INVERSE EFFECTS OF CORTICOSTERONE AND THYROXINE ON GLUCOCORTICOID BINDING SITES IN THE ANTERIOR PITUITARY GLAND

By
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ABSTRACT

The aim of this study was to determine whether pituitary glucocorticoid binding sites are under hormonal control. It was shown that corticosterone and thyroxine exerted antagonistic effects on both the transcortin-like component and true receptor present in the hypophysis: thyroid hormones, in contrast to glucocorticoids which exhibited opposite influence, increased maximum binding without affecting significantly the apparent association constant. Thus, it seems that the concentration of glucocorticoid binding sites is regulated by the glucocorticoid ligands, as well as by a different hormone. Moreover, a striking parallelism was found between plasma transcortin and pituitary transcortin-like capacity, arguing in favour of a plasma origin for this pituitary binder.

The concentration of steroid binding sites in target cells has been reported to be under hormonal control. Thus, some receptors may be regulated by their proper ligand (Milgrom et al. 1973; Cidlowsky & Muldoon 1976) or, in contrast, by different steroids (Milgrom et al. 1973; Leavitt et al. 1974; Roy et al. 1975). As far as interaction of glucocorticoids with pituitary binders is concerned, it has been shown that adrenalectomy increases the concentration of binding sites (Olpe & Mc Ewen 1976) and that the extent of corticosterone binding to the pituitary transcortin-like component is sex-dependent (Koch et al. 1976a).

In this study, we further explore the possibility of a hormone-mediated control of the binding characteristics of both the transcortin-like (TL) compound and the “true” glucocorticoid receptor (compound D). The results indicate that corticosterone and thyroxine exert opposite effects on the concentration of sites of these binding proteins.
MATERIAL AND METHODS

Animals

Male Wistar rats, weighing 250–300 g, were used throughout. The animals were either adrenalectomized or thyroidectomized at various times prior to sacrifice. When specified, 3-week-adrenalectomized animals were given physiological doses of corticosterone\(^1\) in drinking water for the last 14 days (10 mg per 100 ml NaCl 0.15 w-ethanol 5%, v/v). Also, some groups of rats were treated for 10 days with subcutaneous injections of ACTH (Cortrophine-Z\(^\circ\), obtained from Organon) or L-thyroxine sodium at doses of 2 or 5 m\(\mu\)U and 10 \(\mu\)g/100 g body weight per day, respectively; controls were injected with the vehicle. Tissues were blanched by perfusion of 40 ml ice-cold saline through the heart of animals anaesthesized with nembutal. Using this procedure it was shown, by determining the relative distribution of labelled inulin and corticosterone between plasma and pituitary cytosol, that the presence of the TL binder could not be accounted for by mere blood contamination (Koch et al. 1976b).

In order to permit determination of total amount of binding sites (unoccupied + occupied), all the rats were adrenalectomized and hormone replacement therapy was suspended 24 h prior to sacrifice. It has been reported, indeed, that corticosterone disappears from blood within 4 h (Fortier 1959) and that pituitary glucocorticoid receptors are rapidly desaturated (Koch et al. 1975). Thus, in all experiments 24 h-adrenalectomized rats were referred to as controls.

Binding studies

The adenohypophyses were homogenized in TED buffer (10 mM Tris-HCl; 1 mM EDTA; 1 mM dithiothreitol) at pH 7.3 and further spun at 105 000 \(g\) to obtain a cytosol fraction. The cytosol, either untreated or heat-inactivated at 40\(^\circ\)C for 10 min, was incubated at 0\(^\circ\)C for 2 h with \([1,2,6,7\text{-}(n)\text{-3H}]\)corticosterone (112 Ci/mmol) or \([1\text{-}(2)\text{-3H}]\)dexamethasone (20 Ci/mmol), purchased from Amersham. Incubations were carried out using either increasing concentrations of labelled steroids up to 100 \(nM\) or constant amounts of 20 \(nM\). Separation of unbound and bound steroid was performed by filtering the incubates through microcolumns (0.6 x 13 cm) of Sephadex-G25 in TED medium at 4\(^\circ\)C. Specific binding, defined as the difference in binding detected in the presence or absence of a 1000-fold excess of unlabelled steroids, was plotted according to Scatchard (1949). Protein measurements were made as described by Lowry et al. (1951). All experiments were carried out at least two times and yielded qualitatively similar results.

RESULTS

Effect of adrenalectomy and steroid replacement therapy on pituitary glucocorticoid binders and plasma transcortin

Taking advantage of differences in their physicochemical properties, independent determination of compound D and the TL entity could be carried out.

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\(^1\) Abbreviations: corticosterone, 11\(\beta\),21-dihydroxy-4-pregnene-3,20-dione; dexamethasone, 9a-fluoro-16a-methyl-11\(\beta\),17,21-trihydroxy-1,4-pregnadiene-3,20-dione; TL compound, transcortin-like compound.
Indeed, in agreement with studies on thermal stability of glucocorticoid binders in liver cytosol (Koblinsky et al. 1972), we have shown that the former combines both corticosterone and dexamethasone and is inactivated at 40°C, whereas the latter is heat-resistant and bound only the natural steroid (Koch et al. 1976b). This study reported that (1) heat-treatment caused a complete loss of [³H]dexamethasone binding to pituitary cytosol, as revealed by sucrose density centrifugation, with no appearance of a radioactive peak in the transcortin region; (2) dexamethasone did not compete for transcortin binding sites (confirming earlier findings by Rousseau et al. 1972); (3) inactivation of receptor D by either heat-treatment or masking of sites with excess radio-inert dexamethasone resulted in almost the same degree of [³H]corticosterone binding. Moreover, using an antibody raised against rat transcortin, we established that the TL compound exhibited immunological identity with plasma transcortin (Koch et al. 1977). As shown in Fig. 1, adrenalectomy caused a rapid dramatic elevation of pituitary TL binding sites, detectable by 36 h and plateau, about 350% over control

![Fig. 1.](image_url)

Time course of the effect of adrenalectomy (Adrx) on maximum glucocorticoid binding to pituitary compound D and TL component, as well as to plasma transcortin. Each point is the mean from 4 determinations on pooled cytosol fractions. In this, as well as in all other experiments, controls refer to 24 h-adrenalectomized rats (see Material and Methods). The inset shows that binding was linear with respect to concentration of cytosol protein (stippled area indicates working range). DEX: dexamethasone; CORT: corticosterone.
Scatchard plots of glucocorticoid binding to the TL component and compound D of hypophyses from control, 3 weeks-adrenalectomized (Adrx) and adrenalectomized rats treated with corticosterone (Adrx + Cort). The slope of the straight line yields the association constant and intercept with the abscissa maximum binding. In Figs. 2 and 3 apparent positive cooperative binding seems to be detected at low steroid concentrations.

level, by 6 days. Strikingly, these values closely paralleled the plasma transcortin increment. Compound D, on the other hand, increased less rapidly and reached a plateau value about 150% over the control. The mean concentration of these sites from 3 independent experiments yielded 166 ± 7 and 225 ± 14 pm/mg cytosol protein, for pituitaries from control and adrenalectomized rats, respectively (P < 0.02). Scatchard plots of binding data (Fig. 2) further confirmed these observations and revealed that adrenalectomy actually increased the concentration of sites, without altering significantly the apparent dissociation constants, as derived from the slopes of the straight lines. In addition, it emerges from Fig. 2 that administration of physiological doses of corticosterone to adrenalectomized rats completely abolished the increment in number of binding sites of both the TL binder and compound D.

On the other hand, it had to be ascertained whether those high levels of
circulating corticotrophin which are triggered by adrenalectomy could not interfere with the regulation of pituitary glucocorticoid binding sites. In order to test this hypothesis and obviate enhanced secretion of adrenocortical steroids that would result from ACTH treatment and thus mask any direct effect, corticotrophin was injected into adrenalectomized animals given physiological doses of corticosterone, capable of restoring normal plasma resting levels. Results in Table 1 indicate that ACTH therapy does not significantly affect either [3H]dexamethasone or [3H]corticosterone binding, thus excluding a possible influence of this hormone.

**Influence of thyroxine treatment and thyroidectomy on concentration of pituitary glucocorticoid binding sites**

Since it has been reported that thyroxine administration elevates, while thyroidectomy decreases plasma transcortin levels (Labrie 1967), it seemed of interest to assess the influence of the thyroid function on the pituitary TL component, as well as on compound D. As shown in Fig. 3, we found that thyroxine treatment not only increases the number of binding sites of the TL entity, but interestingly enough also that of compound D. Moreover, when thyroxine therapy was combined with adrenalectomy, maximum binding of both components was enhanced. Finally, it appeared that although thyroidectomy resulted in reduction of the amount of pituitary TL binder, it seemed to be without any detectable effect on compound D.

**Table 1.**

Effect of corticosterone and ACTH treatment on binding of radioactive dexamethasone (Dex) and corticosterone (Cort) to pituitary cytosol from adrenalectomized (Adrx) and control rats. When incubated with the latter steroid the cytosol was heated at 40°C for 10 min. Doses and administration schedule of hormones are given under Material and Methods. ACTH₁ = 2 mU; ACTH₂ = 5 mU.

Results are means ± sem.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of animals</th>
<th>Maximum binding fmol/mg protein</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dex</td>
</tr>
<tr>
<td>Vehicle</td>
<td>9</td>
<td>135 ± 6</td>
</tr>
<tr>
<td>Adrx + Cort</td>
<td>9</td>
<td>116 ± 6</td>
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<tr>
<td>Adrx + Cort + ACTH₁</td>
<td>9</td>
<td>110 ± 10</td>
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<tr>
<td>Adrx + Cort + ACTH₂</td>
<td>9</td>
<td>141 ± 11</td>
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Fig. 3.
Scatchard plots of glucocorticoid binding to pituitary cytosol from control, 3 weeks-thyroidectomized (Thyrx), thyroxine treated (T₄) and adrenalectomized rats injected with T₄ (Adrx + T₄). Panel A: binding to the TL component and panel B to compound D.

Fig. 4.
Effect of various experimental treatments on binding capacity of plasma transcortin and pituitary TL component. TX, thyroidectomy; C, control; AX, adrenalectomy; T₄, thyroxine treatment. Data in the left-hand panel are means from 5 determinations (SEM was less than ± 10 %) and those in the right-hand panel are derived from Scatchard plots of Figs. 2 and 3.
The fact that a close relationship seemed to exist between the pituitary TL binder and plasma transcortin level clearly emerges from Fig. 4, which summarizes data obtained under various experimental conditions. In all cases, plasma transcortin binding capacity strikingly paralleled pituitary TL binding concentration. In addition, it was observed that the increment in pituitary binding sites (400 to 700%/o over control) always exceeded that exhibited by plasma (200 to 300%/o above control); thus suggesting some kind of a storage process within the gland.

**DISCUSSION**

The experiments reported here indicate that glucocorticoids and thyroxine exert antagonistic effects on the concentration of pituitary binding sites for both labelled corticosterone and dexamethasone, without affecting the apparent affinity of the binders for these steroids. It appeared, indeed, that adrenalectomy caused an increase in binding capacity of the TL compound as well as component D. This agrees well with reports by Beato et al. (1974) and Olpe & Mc Ewen (1976), describing similar findings with such tissues as the liver, pituitary and various brain structures. In addition, we showed that corticosterone replacement therapy, but not ACTH treatment, was capable of completely reversing the effect of adrenalectomy. This, however, does not seem to be due to occupancy of pituitary binding sites by administered corticosterone, as might be concluded from previous experiments (Koch et al. 1975). It was shown, indeed, that after administration of doses of corticosterone within the physiological level, the steroid was cleared from blood and pituitary binding sites were desaturated within a few hours.

It is worthy of note that thyroid hormones not only elevate maximum binding of the TL component as could be expected from previous findings (Koch et al. 1976b), but also that of compound D. However, whereas thyroidectomy resulted in lowering of TL sites, it seemed to be without detectable influence on binding sites of receptor D. This property of thyroid hormones to modulate concentration of binding sites is not restricted to glucocorticoid binders, since oestrogen receptor content of hypophyses from ovariectomized rats was likewise reported to be altered under these conditions (Cidlowsky & Muldoon 1975). In addition, high affinity binding sites for thyroid hormones have been demonstrated in the adenohypophysis (Schadlow et al. 1972; Sufi et al. 1973).

The observation that corticosterone and thyroxine modulated the concentration of pituitary binders could be explained by: 1) a direct effect on synthesis as suggested by studies dealing with oestadiol binding to the uterus (Cidlowsky & Muldoon 1976); 2) an activation of pre-existing masked sites;

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3) an effect on receptor breakdown and/or translocation into the nucleus, as demonstrated in studies on glucocorticoid binding to the liver (Beato et al. 1974). Further studies will be necessary to ascertain the biochemical mechanisms underlying these observations.

With respect to the TL binding material, the possibility of transfer from the blood also deserves consideration. This hypothesis seems to be supported by our demonstration of a parallel occurrence of pituitary TL and plasma transcortin concentration. It should be emphasized that the presence of the TL binding molecule within the pituitary was shown not to be accounted for by mere blood contamination, as concluded from experiments conducted with isolated pituitary cells (Koch et al. 1977) and determination of haemoglobin content of cytosol (De Kloet & Mc Ewen 1976) or labelled inulin distribution (Koch et al. 1976b). However, in order to more precisely ascertain the origin of pituitary TL material, biosynthetic studies with radioactive precursors would be required.

The presence of this TL component within the adenohypophysis, as well as in tissues such as the liver (Beato & Feigelson 1972), the uterus (Milgrom & Baulieu 1970; Rosenthal et al. 1974) and the kidney (Feldman et al. 1973), raises several questions. The first one deals with its physiological role. In this context, it has already been shown that the binder does not seem to be a true receptor, being unable to bind to DNA-cellulose (De Kloet & Mc Ewen 1976) or to translocate into the nucleus (Koch et al. 1976b). However, earlier findings indicate that it seems to compete with receptor D for corticosterone binding; thus modulating the extent of receptor-steroid complex transfer to the nucleus (Koch et al. 1976a).

A second question refers to the intra- or extracellular localization of the binding protein. The report of Werthamer et al. (1973), showing the existence of TL material within lymphocytes, lend support to the former hypothesis. Without precluding this possibility, recent observations argue in favour of an extracellular location in providing indirect evidence, based on steroid-specificity of binding, of firm attachment of the TL binder onto the plasma membrane of pituitary cells (Koch et al. 1977). This view seems to be strengthened by the detection of corticosterone and oestriadiol binding sites in plasma membranes from liver (Suyemitsu & Terayama 1975) and on the external surface of endometrial cells (Pietras & Szego 1977), respectively. The question may be raised of whether this binding did not occur indirectly through sticking of either plasma transcortin or sex-binding-protein to the cellular membranes. Moreover, recent studies by Harrison et al. (1977) support the view that glucocorticoid interaction with the plasma membrane of pituitary tumour cells may be an important step in the mechanism of steroid action.
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REFERENCES


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