CORRELATION BETWEEN [^{3}H]DEXAMETHASONE BINDING TO A RAT LIVER CYTOSOL RECEPTOR PROTEIN AND STIMULATION OF RNA SYNTHESIS BY GLUCOCORTICOSTEROIDS

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1. Introduction

Glucocorticosteroids affect RNA synthesis in target organs. Well studied is the action of these hormones on rat liver (for review see [1]) the first effect being a stimulation of extranucleolar, α-amanitin sensitive, RNA synthesis [2]. Recent evidence [3] suggests that specific glucocorticosteroid binding proteins present in the cytosol are instrumental in transporting the hormones from the cytoplasm into the nucleus as well as in the stimulation of transcription. Three receptor proteins have been isolated [4–6] named A, B and G. Binder B is very similar in its characteristics to the serum corticosteroid binding globulin transcortin [4]. The receptors A and G bind glucocorticosteroids specifically and with high affinity. However, only receptor G is able to bind the synthetic glucocorticoid dexamethasone and can thus be differentiated from binder A [5]. On the basis of correlation of *in vivo* saturation of binder G to the induction of the hepatic enzymes tyrosine aminotransferase and tryptophane oxygenase [7] as well as on the basis of very recent direct findings of *in vitro* effects of receptor G preparations on transcription [8] strong indications have been obtained suggesting that binder G is the functionally significant receptor.

A further approach to the resolution of this basic problem is based on the inability of rats to respond to glucocorticosteroids with increased RNA synthesis before a certain critical period [9]. One possibility for this insensitivity to the steroid hormones could be the absence at the refractory period of the cytosol receptor protein G. We have therefore estimated the receptor G concentration during postnatal development of rats and have observed a striking correlation between binder G concentration and the ability of rats to respond to the glucocorticosteroids with increased RNA synthesis.

2. Materials and methods

Male Wistar BR II rats kept under standard conditions were used. [^{3}H]Dexamethasone (22 Ci/mmol) and [^{3}H]uridine-5-triphosphate (1 Ci/mmol) were purchased from the Radiochemical Centre, Amersham, England. Unlabelled dexamethasone was purchased from Sigma Chemical corporation, St. Louis, Mo., USA, the unlabelled nucleoside triphosphates from Boehringer, Mannheim, GFR. The rest of the chemicals were reagent grade and were obtained from Merck, Darmstadt, GFR.

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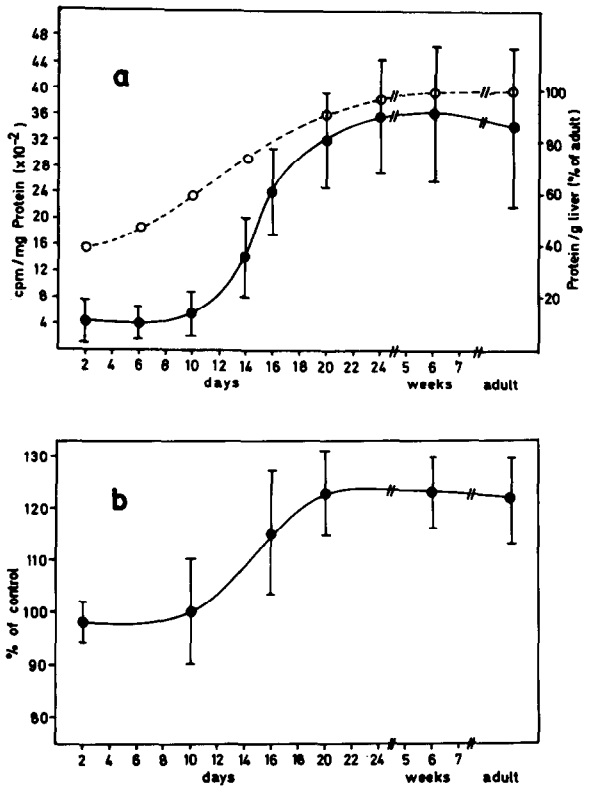

Fig. 1. a) Binding capacity for [^{3}H]dexamethasone of liver cytosol from rats of different ages (see Methods). (•—•—•) cpm [^{3}H]dexamethasone bound per mg protein. (o--o--o--o) Protein content per gram liver expressed as % of that of adult animals. Each point represents the values of 5–10 individual determinations. b) RNA synthetic capacity of isolated liver chromatin from rats pretreated for 2 hr with 2 mg/100 g body weight dexamethasone (see Methods). RNA synthesis is expressed as % of control. Each point represents the values of 2–4 individual experiments.

2.1. *Assay of the binding capacity of rat liver cytosol for [^{3}H]dexamethasone*

Livers of rats of different age were perfused through the vena porta with ice cold buffer consisting of 0.25 M sucrose, 0.067 M Tris, 0.025 M KCl and 0.01 M $MgCl_2$, pH 7.55, weighed and after mincing with scissors homogenized in equal volume of the same buffer. Approximately the same amount of tissue was processed in all the experiments. Rat liver cytosol was then prepared as described earlier [10]. The cytosol was incubated for 4 hr at 0–4°C with 5×10^{-8} M [^{3}H]dexamethasone under continuous

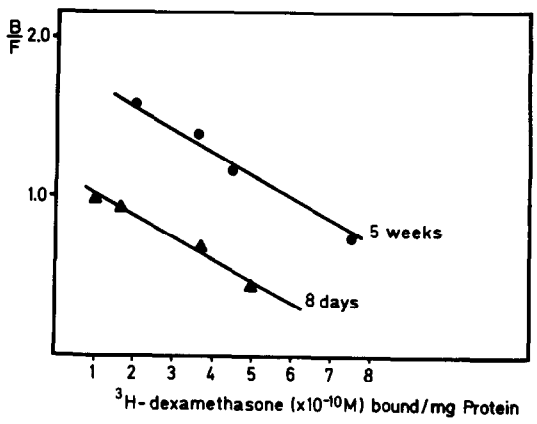

Fig. 2. Scatchard plot of cytosols from 8 days and 5 weeks old rats.

shaking. Specific binding was estimated by performing parallel incubations in the presence of a 5000-fold excess of non-labelled dexamethasone [5]. Free steroid was removed by the charcoal–dextran method [11]. The data were evaluated according to Scatchard [12] and yielded the concentration of steroid binding sites.

2.2. *Assay of RNA synthetic capacity of liver chromatin from rats treated with dexamethasone*

Groups of two to three rats of different ages were injected i.p. with 2 mg dexamethasone per 100 g body weight. After 2 hr chromatin was prepared according to [13]. RNA synthesis was performed in a standard RNA synthesizing mixture [14]. Incubations lasted 25 min at 37°C. The supernatants derived from control animals were used for the estimation of the binding capacity towards [^{3}H]dexamethasone (see sect. 2.1).

Protein was determined according to Lowry et al. [15], DNA according to Burton [16].

3. Results and discussion

Binder G, the specific glucocorticosteroid receptor protein, was quantitated in the cytosol of rats during postnatal development on the basis of its capacity to bind [^{3}H]dexamethasone. The results are shown in fig. 1a. The capacity of the cytosol to bind the syn-

thetic steroid is low in the first 10 days after birth, increases significantly in the next ten days and reaches maximal values at 22 days after birth. Thereafter the binding capacity remains constant being similar to that of the adult animals.

In order to show that the increase in binding sites per unit protein is due to changes in concentration of the receptor and not of its physical state we have determined the dissociation constants (see fig. 2). The Scatchard plots appear to be similar for both 8 day and 5 week old animals, which speaks for an increase in binding sites. Binding studies were also made with cytosol which was treated in such a way as to dissociate receptor–hormone complexes preformed *in vivo*. Under these conditions fluctuation in the concentration of endogenous hormones cannot influence the results. Although the total binding decreases, the form of the curve remains the same. In parallel experiments we have measured the *in vitro* RNA synthetic capacity of liver chromatin after *in vivo* treatment of the animals with dexamethasone. As seen from fig. 1b the curve of stimulation of RNA synthesis by cortisol shows striking similarities to the curve of dexamethasone binding capacity and therefore to the concentration of binder G. As already reported by Sereni and Barnabei [9] the rats do not respond to glucocorticosteroids in respect to RNA synthesis within the first ten days of postnatal life but develop this capacity during the 10–20th day after birth. At the end of this time period the rats respond maximally and this capacity remains constant throughout to the adult stage.

Although the present results are only indicative, taken together with findings of Beato et al. [7] and with our recent direct effects of receptor G fractions on *in vitro* synthesis by extranucleolar chromatin, they strongly suggest that the binder G is the receptor protein principally involved in the stimulation of transcription by glucocorticosteroids.

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