct inhibition of cortisol synthesis may have occurred through inhibition of angiotensin II production. The mechanism of this response requires further study.

The magnitude of urinary prostaglandin E and F excretion rate responses to hypoxemia in the control lambs of the current study clearly were related to renal mass, as the correlation of the change in these variables with postnatal age was nonsignificant when corrected for kidney weight. The reason(s) for the relatively discordant responses of urinary prostaglandin E and F excretion rates to hypoxemia in the current study is unknown. There have been previous reports of increased renal venous prostaglandin E concentrations in response to renal arterial constriction (McGiff et al., 1970a), angiotensin II infusion (McGiff et al., 1970b; Needleman et al., 1973; Isakson et al., 1976), and bradykinin infusion (McGiff et al., 1972) associated with no change in renal venous prostaglandin F2α values. Others have also demonstrated discordant stimulation of prostaglandin E2 and prostaglandin F2α secretion rates in response to renal artery constriction (Dunn et al., 1978), with prostaglandin E2 secretion rate increased earlier than prostaglandin F2α under these conditions. Thus, the brevity of the urinary collection period during hypoxemia in the current study may have accentuated these differences in urinary prostaglandin E and F responses. Furthermore, Dunn et al. (1978) have speculated that rates of renal tubular secretion and reabsorption of these prostaglandins from the tubular lumen may be responsible for discordant prostaglandin E and F excretory rates in short-term experiments.

In summary, inhibition of angiotensin-converting enzyme with captopril in chronically catheterized lambs was associated with decreased baseline mean arterial pressure, decreased baseline urinary sodium excretion rate, and increased baseline arterial plasma renin activity, but did not alter renal hemodynamic and functional responses to hypoxemia. Furthermore, captopril did not alter the increase in arterial plasma renin activity, vasopressin, epinephrine, norepinephrine, and urinary prostaglandins in response to hypoxemia. However, captopril treatment blocked the increase in cortisol in response to hypoxemia. These data suggest that the products of angiotensin-converting enzyme activity are not important regulators of renal responses to hypoxemia, but may be involved in cortisol responses to normocapnic hypoxemia in immature lambs.

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Address for reprints: Douglas N. Weismann, M.D., Department of Pediatrics, University Hospital, Iowa City, Iowa 52242.

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D N Weismann, J E Herrig, O J McWeeny, N A Ayres and J E Robillard

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