NOTE

Biosynthetic Pathways for Corticoids and Androgen Formation in Human Fetal Adrenal Tissue in Vitro

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Synopsis

Eleven incubations of minced human fetal adrenal tissue from two fetuses at mid trimester of pregnancy terminated for socio-economic reasons, were performed for different periods of time with labelled pregnenolone, progesterone, 17α-hydroxyprogesterone and deoxycorticosterone (DOC). The order of magnitudes of enzyme activity when progesterone was used as a substrate and incubated with midterm fetal adrenals was 17α-hydroxylase, 21-hydroxylase and 16α-hydroxylase. The activity of 3β-hydroxysteroid dehydrogenase and 3α,4α isomerase was almost negligible as compared to other enzymic activities as mentioned above. Similar biosynthetic pathways for adrenal corticoids formation were observed in human fetal adrenals as reported in human adults and animals; once progesterone was converted into DOC, further 17α-hydroxylation did not take place.

The human fetal adrenal gland is considered to be an important source of steroid hormones or their precursors in pregnancy (Solomon et al., 1958; Wilson et al., 1966). Our previous data demonstrate the increased activity of the pituitary adrenal axis in human fetuses during delivery resulting in augmented titers of estriol (Arai et al., 1972) and of fetal adrenocorticotropic hormone (ACTH) (Arai et al., 1976). Others emphasized the role of fetal cortisol as a trigger to initiate labor (Liggins, 1968). De novo synthesis of steroids in human fetal adrenals incubated in vitro is reported (Bloch and Benirschke, 1959; Jaffe et al., 1968). Similar results were obtained in perfusion study of preivable fetuses (Diczfalousy, 1969). However, detailed information concerning biosynthetic pathways leading to corticoid in human fetal adrenals, are still insufficient. The following study was undertaken to investigate these problems further.
Materials and Methods

Tissue
Human fetal adrenal tissue was obtained from two fetuses at 22 weeks, which were aborted for socio-economic reasons.

Steroids
Pregnenolone-4-14C (SA 55.7 mCi/m mole, NEC-375, Lot No. 746-100), progesterone-4-14C (SA 59 mCi/m mole, NEC-081, Lot No. 746-118), 17α-hydroxyprogesterone-4-14C (SA 50 mCi/m mole, NEC-148, Lot No. 467-128) and DOC-4-14C (59.8 mCi/m mole, NEC-115, Lot No. 695-249) were obtained from New England Nuclear Corporation and purified by a thin-layer chromatography (TLC) prior to use.
Non-radioactive steroids were purchased from commercial sources (Merck, Steraloids, Ikapharm and Sigma Chemical Co.) and their chemical purity was checked by TLC prior to use.

Incubation
The adrenal gland was minced with scissors and 100 mg of tissue were incubated with labelled precursors in 2 ml of Krebs-Hensleit glucose solution, pH 7.4 at 37°C, without cofactors. Incubations were carried out for various periods of time in a metabolic shaker at 37°C. Gas phase was 95% of O2 and 5% of CO2. Experiments were started within 2 hr after delivery of fetuses. At the end of the incubation, 5 ml of ethanol was added to stop the reaction. Four series of incubations were performed.

Incubation I
Five μg of 14C-pregnenolone (0.3 μCi) was incubated for 5, 15, 30 and 60 min.

Incubation II
Five μg of 14C-progesterone (0.06 μCi) was incubated for 15, 30 and 60 min.

Incubation III
The same as in Incubation I except 5 μg of 14C-17α-hydroxyprogesterone (0.15 μCi) were incubated.

Incubation IV
In this series, 4.1 μg of 14C-DOC (0.2 μCi) was incubated for 2 hr.

Measurement of radioactivity
The samples were dissolved in 10 ml of scintillation fluid containing 2.38 g of PPO and 29.7 mg of POPOP/toluene and counted in a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3330). The counting efficiency was about 90% for 14C. To detect the radioactive areas on chromatograms, an Aloka Radio Chromatogram Scanner (Model TLC-4, 04R069) and autoradiography were used.

Extraction, separation and identification
Following incubation, the minced tissues were homogenized with ethanol in glass homogenizers and centrifuged. The supernatant was evaporated and the lipid was removed in cold 70% methanol. Methylene-dichloride-water partition was performed to separate “free” and “conjugate” steroid fractions. To the free fraction, steroid carriers were added and subjected to thin layer chromatography on 0.25 mm silica gel (Merck GF 254) to separate the metabolites. Chromatographic systems used in this study were; I. benzene : methanol (9 : 1, by vol), II. cyclohexane : ethyl acetate (1 : 1), III. chloroform : ether (9 : 1). The several radioactive metabolites were isolated and identified. The final identification of each metabolite was accomplished by recrystallization with authentic crystalline steroids to constant specific activity (Table 1).

Table 1. Radiochemical purity of radioactive steroids derived from incubation of fetal adrenal tissue with 14C-progesterone and 14C-pregnenolone.

<table>
<thead>
<tr>
<th>Products</th>
<th>Specific activities (dpm/mg)</th>
<th>Before crystallization</th>
<th>1st crystal</th>
<th>2nd crystal</th>
<th>3rd crystal</th>
<th>4th crystal</th>
<th>Mother liquid</th>
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<tr>
<td>17α-hydroxyprogesterone</td>
<td></td>
<td></td>
<td>774</td>
<td>691</td>
<td>684</td>
<td>685</td>
<td>681</td>
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<td>11-deoxy cortisol*</td>
<td></td>
<td></td>
<td>614</td>
<td>585</td>
<td>574</td>
<td>566</td>
<td>561</td>
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<tr>
<td>cortisol*</td>
<td></td>
<td></td>
<td>806</td>
<td>705</td>
<td>692</td>
<td>703</td>
<td>—</td>
</tr>
<tr>
<td>11-deoxycorticosterone*</td>
<td></td>
<td></td>
<td>839</td>
<td>852</td>
<td>846</td>
<td>875</td>
<td>—</td>
</tr>
<tr>
<td>corticosterone*</td>
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<td></td>
<td>321</td>
<td>296</td>
<td>279</td>
<td>280</td>
<td>—</td>
</tr>
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<td>androstenedione</td>
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<td>1042</td>
<td>1068</td>
<td>1089</td>
<td>1069</td>
<td>—</td>
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<tr>
<td>11β-hydroxyandrostenedione</td>
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<td>465</td>
<td>460</td>
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<td>413</td>
</tr>
<tr>
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<td>1166</td>
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<td>1198</td>
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<td>—</td>
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<td>457</td>
<td>439</td>
<td>454</td>
<td>—</td>
</tr>
<tr>
<td>17α-hydroxypregnenolone</td>
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<td></td>
<td>1007</td>
<td>1017</td>
<td>1055</td>
<td>1006</td>
<td>—</td>
</tr>
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</table>

* Recrystallized as acetate.
Results

Metabolism of $^{14}$C-pregnenolone (I)

Fig. 1 shows the results when $^{14}$C-pregnenolone was incubated for varying periods. The precursor pregnenolone decreased gradually with incubation time, whereas $^{14}$C-DHA steadily increased. It is noteworthy that a rapid increase in the radioactive intermediate 17α-hydroxypregnenolone occurred in earlier stage, followed by declining amounts with further incubation. In this experiment, it is apparent that 17α-hydroxypregnenolone showed a rapid increase with a subsequent decrease, suggesting its possible role as an intermediary metabolite in DHA formation. The low recovery rates of Δ4-pregnene-3-oxo compounds (1.8% at 15 min) were observed.

Fig. 1. Amounts of steroids formed from $^{14}$C-pregnenolone, at different time intervals. Abbreviations used: Δ4P; pregnenolone, 17α-OH-Δ4P; 17α-hydroxypregnenolone, DHA; dehydroepiandrosterone.

Metabolism of $^{14}$C-progesterone (II)

Fig. 2 shows the results when $^{14}$C-progesterone was incubated for varying periods. The precursor progesterone decreased gradually with incubation time, whereas corticosterone and cortisol steadily increased. It is noteworthy that increases in intermediates such as DOC, 17α-hydroxyprogesterone occurred early in the incubation followed by declining amounts with further incubation. In total, 20.9% of the precursor progesterone was converted into 17α-hydroxylated compounds and 3.8% 16α-hydroxylated compounds respectively at 15 min.

Fig. 2. Amounts of steroids formed from $^{14}$C-progesterone at different time intervals. Abbreviations used: Δ4P; progesterone, 17α-OH-Δ4P; 17α-hydroxyprogesterone, 16α-OH-Δ4P; 16α-hydroxyprogesterone, S; 11-deoxycortisol, B; corticosterone, F; cortisol.
Metabolism of 17α-hydroxyprogesterone (III)

Fig. 3 shows the results when 14C-17α-hydroxyprogesterone was incubated for varying periods. The precursor 17α-hydroxyprogesterone decreased gradually with incubation time, whereas cortisol increased. A rapid increase in intermediate 11-deoxy-cortisol was observed early in the incubation by declining amounts with further incubation. A total amount of 38.9% of the precursor 17α-hydroxyprogesterone was converted into 21-hydroxylated compounds and 3.8% into Δ4-androstene compounds respectively at 15 min.

Metabolism of 14C-DOC (IV)

As 14C-DOC was incubated for 2 hr, corticosterone was formed (10.0%) but neither 11-deoxycortisol nor cortisol could be detected. No radioactive metabolite which behaved like aldosterone on TLC was formed under the present experimental conditions.

Discussion

The human fetal adrenal is an important part of steroid biosynthesis during pregnancy. Villee et al. (1959 and 1961) examined the activity of steroid hydroxylases of the fetal adrenals at various gestational ages and found that the metabolism of progesterone differed with the age of gestation. They postulated that the older fetuses formed more cortisol and corticosterone than did the earlier fetuses, whereas the 16α-hydroxylases of the human fetal adrenal were very active at all stages of gestation. The minced human fetal adrenals from twin fetuses incubated in vitro with pregnenolone and progesterone formed adrostenedione, 11β-hydroxyandrostenedione, cortisol and 16α-hydroxyprogesterone as major products (Villee et al., 1965). Aldosterone is also reportedly produced from corticosterone in vivo (Dufau and Villee, 1969).

Thus it is well documented that the human fetal adrenal gland is capable of forming a variety of steroids from precursors, but there has been no systematic elucidation of the preferred pathway, if any, for the formation of corticoids and androgen. Some of the published data would indicate divergent results depending upon experimental conditions. Solomon et al. (1958, 1967) claim that perfusion studies and injection of the steroid into umbilical vein may be more representative of the biosynthetic pathways in the intact cell, while slices, minces and homogenates reflect the biochemical conversion of the cells whose architecture has been disrupted.

Minced tissue of the fresh human fetal adrenal gland was utilized in the present experiment without added cofactors. DHA was the main metabolite as pregnenolone
was used as a precursor, and the intermediary metabolite 17α-hydroxypregnenolone accumulated rapidly at 15 min with a bell-shaped curve. The human fetal adrenals would also produce DHA from pregnenolone after 17α-hydroxylation and sidechain cleavage occurred. The low recovery rates of Δ4-3-oxo compounds from pregnenolone seem to indicate the lower activity of 3β-hydroxysteroid dehydrogenase and Δ5-4 isomerase in this tissue. Other studies also reported the very low activity of this enzyme, if any, in the human fetal adrenal gland (Solomon et al., 1967).

Cortisol and corticosterone seemed to be the major endproduct as progesterone was used as a substrate in this experiment. Whereas 17α-hydroxyprogesterone and DOC increased rapidly at 15 min followed by a gradual decrease with a bell-shaped curve suggesting their role as intermediary metabolites. Formations of 11-deoxycortisol resumed a similar pattern but with the peak appearing slightly later. Another metabolite, 16α-hydroxyprogesterone seemed to reach the plateau under the present experimental condition and it is not clear whether further metabolism takes place or not. When 17α-hydroxyprogesterone was a precursor, a significant proportion of cortisol and to a less extent 11β-hydroxyandrostenedione were identified as final metabolites. In this case, 11-deoxycortisol and androstenedione showed a pattern as intermediary metabolites. It seems probable that 11β-hydroxylation occurs after the side-chain cleavage of progesterone and thus 11β-hydroxyandrostenedione is formed. A minor pathway to this steroid from cortisol is also reported (Hudson and Killinger, 1972). Only corticosterone could be identified after the incubation of fetal adrenals with DOC as a substrate. Our results reconfirm the finding by Eichhorn and Hechter (1957) who claimed no hydroxylation at position 17 occurred when 21 hydroxylation had taken place first.

The present results confirm that all the enzymic systems involved in androgen and corticoid biosynthesis, which have been reported in animals, human adults or partly in the human fetus, do also exist in human fetal adrenal glands at mid-gestation. The biosynthetic pathways to corticoids and androgens in this tissue are shown to be practically the same as those of adults and other species.

References