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

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

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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.

Analytical 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 electrodes 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 (Ha-ber 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 PRA 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>
<thead>
<tr>
<th>Table 1</th>
<th>Percentage (%) of Fetoplacental Blood Volume Removed</th>
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<tbody>
<tr>
<td></td>
<td>Fetoplacental blood volume depletion (%)</td>
</tr>
<tr>
<td>n</td>
<td>Level I</td>
</tr>
<tr>
<td>&lt;120 days</td>
<td>7</td>
</tr>
<tr>
<td>&gt;130 days</td>
<td>7</td>
</tr>
<tr>
<td>t</td>
<td>2.273</td>
</tr>
<tr>
<td>P</td>
<td>0.05</td>
</tr>
</tbody>
</table>

n, number of animals; t, values for the t distribution. Values are mean ± SEM.
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 H$_2$O, and hematocrit decreased in all fetuses from 29 ± 9 to 22 ± 7% ($P < 0.05$). No significant changes in arterial blood gases (Pco$_2$ and Po$_2$), 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

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>&lt;120 days (n = 7)</th>
<th>&gt;130 days (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Level I</td>
</tr>
<tr>
<td>pH</td>
<td>7.36 ± 0.01</td>
<td>7.37 ± 0.01</td>
</tr>
<tr>
<td>Pco$_2$ (mm Hg)</td>
<td>43 ± 1</td>
<td>47* ± 1</td>
</tr>
<tr>
<td>Po$_2$ (mm Hg)</td>
<td>26 ± 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>±1 ± 1</td>
<td>±1 ± 1</td>
</tr>
<tr>
<td>OSM (mOsm/kg H$_2$O)</td>
<td>±0.11</td>
<td>±0.08</td>
</tr>
<tr>
<td>Htc (%)</td>
<td>105 ± 1</td>
<td>105 ± 1</td>
</tr>
</tbody>
</table>

* For $P < 0.05$ when values during hemorrhage are compared to control values. Percentages of fetoplacental blood volume removed for each level (levels I to III) of hemorrhage are presented in Table 1. n, number of animals; OSM, plasma osmolality; Htc, hematocrit. Values are mean ± SEM.

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>&lt;120 days</th>
<th>&gt;130 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n Control</td>
<td>Level I</td>
</tr>
<tr>
<td>PRA (ng/ml per hr)</td>
<td>2.22 ± 0.54</td>
<td>2.66 ± 0.78</td>
</tr>
<tr>
<td>All (pg/ml)</td>
<td>40.64 ± 5.32</td>
<td>36.92 ± 5.54</td>
</tr>
<tr>
<td>Aldosterone (pg/ml)</td>
<td>41 ± 1</td>
<td>42 ± 1</td>
</tr>
<tr>
<td>MABF (mm Hg)</td>
<td>7 ± 2</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>186 ± 5</td>
<td>183 ± 5</td>
</tr>
</tbody>
</table>

* For $P < 0.05$ when values during hemorrhage are compared to control values. Percentages of fetoplacental blood volume removed for each level (levels I to III) of hemorrhage are presented in Table 1. n, number of animals; PRA, plasma renin activity; All, angiotensin II; MABF, mean arterial blood pressure. Values are mean ± SEM.
less than in the group of non-nephrectomized fetuses (147.4 ± 16.9%) \( (P < 0.02) \). This small but significant rise in plasma aldosterone concentration in nephrectomized fetuses correlates significantly with changes in plasma potassium concentration \( (r = 0.50, P < 0.05) \). The plasma aldosterone concentrations in the mothers of the nephrectomized fetuses did not change. They were 49 ± 6 pg/ml before hemorrhage and 41 ± 1 pg/ml at the peak of fetal hemorrhage \( (P > 0.1) \).

The effects of blood volume depletion on mean arterial blood pressure (MABP) and heart rate in non-nephrectomized and nephrectomized fetuses are presented in Tables 3 and 4. MABP decreased significantly during hemorrhage in fetuses <120 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 Ald concentration at the peak of fetal blood volume depletion, as suggested previously \( (P < 0.05) \).

The present study also demonstrates that plasma aldosterone concentration increases significantly following blood volume reduction in fetuses >130 days gestation, whereas no significant rise is observed in those <120 days gestation.

The present study demonstrates that the rise in plasma aldosterone concentration 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 significant increase in aldosterone. Furthermore, contrary to these studies, previous "in vitro" work has demonstrated in sheep \( (P < 0.01) \) 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 gestation. 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 \( (P < 0.001) \), the basal plasma All concentrations did not differ significantly. The presence of immunoreactive All in the plasma of nephrectomized fetuses 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 \( (Broughton-Pipkin et al., 1974b) \) demonstrated that the rise in plasma aldosterone concentration is related to the very small increase in plasma All concentration at the peak of fetal blood volume depletion, as suggested previously \( (P < 0.05) \).

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et al., 1977) suggest that All does not cross the placa-
enta. 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 concen-
tration 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 deple-
tion (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 concentra-
tions 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 reg-
ulation of blood pressure following fetal hemorrhage.
However, since the degree of hemodilution reflected
by changes 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. Fur-
thermore, Iwamoto and Rudolph (1979), using the
competitive antagonist [Sar$^1$, Ala$^8$]-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 vasoac-
tive 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 to near-term than
younger fetuses (Robillard et al., 1981). Moreover,
fetal end-organ responsiveness to these vasoactive
substances increases as gestation progresses (Nuway-
hid et al., 1975; Su et al., 1977; Robillard and Weitz-
man, 1980).

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References
Alexander DP, Britton HC, James VHT, Nixon DA, Parker RA,
Wintour EM, Wright RD (1968) Steroid secretion by the adren-
Behrman RE, Kittinger GW (1968) Fetal and maternal responses to
in utero angiotensin infusions in Macaca mulatta. Proc Soc Exp
Biol Med 129: 305-308
Broughton-Pipkin F, Kirkpatrick SML, Lumbers ER, Mott JC
(1974a) Renin and angiotensin-like levels in foetal, newborn and
adult sheep. J Physiol (Lond) 241: 575-588
Broughton-Pipkin F, Lumbers ER, Mott JC (1974b) Factors influ-
encing plasma renin and angiotensin II in the conscious pregnant
ewe and its foetus. J Physiol (Lond) 243: 619-636
Broughton-Pipkin F, Benjamin N, Macallan C (1977) Placental
transfer of a large angiotensin fragment in the guinea pig. Am J
Obstet Gynecol 128: 904-906
Cain MD, Coghlan JP, Catt KJ (1972) Measurement of angiotensin
mination of plasma renin parameters and circulating angiotensin
II. In Oral Contraceptives and High Blood Pressure, edited by M
Creasy RK, Drost M, Green MV, Morris JA (1970) Determination of
fetal, placental and neonatal blood volumes in the sheep. Circ
Res 27: 487-494
Dufau ML, Villez DB (1969) Aldosterone biosynthesis by human


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