Developmental Aspects of the Renal Responses to Hemorrhage during Converting-Enzyme Inhibition in Fetal Lambs

R. Ariel Gomez and Jean E. Robillard

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

SUMMARY. The role of the renin-angiotensin system in modulating the renal hemodynamic and functional responses to reductions of fetoplacental blood volume (8.8–35.5%) was studied in two groups of fetal lambs (<120 days and >130 days gestation; term 145 days) during infusion of either captopril (experimental fetuses) or dextrose 5% in water (control fetuses). At high hemorrhage levels (level III), renal blood flow decreased and renal vascular resistance increased significantly in both groups of fetuses (<120 days and >130 days), either treated or not treated with captopril. However, at low hemorrhage levels (levels I and II), and contrary to what was observed in young fetuses (<120 days), near-term fetuses (>130 days) receiving captopril showed neither significant decreases in renal blood flow nor increases in renal vascular resistance, whereas untreated fetuses of the same gestational ages demonstrated significant decreases in renal blood flow and increases in renal vascular resistance. It was found in both <120 day and >130 day fetuses that hemorrhage is associated with a decrease in urinary flow rate and free water clearance accompanied by an increase in urine osmolality and sodium reabsorption. It was shown that captopril does not modify this response. The present study also demonstrated that the blood pressure response to hemorrhage was characterized by a similar decrease in <120 day fetuses, whether treated or untreated with captopril. On the other hand, blood pressure did not change in control fetuses >130 days, but decreased slightly in captopril-treated fetuses during hemorrhage. Taken together, the present results tend to suggest that the renin-angiotensin system may be an important modulator of the renal hemodynamic response to low level hemorrhage as fetuses approach term, and may be more important in controlling blood pressure in near-term than in young fetuses. (Circ Res 54: 301–312, 1984)

THE renal hemodynamics and functional responses to sequential reductions of fetoplacental blood volume have been studied in chronically instrumented fetal lambs (Gomez et al., 1984). It was found that fetal hemorrhage is accompanied by a decrease in renal blood flow and an increase in renal vascular resistance not associated with significant changes in glomerular filtration rate. Moreover, it was observed that these changes correlate with rises in vasoactive substances and tend to be greater in near-term fetuses than in younger fetuses.

Previous investigations have also demonstrated that the renin-angiotensin-aldosterone system (RAAS) is stimulated during hemorrhage in fetal lambs, and that the degree of stimulation is age-dependent (Iwamoto and Rudolph, 1979, 1981; Robillard et al., 1982a; Gomez et al., 1984). Furthermore, it has been suggested that the RAAS may be more important in controlling cardiovascular homeostasis early during development than later in life (Mott, 1965, 1968, 1969). More recently, Iwamoto and coworkers (1981) demonstrated, using saralasin (a competitive antagonist of angiotensin II), that the RAAS is an important regulator of the cardiovascular response to hemorrhage in fetal sheep.

The role of the RAAS in modulating the renal hemodynamics and functional responses to hemorrhage has not been studied during fetal development. The present protocol was designed to determine the role of the RAAS in these responses by testing the hypothesis that the products of angiotensin-converting enzyme (ACE) activity may modulate renal hemodynamics and renal function during sequential fetoplacental blood volume depletion in chronically instrumented fetal lambs. Moreover, the role of angiotensin II (All) in modulating aldosterone secretion during fetal hemorrhage was investigated. Finally, the role of ACE in modulating urinary kaliurexion was determined.

Methods

Animal Preparation and Surgical Procedures

Pregnant sheep of Dorset and Suffolk mixed breeding were obtained from a local source, and the gestational age
was based on the induced ovulation technique (Jennings and Crowley, 1972) and calculated from the date of mating. Fetal weight at the time of the experiment was calculated as previously described (Robillard and Weitzman, 1980). Before surgery, the animals were fasted for 48 hours. In all fetuses, the chronic indwelling catheter preparation was used as described previously (Robillard and Weitzman, 1980; Robillard et al., 1981). Briefly, when the pregnant ewe was under general anesthesia (Robillard et al., 1981), catheters were inserted into the fetal femoral artery and vein and secured with silk ligatures. Through the same uterine incision, a 3/4 French feeding tube was introduced into the urachus and advanced into the fetal bladder. An additional catheter was secured in the amniotic cavity for intrauterine pressure recording. After surgery, the ewes were kept in a restricted area and fed a standard diet. All vascular catheters were flushed daily with heparin for the first 3 days and every other day henceforth. A recovery period of at least 6 days was required prior to performing experiments.

**Physiological Studies**

The renal response to sequential fetoplacental blood volume reduction was studied in two groups of chronically catheterized fetuses (<120 days and >130 days gestation; term 145 days) receiving a constant infusion of either captopril (5 μg/min per kg), a converting enzyme inhibitor (experimental fetuses) (SQ14225, Squibb), or dextrose 5% in water (0.09 ml/min) used as vehicle for the captopril infusion (control fetuses). Eighteen fetuses between 103 and 119 days gestation were studied; nine received captopril and nine received the vehicle only. Thirty fetuses between 132 and 144 days gestation were studied; six received captopril and seven received the vehicle only.

The infusion of captopril was started 60 minutes before the first urine collection period. In each experiment, a bolus infusion of angiotensin I (AI) was given intravenously to fetuses, both before—and 60 minutes after—captopril infusion was begun, to determine whether the amount of captopril was adequate to inhibit the conversion of AI to angiotensin II (All). Before the infusion of captopril, the administration of a bolus of AI increased arterial blood pressure by 23.6 ± 3.5% in fetuses <120 days, and by 26 ± 2.3% in fetuses >130 days. Sixty minutes after infusion of captopril was begun, a second bolus of AI increased arterial blood pressure by only 2.0 ± 0.8% in fetuses <120 days, and by 2.5 ± 1.4% in fetuses >130 days, thereby showing a significant inhibition (P < 0.001) in the conversion of AI to All during captopril infusion.

Before the fetoplacental blood volume was reduced, a 20-minute prehemorrhage urine collection period was performed in both experimental and control fetuses. After the prehemorrhage urine collection period, blood was withdrawn from the arterial catheter to produce, sequentially, three different levels of fetoplacental blood volume depletion. The fetal arterial blood was removed over 5 minutes with a Harvard infusion-pump. The percentages of fetoplacental blood volume removed for each level of hemorrhage are presented in Table 1. The total fetoplacental blood volume (135 ml/kg) was estimated from the data of Creasy et al. (1970). However, recent investigations published after the end of the present study have shown that the fetoplacental blood volume is probably closer to 110 ml/kg than to 135 ml/kg, in fetal lambs (Brace, 1983a).

<table>
<thead>
<tr>
<th>Percentage (%) of Fetoplacental Blood Volume Removed</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;120 days, control</td>
<td>9</td>
<td>8±0.5</td>
<td>17.7±1.6</td>
</tr>
<tr>
<td>&gt;130 days, control</td>
<td>7</td>
<td>9±0.5</td>
<td>18.8±0.9</td>
</tr>
<tr>
<td>&lt;120 days, captopril</td>
<td>10±2±8</td>
<td>20±2±1.7</td>
<td>35.5±3.0</td>
</tr>
<tr>
<td>&gt;130 days, captopril</td>
<td>6</td>
<td>8±1±1.0</td>
<td>18.2±2.1</td>
</tr>
<tr>
<td>F</td>
<td>0.55</td>
<td>0.50</td>
<td>0.49</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

n = number of animals; F = values for the F distribution. Values are mean ± SEM

Experimental urine collection periods (20 minutes each) were performed at each level of hemorrhage after a 15-minute period had been allowed for stabilization of blood pressure and heart rate after removal of the blood.

In all animals, the fetal glomerular filtration rate (GFR) was determined by a constant infusion (0.09 ml/min) of [125I]-sodium iothalamate (Glofil, Iso-tex Diagnostics, Inc.), as previously described (Robillard and Weitzman, 1980). An equilibration period of 1 hour was observed before the start of the first renal clearance period.

At the midpoint of each urine collection period, arterial blood was obtained for determination of plasma concentration of [125I]-sodium iothalamate, plasma electrolyte concentrations (Na+, K+, Cl-), plasma osmolality, hematocrit, total plasma proteins, arterial blood pH, and arterial blood gases (PO2 and Pco2). Arterial blood was also collected for determination of plasma renin activity (PRA), and of plasma concentrations of angiotensin II (All), aldosterone, arginine vasopressin (AVP), and catecholamines (epinephrine and norepinephrine). To avoid any hemodynamic effects of sampling, we replaced fetal blood samples with equal amounts of maternal blood during the first two periods and thereafter with fetal blood obtained during hemorrhage.

At the end of each urine collection period, urine was collected for determination of electrolytes (Na+, K+, Cl-), [125I]-sodium iothalamate, kallikrein concentrations, and urine osmolality.

We determined renal blood flow (RBF) and renal vascular resistance (RVR) at the end of the control urine collection period and after each experimental urine collection period by infusing approximately 2.0 × 106 radioactive (14Ce, 35Sc, 85Sr, or 99Nb) microspheres (15 ± 3 μm) (3M Co., Minn.), as previously described (Robillard and Weitzman, 1980). In all fetuses, blood pressure and fetal amniotic pressure were recorded continuously with a Statham P23Db pressure transducer (Statham Instruments Div., Gould Inc.) and a Beckman R-611 recorder. The fetal mean arterial blood pressure readings were corrected relative to concomitant amniotic pressures. Heart rate was monitored with a cardiotachometer triggered from the arterial pulse wave.

At the end of the experiment, the ewe and the fetus were killed with a lethal dose of sodium pentobarbital (Somethyl, Mid-Tech, Inc.).

**Analytical Methods**

Arterial pH, PaCO2, and PaO2 were determined at 39°C with a Radiometer PHM 72 MK2 acid-base analyzer (Radiometer Co.). The concentrations of sodium and potassium in blood and urine were measured with a flame
between two different populations of animals. Regression analysis was performed as described recently (Robillard et al., 1982).

The reliability of this assay in our laboratory have been determined by radioimmunoassay as described previously (Haber et al., 1969; Ito et al., 1972; Skowsky et al., 1974; Weitzman et al., 1978). Norepinephrine and epinephrine were determined by means of radioenzymatic assay (Catt, a-Kit, Upjohn Diagnostics), as described previously by Passon and Peuler (1973).

Blood samples for plasma PRA determinations were collected in chilled tubes containing 0.3 M EDTA and 0.025 M O-phenanthroline. Thereafter, the cells and proteins were immediately precipitated with acetone 65% and the supernatant was dried under air for subsequent chromatographic isolation of all on SP sephadex in sodium acetate buffer. Angiotensin II was then measured by radioimmunoassay (Catt et al., 1974). The technique and reliability of this assay in our laboratory have been described recently (Robillard et al., 1982).

Urine samples for kallikrein determinations were collected under ice, and NaCl 0.2% was used as a preservative. The specimens were stored at −70°C. Before assaying for kallikrein, urine samples were desalted on Sephadex G-25 columns. Urinary kallikrein was determined by a radiochemical chotographic assay utilizing the artificial substrate of N-(p-tosyl)-L-arginine[3H]methyl ester-hydrochloride ([H]TAME) (Amersham), as previously described (Robillard et al., 1982).

Gamma emissions generated from the microspheres were measured from both fetal kidneys and reference femoral arterial blood samples with a Beckman 300 γ spectrometer. Energy window ranges were set between 74 and 102 keV for 112 Ce, 210 and 275 keV for 85 Sr, 320 and 410 keV for 35 Nb, and between 420 and 580 keV for 35 Sc. Kidney and isotope separation was performed as described previously (Robillard and Weitzman, 1980).

Computations and Data Analysis

Renal blood flow (RBF), renal vascular resistance (RVR), filtration fraction (FF), osmolar clearance (Cosm), and free water clearance (C1w) were determined as described previously (Robillard and Weitzman, 1980; Robillard et al., 1981).

Statistical analysis of the data within any given population of animals was performed by using Duncan's test and analysis of variance for randomized block design (Ott, 1977). The unpaired t-test was used to compare the means between two different populations of animals. Regression lines and associated correlation coefficients were computed by the least squares formula. The term 'significant' is used throughout the paper to describe changes with a total P value of less than 0.05 in a two-sided significance limit. The results are presented as mean ± se.

Results

Effect of Hemorrhage on Fetal Arterial Values (Table 2, a and b)

During hemorrhage, arterial pH, total protein concentrations, and hematocrit decreased significantly in both control and captopril-treated groups of fetuses of either <120 days or >130 days gestation. No difference was found when the percent changes for each of the above parameters were compared between groups.

Arterial PO2 and PCO2 did not change in the control or in the captopril-treated fetuses >130 days gestation. However, in fetuses <120 days gestation, PCO2 increased in both control and captopril-treated fetuses. The percent changes in PCO2 at each level of fetal hemorrhage were not different when treated or untreated groups were compared; the values were respectively 4.99 ± 2.3% and 3.87 ± 1.5%. Similarly, at the highest level of hemorrhage (level III), arterial PO2 increased slightly but significantly in fetuses <120 days gestation. The rise was similar in both control and captopril-treated fetuses.

Small but significant and similar changes in plasma potassium concentrations were observed in both groups of fetuses (<120 days and >130 days gestation) during captopril infusion. Also, a small but significant rise in plasma osmolality was seen in both control and captopril-treated fetuses >130 days gestation.

Effect of Hemorrhage on Fetal Vasoactive Substances: (Figs. 1 and 2)

During the prehemorrhagic period (Pre-H), no differences were found when the plasma levels of angiotensin II (AI), aldosterone, arginine vasopressin (AVP), epinephrine, and norepinephrine were compared between control and captopril-treated fetuses of either <120 days or >130 days gestation. However, inhibition of angiotensin-converting enzyme (ACE) increased plasma renin activity (PRA) values significantly (P < 0.05) in both groups of fetuses <120 days and >130 days, compared to the control group.

During hemorrhage significant rises in PRA were observed in both control and captopril-treated fetuses (Fig. 1). Although the PRA values were higher in both groups (<120 days and >130 days) of captopril-treated fetuses than in control fetuses, the rise in PRA, expressed as percent changes from Pre-H values, was similar when control fetuses <120 days or >130 days were compared to captopril-treated fetuses of similar age. However, the mean PRA values at the peak of fetal hemorrhage (level III) were significantly higher in >130 day than in <120 day control fetuses.

All increased significantly in control fetuses (<120 days and >130 days) (P < 0.05) during hemorrhage, whereas no changes were observed in captopril-treated fetuses (Fig. 1). Furthermore, the mean
TABLE 2
Arterial Blood Values for Different Levels of Hemorrhage in Fetuses less than 120 days gestation

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 9)</th>
<th>Captopril (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-H</td>
<td>Level 1</td>
</tr>
<tr>
<td>pH</td>
<td>7.38±0.01</td>
<td>7.37±0.01</td>
</tr>
<tr>
<td>PCO₂ (mm Hg)</td>
<td>48±1</td>
<td>47±1</td>
</tr>
<tr>
<td>PO₂ (mm Hg)</td>
<td>23±1</td>
<td>26±1*</td>
</tr>
<tr>
<td>Na⁺ (mEq/liter)</td>
<td>143±1</td>
<td>143±1</td>
</tr>
<tr>
<td>K⁺ (mEq/liter)</td>
<td>3.7±0.1</td>
<td>3.7±0.1</td>
</tr>
<tr>
<td>Cl⁻ (mEq/liter)</td>
<td>105±1</td>
<td>105±1</td>
</tr>
<tr>
<td>Osmolality (mOsm/kg H₂O)</td>
<td>287±2</td>
<td>289±2</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>33±1</td>
<td>31±1*</td>
</tr>
<tr>
<td>Total proteins (g/100 ml)</td>
<td>3.3±0.1</td>
<td>3.2±0.1</td>
</tr>
</tbody>
</table>

* P < 0.05 when values during hemorrhage are compared to prehemorrhage (Pre-H) values. Percentages of blood volume removed

plasma. All concentration at the peak of hemorrhage (level III) was greater in control fetuses >130 days gestation (145 ± 44 pg/ml) than in control fetuses <120 days gestation (59 ± 6 pg/ml) (P < 0.05).

Plasma aldosterone concentration increased significantly (P < 0.05) during hemorrhage in fetuses <120 days and >130 days gestation, either treated or untreated with captopril (Fig. 1). However, no differences were found when either the aldosterone concentrations or the percent changes in aldosterone
Table 2 (cont'd)

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 7)</th>
<th>Captopril (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-H</td>
<td>Level 1</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>7.37±0.01</td>
<td>7.38±0.01</td>
</tr>
<tr>
<td><strong>PCO₂</strong> (mm Hg)</td>
<td>49±2</td>
<td>47±2</td>
</tr>
<tr>
<td><strong>PO₂</strong> (mm Hg)</td>
<td>25±2</td>
<td>25±1</td>
</tr>
<tr>
<td><strong>Na⁺</strong> (mEq/liter)</td>
<td>145±1</td>
<td>145±1</td>
</tr>
<tr>
<td><strong>K⁺</strong> (mEq/liter)</td>
<td>4.3±0.1</td>
<td>4.3±0.1</td>
</tr>
<tr>
<td><strong>Cl⁻</strong> (mEq/liter)</td>
<td>102±1</td>
<td>103±1</td>
</tr>
<tr>
<td><strong>Osmolality</strong> (mOsm/kg H₂O)</td>
<td>280±2</td>
<td>282±1</td>
</tr>
<tr>
<td><strong>Hematocrit</strong> (%)</td>
<td>37±3</td>
<td>36±2</td>
</tr>
<tr>
<td><strong>Total proteins</strong> (g/100 ml)</td>
<td>3.5±0.1</td>
<td>3.5±0.1</td>
</tr>
</tbody>
</table>

For each level (levels 1 to 3) are presented in Table 1. n = number of animals. Values are means ± SEM.

concentrations for each level of hemorrhage were compared between young and old fetuses treated with captopril, or when control fetuses were compared to captopril-treated fetuses of comparable age.

Strong correlations between percent changes in plasma potassium concentrations and percent changes in plasma aldosterone concentrations were found in <120 days (r = 0.53, P < 0.05) and >130 days (r = 0.91, P < 0.05) fetuses treated with captopril, whereas no correlation was found in control, untreated fetuses. Moreover, in fetuses treated with captopril, the slope of the regression lines between percent changes in potassium and percent changes in aldosterone was significantly steeper (P < 0.005) in >130 days (9.6) than in <120 days (3.5) fetuses.

Plasma AVP increased significantly (P < 0.05) in both groups (<120 days and >130 days) of control and captopril-treated fetuses during hemorrhage (Fig. 2). The rise in mean plasma AVP values during hemorrhage, expressed as percent changes from pre-hemorrhage values, was similar in both control and captopril-treated fetuses (<120 days and >130 days).

Plasma norepinephrine (NE) and epinephrine (E) concentrations increased (P < 0.05) in fetuses <120 days gestation and in all fetuses >130 days gestation either treated or untreated with captopril during hemorrhage (Fig. 2). In fetuses >130 days gestation, no differences were found when either the mean plasma values or the percent changes in NE or E concentrations were compared between treated and untreated groups. In fetuses <120 days gestation, the mean values for plasma NE and E concentrations at each level of hemorrhage were significantly (P < 0.05) higher in the captopril-treated fetuses than in the control fetuses; however, when the rise in NE and E concentrations were expressed as percent changes, no significant differences were observed between control and captopril-treated fetuses.

**Effect of Hemorrhage on Fetal Renal Hemodynamics**

During hemorrhage, renal blood flow (RBF) decreased and renal vascular resistance (RVR) increased significantly (P < 0.05) in a similar fashion in both groups of fetuses (<120 days and >130 days gestation), either treated or untreated with captopril (Fig. 3).

Moreover, no significant changes in filtration fraction (FF) were found in either control or captopril-treated fetuses <120 days and >130 days gestation during hemorrhage. In the older group of fetuses (>130 days gestation) untreated with captopril, FF tended to increase during hemorrhage (from 11.4 ± 1.8% to 21.2 ± 3.5%), but the difference between
the means was not statistically significant. However, when we examine closely the data during the first level of hemorrhage (level 1) in fetuses >130 days gestation, we found increases in RVR (11 ± 8%) and FF (8 ± 5.5%) in control fetuses and decreases in RVR (-2.6 ± 9%) and FF (-19 ± 9%) in captopril-treated fetuses. These changes were found to be statistically significant (P < 0.05) when control and captopril-treated fetuses were compared.

The blood pressure response to hemorrhage (Fig. 4) was characterized by a similar decrease in fetuses <120 days gestation whether treated with captopril or not. These findings were associated with a decrease in heart rate in the captopril-treated group of fetuses, and no changes in the control fetuses. On the other hand, blood pressure did not vary significantly in control fetuses >130 days gestation, but decreased slightly (12%) in captopril-treated fetuses during hemorrhage. These results were associated with a significant increase in heart rate in untreated fetuses and no change in captopril-treated fetuses.

Effects of Hemorrhage on Fetal Renal Function

The effects of hemorrhage on fetal renal function in control and captopril-treated groups are presented in Table 3, a and b. No significant changes in GFR...
Gomez and Robillard/Captopril and Fetal Renal Response to Hemorrhage

were observed during hemorrhage in any of the groups of fetuses; however, GFR tended to decrease in both control and captopril-treated fetuses <120 days and >130 days gestation. Significant (P < 0.05) decreases in urinary flow rate (V) and free-water clearance (C\text{H}_2O) and increases in urine osmolality (Uosm) were observed in all groups of fetuses during hemorrhage. Osmolar clearance (Cosm) decreased significantly (P < 0.05) in fetuses >130 days gestation, whether treated with captopril or not. In fetuses <120 days gestation, Cosm decreased in 5 out of 6 control fetuses and in 5 out of 7 captopril-treated fetuses at levels II and III of hemorrhage.

Fractional sodium excretion (\text{FE}_{\text{Na}^+}) decreased significantly (P < 0.05) in all groups of fetuses. This was associated with parallel decreases in urinary excretion of sodium (U_{\text{Na}^+}-V), fractional excretion of chloride (\text{FE}_{\text{Cl}^-}), and urinary excretion of chloride (U_{\text{Cl}^-}-V). Potassium excretion did not change significantly in any of the groups studied. No differences were found when percent changes for the above parameters were compared between treated and untreated fetuses.

Urinary kallikrein excretion rate was also determined in both control and captopril-treated fetuses (<120 days and >130 days gestation). Urinary kallikrein excretion rate, expressed in milli-esterase units (mEU) per hour, did not change during hemorrhage in either control or captopril-treated fetuses. Urinary kallikrein levels were, respectively, 55.1 ± 17.1 and 83.9 ± 23.9 mEU/hr in control and captopril-treated fetuses <120 days before hemorrhage and 53.8 ± 22 and 88.3 ± 57 mEU/hr at the peak of fetal hemorrhage. In fetuses >130 days, urinary kallikrein levels were, respectively, 421 ± 213 and 271 ± 102 mEU/hr in control and captopril-treated fetuses before hemorrhage and 356 ± 210 and 183 ± 115 mEU/hr, respectively, at the peak of fetal hemorrhage.

Discussion

Effect of Angiotensin-Converting Enzyme Inhibition on Vasoactive Substances and Blood Pressure during Hemorrhage

The present study demonstrates that the PRA and plasma All responses to fetoplacental blood volume depletion are significantly greater in near-term fetuses (>130 days) than in younger fetuses (<120 days). These findings suggest that the level of stimulation of the renin-angiotensin system following fetal hemorrhage is age-dependent during the last trimester of gestation and confirms previous results (Robillard et al., 1982a; Gomez et al., 1984). One may speculate that factors such as anatomical and physiological immaturity of the juxtaglomerular apparatus, combined with a decrease in angiotensin converting enzyme (Wallace et al., 1978; Stalcup et al., 1978), may explain, at least partially, the lesser response of the renin-angiotensin system to hemorrhage in young fetuses.

It is also demonstrated that PRA levels were significantly higher in captopril-treated fetuses, supporting previous findings that All is an important modulator of renin secretion during fetal life (Iwamoto and Rudolph, 1979; Robillard et al., 1983). Moreover, the further rise in PRA in captopril-treated fetuses during hemorrhage demonstrates that, in spite of the inhibition of the All feedback mechanism, renin secretion did not reach a maximal stimulation level and that the juxtaglomerular apparatus can be further stimulated. Similar findings have been observed recently during ACE inhibition in newborn lambs submitted to hypoxemia (Weismann et al., 1983).

Since potentiation of vasopressin (AVP) secretion and sympathetic and adrenal catecholamine releases have been found to be associated with captopril
Circulation Research / Vol. 54, No. 3, March 1983

TABLE 3

Effect of Hemorrhage on Renal Function in Fetuses less than 120 days gestation

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>Captopril (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-H</td>
<td>Level 1</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>2.57±0.41</td>
<td>2.11±0.47</td>
</tr>
<tr>
<td>V (ml/min)</td>
<td>0.66±0.15</td>
<td>0.29±0.08*</td>
</tr>
<tr>
<td>Uosm (mOsm/kg H2O)</td>
<td>111±10</td>
<td>188±32*</td>
</tr>
<tr>
<td>Co(s) (ml/min)</td>
<td>0.26±0.07</td>
<td>0.18±0.04</td>
</tr>
<tr>
<td>C_H2O (ml/min)</td>
<td>0.40±0.08</td>
<td>0.11±0.05*</td>
</tr>
<tr>
<td>U_LV (µEq/min)</td>
<td>30.1±8.7</td>
<td>16.3±4.5*</td>
</tr>
<tr>
<td>FE_LV (%)</td>
<td>7.9±1.8</td>
<td>5.4±1.1*</td>
</tr>
<tr>
<td>U Fet (µEq/min)</td>
<td>16.5±7.0</td>
<td>10.0±3.7</td>
</tr>
<tr>
<td>FE Fet (%)</td>
<td>5.8±2.2</td>
<td>4.3±1.3</td>
</tr>
<tr>
<td>U_LV (µEq/min)</td>
<td>2.2±0.9</td>
<td>1.5±0.7</td>
</tr>
<tr>
<td>FE Fet (%)</td>
<td>19.3±5.1</td>
<td>37.8±21.4</td>
</tr>
</tbody>
</table>

* P < 0.05 when values during hemorrhage are compared to prehemorrhage (Pre-H) values. Percentages of fetoplacental blood volume urinary osmolality; Co(s), osmolar clearance; C_H2O, free water clearance; UV, urinary excretion of electrolytes; FE, fractional excretion of

therapy in adult animals (Zerbe et al., 1980), we looked at possible changes in these humoral and neurogenic responses during fetal hemorrhage. First, plasma AVP and plasma catecholamine concentrations increased in both control and captopril-treated fetuses, suggesting that ANP is not essential for the vasopressin and catecholamine responses to fetal hemorrhage as previously observed in adult animals (Zerbe et al., 1980; Morton et al., 1977). Moreover, the rise in plasma AVP in captopril-treated fetuses (<120 days and >130 days of gestation) is of special interest in view of the suggestion that ANP has a stimulating effect on vasopressin secretion in immature animals (Siegel et al., 1979). However, since captopril, which may cross the blood-brain barrier in fetal lambs (Broughton-Pipkin et al., 1982), is also known to inhibit enkephalinase activity and opioid peptides degradation (Swerts et al., 1979), one may speculate that a rise in α-endorphin may contribute to the rise in vasopressin secretion following ACE inhibition as previously suggested in adults (Weitzman et al., 1977). Second, the rise in plasma AVP during hemorrhage in fetuses <120 days gestation tends to be of greater magnitude in captopril-treated than in control fetuses. Only one fetus receiving captopril had a plasma AVP concentration lower than the highest plasma AVP value seen in control fetuses at the highest level of hemorrhage (level III). Similarly, the catecholamine response to hemorrhage in fetuses <120 days gestation was significantly greater in captopril-treated than in control fetuses. The potentiation of vasopressin and catecholamine secretion in captopril-treated fetuses <120 days gestation is difficult to explain. One may speculate that the persistent hypotension associated with ACE inhibition in fetuses <120 days may have contributed to these changes. Alternatively, possible stimulation of the prostaglandin system following ACE inhibition (Brunner and Gavras, 1980), may have potentiated both vasopressin (Casals-Stenzel and Morton, 1979) and catecholamine (Feuerstein et al., 1981) releases. However, further studies will need to be done since the present protocol was not designed to study this aspect.
Table 3 (cont’d)

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>Captopril (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-H</td>
<td>Level 1</td>
</tr>
<tr>
<td><strong>GFR</strong> (ml/min)</td>
<td>4.78±0.47</td>
<td>4.09±0.45</td>
</tr>
<tr>
<td><strong>V</strong> (ml/min)</td>
<td>1.07±0.16</td>
<td>0.50±0.14*</td>
</tr>
<tr>
<td><strong>Uosm</strong> (mOsm/kg H2O)</td>
<td>142±29</td>
<td>277±52*</td>
</tr>
<tr>
<td><strong>Cosm</strong> (ml/min)</td>
<td>0.51±0.05</td>
<td>0.32±0.04*</td>
</tr>
<tr>
<td><strong>C15O2</strong> (ml/min)</td>
<td>0.58±0.17</td>
<td>0.12±0.10*</td>
</tr>
<tr>
<td><strong>Ua-V</strong> (μEq/min)</td>
<td>46.2±12.9</td>
<td>24.8±6.3*</td>
</tr>
<tr>
<td><strong>FeNa+</strong> (%)</td>
<td>7.4±2.7</td>
<td>4.3±1.1*</td>
</tr>
<tr>
<td><strong>Uc-V</strong> (μEq/min)</td>
<td>22.6±5.4</td>
<td>12.9±3.8</td>
</tr>
<tr>
<td><strong>FeCl-</strong> (%)</td>
<td>4.8±1.4</td>
<td>2.9±0.9</td>
</tr>
<tr>
<td><strong>Ua-V</strong> (μEq/min)</td>
<td>10.8±4.0</td>
<td>10.2±2.8</td>
</tr>
<tr>
<td><strong>FeK+</strong> (%)</td>
<td>47.2±14</td>
<td>54.9±14</td>
</tr>
</tbody>
</table>

 removed for each level (levels 1 to 3) of hemorrhage are presented in Table 1. GFR, glomerular filtration rate; V, urinary flow rate; Uosm, electrolytes, n, number of animals. Values are means ± SEM.

Effect of Angiotensin-Converting Enzyme Inhibition on Renal Hemodynamics, Renal Function, and Arterial Blood Pressure during Hemorrhage

It has been suggested that the renin-angiotensin system plays an important role in the response to hemorrhage in fetal lambs (Iwamoto and Rudolph, 1981; Mattioli et al., 1979). Iwamoto and Rudolph (1981) demonstrated that saralasin infusion during fetal hemorrhage resulted in a greater decrease in renal blood flow (RBF) and increase in renal vascular resistance (RVR) than hemorrhage alone. However, these studies (Iwamoto and Rudolph, 1981) did not determine whether the role of the renin-angiotensin system in controlling renal circulation was dependent on fetal age and varied with different levels of hemorrhage.

The present results demonstrate that the renal hemodynamic response to hemorrhage during ACE inhibition is different between young (<120 days) and near-term fetuses (>130 days gestation) (Fig. 3). Inhibition of ACE in fetuses >130 days gestation does not produce significant decreases in RBF nor increases in RVR at low levels of hemorrhage, contrary to results observed in control fetuses. Moreover, filtration fraction (FF) tends to decrease in near-term fetuses treated with captopril. Taken together, the present results tend to suggest that the renin-angiotensin system is an important modulator of the renal hemodynamic response to low-level hemorrhage in near-term fetuses.

However, since captopril potentiates bradykinin vasodilator activity by inhibiting kininase II, it is conceivable that the renal hemodynamic effects of captopril during hemorrhage in near-term fetuses might be secondary to direct or indirect effects of bradykinin potentiation. The present study was not designed to investigate these factors. However, the
absence of significant changes in urinary kallikrein excretion during ACE inhibition, before (Robillard et al., 1983) and during hemorrhage, suggests that this system is not involved in this response. Similar results have also been observed in adult animals (Wong and Zimmerman, 1980; Golub et al., 1981).

Finally, contrary to what we observed in near-term fetuses, the present study does not show any significant effect of captopril on renal hemodynamics during hemorrhage in young fetuses (<120 days). There is no clear explanation for this difference, but it can be speculated that the renal vasculature in young fetuses is less sensitive to ALL, as previously suggested (Robillard, 1983). Moreover, concentration of renal ACE and/or intrarenal formation of ALL may be low early in gestation (Wigger and Stalcup, 1978) and may increase as the kidney matures. Furthermore, since plasma ALL levels are significantly lower in young than in near-term fetuses before and during hemorrhage, one may speculate that the tonic effect of ALL is minimal early in gestation.

The renal function response to ACE inhibition during hemorrhage was also studied (Table 3, a and b). The present results demonstrate in both young and near-term fetuses that hemorrhage is associated with a decrease in urinary flow rate and free water clearance accompanied by an increase in urine osmolality and sodium reabsorption and confirms previous results (Gomez et al., 1984). It is also shown that ACE inhibition does not modify this response. Taken together, it is demonstrated that products of ACE activity are not important in modulating the increase in urine osmolality and the decrease in sodium and chloride excretion associated with hemorrhage. It has been previously suggested that vasopressin is an important mediator of the antidiuresis observed during fetal hemorrhage (Gomez et al., 1984) and that changes in renal hemodynamics associated with hemorrhage are probably the main contributors in controlling sodium and chloride excretion.

The present study also demonstrates that hemorrhage is associated with a significant decrease in arterial blood pressure in control fetuses <120 days gestation, whereas no changes are observed in control fetuses >130 days gestation (Fig. 4). Administration of the ACE inhibitor, captopril, tends to decrease baseline blood pressure in both groups of fetuses (<120 days and >130 days gestation), as previously shown (Robillard et al., 1983). However, in fetuses >130 days gestation, the blood pressure response to hemorrhage during ACE inhibition was different than in control fetuses of similar gestational age; blood pressure decreased in captopril-treated fetuses, whereas no changes were seen in control fetuses. On the other hand, the decline in blood pressure seen in control fetuses <120 days gestation was similar during ACE inhibition. Taken together, the present results suggest that the renin-angiotensin system may have a more important role in controlling blood pressure in near-term (>130 days) than in young (<120 days) fetuses.

Factors other than the renin-angiotensin system may have also contributed to this response. First, one may suggest that a decline in bradykinin degradation secondary to ACE inhibition may have modulated the blood pressure response to hemorrhage. However, this is unlikely; a previous study by our group has shown that ACE inhibition is not associated with a hypotensive response in nephrectomized fetuses (Robillard et al., 1983). We have also demonstrated that bilateral nephrectomy in fetuses >130 days gestation is associated with a decrease in blood pressure during hemorrhage (Robillard et al., 1982a). But, since plasma bradykinin was not determined in the present study, a possible contribution of this peptide to the hypotensive effect observed during hemorrhage in fetuses >130 days receiving captopril cannot be ruled out entirely.

Second, the observation that the tachycardia seen during hemorrhage in control fetuses >130 days was abolished following ACE inhibition suggests that these changes in heart rate may have contributed to the decrease in blood pressure in this group of fetuses. Interestingly, this observation also suggests that angiotensin may be responsible for the maintenance of heart rate during hemorrhage. Previous studies by Iwamoto and Rudolph (1981) have shown that administration of saralasin during fetal-placental blood volume depletion produced fetal bradycardia, supporting the present results. Mechanisms explaining these changes were not investigated in the present study. However, previous findings in fetal (Ismay et al., 1979) and adult animals (Lumbers et al., 1979; Hatton et al., 1981) tend to suggest that ALL reduces vagal tone and that inhibition of ALL activity enhances vagal influence over the heart and interferes with baroreflex function.

Finally, one may suggest that the rate of restitution of blood volume following hemorrhage, which is secondary to the absorption of fluids from the interstitial space (Brace, 1983b; Robillard et al., 1979), varies with gestation and may account at least partially for the gestation-related differences in blood pressure response. However, since the percent changes in hematocrit and total protein were not different between young and near-term fetuses, it is unlikely that this mechanism governing fluid transfer into the fetal circulation differs during the last trimester of gestation.

Influence of Angiotensin-Converting Enzyme Inhibition on Aldosterone Secretion During Hemorrhage

Previous studies by our group have shown that plasma aldosterone concentration increases significantly following fetal blood volume reduction, and that this rise correlates closely with the increase in plasma ALL levels (Robillard et al., 1982a). The present study confirms these results. Furthermore, the present study demonstrates that inhibition of ACE did not blunt the rise in plasma aldosterone secretion associated with hemorrhage. These results suggest that the integrity of the renin-angiotensin system is
not an important component in the fetal adrenal response to hemorrhage but that factors other than AII are modulating this response.

Factors regulating the fetal adrenal response to hemorrhage during ACE inhibition were not investigated in the present study. However, a significant correlation between changes in plasma aldosterone concentration and changes in plasma potassium (K⁺) concentration is described, suggesting that K⁺ is an important factor modulating this response. In this regard, there is evidence that K⁺ can stimulate aldosterone secretion in absence of the renin-angiotensin system in anephric man (Cooke et al., 1973) and in nephrectomized and decapitated animals (McCaa et al., 1973). However, it should be remembered that the regulation of aldosterone biosynthesis is complex and multifactorial, and that factors such as ACTH (Edwards et al., 1982; Rose et al., 1978), sympathetic action of potassium with ACTH (Fredlund et al., 1977), and indoleamines (Edwards et al., 1982) may have contributed also to the rise in plasma aldosterone concentration during fetal hemorrhage.

In summary: (1) the present study demonstrates that there is a difference between the renal hemodynamic responses to hemorrhage during ACE in young and near-term fetuses. It is suggested that the renin-angiotensin system is more important in modulating renal blood flow in near-term than in young fetuses during low-level hemorrhage. (2) It is demonstrated that products of ACE activity are not important in modulating the increase in Uosm and the decrease in sodium and chloride excretion associated with hemorrhage. (3) It is suggested that the renin-angiotensin system may have a more important role in controlling blood pressure and heart rate in near-term than in young fetuses. (4) The present study shows that inhibition of ACE does not blunt the rise in plasma aldosterone secretion associated with hemorrhage, suggesting that the renin-angiotensin system is not an important component in the fetal adrenal response to hemorrhage.

The present work was supported by U.S. Public Health Service Grants HD-11466 and HL-14388 and American Heart Association Grant 78-809. R. Arsel Gomez is supported by a fellowship from the Iowa Kidney Foundation. Jean E. Robillard is the recipient of a Research Career Development Award.

Address for reprints: Jean E. Robillard, M.D., Professor, Department of Pediatrics, University of Iowa College of Medicine, 225 Med Labs, Iowa City, Iowa 52242

Received August 22, 1983; accepted for publication January 19, 1984.

References


Ismay MJA, Lumbers ER, Stevens AD (1979) The action of angiotensin II on the baroreflex response in the conscious ewe and the conscious fetus J Physiol (Lond) 288: 467-479

Ito T, WOo J, Haning R, Horton R (1972) A radioimmunoassay for aldosterone in human peripheral plasma including a comparison of alternate techniques J Clin Endocrinol Metab 34: 106-112


Lumbers ER, McCloskey DI, Potter EK (1979) Inhibition by angiotensin II of baroreceptor-evoked activity in cardiac vagal efferent nerves in the dog. J Physiol (Lond) 294: 69-80


Mott JC (1965) Haemorrhage as a test of the function of the cardiovascular system in rabbits of different ages. J Physiol (Lond) 181: 728-752


INDEX TERMS: Renal blood flow • Angiotensin II • Aldosterone • Vasopressin • Catecholamines • Kallikrein • Blood pressure
Developmental aspects of the renal responses to hemorrhage during converting-enzyme inhibition in fetal lambs.
R A Gomez and J E Robillard

Circ Res. 1984;54:301-312
doi: 10.1161/01.RES.54.3.301

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/54/3/301

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/