Role of Angiotensin II on the Adrenal and Vascular Responses to Hemorrhage during Development in Fetal Lambs

Jean E. Robillard, R. Ariel Gomez, John G. Meernik, William D. Kuehl, and Dianna VanOrden

With the technical assistance of Ellen VanBell, Bridget Consamus, Robert Schmidt, and Kevin Taylor

From the Departments of Pediatrics and Obstetrics/Gynecology, and the Cardiovascular Research Center, University of Iowa, Iowa City, Iowa

SUMMARY. Developmental aspects of fetal adrenal and vascular responses to endogenous increase in plasma angiotensin II (All) following sequential reductions of fetoplacental blood volume were studied in two groups of chronically catheterized fetal lambs (seven were <120 days and seven were >130 days of gestation, term being 145 days). At similar levels of hemorrhage, the rise in plasma renin activity (PRA) was found to be greater in fetuses >130 days than in those <120 days (P < 0.025). Similarly, the effect of hemorrhage on plasma All was more pronounced in fetuses >130 days than in those <120 days (P < 0.05). No changes in plasma aldosterone were seen during hemorrhage in fetuses <120 days, whereas plasma aldosterone increased (P < 0.001) in those >130 days. This increase correlated with the rise in plasma All (r = 0.70, P < 0.001). In order to determine whether factors other than the rise in plasma All were responsible for the increase in plasma aldosterone in fetuses >130 days, these results were compared to results obtained in four nephrectomized fetuses >130 days submitted to similar degrees of hemorrhage. No changes in PRA or plasma All were observed. However, a small increase in plasma aldosterone (from 31 ± 13 to 47 ± 11 pg/ml, P < 0.01) was found, and this correlated with changes in plasma potassium concentration (r = 0.50, P < 0.05). Finally, mean arterial blood pressure decreased during hemorrhage in fetuses <120 days (P < 0.05), whereas no changes were observed in those >130 days unless their kidneys were removed. This suggests that the renin-angiotensin system is an important modulator of fetal blood pressure during hemorrhagic stress. (Circ Res 50: 645-650, 1982)

THE activity of the fetal renin-angiotensin system has been found to be elevated during the last trimester of gestation, compared to adult values (Mott, 1979). Furthermore, it has been demonstrated that the fetal renin-angiotensin system responds to stimulation in a manner similar to the adult system (Lumbers and Lewes, 1979; Robillard et al., 1979, 1981; Siegel and Fisher, 1980a).

However, the ability of angiotensin II (All) to stimulate aldosterone secretion during fetal life has been questioned (Alexander et al., 1968: Siegel and Fisher, 1980a). Alexander et al. (1968), using acute fetal sheep preparations, and Siegel and Fisher (1980a), in chronically catheterized fetal lambs, were unable to demonstrate a rise in fetal plasma aldosterone concentration after either exogenous All infusion or furosemide stimulation of the renin-angiotensin system. However, we demonstrated that there is a close relationship between fetal plasma renin activity (PRA) and plasma aldosterone concentration, suggesting that aldosterone secretion is under the influence of the renin-angiotensin system during fetal life (Robillard et al., 1980). More recently, we also demonstrated that infusion of exogenous All stimulates aldosterone secretion in the fetus but to a lesser degree than in adult ewes (Robillard et al., 1982).

The present protocol was designed to study developmental aspects of the fetal adrenal response to endogenous increases in plasma All following hemorrhage. Furthermore, in order to determine whether factors other than rising plasma All levels might be responsible for the increase in plasma aldosterone concentration, the effects of hemorrhage were studied in binephrectomized fetuses. Finally, blood pressure responses to sequential fetoplacental blood volume reductions were evaluated in groups of fetal lambs of different gestational ages.

Methods

Animal Preparation and Surgical Procedures

Pregnant sheep of Dorset and Suffolk mixed breeding were obtained from a local source and the gestational age based on the induced ovulation technique (Jennings and Crowley, 1972). Prior to surgery, the animals were fasted for 48 hours. Anesthesia of the ewe and surgery of the fetus were performed as described previously (Robillard et al., 1980, 1981). Following surgery, a recovery period of at least 6 days was required prior to performing experiments.

Physiological Studies

Two groups of chronically catheterized fetal lambs were studied. In the first group (n = 7), studies were performed...
between 103 and 119 days of gestation. The second group (n = 7) was studied between 132 and 144 days of gestation (term being 145 days).

In all fetuses, blood pressure and amniotic pressure were recorded continuously with Statham P23Db pressure transducers (Statham Instruments Div., Gould Inc.) and a Beckman R-611 recorder. The mean arterial blood pressure readings were corrected relative to concomitant amniotic pressures. Heart rate was monitored with a cardiocapnometer triggered from the arterial pressure pulse wave. Following a 45-minute stabilization period, a control arterial blood sample was withdrawn for determination of pH, blood gases (Pco2 and Po2), plasma electrolytes (Na+, K+, Cl−), plasma osmolality and hematocrit and for assay of plasma renin activity (PRA), angiotensin II (All), and aldosterone. Thereafter, blood was gradually withdrawn from the arterial catheter to sequentially produce three different levels of fetoplacental blood volume depletion—5 to 10%, 15 to 20%, and 30%. The unpaired t test was used to compare the means ml/kg) was estimated from the data of Creasy et al. (1970). Following each level of hemorrhage, a 15-minute period was allowed for stabilization of blood pressure and heart rate before taking any blood samples.

In another four chronically catheterized fetuses (131-141 days gestation), bilateral nephrectomies were performed in order to determine the effect of similar sequential fetoplacental blood volume reductions on fetal aldosterone secretion in the absence of fetal All stimulation.

**Analitical Methods**

Blood for pH, Pco2, and Po2 was collected anaerobically in heparinized glass syringes, and measurements were immediately determined with the appropriate pH, Pco2 and Po2 electrode at 39°C with a Radiometer PHM 72 MK2 acid-base analyzer (Radiometer Co.). Plasma electrolyte (Na+, K+, Cl−) concentrations and plasma osmolality were determined as previously described (Robillard et al., 1980). PRA and plasma aldosterone concentrations were determined by radioimmunoassays as described previously (Haber et al., 1969; Ito et al., 1972; Robillard et al., 1980).

Plasma All concentrations were determined as described previously (Robillard et al., 1982) using the method of Catt et al. (1974) and Cain et al. (1972). Cross-reactivity of the All antiserum based on All as 100% reactive is 130% for the heptapeptide, 156% for the hexapeptide, 103% for the pentapeptide, and less than 3% for angiotensin I.

**Statistical Analysis**

Statistical analysis of the data within any given population of animals was performed by using Student’s paired t-test and analysis of variance. When multiple comparisons were done on the same group of data, the critical value of t was corrected using the Bonferroni method (Wallenstein et al., 1980). 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**

Three different levels of hemorrhage were studied in fetal lambs <120 days and >130 days gestation. The percentages of fetoplacental blood volume removed for each level of hemorrhage are presented in Table 1. The degree of volume depletion was slightly higher in fetuses >130 days gestation than in younger fetuses.

Effects on arterial blood values in fetuses <120 days and in those >130 days of gestation are presented in Table 2. With maximal (level III) hemorrhage, arterial pH decreased in five of seven fetuses <120 days and in six of seven fetuses >130 days gestation. Arterial Pco2 increased in fetuses <120 days, whereas no changes were observed in those >130 days of gestation. During hemorrhage, plasma osmolality did not vary in fetuses <120 days gestation, but a consistent rise in plasma osmolality was observed in all fetuses >130 days gestation. There were significant decreases in hematocrit in both groups during hemorrhage.

Responses of the fetal renin-angiotensin-aldosterone system are presented in Table 3. Plasma renin activity (PRA) increased significantly in both groups during hemorrhage. The rise in PRA at the peak of hemorrhage was found to be of greater magnitude in fetuses >130 days than in those <120 days gestation (P < 0.025).

The effect of hemorrhage on plasma All concentration was also more pronounced in fetuses >130 days than in the younger group (P < 0.05) (Table 3). On the other hand, the All response to an increase in PRA, expressed as the slope of the regression line between PRA and plasma All concentration, was similar (P > 0.1) in both groups (Fig. 1).

No significant changes in plasma aldosterone concentrations were observed during hemorrhage in fetuses <120 days gestation despite a small but significant increase in plasma All levels at the peak of blood volume depletion (Table 3). In fetuses >130 days gestation, plasma aldosterone concentration increased significantly (P < 0.001) during hemorrhage (Table 3). This increase in plasma aldosterone concentration correlated closely with the rise in plasma All (r = 0.70, P < 0.001) (Fig. 2). Multiple regression analysis of plasma potassium, plasma All, and plasma aldosterone concentration demonstrated a high partial correlation between plasma aldosterone and plasma All (r = 0.697) and a low partial correlation when plasma...
Table 2
Arterial Blood Values for Different Levels of Fetal Hemorrhage

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Level I</th>
<th>Level II</th>
<th>Level III</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.36±0.01</td>
<td>7.37±0.01</td>
<td>7.36±0.01</td>
<td>7.34±0.01</td>
</tr>
<tr>
<td>Pco₂ (mm Hg)</td>
<td>43±1</td>
<td>47±1</td>
<td>48*±1</td>
<td>49*±1</td>
</tr>
<tr>
<td>Pο₂ (mm Hg)</td>
<td>26±1</td>
<td>26±1</td>
<td>25±1</td>
<td>26±1</td>
</tr>
<tr>
<td>Na⁺ (mEq/liter)</td>
<td>143±1</td>
<td>143±1</td>
<td>142±1</td>
<td>142±1</td>
</tr>
<tr>
<td>K⁺ (mEq/liter)</td>
<td>7±1</td>
<td>7±1</td>
<td>7±1</td>
<td>7±1</td>
</tr>
<tr>
<td>OSM (mosm/Kg H₂O)</td>
<td>3.8±1</td>
<td>3.70±0.08</td>
<td>3.67±0.06</td>
<td>3.71±0.07</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>105±0.11</td>
<td>105±0.08</td>
<td>106±0.06</td>
<td>107±0.07</td>
</tr>
<tr>
<td>Cl⁻ (mEq/liter)</td>
<td>105±0.13</td>
<td>105±0.13</td>
<td>106±0.13</td>
<td>107±0.13</td>
</tr>
<tr>
<td>OSM (mosm/Kg H₂O)</td>
<td>26.7±2</td>
<td>288±3</td>
<td>286±3</td>
<td>287±3</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>34±3</td>
<td>32*±2</td>
<td>31*±2</td>
<td>28*±2</td>
</tr>
</tbody>
</table>

For P < 0.05 when values during hemorrhage are compared to control values.

Table 3
Effect of Fetal Hemorrhage on the Fetal Renin-Angiotensin-Aldosterone System and Arterial Blood Pressure

<table>
<thead>
<tr>
<th></th>
<th>120 days (n = 7)</th>
<th>130 days (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRA (ng/ml per hr)</td>
<td>7.22±0.54</td>
<td>8.31±2.79</td>
</tr>
<tr>
<td>All (pg/ml)</td>
<td>40.64±3.32</td>
<td>45.14±2.87</td>
</tr>
<tr>
<td>Aldosterone (pg/ml)</td>
<td>47±3.53</td>
<td>46±3.62</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>47±2.79</td>
<td>46±3.48</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>47±2.53</td>
<td>47±2.53</td>
</tr>
</tbody>
</table>

For P < 0.05 when values during hemorrhage are compared to control values.

 aldosterone was related to plasma potassium concentration (r = 0.379) in fetuses >130 days gestation.

In order to determine whether factors other than a rise in plasma All concentration were responsible for the increase in plasma aldosterone in the older group, the same protocol was repeated in binephrectomized fetuses (131–144 days gestation). At the peak of blood volume depletion (34.2 ± 1.7% of the fetoplacental blood volume removed), arterial pH decreased from a mean value of 7.36 ± 0.02 to 7.30 ± 0.01 (P < 0.01), plasma potassium concentration increased in three of four fetuses from a mean value of 4.72 ± 0.32 to 5.15 ± 0.43 mEq/liter, plasma osmolality increased in two of four fetuses from a mean value of 281 ± 2 to 297 ± 11 mOsm/kg H₂O, and hematocrit decreased in all fetuses from 29 ± 9 to 22 ± 7% (P < 0.05). No significant changes in arterial blood gases (Pco₂ and Pο₂), plasma sodium or chloride concentrations were observed. The effects on the renin-angiotensin-aldosterone system are presented in Table 4. Control PRA values were significantly lower (P < 0.01) in the nephrectomized group (Table 4) than in those >130 days gestation with intact kidneys (Table 3). During hemorrhage, no significant changes in PRA or plasma All concentrations were observed in nephrectomized fetuses. A small but significant increase in plasma aldosterone concentration was demonstrated at the peak of fetoplacental blood volume depletion (level III) (Table 4). The percent change in plasma aldosterone concentration (60.5 ± 15.3%) was significantly
The plasma aldosterone concentrations in the arteri- 
al blood pressure (MABP) and heart rate in non-
nephrectomized and nephrectomized fetuses are pre-
sented in Tables 3 and 4. MABP decreased signifi-
cantly during hemorrhage in all nephrectomized ani-
imals (Table 4).

The effects of blood volume depletion on mean 
arterial blood pressure (MABP) and heart rate in non-
nephrectomized and nephrectomized fetuses are pre-
sented in Tables 3 and 4. MABP decreased signifi-
cantly during hemorrhage in fetuses <120 days ges-
tation (P < 0.05) while no changes were observed in 
fetuses >130 days (Table 3). Heart rate did not change 
in the younger group but increased significantly in 
older fetuses (P < 0.05). MABP decreased during 
hemorrhage in all nephrectomized animals (Table 4).

Discussion

Previous studies using both short-term (Smith et 
al., 1974) and long-term (Broughton-Pipkin et al., 
1974a; Robillard et al., 1979) fetal lamb prepara-
tions have shown that fetal PRA increases signifi-
cantly during blood volume reduction. The present study 
confirms these previous results. Moreover, it dem-
strates that the All response to the rise in PRA is 
similar in young and older lamb fetuses (Fig. 1), but 
that the level of stimulation of the renin-angiotensin 
system following fetoplacental blood volume deple-
tion is age-dependent during the last trimester of 
gestation (Table 3). Previous studies by Siegel and 
coworkers (1980a, 1980b) and by our group (Robillard 
et al., 1981) demonstrated that the PRA response to 
either furosemide stimulation or fetal hypoxemia is 
significantly smaller in fetal lambs <120 days ges-
tation than in near-term fetuses. Factors that may 
account for this difference have not yet been studied. 
However, the degree of anatomical development of 
the juxtaglomerular apparatus, the state of activation 
of the fetal renin-angiotensin system during basal 
conditions, and the possibly higher sympathetic re-
sponse to hemorrhage in near-term fetuses, as previ-
ously shown during fetal hypoxemia (Robillard et al., 
1981), may contribute to the smaller response to 
hemorrhage in fetuses <120 days gestation.

The present study also demonstrates that plasma 
aldosterone concentration increases significantly fol-
lowing blood volume reduction in fetuses >130 days 
gestation, whereas no significant rise is observed in 
those <120 days gestation. This difference probably 
is related to the very small increase in plasma All 
centration at the peak of fetal blood volume deple-
tion, as suggested previously (Robillard et al., 
1981). Contrary to previous reports (Alexander et al., 
1968; Siegel and Fisher, 1980a), the present study 
demonstrates that the rise in plasma aldosterone con-
centration correlates closely with the increase in All 
levels (Fig. 2). One may speculate that, since plasma 
All levels were not determined in the studies of 
Alexander et al. and Siegel and Fisher, the rise in 
plasma All was not great enough to produce a sig-
nificant increase in aldosterone. Furthermore, con-
trary to those studies, previous "in vitro" work has 
demonstrated in sheep (Wintour, 1977) and humans 
(Dufau and Villee, 1969; Pasqualini et al., 1966) that 
the fetal adrenal gland has the ability to synthetize 
and secrete aldosterone.

To determine the relative influence of All on the 
rise in plasma aldosterone concentration in fetuses 
>130 days gestation, blood volume depletion was 
studied in nephrectomized fetuses >130 days ges-
tation. Basal PRA values were significantly lower in 
the nephrectomized fetuses, as previously described 
(Broughton-Pipkin et al., 1974b; Oakes et al., 1977). 
However, contrary to previous results (Broughton-
Pipkin, 1974b), the basal plasma All concentrations 
did not differ significantly. The presence of immu-
noreactive All in the plasma of nephrectomized fe-
tuses is difficult to explain. Previous studies in sheep 
(Alexander et al., 1968; Broughton-Pipkin et al., 
1974b; Robillard et al., 1982), monkeys (Behrman and 
Kittenger, 1968), and guinea pigs (Broughton-Pipkin
et al., 1977) suggest that All does not cross the placenta. Furthermore, since fetoplacental blood volume depletion does not change levels of immunoreactive All in nephrectomized fetuses, it is unlikely that these levels represent the biologically active octapeptide (All). The antibody used to measure All also reacts with other All metabolites, and one may speculate that such fragments originate either from the maternal circulation or from the uterus itself, as previously suggested (Broughton-Pipkin et al., 1977).

During blood volume depletion (level III), a small but significant increase in plasma aldosterone concentration is observed in nephrectomized fetuses, despite the fact that PRA and plasma immunoreactive All do not change. This rise is significantly smaller (P < 0.02) than in the group of non-nephrectomized fetuses matched for age and for level of blood volume depletion (level III), suggesting that the integrity of the renin-angiotensin system is an important component of the response. However, other factors such as changes in ACTH and plasma potassium concentrations may also contribute, though to a lesser extent than All.

The hemodynamic responses to fetoplacental blood volume depletion are characterized by a decrease in blood pressure in fetuses <120 days gestation but by no change in those >130 days gestation. It has been previously suggested (Faber et al., 1974; Robillard et al., 1979) that rapid restoration of blood volume by hemodilution is an important mechanism in the regulation of blood pressure following fetal hemorrhage. However, since the degree of hemodilution reflected in hematocrit was similar in both groups, factors other than differences in the rate of restoration of blood volume must be considered. The present results suggest that the higher All response in near-term fetuses may be important. The finding that the four nephrectomized fetuses >130 days gestation demonstrated a decrease in blood pressure 15 minutes following hemorrhage supports this hypothesis. Furthermore, Iwamoto and Rudolph (1979), using the competitive antagonist [Sar1, Ala8]-All, found that blood pressure decreases significantly in fetal sheep between 115 and 133 days of gestation. Other factors such as the degree of stimulation of the adrenergic system, the level of vasopressin secretion, and the development of end-organ responsiveness to vasoactive hormones may also contribute to maturation of the fetal blood pressure responses to hemorrhage. Stimulation of the adrenergic system and secretion of vasopressin are more important than younger fetuses (Robillard et al., 1981). Moreover, fetal end-organ responsiveness to these vasoactive substances increases as gestation progresses (Nuwayhid et al., 1975; Su et al., 1977; Robillard and Weitzman, 1980).

The present work was supported by U.S. Public Health Service Grants HD-11466 and HL-14388 and American Heart Association Grant 79-809. Jean E. Robillard is the recipient of Research Career Development Award HD-00254. R. Ariel Gomez is the recipient of a research fellowship from the National Kidney Foundation.

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

Decisions with regard to this paper were made by John T. Shepherd, Consulting Editor.

Received July 22, 1981; accepted for publication January 25, 1982.

References

Circulation Research/Vol. 50, No. 5, May 1982


Role of angiotensin II on the adrenal and vascular responses to hemorrhage during development in fetal lambs.
J E Robillard, R A Gomez, J G Meernik, W D Kuehl and D VanOrden

Circ Res. 1982;50:645-650
doi: 10.1161/01.RES.50.5.645

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/50/5/645.citation