INTERACTION BETWEEN STEROID HORMONES AND MEMBRANES OF HEPATOCYTES AND ERYTHROCYTES

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UDC 612.453.018.2:[612.111+612.35].014.2

The uptake of tritiated steroid hormones and their passage through the dense layer of the plasma membranes of rat hepatocytes and rabbit ghost erythrocytes was investigated. The degree of uptake of steroid hormones by the plasma membranes diminishes in the following order: progesterone > testosterone > estrone > prednisolone > estriol. The degree of uptake of the hormones correlates with their solubility in lipids and with the resulting release of enzymes from the lysosomes. Meanwhile it shows negative correlation with the rate of passage through the dense layer of the membranes. The character of uptake of steroid hormones is independent of the role of the plasma membranes.

KEY WORDS: steroid hormones; erythrocytes; hepatocytes; membranes.

There are isolated items of information in the literature on the action of steroid hormones on biological membranes [1-3], but the character of their action on the plasma membrane remains unexplained.

The object of this investigation was to study the character of uptake of steroid hormones by membranes of erythrocytes and hepatocytes on the comparative plane. Unlike other workers [3, 4] who studied the binding of individual steroid hormones by membranes, and whose results are comparable, the present author studied the action of several hormones on membranes of hepatocytes and also on ghost erythrocytes, by means of a new technique.

EXPERIMENTAL METHOD

Membranes of rat liver cells and rabbit erythrocyte ghosts were used. The erythrocytes were obtained by washing rabbits' blood with isotonic saline and subsequent centrifugation (at 4°C). The erythrocyte ghosts and the plasma membranes of the liver cells were isolated and their purity verified by the methods described elsewhere [5, 6] with certain modifications. The protein concentration, determined by Lowry's method, was 5 mg/ml in Tris-HCl buffer solution. The buffer solution with the membranes was used later to prepare special filters. Solutions were made up in distilled water, additionally deionized on a column with ion-exchange resins.

A rectangular cuvette with open top, divided in the middle by a vertical partition made from a special filter into two compartments each with a working volume of 15 ml was used for the experiments. The special filter was made by passing 2 ml of the buffer solution containing biological membranes through a "Synpor" No. 6 filter under a pressure of about 1 atm. The membranes blocked the pores of the filter to such a degree that the buffer solution almost ceased to pass through the filter. The area of the filter was about 5 cm^2. The number of cuvettes corresponded to the number of hormones used. Tris-HCl buffer solution with human serum albumin in a concentration of 6 mg/ml was poured into each compartment of the cuvettes. Labeled hormone was added to one compartment until the concentration in the compartment was about 10^{-9} M. The cuvettes thus prepared had almost identical properties (for membranes of the same type). This was tested by adding the same hormone to different cuvettes. Samples taken after a certain time from other parts of these cuvettes contained equal amounts of hormone.

Tritiated steroid hormones used in the work were obtained from J. R. E. (Belgium) and the Radiochemical Centre, Amersham (England): [1,2-^{3}H] testosterone, [2,4-^{3}H(n)] oestriol, [2, 4, 6, 7-^{3}H] oestrone, [1^{α}, 2^{α}, ^{3}H(n)] progesterone, and [6, 7(n)-^{3}H] prednisolone.

N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 1, pp. 31-33, January, 1978. Original article submitted June 30, 1977.

0007-4888/78/8501-0035 $07.50

TABLE 1

Hormone	Membranes of hepatocytes, F	Erythrocyte ghosts, F	β-glucuronidase		Acid phosphatase P‡
			f*	Q†	
Progesterone	89,0	36,7	99	486	533
Testosterone	21,4	14,2	41,6	260	224
Estrone	20,6	6,4	33,6	125	155
Prednisolone	4,98	4,1	15,5**	—	—
Estriol	3,51	1,8	—	111	153

Legend. One asterisk denotes coefficient of solubility of steroid hormones in lipids (n moles/μ mole lipid) [7]; one and two daggers denote coefficients of liberation of lysosomal enzymes of rabbit liver by steroid hormones [8], t = 37°C; two asterisks denote data for cortisol [7].

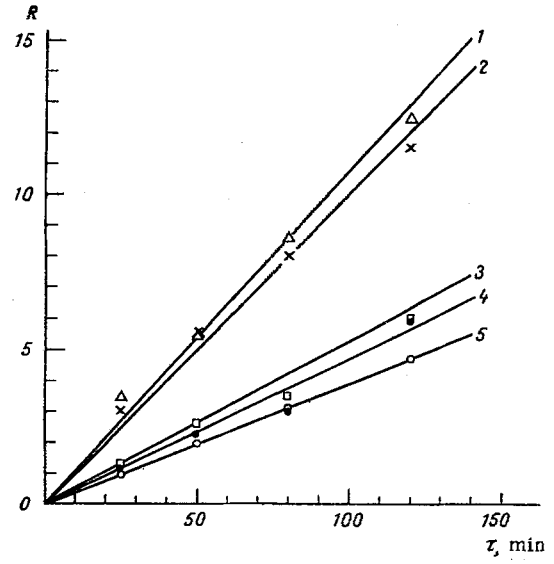

Fig. 1

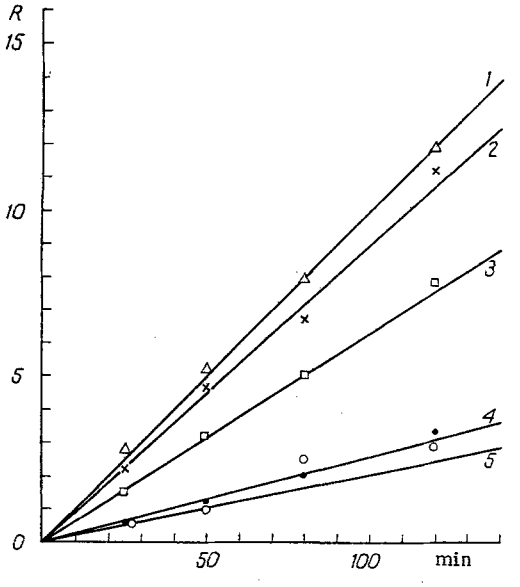

Fig. 2

Fig. 1. Degree of passage of steroid hormones through partition containing plasma membranes of erythrocytes as a function of time. t = 37°C, pH 7.3. R) Coefficient of passage, equal to ratio (in %):

$$\frac{\text{concentration of hormone passing through}}{\text{initial concentration of hormone added}}$$

1) Estriol; 2) prednisolone; 3) estrone; 4) testosterone; 5) progesterone.

Fig. 2. Degree of passage of steroid hormones through partition with plasma membranes of hepatocytes as a function of time. Legend as in Fig. 1.

Samples were added to scintillation fluid containing POPOP, naphthalene, toluene, dioxan, and ethanol. Radioactivity was counted with an Intertechnique 8L30 apparatus (France).

Experiments were carried out in an incubator at 38°C. The pH of the buffer solutions was 7.3.

EXPERIMENTAL RESULTS

The steroid hormone, added to one half of the cuvette filled with buffer solution containing serum albumin, was able to diffuse into the other half by passing through the partition containing densely packed plasma mem-

branes. Under these circumstances one part of the added hormone passed through the membranes, another part passed through the gaps between them, whereas the third part was absorbed by the membranes. Measurement of the activity of the special filter of the partition enabled the degree of binding of the hormones by the membranes to be judged without the need for separating the membranes from the solution containing the hormone, which might have distorted the true results. Binding of the hormones by the membranes was determined by the ratio $C_f/C_s = F$, where C_f is the concentration of the hormone in the membranes (with a correction for the volume of cellulose in the filter) for the given time interval (2 h); C_s is the initial concentration of the hormone in the buffer solution.

As Table 1 shows, the degree of uptake of hormones by the plasma membranes decreased in the following order: progesterone > testosterone > estrone > prednisolone > estriol.

Changes with time in the ratio between the quantity of hormone passing through the dense layer of the hepatocyte and erythrocyte membranes and the quantity added initially are illustrated in Figs. 1 and 2.

These curves can be used to determine the rate at which the hormones pass through the partition with membranes, by differentiating with respect to time. The curves in Figs. 1 and 2 are almost linear in character over the time interval from 0 to 2 h, a result which can be attributed to simultaneous diffusion and absorption.

The experimental results showed that binding of steroid hormones by the hepatocyte membranes took place to a somewhat greater degree than by erythrocyte ghosts; the degree of binding, moreover, correlates with the solubility of the steroid hormones in lipids [7] and is identical in character both for hepatocyte membranes and for erythrocyte ghosts. Correlation between the coefficient F and the degree of release [8] of lysosomal enzymes by steroid hormones also is interesting.

When the data in Table 1 are compared with the graphs in Figs. 1 and 2 it will be noted that the tangent of the angle of slope of the straight lines (the rate of passage of the hormones) decreases with an increase in F.

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