BINDING OF CORTICOSTERONE TO LIVER CHROMATIN ACIDIC PROTEINS

B. DASTUGUE, N. DEFER and J. KRUH

Institut de Pathologie Moléculaire *, 24, rue du Faubourg, Saint-Jacques, Paris 14e, France

1. Introduction

When incubated with liver chromatin acidic proteins, (^{3}H) corticosterone become bound to them. This binding requires intact SH groups and the presence of Mg^{2+}, K^+. The protein bound steroid was isolated by gel filtration chromatography. The apparent dissociation constant is 5×10^{-9} M/l. This binding is specific, corticosterone is displaced only by high concentrations of progesterone, hydrocortisone acetate, calciferol and ouabaïn.

2. Methods

(1–2 ^{3}H) corticosterone was obtained from Amersham Radiochemical Center (Specific Activity: 32 Ci/mmole). Its purity was controled by two dimensional Silica gel thin layer chromatography with the use of chloroform–acetone (7:3) and of chloroform–methanol–water (90:9:1) [1]. Chromatin acidic proteins were prepared from non-adrenalectomized rats. Nuclei were obtained from liver by the technique of Chauveau [2]. Total acidic proteins were extracted from the chromatin according to the procedure of Wang [3]. The incubation medium usually contained: 1 mg of acidic proteins, 2×10^{-8} M (^{3}H) corticosterone, 1 mM $MgCl_2$, 50 mM KCl, 1 mM mercaptoethanol, 0.05 M Tris HCl buffer (pH 7.5 or 8.5), to a final volume of 2 ml. Protein bound corticosterone was isolated either on Sephadex G 25 or on Biogel P_4 chromatography (30 $\times$ 1.5 cm) by elution with the

same buffer as for the incubation. Two ml fractions were collected. Radioactivity of each fraction was measured after the addition of 10 ml of Instagel in a Nuclear Chicago Mark 1 liquid scintillator. The protein concentration was automatically recorded at 280 nm. Protein bound corticosterone was eluted in the excluded volume (fig. 1).
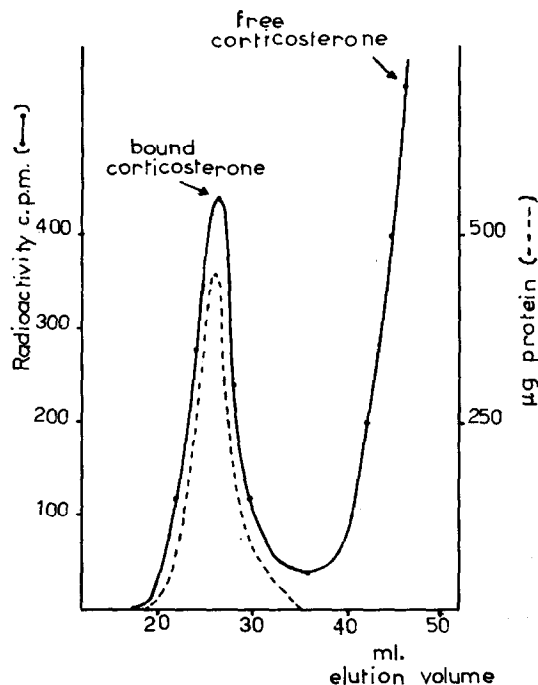

Fig. 1. Isolation of protein bound corticosterone. A Biogel P_4 chromatography column is used. Elution was carried out with 0.05 M Tris HCl buffer (pH 8.5) containing 1 mM $MgCl_2$, 50 mM KCl. Protein concentration was estimated at 280 nm. Radioactivity was measured in a liquid Scintillation counter. Protein bound corticosterone was eluted in the excluded volume.

* Université de Paris, Groupe U 15 de l'Institut National de la Santé et de la Recherche Médicale, Laboratoire associé au Centre National de la Recherche Scientifique.

Table 1

(^{3}H) corticosterone were incubated with 1 mg of various macromolecules and submitted to Biogel chromatography. Bound radioactivity was measured.

Nature of the macromolecule	Bound radioactivity (%)
Acidic protein	100
Total histone	29
DNA	0
Nuclear RNA	12
Serumalbumin	15
Acidic proteins treated with pronase	20

3. Results

3.1. Nature of the bound macromolecule

In order to ascertain that corticosterone was actually bound to acidic proteins and not to an impurity present in the preparation, the incubation was performed with various components of the nuclei: DNA, RNA, histone and also with serumalbumin which could have contaminated some of our preparations. In another control the acidic proteins were digested with pronase (table 1).

It is clear that corticosterone was bound to the acidic proteins and to none of the other components tested. The SH group play an important role in the binding, since this binding was prevented by the addition of 5 mM parahydroxymercuribenzoate (PHMB) and restored by 5 mM β mercaptoethanol.

Control	100
+ PHMB	37
+ PHMB + β mercaptoethanol	97

3.2. Conditions of the binding

Mg^{2+} is required for the binding with the optimal 1 mM concentration. In its absence the bound corticosterone was reduced to 18% of the control. In the presence of 1 mM Mg^{2+} and 1 mM EDTA the binding was reduced to 33%.

K^+ is also required. In its absence only 50% of the corticosterone was bound. The optimal pH was 8.5–9.5. At pH 7.5 the binding was reduced to 70%. The optimal temperature was from 18 to 40°. The

binding was highly reduced at higher temperature. At 20° the binding was instantaneous, at 4° the optimal binding was obtained in about 3 hr. The binding remained unchanged for at least several hours.

3.3. Kinetics

The amount of corticosterone bound is proportional to the amount of added acidic proteins between 125 and at least 1,000 μg. For an average molecular weight of 50,000, about 2 μmole of corticosterone are bound to 1 mole of acidic proteins.

Various amounts of corticosterone were incubated with 1 mg of acidic proteins and the bound corticosterone was measured each time. The use of the Lineweaver and Burk plot allowed the estimation of an apparent dissociation constant K_D of 5×10^{-9} M/l which corresponds to the physiological concentration of the hormone in liver (fig. 2).
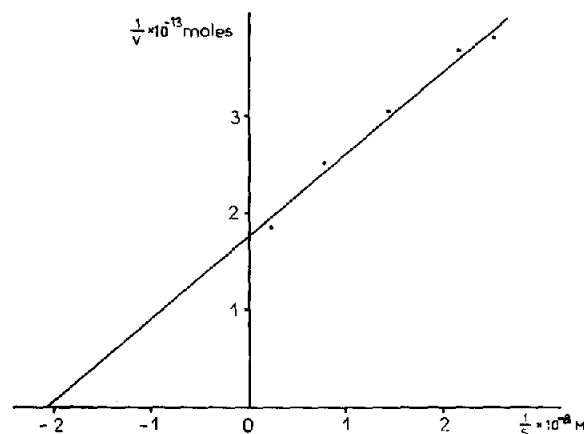

Fig. 2. Amount of protein bound corticosterone as a function of (^{3}H) corticosterone incubated with 1 mg of chromatin acidic proteins. Lineweaver and Burk plot.

3.4. Specificity

At a concentration equal to the concentration of (^{3}H) corticosterone no non-radioactive steroid other than corticosterone itself altered the amount of bound radioactivity. At 100 times higher concentrations, a diminution of the binding occurred with progesterone, hydrocortisone acetate, calciferol and ouabain (table 2).

Table 2
Action of the addition of various steroids on the binding of
(^{3}H) corticosterone to acidic protein.

Added non-radioactive steroid	Bound radioactivity	
	Ratio	Added steroid (^{3}H) corticosterone
	1	100
0	100	100
Corticosterone	42	
Oestradiol	96	125
Deoxycorticosterone		112
Testosterone		108
Progesterone	102	68
Hydrocortisone acetate	102	45
Calciferol	106	54
Ouabaïn		45

4. Discussion

It is usually assumed that steroid hormones, including corticosterone, the physiological glucocorticoid in rat, act at the gene level. Beato et al. [4] observed that injected (^{3}H) corticosterone enter liver cell nuclei. It is first bound to a cytosol receptor [5] and it is translocated into the nucleus. The nuclear receptor appears to be a protein [6, 7]. Several studied were devoted to the nature of the nuclear corticosteroid binding protein. Several groups reported that corticosterone is able to bind to histones *in vitro* [8–11]. Very recently Tsai and Hnilica found that the arginine-rich F_3 histone has the highest affinity toward the glucocorticoid [12], but they suggest in their discussion that the binding could well have occurred with a non-histone protein present as an impurity in their histone preparation, which is an agreement with our observation. The non-histone proteins are probably also involved in the binding of aldosterone in rat kidney [13]. It is clear from our data that the chromatin acidic proteins have a much higher affinity toward corticosterone than histones. The kinetic data favor the presence of well defined acceptor sites specific for this steroid. A competition occurs with few other steroids, only at high concentrations. The competition of ouabaïn with corticosterone has already been observed [14].

The mechanism of action of the glucocorticoid at

the molecular level is still an open question. Acidic proteins represent a very complex class of protein which includes enzymes like RNA polymerase, protein kinase [15]. They diminish the ability of histones to bind to DNA [16, 17] and to RNA [18]. Corticosterone could act either by modifying the action of one of these enzymes or by changing the affinity of the acidic proteins toward histones.

Beato, Seifart and Sekeris [19] have already shown that the association of chromatin with cortisol increases its ability to act as a template for RNA synthesis.

Acknowledgements

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