EFFECTS OF THYROIDECTOMY ON GLUCOCORTICOID RECEPTORS IN RAT LIVER

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1. Introduction

Glucocorticoid and thyroid hormones regulate physiological and developmental processes in a number of tissues. Both hormones have been shown to regulate RNA and protein synthesis. They induce the synthesis of α_{2u} globin in liver parenchymental cells [1,2] and of growth hormone in pituitary cells [3–6]. In both cases, the presence of thyroid hormone is required for the effect of glucocorticoids.

The specific effects of steroid hormones have been shown to result from the cytosol–nuclear translocation of a hormone–receptor complex. Moreover, it has been shown [7] that the concentration of estrogen receptor in uterus cytosol is modulated by thyroid hormone. This led us to study glucocorticoid receptors in rat liver in relation with the thyroid status of the animals.

Here we describe a decrease in the concentration of glucocorticoid receptors and a modification of the receptor properties after thyroidectomy.

2. Material and methods

2.1. *Chemicals*

[1,2-^{3}H]Dexamethasone was obtained either from CEA Saclay (19 Ci/mM) or from Amersham Radiochemical Center (25 Ci/mM). Unlabeled dexamethasone was obtained from Roussel-Uclaf.

2.2. *Animals*

Normal male Sprague-Dawley rats (Charles River) were used. Thyroid gland was destroyed by an intraperitoneal injection of ^{131}I (800 μCi/200 g body wt). TSH (2 IU/animal) was injected 10 h prior to the ^{131}I treatment. Animals were controled by measuring the decrease in heart rate. Two populations of animals were studied: animals of group A with 30–50% decrease in heart rate; animals of group B with 120–150% decrease in heart rate. Untreated animals of the same age were used as control.

2.3. *Preparation of cytosol*

Animals were killed by decapitation, the livers were perfused with ice cold 0.9% NaCl. All subsequent operations were performed at 4°C. Livers were homogenized in buffer I: 20 mM Tris–HCl (pH 7.4), 25 mM KCl, 2.5 mM $MgCl_2$, 1 mM β-mercaptoethanol. Cytosol was prepared as in [8]. Protein concentration was estimated by the Lowry method [9].

2.4. *Steroid binding assays*

Incubations were performed in buffer I in 0.5 ml final vol. at 0°C for 2 h or at 20°C for 20 min. Then the complexes were submitted to filtration on Sephadex G-25 columns (17 × 0.9 cm). The radioactivity of the eluates was measured. In some experiments the dextran–charcoal method [10] was used to remove unbound hormone. Correction for non-specific binding was obtained by substracting the binding obtained in the presence of a 10^3-fold excess of unlabeled hormone.

2.5. *Gel filtration on Sepharose 6B column*

Sepharose 6B chromatography was performed as in [11], except that the Sepharose was equilibrated with buffer I.

A_{280} was automatically recorded with an elugraph (Seive system).

3. Results and discussion

3.1. *Specific binding of [^{3}H]dexamethasone to liver cytosol from normal and thyroidectomized animals*

After thyroidectomy, animals were ranged in two main groups: animals of group A have a low decrease in heart rate; animals of group B have a more marked bradycardia. Liver cytosol from normal and thyroidectomized rats was incubated at 0°C with increasing amounts of radioactive dexamethasone. Figure 1 shows the binding data expressed according to Scatchard [12].

In cytosol from group A animals at least 2 populations of dexamethasone binding sites were found: one with K_d 4 × 10^{-9} M identical to the K_d of the receptor from control animals; one with a lower affinity (K_d 2 × 10^{-8} M). In cytosol from group B animals, only one type of dexamethasone-binding site was found corresponding to the lower affinity sites. Moreover the average number of specific binding sites decreased from 20–25 × 10^3 sites/cell in the liver cytosol from control animals to 7–10 × 10^3 sites/cell in the liver cytosol from group B animals. This difference cannot be attributed to a change in the plasma level of glucocorticoid which is not significantly modified after thyroidectomy [14].

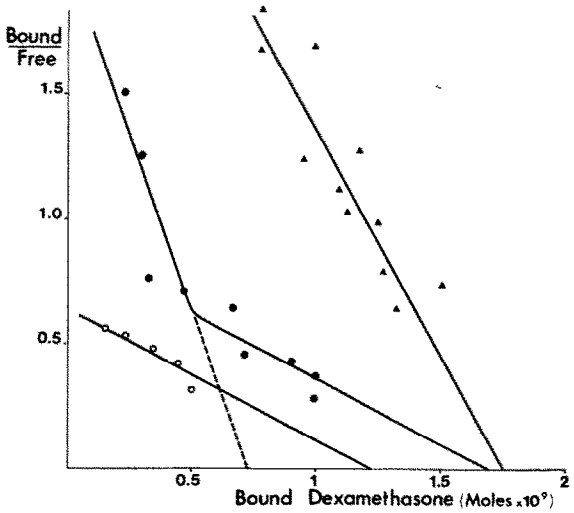

Fig.1. Scatchard plot of the dexamethasone-binding cytosol proteins. Cytosol proteins (3–5 mg/ml) were incubated for 120 min at 0°C with various concentrations of [^{3}H]dexamethasone in the presence and in the absence of an excess of unlabeled hormone. Bound radioactivity was separated by gel filtration. Only the specific binding is shown. Cytosol from control animals (▲); cytosol from animals of group A (●); cytosol from animals of group B (○).

At 20°C the same heterogeneity in the binding sites of thyroidectomized animals was observed (fig.2) with a slight increase of the K_d value.

Heterogeneity in glucocorticoid receptors has been described in normal liver cytosol and in hepatoma [10,11,13]. Since the binding proteins can be identified by Sepharose 6B chromatography [11], this method was used to analyse the glucocorticoid receptors present in the liver cytosol from thyroidectomized rats.

3.2. *Sepharose 6B filtration of the dexamethasone binding proteins*

Gel filtration on Sepharose 6B of cytosol from normal liver incubated 2 h at 0°C with [^{3}H]dexamethasone shows three main peaks (fig.3a): one peak of excluded material which may correspond to aggregated forms of the receptor in the absence of EDTA [15] and two peaks (1,2) eluted in the same volume as DI and DII described [11]; these peaks probably correspond respectively to binders II and I_b obtained [13] on DEAE–Sephadex.

After thyroidectomy the excluded peak disappeared almost completely (fig.3b,3c). Moreover peak 1 was strongly diminished in cytosol from

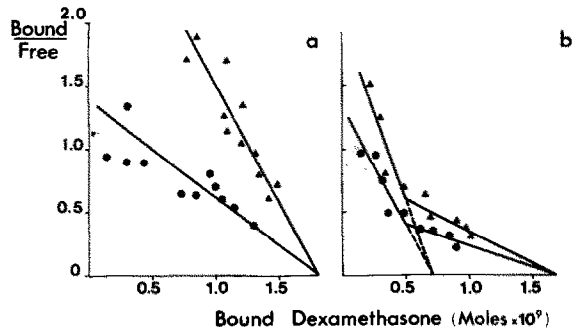

Fig.2. Comparison of the Scatchard plot of the dexamethasone-binding cytosol proteins at 0°C and 20°C. Experiments were performed at 0°C for 120 min (▲) and at 20°C for 20 min (●). (2a) Cytosol from control animals. (2b) Cytosol from animals of group A.

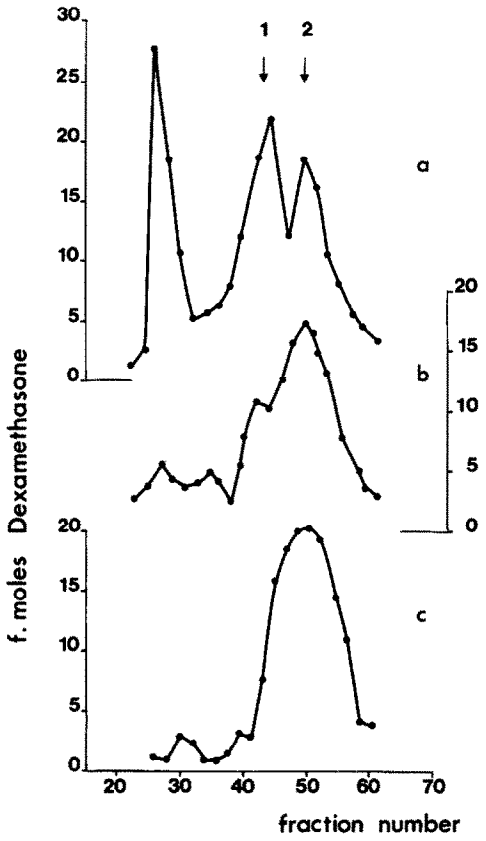

Fig.3. Sepharose 6B chromatography of dexamethasone-binding proteins. Incubation medium (1 ml) containing 10 mg cytosol proteins and 2×10^{-8} M [^{3}H]dexamethasone was submitted to gel filtration on a Sepharose 6B column pre-equilibrated with buffer I. In parallel experiments, 2×10^{-5} M unlabeled dexamethasone was added to the incubation medium. Fractions of 0.6 ml were collected and treated with dextran–charcoal according to [10]. Only the specific binding is shown. (3a) Cytosol from control animals. (3b) Cytosol from animals of group A. (3c) Cytosol from animals of group B.

group A animals (fig.3b) and was not found when cytosol from group B animals was chromatographed under the same conditions (fig.3c).

It might be assumed that the high affinity constant corresponds to the binding protein present in peak 1 and the lower affinity constant to the binding protein present in peak 2.

When cytosol receptor–hormone complexes from control animals were treated with 1 mM EDTA prior to gel filtration performed in the presence of EDTA, the excluded peak was found no longer; peak 1 represents ~80% of the hormone–receptor complexes; peak 2 represents 20% as in [11]. Under the same conditions peak 1 disappears completely in cytosol from hypothyroid type A animals (data not shown).

Our data show that after thyroidectomy, some of the properties of the glucocorticoid receptor are modified; this modification could be correlated either with the degree of aggregation of the receptor or with its ability to bind to other cytoplasmic components.

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