CONVERSION OF PROGESTERONE TO CORTICOSTEROIDS BY THE MIDTERM FETAL ADRENAL AND KIDNEY

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ABSTRACT

Midterm fetal adrenal and kidney tissue homogenates were incubated with ^{3}H-progesterone (1 μM) and its conversion to the ^{3}H-corticosteroids metabolites studied. Cortisol (36.3%) and corticosterone (4.7%) were isolated from the adrenal, and 11-deoxycortisol (32.5%) and deoxycorticosterone (21.1%) from the kidney. The results of these incubations confirmed the presence of 17- and 21-hydroxylase activities in both fetal tissues, and that of 11β-hydroxylase activity only in fetal adrenal tissue. We conclude that during pregnancy when progesterone levels are high, biosynthesis by the fetal kidney of 11-deoxycortisol, the most abundant corticosteroid formed by this tissue in this investigation, might provide to the fetal adrenal an important precursor for cortisol biosynthesis within the fetal compartment.

The control of parturition and the induction of fetal organ maturation in some mammalian species such as the sheep, goat and rabbit is associated with an increase of cortisol in the fetal blood near term, and this increase is a result of increased cortisol secretion from the fetal adrenal gland (1)(2). Studies of cortisol in human umbilical cord blood have revealed no such increase at the end of pregnancy, but information obtained in this way is difficult to interpret because of transplacental passage of maternal cortisol into the fetal circulation (3)(4). Furthermore, maternal cortisol levels are affected by the stress of delivery and this in turn will be reflected in the fetal blood cortisol (5). However,

other corticosteroids such as corticosterone sulfate, deoxycorticosterone, and deoxycorticosterone sulfate are present in cord blood in much higher concentrations than in maternal plasma at term (6), suggesting fetal involvement in the synthesis of corticosteroids.

Two recent findings implicate the fetal adrenal as the main source of corticosterone sulfate: 1) the levels of corticosterone sulfate were lower in maternal and fetal fluids of anencephalic pregnancies than in normal pregnancies, and 2) normal levels were found in umbilical cord blood and maternal plasma of an adrenalectomized gravida (7). However, extraadrenal sources of corticosteroids have also been demonstrated recently, including synthesis of deoxycorticosterone and deoxycorticosterone sulfate by the fetal kidney (8)(9), and synthesis of cortisol by amniotic membranes (10). In this study, we have evaluated C-17, C-21 and C-11 hydroxylations in midterm fetal adrenal and kidney by incubating these tissues with ^{3}H-progesterone and identifying the tritiated C_{21} steroids formed.

MATERIALS AND METHODS

Reagents

[1,2-^{3}H] Progesterone (specific activity, 57 Ci/mmol, [4-^{14}C] 17-hydroxyprogesterone and [4-^{14}C] deoxycorticosterone (specific activities 58 mCi/mmol) were obtained from New England Nuclear (Boston, MA). [4-^{14}C] Corticosterone (specific activity 52 mCi/mmol) was purchased from Amersham Corp. (Arlington, IL) and reference nonradioactive steroids from Sigma Chemical Co. (St. Louis, MO) and Steraloids Inc. (Wilton, NH). Silica gel thin layer chromatography (TLC) plates (F-254) were obtained from Brinkmann. All organic solvents were of spectroquality (Matheson, Coleman and Bell). Absolute ethanol, USP quality, was obtained from Commercial Solvent Corp. Ready-solv solution VI (Beckman) was used for liquid scintillation counting.

Tissue Source

Fetal human adrenal and kidney tissues were obtained from a midterm female fetus within half an hour after therapeutic abortion

by intramniotic infusion of prostaglandin $F_2\alpha$ and after written consent was obtained from the patient. Tissues were dissected and frozen at -80°C until incubations were performed.

Tissue Incubations

Tissue samples from fetal adrenal and kidney were homogenized in 0.1 M phosphate buffer (pH 7.4) containing $MgCl_2$ (3mM). Aliquots of the homogenates equivalent to 200 mg of tissue were added to the vessels containing 2.2 µCi ^{3}H-progesterone and nonradiolabeled progesterone (1 µM) and a NADPH generating system which consisted of NADP (5 mM), glucose-6-phosphate (12 mM) and glucose-6-phosphate dehydrogenase (1.2 units/ml). The final volume in the vessels was adjusted to 2 ml, and incubations were conducted at 37°C in an oscillating water bath for two hours in room air. Control incubations to which boiled tissue or no tissue were added were performed. The incubations were terminated by the addition of methanol to a final concentration of 70%, and samples were kept at -20°C overnight. The methanol extracts were centrifuged, the supernatant transferred to clean tubes and evaporated in a water bath under nitrogen. The residue was then brought up to 2 ml with distilled water and extracted twice with 5 ml dichloromethane ($MeCl_2$). The tritiated radioactivity present in the $MeCl_2$ was regarded as unconjugated steroids.

Identification of Metabolites

The $MeCl_2$ was evaporated, ^{14}C-steroids (approximately 1000 cpm of each) and nonradiolabeled carriers were added as internal standards, and the $MeCl_2$ extract dissolved in 200 µl of ethanol was chromatographed on TLC employing the solvent system methanol: $MeCl_2$: water (6:100:0.3 by vol), hereafter designated solvent system #1. The areas of the chromatogram containing the tritiated radioactivity that co-migrated with the standards 17-hydroxyprogesterone, cortisol, corticosterone, 11-deoxycortisol, deoxycorticosterone, 11β-hydroxyprogesterone and progesterone run simultaneously on TLC were scraped from the plate, eluted with 80% ethanol, and an aliquot of each sample was assayed for radioactivity. To facilitate further purification and separation of the various tritiated steroids formed in these incubations with ^{3}H-progesterone, TLC in solvent system #2, isooctane: ethyl acetate (1:1, v/v), and derivative formation with a mixture of pyridine and acetic anhydride (1:1 v/v) for 2 hours at 37°C followed by TLC system #1 of the reaction products were performed. To establish radiochemical homogeneity of the purified ^{3}H-steroids isolated, nonradiolabeled steroid or its acetate whichever was appropriate, were added (1-3 mg) to each sample after the last TLC purification, and samples crystallized in the solvent pair acetone/water or ether/petroleum ether until ^{3}H to ^{14}C (or ^{3}H/µg standard) ratios in successive mother liquors and crystals were constant within ± 10 percent. Unlabeled steroids with 4-pregnene-3-one groups were quantified by their absorption at 240 mµ using Allen correction (11).

RESULTS

After incubation of human fetal adrenal and kidney tissue homo-

genates with ^{3}H-progesterone, unconjugated and conjugated steroids were separated by partition between $MeCl_2$ and water as described in Materials and Methods.

Most of the total radioactivity was $MeCl_2$-soluble in both fetal tissues: 76.4% in fetal adrenal, and 93.8% in fetal kidney. A smaller percentage of the total tritiated radioactivity was found in the water-soluble fraction of both fetal tissues, although this percentage was much higher in the fetal adrenal (23.5%) than in the fetal kidney (6.2%). This water fraction, which presumably contains polar and conjugated steroids, was not analyzed further. In the control incubations with boiled tissue or without tissue, nearly all of the radioactivity (99%) was $MeCl_2$-soluble.

1. $MeCl_2$-soluble steroids formed in the incubation of fetal adrenal tissue homogenate with ^{3}H-progesterone. Chromatography of the $MeCl_2$ extract on TLC #1 distributed the carrier steroids in six zones. The first zone nearest the origin (Rf=0.13) that represented 52% of the total $MeCl_2$-soluble tritiated radioactivity corresponded in mobility to the cortisol standard. To facilitate further purification, the product was acetylated and purified again by TLC on system #1. The area of the chromatogram migrating with authentic cortisol acetate (Rf=0.49) was then purified further by crystallizations, until constant specific activity (^{3}H/µg ratio) was achieved. The radiochemical homogeneity of this compound was confirmed to be cortisol, representing 47.5% of the total $MeCl_2$-soluble tritiated radioactivity.

Zone two on TLC #1 (Rf=0.33) that represented 13% of the total $MeCl_2$-soluble tritiated radioactivity corresponded in mobility to the corticosterone and 11-deoxycortisol standards. When this area of the

chromatogram was rechromatographed on TLC system #2, 6.2% of the tritiated radioactivity comigrated with corticosterone carrier (Rf=0.09), 0.4% with 11-deoxycortisol carrier (Rf=0.20), and 6.4% represented another unidentified metabolite or metabolites. To facilitate further purification of the corticosterone-like material isolated, the product was acetylated and chromatographed on TLC system #1. The area of the chromatogram migrating with authentic corticosterone acetate (Rf=0.54) was purified further by crystallizations until constant specific activity ($^{3}H/^{14}C$ ratio) was achieved. The radiochemical homogeneity of this compound was confirmed to be corticosterone, representing 6.2% of the total $MeCl_2$-soluble tritiated radioactivity. In a similar fashion, further purification of the 11-deoxycortisol-like material isolated on TLC system #2 was also carried out by acetylation followed by TLC on system #1, and by crystallizations. Most of the tritium counts associated with 11-deoxycortisol acetate carrier on the last TLC were dissociated from carrier during crystallization and no constant specific activity was achieved. Thus, 11-deoxycortisol was ruled out.

Zone three on TLC #1 (Rf=0.47) that represented 4% of the total $MeCl_2$-soluble tritiated radioactivity corresponded in mobility to the marker 11β-hydroxyprogesterone. However, further purification of this labeled material on TLC led to a complete dissociation of tritium counts from carrier 11β-hydroxyprogesterone.

Zone four on TLC #1 (Rf=0.49) that represented 2.1% of the total $MeCl_2$ soluble tritiated radioactivity corresponded in mobility to the standard 17-hydroxyprogesterone. To facilitate further purification of the 17-hydroxyprogesterone-like material, this area of the chromatogram was eluted and rechromatographed again on TLC system #1, and purified

further by crystallizations. Complete dissociation of tritium counts from carrier ^{14}C-17-hydroxyprogesterone during the purification techniques demonstrated that other unidentified metabolites were present in this fraction.

Zone five on TLC #1 (Rf=0.54) that represented 0.8% of the total $MeCl_2$-soluble tritiated radioactivity corresponded in mobility to the deoxycorticosterone marker. However, most of the tritium counts were dissociated from carrier deoxycorticosterone after the material was acetylated, rechromatographed on TLC system #1, and crystallized. No constant specific activity ($^3H/^{14}C$ ratio) was achieved. Therefore, deoxycorticosterone was ruled out.

Zone six on TLC #1 (Rf=0.61) that represented 1.1% of the total $MECl_2$-soluble tritiated radioactivity corresponded in mobility to the progesterone marker. Since this steroid was the substrate of the tissue incubation, no further analysis of this material was performed. Table 1 shows the crystallization data and the percent yield of the various tritiated C_{21}-steroids formed from ^{3}H-progesterone in the fetal adrenal under the described experimental conditions. Results are corrected for final recovery from carriers added to the initial $MeCl_2$ fraction.

2. $MeCl_2$-soluble steroids formed in the incubation of fetal kidney tissue homogenate with ^{3}H-progesterone. Chromatography of the $MeCl_2$ extract on TLC #1 distributed the carrier steroids in six zones. The first zone nearest the origin (Rf=0.13) that represented 3.3% of the $MeCl_2$-soluble tritiated radioactivity corresponded in mobility to the cortisol standard. However, all the tritium counts were dissociated from carrier cortisol after the material was acetylated, chromatographed

and crystallized.

Zone two on TLC #1 (Rf=0.33) that represented 47.6% of the total $MeCl_2$-soluble tritiated radioactivity corresponded in mobility to the 11-deoxycortisol and corticosterone markers. When this eluted material was rechromatographed on TLC system #2, 34.7% of the tritiated radioactivity comigrated with 11-deoxycortisol carrier (Rf=0.2), 0.7% with corticosterone carrier (Rf=0.09) and 12.2% represented another unidentified metabolite or metabolites. To facilitate further purification of the 11-deoxycortisol-like material isolated, the product was acetylated and chromatographed on TLC system #1 again. This area of the chromatogram migrating with authentic 11-deoxycortisol acetate (Rf=0.63), was purified further by crystallizations until constant specific activity ($^3H/\mu g$ ratio) was achieved. The radiochemical homogeneity of this compound was confirmed to be 11-deoxycortisol and represented 34.7% of the total $MeCl_2$-soluble tritiated radioactivity. In a similar fashion, further purification of the corticosterone-like material isolated on TLC system #2 was also carried by acetylation and crystallizations. However, most of the tritium counts associated with corticosterone acetate carrier were dissociated during crystallizations without achieving constant specific activity. Thus, corticosterone was ruled out.

Zone three on TLC #1 (Rf=0.47) that represented 5.2% of the total $MeCl_2$-soluble tritiated radioactivity corresponded in mobility to the marker 11β-hydroxyprogesterone. However, further purification of this tritiated material on TLC led to a complete dissociation of tritium counts from carrier 11β-hydroxyprogesterone.

Zone four on TLC #1 (Rf=0.49) that represented 7.3% of the total

$MeCl_2$-soluble tritiated radioactivity corresponded in mobility to the 17-hydroxyprogesterone marker. To facilitate further purification of the 17-hydroxyprogesterone-like material, this area of the chromatogram was eluted, rechromatographed on TLC system #1, and purified further by crystallizations until constant specific activity ($^3H/^{14}C$ ratio) was achieved. Although part of the tritium counts dissociated from carrier during the purification procedures, radiochemical homogeneity of this compound was confirmed and represented 2.5% of the total $MeCl_2$-soluble tritiated radioactivity.

Zone five on TLC #1 (Rf=0.54) that represented 24.1% of the total $MeCl_2$-soluble tritiated radioactivity corresponded in mobility to the deoxycorticosterone marker. To facilitate further purification of the deoxycorticosterone-like material, this product was acetylated and purified again by TLC on system #1. The area of the chromotogram migrating with deoxycorticosterone acetate carrier (Rf=0.83) was purified further by crystallizations until constant specific activity ($^3H/^{14}C$ ratio) was achieved. The radiochemical homogeneity of this compound was confirmed to be deoxycorticosterone and represented 22.5% of the total $MeCl_2$-soluble tritiated radioactivity.

Zone six on TLC #1 (Rf=0.61) that represented 6.3% of the total $MeCl_2$-soluble tritiated radioactivity corresponded in mobility to the progesterone marker. Since this steroid was the substrate of the tissue incubation no further analysis of this material was performed. Table 1 shows the crystallization data and the percent yield of the various tritiated C_{21}-steroids formed from ^3H-progesterone in the fetal kidney under described experimental conditions. Results are corrected for final recovery from carriers added to the initial $MeCl_2$ fraction.

DISCUSSION

Utilizing an in vitro tissue homogenate assay, the present study shows that the major tritiated corticosteroids formed from ^{3}H-progesterone by the midterm fetal adrenal were cortisol (36.3%) and corticosterone (4.7%); and those formed by the midterm fetal kidney were 11-deoxycortisol (32.5%) and deoxycorticosterone (21.1%).

The generally accepted pathway of cortisol and corticosterone biosynthesis in the adrenal includes hydroxylations at C-17, C-21, and C-11, via 17-hydroxyprogesterone and 11-deoxycortisol for cortisol biosynthesis, and C-21,-11 hydroxylations, via deoxycorticosterone for corticosterone biosynthesis. Since a two-hour incubation rather than a dynamic study was performed, accumulation of the end products cortisol and corticosterone was observed; intermediate metabolites were undetectable. These in vitro results indicate the presence in midterm fetal adrenal tissue of an efficient 11β-hydroxylase activity which seems to have a preferential affinity for 21, 17-dihydroxylated compounds. Cortisol biosynthesis in this fetal tissue was eight times higher than that of corticosterone, and 11β-hydroxyprogesterone was not isolated. The possibility of further metabolism of corticosterone to corticosterone sulfate or aldosterone cannot be discarded, since the conjugated fraction was not analyzed and isolation of aldosterone was not pursued.

In fetal kidney tissue incubation with progesterone, the accumulation of 11-deoxycortisol and deoxycorticosterone, and complete absence of cortisol and corticosterone formation indicate the lack of 11β-hydroxylase activity in this fetal tissue. Steroid-21-17-

hydroxylase activities, as well as in vitro formation of deoxycorticosterone and 17-hydroxyprogesterone from progesterone in fetal kidney tissue have been previously reported (8)(12). However, to our knowledge, in vitro biosynthesis of 11-deoxycortisol, the major metabolite formed from progesterone in our midterm fetal kidney tissue incubation, has not been previously reported.

Extraadrenal steroid 17- and 21-hydroxylase activities have been demonstrated in vitro not only in the fetal kidney, but also in various human fetal tissues such as testes (13), thymus, and spleen (12)(14). In addition, 21-hydroxylation of progesterone has been found in other fetal tissues such as skin, urinary bladder, pancreas and ovary (14). However, steroid-11β-hydroxylase has been demonstrated only in the adrenal (15)(16). In other fetal tissues which have been shown to produce cortisol, such as lung (17) and amniotic membranes (10)(18), an 11-oxidoreductase enzyme is present which converts 11-oxo-hormones such as cortisone to cortisol.

We have demonstrated an efficient conversion of progesterone to cortisol by midterm fetal adrenal, and to 11-deoxycortisol by the midterm fetal kidney. It seems reasonable to speculate that during pregnancy, when progesterone levels are high and fetal renal blood flow increases, 11-deoxycortisol formed in situ by the fetal kidney might provide the fetal adrenal with an important and highly specific substrate for 11β-hydroxylase activity, resulting in the synthesis of biologically active cortisol within the fetal compartment.

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19. Trivial names used for steroids in this paper are:

Cortisol	11β, 17,21-trihydroxy-4-pregnene-3,20-dione
Corticosterone	11β, 21-dihydroxy-4-pregnene-3,20-dione
Deoxycorticosterone	21-hydroxy-4-pregnene-3,20-dione
11-deoxycortisol	17,21-dihydroxy-4-pregnene-3,20-dione
Progesterone	4-pregnene-3,20-dione
17-hydroxyprogesterone	17-hydroxy-4-pregnene-3,20-dione
11β-hydroxyprogesterone	11β-hydroxy-4-pregnene-3,20-dione

TABLE I

Establishment of Radiochemical Homogeneity of ^{3}H-C_{21}-Steroids Isolated after Incubation of Fetal Adrenal and Kidney with ^{3}H-Progesterone.

STEROID	ADRENAL					KIDNEY					
	$^{3}H/^{14}C$ or $^{3}H/\mu g$ Ratios				Yield from ^{3}H-substrate %	$^{3}H/^{14}C$ or $^{3}H/\mu g$ Ratios					Yield from ^{3}H-substrate %
	Before Cryst.	ML_1	ML_2	CR		Before Cryst.	ML_1	ML_2	ML_3	CR	
17-Hydroxyprogesterone[1)]	-	1.4	0.6	0.05	<0.01	38.3	33.9	36.5	-	32.6	2.3
Deoxycorticosterone[1,3)]	-	1.7	1.8	0.4	<0.15	79.3	84.5	91.6	-	78.8	21.1
11-Deoxycortisol[2,3)]	-	1.8	1.2	0.2	<0.1	31.3	43.7	37.5	34.3	39.7	32.5
Corticosterone [1,3)]	11.7	11.9	11.3	10.7	4.7	-	2.1	2.1	-	0.8	<0.2
Cortisol[2,3)]	17.2	15.2	15.8	14.7	36.3	-	1.7	0.4	-	0.1	0

ML mother liquor
CR crystals
1) $^{3}H/^{14}C$ ratios
2) $^{3}H/\mu g$ ratios
3) acetate derivative