Selective alterations in hepatic nuclear T₃-receptors and enzyme responses by glucocorticoid deficit or excess

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Abstract. Glucocorticoid deficit in rats induced by adrenalectomy for a 10-day period resulted in an increase in the apparent association constant (Kₐ) of the liver T₃-receptor while maximal binding capacity (MBC) remained unaltered compared to the intact (sham-operated) animals and to adrenalectomy plus dexamethasone (Sx + D) animals. In addition, a significant increase in the basal activity of liver mitochondrial α-glycerophosphate dehydrogenase (α-GPD) was observed, while cytosolic malic enzyme (ME) activity was not modified in this situation. Dexamethasone injection (5 mg/kg/day for 4–5 days) to previously adrenalectomized animals (Sx + D) induced a significant increase in MBC of nuclear T₃-receptors. On the other hand, Kₐ value and α-GPD activity were restored to the values found in the control group. However, basal activity of ME as well as the response of this enzyme to a saturating dose of T₃ was substantially increased by dexamethasone treatment.

A non-specific in vitro effect of dexamethasone on MBC and ME was excluded as these parameters were not modified when dexamethasone was added immediately before the in vitro assays. The present study indicates that glucocorticoid deficit or excess is able to induce changes at the level of the nuclear T₃-receptor site. The increase in the activity of cytosolic ME induced by dexamethasone injection was associated with a simultaneous increase in the T₃-receptor capacity. Additionally, the increment in mitochondrial α-GPD activity induced by adrenalectomy was associated to an increase in the affinity constant but not in the number of nuclear T₃-binding sites.

A substantial body of evidence has accumulated to indicate that specific nuclear binding sites for triiodothyronine (T₃) are important in the initiation of thyroid hormone action at the cellular level (Oppenheimer & Dillmann 1978). In the intact rat, several studies have shown that the hepatic receptor concentration can change under certain pathophysiological circumstances. Starvation (DeGroot et al. 1977; Bavli 1980), partial hepatectomy (Dillmann et al. 1978), experimentally produced diabetes and glucagon administration (Dillmann & Oppenheimer 1979; Kaiser et al. 1980), all result in a substantial reduction in T₃-receptor number.

Samuels et al. (1978) have shown that in GH₁ tissue culture, T₃ effects a prompt reduction in the number of its receptor sites with a simultaneous decrease in the rate of growth hormone synthesis, although in intact animals, T₃-receptor number in liver does not appear to change with varying thyroidal status (Bernal et al. 1978b; Oppenheimer et al. 1976). Thus, the possibility exists that regulation of pituitary nuclear receptors may differ from that of receptors in the liver. Another characteristic of T₃ action is the multihormonal interaction in the final expression of a specific metabolic effect. Thus, the hormonal requirement for the produc-
tion of α-2μ globulin includes not only T3 but cortisol, dihydrotestosterone and growth hormone as well (Roy 1973). In GH1 and GC pituitary cell culture, a synergistic interaction of glucocorticoids and T3 has been demonstrated in the synthesis of growth hormone (Samuels et al. 1977; Martial et al. 1977).

In view of the importance of the interaction of T3 with other hormonal systems in the expression of certain metabolic effects, we attempted to characterize hepatic nuclear T3-receptors under the influence of glucocorticoid hormones. The effect of glucocorticoid deficit or excess on receptor number and affinity provided an opportunity to assess the physiological consequences of these modifications on the activity of hepatic T3-responsive enzymes.

Materials and Methods

Experiments were conducted on 3 months old male Wistar rats. The animals were adrenalectomized surgically (Sx group). Half of them were injected with dexamethasone for a 4–5 days period (Sx + D group). Normal sham-operated animals were included in the studies. After adrenalectomy, the animals were maintained on 1.0% NaCl in the drinking water.

At the end of each experiment, the rats were fasted for 12 h and then killed by cervical dislocation. Serum was retained for hormonal determinations and the liver removed. A 3 g aliquot was minced and rinsed free of blood and homogenized in 0.32 M sucrose and 1 mM MgCl2 and centrifuged at 700 × g for 10 min at 4°C to obtain a crude nuclear pellet. The supernatant was centrifuged at 8500 × g for 10 min to obtain the mitochondrial fraction. The supernatant from 8500 × g was used to obtain the cytosol fraction, by centrifugation at 105,000 × g for 60 min. The crude nuclear pellet was purified by ultracentrifugation through 2.2 M sucrose, 1 mM MgCl2 and 2 mM dithiothreitol as previously described (Bernal et al. 1978b).

Maximal binding capacity (MBC) and apparent affininty constant (Ka) of T3-nuclear receptors were determined in isolated nuclei by the procedure previously described Bernal et al. 1978a).

Hepatic α-glycerophosphate dehydrogenase activity (α-GPD) was measured according to the method of Lee & Lardy (1965). Malic enzyme activity (ME) was determined in liver cytosol by the method of Hsu & Lardy (1969). The plasma concentrations of T3 and thyroxine (T4) were measured by RIA (Diagnostic Products Corporation) and corticosterone levels by the method of Rerup & Hedner (1961).

Protein was determined by the method of Lowry et al. (1951) and DNA by the diphenylamine method of Burton (1956). Statistical significance of means was established by Student’s t-test.

Results

Adrenalectomy for a 7–10 day period significantly reduced the plasma corticosterone levels (Sx 3.9 ± 1.9 μg/100 ml, control 43.2 ± 19.9 μg/100 ml; P < 0.01).

The effect of adrenalectomy and dexamethasone injection to adrenalectomized animals on hepatic nuclear T3 concentration is indicated in Table 1. MBC was not modified in the Sx group when compared to the control (sham-operated) animals. Glucocorticoid excess induced by the administration of 5 mg dexamethasone/kg body weight for a 4–5 day period to adrenalectomized rats caused a marked increase in the T3-receptor concentration (P < 0.01).

As shown in Table 1, Ka was significantly increased by adrenalectomy while dexamethasone administration to previously adrenalectomized animals restored this value to that found in the control group.

A representative Scatchard plot for nuclear binding measurement is shown in Fig. 1. As shown in Table 1, MBC was significantly higher in the

<table>
<thead>
<tr>
<th>Group</th>
<th>MBC fmoles T3/100 μg DNA</th>
<th>Ka (× 10² M⁻¹)</th>
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</thead>
<tbody>
<tr>
<td>Sx</td>
<td>64.1 ± 12.9 (9)</td>
<td>6.03 ± 0.81 (3)*</td>
</tr>
<tr>
<td>Sx + D</td>
<td>98.7 ± 23.6 (9)</td>
<td>2.97 ± 0.40 (3)*</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.02</td>
</tr>
<tr>
<td>Control</td>
<td>52.3 ± 5.0 (6)</td>
<td>3.00 ± 0.95 (3)*</td>
</tr>
</tbody>
</table>

Values are mean ± sd. Number of animals in parentheses. * Pool of 3 animals per group. P value between Sx and Sx + D. Dexamethasone was injected ip (5 mg dexamethasone phosphate/kg body weight during 4–5 days).

Table 1.

Effect of adrenalectomy (Sx) (10 days duration) and dexamethasone injection to Sx animals (Sx + D) on maximal binding capacity (MBC) and equilibrium association constant (Ka) of liver nuclear T3-receptors.
Sx + D group. On the other hand, the mean apparent association constant (Ka) was substantially increased in Sx animals.

Liver enzyme activity obtained in control, Sx and Sx + D, is indicated in Table 2. A significant increase in the basal activity of α-GPD was observed in the Sx group while basal ME activity was not modified when compared to the value found in the control group. On the other hand, α-GPD activity in the dexamethasone-injected adrenalectomized group. However, the basal ME activity was substantially increased after glucocorticoid administration. The capacity of ME to respond to an ip dose of T₃ (200 µg/100 g body weight) designed to fully saturate the nuclear sites for a period of 24 h (Kaiser et al. 1980) was compared in the control, Sx and Sx + D groups. The increment in enzyme activity was assessed from the difference between the enzyme activity achieved 24 h after the injection of T₃ and the level in uninjected animals. The increment in ME activity after T₃ was 0.016 ± 0.008 (ΔA min/mg protein) in the control group, 0.014 ± 0.0007 in the Sx group and 0.039 ± 0.018 in the Sx + D group (P < 0.01, compared to Sx or control group). These results indicate that both basal and T₃-stimulated activity of ME was significantly increased after dexamethasone administration.

In order to ascertain whether the observed rise in T₃-receptor number and ME activity elicited by dexamethasone injection was not simply caused by a non-specific action of the synthetic glucocorticoid during the in vitro determinations, different doses of dexamethasone (0.1, 0.25, 2.5, 25 µg/ml) were added immediately before the in vitro assay and determinations in the presence of the drug were conducted in the usual way. No difference in MBC and in the activity of ME was observed in the presence or in the absence of dexamethasone (data not shown).

Table 3 summarizes the effect of adrenalectomy and dexamethasone injection to adrenalectomized animals on T₄ and T₃ blood levels. T₄ showed no changes under the different experimental conditions. T₃ level was reduced in the adrenalectomized group compared to the control as well as to the dexamethasone treated group.

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**Table 2.**

Effect of adrenalectomy (Sx) and dexamethasone injection to adrenalectomized animals (Sx + D) on the basal activity of liver mitochondrial α-glycerophosphate dehydrogenase (α-GPD) and cytosolic malic enzyme (ME).

<table>
<thead>
<tr>
<th>Group</th>
<th>α-GPD</th>
<th>ME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sx</td>
<td>0.106 ± 0.018 (10)</td>
<td>0.016 ± 0.002 (8)</td>
</tr>
<tr>
<td>Sx + D</td>
<td>0.076 ± 0.016 (10)</td>
<td>0.072 ± 0.021 (8)</td>
</tr>
<tr>
<td>Control</td>
<td>0.077 ± 0.021 (10)</td>
<td>0.018 ± 0.006 (8)</td>
</tr>
</tbody>
</table>

Values are mean ± sd. Number of animals in parentheses. Dexamethasone was injected ip (5 mg/kg body weight during 4–5 days). P value between Sx and Sx + D.

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**Fig. 1.**

Representative Scatchard plots for the binding of T₃ by nuclei isolated from adrenalectomized (Sx) and dexamethasone-injected adrenalectomized (Sx + D) rats. Incubation contained 100–200 µg DNA. The curves were drawn by least squares computation. MBC and Ka values are given in Table 1. The ordinate represents the ratio of the amount of T₃ bound to the concentration of T₃ free in the assay tubes. The error bars indicate the standard deviations of the data determined in 3 pools of 3 animals per group.
Thyroxine (T₄) and triiodothyronine (T₃) blood levels in adrenalectomized (Sx) and dexamethasone-injected adrenalectomized rats (Sx + D).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>T₄ (nmol/l)</th>
<th>T₃ (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>56.1 ± 14.6</td>
<td>2.15 ± 0.27&lt;sup&gt;1,2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sx</td>
<td>6</td>
<td>44.4 ± 8.2</td>
<td>1.57 ± 0.24&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sx + D</td>
<td>5</td>
<td>51.5 ± 20</td>
<td>1.94 ± 0.20</td>
</tr>
</tbody>
</table>

Values are mean ± sd. Dexamethasone was injected ip (5 mg/kg body weight/4–5 days).

n refers to the number of samples assayed.

1 P value between control and Sx < 0.01.
2 P value between control and Sx + D not significant.
3 P value between Sx and Sx + D < 0.05.

Discussion

The present study indicates that glucocorticoid deficit and excess were able to induce modifications at the level of the nuclear T₃-binding sites; thus Ka increased after adrenalectomy and T₃-binding capacity after dexamethasone treatment.

An increase in the basal level of ME was associated with the increment in T₃-receptor number. In this situation α-GPD activity was found to be normal. In addition an increment in the basal level of α-GPD was associated with a simultaneous increase in Ka value, an effect seen in glucocorticoid deficiency. In this situation, T₃-binding capacity was normal.

It is of interest that diverse factors such as partial hepatectomy, starvation, experimentally induced diabetes, glucagon administration etc., are accompanied by a diminution in nuclear T₃-receptor sites and in the basal and T₃-stimulated activity of ME. In these circumstances the Ka of T₃-receptors and α-GPD are not impaired. A dissociation between receptor number and the metabolic response of T₃ measured by liver α-PGD is evident. Under these circumstances, a marked decrease in circulating T₃ concentration which could account for the diminished basal ME activity was observed. However, the response of this enzyme to injection of T₃ in doses known to saturate the nuclear sites, remained impaired (Dillmann et al. 1978; Dillmann & Oppenheimer 1979).

The changes observed in the activities of α-GPD and ME induced by adrenalectomy and dexamethasone injection, do not appear to be related to parallel changes in serum T₄ or T₃ levels (Table 3), although modifications of these hormones at the nuclear level are not excluded. A higher endogenous saturation of T₃-receptors could explain the increased basal activity of ME observed after dexamethasone treatment. However, when the receptors were fully saturated after injection of T₃, the maximal response of ME was higher than in the control or Sx animals. Increased basal ME activity could be explained in terms of the increased binding capacity of T₃ induced by dexamethasone administration.

In agreement with previous reports (Zellinger et al. 1966), our results indicate that adrenalectomy is accompanied by an increase in the activity of α-GPD, although the mechanism of this activation is not known. An increment in the affinity of nuclear T₃-receptors seen in this situation could be a plausible explanation.

The relative contribution of the number of receptors and affinity on the metabolic effect of the hormone is poorly understood. Our experimental procedure provided a way to induce a particular modification at the nuclear receptor and simultaneously to study the impact of such a modification upon hormone action at the tissue level.

Although the present result seems to indicate that the induction of a metabolic thyroid hormone response could be under the control of a particular characteristic of its nuclear receptor, such an assertion deserves further consideration. A particular substance may act primarily by modifying a specific characteristic of the receptor and simultaneously may exert an additional effect distal to the hormone-receptor interaction, which may alter the hormonal effect expected according to the primary modification on the receptor.

References


Bernal J, Coleoni A H & DeGroot L J (1978b): Thyroid hormone receptors from liver nuclei: characteristics of receptors from normal, thyroidectomized and T₃-treated rats; measurement of occupied and unoccupied receptors, and chromatin binding of receptors. Endocrinology 103: 403–413.


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