ESTIMATION OF ABILITY OF THE THYMUS TO ACCUMULATE TESTOSTERONE AND CHARACTER OF ITS INTERACTION WITH THYMOCYTES

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Androgens participate in the regulation of lymphoid tissue metabolism. Castration of sexually mature male rats leads to marked hypertrophy of the thymus, whereas an excess of androgens in the body causes a decrease in its weight [7, 9, 11], and this is reflected in the state of immune processes [1, 6]. Although many aspects of the mechanism of realization of the thymolytic effect of glucocorticoids have now been discovered [2, 5], the mechanism of action of androgens on the thymus has received little study. There is evidence that the cytosol obtained from thymus homogenate contains specific receptors for androgens [8-11]. According to some investigators [11], androgen receptors are located in a subpopulation of thymocytes resistant to glucocorticoids, whereas others consider that it is not the thymocytes, but the epithelial cells of the thymus which specifically bind androgens [8, 9].

The aim of this investigation was to study the testosterone-accumulating capacity of the thymus and to compare it with that of other organs, and also to determine the localization of the hormone in the thymus and the character of its interaction with thymocytes.

EXPERIMENTAL METHOD

Male rats weighing 150-200 g were used in the experiments on the 5th day after bilateral gonadectomy. ^{3}H-testosterone (from the "Izotop" Combine, USSR) with specific radioactivity of 3100 TBq/mole and unlabeled testosterone (from "Merck," West Germany) were used.

To study the distribution of testosterone among the organs of the castrated rats ^{3}H-testosterone was injected intravenously in a dose of 4×10^5 Bq (1.2×10^{-10} mole)/100 g body weight. The animals were decapitated 2, 5, and 15 min later under ether anesthesia. Blood was collected in tubes previously treated with heparin solution and plasma was obtained by removing the blood cells by centrifugation. After thoracotomy and laparotomy the heart was cannulated and the organs perfused with a large volume of cold 0.9 N NaCl solution to remove all the blood from them. The thymus, liver, prostate gland, and a skeletal muscle (soleus) were removed, weighed, and dried. The tissues were ground to powder in a mortar with crushed glass, and 0.05 ml of a 2% solution of sodium dodecylsulfate (SDS) was added to each sample; the specimens thus prepared were put into flasks containing liquid scintillator. The samples were kept for 30 min before radiometry began — long enough for complete extraction of hormone by the scintillator.

To obtain the total fraction of thymocytes, the previously minced thymus tissue was homogenized in a glass/glass homogenizer with finely ground pestle. Hanks' solution (pH 7.2) was used as isolation medium. The resulting tissue homogenate was filtered through Kapron gauze to separate the stroma, and the thymocytes were sedimented by centrifugation at 800g for 10 min. The cells were washed twice to remove adsorbed label with 10 ml of isolation medium and resuspended. All procedures were performed at 0-4°C. The cells were counted in a Goryaev counting chamber. Viability of the thymocytes was determined by vital staining with trypan blue: it was 85-90%. Thymocytes and stroma treated with SDS and also the supernatant obtained after sedimentation of the thymocytes were subjected to radiometry.

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TABLE 1. Specific Radioactivity of Organs (in cpm/mg tissue) and Blood Plasma (in cpm/μl) at Various Times after Intravenous Injection of ^{3}H-Testosterone (M ± mt; p = 0.05)

Organ	Time of testing, min		
	2	5	15
Thymus	21,0±0,4	6,9±0,9	2,9±0,2
Liver	22,6±0,8	20,3±0,9	26,8±1,2
Prostate	23,0±0,9	7,1±0,5	7,5±0,9
Muscle	22,1±0,5	5,3±0,3	2,6±0,3
Blood plasma	49,8±2,4	11,9±0,9	8,1±0,5

TABLE 2. Binding of ^{3}H-Testosterone by Thymocytes Depending on Its Concentration in Incubation Medium in the Absence (A) and Presence (B) of a 300-fold Excess of the Unlabeled Hormone (M ± mt; p = 0.05)

Concentration of ^{3}H-testosterone in medium, nM	Level of bound ^{3}H-testosterone, fmoles/2.5·10^7 cells	
	A	B
0,5	0,93±0,27	0,80±0,33
1,0	1,47±0,29	1,60±0,27
5,0	2,80±0,47	2,53±0,60
10,0	5,33±1,07	4,53±1,23
20,0	14,67±3,33	14,00±2,67
40,0	32,00±5,60	30,00±7,33

To study the character of binding of testosterone with thymocytes investigations were carried out in vitro: 0.22 ml of a suspension of thymocytes (8×10^7 cells/ml) obtained from the thymus of castrated rats by the method described above was incubated with 0.02 ml of ^{3}H-testosterone solution in a final concentration of 5×10^{-10}–4×10^{-8} M in the absence and in the presence of a 300-fold excess of unlabeled hormone. The incubation time was 30 min (37°C). The cells were then washed 3 times to remove adsorbed hormone with 10 ml of cold Hanks' solution, and prepared for subsequent radiometry as mentioned above.

The following mixture was used as the scintillator: PPO 4 g, POPOP 200 mg, methylcellosol 300 ml, toluene to 1 liter. Radiometry was carried out on an SL-30 liquid scintillation counter (Intertechnique, France). The counting efficiency relative to ^{3}H was 30%.

The results were subjected to statistical analysis by Student's test, using Strelkov's tables [3].

EXPERIMENTAL RESULTS

Table 1 gives data on the character of changes in specific radioactivity of the thymus, liver, prostate, and skeletal muscle depending on time after intravenous injection of ^{3}H-testosterone into the animals. The experiments showed that all organs had virtually identical specific radioactivity after 2 min, which subsequently fell in the thymus, prostate, and muscle sharply (by 3-4 times) by the 5th minute, whereas in the liver it remained at its previous level. After 15 min a further decrease in radioactivity of the thymus and muscle was observed, in the prostate it was unchanged compared with the 5th minute, and in the liver it was increased by 18% above the initial level.

The results are evidence that the thymus accumulates ^{3}H-testosterone from the blood to the same degree as the other organs studied, but unlike the liver and prostate, in whose cells testosterone undergoes metabolic transformation [4], the hormone is rapidly excreted from the thymus and skeletal muscle. The possibility cannot be ruled out that the radioactivity of the liver and prostate after 5-15 min was due to the presence not only of labeled testosterone, but also of its metabolites.

Thymocytes, like the stroma of the organ, bind a small proportion (about 5%) of hormone accumulated by the thymus, and most of it can be detected in the intercellular fluid (Fig. 1). No information on the distribution of androgens within the thymus could be found in the literature.

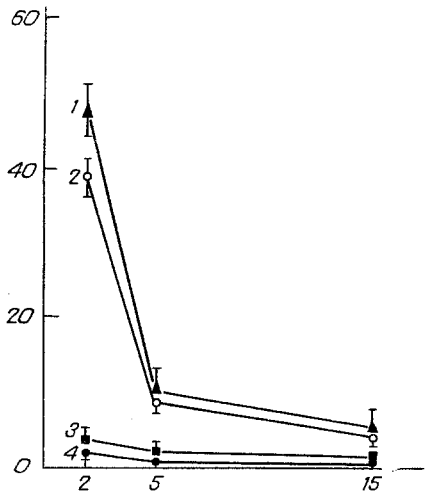

Fig. 1. Accumulation of ^{3}H-testosterone by various fractions of the thymus of castrated rats. Abscissa, time (in min); ordinate, concentration (in fmoles). 1) Thymus (130 mg), 2) intercellular fluid, 3) stroma (6 mg dry weight), 4) thymocytes (8×10^7 cells).

In the next series of experiments, conducted in vitro, dependence of binding of ^{3}H-testosterone by the thymocytes on its concentration in the incubation medium was studied in the absence and in the presence of a 300-fold excess of the unlabeled hormone, which would allow the character of binding of the androgen by the cells to be judged. The results, given in Table 2, point to directly proportional dependence of binding of the labeled hormone on its concentration in the medium within the 5×10^{-10}–4×10^{-9} M range and of the absence of competition between labeled and unlabeled forms of the hormone. The unsaturated character of binding of testosterone by thymocytes indicates that no systems specifically binding androgens are present in these cells.

The results confirm those of an investigation [9] which showed the absence of specific androgen receptors in cytosol isolated from thymocytes. In the opinion of the authors cited, androgen receptors detected in the cytosol from homogenates of whole thymus belong to epithelial cells which disintegrated during isolation of the thymocytes. However, this hypothesis requires experimental confirmation.

It can thus be concluded from the results of this investigation that the concentration of testosterone in the thymus is determined by its concentration in the blood plasma; the thymocytes bind a small proportion of the testosterone accumulated by the organ, probably because of the absence of any systems specifically binding androgens in them. Since thymocytes in vitro do not exhibit sensitivity to androgens [9], it can be postulated that the thymolytic effect of androgens in the intact organism is mediated through other endogenous regulators of lymphocyte metabolism.

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