Antenatal steroids decrease blood-brain barrier permeability in the ovine fetus

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Antenatal steroids decrease blood-brain barrier permeability in the ovine fetus. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R283–R289, 1999.—Antenatal corticosteroid therapy reduces the incidence of intraventricular hemorrhage in premature infants. Enhanced microvascular integrity might provide protection against intraventricular hemorrhage. In the adult, there is evidence to suggest that the blood-brain barrier may be under hormonal control. We hypothesized that antenatal corticosteroids decrease blood-brain barrier permeability in the preterm ovine fetus. Chronically instrumented 120-day-gestation fetuses were studied 12 h after the last of four 6-mg dexamethasone (n = 5) or placebo (n = 6) injections had been given over 48 h to the ewes. Blood-brain barrier function was quantified with the blood-to-brain transfer constant (K) for α-aminoisobutyric acid (AIB). K was significantly lower across brain regions in the fetuses of ewes that received antenatal dexamethasone compared with placebo (ANOVA; interaction, F = 2.54, P < 0.004). In fetuses of dexamethasone- and placebo-treated ewes, K (µl·g brain wt⁻¹·min⁻¹, mean ± SD) was, respectively, 2.43 ± 0.27 vs. 3.41 ± 0.74 in the cortex, 4.46 ± 0.49 vs. 5.29 ± 0.85 in the cerebellum, and 3.70 ± 0.49 vs. 5.11 ± 0.70 in the medulla. We conclude that antenatal treatment with corticosteroids reduces blood-brain permeability in the ovine fetus.

α-aminoisobutyric acid; brain; corticosteroids; sheep

THE BLOOD-BRAIN BARRIER is composed of a continuous layer of cerebrovascular endothelial cells connected by tight intercellular junctions (2, 4, 6, 15). This specialized barrier serves as an interface between the circulating blood and the 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. We have recently shown that, in the ovine fetus, the blood-brain barrier is relatively impermeable to a small hydrophilic molecule, α-aminoisobutyric acid (AIB) (34).

Maternally administered antenatal corticosteroids have been widely used for the prevention of respiratory distress syndrome in low-birth-weight infants (8). This therapy has also been shown to facilitate the transition from fetal to neonatal life by beneficial effects on multiple organ systems (1, 19, 26, 27, 32). These beneficial effects might be explained in part by accelerated vascular maturation. 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 (14, 21, 31). It has been suggested that antenatal steroids stimulate perinatal maturation of the germinal matrix microvasculature, thereby increasing its resistance to adverse perinatal and postnatal events that predispose premature infants to intraventricular hemorrhage (14, 21, 31, 36). The potential mechanism(s) by which antenatal steroids afford protection from intraventricular hemorrhage remain to be defined.

In adult rats, adrenalectomy has been shown to increase blood-brain barrier permeability; corticosterone replacement reverses this effect on the barrier (20). These findings suggest that the entry of substances into the CNS may be responsive to circulating glucocorticoids and that the pituitary-adrenal cortical axis may function as a physiological regulator of blood-brain barrier permeability (20). In the normal adult rat, pharmacological doses of dexamethasone have been reported to reduce barrier permeability to AIB (39). In adult mice, dexamethasone also decreased the permeability of cerebral blood vessels to horseradish peroxidase (16). In several pathological conditions, such as tumors, subarachnoid hemorrhage, and osmotic barrier modification, steroids have been shown to attenuate increases in barrier permeability (24, 40). Therefore, in the adult, dexamethasone decreases blood-brain barrier permeability and protects the barrier from a variety of pathological conditions (18, 24, 40). Thus it is likely that the protective effect of antenatal steroids in the premature brain may also, in part, relate to enhanced barrier maturation and decreased barrier permeability in normal and pathological conditions.

Given the above considerations, we hypothesized that maternally administered antenatal corticosteroids decrease blood-brain barrier permeability in the ovine fetus. To test this hypothesis, we examined the effects of maternal antenatal corticosteroid treatment, in clinically relevant doses, on blood-brain barrier permeability in preterm ovine fetuses at 80% of gestation.

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 National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Animal preparation. As previously described in detail (33, 34), surgery was done under 1–2% halothane and oxygen anesthesia on 11 mixed-breed ewes at 112–113 days of gestation. Briefly, in each of the fetuses, polyvinyl catheters were placed into a brachial vein for isotope administration, a brachial artery advanced to the thoracic aorta for blood sample withdrawal, and a femoral artery advanced to the upper abdominal aorta for heart rate and blood pressure monitoring. Amniotic fluid catheters were placed for pressure monitoring and to correct fetal arterial blood pressures. Femoral artery catheters were also placed in the ewes.

After 2–4 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.

Dexamethasone was chosen for use in our studies because it is one of the most extensively studied corticosteroids for accelerating fetal maturation, has been widely used in experimental studies of the CNS, and is also used to treat CNS disorders (5, 13, 16, 18, 23, 24, 40). The dose of dexamethasone used in these studies was based on current recommendations for fetal maturation in pregnant women with 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). Moreover, fetal sheep that have received different doses, by either direct fetal or maternal injections, had comparable fetal plasma corticosteroid levels by 6 h after either treatment (1). 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 that we utilized 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 (10, 19, 23).

Experimental protocol and methodology. Studies were performed 5–7 days after recovery from surgery at 117–120 days or 80% of gestation. Although the maturation of fetal sheep at this time in gestation is not directly comparable to premature infants at risk for intraventricular hemorrhage, we selected this gestational age to perform our studies because antenatal corticosteroids have been shown to have significant cardiovascular and renal effects at a similar time in gestation (1, 27).

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-New England Nuclear, Boston, MA). The blood-to-brain transfer constant (\(K_i\)) was measured as previously described (9, 25, 34). After baseline physiological determinations, [14C]AIB was rapidly injected intravenously and arterial plasma concentrations were obtained at fixed times (in min) before and after injection as follows: \(-1, 0.5, 1, 2, 3, 5, 7, 15, 30, 45, 60\), and at termination within 8 to 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 (9) and preliminary mathematical analysis of AIB in fetal sheep, the 60-min study interval and this sampling regimen were determined to accurately characterize the plasma profile needed for calculation of \(K_i\) (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 \(K_i\) as described by Ohno et al. (25). In our experiments, the unidirectional \(K_i\) was quantified for [14C]AIB in the fetal sheep. Brain vascular volume was determined by giving [14C]polyethylene glycol (PEG; Amersham, UK) 2 min before the end of the experiment to separate fetuses of dexamethasone- and placebo-treated ewes. The brain vascular volume values did not differ between the two groups and were similar to our previously reported values (34).

[14C]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, 25, 34, 35). The fetuses received either 60 µCi of [14C]AIB or 90 µCi of [13C]PEG. Blood samples were withdrawn as outlined above. Intermittent samples were withdrawn rather than a single integrated sample so that the plasma radioactivity profile could be compared between groups to ensure accuracy of the methodology. The pattern of the plasma concentration profile (Fig. 1) was similar between the groups and to our previous report (34). However, the plasma peak (dpm/µl) appeared higher in the fetuses of the dexamethasone-treated than of the placebo-treated ewes. The higher plasma counts are most likely related to the lower fetal weight in this group (RESULTS). The relatively higher counts in this group would not have affected the calculation of \(K_i\).

At the end of the study, each 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 cord intact. The fetus was then placed in a sterile container and allowed to breatheroom air until death occurred. Brain vascular volume values did not differ between the two groups and were similar to our previously reported values (34).

![Fig. 1. Plasma radioactivity profile of \(\alpha-[14C]a\)-aminoisobutyric acid (\([14C]AIB\)) (in dpm/µl) is plotted against study time (in min) for fetuses of dexamethasone-treated (●) and placebo-treated ewes (○). [14C]AIB is given intravenously at study time zero. Values are means ± SD. See Experimental protocol and methodology for details.](http://ajpregu.physiology.org/Downloadedfrom/10.220.33.5.onNovember7,2017)
circulation intact and decapitated to immediately terminate blood flow to the brain. The brain was removed within 3–5 min for regional brain tissue samples. The weight of the fetus was determined. The ewe was then killed with pentobarbital sodium (300–200 mg/kg).

The brains were dissected into the following regions: cortex, coro- silum, caudate nucleus, hippocampus, superior colliculus, inferior colliculus, thalamus, pons, medulla, and cervical spinal cord. The cortex was further divided into the frontal, parietal, and occipital cortex. Although histological examination of the germinal matrix was not performed in the present study, we have previously examined fetuses at 87, 130, and 135 days of gestation. At 87 days of gestation the germinal matrix was present, at 130 days of gestation it appeared partially involuted, and at 135 days of gestation a scant amount of germinal matrix remained. Therefore, there was probably more germinal matrix at 117–120 days than at 130 days of gestation. Even though the germinal matrix was not dissected separately in the present study, the caudate nucleus represents the anatomic location of the germinal matrix in the floor of the lateral ventricles, where it exists in an immature fetus. This area would have been included in our dissection of the caudate nucleus.

Tissue samples were treated as previously described (9, 34). Briefly, Solvable (Packard Instruments, Downers Grove, IL) was added to the vials containing the tissue samples, which were then placed in a shaking water bath at 50°C overnight. Tissue sample decoloration was achieved with 30% hydrogen peroxide. Atomlight (Dupont-New England Nuclear) was added to each vial before the radioisotope was quantified with a TM analytic beta counter (model 6895, Elk Grove Village, IL). All samples were corrected for background, sample spillover, and quenching. The plasma from the arterial blood samples was measured into scintillation vials. The scintillation cocktail (Atomlight) was added to each vial before counting. The plasma radioactivity was quantified as described for the tissue samples. $K_i (\mu l \cdot g \text{ brain wt}^{-1} \cdot \text{min}^{-1})$ is given by

$$K_i = A_{tr} \int_{0}^{t} \frac{C(t)}{V_{pcp}} \, dt$$

where $A_{tr}$ is the amount of tracer (dpm/g) that crossed the blood-brain barrier from blood to brain during the tracer study, and $C_i$ is the tracer concentration in plasma (dpm/ul) at time $T$ (min). $A_{tr}$ is obtained by correcting the total amount of isotope measured in the tissue ($A_{tiss}$ dpm/g) for that residual part remaining in the brain vasculature space, which is measured by the $[^{14}C]PEG$. Thus $A_{tr} = A_{tiss} - V_{pcp} C_i$, where $V_p = A_{tr} / C_i$, and $A_{tiss}$ and $C_i$ have the same definitions as $A_{tiss}$ and $C_i$ above, respectively, except that they apply to $[^{14}C]PEG$ (9).

The integral of the plasma concentrations was calculated by determining the area under the curve using the trapezoidal rule. The AIB disappearance curve can be fitted to three exponentials, the slowest of which yields a half-time of 0.032 min in the ovine fetus. There were no differences in the disappearance curves between the fetuses of the dexamethasone- and placebo-treated ewes. Our sampling protocol was sufficient to characterize the disappearance curve during the study, which was the time interval required by this method.

Arterial pH, blood gases, oxygen saturation, hematocrit, heart rate, and mean arterial blood pressure were measured on the fetuses and ewes at baseline and at 30 and 50 min of study. Arterial plasma osmolality and 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% (15 ml) of the fetal blood volume. Heart rates and 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, 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. Hemoglobin and oxygen saturation were measured on a Radiometer hemoximeter (model OSM2, Copenhagen, Denmark). Hematocrit was measured in duplicate by the microhematocrit method. Plasma osmolality was measured in duplicate on a vapor pressure osmometer (Vapro model 5520, Wescon, Logan, UT), glucose on a glucose-lactate analyzer (YSI 2300, STAT, Yellow Springs, OH), and insulin and cortisol concentrations by $^{125}$I RIA (DPC, Los Angeles, CA; INCSTAR, Stillwater, MN).

Statistical analysis. All results were expressed as means ± SD. Serial measurements of physiological variables were compared by two-factor ANOVA for repeated measures. Two-factor ANOVA for repeated measures was used to compare regional brain permeability within and between the fetuses of the dexamethasone- and placebo-treated ewes. To further describe the statistically significant group-region interaction, a separate ANOVA for repeated measures was performed on the fetuses of dexamethasone- and placebo-treated ewes. When a significant interaction was present by ANOVA, the two groups were further compared by Bonferroni corrected two-group t-tests. Within each group all other brain regions were compared with the cortex by Bonferroni corrected paired t-tests. When physiological and hormonal variables were compared between the fetuses of the dexamethasone- and placebo-treated ewes and between the two groups of ewes, the Bonferroni corrected two-group t-test was used. $P < 0.05$ was considered statistically significant.

RESULTS

The fetuses of the dexamethasone-treated ewes were 119 ± 1 and those of the placebo-treated ewes were 118 ± 1 days of gestation at the time of study. Fetuses of the dexamethasone-treated ewes were significantly smaller (2.21 ± 0.17 kg) than those of the placebo-treated ewes (2.58 ± 0.27 kg). The weight of the dexamethasone-treated ewes (69 ± 12 kg) did not differ significantly from that of the placebo-treated ewes (59 ± 7 kg). The dexamethasone-treated ewes received 0.09 ± 0.01 mg/kg for each dose of dexamethasone. None of the dexamethasone-treated ewes developed premature labor.

Arterial pH, oxygen tension, carbon dioxide tension, base excess, oxygen saturation, hematocrit, heart rate, mean arterial blood pressure, plasma osmolality, and glucose, insulin, and cortisol concentrations did not differ significantly between the two groups of fetuses (Table 1). Plasma arterial insulin concentrations were significantly higher and cortisol concentrations significantly lower in the dexamethasone- than in the placebo-treated ewes. The pH, blood gas, heart rate, and mean arterial blood pressure values of the fetuses and ewes did not change during the 1-h study.

The regional brain $K_i$ values in fetuses of the dexamethasone- and placebo-treated ewes are illustrated in Fig. 2. The $K_i$ values were significantly lower across the
Table 1. Physiological and hormonal variables of fetuses and ewes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dexamethasone-Treated Ewes (n = 5)</th>
<th>Placebo-Treated Ewes (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetuses</td>
<td></td>
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</tr>
<tr>
<td>pH</td>
<td>7.32 ± 0.03</td>
<td>7.32 ± 0.04</td>
</tr>
<tr>
<td>PaO2, mmHg</td>
<td>29 ± 3</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>PaCO2, mmHg</td>
<td>44 ± 4</td>
<td>50 ± 5</td>
</tr>
<tr>
<td>Base excess, meq/l</td>
<td>−2.6 ± 2.8</td>
<td>0.2 ± 1.6</td>
</tr>
<tr>
<td>O2 saturation, %</td>
<td>81.1 ± 4.7</td>
<td>75.4 ± 11.0</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>34 ± 4</td>
<td>36 ± 5</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>213 ± 20</td>
<td>184 ± 25</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>53 ± 8</td>
<td>45 ± 9</td>
</tr>
<tr>
<td>Osmolarity, mosmol/kgH2O</td>
<td>298 ± 6</td>
<td>296 ± 7</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>41.1 ± 9.50</td>
<td>26.7 ± 9.56</td>
</tr>
<tr>
<td>Insulin, log µU/ml</td>
<td>1.67 ± 0.32</td>
<td>1.39 ± 0.33</td>
</tr>
<tr>
<td>Cortisol, nmol/l</td>
<td>17.2 ± 3.6</td>
<td>24.5 ± 9.2</td>
</tr>
<tr>
<td>Ewes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.55 ± 0.04</td>
<td>7.50 ± 0.02</td>
</tr>
<tr>
<td>PaO2, mmHg</td>
<td>103 ± 4</td>
<td>105 ± 13</td>
</tr>
<tr>
<td>PaCO2, mmHg</td>
<td>31 ± 4</td>
<td>34 ± 4</td>
</tr>
<tr>
<td>Base excess, meq/l</td>
<td>6.8 ± 2.0</td>
<td>5.0 ± 3.1</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>26 ± 2</td>
<td>27 ± 4</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>99 ± 22</td>
<td>89 ± 10</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>84 ± 13</td>
<td>85 ± 11</td>
</tr>
<tr>
<td>Osmolarity, mosmol/kgH2O</td>
<td>304 ± 11</td>
<td>304 ± 10</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>108.2 ± 17.9</td>
<td>75.1 ± 12.4</td>
</tr>
<tr>
<td>Insulin, log µU/ml</td>
<td>1.79 ± 0.25*</td>
<td>1.21 ± 0.1</td>
</tr>
<tr>
<td>Cortisol, nmol/l</td>
<td>16.7 ± 3.4*</td>
<td>112.3 ± 45.4</td>
</tr>
</tbody>
</table>

Values are means ± SD. PaO2 and PaCO2, arterial oxygen and carbon dioxide tension, respectively; MAP, mean arterial blood pressure. *P < 0.05 vs. values of placebo group.

brain regions in the fetuses of the dexamethasone-treated ewes than in those of the placebo-treated ewes (ANOVA; interaction, F = 2.54, P < 0.004). The magnitude of the decreases in permeability was not uniform across the brain regions. In the cortex, there was a 29% reduction in the Kt in the fetuses of the dexamethasone-compared with the placebo-treated ewes; in the caudate nucleus it was 26%; in the hippocampus it was 26%; in the cerebellum it was 16%; in the thalamus it was 27%; in the superior colliculus it was 25%; in the inferior colliculus it was 34%; in the pons it was 30%; in the medulla it was 28%; and in the cervical spinal cord it was 31%. The hippocampus, pons, medulla, and cervical spinal cord proved to be the regions with the most difference between the two groups of fetuses by Bonferroni corrected two-group t-tests (Fig. 2).

The Kt values for AIB exhibited significant regional heterogeneity within the fetuses of the dexamethasone-treated (ANOVA; F = 29.43, P < 0.001) and placebo-treated (ANOVA; F = 46.27, P < 0.001) ewes. In the fetuses of the dexamethasone-treated ewes, the Kt values for AIB in the cerebellum differed significantly from those in the cortex; in the fetuses of the placebo-treated ewes, the values in the cerebellum, pons, medulla, and cervical spinal cord differed significantly from those in the cortex (P < 0.01; Fig. 2).

The Kt values of the frontal, parietal, and occipital cortices (2.37 ± 0.39, 2.42 ± 0.47, and 2.51 ± 0.26 µl·g brain wt⁻¹·min⁻¹, respectively) in the fetuses of the dexamethasone-treated ewes were significantly lower than the values (3.43 ± 0.93, 3.20 ± 1.07 and 3.60 ± 0.60 µl·g brain wt⁻¹·min⁻¹, respectively) in the fetuses of the placebo-treated ewes (ANOVA; F = 7.89, P < 0.02). Differences were not observed among these cortical regions within the two groups of fetuses.

**DISCUSSION**

This study examined the effects of maternal antenatal corticosteroid treatment in doses similar to those used clinically on blood-brain barrier permeability in ovine fetuses at 80% of gestation. The major finding of our study was that the blood-brain barrier permeability was lower across brain regions in the fetuses of ewes treated with dexamethasone.

Although dexamethasone concentrations were not measured in our study, the lower plasma cortisol concentrations suggest that dexamethasone had suppressed the adrenocortical axis in the dexamethasone-treated ewes. Likewise, the higher insulin concentrations in the dexamethasone-treated ewes are consistent with glucocorticoid-related glucose intolerance (7). Similar to findings in rodents, this dexamethasone regimen was associated with smaller fetuses (11). In contrast to findings with other antenatal and fetal corticosteroid treatment regimens, the fetuses and ewes in our study did not have significant elevations in systemic arterial blood pressure at the time of study. However, it is important to point out that our dexamethasone dosage was lower than in previous reports and we did not measure fetal or maternal arterial blood pressures during drug treatment (1, 10).

Antenatal corticosteroids have been shown to enhance cardiovascular stability, improve renal functional maturation, and reduce the incidence of necrotizing enterocolitis and intraventricular hemorrhage (1, 14, 21, 23, 27, 31, 32). These diverse organ effects might...
be explained by enhanced vascular maturation. The reduction in the incidence of intraventricular hemorrhage in premature infants (14, 21, 31) suggests that corticosteroids might have important effects on the cerebral vasculature. These concepts are consistent with our findings that maternal administration of corticosteroids reduced blood-brain barrier permeability and enhanced brain microvascular maturation in the fetus. However, extrapolation of our findings to the human fetus must be done with caution, because the maturation of the ovine fetus at 80% of gestation is not directly comparable to human premature fetuses at risk for intraventricular hemorrhage.

In adult rats, changes in endogenous adrenocortical function have been shown to alter blood-brain barrier permeability (20). Adrenalectomy, but not adrenal demedullation, increased the permeability of brain tissue to albumin, and corticosterone replacement reversed these effects on the barrier (20). Chronic oral dexamethasone administration was shown to reduce water flux across the blood-brain barrier in adult rats (29). Pharmacological doses of dexamethasone reduced horseradish peroxidase transit across the cerebral endothelium of adult mice and blood-brain-barrier permeability to A1B by 30–50% in most brain regions of adult rats (16, 39). Our findings of reduced blood-brain barrier permeability in the ovine fetus are consistent with these findings in adult rodents (16, 20, 29, 39). However, the magnitude of the reduction in barrier permeability was lower (16–34%) in our fetuses exposed to antenatal corticosteroids compared with the findings in adult rats using the same methodology (39). Although the reasons for the smaller reductions in permeability in our study cannot be determined with certainty, differences could be related to the pharmacological dose (39) vs. our lower dose, the treatment regimen, species, or age at study, e.g., fetuses vs. adults. In addition, during early postnatal development corticosteroid receptor expression in the brain is reduced compared with that of the adult (30). This may modify the response of the immature brain to steroids. Another important consideration is that in our study dexamethasone was given to the ewes. Corticosteroids are known to affect placental hormonal responses (17) and might also affect other placental factors, which might in turn alter the barrier's response to corticosteroids (1, 17). Nevertheless, the findings of our study combined with those in adult rodents strongly suggest that the adrenocortical axis is an important regulator of blood-brain barrier function in the fetus and adult (16, 20, 29, 39).

Consistent with our previous findings, heterogeneity in regional barrier permeability was observed in the fetuses of both the dexamethasone- and placebo-treated ewes, such that regional permeability was highest in the cerebellum and more caudal brain structures, including the pons, medulla, and cervical spinal cord (34). The effect of antenatal corticosteroids was not uniform in all regions, such that the percent decrease compared with the fetuses of placebo-treated ewes varied between 16 and 34%. The dexamethasone-related reduction in barrier permeability appeared to be greater in the more caudal brain structures. The reasons for these differential effects on barrier function might relate to the nonuniform distribution of glucocorticoid receptors in the brain (22). In the rodent brain, although glucocorticoid receptors appear to be present throughout the brain before the time of birth, regional ontogenetic changes have been reported (30). In the hippocampus, receptor concentrations rise slowly and do not achieve adult levels until the second to third week of life (30), whereas in the cerebellum, receptor levels are relatively high and remain more or less constant between postnatal days 8 and 16 (30). In contrast, the dexamethasone-related reduction in barrier permeability was larger in the hippocampus than in the cerebellum in our fetal sheep. Therefore, the relative changes in the immature rodent brain glucocorticoid receptor density and mRNA (30) cannot entirely account for the differential effects of dexamethasone on barrier function in the ovine fetus. However, although it is clear that receptor binding site density and mRNA regional distribution change with ontogeny in the immature rodent brain, it remains uncertain whether direct comparisons can be made with the premature fetal sheep brain at 80% of gestation.

The blood-brain barrier findings in the present study deserve comparison with our previous work examining the ontogeny of barrier function (34). In the present study, the K values in the fetuses of the placebo-treated ewes appeared similar to our previous values at 90% gestation (34). This is expected, because the age difference was only 15 days between the two groups of fetuses. Large differences were not observed between fetuses at 60 and 90% gestation, except in the more caudal brain structures (34). Similarly, the medulla and cervical spinal cord permeability appeared somewhat higher at 80% than at 90% gestation (34). Maternal treatment with dexamethasone resulted in precocious development of barrier function in fetal sheep such that the values at 80% gestation were only slightly higher than those observed in 1- to 3-day-old newborn lambs (34).

Antenatal corticosteroids have been shown to reduce the risk of early-onset intraventricular hemorrhage and the risk for more severe grades of intraventricular hemorrhage (14, 21, 31, 36). It has been suggested that corticosteroids stimulate the maturation of the germinal matrix microvasculature, making it more resistant to perinatal and postnatal perturbations known to cause intraventricular hemorrhage (21, 36). Although the effects of antenatal corticosteroids on barrier function were examined in chronically catheterized fetal sheep under homeostatic conditions, steroids have been shown to attenuate increases in barrier permeability during pathophysiological conditions such as tumors, cold injury to the brain, osmotic barrier opening, and subarachnoid blood in adult rodents (18, 24, 40). Therefore, the effect of antenatal corticosteroids in our study, combined with previous findings that perturbations in barrier function are attenuated by steroids, might be interpreted to suggest that the protective effect of this maternal treatment is based on maturation of the
blood-brain barrier microvascular endothelium in premature infants, which might afford protection under conditions of perinatal and postnatal stress (14, 21, 31, 36).

The site of action of corticosteroids on the microvasculature of the fetal blood-brain barrier cannot be determined by our study. However, several possibilities exist. At the microvascular level, dexamethasone might act on the brain endothelia and adjacent microglia to accelerate tight junction development by inducing signals for endothelial differentiation (28, 37). Thus tight junction integrity might be strengthened earlier in gestation, resulting in larger transmembrane resistance and reductions in barrier permeability (28, 37). The tight junction region is characterized by a network of intramembrane fibrils that surround the apices of cells and seal the intercellular space separating the apical and basolateral fluid compartment of the endothelia (12). Occludin, an integral membrane protein, has recently been shown to be important in the tight junction structure (12). Although it is not known if the expression of occludin is hormonally regulated, it remains possible that the reduction in barrier permeability in our fetuses might have been related to dexamethasone receptor-mediated responses that augment the synthesis of novel proteins localized to tight junctions (12, 38).

In summary, a course of antenatal corticosteroids similar to that which is given to women in preterm labor reduced blood-brain barrier permeability in the ovine fetus.

Perspectives

Antenatal corticosteroid therapy is widely used to treat pregnant women in premature labor. The relatively low dose and treatment regimen that was given to the ewes in our study was similar to that used in the clinical setting. Our findings may be interpreted to suggest that this extensively used treatment accelerates a vital aspect of brain development in the fetus. The finding that antenatal corticosteroids reduce blood-brain barrier permeability in the fetus cannot, because of gestational age and species-related differences, directly account for reductions in the incidence of intraventricular hemorrhage in human premature infants whose mothers received this drug (8, 13, 21, 36). However, on the basis of our findings, we speculate that the doses of corticosteroids used clinically might accelerate microvascular maturation and affect blood-brain function in the human premature fetus. Moreover, it is important to emphasize that our findings are the first to suggest that the blood-brain barrier is hormonally responsive in the fetus of any species and that corticosteroids are important in regulation of the maturation of this barrier.

We acknowledge the technical assistance of Edward Lai, Amanda J. McKnight, Kaushal B. Mehta, Joyce M. Oen, Christopher B. Reilly, and Catherine Yan, and the secretarial assistance of Betsey Mottershead.

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

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Received 21 April 1998; accepted in final form 25 September 1998.

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