Trend Analysis of Serum Progesterone, Deoxycorticosterone, Deoxycorticosterone Sulfate, Cortisol, Corticosterone, 18-Hydroxydeoxycorticosterone and Estradiol in Early Neonates

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Abstract

To elucidate the origin and regulatory mechanism of deoxycorticosterone (DOC) and deoxycorticosterone sulfate during fetal life, the levels of serum DOC, DOC sulfate, progesterone, cortisol, corticosterone and 18-hydroxydeoxycorticosterone (18OH-DOC) were determined in the fraction separated on high performance liquid chromatogram (HPLC) by radioimmunoassay (RIA) using the serum from normal newborn. Elimination curves both of serum DOC and DOC sulfate showed two phases: rapidly decreasing and slowly decreasing ones. Both serum DOC and DOC sulfate correlated with progesterone (r=0.340, p<0.01; r=0.737, p<0.01, respectively). They also correlated with cortisol (DOC, r=0.467, p<0.01; DOC sulfate, r=0.549, p<0.01, respectively). Serum DOC reached normal adult levels by 16 hrs after birth. However serum DOC sulfate concentration was maintained high throughout the entire early neonatal period. On the contrary, the changes in serum cortisol, corticosterone and 18OH-DOC showed a peak surge in the initial phase after delivery. Both serum corticosterone and 18OH-DOC correlated with cortisol (r=0.518, p<0.01; r=0.410, p<0.01, respectively).

These findings suggest that, in the fetus, serum DOC and DOC sulfate are mainly produced at extraadrenal sites isolated from normal mineralocorticoids synthesis and after birth they begin to be formed at adrenal glands.
droxy-steroid sulfotransferase are present in extraadrenal tissues (Ottoson et al., 1984; Winkel et al., 1980; Casey et al., 1983; Casey et al., 1981). Extraadrenal production as the main site of these steroids was suggested by many authors (Pakravan et al., 1974; Dorr et al., 1986; Parker et al., 1983a; Parker et al., 1983b; Craig et al., 1980). However, Voutilainen and Miller showed that P450c21 mRNA from fetal tissues was detected only in the adrenal gland (Voutilainen R. and W. L. Miller, 1986). The origin of these steroids in the early neonatal period is still controverted.

In order to answer these questions, this study was conducted to determine the levels of DOC, DOC sulfate, progesterone, cortisol (F), corticosterone (B), 18-hydroxycorticosterone (180H-DOC) and 17β-estradiol (E2) using serum immediately taken from normal neonates by RIA following separation on HPLC, and to follow the quantitative changes in these steroids and to analyze the mutual correlation of these steroids during the neonatal period. If the DOC and DOC sulfate were reduced proportionately to the progesterone, they would be formed at the extraadrenal sites.

Materials and Methods

Subjects

Eight randomly selected, healthy newborns with uneventful delivery (39 weeks±1.8 days) at the lying-in ward of Teikyo Univ. Hospital were chosen subjects. No problems were reported after birth, and there was no evidence of systemic or endocrine disease. They were started at 6-8 hrs after birth with 5% glucose and replaced with breast milk at 8-12 hrs. The average intake was 50-60 ml/kg BW on the first day, 70-80 ml/kg BW on day 2 and 100-150 ml/kg BW thereafter. Blood samples were obtained from the antecubital or dorsal veins of the hand at 0 hr, 2 hrs, 4 hrs, 8 hrs, 16 hrs, 24 hrs, 48 hrs, 72 hrs, 144 hrs (6 days after delivery). Informed consent was given and approved by each parent before the protocol. The blood samples at 0 hr were of umbilical arterial blood. Some samples were missed because of practical procedures. The number of samples taken each time was 4-8. The samples were immediately centrifuged at 4°C, and stored at -20°C until used.

Assay procedure for serum steroids

1) Radiolabeled or non-radiolabeled steroids

Radiolabeled isotopes including [1, 2-3H] DOC (1.813TBq/mmol; 49 Ci/mmol), [1, 2, 6, 7-3H] progesterone (3.763 TBq/mmol; 101.7 Ci/mmol), [1, 2-3H] hydrocortisone (1.58 TBq/mmol; 42.7 Ci/mmol), (1, 2-3H] corticosterone (2.22 TBq/mmol; 60.0 Ci/mmol), [1, 2-3H] 18OH-DOC (1.11 TBq/mmol; 30.0 Ci/mmol) and (2, 4, 6, 7-8H] 17β-estradiol 4.144 TBq/mmol; 112.0 Ci/mmol) were purchased from New England Nuclear Company. All non-radiolabeled steroids were purchased from Sigma Chemical Company. [1, 2-3H] DOC sulfate was synthesized from [1, 2-3H] DOC as follows: [1, 2-3H] DOC (0.37 MBq; 10 µCi) was dissolved in 50 µl pyridine and 150 µl pyridine chlorosulfonic mixture (pyridine: chlorosulfonic acid= 10 : 3.5) added. The solution was mixed at 60°C for 30 min, then put into a Sep-Pak C18 cartridge (Waters Associates) and washed with 10 ml of distilled water. The Sep-Pak column was then neutralized with 1 ml of 0.2 M NaOH. After a 4 ml acetone elution, [1, 2-3H] DOC-SO4Na was obtained. The solvent was evaporated and dissolved in 1 ml methanol. The [1, 2-3H] DOC-SO4Na was purified further by TLC steps in silica gel 60 F 254 (Merk) with chloroform- methanol-ethylacetate (80: 20: 4 by vol). The [1, 2-3H] DOC sulfate was detected with a TLC gas flow scanner.

2) Serum steroid measurement

Each sample (0.3-0.5 ml serum) was mixed with dexamethasone (DEX) and [1, 2-3H] DOC sulfate as the internal standards prior to extraction with a Sep-Pak C19 cartridge. The serum sample was washed with 4 ml of distilled water and the steroid fraction was eluted with 2 ml of acetone. The solvent was mixed with 1 ml of sodium acetate buffer (0.1 M, pH 5.0) and extracted twice with diethyl ether (3 ml). The ether extract contained less than 5% of DOC sulfate, whereas the residue had less than 1% of nonconjugated DOC. The ether extract was evaporated under a stream of nitrogen gas. The sample extract obtained was redissolved in
acetonitrile and applied to reverse HPLC (ODS column, acetonitrile: H₂O=50:50 by vol) for steroid separation, this process having been developed by our laboratory. Each appropriately isolated steroid fraction was quantified by radioimmunoassays (RIA). The RIAs were performed as follows. An aliquot of fractions was evaporated. Standards, in concentrations which ranged from 0 to 500 pg per tube, were prepared by evaporation. About 10,000 dpm of each radiolabeled steroid was added to the dried samples and standards. Then, 250 μl antibody in 0.05 M borate buffer (PH 7.8) containing 0.05 W/V % of BSA was added. The mixture were incubated for 20 min at 25°C and 250 μl saturated ammonium sulfate was added. After centrifugation, the radioactivity of the supernatant, representing the free steroid, was measured in a liquid scintillation spectrophotometer. The limit of sensitivity of these assays was between 5 and 10 pg/tube. The cross reactivities of all antisera investigated are shown in Table 1. All antisera to steroids were kindly supplied by Dr. Kanbegawa (Dept. of Obstetrics and Gynecology, Teikyo Univ. School of Med.). All values were corrected for procedure loss which was calculated from the area of the DEX peak. The inter- and intraassay coefficients of variation throughout the complete assay procedure were less than 10% for all of the six steroids.

The residual serum after the diethyl ether extraction was diluted to 3.0 ml with sodium acetate buffer (0.1 M, pH 5.0) that contained 20 U of sulfatase (Sigma Co.). The mixture was incubated at 37°C for 4 hours. The DOC sulfate was enzymatically hydrolyzed. After the hydrolyzation, DOC derived from the DOC sulfate was extracted into diethyl ether (6 ml). From the aliquot the recovery rate was estimated, and the residual was applied to RIA. The mean hydroxylation of DOC sulfate was estimated to be 85%. The mass of DOC sulfate relative to the molecular weight of DOC was calculated. The levels of steroids in the early neonatal period are shown as the mean±SD. Statistical analysis was made by a non-parametric test (Wilcoxon signed ranks test) for the serum levels of steroids and by linear regression analysis for the mutual correlations among them.

**Results**

**Changes in the levels of serum steroids in early neonatal period (Table 2, Fig. 1, 2)**

As shown in Fig. 1, serum progesterone decreased showing elimination like a two-compartment model which had a rapidly decreasing phase and a slowly decreasing one. Serum progesterone decreased rather rapidly from the mean cord level of 251.8±175.5 ng/ml to 21.2±4.8 ng/ml at 8 hrs. Between 8 and 16 hrs the serum progesterone curve reached a plateau. Thereafter serum progesterone gradually declined. Mean serum progesterone had reached normal adult levels by 6 days after birth (0.5±0.3 ng/ml).

Serum DOC decreased very rapidly within 8 hrs after birth from 7.2±1.3 ng/ml in umbilical cord blood to 1.4±0.2 ng/ml at 8 hrs after delivery. Sixteen hours after delivery, the logarithmic serum DOC decreased linearly. The serum DOC level dropped to below the adult level 16 hrs after delivery.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>anti-P</th>
<th>anti-DOC</th>
<th>anti-F</th>
<th>anti-B</th>
<th>anti-18OH-DOC</th>
<th>anti-E₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone (P)</td>
<td>100</td>
<td>9</td>
<td>2.42</td>
<td>—</td>
<td>0.05</td>
<td>&lt;0.08</td>
</tr>
<tr>
<td>Deoxycorticosterone (DOC)</td>
<td>0.01</td>
<td>100</td>
<td>28.1</td>
<td>4.80</td>
<td>2.00</td>
<td>&lt;0.08</td>
</tr>
<tr>
<td>Cortisol (F)</td>
<td>0.01</td>
<td>0.06</td>
<td>100</td>
<td>2.20</td>
<td>&lt;0.06</td>
<td>&lt;0.08</td>
</tr>
<tr>
<td>Corticosterone (B)</td>
<td>0.01</td>
<td>0.30</td>
<td>8.20</td>
<td>100</td>
<td>0.09</td>
<td>&lt;0.08</td>
</tr>
<tr>
<td>Aldosterone (ALdo)</td>
<td>0.01</td>
<td>0.03</td>
<td>2.88</td>
<td>0.17</td>
<td>20.8</td>
<td>—</td>
</tr>
<tr>
<td>Pregnenolone</td>
<td>2.00</td>
<td>1.66</td>
<td>&lt;0.04</td>
<td>0.07</td>
<td>—</td>
<td>&lt;0.08</td>
</tr>
</tbody>
</table>
As shown in Fig. 1, the serum DOC sulfate elimination curve was classified into two periods at 16 hrs after delivery. In the former period (for 16 hrs after delivery) the serum DOC sulfate level dropped abruptly. In contrast, the latter phase (after 16 hrs after delivery) showed a gradual decrease in serum DOC sulfate. In both phases, the elimination curve was almost a straight line. The serum DOC sulfate concentration decreased to 50% of its cord level at 16 hrs after delivery (74.6 ± 12.7 ng/ml) and to 30% of its cord level at 6 days after delivery (48.3 ± 12.3 ng/ml). Even at 6 days after delivery the serum DOC sulfate concentration was still 30 times higher than the normal adult level. On the other hand, the serum progesterone and DOC had already reached the normal adult levels within 6 days after delivery.

Serum E\textsubscript{2} also decreased abruptly from birth (30.0 ± 15.1 ng/ml) to 1 hr after delivery (0.23 ± 0.20 ng/ml). Thereafter it decreased gradually and maintained regained prepubertal levels by 8 hrs after delivery.

The postnatal trend of serum F was different from the abovementioned steroids. Serum F significantly increased from 30.4 ± 7.7 ng/ml in the umbilical artery to 160.7 ± 64.8 ng/ml at 2 hrs after delivery (P < 0.05). Some fluctuations were noted until 8 hrs after delivery. Mean cortisol was 39.5 ± 31.7 ng/ml at 3 days and 74.1 ± 52.4 ng/ml at 6 days.

The change in serum B and 180H-DOC was similar to that of F. These two steroids showed a small surge from birth to 8 hrs after delivery.

**Correlation between these steroids**

The statistical correlations among these steroids after delivery (1-144 hrs) were analyzed. Both the serum DOC and DOC sulfate correlated with serum progesterone (DOC, r=0.34, p<0.05; DOC sulfate, r=0.73, p<0.01). Besides there was signifi-
Fig. 1. Mean (±SD) serum levels of progesterone (P ●), deoxycorticosterone (DOC ▲), deoxycorticosterone sulfate (DOC-S ○) and estradiol (E2 △) in 8 normal neonates from birth to 6 days of age.

Fig. 2. Mean (SD) serum levels of cortisol (F ■), corticosterone (B □), and 18-hydroxydeoxycorticosterone (18 OH-DOC △) in 8 normal neonates from birth to 6 days of age.

cant correlation with the serum DOC and serum F (r=0.467, p<0.01), serum DOC sulfate and serum F (r=0.549, p<0.01). On the other hand, serum 18OH-DOC hand no correlation with progesterone, but F (r=0.410, p<0.01). Serum B significantly correlated with progesterone (r=0.760, p<0.01), as well as with F (r=0.518, p<0.01).

Discussion

To date, there have been few reports available dealing with the analysis of serum
steroid concentrations in the early neonatal period. Sippell et al., (1978) reported a detailed time trend analysis of plasma DOC, progesterone and F in this specific period. Klein et al. (1972) reported the measurement of the DOC sulfate concentration in umbilical cord blood on days 3 and 5. Brown et al. (1972) also reported the determinations of the plasma DOC concentrations in this period. The present study was the first detailed report to analyze these specific steroids.

In the present study, the elimination curve of serum progesterone, DOC and DOC sulfate had two phases: rapidly and slowly decreasing ones. It looked like a two-compartment model which shows the elimination of an injected substance. These curves meant that the production of these steroids stopped abruptly. There were significant correlations among serum progesterone, DOC and DOC sulfate. The decreased part of serum DOC and DOC sulfate could be derived from peripheral progesterone. The decreased parts of DOC and DOC sulfate produced in the adrenal gland in this period cannot explain the changes in serum F in the same period. The serum F concentration increased immediately after delivery, whereas DOC and DOC sulfate declined rapidly. This high F level in early neonatal life could be explained by the fact that 3β- hydroxysteroid dehydrogenase (3β-HSD) in the adrenal gland is suppressed by massive estrogen (Fujieda et al., 1982). However after birth, in this study, the serum levels of E₂ decreased rapidly from birth in the early hours of age. This change in serum E₂ agrees with the finding of Winter et al.. Decreasing estrogen can induce acceleration of Δ⁴-3 ketosteroid (oxo-steroid) secretion. In addition, the human fetal adrenal cell culture demonstrated that cortisol and corticosterone can be formed from progesterone under no estrogen medium (Fujieda et al., 1982; Voutilainen et al., 1979). Besides, the fetal zone of the adrenal cortex, which is deficient in 3β-HSD, is involuted and the adult one becomes predominant after delivery. Thus, the change in the serum F concentration during these periods demonstrated an increase in the production of steroids by the adrenal glands. It is well known that the placenta produces a large amount of progesterone. After birth, the rapid elimination of this placental progesterone can overcome the increased production of progesterone by the adrenal gland. It might be the same with the changes in serum DOC and DOC sulfate as with progesterone. The decrease in serum DOC and DOC sulfate might not be due to the adrenal gland but to progesterone, because some of the decreased quantity of DOC and DOC sulfate was produced mainly at extraadrenal sites. If DOC and DOC sulfate were produced at the adrenal gland, serum DOC and DOC sulfate concentrations would show a peak surge like that of serum F. This results agreed with the report by Sippell et al., (1978) in which serum DOC and DOC sulfate decreased in a significant correlation with serum progesterone.

On the other hand, the changes in serum B and 18OH-DOC, which are metabolites of DOC, were different from those of DOC sulfate. These two steroids significantly correlated with F and showed a peak surge in the initial phase after delivery. This synchronization with serum F suggested that serum B and 18OH-DOC in the fetus could be produced by the adrenal gland. These data demonstrated that only DOC and DOC sulfate could be formed at extraadrenal sites in the fetal compartment.

The correlation among serum DOC and DOC sulfate and F is interesting. In human umbilical arterial blood, there was no positive correlation with serum DOC and F (Igarashi et al., 1988). In the sheep, the fetus did not have a correlation with
serum DOC and F (Magyar et al., 1981). On the other hand, both serum DOC and DOC sulfate had a positive correlation with serum F during the early neonatal period. These results suggested that the adrenal gland was playing the main role as the production site of DOC and DOC sulfate to replace the extraadrenal sites after delivery.

The serum concentration of DOC decreased to the adult levels by 16 hrs after birth. Thereafter, the main production site of serum DOC could be in the adrenal gland. In contrast to DOC, the serum concentration of DOC sulfate was maintained much higher than the adult one. It has to be considered that conjugated steroids have a slow metabolic clearance rate (Guerami et al., 1983). It is hard to determine precisely how adrenal glands produced DOC sulfate at 6 days after birth. However, it is remarkable that the serum DOC sulfate concentrations were high through the entire early neonatal period. DOC sulfate has a mineralocorticoid action when it hydrolyzes to DOC at local sites such as the aorta and kidney. DOC sulfate would therefore be an important steroid to consider with regard to electrolyte and water-balance in this period.

In conclusion, the author suggests that in the fetus serum DOC and DOC sulfate are mainly produced at extraadrenal sites and after birth, these steroids begin to be produced at adrenal glands.

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References


