PHARMACOLOGY AND TOXICOLOGY

The Role of Membrane Stage in the Interaction of Cortisol with Hepatocytes

S. I. Ogurtsov and A. S. Dukhanin

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We studied the effect of polyvinylpyrrolidone-immobilized cortisol on the distribution of glucocorticoid-receptor complexes in hepatocytes. Immobilized cortisol 1.2-1.5-fold increased the number of glucocorticoid-receptor complexes associated with the nuclei in a dose-dependent manner. The transport of glucocorticoid-receptor complexes into hepatocyte nuclei induced by immobilized cortisol is associated with their activation. Moreover, the ratio between nuclear pools of glucocorticoid-receptor complexes resistant to 0.2 and 0.4 M KCl extraction was changed. It was concluded that glucocorticoid binding to specific plasma membrane sites induced further stages of interaction between the hormone and competent cell.

Key Words: *cortisol; polyvinylpyrrolidone immobilization; hepatocytes; glucocorticoid receptors*

Recent studies showed that plasma membrane plays a key role in the realization of biological effect of steroid hormones [6]. Phospholipids and regulatory and functional proteins are membrane targets for steroids. It is known that steroids (androgens, estrogens, gestagens, active vitamin D metabolites, and mineralocorticoids) regulate activity of serotonin, N-methyl-D-aspartate, opioid, and $GABA_A$ receptors, modulate activity of tyrosine kinase, Na^+,K^+-ATPase, adenylate cyclase, phospholipase C, and second messenger enzyme systems, and change ionic permeability for calcium, chlorine, potassium, and sodium [3,4,8,11].

Our previous study of membranotropic activity of glucocorticoids in isolated hepatocytes showed that polyvinylpyrrolidone-immobilized cortisol (PVP-cortisol) potentiated the inhibitory effect of free hormone on D-glucose absorption in hepatocytes by decreasing the maximum rate of absorption [1]. Since the effect of PVP-cortisol could be realized only at the level of the plasma membrane, the purpose of the present study was to elucidate possible role of the membrane stage of cortisol-cell interaction in nuclear effects of the hormone.

Russian Medical State University, Moscow

MATERIALS AND METHODS

Hepatocytes were isolated after *in situ* collagenase treatment as described elsewhere [2]. Viability of isolated cells evaluated by trypan blue exclusion test [9] was >80%.

The interaction of ^3H- and PVP-cortisol with hepatocytes was studied as follows: 400 μl cell suspension (1.5×10^6 cells/ml) in Hanks solution was incubated with 20 μl 10^{-8} M ^3H-cortisol and 10 μl unlabelled cortisol at 37°C for 2 min. Incorporation was stopped by adding 10 ml cold (0°C) Hanks solution.

Isolation of cytoplasmic and nuclear hormone-receptor complexes, separation of activated and inactivated subpopulations of cytoplasmic complex on diethylaminoethyl cellulose (DEAE cellulose), and differential extraction of nuclear complexes were carried out as described elsewhere [10].

Radioactivity was measured on a Beta-2 scintillation counter. The selected time of measurement al-

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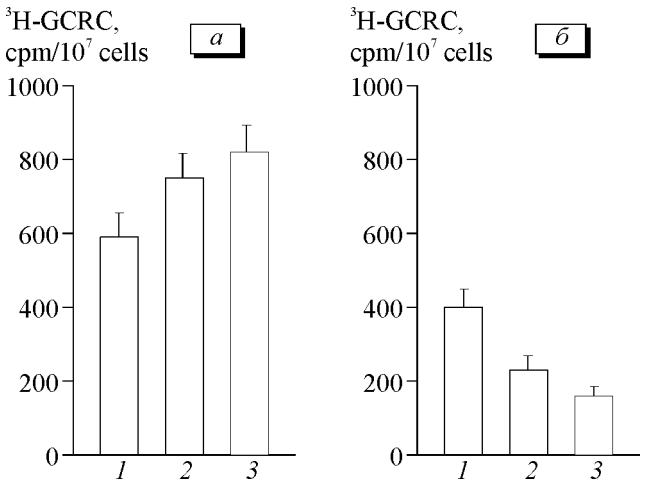

Fig. 1. Effect of polyvinylpyrrolidone-immobilized cortisol (PVP-cortisol) on specific binding of ^{3}H-cortisol (5×10^{-8} M) with nuclear (*a*) and cytoplasmic (*b*) hepatocyte fractions (number of bound ^{3}H-glucocorticoid-receptor complexes; ^{3}H-GCRC). *1*) control; *2*) PVP-cortisol, 10^{-6} M; *3*) PVP-cortisol, 3×10^{-6} M.

lowed to count over 3000 impulses and to decrease counting error to 4%.

Statistical processing of the results was performed using Student's nonparametric *t* test.

RESULTS

The study of the effect of PVP-cortisol on the distribution of ^{3}H-cortisol-receptor complexes in hepatocytes showed that the immobilized hormone in concentrations of 1 and 3 μM significantly (by 30-45%, $p<0.05$) increased specific binding of ^{3}H-cortisol to hepatocyte nuclei. At the same time, the number of ^{3}H-glucocorticoid-receptor complexes (GCRC) in the cytoplasmic fraction decreased by 40%, which corresponded to similar increase in the number of nuclear ^{3}H-GCRC. The total specific binding of ^{3}H-cortisol in cytoplasmic and nuclear fractions did not change in the presence on PVP-cortisol.

Experimental value of parameter A [7] for ^{3}H-cortisol was 0.57±0.03, which indicates that by its binding capacity cortisol is inferior to prednisolone (0.59±0.03), but superior to corticosterone (0.55±0.02) [5]. Parameters A for hypothetic complete agonist and antagonist are 1 and 0, respectively [7].

PVP-cortisol 1.2-1.5-fold increased the ratio of nuclear ^{3}H-GCRC to total GCRC in hepatocytes (to 0.68-0.85). In this case, parameter A for PVP-cortisol surpassed that for highly active fluorinated synthetic glucocorticoids triamcinolone and dexamethasone (Fig. 1).

The study of the effect of PVP-cortisol on the content of activated and inactive ^{3}H-GCRC in hepatocyte cytoplasm showed that immobilized hormone induced transition of inactive GCRC into its active form.

Figure 2 demonstrates ^{3}H-GCRC elution profile of cytosol from hepatocytes incubated with 33 nM ^{3}H-cortisol and 1 μM immobilized cortisol at 37°C for 30 min. Elution of cytosol from activated hepatocytes with KCl gradient revealed the presence of two fractions of hormone-receptor complexes eluted with 50 mM and 200 mM KCl. These fractions corresponded to activated and inactive forms of cortisol receptor complex.

Addition of PVP-cortisol to cell suspension reduced the pool of inactive receptors, while the content of activated GCRC increased insignificantly (Fig. 2). In the absence of PVP-cortisol the ratio between activated and inactive GCRC was 0.63± 0.05 (n=6). The presence of 1 μM PVP-cortisol significantly ($p<0.05$) in-

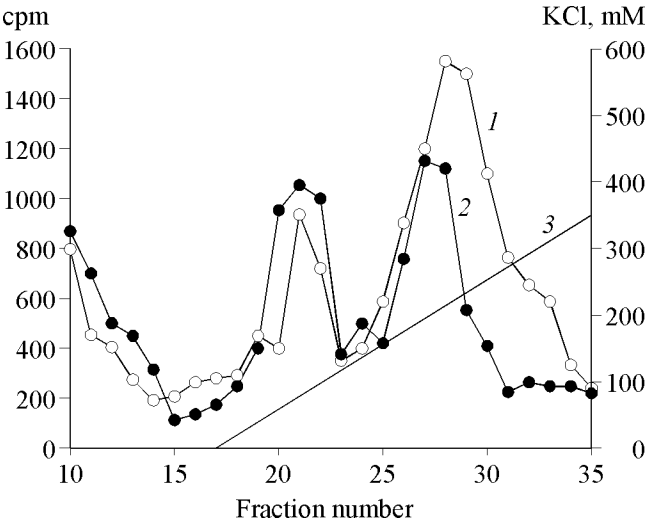

Fig. 2. Elution profile of ^{3}H-GCRC in the absence and (*1*) and presence (*2*) of 10^{-6} M PVP-cortisol (chromatography on DEAE cellulose). *3*) KCl gradient.

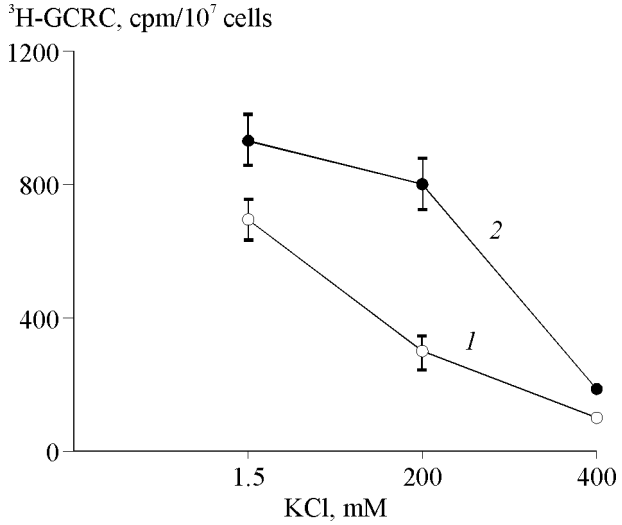

Fig. 3. Content of ^{3}H-glucocorticoid-receptor complexes (^{3}H-GCRC) in hepatocyte nuclei after extraction with KCl solutions of different ionic strength. Incubation in the presence of 10 nm ^{3}H-cortisol without (*1*) and with (*2*) 3×10^{-6} M PVP-cortisol.

creased this ratio to 0.90±0.04 (n=6). Since PVP-cortisol caused no significant changes in the number of transformed receptors, the observed shift in the ratio between these receptor forms could depend on the transpart of activated GCRC into hepatocyte nuclei.

Analysis of intracellular ^{3}H-GCRC heterogeneity revealed the following ^{3}H-GCRC distribution in control samples (without PVP-cortisol): 40% ^{3}H-GCRC resistant to 0.2 M KCl extraction and 15% GCRC resistant to 0.4 M KCl (Fig. 3).

In the presence of PVP-cortisol, the portion of GCRC resistant to 0.2 M KCl extraction increased to 62%. The pool of GCRC resistant to 0.4 M KCl was 14±2%, which did not differ from the control. Thus, PVP-cortisol increased specific ^{3}H-cortisol binding to hepatocyte nuclei and modulated the interactions between hormone-receptor complexes and nuclear acceptors.

These data suggest that the membrane stage plays an important role in the realization of intracellular reactions mediating the interaction of cortisol and competent cell.

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