^{3}H-CORTICOSTERONE RECEPTION BY RAT HEPATOCYTES

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Glucocorticoids are involved in the regulation of expression of genes responsible for biosynthesis of inducible enzyme proteins such as tryptophan oxygenase, tyrosine aminotransferase, phosphoenolpyruvate carboxykinase, etc. [1]. An important role in this mechanism is played by cytosol receptors. They are found in the liver, kidneys, heart, skeletal muscles, thymus, and other organs [2-4]. Glucocorticoids evoke a whole range of effects unconnected with this mechanism [5]. They include inhibition of hexokinase and phosphorylase in the liver [6], labilization of lysosomal membranes [6, 7], and an increase in tissue cAMP concentration, which is particularly marked in the presence of low- and very low-density lipoproteins [6]. These facts suggest the possible existence of glucocorticoid receptors in the structure of the plasma membranes. This has been virtually proved in cells of the thymus [9]. As regards hepatocytes, however, this problem remains unsolved. The receptor itself has not yet been isolated, nor has its structure been determined.

In this investigation we studied specific binding of ^{3}H-corticosterone with rat hepatocytes and also the possibility of interaction of the hormone with β-adrenoreceptors.

EXPERIMENTAL METHOD

Experiments were carried out on isolated hepatocytes from Wistar rats. The cells were obtained by the method in [9] in our own modification [10]. After decapitation of the animals the liver was perfused initially in situ for 5-10 min with Ca^{2+}-free Hanks's solution, after which it was connected to a perfusion system and recirculating perfusion carried out through the hepatic vein for 20-30 min with Ringer–Krebs solution (pH 7.4; 37°C), containing 0.03% type I collagenase ("Sigma"). The tissue was dissociated by means of a spatula. Hepatocytes were isolated by differential centrifugation from the resulting suspension. The yield of cells from 1 g of tissue was $40 \cdot 10^6$ hepatocytes. The trypan blue test showed that not less than 90% of the cells remained viable. The isolated cells were suspended in incubation medium (medium 199, containing 4% of albumin) and preincubated at 25°C for 30 min. The medium contained 10^6 cells in 1 ml. All subsequent operations including incubation of the cells with the hormone were carried out at 0-4°C. After preincubation the cell suspension was divided into four parts. The 1st part contained only hepatocytes, the 2nd contained hepatocytes and unlabeled corticosterone in a final concentration of 10^{-5} M, whereas the 3rd contained hepatocytes and propranolol (Obsidan) in a final concentration of 10^{-5} M, and

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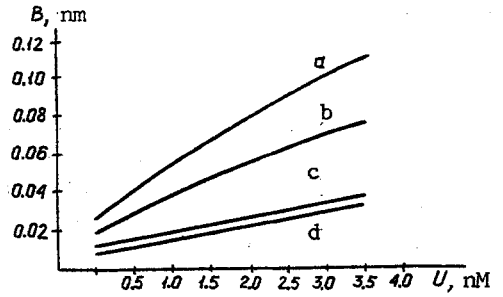

Fig. 1

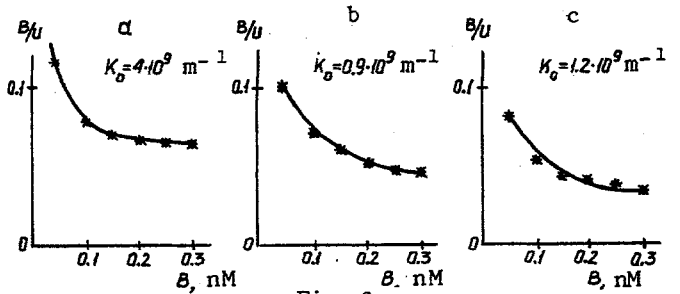

Fig. 2

Fig. 1. Binding of ^{3}H-corticosterone by isolated hepatocytes as a function of hormone concentration in incubation medium: a) binding of ^{3}H-corticosterone depending on concentration; b) binding of ^{3}H-corticosterone in presence of excess of propranolol; c) binding of ^{3}H-corticosterone in presence of excess of corticosterone; d) binding of ^{3}H-corticosterone in presence of an excess of corticosterone and propranolol. Abcissa, concentration of added ^{3}H-corticosterone; ordinate, quantity of bound corticosterone.

Fig. 2. Specific binding of ^{3}H-corticosterone by rat hepatocytes, calculated by Scatchard plot: a) binding of ^{3}H-corticosterone with specific glucocorticoid receptors; b) specific binding of ^{3}H-corticosterone with averaged kinetic characteristics; c) binding of ^{3}H-corticosterone with β-adrenoreceptors.

the 4th contained hepatocytes, propranolol, and unlabeled corticosterone in the above-mentioned concentrations. The cells (100 μl) thus prepared were mixed with 100 μl of a solution of ^{3}H-corticosterone in different concentrations (from 0.2 nm to 1.5 nm). Incubation lasted 18 h. The cells, washed with 20 volumes of cold buffer, were sedimented by centrifugation at 2000 rpm for 3 min. The washing procedure was repeated twice. The cells, washed free from unbound hormone, were treated with 400 μl of 5%. Triton X-100 transferred to vials, and covered with dioxan scintillator. Radioactivity activity was counted on a "Multimat" counter (France). The results given below are mean values of three measurements.

EXPERIMENTAL RESULTS

Binding curves of ^{3}H-corticosterone were obtained from the experimental results (Fig. 1a-d). Curve a (Fig. 1) demonstrates binding of the labeled hormone with hepatocytes, when the hormone interacts with all possible binding sites. This type of interaction is called total binding.

On the addition of an excess of unlabeled hormone to the incubation medium, competing with the labeled hormone for all binding sites except saturated and nonspecific, curve c is obtained. In this case binding of ^{3}H-corticosterone falls by 60%.

Interesting results were obtained against the background of an excess of propranolol (Fig. 1b), when binding of ^{3}H-corticosterone was reduced by 30%. Propranolol can compete with corticosterone only for β-adrenoreceptors, for it is blocker of receptors of this type.

Curve d repeats the course of c and reflects interaction of ^{3}H-corticosterone in the presence of two competitors, namely unlabeled corticosterone and propranolol, taken in equal and excessive quantities.

Specific binding is the difference between total binding (Fig. 1a) and nonspecific binding (Fig. 1c). By subtracting nonspecific from total binding and converting to Scatchard coordinates (Fig. 2b) we obtain a Scatchard plot, which shows that we are dealing with a specific, saturable process of binding of ^{3}H-corticosterone with hepatocytes, and the curvilinear nature of the graph indicates that there are at least two types of specific sites of interaction with different kinetic characteristics. Only the averaged value of all binding constants can be obtained from such a graph, and the number of binding sites will be the total number of all specific binding sites. The averaged constant (K_a) calculated in this way has the value $K_a = 0.9 \cdot 10^9\ M^{-1}$, whereas the number of sites (n) is given by the equation $n = 1.5 \cdot 10^{-14}$ moles/mg protein. Closely similar results were obtained previously [11] in a study of binding of steroid hormones by plasma membranes isolated from rat liver homogenate: $K_a = 0.88 \cdot 10^9\ M^{-1}$, $n = 1.04 \cdot 10^{-12}$ moles/mg protein.

The decrease in total binding of ^{3}H-corticosterone in the presence of an excess of propranolol suggested that binding of the hormone can take place at β-adrenoreceptors. In that case the difference between total binding and binding in the presence of an excess of propranolol, converted to Scatchard coordinates (Fig. 2c) enabled the parameters of corticosterone binding with β-adrenoreceptors to be determined: $K_a = 1.2 \cdot 10^9\ M^{-1}$ and $n = 0.9 \cdot 10^{-14}$ moles/mg protein. Interaction of glucocorticoids with β-adrenoreceptors evidently makes its own contribution to hormonal regulation of metabolism in the liver cells. It has been shown, for instance, that propranolol, if added to incubation medium containing surviving slices of rat liver, completely abolished the increase in free acid phosphatase activity, due to the action of hydrocortisone [8].

In order to characterize yet another type of binding, excluding interaction with β-adrenoreceptors, it was necessary to subtract from the values of curve b (Fig. 1), reflecting binding of the hormone with hepatocytes under conditions when the β-adrenoreceptors were blocked and only nonspecific interaction and interaction specific for a different type of receptors was possible, the values of curve b (Fig. 1) representing only nonspecific interaction of ^{3}H-corticosterone with hepatocytes. The difference thus obtained between Scatchard coordinates, gives us the kinetic characteristics of the following type of receptors. They may be called intrinsic glucocorticoid receptors: $K_a = 4 \cdot 10^9\ M^{-1}$, $n = 0.52 \cdot 10^{-14}$ mole/mg protein (Fig. 2a).

Proof of the existence of specific glucocorticoid receptors on plasma membranes of rat hepatocytes, with high affinity for hormones, has thus been obtained. Their number per milligram cell protein was calculated. It is shown that, besides these intrinsic receptors, glucocorticoids can interact with β-adrenoreceptors, affinity for which is significantly lower.

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ROLE OF NEUTROPHIL-PRODUCED MYELOPEROXIDASE IN THE PATHOGENESIS OF CATARACT

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Polymorphonuclear leukocytes (neutrophils) are cells specialized morphologically and biochemically for undertaking defensive reactions by phagocytosis and also by secretion of biologically active substances into the pericellular space. However, stimulated neutrophils not only have a bactericidal effect, but they also induce nonspecific damage to nearby tissues and cells [1]. Antimicrobial factors of neutrophils are generally divided into oxygen-dependent and oxygen-independent [9]. The first group includes oxygen derivatives with varying degrees of reduction: the $^1O_2$-superoxide-radical, hydrogen peroxide (H_2O_2), the hydroxyl radical (OH), and its active form – singlet oxygen ($^1O_2$). The hydroxyl radical possesses the highest degree of oxidative capacity in living nature, enabling it to attack and destroy virtually all biomolecules. Singlet oxygen interacts particularly actively with unsaturated fatty acids of membrane phospholipids and induces their peroxidation. Singlet oxygen and the hydroxyl radical rupture peptide bonds in proteins, decarboxylate amino acids, induce peroxidation of membrane lipids, and degrade nucleic acids [5, 11]. Oxygen-independent antimicrobial factors of neutrophils include cationic proteins: myeloperoxidase, defensins, bactericidal permeability-increasing protein, cathepsin G, elastase, and lactoferrin. The action of the defensins [8], of bactericidal permeability-increasing protein [13], cathepsin G, and elastase [10] is associated with disturbance of permeability of biological membranes, whereas that of lactoferrin is dependent on production of the hydroxyl radical

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