THE PARATHYROIDS IN CORTICOSTEROID-TREATED PREGNANT RATS AND THEIR OFFSPRING

II. Effect of deoxycorticosterone acetate (DOCA)

By

C. Göran Hansson and Lennart Angervall

ABSTRACT

DOCA administered daily to rats during the second half of pregnancy significantly increased the mitotic rate in both maternal and foetal parathyroids as well as the foetal parathyroid volume suggesting a stimulation of the parathyroid glands. The morphological changes in the parathyroids of foetuses of the DOCA treated rats were similar to those previously observed in foetuses of alloxan diabetic rats.

Alloxan diabetic rats not treated with insulin and their offspring have enlarged parathyroids with increased mitotic rate, suggesting stimulation of the parathyroids. It is likely that diabetic rats may have an enhanced corticosteroid production and that their offspring are exposed to the action of increased amounts of maternal corticosteroids (Angervall 1959; Hansson & Angervall 1966 a). The question was raised whether such steroids could be of pathogenetic significance in the parathyroid changes in alloxan diabetes in the rat. In a previous investigation (Hansson & Angervall 1966 b) pregnant rats were given geometrically increasing doses of cortisone, but it was found that cortisone had little or no effect on the development of the foetal parathyroids or on the morphology of the maternal parathyroids.

In the present investigation the morphology of maternal and foetal parathyroids was studied after administration of a mineral corticosteroid – deoxycorticosterone acetate (DOCA).
MATERIALS AND METHODS

Virgin albino rats of a Wistar strain bred for many years at the Department of Pathology, Gothenburg, were used, the time of conception being ascertained as stated previously (Hansson & Angervall 1966 a).

The pregnant rats were divided into the following groups:
- Control Group comprising 15 untreated rats,
- DOCA\textsubscript{1} Group comprising 8 rats given 0.25 mg DOCA i. m. twice daily,
- DOCA\textsubscript{2} Group comprising 7 rats given 2.25 mg DOCA i. m. twice daily.

All the animals were fed rat bread (cf. Angervall 1959) and water ad libitum. DOCA (Pharmacia) was given daily at 9 a.m. and 4 p.m. from the 10th day of pregnancy to the interruption of pregnancy, which was done on the 22nd day at 3 p.m. The duration of pregnancy was then 522 hours for all rats. Excision of maternal and foetal endocrine organs and morphological examinations were all performed as described previously (Hansson & Angervall 1966 a). The kidneys were weighed and fixed in 10 per cent neutral formalin solution and 4 μ thick paraffin sections were stained by Weigert's modification of van Gieson's haematoxylin procedure. The chemical serum calcium and serum phosphorus estimations were also the same as in the latter investigation. The statistical methods were as used previously and according to the principles given by Hald (1952).

RESULTS

Effect on Mothers

Body weights at conception and parturition as well as weights of adrenals and kidneys are given in Table 1. The body weight of the controls and of the DOCA-treated rats increased during pregnancy, although the increase tended to be somewhat lower in the DOCA\textsubscript{2} Group. The adrenal weights were not significantly affected by DOCA. The DOCA\textsubscript{2} Group showed a significantly increased renal weight but the kidneys exhibited no consistent morphological changes. The rats tolerated the DOCA treatment well and no animal died during the experiment.

Table 1.

<table>
<thead>
<tr>
<th>Series</th>
<th>Body weight at conception (g)</th>
<th>Body weight at parturition (g)</th>
<th>Adrenals (mg)</th>
<th>Kidneys (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>178 ± 6</td>
<td>201 ± 8</td>
<td>64.0 ± 3.3</td>
<td>1.25 ± 0.05</td>
</tr>
<tr>
<td>DOCA\textsubscript{1}</td>
<td>183 ± 4</td>
<td>214 ± 8</td>
<td>67.4 ± 5.3</td>
<td>1.33 ± 0.06</td>
</tr>
<tr>
<td>DOCA\textsubscript{2}</td>
<td>196 ± 4\textsuperscript{1)}</td>
<td>210 ± 6</td>
<td>62.8 ± 6.1</td>
<td>1.53 ± 0.06\textsuperscript{2)}</td>
</tr>
</tbody>
</table>

\textsuperscript{1)} \( t = -2.37 \) (\( P < 0.05 \)).

\textsuperscript{2)} \( t = -3.39 \) (\( P < 0.01 \)).
Table 2 gives the data on parathyroid volume, nuclear diameters, mitotic rate and serum calcium and phosphorus in the controls and the two DOCA-treated Groups. Neither parathyroid volume nor nuclear diameters, seemed to be affected by DOCA, but the mitotic rate showed a tendency to increase in the DOCA₁ Group and was significantly increased in the DOCA₂ Group. The serum calcium and serum phosphorus levels in the controls and the two DOCA Groups did not differ significantly.

Effect on Offspring

The average number of offspring per litter was similar in all groups (C Group, 7.9 ± 0.5; DOCA₁ Group, 8.7 ± 0.6; DOCA₂ Group, 8.1 ± 1.0). The mean litter weights were 4.23 ± 0.13 g for the controls and 4.36 ± 0.06 g and 4.15 ± 0.20 g respectively for the DOCA₁ and DOCA₂ Groups, the corresponding mean birth weights being 4.23 ± 0.05, 4.36 ± 0.03, and 4.23 ± 0.04 g. There were no significant differences between the groups and there was no mortality.

The adrenals were significantly lighter in the DOCA Groups weighing: C Group, 3.82 ± 0.14 mg, DOCA₁ Group 2.89 ± 0.05 mg, DOCA₂ Group 3.15 ± 0.06 mg (t = 6.20; P < 0.001 and t = 4.47; P < 0.001 resp.). Fig. 1 illustrates the regressions of the weight of the adrenals on the body weight. It will be seen that there is a significant relationship between the weight of the adrenals and body weight in all groups.

Birth weight, for those young whose parathyroid volume was determined, as well as the parathyroid volume and mitotic rate are given in Table 3, and the parathyroid volume has been plotted against birth weight in Fig. 2. The offspring of DOCA-treated rats showed a significantly greater parathyroid volume and a higher mitotic rate. There was no significant rela-

Table 2.
Parathyroid volume, nuclear diameters and mitotic rate and serum calcium and phosphorus in C- and DOCA-series.

<table>
<thead>
<tr>
<th>Series</th>
<th>Parathyroids mm³</th>
<th>D₁</th>
<th>D₂</th>
<th>Mitotic rate</th>
<th>Serum calcium mEq./l</th>
<th>Serum phosphorus mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.224 ± 0.012</td>
<td>68 ± 1</td>
<td>95 ± 1</td>
<td>1.3 ± 0.5</td>
<td>4.7 ± 0.2</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td>DOCA₁</td>
<td>0.230 ± 0.022</td>
<td>69 ± 1</td>
<td>96 ± 2</td>
<td>3.6 ± 1.3¹</td>
<td>4.9 ± 0.2</td>
<td>5.0 ± 0.4</td>
</tr>
<tr>
<td>DOCA₂</td>
<td>0.226 ± 0.015</td>
<td>67 ± 1</td>
<td>94 ± 2</td>
<td>4.1 ± 0.9²</td>
<td>4.8 ± 0.1</td>
<td>4.2 ± 0.4</td>
</tr>
</tbody>
</table>

¹) t = -1.63.
²) t = -2.77 (P < 0.02).
Fig. 1.
The regression of adrenal's weight on body weight in control and DOCA-treated rats.

- **C**
  \[ y = -1.33 + 1.22 \cdot x \quad (t_b = 5.55; \ P < 0.001) \]

- **DOCA**
  \[ y = 1.02 + 0.43 \cdot x \quad (t_b = 3.07; \ P < 0.01) \]
  \[ y = 0.87 + 0.54 \cdot x \quad (t_b = 3.38; \ P < 0.01) \]

Table 3.
Birth weight, parathyroid volume and mitotic rate in C- and DOCA-series.

<table>
<thead>
<tr>
<th>Series</th>
<th>Birth weight (g)</th>
<th>Parathyroids (mm³)</th>
<th>Mitotic rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>4.10 ± 0.13</td>
<td>0.0093 ± 0.0004</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>DOCA₁</td>
<td>4.40 ± 0.07¹)</td>
<td>0.0119 ± 0.0005²)</td>
<td>16 ± 2</td>
</tr>
<tr>
<td>DOCA₂</td>
<td>4.18 ± 0.13</td>
<td>0.0121 ± 0.0005³)</td>
<td>22 ± 3⁴)</td>
</tr>
</tbody>
</table>

¹) \( t = -2.04 \) (\( P = 0.05 \)).
²) \( t = -3.71 \) (\( P < 0.001 \)).
³) \( t = -4.16 \) (\( P < 0.001 \)).
⁴) \( t = -2.58 \) (\( P < 0.02 \)).

There was no significant relationship between parathyroid volume and birth weight in either group, but a tendency to negative relationship in the DOCA₂ Group.

Multiple regression analysis of the relationship between parathyroid volume and serum calcium and phosphorus and birth weight disclosed a significant and positive relationship between parathyroid volume and serum phosphorus in the C Group (\( t \) for the partial coefficient of regression (\( t_b \)) = 2.25;
The regression of parathyroid volume on birth weight in control and
DOCA-treated rats.

C     \[ y = 0.0097 - 0.0001 x \]  \( (t_b = -0.12; P > 0.9) \)

DOCA\(_1\) \[ y = 0.0089 + 0.0007 x \]  \( (t_b = 0.31; P < 0.8) \)

DOCA\(_2\) \[ y = 0.0241 - 0.0029 x \]  \( (t_b = -1.35; P < 0.2) \)

\( P < 0.05 \) and a significant and negative relationship between parathyroid
volume and birth weight in the DOCA\(_2\) Group \( (t_b = -3.06; P < 0.01) \). No
significant relationship between foetal parathyroid volume and maternal
serum calcium and phosphorus was demonstrated in the DOCA-treated groups.

**DISCUSSION**

**Effect on Mothers**

The rate of body weight increase in the DOCA\(_2\) rats during pregnancy was
slightly reduced, but the DOCA\(_1\) rats showed a body weight increase similar
to that seen in the controls. *Wexler* (1963) observed a slightly retarded body
weight increase in male rats given 1 mg DOCA daily for 63 consecutive days.
This author also observed reduced weight of the adrenals and lipid depletion
in the glomerular zone in male rats after DOCA administration, but in the
present investigation there was no significantly reduced adrenal weight in the
DOCA-treated rats.

While the parathyroid volume and nuclear diameters were not significantly
changed in the DOCA-treated rats, the mitotic rate tended to be increased in
the DOCA₁ Group and was significantly enhanced in the DOCA₂ Group. Thus, like alloxan diabetic pregnant rats (Hansson & Angervall 1966 a), DOCA-treated pregnant rats showed morphological changes in the parathyroids which suggest a stimulation of the parathyroids. A stimulation of the parathyroids might be due either to DOCA acting directly on the parathyroids or to a DOCA-induced change in the calcium-phosphorus homeostasis.

Lehr (1959) postulated the existence of an adrenal cortex-parathyroid axis with the adrenal cortex and particularly the mineral corticosteroids exerting a trophic effect upon the parathyroids, but so far without convincing evidence.

It is well known that corticosteroids and especially mineral corticosteroids tend to induce hypokalaemia and alkalosis. Hypokalaemia and alkalosis lower the plasma ionized calcium level (Ganong 1963). A fall in plasma ionized calcium is a stimulus to parathyroid secretion (e.g. Fourman 1960). The serum calcium and serum phosphorus levels in the DOCA-treated rats remained unchanged in the present investigation, but the concentration of ionized plasma calcium may be depressed without changes in the total serum calcium level (Danowski 1962). Therefore, it is possible that increased parathyroid activity in DOCA-treated rats is a consequence of lowered ionized plasma calcium.

Effect on Offspring

The mean number of young per litter, the mean weight of all offspring and the mean litter weight were not significantly different in the three groups, and the death rate was nil in all groups. Douglas & Langford (1965) found a significantly reduced number of young per litter and an increased mortality in the offspring of rats given DOCA and NaCl during or before pregnancy. This difference between the latter investigation and ours may to some extent be explained by the fact that in the present investigation pregnancy was terminated on the 22nd day, while the young were born spontaneously in Douglas’ investigation.

It seems established that the foetal pituitary-adrenocortical system in the rat (and other species) is functioning towards the end of gestation and also that the transplacental passage of maternal corticosteroids is a factor in controlling the function of the foetal pituitary-adrenocortical system (e.g. Noumura 1959; Jost et al. 1962; Diczfalusy 1962). The significantly reduced weight of the foetal adrenals after DOCA administration to the mothers indicates that this steroid passed to the foetuses and inhibited the foetal ACTH production.

Previous studies on the effect on parathyroid morphology of hormones derived from the mothers have in most cases been negative, i.e. the foetal parathyroid glands have been unaffected by maternal hormones. Thus growth hormone (Engfeldt & Hultquist 1953) and cortisone (Hansson & Angervall 1966 b) given to pregnant rats have had no apparent effect on the foetal
parathyroids. Angervall (1962) found no effect on the foetal parathyroid volume when the mothers were adrenalectomized on the 12th or 13th day of pregnancy. Nor did hypophysectomy of pregnant rats have any effect on parathyroid volume in the foetuses (Angervall & Lundin 1963). In maternal diabetes induced either by pancreatectomy (Hultquist 1950) or with alloxan (Angervall 1959; Hansson & Angervall 1966a), the foetuses had enlarged parathyroids. The increase in parathyroid volume and in mitotic rate in the present investigation were of an order similar to those observed in foetuses of alloxan diabetic rats (Hansson & Angervall 1966a), but compared to the diabetic rats there was no significant relationship between foetal parathyroid volume and maternal serum calcium and phosphorus, nor any significant changes in the serum calcium and phosphorus by comparison with the controls. However, as stated previously, the ionized plasma calcium may be depressed after DOCA treatment without any changes in total serum calcium level and therefore it is conceivable that the mechanism for parathyroid stimulation is the same in mother and offspring, i.e. a lowered ionized plasma calcium.

In infants of diabetic mothers hyperplasia of the parathyroids has been observed (Hultquist et al., abstract, 1965) and diabetic women have an increased urinary output of aldosterone (Rinsler & Rigby 1957) and a two to fourfold increase in aldosterone production has been observed during the last trimester of diabetic pregnancy (see Kyle 1963). Thus, mineral corticosteroids possibly contribute to foetal parathyroid stimulation when the mother has diabetes.

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