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STEROID-BINDING PROPERTIES OF CORTICOSTEROID RECEPTORS IN DIFFERENT TARGET TISSUES OF THE RAT

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SUMMARY

Specific corticosteroid binding receptors, characterized by high affinity and low capacity, were demonstrated in the cytoplasm of the liver, the thymus and the diaphragm of adult male rats. Corticosterone metabolite without biological activity is not bound by these receptors.

The receptors in the liver cytoplasm appear to be similar to those in the thymus cytoplasm, an assumption based on similarities of corticosteroid binding properties.

INTRODUCTION

The current hypothesis on the mechanism of corticosteroid action assumes the binding of the steroid to the specific receptors in the cytoplasm of target tissue cells [1, 2].

Corticosteroids affect many tissues and the responses are as diverse as the induction of gluconeogenesis in the liver [3] and death in lymphoid cells [4].

The specific receptors in corticosteroid target tissues, mainly in the liver [5, 6] and in the thymus [7–9] have been intensively studied, mainly by using the synthetic corticosteroid, dexamethasone [6], or by cortisol [5, 7] non-specific to rats. All these investigations lead to rather discrepant results, probably due to methodological and computational differences.

Therefore, it has seemed necessary to us to compare the corticosteroid binding characteristics of receptors in the various target tissues, never attempted before, particularly since, at a given plasma corticosterone level, the amount of corticosterone taken up by the target organ might be decisively influenced by the relative corticosteroid binding of the target tissues.

As corticosterone is the species-specific corticosteroid of the rat, we have attempted to characterize the corticosterone binding properties of receptors in the liver, thymus and diaphragm of the rat by determining the number of binding sites and their corticosterone binding affinity.

EXPERIMENTAL

The number of binding sites and the association constant of the receptor–corticosterone complex in liver, thymus and diaphragm slices were determined by measuring the "displaceable binding" according to Sharp *et al.* [10] and Snart *et al.*[11].

Biological materials

Male albino rats from the CFE strain, weighing 180–220 g were kept on a standard diet with free access to water and acclimatized to animal room conditions of uniform temperature (24 ± 1°C) and controlled r.h. (50–65%).

Sixteen hours after bilateral adrenalectomy the organs studied were briefly perfused with ice-cold physiological saline, under ether anaesthesia, to remove blood.

Incubation procedure

In a special plastic slicer the tissues were sliced to 350 μm thickness in cold with a razor blade. Between 200 and 400 mg of tissue were preincubated in 50 ml of Krebs–Ringer phosphate buffer (KRP), pH 7·4, containing glucose (100 mg% w/v), at 37°C under O_2 for 2 h to promote the breakdown of endogenous corticosterone possibly present in the tissue despite previous adrenalectomy. The preincubation tissue was distributed into two Erlenmayer flasks containing 50 ml of ice-cold KRP with [^{3}H]-corticosterone (36 Ci/mmol, Amersham, England) in concentrations $2{\cdot}5 \times 10^{-11}$ mol/l – 10^{-9} mol/l, either alone or in the presence of competing 10^{-6} mol/l of non-radioactive corticosterone (Fluka).

Equilibrium was found to be established after 60 min of incubation in cold, under constant stirring. After this, the surface of the tissue slices was rinsed with cold physiological saline, blotted and the slices were weighed.

Corticosterone from the slices was extracted with chloroform (Reanal, analytical grade)–methanol (Reanal, analytical grade) (2:1 v/v) at 37°C overnight. The extract was evaporated to dryness and the radioactivity measured in a liquid scintillation counter (Packard, Model 2420).

In one series of experiments 10^{-6} mol/l tetrahydrocorticosterone (Mann Research 3α, 11β, 21-trihydroxy-5β-pregnan-20-one) was used as competing steroid.

Mathematical analysis of data

The amount of corticosterone bound after incubation at different [^{3}H]-corticosterone concentrations

Table 1. Bound [^{3}H]-corticosterone in tissue slices

Concentration of [^{3}H]-corticosterone in the medium $\times 10^{-9}$ mol/l	[^{3}H]-Corticosterone bound $\times 10^{-9}$ mol/1000 g wet weight		
	Liver	Thymus	Diaphragm
0·025	0·06 ± 0·01* (12)	0·02 ± 0·01 (7)	0·03 ± 0·005 (7)
0·050	0·19 ± 0·02 (15)	0·04 ± 0·02 (3)	0·05 ± 0·02 (7)
0·075	0·31 ± 0·04 (9)	0·06 ± 0·02 (11)	0·12 ± 0·01 (13)
0·10	0·36 ± 0·08 (9)	0·09 ± 0·03 (5)	0·12 ± 0·04 (13)
0·25	0·63 ± 0·09 (9)	0·15 ± 0·05 (5)	0·17 ± 0·05 (7)
0·50	1·42 ± 0·17 (8)	0·24 ± 0·07 (11)	0·16 ± 0·04 (6)
0·75	1·60 ± 0·39 (9)	0·32 ± 0·12 (7)	0·23 ± 0·15 (18)
1·0	1·92 ± 0·30 (13)	0·58 ± 0·28 (21)	0·52 ± 0·14 (15)

* Mean ± S.E.
Number of determinations in parentheses.

was estimated from the differences between [^{3}H]-corticosterone concentrations in slices incubated in the absence or in the presence of competing, non-radioactive corticosterone, respectively. The number of corticosterone binding sites and the association constant of the receptor–corticosterone complex were calculated by the function based on the law of mass action:

$$S_B = \sum_{i=1}^{I} \frac{n_i k_i S}{1 + k_i S}$$

where: S_B = concentration of bound corticosterone; S = concentration of free corticosterone; I = number of set of sites; n = number of corticosterone binding sites; and k = association constant.

This function was fitted by applying the least squares method. This gives a non-linear system of equations, solved by Newton's multidimensional iteration. A CDC 3300 computer was used.

RESULTS

Corticosterone was bound by all the three tissues studied, the concentration of bound hormone being the highest in the liver and of about the same level in the thymus and the diaphragm (Table 1).

To test whether the radioactivity measured in the incubated tissues represented unchanged corticosterone or a metabolite produced during the incubation procedure, the liver slices incubated with 10^{-9} mol/l [^{3}H]-corticosterone were subjected to paper chromatography. The solvent system Bush B_5 was used [12]. According to the radiochromatogram (Packard Scanner, Model 7201) radioactivity was localized in a single spot corresponding to the R_F value of the authentic [^{3}H]-corticosterone (Fig. 1.).

Tetrahydrocorticosterone, a metabolite without biological activity, does not displace the [^{3}H]-corticosterone bound by the receptors of the liver slices (Table 2).

Evaluation of binding data revealed one set of binding sites in all the three corticosteroid target tissues under study. The corticosteroid receptors of these tissues are characterized by high affinity and low capacity (Table 3). The number of binding sites and the value of the association constant are the estimate of the parameters of the optimal curve fitting to the measurement results given in Table 1. The *SD* values of the curves given by these parameters, with S^{-2} weighting of each point, are: liver—$S = 0{\cdot}029 \times 10^{-9}$; thymus—$S = 0{\cdot}011 \times 10^{-9}$; and diaphragm—$S = 0{\cdot}022 \times 10^{-9}$.

The association constant of the corticosterone–receptor binding was found to be of the same order of magnitude in the liver and in the thymus.

DISCUSSION

It has been shown that the binding of corticosteroid to the cytoplasmic receptor proceeds both at 4 and 37°C, whereas the translocation of cytoplasmic to nuclear receptor is temperature-sensitive, it does not occur at low (0–4°C) temperatures [6, 8, 13]. Having performed the binding studies at low temperatures (0–4°C), our results probably relate to cytoplasmic receptors.

The association constant of these receptors falls in the order of magnitude of $10^9/M^{-1}$, the corticosterone binding is of high affinity.

Since we have found low binding capacity and high binding affinity, the cytoplasmic corticosteroid receptors demonstrated in the liver, thymus and diaphragm

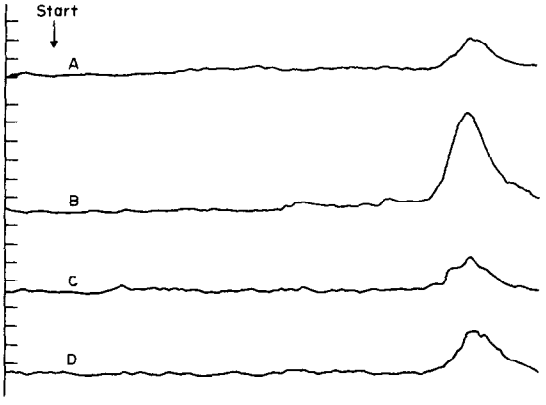

Fig. 1. Radioactivity measured in liver slices incubated with [^{3}H]-corticosterone (10^{-9} mol/l). On the "Start" point: A—0·008 μCi [^{3}H]-corticosterone; B—0·02 μCi [^{3}H]-corticosterone; C and D—united extracts of about 1 g of incubated liver slices, were applied on the paper.

Table 2. Attempts to displace [^{3}H]-corticosterone bound to liver cytoplasmic corticosteroid receptors by tetrahydrocorticosterone

In the medium	[^{3}H]-corticosterone concentration $\times 10^{-9}$ mol/1000 g (wet weight) In the tissue	Displaced
10^{-9} mol/l [^{3}H]-corticosterone	5·22 ± 0·37* (12)	
10^{-9} mol/l [^{3}H]-corticosterone + 10^{-6} mol/l corticosterone	3·72 ± 0·12 (12)	1·54 ± 0·31
10^{-9} mol/l [^{3}H]-corticosterone + 10^{-6} mol/l tetrahydrocorticosterone	5·20 ± 0·42 (11)	0

* Mean ± S.E.
Number of determinations in parentheses.

Table 3. Steroid-binding properties of cytoplasmic corticosteroid receptors

	Number of binding sites $\times 10^{-9}$ mol/1000 g (wet weight)	Association constant (0°C) $\times 10^9 M^{-1}$	$n \times k$
Liver	4·55	0·74	3·37
Thymus	0·61	1·49	0·91
Diaphragm	0·29	5·83	1·69

slices meet the criteria of specificity stated by Kornel[2] and Litwack and Singer[14], and should be considered as being involved in the specific corticosteroid action.

The physiological significance of these receptors is underlined by their failing to bind the biologically inactive tetrahydrocorticosterone.

Beato *et al.*[5, 15] and Baxter and Tomkins[6, 16, 17] demonstrated a cytoplasmic corticosterone receptor in the liver with an association constant of the same order of magnitude as the one described in the present paper. Snart *et al.*[18] found the liver cytoplasmic receptors to be characterized by the association constant 10^8 M^{-1}, a difference probably due to different experimental conditions.

We have demonstrated only one set of high affinity binding sites in the liver. This is in good agreement with the results of Beato *et al.*[15] and Litwack and Singer[14]. They detected several corticosteroid binding proteins in the liver cytoplasm, out of which only one bound corticosterone with high affinity and low capacity.

Our results comply with the corticosteroid binding characteristics described under identical experimental conditions for the liver and the thymus cytoplasmic receptors by other workers [5, 16, 19–23].

Neither the number of corticosteroid binding sites of the receptor nor the value of the association constant alone define the quantity of corticosterone taken up by the target tissues under identical experimental conditions. To be able to compare the corticosteroid affinities of the various tissues, we have introduced the term "corticosteroid-binding ability" by which we mean the multiplication product of the number of binding sites and the value of the association constant. The "corticosteroid-binding ability" in the liver is higher than in the other two tissues studied (Table 3). This is in good agreement with our finding that, under identical experimental conditions, the concentration of bound corticosterone in the liver is higher than in the other two organs studied (Table 1). Lippman and Thompson[24] confirmed quite recently that the corticosteroid-binding cytoplasmic receptors isolated from mouse fibroblast and rat hepatoma cell cultures differ from each other. However, this does not contradict the statement that the rat liver cytoplasmic receptors appear to be similar to the cytoplasmic receptors in the thymus, confirmed by the respective association constants being of the same order of magnitude (Table 3). The presence of specific receptors with similar binding properties in the different corticosteroid-responsive tissues could mean that the corticosteroid–cytoplasmic receptor binding is one of the prerequisites of the specific corticosteroid action by initiating the events leading to the specific target cell response to the corticosteroid.

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