Peripheral Chemoreceptor Control of Fetal Renin Responses to Hypoxia and Hypercapnia

Charles E. Wood, Curtis Kane, and Hershel Raff

The renin response to hypoxia in late gestation fetal sheep has been well characterized. However, the renin response to asphyxia—the combination of hypoxia and hypercapnia—has not been extensively studied. The purpose of this study was to determine 1) the interaction of hypoxia and hypercapnia in the control of renin secretion in late gestation fetal sheep and 2) the role of peripheral arterial chemoreceptors therein. Chronically catheterized fetal sheep (intact or sinoaortie denervated) were exposed to hypoxia and/or hypercapnia for 30 minutes. Hypercapnia alone had no effect on plasma renin activity or aldosterone but did result in a significant increase in angiotensin II. Hypercapnia combined with hypoxia resulted in a significant increase in renin activity, angiotensin II, and aldosterone. Sinoaortic denervation attenuated the renin and angiotensin II responses to hypercapnia plus hypoxia. The increase in renin and angiotensin II in response to hypercapnia with or without concomitant hypoxia strongly correlated with the magnitude of the decrease in arterial pH in intact fetuses only. Hypoxia alone and in concert with hypercapnia increased mean arterial pressure and decreased heart rate in intact but not sinoaortic denervated fetuses. We conclude that 1) hypercapnia more potently increases plasma renin activity than does hypoxia in late gestation fetal sheep, 2) arterial pH may be the relevant signal perceived by the peripheral arterial chemoreceptors for the control of the renin-angiotensin system during asphyxia, and 3) the cardiovascular response to hypoxia is mediated, in part, by peripheral arterial chemoreceptors. (Circulation Research 1990;67:722–732)

Fetal animals respond to acute hypoxia or asphyxia with increased plasma catecholamine concentrations and increases in the rate of secretion of renin, vasopressin, adrenocorticotropic hormone, and other hormones thought to aid in the redistribution of cardiac output toward vascular beds, which require maintenance of oxygen delivery (in particular the coronary circulation and cerebral circulation). Investigation of the responses of the fetus to asphyxia is particularly important because asphyxia is probably the most common form of fetal distress in utero, often resulting from compression of the fetal umbilical cord.

Experiments performed in several laboratories have investigated the response of the renin-angiotensin system to asphyxia or hypoxia in the fetus. The responsiveness of the fetal renin-angiotensin system to hypoxia develops over the last 20% of fetal life. In sheep, parturition occurs at approximately 147 days. The plasma renin activity (PRA) response to hypoxia is less in fetal sheep younger than 120 days of gestation as compared with fetal sheep older than 130 days. This may be attributable to the lack of responsiveness of the younger fetal juxtaglomerular cells to adrenergic stimulation. In a recent report by Rawashdeh and coworkers, renin secretion in vivo was stimulated by isoproterenol in fetuses between 116 and 134 days of gestation but not in fetuses between 93 and 107 days of gestation. Furthermore, recent studies using fetal renal cortical slices in vitro have found secretion of active and inactive renin even at 107 days of gestation.

Although much is known about the development of the fetal renin response to hypoxia, little is known about the interaction between changes in arterial oxygen tension (PaO₂) and arterial carbon dioxide tension (PaCO₂). Investigators demonstrating renin responses to fetal hypoxia have most often produced isocapnic or hypocapnic hypoxia and have not, therefore, studied the effect of concomitant hypercapnia on the fetal renin response to hypoxia. In adult animals, hypercapnia modulates the renin response
to hypoxia; it would seem logical that hypercapnia and hypoxia interact in the control of fetal renin secretion as well. One goal of the present experiments is to investigate the possible interaction between PaO2 and PaCO2 in the control of fetal renin secretion.

Peripheral chemoreceptors are active in fetal sheep. Results of experiments by Itskovitz and Rudolph indicated that the heart rate responses to uterine artery compression are mediated by peripheral chemoreceptors in the carotid sinus and by chemoreceptors with afferent fibers in the aortic depressor nerves. No experiments have been performed, however, to investigate the role of the peripheral chemoreceptors in the control of endocrine responses to acute hypoxia and/or hypercapnia in the fetus. Therefore, a second goal of these experiments is to investigate the influence of chronic sinoaortic denervation on the renin, angiotensin, and aldosterone responses to hypoxia and/or hypercapnia in fetal sheep.

Materials and Methods

We studied 25 pregnant ewes of mixed Western and Florida native breeds. Two sheep carried twins; the others carried single fetuses. On the day of study, the fetuses were between 123 and 144 days of gestation.

Surgical Preparation

We prepared the fetuses between 4 and 6 days before the first study. For 24 hours before surgery, ewes were not fed but were allowed free access to drinking water. During surgery, ewes were anesthetized with halothane (1.0–2.5%) in oxygen. By use of strictly aseptic techniques, the uterus was exposed via a midline incision and incised; a fetal hind limb was delivered. A polyvinyl chloride catheter (0.030 in. i.d., 0.050 in. o.d.) was inserted into the tibial artery, and a larger catheter (0.040 in. i.d., 0.070 in. o.d.) was inserted into the saphenous vein. A second fetal hind limb was delivered and catheterized. A polyvinyl chloride catheter (0.050 in. i.d., 0.090 in. o.d.) with side holes cut in the tip was sutured to the skin before returning the second hind limb to the amniotic cavity. The amniotic catheter was used for measurement of amniotic fluid pressure during experiments. Before closing the incision in the uterus, 500 mg ampicillin (Polyflex, Veterinary Products, Bristol Laboratories, Syracuse, N.Y.) was injected into the amniotic fluid. Catheters were filled with heparin (1,000 units/ml; Elkins-Sinn, Cherry Hill, N.J.), plugged, and exteriorized through a stab wound in the flank, where they were protected by a cloth pouch sutured to the skin. Twin fetuses were both catheterized.

Sixteen fetal sheep were prepared with vascular catheters only. Eleven fetal sheep were subjected to bilateral section of the carotid sinus and aortic depressor nerves. After placement of the vascular catheters, the fetal head was located, and the uterus and the fetal skin were incised over the trachea. The fetal skin was attached to the wall of the uterus with clamps to prevent leakage of amniotic fluid; then sinoaortic denervation was performed. Complete- ness of denervation was not tested intraoperatively but was confirmed after recovery by the lack of change in heart rate in response to hypoxia. The fetal skin was closed; then the wall of the uterus was closed.

Lastly, the incision in the abdomen was sutured. After completion of the abdominal surgery, polyvinyl chloride catheters (0.050 in. i.d., 0.090 in. o.d.) were inserted into the maternal femoral artery and vein at the level of the femoral triangle, and the tips were advanced to the abdominal aorta and inferior vena cava, respectively. The maternal catheters were filled with heparin, plugged, and routed subcutaneously to the flank, where they were protected by the pocket. The ewe was treated with ampicillin (500 mg i.m.) and returned to her pen. Ewes were treated with ampicillin for 5 days after surgery. All catheters were flushed and reheparinized at least once every 4 days. Fetuses were treated with ampicillin (500 mg via the amniotic fluid catheter) once every day that the catheters were flushed.

Experimental Protocol

All experiments were started between 8:00 AM and noon. Four fetuses participated in three studies, 14 fetuses participated in two studies, and nine fetuses participated in one study. The order of experiments performed was randomized.

On the morning of an experiment, the ewe to be studied was placed in a study cart and transported to the laboratory from the Health Center Animal Resources Department. One fetal arterial catheter, one fetal venous catheter, and the amniotic fluid catheter were connected to pressure transducers (model P23Db, Statham Instruments, Oxnard, Calif.). Pressures were continuously monitored using a Beckman (model R611, Beckman Instruments, Inc., Fullerton, Calif.) or Grass (model 7, Grass Instrument Co., Quincy, Mass.) direct-writing recorder. Fetal heart rate was calculated from the phasic arterial pressure signal using appropriate Beckman or Grass cardiofetometers. Mean fetal arterial pressure was measured as the damped output of a channel slaved from the phasic arterial pressure signal. Fetal mean arterial, central venous, and amniotic pressures and fetal heart rate were sampled with analog-to-digital conversions performed at a rate of 1 Hz using an analog-to-digital converter (System 500, Keithley Instruments, Inc., Cleveland).

Each experiment consisted of two concatenated study periods of 30 minutes each. The ewes and their fetuses were subjected to four protocols: 1) normoxia in the first experimental period and normoxia in the second experimental period (NX/NX), 2) normoxia in the first period and isocapnic hypoxia in the second (NX/HX), 3) hypercapnia in the first period and hypercapnia in the second (HC/HC), and 4) hypercapnia in the first period and hypercapnia plus hypoxia in the second (HC/HX). In this way, the
effects of hypoxia and/or hypercapnia could be examined.

Five-milliliter blood samples were withdrawn from a fetal arterial catheter, and 10-ml blood samples were drawn from the maternal arterial catheter at 30-minute intervals for 60 minutes. Each blood sample was placed in a chilled plastic centrifuge tube containing 0.3 M Na2EDTA (0.05 ml/ml blood; Sigma Chemical Co., St. Louis). All of these tubes were kept on ice until the end of the experiment, when they were centrifuged at 3,000g for 20 minutes in a refrigerated (4°C) centrifuge. After centrifugation, the plasma was stored at −20°C until hormones were assayed. Additional blood samples (2 ml) were drawn anaerobically from a fetal arterial catheter for analysis of fetal arterial pH (pHi), Pao2, Paco2, hematocrit, and plasma electrolytes. Fetal blood gases were measured in all experiments at 30-minute intervals.

Immediately after drawing the first fetal and maternal blood samples, a polyethylene bag was fitted over the ewe’s head and neck, held loosely in place with elasticized cloth. Normoxia was achieved by passing room air through the bag. Isocapnic hypoxia was achieved by passing through the bag a gas mixture of 8–10% O2 and 0.5–3% CO2 (adjusted to maintain maternal Paco2 at initial levels). Hypercapnia was achieved by using a gas mixture of 21% O2 and 3–6% CO2 (adjusted to increase fetal Paco2 by at least 10 mm Hg). Hypercapnia plus hypoxia was achieved by using a gas mixture of 8–10% O2 and 3–6% CO2. All gases were delivered at a minimum flow of 30 l/min.

Room air was supplied from compressed air. Other gas mixtures were made from individual tanks of O2, CO2, and N2 and were mixed using a three-tube mixer and flowmeter (model 7400, Matheson Gas Products, East Rutherford, N.J.), with flowmeter tubes 603, 604, and 605 for CO2, O2, and N2, respectively. The FiO2 and FiCO2 of the output of the gas mixer were measured using an airway gas monitor (model PB252, Puritan-Bennet, Wilmington, Mass.).

Analysis of Blood Samples

PRA was measured using a modification of the method of Haber and coworkers.13 Angiotensin I (Ang I) was generated at pH 5.7 at both 37°C and 0°C. Before generation of Ang I, pH was adjusted by addition of 0.5 M citric acid plus 1 M phosphate buffer (1:1.1 dilution of the generated sample), and protease activity was inhibited by addition of phenylmethylsulfonyl fluoride (3% solution in ethanol, 1:1.01 dilution; Sigma). Ang I was generated for either 1 or 2 hours. The generation was linear over the total time of the incubation. Ang I was measured by radioimmunoassay (RIA), using rabbit anti-Ang I generated in the laboratory of Dr. M. Ian Phillips, Department of Physiology, University of Florida. The range of the standard curve was from 3.9 to 250 pg Ang I/tube. This allowed determination of PRA in plasma samples as small as 50 μl. PRA was expressed as the rate of generation of Ang I at 37°C minus the generation at 0°C. In one plasma pool, measured once in each of 20 assays, PRA was 7.4±1.8 (mean±SD) ng Ang I/ml·hr. In another plasma pool, measured 20 times in one assay, PRA was 15.8±2.8 ng Ang I/ml·hr. Considering both the generation and RIA of Ang I, therefore, the interassay coefficient of variation was 24.4%, and the intra-assay coefficient of variation was 17.5%. In previous studies from this laboratory,14 we determined PRA using a kit from Clinical Assays (Baxter Healthcare, Cambridge, Mass.). In 21 plasma samples from other experiments in adult sheep, the relation between the two assays was as follows: Clinical Assays kit=(new assay)×(0.70±0.06) r2=0.93. Plasma angiotensin II (Ang II) was measured by RIA after extraction on bentonite. This assay has been described more fully elsewhere.15,16 The antisera used has less than 0.3% cross-reactivity with Ang I and was a generous gift from Dr. Ian A. Reid, Department of Physiology, University of California, San Francisco. Plasma aldosterone concentration was measured by RIA in unextracted plasma using a kit from Diagnostic Products Corp., Los Angeles.

Blood gases were measured using a BMS3Mk2 blood microsystem and PHM73 blood gas analyzer (Radiometer, Copenhagen, Denmark). Plasma sodium and potassium concentrations were measured in blood samples after measurement of blood gases using a Nova I ion-specific electrode system (Nova Biomedical, Waltham, Mass.).

Calculations and Statistical Analyses

All fetal intravascular pressures were calculated using amniotic fluid pressure as zero reference. Changes in the values of fetal hormonal and hemodynamic variables over time and between groups were assessed using two-way and four-way analysis of variance (ANOVA) corrected for repeated measures in the time dimension.17 A posteriori comparison of individual means was performed using Duncan’s multiple range test.18 Correlations among variables were tested using least-squares correlation analysis. A significance level of 0.05 was used to reject the null hypothesis in all tests.

Results

The Stimuli to Renin Secretion

During 60 minutes of normoxia (NX/NX) in intact fetal sheep, Pao2, Paco2, and pH did not change significantly (Figure 1). The values of these variables were also unchanged in their mothers (Pao2=90.0±2.0, Paco2=32.5±1.0, and pH=7.52±0.01). Blood gases were unaltered in sinoaortic denervated fetuses (Figure 1) and their mothers.

Isocapnic hypoxia (NX/HX) was achieved by reducing fetal Pao2 from 19.7±0.6 mm Hg at 0 minutes to 14.1±1.2 mm Hg at +30 minutes in intact fetuses and from 19.8±1.8 mm Hg at 0 minutes to 12.4±0.7 mm Hg at +30 minutes in the sinoaortic denervated fetuses, while holding Paco2 constant in both groups (Figure 1). Fetal pH was decreased...
during the period of hypoxia in intact fetuses, indicating the development of metabolic acidemia. pHa was not altered by hypoxia alone in the sinoaortic denervated fetuses or in the mothers’ circulations. Maternal PaO2 was decreased from 84.3±5.8 and 94.2±4.7 mm Hg at 0 minutes to 46.9±4.7 and 45.5±2.9 mm Hg at +30 minutes in ewes carrying intact and sinoaortic denervated fetuses, respectively.

During the period of hypercapnia (HC/HC), fetal PaCO2 was increased in the intact and sinoaortic denervated fetuses (Figure 1). The increase in fetal PaCO2 was associated with decreased pHa in both intact and sinoaortic denervated fetuses. Maternal PaCO2 increased from 34.3±1.5 (at -30 minutes) to 40.3±2.9 (at +30 minutes) mm Hg in the ewes carrying intact fetuses and from 31.4±1.3 to 37.4±1.3 mm Hg in the ewes carrying sinoaortic denervated fetuses. Fetal PaO2 increased in the intact and sinoaortic denervated fetuses (Figure 1). Maternal PaO2 increased from 87.1±3.9 (at -30 minutes) to 118.6±3.2 (at +30 minutes) mm Hg and from 93.0±2.2 to 108.2±4.9 mm Hg in the ewes carrying intact and sinoaortic denervated fetuses, respectively.

Hypercapnia plus hypoxia (HC/HX) resulted in changes in PaO2 and PaCO2 that were similar to those in intact and sinoaortic denervated fetuses and similar to changes with hypoxia (NX/HX) and hypercapnia (HC/HC) alone, respectively. The induced changes in maternal PaO2 and PaCO2 were similar to those induced during hypoxia and hypercapnia alone.

Responses in Intact Fetal Sheep

Fetal PRA (Figure 2) and plasma concentrations of Ang II (Figure 3), aldosterone (Figure 4), and K+ (Table 1) were not significantly altered during normoxia (NX/NX). Plasma Na+ was significantly lower at 0 minutes than at -30 minutes (Table 1). Fetal mean arterial blood pressure (Figure 5) and heart rate (Figure 6) were also not significantly altered during normoxia. Fetal hematocrit was significantly reduced at +30 minutes relative to -30 minutes (Table 1).

Isocapnic hypoxia (NX/HX) did not significantly alter any of the measured hormonal variables except for a small but significant increase in plasma aldosterone concentration at +30 minutes (Figures 2–4). Statistical analysis of blood pressure and heart rate is presented in the legends of Figures 5 and 6. Briefly, fetal arterial blood pressure increased from 47.2±2.0 mm Hg at the onset of hypoxia to a peak of 51.1±2.3 mm Hg at 24 minutes after the onset of hypoxia (Figure 5). Fetal heart rate was decreased from 164.6±6.1 beats/min at the onset of hypoxia to a nadir of 129.7±9.4 beats/min at 23 minutes after the onset of hypoxia (Figure 6). Plasma K+ increased from 4.28±0.17 to 4.65±0.19 meq/l, and hematocrit increased from 36±2% to 38±2% (Table 1). Plasma Na+ was not significantly altered (Table 1).

Sixty minutes of hypercapnia (HC/HC) increased Ang II from 48±18 to 154±75 pg/ml (Figure 3) without
a statistically significant change in PRA or aldosterone in the intact fetuses. Neither plasma Na⁺, K⁺, nor hematocrit was significantly altered (Table 1). Hypercapnia did not significantly alter mean arterial blood pressure (Figure 5) or heart rate (Figure 6).

Hypoxia plus hypercapnia (HC/HX) increased PRA from 6.4±2.1 to 12.9±4.3 Ang l/ml·hr (Figure 2), Ang II from 64±15 to 268±111 pg/ml (Figure 3), and aldosterone from 352±35 to 426±65 pg/ml (Figure 4) at -30 and +30 minutes, respectively. As

**Figure 2.** Graphs showing fetal plasma renin activity measured during experiments in which intact and sinoaortic denervated (SAD) fetuses were exposed to 60 minutes of normoxia (NX/NX, top left panel), 60 minutes of hypercapnia (HC/HC, top right panel), 30 minutes of normoxia followed by 30 minutes of hypoxia (NX/HX, bottom right panel), and 30 minutes of hypercapnia followed by 30 minutes of hypoxia (HC/HX, bottom right panel). Data are represented as mean values; vertical bars represent ±1 SEM. The number of experiments in each group is shown in parentheses. *Significant difference from the value at -30 minutes, as tested by Duncan’s multiple-range test.

**Figure 3.** Graphs showing fetal plasma angiotensin II concentrations measured during experiments in which intact and sinoaortic denervated (SAD) fetuses were exposed to 60 minutes of normoxia (NX/NX, top left panel), 60 minutes of hypercapnia (HC/HC, top right panel), 30 minutes of normoxia followed by 30 minutes of hypoxia (NX/HX, bottom right panel), and 30 minutes of hypercapnia followed by 30 minutes of hypercapnia and hypoxia (HC/HX, bottom right panel). Data are represented as mean values; vertical bars represent ±1 SEM. The number of experiments in each group is shown in parentheses. *Significant difference from the value at -30 minutes, as tested by Duncan’s multiple-range test.
**ALDOSTERONE (pg/ml)**

**FIGURE 4.** Graphs showing fetal plasma aldosterone concentrations measured during experiments in which intact and sinoaortic denervated (SAD) fetuses were exposed to 60 minutes of normoxia (NX/NX, top left panel), 60 minutes of hypercapnia (HC/HC, top right panel), 30 minutes of normoxia followed by 30 minutes of hypoxia (NX/HX, bottom right panel), and 30 minutes of hypercapnia followed by 30 minutes of hypercapnia and hypoxia (HC/HX, bottom right panel). Data are represented as mean values; vertical bars represent ±1 SEM. The number of experiments in each group is shown in parentheses. *Significant difference from the value at −30 minutes, as tested by Duncan's multiple-range test.

**TABLE 1.** Hematocrit and Plasma Electrolytes in Fetal Sheep

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (min)</th>
<th>Na⁺ (meq/l)</th>
<th>K⁺ (meq/l)</th>
<th>Hct (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intact fetal sheep</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NX/NX</td>
<td>−30</td>
<td>142.8±1.7</td>
<td>4.08±0.19</td>
<td>32±1</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>141.4±1.5*</td>
<td>4.09±0.13</td>
<td>31±1</td>
</tr>
<tr>
<td></td>
<td>+30</td>
<td>142.4±1.5</td>
<td>4.05±0.13</td>
<td>30±1*</td>
</tr>
<tr>
<td>NX/HX</td>
<td>−30</td>
<td>142.2±0.7</td>
<td>4.28±0.17</td>
<td>36±2</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>141.8±0.8</td>
<td>4.23±0.18</td>
<td>37±2</td>
</tr>
<tr>
<td></td>
<td>+30</td>
<td>142.0±0.6</td>
<td>4.65±0.19*</td>
<td>38±2*</td>
</tr>
<tr>
<td>HC/HC</td>
<td>−30</td>
<td>143.8±1.0</td>
<td>4.12±0.14</td>
<td>34±2</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>143.8±1.0</td>
<td>4.20±0.11</td>
<td>35±2</td>
</tr>
<tr>
<td></td>
<td>+30</td>
<td>143.5±1.0</td>
<td>4.16±0.12</td>
<td>34±2</td>
</tr>
<tr>
<td>HC/HX</td>
<td>−30</td>
<td>143.9±0.7</td>
<td>4.26±0.19</td>
<td>36±2</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>143.9±1.0</td>
<td>4.26±0.20</td>
<td>36±3</td>
</tr>
<tr>
<td></td>
<td>+30</td>
<td>143.9±0.9</td>
<td>4.81±0.24*</td>
<td>37±3</td>
</tr>
<tr>
<td><strong>Sinoaortic denervated fetal sheep</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NX/NX</td>
<td>−30</td>
<td>144.0±0.9</td>
<td>4.23±0.11</td>
<td>32±1</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>144.3±1.0</td>
<td>4.21±0.09</td>
<td>33±1</td>
</tr>
<tr>
<td></td>
<td>+30</td>
<td>144.1±0.9</td>
<td>4.17±0.09</td>
<td>32±1</td>
</tr>
<tr>
<td>NX/HX</td>
<td>−30</td>
<td>144.5±0.8</td>
<td>4.13±0.13</td>
<td>33±1</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>144.5±0.8</td>
<td>4.11±0.10</td>
<td>33±1</td>
</tr>
<tr>
<td></td>
<td>+30</td>
<td>144.6±0.8</td>
<td>4.41±0.10*</td>
<td>34±1</td>
</tr>
<tr>
<td>HC/HC</td>
<td>−30</td>
<td>144.6±1.0</td>
<td>4.14±0.13</td>
<td>33±2</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>145.1±1.1</td>
<td>4.07±0.15</td>
<td>33±2</td>
</tr>
<tr>
<td></td>
<td>+30</td>
<td>145.4±1.2</td>
<td>4.11±0.18</td>
<td>32±2</td>
</tr>
<tr>
<td>HC/HX</td>
<td>−30</td>
<td>143.4±0.4</td>
<td>4.18±0.24</td>
<td>33±2</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>143.5±0.5</td>
<td>4.17±0.24</td>
<td>33±2</td>
</tr>
<tr>
<td></td>
<td>+30</td>
<td>143.5±0.8</td>
<td>4.48±0.26*</td>
<td>34±2</td>
</tr>
</tbody>
</table>

Hct, hematocrit; NX/NX, normoxia in the first experimental period (from −30 to 0 minutes) and normoxia in the second experimental period (from 0 to +30 minutes); NX/HX, normoxia in the first period and isocapnic hypoxia in the second; HC/HC, hypercapnia in the first period and hypercapnia in the second; HC/HX, hypercapnia in the first period and hypercapnia plus hypoxia in the second.

*Significantly different from −30 minutes.
during hypercapnia alone (HC/HC), pH$_4$ decreased (Figure 1). Plasma K$^+$ increased, but plasma Na$^+$ did not change (Table 1). In this group (HC/HX), hypercapnia alone had no significant effect on mean arterial blood pressure (Figure 5) but increased heart rate from 152.2±14.1 to 173.4±10.3 beats/min at −30 and 0 minutes, respectively (Figure 6). Hypoxia superimposed on hypercapnia (HC/HX) increased mean arterial blood pressure from 45.8±3.9 to 52.6±5.2 mm Hg at 0 and +30 minutes, respectively (Figure 5), and decreased heart rate to 113.7±9.5 beats/min at +5 minutes (Figure 6).

**Responses in Sinoaortic Denervated Fetal Sheep**

The induced changes in blood gases and pH$_4$ in the sinoaortic denervated fetal sheep were similar to those induced in the intact fetal sheep (Figure 1). Also consistent with the intact fetal sheep is the lack of stimulation of PRA (Figure 2), Ang II (Figure 3), and aldosterone (Figure 4) by normoxia (NX/NX) or hypoxia (NX/HX). Sinoaortic denervation attenuated the increase in PRA and Ang II induced by the combination of hypercapnia and hypoxia (HC/HX).

Sinoaortic denervation attenuated the responses of mean arterial blood pressure and heart rate (Figures 5 and 6) to hypoxia (NX/HX). Neither hypercapnia alone (HC/HC) nor hypercapnia plus hypoxia (HC/HX) changed fetal arterial blood pressure or heart rate (Figures 5 and 6).

**Comparison of Responses in Intact and Sinoaortic Denervated Fetal Sheep**

The effect of denervation could be demonstrated by plotting the relation between PRA and pH$_4$ and Ang II and pH$_4$ in intact and sinoaortic denervated fetal sheep (Figure 7). There were significant relations between pH$_4$ and PRA ($r=0.52$, $n=72$, $p<0.0005$) and pH$_4$ and Ang II ($r=0.63$, $n=72$, $p<0.0005$) in intact, but not in sinoaortic denervated fetal sheep ($r=0.04$, $n=72$, $p>0.9$).
p>0.5 [NS], and r=0.21, p>0.1 [NS], respectively; n=69). Therefore, denervation eliminated the relation between pH and PRA and pH and Ang II.

**Responses in the Pregnant Ewes**

Neither hypoxia (NX/HX) nor hypercapnia (HC/HC) stimulated increases in PRA or Ang II (Table 2). PRA actually decreased during hypoxia (NX/HX). On the other hand, aldosterone was increased significantly by hypercapnia plus hypoxia (HC/HX, Table 2). Plasma K+ was increased significantly during HC/HX, while plasma Na+ was not altered by either hypoxia (NX/HX) or hypercapnia (HC/HC, Table 3). Hematocrit was significantly increased during hypercapnia (HC/HC) and during hypoxia plus hypercapnia (HC/HX, Table 3).

**Discussion**

One of the most common forms of fetal distress is asphyxia, often produced by compression of the umbilical cord. The fetal reflex responses to asphyxia (e.g., redistribution of combined ventricular output10 and increased secretion of several hormones including adrenocorticotropic hormone,3 vasopressin, and renin2) are appropriate for survival. The present study is, to our knowledge, the first designed to test the interaction between hypoxia and hypercapnia in the control of fetal renin secretion and to identify the afferent pathways involved in the generation of the response. When PRA was increased by hypercapnia, it was not further increased by the addition of hypoxia, indicating that the PRA response to asphyxia was mainly the result of the response to the

**Table 2. Plasma Renin Activity and Plasma Angiotensin II and Aldosterone Concentrations in Pregnant Ewes**

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (min)</th>
<th>PRA (ng Ang I/ml·hr)</th>
<th>Ang II (pg/ml)</th>
<th>Aldo (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NX/NX</td>
<td>-30</td>
<td>1.8±0.3</td>
<td>36±6</td>
<td>91±19</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.9±0.3</td>
<td>38±6</td>
<td>100±20</td>
</tr>
<tr>
<td></td>
<td>+30</td>
<td>1.8±0.3</td>
<td>35±5</td>
<td>127±31</td>
</tr>
<tr>
<td>NX/HX</td>
<td>-30</td>
<td>4.3±2.9</td>
<td>59±31</td>
<td>130±63</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>4.6±3.0</td>
<td>62±31</td>
<td>141±87</td>
</tr>
<tr>
<td></td>
<td>+30</td>
<td>3.3±1.7</td>
<td>65±32</td>
<td>125±80</td>
</tr>
<tr>
<td>HC/HC</td>
<td>-30</td>
<td>1.8±0.2</td>
<td>27±2</td>
<td>88±19</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.4±0.2</td>
<td>27±3</td>
<td>129±21</td>
</tr>
<tr>
<td></td>
<td>+30</td>
<td>1.2±0.2</td>
<td>27±3</td>
<td>93±63</td>
</tr>
<tr>
<td>HC/HX</td>
<td>-30</td>
<td>2.8±1.2</td>
<td>42±10</td>
<td>85±27</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>2.8±1.3</td>
<td>38±8</td>
<td>142±33*</td>
</tr>
<tr>
<td></td>
<td>+30</td>
<td>3.0±1.2</td>
<td>43±9</td>
<td>147±44*</td>
</tr>
</tbody>
</table>

PRA, plasma renin activity; Ang I, angiotensin I; Ang II, angiotensin II; Aldo, aldosterone; NX/NX, normoxia in the first experimental period (from -30 to 0 minutes) and normoxia in the second experimental period (from 0 to +30 minutes); NX/HX, normoxia in the first period and isocapnic hypoxia in the second; HC/HC, hypercapnia in the first period and hypercapnia in the second; HC/HX, hypercapnia in the first period and hypercapnia plus hypoxia in the second.

*Significantly different from -30 minutes.
hypercapnia. We also found that sinoaortic denervation attenuated the PRA response to asphyxia, indicating that much of the PRA response was mediated by the peripheral chemoreceptors.

Renin Responses in Fetal Sheep

Results of other studies from this laboratory have suggested that the renin, adrenocorticotrophic hormone (ACTH), vasopressin, and heart rate responses to hypoxemia and to hypotension produced by vena caval obstruction may be mediated in part by chemoreceptors.12 Hemorrhage in the fetus decreases umbilical-placental blood flow,20 which produces hypercapnic acidosis.21 We and others have found that the PRA, ACTH, and vasopressin responses to fetal hemorrhage correlate with the induced changes in pH,21,22 The correlation with pH was stronger than the correlation with induced changes in mean arterial pressure or central venous pressure,21 suggesting involvement of the peripheral or central chemoreceptors. Vena caval obstruction stimulates increases in fetal plasma PRA, ACTH, and vasopressin and decreases in fetal heart rate, which correlate with the degree of hypotension during the obstruction.12,23 Sinoaortic denervation attenuated these responses, suggesting involvement of either the arterial baroreceptors or chemoreceptors.12 The blockade of vena caval obstruction--induced bradycardia by sinoaortic denervation suggested that the bradycardia often observed during hypotension in the fetus may be mediated by the peripheral chemoreceptors rather than by ventricular C fiber afferent nerves, as has been suggested previously.23

It is unlikely that the PRA response to hemorrhage is entirely accounted for by changes in PaO2 or PaCO2 or pH. The ovine fetal PRA response to acute hypoxia is small compared with PRA responses to hemorrhage. In the study by Robillard and coworkers,2 fetal sheep older than 120 days of gestation responded to hypoxemia (PaO2 decreased from 22±1 to 13±1 mm Hg) with increases in PRA from 3.7±1.1 to 6.9±2.3 ng Ang I/ml·hr. In the present study, decreases in PaO2 to 14.1±1.2 mm Hg did not significantly increase PRA, and increases in PaCO2 from 44.5±1.3 to 57.5±3.7 mm Hg increased PRA from 5.0±1.5 to 6.4±1.8 ng Ang I/ml·hr (in intact fetuses in the HC/HX group). During slow hemorrhage, on the other hand, PRA increased from 5.0±1.5 to 24.8±2.9 ng Ang I/ml·hr.21

In the present study, hypercapnia was more effective than hypoxia as a stimulus to the fetal renin-angiotensin-aldosterone axis. We could not demonstrate a significant interaction or synergism between hypoxia and hypercapnia in the stimulation of fetal renin. Sinoaortic denervation attenuated the renin-angiotensin-aldosterone response, suggesting a role of the peripheral chemoreceptors. However, it did not eliminate the response, suggesting that central chemoreceptors may be involved. The role of the peripheral chemoreceptors in the renin and Ang II responses to hypercapnia is best illustrated by the correlation between pH and PRA or Ang II. The lack of effect of hypoxia suggests that peripheral chemoreceptors mediate PRA responses to hypercapnia by sensing pH or PaCO2. That is, the primary stimulus to PRA during hypoxia may be the resultant acidosis rather than the change in PaO2. In a previous study,24 we infused HCl intravenously into late-gestation fetal sheep to test the hypothesis that acute acidemia stimulates reflex responses often associated with hypotension and/or hemorrhage in fetal sheep. We found that the acute acidemia stimulated increases in ACTH and vasopressin but did not produce any change in PRA. Those results would therefore appear to conflict with the present results. It is possible that fetal sheep respond to metabolic acidemia differently than to respiratory acidemia. During infusion of H+, for example, PaCO2 increased to levels similar to those observed during ventilatory hypercapnia in the present study. However, the decrease in pH was greater (to less than 7.2 after 30 minutes and to near 7.0 after 60 minutes of infusion).

The finding that hypercapnia is more effective in stimulating renin secretion than hypoxia is consistent
with the results of studies in dogs and rats.9,25 In adult dogs anesthetized with sodium pentobarbital, renin secretion was stimulated by hypoxia and by hypercapnia.9 It is possible that aortic chemoreceptor afferent fibers are important in this regard, or it is possible that some other mechanism mediates the response. It is unlikely that hypoxia had any direct effect on the kidney to secrete renin.26 It is also now well established that hypoxia does not decrease angiotensin converting enzyme activity.16,27

The efferent mechanism generating the renin response to asphyxia may be a combination of sympathetic and sympathoadrenal inputs to the kidney. The study of Robillard et al28 on the effect of unilateral renal denervation on the renal responses to hypoxia suggests a possible role for renal efferent nerves in the control of fetal renal function and possibly renin secretion. The relative roles of the aortic and carotid sinus chemoreceptor afferent fibers in the control of renin secretion in the fetal sheep remains to be tested. Furthermore, it is possible that there is some interaction between arterial chemoreceptors and baroreceptors in the control of the renin responses to hypoxia. Because hypoxia increases fetal blood pressure and because increases in fetal blood pressure are known to inhibit fetal renin secretion,29 renin secretion during hypoxia may represent the sum of stimulation by hypoxia and inhibition by increased arterial blood pressure. It is possible that control of fetal arterial blood pressure during the period of hypoxia may allow a larger PRA response.

Renin Responses in Ewes

In adult rats,25 a 20-minute exposure to normocapnic hypoxia (Pao2 = 34 ± 1 mm Hg) was effective in increasing plasma ACTH and aldosterone concentrations, but not PRA. Hypercapnia (Paco2 = 55 ± 2 and 72 ± 2 mm Hg during exposure to FiCO2 of 4% and 8%) also was ineffective in stimulating PRA. The higher level of Paco2 did increase plasma ACTH and aldosterone concentrations. Only hypoxia plus hypercapnia increased PRA as well as plasma ACTH and aldosterone concentrations.

In the present study, the pregnant ewes did not respond to hypoxia, hypercapnia, or hypoxia plus hypercapnia with increased PRA or Ang II concentration. Only plasma aldosterone concentration was increased during hypercapnia (from -30 to 0 minutes) in the hypoxia plus hypercapnia (HC/HX) study group. In the ewes receiving hypercapnia alone (HC/ HC), aldosterone appeared to increase between -30 and 0 minutes, although the apparent increase was not statistically significant. The relative degree of hypoxia and/or hypercapnia was not great in these experiments. Nevertheless, it is interesting that these ewes responded to a small increase in Paco2 (3.8 - 9.1 mm Hg) with a borderline aldosterone response, whereas the conscious rats of Raff and Roarty25 responded with increased aldosterone only after PaCO2 was increased approximately 30 mm Hg. The increase in aldosterone in the ewes exposed to hypercapnia before hypoxia (HC/HX) was probably secondary to increased maternal plasma ACTH concentration3 and/or increased plasma K+ (Table 3, HC/ HX at 0 minutes).

Heart Rate

The results of the present study confirm previous results implicating the role of the peripheral chemoreceptors in the control of the heart rate response to asphyxia.11 As shown in Figure 6 (left), normocapnic hypoxia decreased fetal heart rate. This response was abolished by sinoaortic denervation (Figure 6, right). These results suggest that hypoxic cardiodeceleration in the fetus, known to be a parasympathetic efferent mechanism,30 is mediated by afferents carried in the carotid sinus and/or aortic depressor nerves.

There was a small increase in heart rate during hypercapnia alone (HC/HX) in the intact but not in the sinoaortic denervated fetuses. This suggests that a large proportion of the chronotropic effects of hypercapnia in the fetus is mediated by peripheral chemoreceptors. However, direct stimulation of central chemoreceptors in fetal sheep increases fetal heart rate.31 Interestingly, hypoxia superimposed on hypercapnia (HC/HX) again decreased heart rate to nadir levels similar to those observed during hypoxia alone (NX/HX). This might represent the sum of reflex bradycardia during hypoxia and a possible direct effect of the asphyxia on the heart itself, since in the sinoaortic denervated group heart rate tended to decrease slightly during hypoxia plus hypercapnia.

Conclusions

We conclude from the results of these experiments that hypercapnia is a more potent stimulus to fetal renin secretion than is hypoxia and that there appears to be no interaction between hypoxia and hypercapnia in the control of fetal renin secretion. The receptors responsible for this response appear to be mainly the peripheral chemoreceptors. The best predictor of PRA during hypoxia and/or hypercapnia was the change in pH, in intact fetuses. This relation was eliminated by sinoaortic denervation, suggesting that the afferent mediators of the PRA responses to changes in pH, are the peripheral chemoreceptors.

Acknowledgments

We gratefully acknowledge the gift of anti-angiotensin I antiserum from M. Ian Phillips, DSc, Department of Physiology, University of Florida, and of anti-angiotensin II antiserum from Ian A. Reid, PhD, Department of Physiology, University of California, San Francisco. We thank Dr. Hong-Gen Chen and Ms. Ellen Manlove for help in running these experiments.

References


**KEY WORDS** renin • angiotensin II • aldosterone • blood pressure • heart rate • chemoreceptors
Peripheral chemoreceptor control of fetal renin responses to hypoxia and hypercapnia.
C E Wood, C Kane and H Raff

Circ Res. 1990;67:722-732
doi: 10.1161/01.RES.67.3.722

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

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/67/3/722

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/