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CHARACTERISTICS OF STEROID HORMONE BINDING SYSTEMS IN HEPATOCYTE PLASMA MEMBRANES

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UDC 612.35.014.2-06:
612.453.018

Parameters of binding of cortisone and estradiol to plasma membranes of rat hepatocytes were studied by the method of liquid scintillation radiometry. The presence of two systems for the binding of these hormones in the membranes was demonstrated. One system is specific (saturable) and binds hormones in physiological concentrations. The capacity and affinity of this system for cortisone are significantly higher than for estradiol. The binding parameters within the temperature range from 4 to 37°C for cortisone and estradiol respectively are: Dissociation constant 2.1-3 and 2.7-4.5 nM, number of binding sites 2-2.4 and 0.14-0.18 nmoles/mg protein. Experiments with p-chloromercuribenzoate demonstrate the role of proteins in the working of this system. The second (unsaturable) system is nonspecific and its function is determined by the lipid component of the membranes. The affinity of corticosteroids for hepatocytes is probably due to the activity of the (saturable) specific system of the plasma membranes of these cells.

KEY WORDS: plasma membranes; steroid hormones; binding systems; binding parameters.

An important role in the realization of the action of steroid hormones on the cell is ascribed to its membranous structures and, in particular, the membranes of the intracellular organelles. Meanwhile one of the factors limiting the entry of steroids into the cell, transport through the plasma membrane, still remains virtually unknown. Only recently has evidence been obtained that the action of steroid hormones is mediated through their specific binding to the plasma membranes [1, 5-7]. In the present writers' opinion, this binding in hepatocyte membranes is due to the functioning of a membrane system which has been called the "system of preference for corticosteroids" [2].

The investigation described below was devoted to a study of certain parameters of the functioning of this system.

EXPERIMENTAL METHOD

Plasma membranes were isolated by the method of Dorling and Le Page [3] from the liver of female albino rats weighing 150-200 g. The purity of the membrane fraction was verified by determining activity of the marker enzyme 5'-nucleotidase [4] and electron-microscopically. Steroid hormones (cortisone-1,2-^{3}H and estradiol-6,7-^{3}H, from IRA) were incubated with a suspension of membranes (protein concentration 200 μg/ml) for 1 h. After sedimentation of the membranes on the VAC-601 centrifuge the supernatant was sampled in doses of 0.1 ml for scintillation analysis on the Intertechnique (France) apparatus. The SH-groups of the membrane proteins were blocked by p-chloromercuribenzoate (PCMB). The results were subjected to statistical analysis by Student's method.

Department of Molecular Pharmacology and Radiobiology, Medico-Biological Faculty, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Yudaev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 2, pp. 172-174, February, 1980. Original article submitted February 6, 1979.

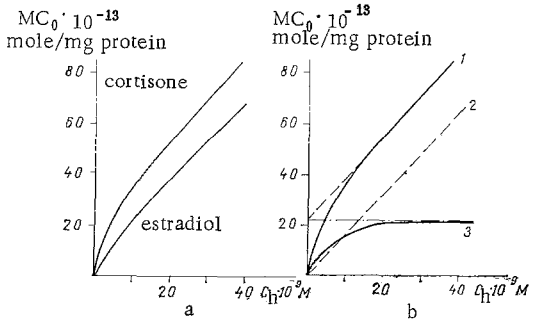

Fig. 1. Binding of cortisone-1,2-^{3}H and estradiol-6,7-^{3}H by plasma membranes (PM) of hepatocytes and role of saturable and unsaturable components in this process: a) binding of cortisone-1,2-^{3}H and estradiol-6,7-^{3}H by PM as a function of concentration of free hormones. Incubation temperature t° = 20°C. MC_0) total quantity of bound hormone, C_h) its free concentration. b) Graphic analysis of binding of cortisone-1,2-^{3}H by PM. 1) as in Fig. 1a; 2) quantity of cortisone bound by unsaturable system (MC_1); 3) quantity of cortisone bound by saturable system (MC_2).

TABLE 1. Effect of Temperature on Parameters of Hormone Binding by Hepatocyte Plasma Membranes

Hormone	Temp., °C	$K_{bc}^{us} \cdot 10^{-4}$ liter/mg	$K_{dis}^{ss} \cdot 10^{-9}$ M	$M_0 \cdot 10^{-9}$, mole/mg
Cortisone	4	0,75	2,1	2,0
	20	1,60	2,4	2,1
	37	2,50	3,0	2,4
Estradiol	4	0,70	2,7	0,14
	20	1,70	3,6	0,16
	37	2,50	4,5	0,18

Legend. K_{bc}^{us}) Binding constant of unsaturable system, K_{dis}^{ss}) dissociation constant of saturable system, M_0) number of binding sites of saturable system.

EXPERIMENTAL RESULTS

The study of binding of cortisone and estradiol by hepatocyte membranes showed that its character differs for these two hormones (Fig. 1a). Compared with estradiol, cortisone is bound more abundantly, especially in the region of low concentrations. The binding curves were nonlinear up to a concentration of 2×10^{-8} M. With an increase in the concentration of cortisone and estradiol in the medium the ratio of the quantity of the former to that of the latter absorbed fell and approached 1. Analysis of the curves shows that each of them can be represented by two components, one of which (a straight line) characterizes the process of free diffusion of the hormone into the lipid phase of the membrane, while the other characterizes specific binding of the hormone. This analysis was carried out graphically and is indicated in Fig. 1b for cortisone. The continuation of the linear part of the curve, characterizing dependence of the quantity of bound and free hormone, to the ordinate gives the number of binding sites of the specific (saturable) system. The tangent of the angle of slope of this straight line characterizes the "binding constant" of the unsaturable system, which is in fact the partition coefficient of the hormone in a lipid–water system in the assigned concentration of membranes (as protein). This constant for cortisone was 1.6×10^{-4} liter/mg protein and for estradiol 1.7×10^{-4} liter/mg. The difference between the total quantity of bound hormone and the quantity taken up by the unsaturable system gives the quantity of hormone accumulated by the specific (saturable) binding system. The results of this analysis are given in Fig. 2a. Clearly the saturable system of the plasma membranes of the hepatocytes had more than 10 times the affinity for cortisone than for estradiol. Analysis by Scatchard plots (Fig. 2b) gave values of the binding

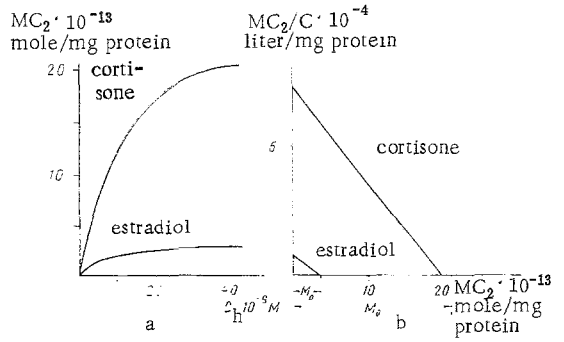

Fig. 2

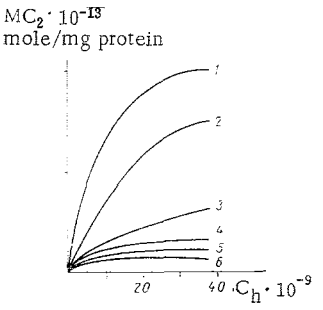

Fig. 3

Fig. 2. Binding of cortisone-1,2-^{3}H and estradiol-6,7-^{3}H by saturable system of hepatocyte PM. a) Binding of cortisone-1,2-^{3}H and estradiol-6,7-^{3}H by saturable system as a function of concentration of free hormones; $t_0 = 20°C$. Legend as in Fig. 1b. b) Values from Fig. 2a transformed to Scatchard plot. Legend as in Fig. 1b; M_0) number of binding sites for corresponding hormone.

Fig. 3. Character of binding of labeled cortisone (1) and estradiol (4) in presence of unlabeled cortisone and estradiol (100×10^{-9} M of each). 2, 3) Binding of labeled cortisone in presence of unlabeled estradiol and cortisone respectively; 5, 6) binding of labeled estradiol in presence of unlabeled cortisone and estradiol respectively. Legend as in Figs. 1 and 2. $t_0 = 20°C$.

parameters for cortisone and estradiol: The dissociation constant and the number of binding sites for the former were 2.4×10^{-9} M and 2.12×10^{-9} mole/mg protein, and for the latter 3.5×10^{-9} M and 0.16×10^{-9} mole/mg respectively.

The process of binding of estradiol and cortisone is temperature dependent; as Table 1 shows, both systems of hormone binding, especially the unsaturable system, are sensitive to changes of temperature.

To detect the specificity of hormone binding by the plasma membranes (by their saturable systems) experiments were carried out with unlabeled analogs of cortisone and estradiol. It will be clear from Fig. 3a that in the presence of 1×10^{-7} M cortisone the quantity of labeled cortisone bound with the saturable system fell sharply; a similar relationship was observed during incubation of labeled estradiol with membranes in the presence of unlabeled estradiol. In the case of crossed incubation (cortisone-1,2-^{3}H +estradiol and estradiol-6,7-^{3}H +cortisone) only a very small change in binding was found. Analysis of the character of interaction between the hormones (plotting the results between modified Lineweaver–Burk coordinates) showed that labeled and unlabeled forms of hormones compete for binding sites in the membrane; interaction between a pair of analogs is competitive in character, whereas interaction between a pair of "crossed" hormones is of the noncompetitive inhibition type.

Binding of the test hormones was considerably reduced also by treatment of the membranes with PCMB, which blocks SH groups. For instance, the number of binding sites of the saturable system was changed by the action of this compound in a concentration of 1 mM (at 20°C) from 2.1×10^{-9} to 1.1×10^{-9} mole/g (cortisone) and from 0.16×10^{-9} to 0.10×10^{-9} mole/g (estradiol), i.e., it was reduced by almost half. Since PCMB interacts specifically only with SH-containing proteins, it can be tentatively suggested that it is these proteins which are components of the saturable system for binding of steroid hormones to hepatocyte plasma membranes. It is instructive that treatment of the membranes with PCMB did not change binding of hormones by the unsaturable system. This fact, together with the characteristics of function of that system already examined, suggests that its structure is determined by lipids, and that proteins (at least SH-containing proteins) are not represented in it. It is also very important that the role of the two systems for specific accumulation of hormones in the plasma membranes is different. In fact, most of the cortisone (and what is important, especially in physiological concentrations) is bound by the specific saturable system, whereas practically all the estradiol interacts with the unsaturable binding system.

On the basis of these characteristics of the binding systems of hormones it can thus be concluded that at the plasma membrane level separation of the functions of these systems takes place for selective accumulation of cortisone, for which there is specific affinity, and the relatively indifferent estradiol. Because of this separation the saturable system can be distinguished as the "system of preference" for corticosteroids.

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CHANGES IN CONTENT OF CYTOCHROMES c and a (a_3) IN LIVER MITOCHONDRIA OF HYPERTHYROID RATS

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UDC 577.7:577.17+577.15.158

Male Wistar rats aged 1, 3, 12, and 24 months were given thyroxine in a dose of 250 μg/100 g body weight daily. After 9 days the content of cytochromes c and a (a_3) was considerably increased. An appreciable increase in the cytochrome c content was observed as early as after 24 h, whereas the content of cytochromes a (a_3) did not exceed normal even after 2 days. The content of cytochromes a (a_3) in rats aged 3, 12, and 24 months was little lower after 24 h than normally. A significant temporary increase in the ratio c/a (a_3) after 1-2 days was observed only in rats aged 12 and 24 months. An increase in the ratio c/a (a_3) with age also was demonstrated. The prospects for the use of thyroid hormones in the study of regulation of biogenesis of the mitochondria are suggested.

KEY WORDS: thyroxine; cytochromes; biogenesis of mitochondria; age.

In hyperthyroidism and thyrotoxicosis an increase is observed in the content of individual cytochromes of the respiratory chain in the liver mitochondria [3, 5, 9]. The cytochrome content is low in thyroidectomized animals [10]. Synthesis of mitochondrial cytochromes is effected by mitochondrial and cytoplasmic systems or protein synthesis, and the contribution of these two systems to the synthesis of different cytochromes differs [7]. For instance, cytochromes a and a_3 are synthesized with the participation of both systems, whereas cytochrome c is synthesized by the cytoplasmic system only. The regulatory mechanisms in both protein-synthesizing systems are sensitive to thyroid hormones [2], but the temporal characteristics of induction of synthesis of individual mitochondrial cytochromes under these circumstances have received little study. The object of this investigation was to study changes in the content of cytochromes c and a (a_3) in the liver mitochondria of rats of different ages in the early stage after administration of thyroxine.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar albino rats of four age groups: 1, 3, 12, and 24 months. The animals were given a daily intraperitoneal injection of L-thyroxine in a dose of 250 μg/100 g body weight. The animals were killed 1, 2, and 9 days after the first injection (after 1, 2, and 9 injections respectively). Mitochondria were isolated by differential centrifugation in medium with final concentrations of: sucrose 0.3 M, Trilon B 1 mM, pH 7.2; they were washed and suspended in medium without Trilon B. The protein concentration in the mitochondrial suspensions was determined by Lowry's method in Miller's modification [13]. The ADP/O coefficient and the respiratory control in the experimental series were indistinguishable from normal during oxidation of succinate, except for a marked decrease in ADP/O in the 24-month-old rats and of the respiratory control in rats aged 3, 12, and 24 months receiving nine injections of the hormone.

The differential spectrum of the cytochromes was recorded as described in [10], with very slight changes. To a test tube containing 3.6 ml of 100 mM K-phosphate buffer, pH 7.4, were added 0.3 ml of mitochondrial

Department of Biochemistry of Ontogeny, Research Institute of Biology, Khar'kov University. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 2, pp. 174-176, February, 1980. Original article submitted April 4, 1979.

 0007-4888/80/8902-0150$07.50