Effect of cortisol on the plasma and lymphoid tissue distributions of tritiated glucocorticoids in C57BL/6 mice

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ABSTRACT

The mechanism of action of very high doses of corticosteroids, such as those administered as bolus doses in the treatment of inflammatory and immune diseases or those currently used in rodents to isolate the small proportion of medullary thymocytes considered to be corticoresistant, is still undefined. The possible existence of selective local concentration by some tissues, particularly lymphoid organs, cannot be excluded. Therefore, using C57BL/6 mice, the kinetics of lymphoid tissue and plasma radioactivities after i.p. injection of steroids, either alone or with an excess of non-radioactive cortisol hemisuccinate (up to 10 mg/animal, i.e. 500 mg/kg) were studied. There was a rapid and dose-dependent retention of $[^3H]$corticosterone and $[^3H]$cortisol in the thymuses of cortisol-treated compared with control animals. The spleen also appeared to be capable of accumulating steroids. However, when the tissue/plasma ratio of $[^3H]$steroid concentration and the change in extracellular space in the presence of an excess of non-radioactive cortisol were taken into consideration, only the thymus was able to concentrate steroids above concentrations in the plasma. Moreover, this effect did not appear to be specific for glucocorticoids, since tracer accumulation was also observed when sex steroids were used as tracers. The cells of the reticuloendothelial system may, in part, be responsible for this phenomenon of steroid concentration in lymphoid organs.


INTRODUCTION

During the last decade a considerable amount of information has been obtained concerning the prominent role of receptors in the mechanism of action of steroid hormones. Extensive investigations have been carried out using rodent and human lymphoid cells to measure the number of glucocorticoid receptors and to determine the physiological actions of these steroids in vitro (Munck & Leung, 1977; Lippman, 1979; Young, Nicholson, Guyette et al. 1979; Homo, Picard, Durant et al. 1980; Homo-Delarche, 1984). These experiments, however, failed to explain fully the differences in the steroid sensitivities between corticosensitive species or, in a given species, among the different lymphoid organs or lymphoid cell types. For example, administration of cortisol to rodents led to the almost complete disappearance of the thymus gland, whereas the effect on spleen and lymph nodes was much less marked (Ishidate & Metcalf, 1963; Cohen & Claman, 1971; Weissman, 1973; Duval, Dardenne, Dausse & Homo, 1977). Moreover, the variability of the clinical response to glucocorticoids may also be related to individual and species differences in the bioavailability of the administered drugs. This parameter depends upon numerous factors, including the intrinsic potency of the molecule, the absorption and distribution of the steroid and its metabolic degradation (Stubb, 1975; Ballard, 1979; Morris, 1980).

Under certain circumstances the doses administered are much greater than the physiological or even the usual steroid concentrations during treatment. Massive doses of glucocorticoids are commonly used for the treatment of septic shock or acute anaphylactic reaction, and as pulse therapy in the treatment of rheumatoid arthritis and lupus nephritis (Fan, Yu, Clements et al. 1978; Baylis, Williams, English et al. 1982; Weiss, 1982). On the other hand, high doses of cortisol (10–20 mg/animal, i.e. 500–1000 mg/kg) have routinely been used to isolate a small population of corticoresistant medullary thymocytes from the rodent thymus (Blomgren & Anderson, 1969; Duval, Dausse & Dardenne, 1976). Little information, however, is available concerning the bioavailability of such high doses of glucocorticoids, particularly how
they are distributed among lymphoid organs. We have thus determined the plasma and tissue distributions of injected radioactive tracers, administered either alone or in conjunction with large amounts of unlabelled cortisol. Tissue distribution was compared between lymphoid organs (thymus and spleen) and the kidneys, thus allowing a study of the kinetics of steroid elimination in more or less vascularized targets for glucocorticoid action.

**MATERIALS AND METHODS**

**Animals**

Intact C57BL/6 female mice, 6–8 weeks old (15–20 g body weight), were purchased from Iffa Credo (Les Oncins, France) and maintained on a normal diet with free access to tap water.

**Reagents**


**Analysis of the tissue distribution of steroids and inulin**

Mice received an i.p. injection of a tritiated steroid (10 μCi/animal which, according to the specific activity of the compound, corresponded to 0·1–0·25 nmol) with either 10 mg cortisol hemisuccinate (unless stated otherwise) in a volume of 0·2 ml, or the diluent alone (17·5%, w/v, glucose in sterile water). At various intervals thereafter, three or four animals from each group were killed by cervical dislocation and their thymuses, spleens and both kidneys removed. These were dissected free of fatty tissue and weighed. After tissue solubilization using Soluene 350 (Packard Instruments, Rungis, France) the radioactivity accumulated in the various organs was determined by liquid scintillation counting. Plasma radioactivities were determined in blood taken from the retro-orbital sinus. Results are expressed as d.p.m./100 mg tissue wet weight or d.p.m./100 μl plasma after the appropriate quench correction. In some experiments the values obtained for animals treated with cortisol were expressed as percentages of those in control animals. The extracellular fluid volume was determined by injecting 10 μCi $[^3]H$inulin/animal and measuring tissue and plasma radioactivities as described above for tritiated steroids.

**RESULTS**

The mean wet weights of thymuses, spleens and kidneys taken 4 h after injection of the diluent alone were 66·7 ± 4·3 (s.d.), 82·6 ± 6·4 and 230·5 ± 13·3 mg respectively in 12 different experiments comprising three animals. These values were very similar between experiments. The weights of these organs in cortisol-treated animals were similar to those of control animals 4 h after injection, thus allowing steroid uptake to be expressed as d.p.m./mg tissue wet weight.

The kinetics of tissue accumulation of radioactivity following i.p. injection of $[^3]H$corticosterone are shown in Fig. 1. Uptake of the tracer was very rapid in the three organs studied, attaining a maximum within 15 min of injection. The radioactivity in the thymus and spleen then decreased to reach very low values 2 h later. On a wet weight basis, tracer uptake was four- to fivefold higher in kidneys than in the lymphoid organs, but the tissue radioactivity decreased more rapidly and became almost negligible 4 h after injection. Injection of 10 mg cortisol together with the tracer induced a significant and sustained increase in the tissue radioactivity of thymus and spleen. In the kidneys the tissue radioactivity of treated animals, which remained almost constant for 4 h, was lower than that of controls during the first 45 min after injection, but much higher thereafter.

The effect of cortisol on tissue radioactivity levels may possibly be accounted for by an alteration in the distribution of the tracer volume in the presence of high concentrations of cortisol hemisuccinate. We therefore studied the tissue accumulation of $[^3]H$inulin, a widely used indicator of extracellular volume, with or without a simultaneous injection of 10 mg cortisol. Cortisol, which did not alter thymic accumulation of $[^3]H$inulin, induced a transient splenic increase in $[^3]H$inulin retention during the first 45 min and a sustained enhancement of renal $[^3]H$inulin retention (results not shown).

The effects of various concentrations of unlabelled cortisol (1–10 mg) on the plasma levels of radioactivity after injection of $[^3]H$corticosterone are shown in Fig. 2. At its peak, i.e. 30 min after injection, the level of plasma radioactivity appeared to be independent of the dose of cortisol administered. Administration of either 1 or 5 mg cortisol hemisuccinate together with the tracer significantly enhanced plasma radioactivity during the first 60 min after injection, but did not alter the kinetics of steroid elimination, since the levels of plasma radioactivity in animals treated with 1 and 5 mg cortisol were equivalent to those of control animals 4 h after injection. In contrast, administration of 10 mg cortisol altered tracer elimination, since the plasma radioactivity remained almost constant during the 4-h experimental period.

The DISCUSSION did not appear to be specific for glucocorticoids, since tracer accumulation was also observed when \(^{3}\text{H}\)testosterone and \(^{3}\text{H}\)oestradiol were used.

DISCUSSION

The kinetics of plasma distribution and elimination of radioactivity observed in these experiments after i.p.

When the results were expressed as the ratio of organ to plasma percentages (Table 1) it appeared that the thymus, and to some extent the spleen, was able to retain the radioactive steroid in the presence of an excess of non-radioactive hormone. In contrast, the ratio determined for the kidneys under the same conditions was consistently less than one.

Similar results were obtained in experiments carried out using another glucocorticoid, \(^{3}\text{H}\)cortisol, as tracer. Administration of increasing concentrations of cortisol hemisuccinate (0.25–10 mg/animal) caused a dose-dependent increase in accumulation of radioactivity in the thymus and spleen 60 min after injection. This increase was significant at a concentration of 0.25 mg/animal and reached a plateau between 5 and 10 mg/animal (results not shown). In contrast, cortisol (10 mg/animal) only minimally increased the thymic retention of the synthetic glucocorticoid tracer \(^{3}\text{H}\)dexamethasone (Fig. 3). Moreover, this effect did not appear to be specific for glucocorticoids, since tracer accumulation was also observed when \(^{3}\text{H}\)testosterone and \(^{3}\text{H}\)oestradiol were used.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Kinetics of tissue radioactivity in mouse (a) thymus, (b) spleen and (c) kidney following i.p. injection of 10 μCi \(^{3}\text{H}\)corticosterone either alone (○) or with 10 mg cortisol hemisuccinate (●) per animal. Values are means ± S.D. of two experiments with three individual determinations in each. The radioactivity recovered in the thymus, spleen and kidney of control animals represents 1.2, 2.2 and 43% respectively of the injected radioactivity 15 min after injection, and 0.15, 0.6 and 1.6% respectively 4 h after injection.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Kinetics of total plasma radioactivity in mice following injection of \(^{3}\text{H}\)corticosterone either alone (○) or in the presence of 1 (■), 5 (○) or 10 (▲) mg cortisol hemisuccinate per animal. Values are means ± S.D. of two experiments with three separate animals/group in each experiment.}
\end{figure}

injection of trace amounts of \(^{3}\text{H}\)corticosterone or \(^{3}\text{H}\)cortisol were very similar to those described by others, with an early peak of plasma radioactivity followed by very rapid elimination (Peterson, Wyngaarden, Guerra et al. 1955; Marandici &
TABLE 1. Effect of simultaneous injection of 10 mg cortisol hemi-
succinate on the plasma and tissue distribution of a tracer dose of 
\([^3\text{H}]\text{corticosterone in mice. Values are means ± S.D. of three}

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Thymus</th>
<th>Spleen</th>
<th>Kidney</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>382 ± 85*</td>
<td>263 ± 86**</td>
<td>56 ± 18</td>
<td>219 ± 72*</td>
</tr>
<tr>
<td>60</td>
<td>940 ± 26**</td>
<td>416 ± 114**</td>
<td>180 ± 84**</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>1591 ± 640**</td>
<td>273 ± 53*</td>
<td>922 ± 856**</td>
<td>839 ± 422**</td>
</tr>
<tr>
<td>240</td>
<td>1484†</td>
<td>350-5†</td>
<td>846†</td>
<td>1220 ± 483*</td>
</tr>
</tbody>
</table>

Radioactivity (organ : plasma ratio)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Thymus</th>
<th>Spleen</th>
<th>Kidney</th>
<th>Plasma</th>
</tr>
</thead>
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<td>1</td>
</tr>
<tr>
<td>120</td>
<td>1.8</td>
<td>0.4</td>
<td>0.6</td>
<td>1</td>
</tr>
<tr>
<td>240</td>
<td>1.3</td>
<td>0.3</td>
<td>0.75</td>
<td>1</td>
</tr>
</tbody>
</table>

*P = 0.064, **P < 0.05 compared with control (Wilcoxon's t-test).
†Two experiments were performed.
Radioactivity in cortisol-treated animals was expressed as a percentage of that in control animals injected with tracer alone.

Monder, 1984). Injection of cortisol hemisuccinate together with the tracer induced a significant increase in the level of plasma radioactivity, which probably reflects the liberation of the tritiated steroid from its binding sites by an excess of non-radioactive steroid. This release may arise not only from the specific receptors in well-perfused organs (for example, kidney, liver and spleen), but may also come from non-specific adsorption sites. The elimination of the tracer was, however, affected differently by the various concentrations of cortisol used, with a rapid return of plasma radioactivity levels to control values at 1 and 5 mg cortisol/animal, and a sustained plasma radioactivity level at 10 mg cortisol/animal. This effect may be linked to impaired excretion of the tritiated steroid and its metabolites by the kidney after administration of high doses of steroids.

With regards to the distribution of the radiolabelled tracers in thymus and spleen after simultaneous injection of non-radioactive cortisol, it appears that this excess of exogenous hormone promotes accumulation of the tracer by the thymus and spleen. This phenomenon may be due, in part, to the increased plasma concentration of the free radioactive tracer. The competing non-radioactive steroid may also displace the radioactive molecule from plasma proteins (transcortin as well as albumin) as indicated by the experiments using radioactive testosterone and oestriadiol, thus increasing the proportion of unbound tracer able to diffuse across the cell membrane. The results obtained in the presence of \([^3\text{H}]\text{dexamethasone, a synthetic glucocorticoid which does not bind to transcortin, suggest that other parameters, such as lipid solubility or metabolism of the molecules, may also affect tissue accumulation. Moreover, the organ/plasma ratio was higher and more sustained in the thymus than in the spleen, thus suggesting that the thymus is able to retain steroids above plasma levels. This effect of cortisol seems to

FIGURE 3. Tissue radioactivity in mouse thymus 30 min after injection of various tracers either alone (open bars) or in conjunction with 10 mg cortisol/animal (stippled bars). Each value represents the mean ± S.D. of three experiments with three separate animals/group in each experiment. B. cortisol; F, corticosterone; DEX, dexamethasone; TEST, testosterone, OE2, oestradiol.
correspond to a real concentration of the tracer in the thymus, since no significant change occurred in the thymic extracellular fluid, as determined by using \(^{3}H\)Jinulin, in contrast with the spleen. Several observations indicate that cells of the reticulo-endothelial system may be involved in this phenomenon in the thymus. Indeed, steroids injected at high doses accumulate in macrophages and reticular cells (Miyata & Takaya, 1983), and macrophages rapidly infiltrate the thymus following glucocorticoid administration (Lundin & Schelin, 1966). Moreover, in the thymic cortex, macromolecules incapable of freely crossing the capillary endothelium are nonetheless promptly sequestered by macrophages along the vessels (Dougherty, 1952; Raviola & Karnovsky, 1972). In addition, fat cells may also represent an important steroid reservoir (Deslypere, Verdonck & Vermeulen, 1985). Moreover, a similar effect of unlabelled steroids on the tissue accumulation of radioactive tracers above plasma levels has previously been observed in mouse brain and human uterus (Coutard, Osborne-Pellegrin & Funder, 1978; Laufer, Gambone, Chaudhuri et al. 1983).

In the spleen, cortisol also produced a retention of radioactivity above the plasma level, but this effect was only transient. There may be a possible relationship between the different capacities of the thymus and spleen to concentrate glucocorticoids administered at high doses and their differential sensitivities to steroid-induced atrophy.

In the kidneys the action of cortisol on tissue accumulation of radioactivity appears complex. During the first 45 min after cortisol injection the total tissue radioactivity was lower than that measured after injection of the tracer alone, suggesting that the unlabelled steroid effectively displaced the tracer from either its specific or adsorption binding sites, while during the rest of the experiment the tissue radioactivity of the kidneys remained higher than that observed in animals injected with tracer alone. This phenomenon appears to due primarily to impaired steroid elimination rather than to tissue accumulation, since the organ/plasma ratio determined for the kidneys remained below one. This decreased elimination, which was also observed when considering the effect of 10 mg cortisol on the plasma radioactivity level (Fig. 2), may be related to an effect of high doses of steroid on the vascular permeability and blood flow within the kidney, as demonstrated by the sustained increase of the renal extracellular space.

Although the various mechanisms responsible for the alterations of steroid distribution and bioavailability after administration of high doses of glucocorticoids are not fully understood, it appears that such treatment may induce the accumulation of the drug above plasma levels in some organs, particularly in lymphoid tissue, and that a similar phenomenon may be of importance in understanding the mechanism of "bolus" corticotherapy.

**REFERENCES**


