LITERATURE CITED

1. A. V. Kaplan, V. A. Landa, V. M. Lirtsman, et al., Ortoped. Travmatol., No. 1, 56 (1980).
2. V. A. Landa, A. N. Polyakov, and V. K. Baranov, Ortoped. Travmatol., No. 10, 55 (1976).
3. V. A. Landa, A. N. Polyakov, and V. K. Baranov, Byull. Éksp. Biol. Med., No. 5, 589 (1977).
4. G. S. Yumashev and B. N. Kryukov, Khirurgiya, No. 12, 62 (1977).
5. C. T. Brighton, L. B. Friedenberg, L. M. Zemsky, et al., J. Bone Jt. Surg., 57-A, 368 (1975).
6. C. T. Brighton, L. B. Friedenberg, E. I. Mitchell, et al., Clin. Orthop., 124, 106 (1977).
7. C. A. Bassett, S. N. Mitchell, L. Norton, et al., Acta Orthop. Belg., 44, 706 (1978).
8. G. Millonig, J. Appl. Physics, 32, 1637 (1961).
9. L. A. Norton and R. R. Moor, J. Dent. Res., 51, 1492 (1972).
10. L. W. M. Janssen, L. M. A. Akkerman, M. Gorissen, et al., Acta Orthop. Belg., 44, 659 (1978).

INTERACTION OF STEROID HORMONES WITH HEPATOCYTES AND THEIR PLASMA MEMBRANES

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UDC 612.35.014.2.014.46:[615.357.453+615.357.631

KEY WORDS: plasma membranes; hepatocytes; steroid hormones.

The plasma membranes of target cells play an important role in the mechanism of the biological signal of the steroid hormones, for they are the primary systems for "recognition," binding, and incorporation of the molecules of these compounds.

Interaction between members of three groups of steroid hormones (corticosterone, estradiol, and testosterone) with rat liver cells and their plasma membranes was investigated. The choice of hormones (estradiol and testosterone, with no affinity for the liver, corticosterone with affinity for the liver) and of test objects was determined by the need to undertake a comparative (correlative) analysis of the response of the membranous structures of the liver to these steroids, for it is in these structures that the "preference systems" are found, at least for corticosteroids [3].

EXPERIMENTAL METHOD

Plasma membranes (PM) were isolated from the liver of female albino rats by the method of Dorling and Le Page [6] with modifications. The yield of PM was 1-1.2 mg protein/g tissue. The degree of purification of PM was verified electron-microscopically and on the basis of the increase in specific activity of PM marker enzymes compared with the homogenate (Table 1).

Isolated hepatocytes were obtained by the method of Kanaeva et al. [2] with modifications. The yield of cells from 5-6 g liver was 30% of the total number of cells, viz. 300-500 million. The protein content was 0.6-0.8 mg/million cells. The viability of the hepatocytes was estimated by staining with trypan blue (the number of viable cells was 90-95%) and by polarographic monitoring of their respiratory activity. Lipids for lysosome formation were isolated by Folch's method from egg yolk. A suspension of lipids for liposome formation was sonicated on an MSE (Sweden) ultrasonic disperser. Liposomes contained cholesterol and phosphatidylcholine in the ratio of 1:1 by weight.

Isolated hepatocytes, PM, and liposomes were incubated with labeled steroid hormones (corticosterone, estradiol, or testosterone, from the Radiochemical Centre, Amersham, England) in concentrations of the latter of between 0.5 and 50 nM for 30 sec or 3, 10, or 30 min. The incubation temperature was 37°C for cells and 20°C for PM and liposomes. Incubation of the cells was interrupted by filtration of the suspension through a

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 6, pp. 722-725, June, 1981. Original article submitted December 12, 1980.

0007-4888/81/9106-0804$07.50

TABLE 1

Test object	Activity, μg P/mg protein × 15 min	
	5'-nucleotidase	Na,K-ATPase
Liver homogenate	2,2±0,8	15,0±1,2
PM of liver cells	51,1±6,7	81±3,1

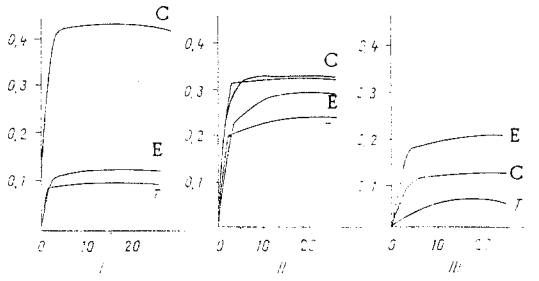

Fig. 1

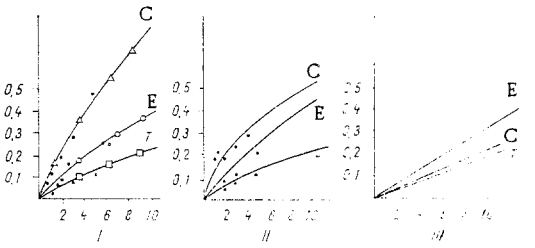

Fig. 2

Fig. 1. Binding of corticosterone (C), estradiol (E), and testosterone (T) by cells (I), PM (II), and liposomes (III) depending on incubation time. Abscissa, incubation time (in min); ordinate, concentration of bound hormones ($\times 10^{-12}$M).

Fig. 2. Quantity of corticosterone (C), estradiol (E), and testosterone (T) bound by cells (I), PM (II), and liposomes (III) depending on concentration of free hormones in medium. Abscissa, concentration of free hormones ($\times 10^{-9}$M); ordinate, the same as in Fig. 1.

No. 89 filter (from Filtrak). Incubation of PM and liposomes during the study of the dependence of hormone binding on time was stopped by filtering the suspension of membranes through a GF/f filter (from Whatman), and for the investigation of dependence on concentration, it was stopped by centrifugation at 40,000g for 10 min in siliconized tubes. The filters already used were transferred to cuvettes with scintillation fluid for subsequent radioactivity counting. A parallel control of adsorption of the steroid hormones by the filters or tubes themselves was set up. The results were subjected to statistical analysis by Student's t-test (at the $P = 0.05$ level).

EXPERIMENTAL RESULTS

The results of the study of dependence of steroid hormone binding by cells, PM, and liposomes on incubation time are given in Fig. 1. Clearly (Fig. 1a) the maximal rate of incorporation of the test steroids into hepatocytes was observed before 30 sec, and by the 3rd minute the quantity of accumulated hormone was constant. It is important to note that the quantity of corticosterone accumulated by the hepatocytes was significantly greater than that of estradiol and testosterone. A similar (although less marked) dependence was observed in the case of steroid hormone binding with PM. In the case of liposomes (Fig. 1, III) two facts must be noted: the slower accumulation of steroids than with cells and PM (the equilibrium state was reached after incubation for 10 min) and the preferential accumulation of estradiol and not of corticosterone, as in the first two cases.

Examination of the results of the study of dependence of binding on concentration (Fig. 2) also reveals two significant differences between the cells and PM, on the one hand, and liposomes on the other. The character of hormone binding in cells and PM was nonlinear and in liposomes it was linear for all steroids studied. Furthermore, greater accumulation of estradiol was observed in liposomes. The nonlinear dependence of binding of hormones on their concentration indicates that the mechanism of steroid accumulation by hepatocytes and their PM may be capable of saturation. The results of experiments to test this hypothesis are shown in Fig. 3. Analysis of accumulation of the hormones by cells in Edie–Hofstie coordinates and of binding by PM in Scatchard plots shows that two systems participate in the binding and accumulation of steroid hormones by these objects: one becomes saturated in the region of concentrations tested, whereas the other does not. Corresponding calculations enable the parameters of function of the saturable system of steroid accumulation in the cells and binding in PM to be estimated quantitatively. The numerical values of the parameters are given in Table 2.

TABLE 2

Hormone	Cells		PM	
	$K_M \cdot 10^9 M^{-1}$	Vmax, mg protein/min	$K_{dis} \cdot 10^9 M^{-1}$	M, pmoles/mg protein
Corticosterone	0,1	3,0	6,6	1,5
Estradiol	0,8	0,2	0,6	0,15
Testosterone	0,5	0,3	0,4	0,3

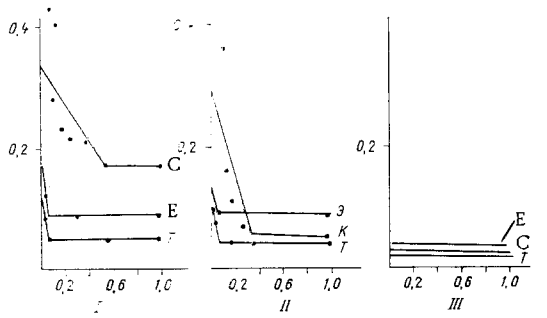

Fig. 3. Hormone binding in Edie–Hofstie coordinates for hepatocytes (I) and Scatchard plots for PM (II) and liposomes (III). Abscissa, concentration of bound hormone ($\times 10^{-12}$M); ordinate, ratio of concentration of bound hormone to that of free hormone.

It will be clear from Table 2 that the maximal rate of absorption of corticosterone by the cells is an order of magnitude greater than in the case of estradiol and testosterone. It can be tentatively suggested that the difference in the rate of steroid absorption is due to the different number of binding sites for them in the hepatocyte PM. In fact, judging from the results of determination of the number of binding sites for hormones in isolated PM, the number of such sites for corticosterone is significantly greater than for the other two steroids. As regards the unsaturable component of steroid absorption by cells and the unsaturable system of hormone binding in PM and liposomes, attention is drawn (Fig. 3) to the identical character of the straight lines reflecting the activity of these components: the presence of correlation between the quantity of hormone absorbed by the unsaturable component and its lipid-solubility, and the linear relationship between the quantity of bound hormone and its concentration in the medium (the straight lines in Fig. 3, II parallel to the abscissa). The considerable similarity in operation of the unsaturable components of the plasma membrane and liposomes indicates that these components are lipid in nature. Meanwhile the nature of the saturable component is much more complex, and it is probable that protein and other structures are concerned in its function.

A system thus exists in hepatocytes for incorporating steroid hormones. It is represented by a "universal" unsaturable component for incorporating corticosterone, estradiol, and testosterone, and a specific component, distinguished by high affinity for the trophic hormone corticosterone only. Comparison of the data on incorporation of hormones into the cells and their binding by the PM of these cells leads to the conclusion that the system under examination (and its components) is a structure localized in the hepatocyte PM. The essential feature is that the unsaturable component "works" in the presence of steroid concentrations of over 10^{-7}M. Hence it follows that under physiological conditions a sudden shift in the roles of two components of PM in steroid hormone binding must take place, namely: the virtual disappearance of the role of the unsaturable component (because of the low blood levels of the hormones and the presence of plasma protein carriers in the blood which prevent free diffusion of steroids into the membrane), and on the other hand, the dominant influence of the saturable component on binding of the "essential" hormones and their absorption by the cells. In other words, it is suggested that the "preference system" for corticosteroids [1, 3], and estrogens [4, 5] in the corresponding target organs determine the actual existence of the target organs: Membrane structures specifically selecting trophic hormones, because of their physicochemical characteristics,

possess incomparably greater affinity under physiological conditions for steroid hormones that are trophic for the particular organ; under these circumstances the "unconcern" of the cells for other steroids is determined by superposition of "insensitivity" of the saturable component to them and "blocking" of the unsaturable component of PM, for reasons pointed out above.

LITERATURE CITED

1. Yu. P. Denisov, P. V. Sergeev, N. P. Saksonov, et al., Byull. Éksp. Biol. Med., No. 2, 172 (1980).
2. I. P. Kanaeva, A. V. Karyakin, T. V. Alenicheva, et al., Tsitologiya, No. 5, 545 (1975).
3. P. V. Sergeev and Yu. P. Denisov, Farmakol. Toksikol., No. 5, 597 (1978).
4. P. V. Sergeev, Yu. P. Denisov, and S. N. Portugalov, Farmakol. Toksikol., No. 5, 453 (1979).
5. P. R. Dorling and R. N. Le Page, Biochim. Biophys. Acta, 318, 33 (1973).

EFFECT OF SYMPATHETIC IMPULSES ON PARAFOLLICULAR CELLS (C CELLS) OF THE THYROID GLAND

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UDC 612.441.014.2-06:612.89

KEY WORDS: thyroid gland; sympathetic innervation; C cells; serotonin.

The specific activator of the thyroid gland is the thyrotrophic hormone of the pituitary, but it is also dependent on direct nervous impulses travelling along efferent nerve fibers. Sympathetic impulses have an excitatory action on the thyroid gland; they stimulate the uptake of iodine by the gland, intrathyroid hormone production, and secretion of thyroid hormone [1-4]. Besides the follicular and interfollicular epithelium, the thyroid parenchyma also contains parafollicular or C cells, of neural origin, which produce the iodine-free protein hormone calcitonin. The fact that the C cells belong to the neuroendocrine (the "AFUD") system is confirmed by the presence of monoamines (especially serotonin) in them, and also by the ability of C cells to assimilate and decarboxylate monoamine precursors [7, 9-11]. The C cells are not dependent on the pituitary, removal of which is not reflected in their state. Accordingly the question arises whether sympathetic impulses take part in the regulation of functional activity of these cells, and the investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

Experiments were carried out on male rabbits weighing 1.5-1.8 kg, divided into three groups with seven or eight animals in each group. Group 1 (control) consisted of intact rabbits. In the experimental rabbits of group 2 the superior cervical sympathetic ganglia (the main source of the sympathetic innervation of the thyroid gland) was subjected to prolonged stimulation by application of a thin silver wire loop to the ganglion. Animals of group 3 underwent bilateral extirpation of these ganglia (cervical sympathectomy). The experiment lasted 10 days. At the end of that time the animals were autopsied and the thyroid glands removed and stained by Sawicki's method [12]. In parallel tests the serotonin concentration in thyroid gland homogenates was determined by Kulinskii and Kostyukovskaya's method [5]. Since an increase in the secretory activity of the glandular cells is accompanied by swelling of their cytoplasm and nucleus, on examination of thyroid gland sections stained by the above method outlines of the C cells were projected by means of a drawing apparatus on paper, so that their area of cross section could be measured with a planimeter and expressed in square microns.

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(Presented by Academician of the Academy of Medical Sciences of the USSR L. T. Malaya.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 6, pp. 725-727, June, 1981. Original article submitted November 27, 1980.

0007-4888/81/9106-0807$07.50