Exogenous and endogenous corticosteroids modulate blood-brain barrier development in the ovine fetus

BARBARA S. STONESTREET,1 GRAZYNA B. SADOWSKA,1 AMANDA J. MCKNIGHT,1 CLIFFORD PATLAK,2 AND KATHERINE H. PETERSSON1

1Department of Pediatrics, Women and Infants Hospital of Rhode Island, Brown University School of Medicine, Providence, Rhode Island 02905; and 2Department of Surgery, State University of New York at Stony Brook, Stony Brook, New York 11794-8191

Received 1 December 1999; accepted in final form 25 February 2000

Exogenous and endogenous corticosteroids modulate blood-brain barrier development in the ovine fetus. Am J Physiol Regulatory Integrative Comp Physiol 279: R468–R477, 2000.—We previously reported decreases in blood-brain barrier permeability in the ovine fetus at 80% of gestation after antenatal corticosteroids and shown that permeability is not reduced in newborn lambs after postnatal corticosteroids. We now test the hypotheses that exogenous antenatal corticosteroids decrease blood-brain barrier permeability at 60% but not 90% of gestation in ovine fetuses and that endogenous increases in plasma cortisol concentrations are associated with ontogenic decreases in barrier permeability during gestation. Chronically instrumented ovine fetuses were studied 12 h after the last of four 6-mg dexamethasone or placebo injections were given 12 h apart over 48 h to ewes. Fetuses at 80% of gestation from placebo-treated ewes studied under the same protocol were also included. Blood-brain barrier function was quantified with the blood-to-brain transfer constant (Kb) to α-aminoisobutyric acid. Kb values were lower in cerebral cortex, caudate nucleus, hippocampus, superior colliculus, thalamus, medulla, and cervical spinal cord in fetuses of dexamethasone- than placebo-treated ewes at 60% but not 90% of gestation. Regional brain Kb values demonstrated inverse correlations with increases in gestation and plasma cortisol concentrations in most brain regions. We conclude that maternal treatment with exogenous corticosteroids was associated with decreases in blood-brain barrier permeability at 60% but not 90% of gestation and that increases in gestation and endogenous cortisol concentrations were associated with ontogenic decreases in barrier permeability during fetal development.

alpha-aminoisobutyric acid; brain; cortisol; gestation; maturation; sheep; steroids

the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

MATERNALLY ADMINISTERED ANTENATAL corticosteroids have been used widely to reduce the incidence of respiratory distress syndrome in low birth weight infants (9). This therapy also has been shown to facilitate the transition from fetal to neonatal life by beneficial effects on other organ systems (1, 19, 26, 27, 31). Antenatal steroid administration has been reported to have an important role in lowering the risk of early onset and severe intraventricular hemorrhage in premature infants (13, 21, 30). These effects might be explained in part by accelerated vascular maturation.

The blood-brain barrier is composed of a continuous layer of cerebrovascular endothelial cells connected by intercellular tight junctions (2, 4, 6, 15). This specialized barrier serves as an interface among the circulating blood, brain interstitium, and parenchyma, isolating brain tissue from blood constituents. Therefore, the blood-brain barrier maintains central nervous system (CNS) homeostasis by preventing entry of substances that might alter neuronal function in the CNS.

The development of the blood-brain barrier is an important component of brain maturation. The blood-brain barrier is a selectively permeable barrier, which may potentially change during development. We previously have demonstrated ontogenic decreases in blood-brain barrier permeability to α-aminoisobutyric acid (AIB) from 60% of fetal gestation through the neonatal period up to maturity in adult sheep (33). We also have shown that the blood-brain barrier is relatively impermeable to AIB in most brain regions of fetuses and lambs (33). Because the blood-brain barrier is relatively impermeable in the fetus and newborn, the barrier also protects the developing brain from factors that could impair neuronal function.

Evidence in adult subjects suggests that the blood-brain barrier is under hormonal control (16, 20, 24, 40). In adult rats adrenalectomy increases blood-brain barrier permeability, and corticosterone replacement reverses this effect on the barrier (20). These findings suggest that the pituitary-adrenal cortical axis may function as a physiological regulator of barrier function (20). In addition, in adult rodents, pharmacological doses of dexamethasone have been reported to reduce barrier permeability (16, 28, 40).

In the prenatal and perinatal periods, damage to the blood-brain barrier might potentially result from hypoxic-ischemic brain injury and/or intraventricular hemorrhage. In addition, in this setting blood-brain barrier damage might potentially render premature
infants with hyperbilirubinemia more susceptible to brain injury. Consequently, we examined the effects of maternal treatment with antenatal steroids on blood-brain barrier function in the fetus (34). Consistent with findings in adult rodents, we recently have reported decreases in blood-brain barrier permeability in the ovine fetus at 80% of gestation after antenatal corticosteroids had been administered to pregnant ewes (34). In contrast, we have shown that postnatal corticosteroids do not reduce blood-brain barrier permeability in newborn lambs (35). It remains to be determined whether the decreases in blood-brain barrier permeability after maternal treatment with antenatal corticosteroids are present throughout fetal development or whether there is a time before birth when the barrier is no longer responsive to this maternal treatment. It is well known that the pituitary-adrenal cortical axis matures during fetal development and that endogenous cortisol concentrations increase particularly in the latter part of gestation (39). Based on the findings in adult rodents suggesting that the pituitary-adrenal cortical axis may function as a physiological regulator of the blood-brain barrier, it is also likely that developmental changes in this axis might affect barrier function in the fetus.

Given the above considerations, there is evidence to suggest that maternally administered exogenous corticosteroids might exert age-related differential effects on the developing barrier and that the pituitary-adrenal cortical axis may function as a physiological regulator of barrier function in the fetus. Therefore, in the present study, we tested the hypotheses that 1) antenatal corticosteroids decrease blood-brain barrier permeability in early and not in late gestation ovine fetuses and 2) increases in endogenous plasma cortisol concentrations are associated with developmental decreases in blood-brain barrier permeability in the fetus. To test these hypotheses, we examined the effects of maternal antenatal corticosteroid treatment on blood-brain barrier permeability early (60%) and late (90% of gestation) in ovine fetal development. We also examined the association between increases in endogenous plasma cortisol concentrations and decreases in barrier permeability during fetal development.

MATERIALS AND METHODS

This study was conducted after approval by the Institutional Animal Care and Use Committees of Brown University and Women and Infants Hospital of Rhode Island and according to the Guide For the Care and Use of Laboratory Animals, National Institutes of Health.

Animal preparation. As previously described in detail (32, 33), surgery was done under 1–2% halothane anesthesia on 19 mixed breed ewes at 82–83 days, 6 at 112–113 days, and 17 at 128–129 days of gestation. Singleton and twin pregnancies were included. When a twin gestation was present, the surgery and study were performed on one fetus. Briefly, in the fetuses, polyvinyl catheters were placed into a brachial vein for isotope administration and into the thoracic aorta via the brachial artery for blood sample withdrawal and heart rate and blood pressure monitoring. The surgery was modified slightly in the fetuses at 60% of gestation. In this group, catheters were placed in the subclavian vein and artery and advanced to the thoracic aorta (33). An amniotic fluid catheter was placed for pressure monitoring and to correct fetal arterial blood pressures. A femoral artery catheter was also placed in the ewes.

The six fetuses operated on at 112–113 days of gestation were part of a previous report (34). They have been included here to examine the association between increases in endogenous corticosteroids and barrier development in the fetus (see Figs. 3–5). This was done to limit unnecessary animal usage and was justified because those studies were performed within the same time frame as the ones in the current report using identical methods and study design.

After 2–6 days of recovery from surgery, the ewes were randomly assigned to receive either four 6-mg doses of dexamethasone (4 mg/ml; Fujisawa, Deerfield, IL) or placebo (0.9% NaCl) given as intramuscular injections every 12 h for 48 h. The final injection was given 12 h before the onset of the studies. The percent of gestation and treatment groups, number of animals in each group, and duration of recovery from surgery to dexamethasone or placebo treatment are summarized in Table 1.

Experimental protocol and methodology. Four to eight days after recovery from surgery at 87–90 days or 60%, 117–120 days or 60%, 135–137 days or 90% of gestation, the studies were performed. The fetuses were studied while the ewes were standing quietly in a cart after being acclimatized to the laboratory for 2 h. Blood-brain barrier function was measured in the fetuses with [14C]AIB (Dupont-NEN, Boston, MA). The blood-to-brain transfer constant (K<sub>i</sub>) was measured as previously described (10, 25, 33, 34). After baseline physiological determinations were obtained, [14C]AIB was rapidly injected intravenously and arterial plasma concentrations were obtained at fixed times before and after injection as follows: −1, 0.5, 1, 2, 3, 5, 7, 15, 30, 45, 60 min and at termination within 8–10 min after the end of the study. On the basis of our previous analysis of rate constants and exposure times for tracers in adult rats (10) and mathematical analysis of AIB in fetal sheep, the ~60-min interval and this sampling regimen were determined to accurately characterize the plasma profile needed for calculation of the blood-to-brain K<sub>i</sub> (33, 34). Brain parenchymal tracer concentration was determined at the end of the experiment. Knowledge of the plasma concentration profile and the concentration of tracer in the parenchyma allows calculation of the blood-to-brain K<sub>i</sub> as described by Ohno et al. (25). In our experiments, the unidirectional K<sub>i</sub> was quantified for [14C]AIB in the fetal sheep. Brain vascular volume was determined by giving [14C]polyethylene glycol (PEG; American, UK) 2 min before the end of the experiment to fetuses of separate dexamethasone- and placebo-treated ewes at each gestational age examined. The brain vascular volume values did not differ between the fetuses of dexamethasone-

### Table 1. Study groups

<table>
<thead>
<tr>
<th>Gestation, %</th>
<th>Treatment Group</th>
<th>No./Group</th>
<th>Recovery From Surgery to Dexamethasone or Placebo Treatment, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>Dexamethasone</td>
<td>9</td>
<td>3(2–5)</td>
</tr>
<tr>
<td>60</td>
<td>Placebo</td>
<td>10</td>
<td>3(2–5)</td>
</tr>
<tr>
<td>80</td>
<td>Placebo</td>
<td>6</td>
<td>3(2–5)</td>
</tr>
<tr>
<td>90</td>
<td>Dexamethasone</td>
<td>9</td>
<td>5(5–6)</td>
</tr>
<tr>
<td>90</td>
<td>Placebo</td>
<td>8</td>
<td>5(5–6)</td>
</tr>
</tbody>
</table>

Nos. in parentheses are range.
and placebo-treated ewes and were similar to our previous values (33).

AIB is a synthetic amino acid that is not present in mammalian tissues. This amino acid has been used extensively to measure accurately the total and regional blood-brain barrier permeability in a variety of mammals and fetal sheep (3, 33, 34, 38, 40). The fetuses at 87–90 days of gestation received 50 μCi/kg of [14C]AIB or 60 μCi of [14C]PEG, at 117–120 days of gestation they received 60 μCi of [14C]AIB or 90 μCi of [14C]PEG, and at 135–137 days of gestation they received 90–130 μCi of [14C]AIB or 80–100 μCi of [14C]PEG. These isotope doses were selected based on our previous experience and varied slightly in the fetuses at 90% of gestation depending on the estimated fetal weight at surgery (33, 34).

At the end of the study the ewe was given intravenous pentobarbital sodium (15–20 mg/kg) to achieve a surgical plane of anesthesia. A hysterotomy was performed, and the fetus was withdrawn from the uterus with the umbilical circulation intact and decapitated to immediately terminate blood flow to the brain. The brain was removed within 8–10 min for regional brain tissue samples. The weight of the fetus was determined. The ewe was then killed with pentobarbital sodium (100–200 mg/kg).

The brains were dissected into the following regions: cerebral cortex, cerebellum, caudate nucleus, hippocampus, superior colliculus, inferior colliculus, thalamus, pons, medulla, and cervical spinal cord. The cerebral cortex was further divided into the frontal, parietal, and occipital cortices. Additional regions included the perior colliculus, inferior colliculus, thalamus, pons, medulla, and cervical spinal cord. The cerebral cortex was further divided into the frontal, parietal, and occipital cortices. Additional regions included the perior colliculus, inferior colliculus, thalamus, pons, medulla, and cervical spinal cord.

Tissue samples were treated as previously described (10, 33, 34). Briefly, Solvable (Packard Instruments, Downers Grove, IL) was added to the vials containing the tissue samples, and they were placed in a shaking water bath at 50°C overnight. Tissue sample decoloration was achieved through we did not dissect germinal matrix separately, the cerebral cortex, cerebellum, caudate nucleus, hippocampus, superior colliculus, inferior colliculus, thalamus, pons, medulla, and cervical spinal cord. The cerebral cortex was further divided into the frontal, parietal, and occipital cortices. Although we did not dissect germinal matrix separately, the cerebral cortex represents the anatomic location of the germinal matrix in the floor of the lateral ventricles. This area was included in our dissection of the caudate nucleus.

Tissue samples were treated as previously described (10, 33, 34). Briefly, Solvable (Packard Instruments, Downers Grove, IL) was added to the vials containing the tissue samples, and they were placed in a shaking water bath at 50°C overnight. Tissue sample decoloration was achieved through we did not dissect germinal matrix separately, the cerebral cortex, cerebellum, caudate nucleus, hippocampus, superior colliculus, inferior colliculus, thalamus, pons, medulla, and cervical spinal cord. The cerebral cortex was further divided into the frontal, parietal, and occipital cortices. Although we did not dissect germinal matrix separately, the cerebral cortex represents the anatomic location of the germinal matrix in the floor of the lateral ventricles. This area was included in our dissection of the caudate nucleus.

The blood-to-brain $K_i$ (µl·g brain$^{-1}$·min$^{-1}$) is given by

$$K_i = A_{tr} \int_0^t c_p(t) \, dt$$

where $A_{tr}$ is the amount of tracer that crossed the blood-brain barrier from blood to brain during the tracer study (dpm/g) and $c_p$ is the concentration of tracer in plasma (dpm/µl) at the time $t$ (min). $A_{tr}$ is obtained by correcting the total amount of isotope measured in the tissue $A_{tr}$ (dpm/g) for that residual part remaining in the brain vasculature space, which is measured by the [14C]PEG. Thus $A_{tr} = A_m - V_p c_p$, where $V_p$ is the plasma volume of brain tissue (µl/g) and $c_p$ is the concentration of tracer in the terminal plasma sample (dpm/g). $V_p = \frac{A_{tr}}{c_p}$, where $A_m$ and $c_p$ have the same definitions as $A_m$ and $c_p$, above except that they apply to [14C]PEG (10).

Arterial pH, blood gases, hematocrit, heart rate, and mean arterial blood pressure were measured on the fetuses and ewes at baseline, 30 min, and 50 min of study. Arterial plasma osmolality, glucose, insulin, and cortisol concentrations were measured on the fetuses and ewes before the end of the study. Blood removed for study sampling was not replaced because the maximum amount of blood withdrawn for any study was <6% of the fetal blood volume in the three age groups. Heart rates, mean arterial blood, and amniotic fluid pressures in fetal sheep, and heart rates and mean arterial blood pressures in the ewes were measured with pressure transducers (model 1280 C; Hewlett Packard, Lexington, MA) and recorded on a polygraph (model 17758 B series, Hewlett Packard). Blood gases and pH were measured on a Corning blood gas analyzer (model 238; Corning Scientific, Medford, MA) at 39.5°C in fetuses and 38.5°C in ewes. Hematocrit was measured in duplicate by the microhematocrit method. Plasma osmolality was measured in duplicate on a vapor pressure osmometer (Vapro model 5520; Wescor, Logan, UT), glucose on a glucose analyzer (YSI 2300; STAT, Yellow Springs, OH). Insulin concentrations were measured in duplicate using the Coat-A-Count Insulin, a solid phase 125I-radioimmunoassay (DPC, Los Angeles, CA). The Coat-A-Count Insulin antiserum exhibits 100% cross-reactivity with insulin. The observed coefficient of variation for inter- and intra-assay precision were 6.1% and 6.3%, respectively. Cortisol concentrations were measured in duplicate using Clinical Assays GammaCoat Cortisol 125I-radioimmunoassay (DiaSorin, Stillwater, MN). The GammaCoat antiserum exhibits 100% cross-reactivity with cortisol. The observed coef-

Table 2. Physiological and hormonal variables of fetuses of the dexamethasone- and placebo-treated ewes

<table>
<thead>
<tr>
<th>Variable</th>
<th>60% Gestation</th>
<th>90% Gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dexamethasone</td>
<td>Placebo</td>
</tr>
<tr>
<td>pH</td>
<td>7.35 ± 0.06</td>
<td>7.36 ± 0.02†</td>
</tr>
<tr>
<td>PaO₂, mmHg</td>
<td>29 ± 7</td>
<td>26 ± 3*</td>
</tr>
<tr>
<td>PaCO₂, mmHg</td>
<td>46 ± 3</td>
<td>48 ± 4</td>
</tr>
<tr>
<td>Base excess, meq/l</td>
<td>−0.2 ± 5</td>
<td>0.9 ± 3</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>32 ± 5</td>
<td>34 ± 5*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>200 ± 12</td>
<td>196 ± 134*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>44 ± 6*</td>
<td>34 ± 9*</td>
</tr>
<tr>
<td>Osmolality, mosmol/kgH₂O</td>
<td>296 ± 8</td>
<td>293 ± 5</td>
</tr>
<tr>
<td>Cortisol, ng/ml</td>
<td>6 ± 1</td>
<td>7 ± 1*</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>54 ± 15*</td>
<td>33 ± 6*</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>21 ± 7*</td>
<td>13 ± 4*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 9 in dexamethasone group and 10 in placebo group at 60% gestation; n = 8 in the dexamethasone group and 9 in the placebo group at 90% gestation. PaO₂ and PaCO₂, arterial oxygen and carbon dioxide tension, respectively; MAP, mean arterial blood pressure. *P < 0.05 vs. value of placebo-treated group. †P < 0.05 vs. value of placebo-treated group at 90% gestation.
CARTEROSTERDOIDS AND BLOOD-BRAIN BARRIER DEVELOPMENT

Table 3. Physiological and hormonal variables of the dexamethasone- and placebo-treated ewes

<table>
<thead>
<tr>
<th>Variable</th>
<th>60% Gestation</th>
<th>90% Gestation</th>
<th>60% Gestation</th>
<th>90% Gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.55 ± 0.04*</td>
<td>7.47 ± 0.06</td>
<td>7.55 ± 0.03*</td>
<td>7.49 ± 0.02</td>
</tr>
<tr>
<td>PaCO2, mmHg</td>
<td>93 ± 2</td>
<td>100 ± 5</td>
<td>99 ± 9</td>
<td>95 ± 9</td>
</tr>
<tr>
<td>PaO2, mmHg</td>
<td>35 ± 3</td>
<td>37 ± 7</td>
<td>32 ± 2</td>
<td>34 ± 2</td>
</tr>
<tr>
<td>Base excess, meq/l</td>
<td>9.4 ± 4.2</td>
<td>4.4 ± 2</td>
<td>7.4 ± 3*</td>
<td>4.0 ± 2</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>28 ± 3</td>
<td>28 ± 5</td>
<td>29 ± 3</td>
<td>30 ± 3</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>81 ± 13</td>
<td>75  ± 8*</td>
<td>119 ± 25</td>
<td>106 ± 32</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>91 ± 10</td>
<td>88 ± 21</td>
<td>87 ± 8</td>
<td>80 ± 5</td>
</tr>
<tr>
<td>Osmolality, mosmol/kgH2O</td>
<td>299 ± 8</td>
<td>295 ± 5</td>
<td>303 ± 7</td>
<td>297 ± 6</td>
</tr>
<tr>
<td>Cortisol, ng/ml</td>
<td>117 ± 22*</td>
<td>85 ± 15†</td>
<td>110 ± 31*</td>
<td>72 ± 9</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>37 ± 19*</td>
<td>22 ± 10†</td>
<td>31 ± 26</td>
<td>11 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 9 in dexamethasone group and 10 in placebo group at 60% gestation; n = 8 in the dexamethasone group and 9 in the placebo group at 90% gestation. *P < 0.05 vs. value of placebo-treated group. †P < 0.05 vs. value of placebo-treated group at 90% gestation.

One-way ANOVA was used to compare plasma cortisol concentrations among the fetuses of the placebo-treated ewes at 60, 80, and 90% of gestation (see Fig. 3). If a significant difference was found by one-way ANOVA, the Newman–Keuls post hoc test was used to identify specific differences among the groups for plasma cortisol concentrations. The $K_i$ values of the brain regions were compared with plasma cortisol concentrations using the least-squares method (see Figs. 4 and 5). Because plasma cortisol concentrations increased during gestation (see Fig. 3), the $K_i$ values of the brain regions were also compared with gestational age. Multiple regression partial correlational analyses were used to compare the relative strength of the associations between increases in gestation and plasma cortisol concentrations and changes in regional brain $K_i$ during fetal development (Table 4). $P < 0.05$ was considered statistically significant.

RESULTS

The fetuses of the dexamethasone- and placebo-treated ewes were 88 ± 1 and 88 ± 1 days, and 136 ± 1 and 137 ± 1 days of gestation at the time of study, respectively. The weights of the fetuses of the dexamethasone-treated ewes did not differ from those of the placebo-treated ewes at 60% (0.59 ± 0.11 vs. 0.63 ± 0.09 kg) or 90% (4.23 ± 0.72 vs. 3.66 ± 0.63 kg) of gestation. In the dexamethasone-treated ewes 1 of 9 fetuses of the dexamethasone- and placebo-treated ewes and the ewes at 60 and 90% of gestation.

Table 4. Summary of multiple regression: partial correlational analyses comparing regional $K_i$ to gestational age and endogenous plasma cortisol concentrations

<table>
<thead>
<tr>
<th>Regional $K_i$</th>
<th>R</th>
<th>P</th>
<th>Partial Correlation Gestational Age</th>
<th>P</th>
<th>Partial Correlation Cortisol Concentration</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>0.69</td>
<td>0.001</td>
<td>-0.33</td>
<td>0.12</td>
<td>-0.30</td>
<td>0.16</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>0.53</td>
<td>0.003</td>
<td>0.02</td>
<td>0.93</td>
<td>-0.40</td>
<td>0.06</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.82</td>
<td>0.001</td>
<td>-0.46</td>
<td>0.03</td>
<td>-0.43</td>
<td>0.04</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.60</td>
<td>0.010</td>
<td>-0.08</td>
<td>0.72</td>
<td>-0.39</td>
<td>0.07</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.79</td>
<td>0.001</td>
<td>-0.43</td>
<td>0.04</td>
<td>-0.41</td>
<td>0.05</td>
</tr>
<tr>
<td>Superior colliculus</td>
<td>0.66</td>
<td>0.002</td>
<td>-0.14</td>
<td>0.54</td>
<td>-0.42</td>
<td>0.04</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>0.65</td>
<td>0.003</td>
<td>0.03</td>
<td>0.90</td>
<td>-0.50</td>
<td>0.01</td>
</tr>
<tr>
<td>Pons</td>
<td>0.56</td>
<td>0.021</td>
<td>0.002</td>
<td>0.99</td>
<td>-0.40</td>
<td>0.06</td>
</tr>
<tr>
<td>Medulla</td>
<td>0.76</td>
<td>0.001</td>
<td>-0.25</td>
<td>0.25</td>
<td>-0.49</td>
<td>0.02</td>
</tr>
<tr>
<td>Cervical spinal cord</td>
<td>0.94</td>
<td>0.001</td>
<td>-0.82</td>
<td>0.001</td>
<td>-0.46</td>
<td>0.03</td>
</tr>
</tbody>
</table>
and in the placebo-treated ewes 2 of 10 pregnancies were twins at 60% of gestation ($\chi^2$, not significant (ns)). In the dexamethasone-treated ewes 4 of 9 and in the placebo-treated ewes 5 of 8 pregnancies were twins at 90% of gestation ($\chi^2$, ns). The numbers of twin pregnancies also did not differ between the ewes at 60 and 90% of gestation ($\chi^2$, ns). None of the treated ewes developed premature labor.

Arterial pH, oxygen tension, carbon dioxide tension, base excess, hematocrit, plasma osmolality, and cortisol concentrations did not differ between the fetuses of dexamethasone- and placebo-treated ewes at 60 or 90% of gestation (Table 2). Heart rate was higher in the fetuses of dexamethasone- than the placebo-treated ewes at 90% but not 60% of gestation. Mean arterial blood pressure was higher in the fetuses of dexamethasone- than the placebo-treated ewes at 60% but not 90% of gestation. Arterial plasma glucose and insulin concentrations were higher in the fetuses of the dexamethasone- than the placebo-treated ewes at 60 and 90% of gestation. Arterial pH oxygen tension, heart rate, and plasma glucose and insulin concentrations were higher, and hematocrit, mean arterial blood pressure, and plasma cortisol concentration were lower in the fetuses of the placebo-treated ewes at 60 than at 90% of gestation (Table 2).

Arterial oxygen tension, carbon dioxide tension, hematocrit, heart rate, mean arterial blood pressure, and plasma osmolality did not differ between the dexamethasone- and the placebo-treated ewes at 60 or 90% of gestation (Table 3). Whereas, the arterial pH and plasma glucose concentrations were higher and the plasma cortisol concentrations were lower in the dexamethasone- than the placebo-treated ewes at 60 and 90% of gestation. The arterial base excess, carbon dioxide tension, oxygen tension, heart rate, and plasma cortisol concentration were lower in the fetuses of the placebo-treated ewes at 60% but not 90% of gestation. Arterial plasma glucose and insulin concentrations were higher in the fetuses of the dexamethasone- than the placebo-treated ewes at 60% and 90% of gestation. Heart rate was higher in the dexamethasone- than the placebo-treated ewes at 60 but not 90% of gestation. The arterial base excess was higher in the dexamethasone- than the placebo-treated ewes at 90% of gestation. Heart rate was lower, and plasma glucose and insulin concentrations were higher in the placebo-treated ewes at 60% than at 90% of gestation. The arterial pH, oxygen tension, carbon dioxide tension, base excess, hematocrit, heart rate, and mean arterial blood pressure values of fetuses and ewes did not change during the 1-h study in any group.

The regional brain $K_i$ values for AIB in fetuses of the dexamethasone- and placebo-treated ewes are illustrated at 60 (Fig. 1A) and 90% (Fig. 1B) of gestation. The $K_i$ values in the cerebral cortex, caudate nucleus, hippocampus, thalamus, superior colliculus, medulla, and cervical spinal cord were lower at 60% of gestation in the fetuses of the dexamethasone- than in the placebo-treated ewes (ANOVA, main effects for dexamethasone treatment: $F = 6.17$, $P < 0.025$, Fig. 1A). The magnitude of the decreases in permeability was also not uniform across the cerebral cortical regions. In contrast, the $K_i$ values did not differ in the brain regions at 90% of gestation between the fetuses of the dexamethasone- and the placebo-treated ewes (ANOVA, main effects for dexamethasone treatment: $F = 0.73$, $P = 0.41$, Fig. 1B). The $K_i$ values were also lower in the parietal cortex at 60% of gestation in the fetuses of the dexamethasone- than the placebo-treated ewes (ANOVA, main effects for dexamethasone treatment: $F = 13.88$, $P < 0.01$, Fig. 2). The magnitude of the decreases in permeability was also not uniform across the cerebral cortical regions. In contrast, the $K_i$ values did not differ in the cerebral cortical regions at 90% of gestation between the fetuses of the dexamethasone- and the placebo-treated ewes (ANOVA, main effects for dexamethasone treatment: $F = 0.41$, $P = 0.53$).

Endogenous plasma cortisol concentrations were (ANOVA: $F = 19.60$, $P < 0.01$) higher at 90% than at 60 and 80% of gestation in the fetuses of the placebo-treated ewes (Fig. 3). The $K_i$ values for AIB in the fetuses of the placebo-treated ewes demonstrated in-
verse relationships with both increases in gestation and endogenous plasma cortisol concentrations in the cerebral cortex, cerebellum, medulla, caudate nucleus, hippocampus, thalamus, superior colliculus, inferior colliculus, pons, and cervical spinal cord. $K_i$ values for AIB in the fetuses of the placebo-treated ewes are plotted against gestational age and plasma cortisol concentrations for the cerebral cortex (Fig. 4A), cerebellum (Fig. 4B), and medulla (Fig. 4C) and for the hippocampus (Fig. 5A), thalamus (Fig. 5B), and cervical spinal cord (Fig. 5C). The $K_i$ values for AIB for the brain regions that are not illustrated demonstrated similar inverse relationships with both increases in gestation and endogenous plasma cortisol concentrations: caudate nucleus (gestation: $r = -0.39$, $n = 24$, $P < 0.01$; plasma cortisol concentrations: $r = -0.53$, $n = 24$, $P < 0.01$), superior colliculus (gestation: $r = -0.56$, $n = 24$, $P < 0.01$; plasma cortisol concentrations: $r = -0.65$, $n = 24$, $P < 0.01$), inferior colliculus (gestation: $r = -0.47$, $n = 24$, $P < 0.02$; plasma cortisol concentrations: $r = -0.65$, $n = 24$, $P < 0.01$), and pons (gestation: $r = -0.42$, $n = 24$, $P < 0.05$; plasma cortisol concentrations: $r = -0.56$, $n = 24$, $P < 0.01$).

In Table 4 the multiple regression correlation coefficients $R$ and the partial correlation coefficients are summarized for regional brain $K_i$ values vs. gestational age and plasma cortisol concentrations. The multiple regression partial correlational analysis compared the relative strength of the association between the increases in gestation and plasma cortisol concentrations
CORTICOSTEROIDS AND BLOOD-BRAIN BARRIER DEVELOPMENT

Fig. 5. $K_i$ values for AIB in the fetuses of the placebo-treated ewes plotted against gestational age and plasma cortisol concentrations of the hippocampus (A), thalamus (B), and cervical spinal cord (C): hippocampus (gestation: $r = -0.77, P < 0.01$; plasma cortisol concentrations: $r = -0.76, P < 0.01$), thalamus (gestation: $r = -0.75, P < 0.01$; plasma cortisol concentrations: $r = -0.74, P < 0.01$), and cervical spinal cord (gestation: $r = -0.93, P < 0.01$; plasma cortisol concentrations: $r = -0.81, P < 0.01$).

and the decreases in regional brain $K_i$ values. This analysis demonstrated relatively equal strengths of association between the increases in gestational age and plasma cortisol concentrations and the decreases in $K_i$.

DISCUSSION

The novel findings of the present study are that maternally administered exogenous antenatal corticosteroids reduced blood-brain permeability early but not late in fetal development and that increases in endogenous plasma cortisol concentrations were associated with decreases in regional blood-brain barrier permeability during fetal development. The present findings combined with those in our previous report (34) can be interpreted to suggest that there is an age-related differential responsiveness of the fetal blood-brain barrier to maternally administered corticosteroids.

Dexamethasone was used because it is one of the most extensively studied corticosteroids for accelerating fetal maturation, has been used widely in studies of the CNS, and is also used to treat CNS disorders (5, 12, 16, 18, 23, 24, 40). The dose of dexamethasone was based on current recommendations for fetal maturation in pregnant women who are in premature labor (23). Although optimal effects from a complete course of antenatal corticosteroids begin 24 h after the last dose, even short-term exposure to fetal or maternal treatment has been shown to alter cardiovascular and renal function in preterm sheep (1, 27). In addition, women in premature labor often deliver less than 24 h after a complete course of corticosteroids. Therefore, the dexamethasone dose and treatment regimen in this study were similar to those used in pregnant women for fetal maturation and were selected to achieve near maximal corticosteroid effects, while minimizing the risk of premature labor (11, 19, 23).

Maternal antenatal dexamethasone treatment affected the hormonal and metabolic homeostasis of the ewes and the biochemical, metabolic, hormonal, and hemodynamic homeostasis of the fetal sheep. In the ewes, the lower plasma cortisol and higher glucose concentrations suggest that dexamethasone had suppressed the adrenocortical axis and caused glucose intolerance both at 60 and 90% of gestation (7, 34). Likewise, in the fetuses, the higher glucose and insulin concentrations also suggest glucocorticoid-induced glucose intolerance was present at both gestational ages. The dysregulation of fetal glucose homeostasis could have been a result of corticosteroid-induced insulin resistance, hepatic gluconeogenesis, and/or the elevated glucose concentrations in the ewes (37).

Antenatal corticosteroids have been shown to enhance cardiovascular stability and elevate blood pressure in premature lambs (1, 27). Continuous direct infusions of corticosteroids also increase blood pressure in fetal sheep (11, 36). The mean arterial blood pressure values in the fetuses of the dexamethasone-treated ewes were higher than those of the placebo-treated ewes at 60% but not 90% of gestation. The increase in blood pressure observed at 60% of gestation is consistent with previous reports using other antenatal and fetal corticosteroid regimens but differ from our findings at 80% of gestation in which fetal blood pressure was not elevated after the same treatment (1, 11, 27, 34). The increase in blood pressure at 60% and not 90% of gestation and the increase in heart rate at 90% and not 60% of gestation suggest that the fetal hemodynamic responses to exogenous corticosteroids are dependent on the specific hemodynamic measure, treatment regimen, and time in gestation when corticosteroids are given (1, 11, 27, 34). Moreover, at least for the dose and treatment regimen of dexamethasone in this study, age-related differential responses were observed with regard to cardiovascular effects and decreases in regional blood-brain barrier permeability (Figs. 1 and 2), but not for dysregulation of fetal glucose homeostasis (Table 2). These findings emphasize that the effects of maternally administered exogenous corticosteroids may differ depending on the fetal response evaluated and the time in gestation when they are given.
It is also important to point out that although acute hypertension has been reported to result in increases in blood-brain barrier permeability, the increase in blood pressure at 60% of gestation was relatively small and not in the range that would be expected to affect barrier permeability (14).

The findings of the present study suggest that a course of antenatal corticosteroids, similar to that which is used to treat pregnant women in premature labor, reduced blood-brain permeability early but not late in fetal development. Considered together with our previous work (34), these findings suggest that antenatal corticosteroids reduce blood-brain barrier permeability at 60 and 80% of gestation but not in near-term fetal sheep at 90% of gestation. Therefore, our results support the concept that exogenous corticosteroids enhanced cerebral microvascular maturation earlier but not later in gestation.

Although maternal antenatal corticosteroid treatment reduced blood-brain barrier permeability in the fetuses at 60% of gestation, the decreases in barrier permeability were not large. Several factors related to our dexamethasone treatment regimen may have influenced the effects of exogenous corticosteroids on barrier function in the fetus. It remains possible that the fetal dexamethasone plasma concentrations might not have been high enough or elevated for long enough to have had a maximal impact on the fetal barrier, the effect of dexamethasone administration to the ewe might differ from that of direct fetal administration, and the effect of dexamethasone on the fetal barrier is most likely mediated via glucocorticoid receptors, whereas the effects of endogenous corticosteroids might have been mediated by glucocorticoid and mineralocorticoid receptors. Nevertheless, we have confirmed that antenatal corticosteroid treatment reduced barrier permeability earlier but not later in gestation. Similar to our findings at 80% of gestation, the effects of antenatal corticosteroids were not uniform in all brain regions at 60% of gestation (34). The reasons for these differential effects on barrier function might relate to the nonuniform distribution of glucocorticoid receptors in the brain (22).

The findings in this study are important for several reasons. Our earlier work examined blood-brain barrier permeability to AIB at 80% of gestation, which was not comparable to the time in gestation when human premature infants are at the highest risk for intraventricular hemorrhage (34). The infants who are at the greatest risk for this complication of prematurity are the most immature infants at 23–25 wk of gestation, which is 57–63% of the human gestation. Although our findings in the sheep fetus cannot be directly applied to the human fetus, our results can be interpreted to suggest that maternally administered antenatal corticosteroids reduce blood-brain barrier permeability at the earliest time in gestation, when chronic catheterization of the ovine fetus is feasible (33). These findings at 60% of gestation imply that this phenomenon occurs at a very early time in gestation, which might be comparable to the time when very low birth weight infants are at the highest risk for intraventricular hemorrhage.

The findings that maternal antenatal corticosteroid treatment did not influence blood-brain barrier permeability in near-term ovine fetuses are consistent with our recent results in newborn lambs (35). Blood-brain barrier permeability was not reduced even when a dose of 0.5 mg/kg was given directly to the lambs in the same regimen as used for the ewes (35). However, it should be pointed out that pharmacological doses of dexamethasone have been reported to reduce blood-brain barrier permeability even in adult rodents (16, 28, 40). Although the differences between our findings in fetuses at 90% of gestation and in newborn lambs compared with those in adult rodents (16, 28, 40) might be related to differences in species and treatment regimen, we cannot rule out the possibility that a higher dose of dexamethasone administered directly to the late gestation fetus may have reduced blood-brain barrier permeability. Therefore, our recent work in newborn lambs (35), earlier findings in fetal sheep (34), and current results suggest that corticosteroids reduce blood-brain barrier permeability in fetuses at 60 and 80% of gestation but not in the near-term fetuses or newborn lambs. Consequently, there is a time in gestation when the blood-brain barrier vasculature is responsive to exogenous antenatal corticosteroids and a time when this responsiveness is no longer present.

The reason for this age-related differential responsiveness cannot be determined from our study. Although the concentration of glucocorticoid binding sites have been reported to remain relatively constant in the fetus and newborn (29), the brain is a heterogeneous tissue and the majority of glucocorticoid receptors are most likely present on fibroblasts, astrocytes, neurons, and blood vessels rather than endothelial cells. Therefore, we cannot rule out the possibility that changes in regional brain endothelial cell corticosteroid receptors might have occurred during fetal maturation. It is also important to point out that the studies of glucocorticoid binding sites were done in fetuses with intact adrenal glands (8, 29). Because activated glucocorticoid receptors do not rebinding steroids, simple binding characteristics do not necessarily reveal anything about receptor availability or action (8). Therefore, conclusions regarding the contribution of changes in regional brain endothelial cell glucocorticoid receptors await studies that focus on the fetal maturation of these receptors specifically within brain endothelial cells.

In the present study, we have demonstrated inverse correlations between increases in endogenous cortisol concentrations and decreases in $K_i$ values to AIB in most brain regions. These findings strongly suggest that endogenous increases in cortisol concentrations could be in part responsible for the ontogenic decreases in blood-brain barrier permeability that occur during normal fetal life (33). However, because endogenous plasma cortisol concentrations increase during gestation, we used multiple regression partial correlational analysis to examine the relative strength of the asso-
cortisol concentrations and the decreases in regional permeability. Results of this analysis suggested that the strengths of these associations were relatively equal.

Our findings that maternal treatment with exogenous corticosteroids reduces blood-brain barrier permeability early but not later in gestation and that ontogenic decreases in barrier permeability correlate with elevations in endogenous corticosteroids are consistent with the view that early in gestation the barrier is still responsive to exogenous corticosteroids because it has not yet been exposed to endogenous elevations in corticosteroids. Later in gestation, increases in gestational age and endogenous cortisol concentrations are associated with reductions in barrier permeability; thus the barrier is no longer responsive to exogenous corticosteroids. Therefore, the age-related differential responsiveness of the fetal blood-brain barrier to maternally administered exogenous corticosteroids is most likely related to the effects of endogenous corticosteroids on barrier maturation.

The site of action of the exogenous and endogenous corticosteroids on the blood-brain barrier in the fetus cannot be ascertained with certainty from our studies (34). However, in an in vitro model of the blood-brain barrier, physiologically relevant hydrocortisone concentrations were found to increase transendothelial resistance and decrease permeability to sucrose (17). Therefore, it is probable that the site of action of corticosteroids on the blood-brain barrier is the intercellular tight junctions of cerebrovascular endothelial cells.

In summary, maternal treatment with a corticosteroid regimen similar to that used in the clinical setting reduced blood-brain barrier permeability in the ovine fetus at 60% but not 90% of gestation. Both increases in gestation and endogenous plasma cortisol concentrations are associated with the developmental decreases in permeability.

Perspectives

Antenatal corticosteroid therapy is used widely to treat pregnant women in premature labor. The relatively low dose and treatment regimen given in this and our former study (34) were similar to those used in the clinical setting. This antenatal corticosteroid treatment regimen appears to accelerate a vital component of brain maturation in the fetus at 60 and 80% but not 90% of gestation (34). The findings of this study also suggest that at an early time in gestation, which is similar to the time when the most immature infants are at highest risk for intraventricular hemorrhage, antenatal corticosteroids reduce blood-brain barrier permeability. In addition, our findings support the more general concept that the effects of exogenous steroids may differ depending on the stage of maturation, when they are given, and the aspect of fetal development examined.

We acknowledge the excellent technical assistance of Jesse M. Allen, Christopher M. Ellit, Joshua E. Markowitz, and Aimee Till, and the expert statistical advice of Richard H. Lavoie, PhD, Professor of Mathematics, Providence College, Providence, RI.

This work was supported by the National Institute of Child Health and Human Development Grant R01-HD-34618.

REFERENCES


