Changes in the responsiveness of adrenal cells from foetal goats at different times during pregnancy

John R. G. Challis and W. Bruce Currie†

MRC Group in Reproductive Biology, The University of Western Ontario, Departments of Obstetrics & Gynaecology and of Physiology, London, Ontario, Canada and Cornell University†, New York State College of Agriculture and Life Sciences, Ithaca, New York, USA

Abstract. To understand the factors responsible for the increased pre-partum output of cortisol from the adrenal glands of the goat foetus, we measured changes in responsiveness to ACTH in vitro of dispersed adrenal cells from foetal goats of various gestational ages and we determined the output of cortisol (F) and corticosterone (B) in the presence of exogenous progesterone (P4) and 17α-hydroxyprogesterone (17α-OHP4). The increment in F output after ACTH increased 5-fold between day 100 and days 147 or 154 (term). This increase was not associated with changes in the ED₅₀ for ACTH stimulation of F output. The basal and ACTH-stimulated output of F was similar to that of B at day 100 but 5–7 times greater than that of B at day 154. There was a significant effect of ACTH on P4 output on days 77, 100 and 147 but net utilization of P4 by the cells at term. The ratio of exogenous P4: 17α-OHP4 converted to F varied with gestation and increased from 0.11 at day 100 to 0.99 at day 154. Exogenous P4 was converted to B at all stages of pregnancy.

We conclude that in the goat there is an increase in the responsiveness of foetal adrenal cells to ACTH between day 100 of gestation and term. One factor associated with this change in adrenal responsiveness may be an increase in the activity of the enzyme 17α-hydroxylase.

In the goat, as well as in the sheep, parturition is thought to be triggered by the foetus through an increase in the production of cortisol from the foetal adrenal gland (Liggins et al. 1977; Currie & Thorburn 1977a). In foetal sheep, the increase in adrenal cortisol output is attributable, at least in part, to an increase in the responsiveness of the foetal adrenal gland to trophic hormonal stimulation during late pregnancy (Madill & Bassett 1973; Wintour et al. 1975; Glickman & Challis 1980; Magyar et al. 1980). This increase in response is associated with an increase in the activity of adrenal 17α-hydroxylase as well as other enzymes (Anderson et al. 1972; Challis et al. 1981a). In the present study, we have investigated whether the pre-partum rise in plasma cortisol concentrations in the foetal goat (Currie & Thorburn 1977a,b) might also result from increased adrenal responsiveness to ACTH stimulation. We incubated adrenal cells from foetal goats of various gestational ages with ACTH and with different potential substrates for corticosteroid biosynthesis, to determine whether any changes in responsiveness might be associated with alterations in 17α-hydroxylase activity.

Materials and Methods

Animals

Adrenal tissue was obtained from 2 foetuses on day 77 of pregnancy, from 5 foetuses on day 100, from 3 foetuses on day 147 and from 5 foetuses on day 154, at the onset of active labour. The goats were of predominately French Alpine breeding and were housed under typical commercial conditions. They were fed a ration of hay and high protein concentrate to requirements. The stage of pregnancy was dated from artificial insemination and was confirmed from the size of the foetus at the time.
of autopsy. Under comparable management conditions during the previous year, the length of pregnancy ranged between 153 and 155 days.

Collection and preparation of tissues
Goats were anaesthetized with sodium thiamylal, and the foetuses were delivered surgically. The foetal adrenal glands were removed, and dispersed adrenal cells were prepared by using 0.05% collagenase (Type I, Sigma Chemical Co., St. Louis, Mo), essentially as described by Glickman & Challis (1980). The cells were incubated (50,000–100,000 cells/ml) for 6 h under conditions described previously (Glickman & Challis 1980) in the absence or presence of ACTH, (17a-OHP4; Cortrosyn®, Organon, Toronto, Canada), and in some experiments with progesterone (P4) or 17α-hydroxyprogesterone (17α-OHP4; both 100 ng/ml, ~0.3 μM, Steraloids, Wilton, NH). P4 and 17α-OHP4 were added in 5 μl re-distilled ethanol. All other tubes received 5 μl ethanol alone. At the end of the incubation period the tubes were frozen at -20°C until analysis.

Radioimmunoassay
Total concentrations, (i.e. cells and medium) of cortisol, corticosterone and progesterone were determined by RIA as described previously (Challis et al. 1981a,b). The samples were all measured in one assay. The intra-assay coefficients of variation for cortisol, corticosterone and progesterone were 7.7%, 13.7% and 8.1%, respectively. Tubes containing incubation buffer alone (Glickman & Challis 1980), or buffer with either 0.3 μM P4 or 17α-OHP4, but without adrenal cells, were included in each incubation, assayed, and subtracted as RIA cross-reactivity blanks as appropriate.

Analysis of results
The concentration of steroid present in the cells at the start of the incubation period (T0) was measured, and subtracted from that present in the tubes after incubation to give net output. All results are expressed as mean ± SEM for triplicate observations on pooled adrenal tissue from the number of foetuses indicated above. The amount of ACTH required to produce half maximum output of steroid (ED50) was calculated from plots of the arithmetic means of cortisol concentrations. One way analysis of variance was used to determine the significance of ACTH effects on cortisol and corticosterone output. Nested analysis of variance was used to compare ACTH effects on corticosteroid output at different gestational ages. Duncan’s Multiple Range Test was used to compare basal values of steroid output and the effects of P4 and 17α-OHP4 on cortisol and corticosterone production.

Results

Changes in foetal adrenal weight during pregnancy
The mean weight of pairs of foetal adrenal glands increased from 37.2 mg (2 foetuses) on day 77 to 68.8 ± 2.6 mg (mean ± SEM; 5 foetuses) on day 100 and 266.3 ± 18.0 mg (5 foetuses) at term.

Foetal adrenal responsiveness to ACTH
The basal output of cortisol, in the absence of ACTH, was greater on day 77 (21.7 ± 1.32 pg/10³ cells/6 h) and at term (15.8 ± 2.3 pg/10³ cells/6 h) than on days 100 or 147 (Table 1; Fig. 1). The effect of ACTH on cortisol output was significant on days 100, 147 and term, (all P < 0.001), but not on day 77 (P > 0.05). Cortisol output in response to ACTH was significantly greater on day 147 and at term than on day 100 (P < 0.001). The increment (Δ) in cortisol output when the maximum

<table>
<thead>
<tr>
<th>Time of pregnancy (days)</th>
<th>Cortisol output (pg/10³ cells/6 h)</th>
<th>Δ increment (pg/10³ cells/6 h)</th>
<th>ED50 pg ACTH/ml</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Maximum</td>
<td></td>
</tr>
<tr>
<td>77</td>
<td>21.7 ± 1.3*</td>
<td>36.7 ± 9.3</td>
<td>N/A</td>
</tr>
<tr>
<td>100</td>
<td>4.0 ± 0.3</td>
<td>16.6 ± 0.4</td>
<td>12.6</td>
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<tr>
<td>147</td>
<td>5.8 ± 0.3</td>
<td>56.8 ± 3.8</td>
<td>51.0</td>
</tr>
<tr>
<td>154 (term)</td>
<td>15.7 ± 2.3</td>
<td>67.2 ± 10.7</td>
<td>51.5</td>
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* Mean ± SEM for triplicate observations, N/A = not applicable; no significant stimulation by ACTH.
The term foetal from and vitro. The o i-er ÜJ term day -z. basal S ro Oo o-CO ct40 20h 40 60 term output (Table h after 0 goats to 100, (A-A) goats at the of pregnancy IÖ the 31). The day 100, 3 at day 147 and 5 at term (day 154).

values after addition of ACTH were compared to basal outputs increased from 13 pg/10^3 cells/6 h on day 100, to > 50 pg/10^3 cells/6 h on day 147 and at term (Table 1). The ED_{50} did not change signifi-
cantly between day 100 of pregnancy and term (Table 1).

The basal output of corticosterone on day 77 (12.4 ± 1.5 pg/10^3 cells/6 h) was significantly greater than at all other stages of pregnancy (all P < 0.05; Fig. 2). There was a significant effect of ACTH treatment on corticosterone output on days 100 and at term, but not at day 77 nor day 147 (Fig. 2). The increment (Δ pg/10^3 cells/6 h) for corticosterone output was similar on day 100 and at term (Fig. 2).

The ratio for basal outputs of cortisol: corticosterone (F:B) was 1.45 on day 100 and 7.15 at term. The mean F:B output ratio at all concentrations of ACTH added was significantly lower on day 100 (1.09 ± 0.086; sem, n = 6) than at term (5.49 ± 1.00; sem, n = 6; P < 0.01 t-test).

The basal output of progesterone on day 77 (22.6 ± 5.0 pg/10^3 cells/6 h) was greater than at other times in pregnancy (Fig. 3). There was a significant effect of ACTH on progesterone output by foetal adrenal cells on days 77, 100 and 147 (all P < 0.01); (Fig. 3). At term, the mean concentra-
tion of progesterone in the cells at the start of incubation (17.7 pg/10^3 cells), was greater than the concentration in the cells + medium for all incubations in the absence or presence of ACTH, presumably reflecting net utilization of progesterone by the cells at this time.
The output of cortisol by isolated adrenal cells from foetal goats at day 77, 100, 147 and term pregnancy (left to right) in the basal state ([ ]) in the presence of ACTH ([ ]) P4 ([]), P4+ACTH ([]), 17α-OHP4 ([]) and 17α-OHP4+ACTH ([]). For concentrations of hormones added, see text. Each value is the mean ± SEM of triplicate observations for the number of animals indicated in Fig. 1.

**Effects of exogenous P4 and 17α-OHP4 on cortisol and corticosterone output**

The effects of P4 and 17α-OHP4 (0.3 μM) in the absence or presence of ACTH (100 ng/ml) on cortisol and corticosterone output are shown in Figs. 4 and 5. On day 77 there was significant conversion of both P4 (P < 0.05) and 17α-OHP4 (P < 0.01) into cortisol, with no further stimulation by ACTH. Cortisol formation from 17α-OHP4 was significantly greater than that from P4 (P < 0.01).
On day 100, the mean output of cortisol after addition of progesterone was greater than in the control or ACTH-treated incubations, but, by Duncan's analysis, these differences were not statistically significant. There was a significant increase in cortisol output after addition of 17α-OHP4 ($P < 0.01$). On day 147, addition of P4 or 17α-OHP4 caused a significant increase in cortisol output over basal or ACTH-stimulated values (both $P < 0.01$). For each substrate, cortisol output was enhanced further in the presence of ACTH (both $P < 0.05$). Cortisol output after addition of 17α-OHP4 was significantly greater than after the addition of P4 ($P < 0.05$). At term, both P4 and 17α-OHP4 increased cortisol output, but there was no difference between the substrates and no further effects of ACTH ($P < 0.05$). Fig. 4 shows that the ratio of cortisol formed from P4 relative to that formed from 17α-OHP4 rose from 0.11 on day 100 to 0.55 on day 147 and 0.99 at term.

After addition of P4 there was a significant output of corticosterone by foetal adrenal cells at all the times in gestation that were studied (all $P < 0.05$; Fig. 5). At day 147, but not at other times, the output of corticosterone from P4 was lower after addition of ACTH ($P < 0.05$). The output of corticosterone after the addition of 17α-OHP4 with or without ACTH was not different from the basal values, nor from that obtained in the presence of ACTH alone. The mean ratio of progesterone converted to cortisol and corticosterone was 0.13 on day 100, 0.73 on day 147 and 1.52 on day 154.

**Discussion**

At different times in pregnancy we found substantial changes in the capacity of isolated adrenal cells from foetal goats to produce cortisol and corticosterone after the addition of ACTH or of potential precursors for corticosteroid production. We recognize that as in the foetal sheep adrenal (Robinson et al. 1979), these changes could be due to either alterations in the activities of an existing cell type, or to the appearance of new cell types with a different potential for corticosteroidogenesis or possibly both of these explanations.

We found that on day 77 the basal output of cortisol and of corticosterone was higher than at other times in pregnancy. These cells were not stimulated to produce corticosteroids by ACTH although they did produce progesterone. At this time, exogenous P4 and 17α-OHP4 were converted to cortisol, indicating that the cells had some 17α-hydroxylase, 21- and 11β-hydroxylase activities. The failure to demonstrate cortisol output after addition of ACTH but in the absence of exogenous substrate may indicate that under these conditions endogenously produced progesterone is metabolized to other products that were not measured in our studies.

We examined steroid production only from endogenous precursor pools, or from exogenous C_{21} Δ^{4}-3-oxo steroids. In human foetal adrenal glands, Carr et al. (1980) have shown utilization of low density lipoprotein (LDL) for steroidogenesis. However, we have failed to stimulate cortisol output by sheep foetal adrenal cells after addition of LDL in vitro, at times when these cells respond poorly to ACTH (Manchester & Challis, unpublished observations), suggesting that availability of a precursor as LDL may not be the rate-limiting step.

In foetal sheep there is a decrease in adrenal responsiveness between day 50 and 100 which may relate to competition between ACTH and larger peptide(s) for ACTH binding sites on the adrenal (Jones & Roebuck 1980). In foetal sheep the block to cortisol production at day 100 cannot be overcome by provision of exogenous guanylylimidodiphosphate or dbcAMP, suggesting that intracellular steps in steroidogenesis are involved (Challis et al. 1981a). Our present demonstration that ACTH stimulates a significant rise in cortisol output by foetal goat adrenals at day 100, might suggest that in this species any block is not as complete as in the foetal sheep.

The maximum responsiveness of the foetal sheep adrenal to ACTH stimulation increases between days 100–110 of pregnancy and at term (Wintour et al. 1975; Glickman & Challis 1980; Magyar et al. 1980). The present results suggest that a similar pattern of increasing responsiveness occurs in the adrenal of the foetal goat. By day 100, foetal adrenal cells produced cortisol in response to exogenous ACTH, implying the presence of ACTH receptors by this time. However, the ED_{50} for ACTH stimulation of cortisol output remained similar between day 100 and term, indicating that the sensitivity of the cells was relatively unchanged throughout the last 50 days of pregnancy. The increase in responsiveness was expressed best as the increment in cortisol output after addition of ACTH, which is similar to results in the foetal
rabbit (Devskar et al. 1980) and sheep (Magyar et al. 1980). In foetal sheep, it has been suggested that the increase in adrenal responsiveness during late pregnancy may be associated with an increase in the concentration of ACTH receptors (Durand 1979; Durand et al. 1981), adenylate cyclase coupling (Durand et al. 1981), or to an increased enzymatic capacity for cortisol biosynthesis (Anderson et al. 1972; Challis et al. 1981a).

The present results, while not excluding other explanations, suggest that an increase in enzymatic activity, at least in 17α-hydroxylase, attends and may explain the increase in responsiveness. In the absence of exogenous precursors, but in the presence of ACTH, the ratio of cortisol: corticosterone output rose 5-fold between day 100 and term. In addition, the relative conversions of progesterone and 17α-OHP4 to cortisol rose from 0.11 to 0.99 during this period of time. The relative lack of P4 conversion to cortisol at day 100 was not due to an inability of P4 to enter the cells, since P4 was converted readily to corticosterone at all times in pregnancy studied. Furthermore, there was a 10-fold increase in the ratio of progesterone conversions to cortisol and corticosterone between days 100 and 154. Taken together, these results suggest strongly that 17α-hydroxylase activity increases in the goat foetal adrenal in late pregnancy. It is possible that changes in 11β-hydroxylase might also promote corticosteroid production (Anderson et al. 1972). Because other pathways of P4 metabolism in goat foetal adrenals were not examined in this study, and have not been described elsewhere, we are unable to assess the possible importance of alternate fates of progesterone.

It is of interest that on day 147, ACTH increased the output of cortisol from both P4 and 17α-OHP4 while it appeared to decrease the formation of corticosterone from P4. The decrease in corticosterone indicates that the effect of ACTH on cortisol output is unlikely to reflect increased precursor uptake into the cells. These results raise the possibility that ACTH may have short-term effects on hydroxylase systems in directing the pathways to cortisosteroid production, in addition to actions on cholesterol side-chain cleavage (Sayers & Portanova 1975).

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References


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