DIFFERENT BIOLOGICAL ACTION OF CORTICOSTEROIDS, CORTICOSTERONE AND CORTISOL, AS A BASE OF ZONAL FUNCTION OF ADRENAL CORTEX

By
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ABSTRACT

The effects of corticosterone and cortisol in concentrations attainable in the adrenal gland were studied on ACTH-induced steroidogenesis in cultured cortical cells of foetal human and rat adrenals. Corticosterone at a concentration of $5.8 \times 10^{-5}$ mol/l clearly inhibited cortisol production ($65.5\%; P < 0.005$) and simultaneously increased androgen production in tissue culture of foetal human adrenals. Cortisol at a concentration of $2.8 \times 10^{-4}$ mol/l clearly inhibited 18-OH-DOC (74.0\%, $P < 0.001$) and aldosterone (83.7\% $P < 0.005$) production in tissue culture of foetal rat adrenals. In primary culture of foetal human adrenals cortisol did not decrease aldosterone production absolutely, but it significantly decreased the relative amount of aldosterone with respect to corticosterone. Cortisol did not inhibit corticosterone production in either culture.

The results demonstrate that cortisol and corticosterone have qualitatively different effects on adrenal steroidogenesis and that these steroids may play a basic role in the functional zonation of the adrenal gland.

ACTH is the trophic stimulus for adrenal steroidogenesis and elevated levels of serum glucocorticoids inhibit hypophyseal ACTH secretion. The concentrations of adrenal steroids in peripheral blood have hardly any direct effect on steroidogenesis at the adrenal level, but due to the architecture and the centripetal blood flow of the adrenal cortex, its inner zones and the medullary tissue are constantly exposed to between 10- and 100-fold higher concentrations of corticoids than cells generally are (Wherry et al. 1962; Dobbie & Symington

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Besides, the concentration of corticoids in stimulated adrenal cells has been estimated to be 40-fold higher compared to the extracellular medium (Clayman et al. 1970). The inhibitory effect of corticosteroids on RNA (Ferguson et al. 1967) and protein (Morrow et al. 1967) synthesis by adrenal cells in vitro has been demonstrated. Kahri (1970, 1973) has shown that daily addition of corticosterone at a concentration of $6 \times 10^{-3} \text{ M}$ into tissue culture medium of rat foetal adrenals completely inhibited the ACTH-induced mitochondrial differentiation (the change of the mitochondrial inner membrane from a tubular or lamellar configuration to a vesicular one). Salmenperä et al. (1976) have shown that corticosterone at a concentration of $5.7 \times 10^{-5} \text{ M}$ inhibited 11β- and 18-hydroxylations in tissue culture of foetal rat adrenals. Based on these observations the effects of corticosterone and cortisol on steroidogenesis of cortical cells were tested in primary culture of human foetal adrenals. In addition, the effects of cortisol and corticosterone on steroidogenesis of cultured foetal rat adrenals were compared with the effects of the same hormones on human foetal adrenals.

**MATERIALS AND METHODS**

*Tissue culture experiments*

Early midterm foetuses were used for the preparation of primary cultures of human adrenals according to the method described by Kahri (1966). The crown-rump length of the foetuses used in this study ranged from 5 to 17 cm corresponding to the gestational age of 10–18 weeks (Tanimura et al. 1971). They were obtained from abortions induced for sociomedical reasons. Twenty-one-day-old foetal albino Sprague-Dawley rats were used for the preparation of primary cultures of rat adrenals according to the method of Kahri (1966). The tissue culture medium (5 ml/dish) consisted of 50 % Melnick’s solution A (0.5 % lactalbumin hydrolysate in Hanks’ balanced salt solution), 25 % Eagle’s minimum essential medium (both from Pharmaceutical Manufacturer Orion Oy, Finland) and 25 % newborn calf serum (Gibco, U.K.) and it was changed every 6th day. The cultures were studied by phase contrast microscopy for cell viability and morphological changes. After homogenous undifferentiated cell growth had been achieved in rat adrenal culture, ACTH (Synaethen, Ciba-Geigy Ltd., Basel, Switzerland) was added from day 18 for 6 days at a daily dose of 0.2 μg/ml (= 0.02 IU/ml) to the rat adrenal culture. This dose has been tested to cause maximal stimulation of steroidogenesis. In human adrenal culture ACTH stimulation was carried out in a similar way, from the beginning of the 6th day of cultivation when the proliferating cells were of undifferentiated permanent cortex type (Kahri & Halinen 1974; Kahri et al. 1976). To test the effects of corticosterone on the ACTH-induced steroid secretion in human adrenal cultures, the corticosterone (Sigma Co., USA) concentration was adjusted to $5.8 \times 10^{-2}$, $5.8 \times 10^{-6}$, $2.9 \times 10^{-5}$ and $5.8 \times 10^{-5}$ mol/l in the final tissue culture medium at the same time as ACTH stimulation was started (on the 6th day of cultivation). Similarly, in order to test the effects of cortisol in human and rat adrenal cultures, the cortisol (Sigma Co., USA) concentration was adjusted to $5.8 \times 10^{-5}$ and $2.8 \times 10^{-4}$ mol/l in the final tissue culture medium at the same time as ACTH.
stimulation was started (on the 6th day of cultivation in human and on the 18th day in rat adrenal cultures). After stimulating the cultures for 6 days, the tissue culture media were stored at −20°C until analysed.

Steroid measurements from culture media

The steroid measurements from the culture media were made by the method of Salmenperä & Kahri (1976) after slight modification. This method is described in detail elsewhere (Voutilainen 1979). Only a brief description will be given here. After addition of the tracers ([3H]18-OH-DOC (18-hydroxysteroidcorticosterone; 18-21-dihydroxy-4-pregnene-3,20-dione) 10 000 cpm, [14C]corticosterone 1000 cpm and [3H]aldosterone 1000 cpm for rat media and [3H]cortisol, [3H]corticosterone, [3H]DHA (dehydroepiandrosterone) and [3H]aldosterone 1000 cpm of each for human media) the media were extracted with dichloromethane. The dried extracts were dissolved in 80% ethanol and partitioned with 4 vols. of cyclohexane. The samples were then fractioned on a 0.5 g Sephadex LH-20 column. The “androgen fraction” from the Sephadex column was further purified on a 0.15 g Hi-Flosil column. Cortisol, corticosterone, 11-deoxycortisol, 18-OH-DOC, DHA and androstenedione (4-androstene-3,17-dione) were quantitated as their O-methylxime-trimethylsilyl derivatives with gas liquid chromatography using 1% SE-30 packed columns. A flame ionization detector was used and the responses were detected with a digital integrator. Aldosterone was measured radioimmunologically using a highly specific antiserum (New England Nuclear, Mass., USA, prepared in sheep against aldosterone-18-21-dihemisuccinyl-BSA). In the concentrations measured here, the precision of the method was of the order of 2-17% (coefficient of variation). For statistical testing of the results Student's two sided t-test was used.

Endogenous cortisol (F) and 11-deoxycortisol (S) production in ACTH-stimulated (0.02 IU/ml/day for 6 days) tissue culture of human foetal adrenals in the absence and the presence of various doses of exogenous corticosterone (B). Vertical lines indicate ± SEM (n = number of cultures in each group).
RESULTS

Effects of corticosterone on steroidogenesis in ACTH-stimulated human foetal adrenal culture

Fig. 1 shows the effect of added corticosterone on endogenous ACTH-stimulated cortisol and 11-deoxycortisol production. The unstimulated steroid production at this stage of cultivation is very small (about \(1 \times 10^{-7}\) mol/l cortisol and \(<3 \times 10^{-8}\) mol/l corticosterone, 11-deoxycortisol, DHA and androstenedione) compared with the ACTH-stimulated situation. The inhibition of cortisol production increased as the corticosterone concentration was raised from \(5.8 \times 10^{-7}\) to \(5.8 \times 10^{-5}\) mol/l. The inhibition percentage of cortisol secretion at the concentration of \(5.8 \times 10^{-5}\) mol/l of corticosterone was 65.5% (\(P < 0.005\)). No toxic effects or changes in growth could be seen in the corticosterone-treated cultures. The aldosterone content of the cultures was also measured (results not shown in Fig. 1). The aldosterone concentration varied from \(1.1 \times \)
10⁻⁹ to 1.7 × 10⁻⁹ mol/l and there was no significant change in aldosterone secretion in corticosterone-treated cultures.

In the experiment shown in Fig. 2 some additional steroids were measured in order to get a more comprehensive picture of the effects of corticosterone and cortisol on adrenal steroidogenesis. Fig. 2 shows that in addition of decreased cortisol production corticosterone caused an increase in adrenal androgens: DHA increased 88.1 % (P = 0.10) and androstenedione 73.7 % (P < 0.01). 11-Deoxycorticosterone increased slightly (14.0 %). The effect of cortisol on ACTH-stimulated adrenal androgen secretion was less pronounced than that of corticosterone. A slight increase in corticosterone (44.1 %) and 11-deoxycorticosterone (64.5 %) was noted in those ACTH-stimulated cultures where exogenous cortisol was present compared with the mere ACTH-treated cultures (these changes were not statistically significant).

Fig. 3.
Concentrations of corticosterone (B), 18-hydroxydeoxycorticosterone (18-OH-DOC) and aldosterone (Aldost) in ACTH-stimulated (0.02 IU/ml/day for 6 days) tissue culture of foetal rat adrenals in the absence and the presence of exogenous cortisol (F) (2.8 × 10⁻⁴ M). Vertical lines indicate ± SEM (n = number of cultures in each group).

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Effects of cortisol on steroidogenesis in ACTH-stimulated foetal rat and human adrenal culture

Since cortisol did not significantly change the steroid pattern at the concentration of $5.8 \times 10^{-5}$ mol/l (when corticosterone already had clear effects), a higher concentration of cortisol was tested. As human material was scanty, foetal rat adrenal culture was used first (Fig. 3). Cortisol caused a clear decrease in 18-OH-DOC ($74.0\% ; P < 0.001$) and aldosterone ($83.7\% ; P < 0.005$) secretion, simultaneously as corticosterone production increased ($16.1\% ; P > 0.1$). Rat adrenal does not secrete androgens (because of the lack of 17α-hydroxylase) and these steroids were therefore not measured. A human foetal adrenal culture was then subjected to the same cortisol treatment (Fig. 4; the foetus used in this study was the smallest one in our material and it was possible to prepare only six culture dishes: the amount of adrenal tissue per dish was probably smaller than in the other experiments. For these reasons the absolute amounts of produced steroids were smaller than usually and therefore, for example, DHA and androstenedione could not be measured reliably from in-

![Fig. 4.](image)

Concentrations of corticosterone (B), 11-deoxycorticisol (S) and aldosterone (Aldost) in ACTH-stimulated (0.02 IU/ml/day for 6 days) tissue culture of human foetal adrenals in the absence and the presence of exogenous cortisol (F) ($2.8 \times 10^{-4}$ M). Vertical lines indicate $\pm$ SEM ($n =$ number of cultures in each group).
Table 1.
Effect of cortisol (F) on the relative secretion of corticosterone (B), aldosterone and 18-OH-DOC in ACTH-stimulated tissue culture of foetal rat and human adrenals. Ratios calculated from the results of the experiments shown in Figs. 3 and 4.

<table>
<thead>
<tr>
<th></th>
<th>ACTH rat</th>
<th>F + ACTH rat</th>
<th>ACTH human</th>
<th>F + ACTH human</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-OH-DOC/B</td>
<td>0.33 ± 0.012</td>
<td>0.074 ± 0.0045</td>
<td>0.074 ± 0.0045</td>
<td>0.20 ± 0.02</td>
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<tr>
<td></td>
<td><em>P &lt; 0.001</em></td>
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<td><em>P &lt; 0.01</em></td>
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<tr>
<td>10^2 x Aldosterone/B</td>
<td>1.91 ± 0.15</td>
<td>0.25 ± 0.08</td>
<td>0.61 ± 0.08</td>
<td>0.20 ± 0.02</td>
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<tr>
<td></td>
<td><em>P &lt; 0.001</em></td>
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dividual cultures in this experiment). The increase in corticosterone production in the cortisol-treated cultures was much higher (461 %; *P < 0.05) than in the rat culture. 11-Deoxycortisol secretion increased slightly (31.6 %; *P > 1.0). In contrast to the rat adrenal, there was no absolute decrease in aldosterone secretion in the human culture, but the relative amount of aldosterone in respect to corticosterone decreased significantly (*P < 0.01) (Table 1).

DISCUSSION
The concentration range of added corticosterone and cortisol in this study must be discussed in order to draw conclusions on the possible physiological significance of the present results. The concentrations of endogenously produced corticosterone in the tissue culture medium of human foetal adrenals in ACTH-stimulated dishes was 3.3 x 10^-6 mol/l (not shown in Fig. 1). Clayman et al. (1970) have shown that the corticosterone concentration in the adrenal gland was 40-fold compared to the maximal concentration in the incubation medium of ACTH-treated rat adrenals. They measured corticosterone concentrations as high as 4 x 10^-4 mol/l in the rat adrenal gland. The concentration of endogenously produced cortisol in the tissue culture medium of ACTH-stimulated dishes was about 2.6 x 10^-3 mol/l (Fig. 1). The intracellular concentration can therefore be considered to be much higher. It can thus be concluded that the corticosterone and cortisol concentrations used in the present experiments are physiological at the adrenal level (at least intracellularly). In rat adrenal the added cortisol concentration is not, of course, physiological because rat adrenal does not secrete any 17-hydroxylated steroids. Rat adrenals were used in experiments, because 18-hydroxylation is easier to study in rat than human adrenals by the present method.
In this study corticosterone clearly inhibited cortisol production in human foetal adrenal culture, and at the same time it increased the production of DHA and androstenedione. The effect of corticosterone on its own production was not measured, as it cannot be reliably estimated owing to the large amount of added corticosterone. Adrenal androgens are secreted preferentially from the zona reticularis (Ofstad et al. 1961; Jones et al. 1970). The corticosterone concentration in the adrenal gland is probably highest in the zones of lower fasciculata and reticularis owing to the centripetal blood flow in the adrenal gland (Dobbie & Symington 1966). Thus corticosterone may be a factor which shifts the steroid pattern in the zona reticularis towards androgenic type. The exact site of action of corticosterone on steroid hydroxylations is not quite clear. Salmenperä et al. (1976) showed decreased 11β- and 18-hydroxylation in corticosterone-treated rat adrenal culture. 18-Hydroxylation activity is more difficult to measure by the present method in human than in rat adrenals, because human adrenals do not secrete 18-OH-DOC in sufficiently high concentrations to be measured by the present method. Therefore it is not possible on the basis of the present study to draw conclusions about the effect of corticosterone on 18-hydroxylation in human adrenals. The preferential site of action of corticosterone seems to be 11β-hydroxylation since cortisol secretion decreased but 11-deoxycortisol did not.

The addition of cortisol to the rat adrenal culture (Fig. 3) clearly demonstrates the inhibitory effect of this steroid on 18-hydroxylation. Although the absolute aldosterone production did not decrease in cortisol-treated human adrenal culture (Fig. 4), the relative amount of aldosterone in respect to corticosterone decreased significantly (Table 1). The effect of cortisol on its own production was not measured for the same reason as the effect of corticosterone on its own production. Cortisol does not seem to inhibit 11β-hydroxylation generally, because corticosterone is not decreased in either rat or human cortisol-treated cultures (Figs. 3 and 4). This does not, however, exclude the possibility that cortisol has some inhibitory effect on its own synthesis at the step of 11β-hydroxylation. Since 11-deoxycortisol is not decreased in cortisol + ACTH-treated cultures of human adrenals, compared to merely ACTH-treated cultures (Fig. 4), cortisol does not seem to inhibit 17α-hydroxylation. Hornsby & O’Hare (1977) studied [3H]DOC metabolism in rat adrenal culture and found that the addition of cortisol in a concentration of $5.5 \times 10^{-5} \, \text{M}$ decreased the relative conversion of [3H]DOC to aldosterone and 18-OH-corticosterone and increased the relative amount of corticosterone and 18-OH-DOC. In the present study cortisol inhibited rat adrenal steroidogenesis at the concentration of $2.8 \times 10^{-4} \, \text{M}$ and a decrease was noted both in aldosterone and 18-OH-DOC secretion. At a cortisol concentration of $5.8 \times 10^{-5} \, \text{M}$ (results not shown) 18-OH-DOC did not decrease significantly in our study.

The mechanism by which corticosterone and cortisol inhibit certain steps in
steroidogenesis does not fall within the scope of the present work. In the present study corticosterone and cortisol in concentrations attainable physiologically in the adrenal gland had qualitatively different effects on the endogenous production of corticosteroids in ACTH-stimulated cultures of human adrenals. Corticosterone significantly inhibited cortisol production and increased adrenal androgen production. Cortisol inhibited 18-hydroxylation but not corticosterone production. The results show that there is important regulation of steroidogenesis by adrenal steroids at the adrenal level, as has been suggested earlier by numerous investigators (Birmingham & Kurlents 1958; Péron et al. 1960; Hill & Singer 1968; Salmenperä et al. 1976). These findings are in agreement with our suggestion that cortisol and corticosterone are regulators of the functional zonation of the adrenal gland (mineralocorticoids secreted preferentially from zona glomerulosa, glucocorticoids from zona fasciculata + reticularis and androgens from zona reticularis).

REFERENCES


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