CORTISOL RELEASE, DISTRIBUTION
AND METABOLISM IN INDUCED OESTROGENIC
DEFICIENCY

By

Jens Aas Jansen, Jens Schou and H. Singh

ABSTRACT

The biological half-life of exogenous [4-\textsuperscript{14}C] cortisol in guinea pigs is approximately 105 min. After ovariectomy there is a tendency to a prolonged half-life (delayed metabolism) of cortisol. The plasma cortisol in guinea pigs is 314 ± 79 ng/ml with an ultrafiltrable fraction of 38 ± 15 ng/ml. Skin cortisol is 44 ± 12 ng/g tissue. After ovariectomy the plasma cortisol is decreased (195 ± 36 ng/ml) and there is a higher relative diffusible fraction, while the skin cortisol is decreased (23 ± 4 ng/g). During stress release there may be a parallel release of a cortisol-binding non-ultrafiltrable component or an elimination of a protein bound not fluorescent or separated steroid. The tissue cortisol is higher than would be expected from the values of diffusible cortisol in plasma. The low concentration of ultrafiltrable cortisol may explain the reduced rate of cortisol metabolism.

Exogenous administration of oestrogens and the increased oestrogenic activity in pregnancy are associated with increased plasma cortisol levels, both protein bound as well as unbound (for references see Plager et al. 1964). Further, hyper-oestrogenism is accompanied by an increased concentration of transport proteins in the plasma, including transcortin (cortisol binding globulin = CBG, see Beisel et al. 1964).

In a state of assumed oestrogenic deficiency during the menopausal years in women, Schou et al. (1967) demonstrated a decreased cortisol content in skin tissue sampled by needle biopsies accompanied by only minor (insignificant) variations in plasma cortisol concentrations. Consequently, the concentration of
cortisol in the tissue did not seem to depend only on the cortisol concentration in the plasma.

The purpose of the present investigation was to determine the content of cortisol in skin and plasma during induced oestrogenic deficiency, and to determine whether there is a simple relationship between freely diffusible cortisol in plasma and the content of cortisol in the skin and subcutaneous tissue representing mainly connective tissue. Furthermore the metabolism of cortisol was evaluated from the half-life and the chromatographic pattern of labelled metabolites after administration of [4-\(^{14}\)C] cortisol. Guinea pigs were used for the experiments since cortisol in this species as in man is the main glucocorticoid, while in other smaller rodents there is an excess of corticosterone.

To estimate the stress condition of the animals during the experiments with labelled cortisol, the concentration of cortisol in the plasma (free and bound) and in the skin was measured. The results showed an unexpected variation in the fraction of unbound cortisol in the plasma of ovariectomized animals.

METHODS

Young mature white female guinea pigs weighing 350-450 g were used.

Surgery

Ovariectomy was performed under ether anaesthesia by the dorsal approach through a single midline incision. The ovaries were extruded through the dorsal muscles after blunt dissection. They were then removed after careful double ligation of the upper horn. The muscles and skin were closed with silk sutures.

A control group was anaesthetized and operated on in a similar manner except that the ovaries were only extruded and replaced in situ after manipulation without ligation of the horn.

Experimental procedure

Four weeks after the operation when the skin was completely healed the unanaesthetized animal was fixed in the ventral position on a special table. A vein in the hind leg was exposed and the labelled cortisol injected after placing two ligatures around the vein which could be tightened immediately after the injection to prevent bleeding when the needle was removed. The injected dose contained 300 nCi [4-\(^{14}\)C] cortisol (The Radiochemical Centre, Amersham, specific activity: 156 µCi/mg) in 100 µl 10% v/v ethanol in water. The injected dose contained 1.93 µg cortisol which was comparable to the endogenous amount in 6-8 ml of guinea pig plasma. The injection could be performed on the unanaesthetized animals usually without any pain reaction, when only the eyes of the animals were covered by a hand. The skin wound was closed by two silk sutures, and the animal returned to the cage until blood samples were obtained by heart puncture after stunning the animals 80 or 160 min later. In preliminary experiments it was established that complete distribution followed by a mono-exponential decrease in labelled plasma cortisol was reached 40-60 min after [4-\(^{14}\)C] cortisol injection.
**Sampling and analytical procedures**

After stunning the animals by a blow on the neck, 10 ml blood was drawn by heart puncture into heparinized centrifuge tubes after which the guinea pigs were completely bled. The pelt on the lower part of the back was cut with an electric clipper and a skin sample (corium and subcutaneous tissue) was excised. The blood was spun and the plasma and tissue were immediately used for analytical procedure.

The concentration of cortisol was determined in 50 µl plasma, 50-100 mg tissue and 200 µl ultrafiltrate of plasma by the method of Jansen et al. (1967). It should be noted that corticosterone and oestrogenic steroids are removed during the procedure. By means of thin-layer chromatography it has been shown that only cortisol is measured by this method, when applied to skin and plasma from control guinea pigs. The plasma ultrafiltrate was obtained through a Viskinge® cellophane membrane (average pore diameter 24 Å) at 37°C, pressure 1 kg/cm², in an atmosphere of 96% O₂ and 4% CO₂ using the method of Ames & Sakanoue (1964). Approximately 500 µl ultrafiltrate was collected from 2.5 ml plasma. If the Heller test was positive the ultrafiltrate was discarded.

The total amount of proteins in the plasma was determined by the method of Daughaday et al. (1952). For determination of [4-14C] cortisol 1 ml plasma was washed with 5 ml hexane in order to remove lipids and then with 6 ml dichloromethane. Five ml of the dichloromethane containing cortisol and other steroids was evaporated to dryness and re-dissolved in 200 µl dichloromethane for paper chromatography using Deckx et al. (1964) solvent system »H₉« to separate the cortisol from the labelled metabolites. The amount of [4-14C] cortisol was determined by paper strip radiochromatogram scanning (Packard Model 7201) in relation to standard amounts of 1 nCi [4-14C] cortisol carried through the complete procedure.

Using the logarithms of the plasma concentrations of [4-14C] cortisol after 80 and 160 min, the regression line for the monoexponential decrease was calculated for the three groups of results. The linear functions allowed the half-lives (t₁/₂) and the apparent volumes of distribution (extrapolation of the line to t₀) to be determined.

**RESULTS**

The concentrations of cortisol in the plasma, plasma ultrafiltrate and skin from ovariectomized guinea pigs, as well as the control operated and non-operated controls are given in Table 1. In the animals taken directly from the cage, the plasma cortisol was the same in ovariectomized and control operated animals. Both of these groups showed a lower mean concentration than the non-operated controls, but the difference was not significant.

The concentration of cortisol in ultrafiltrate of plasma shows some variation. When considered in relation to the level of total plasma concentrations (Table 1 and Fig. 1), it appears that the concentration of ultrafiltrable (diffusible) cortisol in plasma is significantly increased (P < 0.05) after ovariectomy. It is also noteworthy that the concentration of cortisol in the skin is lower (but not significant, P > 0.05) in the ovariectomized group than in the two control groups (Table 1).

The results of the experiments with [4-14C] labelled cortisol are summarized
The concentrations of cortisol in plasma, plasma ultrafiltrate (both ng/ml ± SEM) and skin (ng/g ± SEM) and the per cent (± SEM) of ultrafiltrable cortisol in plasma of ovariectomized, control operated and non-operated control guinea pigs. Figures are given for animals taken directly from the cage, and 80 and 160 min after the injection of [4-¹⁴C] labelled cortisol (total amount comparable to approximately 1/50 of the endogenous pool). Figures in brackets indicate number of animals.

<table>
<thead>
<tr>
<th></th>
<th>Uninjected</th>
<th>80 min after ¹⁴C-inj.</th>
<th>160 min after ¹⁴C-inj.</th>
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<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td>Ultrafiltr.</td>
<td>Per cent ultrafiltr.</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>314 ± 79</td>
<td>38 ± 15</td>
<td>14 ± 2</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td></td>
<td></td>
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<tr>
<td>Operated controls</td>
<td>218 ± 40</td>
<td>20 ± 5</td>
<td>8 ± 1</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td></td>
<td></td>
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<tr>
<td>Ovariectomized</td>
<td>195 ± 36</td>
<td>42 ± 15</td>
<td>25 ± 6</td>
</tr>
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<td></td>
<td>(4)</td>
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The concentration of ultrafiltrable cortisol in relation to total plasma cortisol from guinea pigs before (indicated by 1), 80 min (indicated by 2), and 160 min (indicated by 3) after intravenous injection of 1.93 µg [4-14C] cortisol to guinea pigs. The concentration of ultrafiltrable cortisol is indicated by vertical bars descending from the line A. The vertical distance between the upper straight (A) and the lower curve (B) indicates the unbound (ultrafiltrable) fraction of cortisol at the total plasma concentrations read on the abscissa under ideal control condition. The curve B is drawn on the basis of our own control findings which are in close agreement with Fig. 1 in Beisel et al. (1964). The vertical distance from the curve B to the abscissa indicates the protein bound fraction in the control condition. The vertical bars ascending from the abscissa denote the skin concentration of cortisol (ng/g) in the nine group of experiments.

in Table 2. The apparent tendency after ovariectomy to a prolonged half-life of cortisol and an increase in the volume of distribution (Vd) is not statistically significant. The scanning pattern of the chromatograms excluded any qualitative differences in the metabolism of cortisol in the three experimental groups.

In the control animals the total plasma cortisol is almost doubled 80 min after the injection, and at 160 min it has decreased to the control level. The same tendency is found in operated controls and in ovariectomized animals.
Table 2. Calculated half-life ($t_{1/2}$, min) and apparent volume of distribution ($V_d$) for [4-$^{14}$C] cortisol in guinea pigs. Groups: operated controls (OC), non-operated controls (NOC), and ovariectomized animals (OVZ) four weeks after the operation. $V_d$ is expressed in per cent of body weight. Figures in brackets indicate degrees of freedom.

<table>
<thead>
<tr>
<th></th>
<th>$t_{1/2} \pm \text{sd}$</th>
<th>$V_d \pm \text{sd}$</th>
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<tbody>
<tr>
<td>OC (9)</td>
<td>95 ± 32</td>
<td>74 ± 8</td>
</tr>
<tr>
<td>NOC (10)</td>
<td>104 ± 30</td>
<td>86 ± 9</td>
</tr>
<tr>
<td>OVZ (8)</td>
<td>125 ± 56</td>
<td>98 ± 14</td>
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</table>

The last mentioned group reaches the highest of all the measured values after 80 min which is more than 3 times the control level of this group.

The diffusible fraction of plasma cortisol is increased in parallel with the changes in total cortisol in both controls and operated controls (Table 1). In ovariectomized animals, however, the percentage of diffusible cortisol is decreased during stress and there is a marked increase in plasma cortisol. From Fig. 1 it is seen that the initial diffusible fraction in ovariectomized animals is much higher than would be expected at the low total plasma concentration, while the values after 80 and 160 min are lower than would be expected in the presence of the excessively high total cortisol concentrations. The percentage of diffusible cortisol after 160 min (see Table 1) is even significantly ($P < 0.05$) lower than the initial value.

The mean concentration of proteins in plasma (Table 3) did not show any significant differences between the three experimental groups, nor did the experimental procedure induce any significant changes.

DISCUSSION

The guinea pig was chosen for our experiments as cortisol is the major glucocorticoid in this species. Unfortunately guinea pigs do not have any superficial veins for intravascular injections or for blood sampling through the skin. A vein was therefore exposed for the labelled injection and this procedure produced a considerable stress reaction.

Ovariectomized animals taken directly from the cage show an increased concentration of freely diffusible cortisol, indicating that the protein bound fraction is lowered as compared to control animals. In plasma, cortisol is partly bound to albumin (CBA, low affinity, high capacity), and partly to transcortin (CBG, high affinity, limited capacity), conf. Daughaday (1956), Beisel et al. (1964). As the total concentration of plasma protein is unchanged after ovariectomy (Table
Table 3.
The concentration of protein in plasma (mg/ml ± SEM) from ovariectomized, control operated and non-operated control guinea pigs. Figures are given for animals taken directly from the cage, and 160 min after the injection of [4-14C] labelled cortisol. Figures in brackets indicate number of animals.

<table>
<thead>
<tr>
<th></th>
<th>Uninjected</th>
<th>160 min after 14C-inj.</th>
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<tbody>
<tr>
<td>Controls</td>
<td>61.4 ± 4.4</td>
<td>64.5 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td>Operated controls</td>
<td>64.3 ± 1.1</td>
<td>69.1 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(5)</td>
</tr>
<tr>
<td>Ovariectomized</td>
<td>67.1 ± 2.5</td>
<td>72.2 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(6)</td>
</tr>
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</table>

3) this points to a decreased concentration of transcortin as basis for the lowered protein bound fraction. Due to the high affinity of transcortin to cortisol, changes in the concentration of transcortin in plasma may significantly change the bound fraction of cortisol although no changes in total plasma protein can be detected. During stress release of cortisol after ovariectomy, the fraction of free cortisol decreases (open column 3 in Fig. 1) so that the protein bound fraction increases significantly. As there is no significant changes in total plasma protein the most likely explanation is a simultaneous release of transcortin during the stress condition. Further investigations are in progress to elucidate whether such changes in transcortin concentrations occur. Changes in transcortin release, however, are not the only possible explanation for our findings. Changes in bound or unbound fluorescent metabolites or changes in bound not fluorescent metabolites could also explain the results. In this connection it should be noted that the specificity of the method for cortisol determination has been verified in control animals. It is moreover remarkable that the tissue cortisol in the ovariectomized group is initially very low while it increases significantly even exceeding the ultrafiltrable plasma cortisol after 80 and 160 min (Table 1). This indicates that the concentration of cortisol in tissue is not in a simple relationship to the ultrafiltrable cortisol in plasma. In parallel with the apparent increase in cortisol binding capacity in the plasma there seems to be an increased peripheral accumulation of cortisol in the skin. The strong binding of cortisol with relatively low concentrations of ultrafiltrable cortisol was accompanied by an apparent delay in the rate of cortisol metabolism following ovariectomy.
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REFERENCES


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