Changes in Fetal Plasma Corticotropin-Releasing Hormone during Androstenedione-Induced Labor in the Rhesus Monkey: Lack of an Effect on the Fetal Hypothalamo-Pituitary-Adrenal Axis*

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ABSTRACT

Androstenedione infusion to pregnant monkeys leads to premature labor and live delivery. Androstenedione-induced labor also increased placental CRH messenger RNA and peptide to concentrations observed at term in pregnant monkeys. Placental CRH may modulate fetal pituitary-adrenal function during pregnancy in primates. This study tested the hypothesis that androstenedione-induced premature delivery in pregnant monkeys results from androstenedione-induced increases in placental CRH, which stimulate premature activation of the fetal pituitary-adrenal axis. The hypothesis was tested by comparing fetal umbilical vein (FUV) plasma CRH, ACTH, dehydroepiandrosterone sulfate, and cortisol concentrations at cesarean section in fetuses from mothers undergoing spontaneous, term labor (group I), with those in fetuses from mothers undergoing androstenedione-induced, premature labor (group II) and with those from mothers not in labor (group III). In addition, gestation-related changes in maternal plasma CRH concentrations were investigated, and CRH immunoactivity was characterized by Sephadex G50 chromatography in pooled maternal plasma extracts.

FUV CRH concentrations were similarly elevated in group I and group II fetuses, compared with group III fetuses. Despite similar FUV blood gases in all fetuses, FUV ACTH and dehydroepiandrosterone sulfate concentrations were higher in group I fetuses than in group II or group III fetuses. The majority of CRH immunoactivity coeluted with synthetic human CRH. Maternal plasma CRH concentrations showed a modest increase with gestation in the rhesus monkey.

These data: 1) demonstrate that androstenedione treatment of pregnant monkeys at 0.8 of gestation elevates fetal plasma CRH to similar concentrations measured at term; 2) do not support the hypothesis that androstenedione-induced delivery in the monkey results from premature activation of the fetal pituitary-adrenal axis by placental CRH; but 3) do support a role for activation of the fetal hypothalamic-pituitary-adrenal axis in association with spontaneous term labor in the monkey; and 4) demonstrate important interprimate species differences in maternal CRH physiology. (Endocrinology 139: 2803–2810, 1998)
androstenedione (20) and/or cortisol (21, 22), may exert direct and/or ACTH-mediated trophic effects on fetal adrenal steroidogenesis. We have thus raised the hypothesis that, in primates, fetal androgen and placental CRH [and Karalis et al. (24) have recently suggested that cortisol and placental CRH] are part of a positive feed-forward loop that would eventually culminate in birth.

In the present study, we have tested the hypothesis that androstenedione-induced labor in the pregnant monkey is caused by placental CRH-induced increases in fetal pituitary-adrenal function, by comparing umbilical vein plasma CRH, ACTH, dehydroepiandrosterone sulfate (DHEAS), and cortisol concentrations in fetuses from mothers undergoing STL, with those in fetuses from mothers undergoing androstenedione-induced, premature labor (APL), and with those from mothers not in labor (NIL).

Among primates, the sparse comparative information demonstrates important differences in placental CRH physiology. For example, in pregnant women, whereas the increase in maternal plasma CRH is exponential with gestation (1, 2, 4), in pregnant baboons, CRH levels peak in the first half of pregnancy and do not increase further at term (9, 10). To date, no information exists on changes in maternal plasma CRH concentrations with gestation in other primate species. Thus, further objectives of these investigations were to characterize CRH immunoactivity in maternal plasma and gestation-related changes in maternal plasma CRH concentrations in chronically instrumented, unanesthetized pregnant rhesus monkeys.

Some of these results have been previously published in abstract form (25).

Materials and Methods

Use of animals

Twenty multiparous pregnant rhesus monkeys (6.9 ± 0.3 kg; mean ± SEM), carrying fetuses of known gestational ages, were obtained from the California Regional Primate Research Center (Davis, CA) and were acclimated to the laboratory conditions, as previously described in detail (26). All procedures were approved by the Cornell University Institutional Animal Care and Use Committee and were performed in facilities approved by the American Association for the Accreditation of Laboratory Animal Care. In brief, at approximately 60 days gestational age (dGA), all animals underwent a full physical examination, were housed in individual cages in rooms with controlled light-dark cycles (14-h light, 10-h dark), and were placed in quarantine. During quarantine, the animals were jacketed, and the tether through which vascular catheters and electrode wires were to be connected after surgical instrumentation was suspended from the top of the cage. One week later, the tether was fixed to the back of the jacket. The animals were fed daily [Purina 5045 High Protein Monkey Chow (Purina, St. Louis, MO) and fresh fruits], and water was continuously available.

Surgical instrumentation and postsurgical management

After food deprivation for 24 h, surgery was performed at 129 ± 3 dGA (mean ± SEM of all instrumented animals, n = 13), under general anesthesia (15 mg/kg ketamine for induction; 1–2% halothane in O2 administered at 2 liters/min for maintenance), the fetus was exteriorized, and care was taken to minimize heat loss and maintain umbilical cord patency. Blood samples were drawn (5 ml) from the umbilical vein for measurement of blood gases and pH (ABL 500, Radiometer, Copenhagen, Denmark; measurements corrected to 38 C) and determination of circulating concentrations of CRH, ACTH, DHEAS, and cortisol (by RIA). In the two animals that delivered from groups I and II, respectively, blood was drawn directly from the right ventricle, under halothane general anesthesia only, for determination of hormone concentrations but not for blood gas or pH analyses. Any blood taken from the umbilical vein during cesarean section was collected within 5 min of fetal exteriorization. Any blood taken from the right ventricle of a viable newborn was taken within 30 min of delivery. At necropsy, the animals were euthanized by exsanguination while still under halothane; and the adrenal glands were isolated, removed, and weighed.

Some of the animals used in this investigation have been subjects of other previously reported studies (18, 19, 20, 28).

Hormone analyses

All maternal and fetal blood samples taken for hormone analyses were immediately transferred into chilled polypropylene collection tubes and centrifuged at 4 C at 1200 × g for 5 min. Plasma was removed, aliquoted, flash frozen, and stored at −20 C until assayed. All hormone assays were performed within 2 months of plasma collection.

CRH RIA and chromatography. CRH was measured by RIA with human CRH standard and antisem to human CRH and was validated for...
analysis of rhesus plasma (9). CRH was extracted from 0.5 ml plasma with Sep-Pak C_{18} cartridges (Waters Associates, Millford, MA) and eluted with triethylamine-formic acid-propranolol, as previously described in detail (9). The extracts were evaporated, reconstituted in assay buffer, and assayed for CRH immunoactivity. CRH extracted from rhesus monkey plasma diluted in parallel with human CRH. The CRH antibody was a midportion antibody that did not cross-react with antibody was a midportion antibody that did not cross-react with monkey plasma diluted in parallel with human CRH. The CRH buffer, and assayed for CRH immunoactivity. CRH extracted from rhedefined with triethylamine-formic acid-propranolol, as previously defined with triethylamine-formic acid-propranolol, as previously described (27). Samples or standards (50 µl) were incubated with antiserum (INCStar, 50 µl) for 24 h at 4 C. Radioiodinated ACTH (INCStar, 50 µl) was then added and incubated for a further 24 h at 4 C. Goat antirabbit precipitating complex (200 µl) was added and incubated for 20 min at room temperature. Cold PBS (0.01 M NaPO₃, 0.9% NaCl, pH 7.2, 1 ml) was added to each tube and centrifuged at 1000 g % of body weight.

**TABLE 1. Details of animals studied**

<table>
<thead>
<tr>
<th>Group</th>
<th>Rhesus no.</th>
<th>dGA @ surgery</th>
<th>dGA @ start of infusion</th>
<th>dGA @ C-sec/Del</th>
<th>Combined adrenal weights</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (STL)</td>
<td>1</td>
<td>118</td>
<td>145</td>
<td>156</td>
<td>0.41 0.08</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>132</td>
<td>138</td>
<td>158</td>
<td>0.30 0.07</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>132</td>
<td>138</td>
<td>159</td>
<td>0.20 0.05</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>136</td>
<td>142</td>
<td>174 (del.live)</td>
<td>0.41 0.08</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>146</td>
<td>153</td>
<td>166</td>
<td>0.64 0.12</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>139</td>
<td>152</td>
<td>158</td>
<td>0.34 0.07</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td></td>
<td>133.8 ± 3.8</td>
<td>144.7 ± 2.7</td>
<td>162.0 ± 2.8</td>
<td>0.38 ± 0.06</td>
</tr>
<tr>
<td>II (APL)</td>
<td>7</td>
<td>121</td>
<td>139</td>
<td>143</td>
<td>0.24 0.06</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>118</td>
<td>139</td>
<td>143</td>
<td>0.23 0.06</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>136</td>
<td>142</td>
<td>146</td>
<td>0.23 0.06</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>132</td>
<td>140</td>
<td>141 (del.live)</td>
<td>0.23 0.06</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td></td>
<td>127.0 ± 3.3</td>
<td>139.8 ± 0.6</td>
<td>143.2 ± 0.8</td>
<td>0.26 ± 0.03</td>
</tr>
<tr>
<td>III (NIL)</td>
<td>12</td>
<td>149</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>152</td>
<td></td>
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<tr>
<td></td>
<td>17</td>
<td>160</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td></td>
<td>150.8 ± 3.9</td>
<td></td>
<td></td>
<td>0.31 ± 0.03</td>
</tr>
</tbody>
</table>

Values shown are mean ± SEM. Cesarean sections were performed under 1–2% halothane in O₂ administered at 2.0 liters/min⁻¹.

**TABLE 2. Umbilical vein blood gases and pH and days gestational age (dGA) at cesarean section (C-sec) or live delivery (Del) for fetal monkeys taken from mothers in groups I–III**

<table>
<thead>
<tr>
<th>Group</th>
<th>pH</th>
<th>PCO₂ (mmHg)</th>
<th>PO₂ (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (STL, n = 6)</td>
<td>7.22 ± 0.03</td>
<td>59.0 ± 3.92</td>
<td>39.9 ± 9.4</td>
</tr>
<tr>
<td>II (APL, n = 5)</td>
<td>7.23 ± 0.03</td>
<td>50.2 ± 2.61</td>
<td>39.1 ± 12.8</td>
</tr>
<tr>
<td>III (NIL, n = 7)</td>
<td>7.21 ± 0.02</td>
<td>56.5 ± 4.64</td>
<td>39.6 ± 10.7</td>
</tr>
</tbody>
</table>

Values shown are mean ± SEM. Cesarean sections were performed under 1–2% halothane in O₂ administered at 2.0 liters/min⁻¹.

**DHEAS. A commercial assay kit (Diagnostic Products Corporation, no. TKDS-1) for human plasma dehydroepiandrosterone was validated for analysis of rhesus plasma (28). Dehydroepiandrosterone recovery was determined by mixing rhesus plasma pool 1:1 with dehydroepiandrosterone of known concentration in human plasma (0.9, 3.5, 8.7, 34.7, 86.7, and 173.4 pmol/tube⁻¹). All dehydroepiandrosterone-spiked samples were diluted 1:1 with kit zero calibrator to ensure measurements from the linear region of the standard curve. Recovery was 100 ± 2%. Parallelism was demonstrated by serial dilution of rhesus plasma in kit zero calibrator. The intraassay CV was 3.0%, and the interassay CV was 14.9%, for a rhesus quality control sample of 3.0 pmol/tube⁻¹ (n = 13). The assay lower limit of detection (90% B/B₀) was 2.0 pg/tube⁻¹, which represents 2 pg/ml⁻¹. Cross-reactivity of the antibody, at 1200 pg/ml⁻¹, was porcine ACTH₁₋₃₉ 100%, human ACTH₁₋₃₉ 100%, AVP < 0.01, oxytocin < 0.01, β-endorphin < 0.01%, αMSH < 0.01%, and human GH < 0.01%. Serial dilutions of pregnant rhesus monkey plasma gave parallel responses in the assay. Accuracy was evaluated by adding known amounts of human ACTH₁₋₃₉ to plasma samples and measuring the final concentrations by RIA.

**Cortisol. A commercial assay kit (Diagnostic Products Corporation, no. KCOD2) for human plasma cortisol was validated for analysis of rhesus plasma (28). Cortisol recovery was determined by mixing rhesus plasma pool 1:1 with cortisol solution of known concentration in human plasma (200, 300, 400, and 500 ng/ml⁻¹). All cortisol-spiked samples were diluted 1:1 with kit zero calibrator to ensure measurements from the linear range.
region of the standard curve. Recovery was 100.2 ± 3.8%. Parallelism was demonstrated by serial dilution of rhesus plasma in kit zero calibrator. The intraassay CV was 9.8% for a quality control sample of 106 ng/ml⁻¹, and 9.0% for a quality control sample of 377 ng/ml⁻¹ (n = 27). The assay lower limit of detection (90% B/B₀) was 4.9 ng/ml⁻¹ (n = 27).

Analyses of myometrial activity

Recording of the myometrial EMG was performed with a computer-based data acquisition system. The signal was sampled at 32 Hz and integrated, and the average over 8 sec was digitized and stored as one data point reading with a time signal.

A switch in myometrial EMG activity from contractures to contractions was determined visually. A switch was defined if at least 6 contractions, each lasting approximately 1 min., occurred sequentially and the bout of contraction activity lasted at least 30 min. We have previously reported that, in the monkey, this switch in myometrial activity, from contractures to contractions, usually occurs around the onset of darkness. The switch is reversible, recurrent, and progressive (the number of nighttime contractions augmenting on consecutive nights until delivery finally occurs) (29). Cesarean section was performed on group I monkeys after intralipid infusion, and on group II monkeys after androstenedione treatment, when at least three consecutive and augmenting nocturnal switches in myometrial activity had occurred. The nature and intensity of the switch in myometrial activity, from contractures to contractions, was similar in groups I and II.

Data analysis

Fetal experiments. Values for hormone concentrations, in the two infants from which blood was drawn from the right ventricle, were similar to values measured in the umbilical vein of fetuses under study. All measured hormone concentrations from these animals were thus pooled within their respective study groups.

Umbilical vein blood gases and pH, and circulating concentrations of CRH, ACTH, DHEAS, and cortisol are expressed as the mean ± sem for each age group: group I (STL), group II (APL), and group III (NIL). Similarly, for each of these study groups, adrenal weights are presented as the mean ± sem of the combined (left and right adrenal gland) absolute weight, as well as the combined adrenal weight, expressed as a percentage of body weight.

For all of these variables, measured statistical comparisons were made between groups I-III, by ANOVA, with the Student Newman-Keuls test for normally distributed data, and with Kruskal-Wallis with Dunn’s test for distribution-free data. Statistical significance was accepted at P < 0.05.

Maternal experiments. Results for daily maternal plasma CRH concentrations in late pregnancy were divided into five gestational age groups: 119–124; 125–135; 136–146; 147–157; and 158–168 dGA. At each age group, the daily CRH concentrations were averaged for each individual animal. Plasma hormone concentrations at each age group represent the mean ± sem of all individual animal means. Trends in plasma hormone concentrations over time were analyzed using linear regression analysis. Differences in plasma hormone concentrations between gestational age groups were assessed with ANOVA for repeated measures with the Student-Newman-Keuls test. The first gestational age group (119–124 days gestation) was omitted for this analysis because it contained only one animal. Statistical significance was accepted at P < 0.05.

Results

Fetal experiments

Fetuses from mothers undergoing STL (group I) were taken at an older gestational age (P < 0.05) than fetuses from mothers undergoing APL (group II) and fetuses from mothers not in labor (group III). Group II and group III fetuses were taken at cesarean section at a similar gestational age. Consequently, group III fetuses served as gestation-matched, NIL controls for group II fetuses (Tables 1 and 2).

Circulating umbilical vein CRH concentration was elevated to 99.0 ± 6.1 pg/ml⁻¹ in fetuses from mothers undergoing STL (group I, n = 6) and to 107.4 ± 13.5 pg/ml⁻¹ in fetuses from mothers undergoing APL (group II, n = 5), compared with fetuses from control mothers (70.3 ± 7 pg/ml⁻¹, n = 7, group III, NIL) (P < 0.05, Fig. 1). Treatment of pregnant monkeys with androstenedione resulted in umbilical vein plasma CRH concentrations similar to those measured at STL (Fig. 1).

Umbilical vein blood gases and pH were similar for all study groups (Table 2). Despite this, umbilical plasma ACTH (439.2 ± 91.4 pg/ml⁻¹, n = 6) and DHEAS (278.4 ± 132.9 ng/ml⁻¹, n = 6) concentrations were higher in group I (STL) fetuses than in group II (APL, n = 5; ACTH = 163.5 ± 74.5 pg/ml⁻¹; DHEAS = 33.1 ± 6.9 ng/ml⁻¹) or group III (NIL, n = 7; ACTH = 223.3 ± 43.3 pg/ml⁻¹; DHEAS = 34.3 ± 6.8

Fig. 1. Umbilical vein plasma CRH, ACTH, DHEAS, and cortisol concentrations (mean ± SEM) for fetuses whose mothers underwent STL (group I, black bars, n = 6), androstenedione-induced labor (APL, group II, gray bars, n = 5), and those whose mothers were not in labor (NIL, group III, white bars, n = 7). Comparisons were made between the three study groups by ANOVA with the Student Newman-Keuls test for normally distributed data and with Kruskal-Wallis with Dunn’s test for distribution-free data. a, P < 0.05 vs. NIL (ANOVA); b, P < 0.05 vs. NIL/APL (ANOVA); c, P < 0.05 vs. NIL/APL (Kruskal-Wallis test); d, P < 0.05 vs. APL (Kruskal-Wallis test).
ng/ml) fetuses \((P < 0.05, \text{Fig. 1})\). Umbilical plasma cortisol concentrations were elevated in group I (STL, 167.5 ± 38.8 ng/ml, \(n = 6\)) fetuses, when compared with group II (APL, 100.4 ± 7.8 ng/ml, \(n = 5\)) fetuses \((P < 0.05, \text{Fig. 1})\), but were not different when they were compared with group III (NIL, 123.0 ± 8.5 ng/ml, \(n = 7\)) fetuses.

The increased combined absolute adrenal weight in group I (STL) fetuses, compared with group II (APL) and group III (NIL) fetuses, fell outside significance \((P < 0.09)\). The lack of statistical significance in this comparison was reinforced, when comparing the combined adrenal weights, expressed as a percentage of body weight, among the three study groups (Table 1).

**Maternal experiments**

**Maternal experiments**

Chromatography of maternal rhesus monkey plasma CRH. Pooled plasma extracts from all animals used in the study between 119–168 dGA revealed that the majority of the CRH immunoactivity coeluted with synthetic human CRH. In addition, a second smaller molecular weight peak was detected, similar to that observed in human placental extracts (30).

**Maternal plasma hormone concentrations in late pregnancy.** All plasma samples for which hormone concentrations are reported were taken when the uterus was in the contractures mode and before the development of labor in all animals. Mean maternal plasma concentrations of CRH between 119–168 dGA, in the pregnant monkey, were 500 ± 99 pg/ml. Linear regression revealed a positive trend in maternal plasma CRH \((R^2 = 98.9, \text{slope} 76.6 \text{pg/ml}^{-1} \cdot \text{group}, P < 0.0005)\) and a modest, but significant, increase with gestation when analyzed by ANOVA for repeated measures with the Student-Newman-Keuls test (Fig. 2). When comparing the four gestational age time groups (125–135, 136–146, 147–157, and 158–168 dGA), maternal plasma CRH between 158–168 dGA (682.1 ± 166.7 pg/ml) was significantly elevated \((P < 0.05)\) from maternal plasma CRH concentrations between 136–146 (512.9 ± 83.4 pg/ml) and 125–135 (308.3 ± 64.9 pg/ml) dGA.

**Discussion**

We (18) and others (31–34) have hypothesized a central role for placental estrogen in linking fetal adrenal androgen production and promotion of parturitional events in the primate. According to this hypothesis, sustained administration of androstenedione to pregnant monkeys leads to persistent elevations in plasma estradiol concentrations, recurrent switching in myometrial activity to contractions, cervical dilatation and effacement, fetal membrane rupture, and live delivery of the monkey fetus (18). However, in another study, we have reported also that androstenedione treatment of the pregnant monkey at 0.8 of gestation is accompanied by an increase in placental CRH mRNA and peptide concentrations, to values similar to those measured at STL (20). Thus, it could be argued that premature labor and delivery, after androstenedione treatment of the pregnant monkey, is induced by premature activation of the fetal pituitary-adrenal axis, resulting from increased placental CRH, instead of or in addition to promoting a direct increase in placental estrogen synthesis.

We report that the androstenedione-induced increases in placental CRH mRNA and peptide concentrations previously measured by molecular and cellular techniques (20) are reflected in a significant increase in umbilical vein plasma CRH concentrations in the monkey. This increase in umbilical vein plasma CRH is of similar magnitude to that measured in monkey fetuses whose mothers were in STL (present study) and to that measured in human fetuses during term labor (35).

However, despite similar elevations in umbilical vein plasma CRH concentrations in androstenedione-induced labor in STL groups in the present study, umbilical vein plasma ACTH, DHEAS, and cortisol concentrations were not elevated in the former group. Therefore, these data do not support the hypothesis that androstenedione-induced labor in the monkey is caused by increased fetal pituitary-adrenal activity resulting from elevated placental CRH, but rather, they emphasize that androstenedione-induced labor in the monkey is caused by a direct increase in placental estradiol biosynthesis, by-passing the fetal hypothalamo-pituitary-adrenal (HPO) axis, as first proposed by Meenes et al. (18).

The dissociation between umbilical plasma CRH and fetal pituitary-adrenal products is interesting, particularly because the immunoreactivity present in human (4) and rhesus monkey (25) maternal plasma corresponds to CRH(1–41), and circulating CRH has been reported to be biologically active in human fetal plasma (35). One possible explanation for this apparent discrepancy could be that circulating umbilical vein plasma CRH concentrations in androstenedione-induced labor or STL are much lower than those present in the fetal monkey hypothyseal portal system and, therefore, insufficient to stimulate corticotrope activity. It is instructive to compare the hypothyseal-portal concentrations reported by Plotsky et al. in the rat (approximately 200 pg/ml) and Liu et al. in the sheep (approximately 100 pg/ml) (36).

Contrary to common assumption, these concentrations are similar to the peripheral plasma concentrations reported here. These observations demonstrate the need for studies that determine the effects of physiological alterations in peripheral CRH concentrations on ACTH release, in both pregnant and nonpregnant primates, in comparison with other species. Alternatively, pituitary-adrenal sensitivity in fetuses from the androstenedione labor group, which were younger,
may be lower than pituitary and/or adrenal sensitivity in term fetuses. However, Berghorn and colleagues (37) reported that, in the baboon, the fetal hypothalamic-pituitary axis was capable of responding to intracarotid injection of CRH with significant increases in fetal plasma ACTH, even at midgestation. The resulting peripheral CRH concentration in the fetus, as a result of exogenous CRH administration, was not measured. Interestingly, in that same study, when CRH was administered into the fetal antecubital vein, fetal plasma ACTH concentrations remained unchanged. In addition (although substantial evidence for maturation of fetal pituitary and adrenal responsiveness in late gestation exists, with development of pituitary responsiveness preceding that of the adrenal gland) (38), the adrenal cortex of the human, baboon, and monkey fetus (even at midgestation) has been repeatedly reported to be responsive (in terms of androgen or cortisol secretion) to a variety of trophic peptides (including ACTH), both in vitro (39–44) and in vivo (33, 45–47). Taken together, these past reports suggest that the lack of fetal pituitary-adrenal stimulation by umbilical plasma CRH after androstenedione treatment in the pregnant monkey is not caused by plasma CRH bioactivity or by insufficient local CRH concentration at the fetal corticotrope level or by insensitivity of the fetal pituitary or the adrenal glands.

More plausible explanations may relate to the presence of binding proteins (BPs) for CRH in fetal plasma or to confounding influences imposed by androgen negative feedback at the fetal HPA axis. To address the first point, a CRH-BP exists in human maternal plasma (48) and has been shown to reduce ACTH-releasing bioactivity of placentally derived CRH, but not hypothalamic CRH (49). Furthermore, plasma CRH-BP concentrations in pregnant women are elevated until late gestation and fall markedly toward term (50). Because CRH-BP is also present in human umbilical blood, albeit at much lower concentrations than those measured in the maternal circulation (51), it could be hypothesized that, in the present study, the bioactivity of umbilical vein plasma CRH in younger fetuses from androstenedione-treated mothers was masked by elevated fetal CRH-BP concentrations at this time. In contrast, umbilical CRH in older fetuses from mothers undergoing STL may possess greater bioactivity, caused by reduced fetal CRH-BP concentrations at term. To date, the presence of CRH-BP in rhesus monkey maternal or fetal plasma and their relationship to gestational age have not been addressed.

Second, evidence exists for negative feedback regulation by androgens at the HPA axis in the pregnant monkey. This evidence is based on a study that reported that, after androstenedione treatment of the pregnant monkey, maternal plasma ACTH concentrations fell significantly (28). Although the operation of androgen feedback mechanisms have not been demonstrated in the primate fetus, it is possible that the capacity of umbilical vein plasma CRH to stimulate pituitary ACTH and adrenal steroidogenesis, in fetuses from androstenedione-treated mothers, may have been masked by opposing influences on ACTH secretion imposed by the androgenic actions of androstenedione itself. In relation to this, it is of interest that Pepe et al. (47) reported that the ability of the fetal adrenal to increase DHA production, in response to an acute infusion of ACTH or PRL, was also abolished after maternal androstenedione treatment in the pregnant baboon. If the fetal adrenal is suppressed by androstenedione treatment, this does not exclude the possibility that, during normal pregnancy, a rise in placental CRH and fetal adrenal products may be causally linked.

Another interesting point arising from the data presented in this manuscript relates to a comparison between umbilical vein hormone concentrations in fetuses whose mothers were in STL and fetuses whose mothers were not in labor. Despite similar blood gas status, umbilical vein plasma ACTH and DHEAS concentrations were higher in the former than in the latter group. It is well established that increased activity of the fetal HPA axis plays an important role in the initiation of parturition in pregnant sheep (38), but no unequivocal evidence exists to support such a role for the fetal HPA axis in primate species. Difficult paradigms, such as experimental anencephaly (52) or adrenalectomy (53) of fetal monkeys, disrupt the timing of parturition but yield inconclusive results to support a role for the fetal HPA axis in promoting parturition in the nonhuman primate. Previous studies on gestation-related hormonal changes in the fetal circulation in primate pregnancy are limited to the elegant and arduous work of Seron-Ferre et al. (54) and Walsh et al. (55), who reported that vaginal delivery in monkeys with live fetuses was preceded by rising exponential concentrations of DHEAS in fetal, but not in maternal, blood. The present study is the first to report that in the pregnant monkey fetal ACTH, in addition to DHEAS, concentrations are elevated in STL. With the provision that these measurements are under the influence of halothane anesthesia, these data support a role for activation of the fetal pituitary-adrenal axis in association with parturition in the primate. Thus, during normal pregnancy, likely sources of increased androstenedione may be the placenta, after the action of placental 3β-HSD on increased androgen synthesis from fetal adrenal origin and/or the fetal adrenal itself, after conversion of DHEAS to androstenedione by 3β-HSD within the adrenal (56, 57). The alternative explanation, that the increased fetal pituitary-adrenal activity in the monkey is the result of STL, cannot be discounted. However, because androstenedione-induced labor was not accompanied by increased fetal HPA activity, this possibility seems unlikely.

The group of fetuses from mothers that were not in labor, presented in the current paper (group III), span a large range of gestational ages. It is of interest that two fetuses from this group were at 160 and 163 days gestation. However, in these two fetuses, umbilical plasma CRH, ACTH, DHEAS, and cortisol concentrations were low and are representative of the mean values reported for this group.

That umbilical vein plasma cortisol concentrations are not significantly elevated in fetuses whose mothers were in STL (compared with fetuses whose mothers were not in labor) is, perhaps, not a surprising result. Walsh et al. (55) suggested that because the primate placenta is readily permeable to cortisol (58), but impermeable to DHEAS (59), plasma cortisol levels in the fetus are less sensitive indicators of short-term changes in fetal adrenal activity than are fetal plasma concentrations of DHEAS. Another factor that may account for the lack of significant change in umbilical cortisol con-
centrations is increased cortisol metabolism by the placenta and fetal liver in late gestation (58).

Finally, it should be emphasized that important differences exist in the nature and magnitude of the maternal plasma CRH increase with gestation amongst primate species. Whereas, in pregnant women, maternal plasma CRH increases exponentially (approximately 100-fold) toward term (1, 2, 4), in pregnant baboons, maternal plasma CRH concentrations peak in the first half of pregnancy and do not increase further at term (9, 10). Our present results suggest, for the first reported, that in the pregnant rhesus monkey, maternal plasma CRH does increase toward term but that this increase is modest, compared with the pregnant woman (8). This further result reveals important interprimate species differences in placental CRH physiology.

In conclusion, the data presented in this study: 1) demonstrate that androstenedione treatment of the pregnant monkey, at 0.8 of gestation, leads to elevated umbilical vein plasma CRH, to concentrations similar to those measured at term; 2) do not support the hypothesis that androstenedione-induced labor in the pregnant monkey is caused by increased fetal adrenal steroidogenesis resulting from increased placental CRH; but 3) do support a role for activation of the fetal HPA axis in association with parturition in the primate; and 4) demonstrate a modest increase in maternal plasma CRH during late gestation in the pregnant rhesus monkey.

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