EFFECT OF ANABOLIC STEROIDS ON PLATELET PROLIFERATION

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Anabolic steroids of varied chemical structure are currently widely used in clinical practice: derivatives of male sex hormones (testosterone, methandrostenolone) and phytoecdysteroids (ecdystene) [1, 3]. The use of anabolic preparations in medicine and sport is accompanied by a number of adverse side effects such as virilization, liver damage, and disturbance of functions of the immune system [1, 3, 4]. A thymolytic effect also has been discovered in cases of repeated administration of large doses of androgen-anabolic steroids to experimental animals [5, 6]. The mechanism of the thymolytic action of anabolic steroids has not been adequately studied. Data in the literature [5, 6] suggest that anabolic steroids can change the proliferative activity of thymic lymphocytes.

The aim of this investigation was to study the action of anabolic steroids on thymocyte proliferation in vitro, incorporation of ^{3}H-thymidine into thymocytes, and the DNA content and weight of the thymus of experimental animals in experiments in vivo.

EXPERIMENTAL METHOD

C57BL/6 mice weighing 18-20 g were kept on the standard animal house diet.

The apparatus used included a microscope, Goryaev counting chamber, centrifuge, Beta-2 scintillation counter, incubator, and SF-46 spectrophotometer. The following reagents were used: liquid nutrient medium 199, fetal calf serum produced by the N. F. Gamaleya Research Institute of Epidemiology and Microbiology, ^{3}H-testerone ("Sigma"), methandrostenolone (All-Union Pharmaceutical Chemical Research Institute), ecdystene (Uzbek Research Institute of Plant Chemistry), TCA, perchloric acid, dimethylsulfoxide, HEPES ("Serva"), ^{3}H-thymidine with specific activity of 37 kBq/mmole ("Izotop"), and Whatman 3MM filter paper. The animals were given intraperitoneal injections of steroid preparations dissolved in dimethylsulfoxide; the quantity of solution injected did not exceed 40 μl/mouse. The substances were given either daily for 10 days or in a single dose 24 h before sacrifice. The solution of ^{3}H-thymidine was injected intraperitoneally 4 h before sacrifice in a dose of 1 μCi/g body weight. The volume of the aqueous solution of the isotope did not exceed 100 μl. The animals were killed by manual cervical dislocation. The thymus glands were weighed and homogenized at 0°C in 5% TCA. The homogenate was transferred quantitatively to test tubes and washed twice with TCA and distilled water, with centrifugation (0-4°C, 2000*g*, 10 min) and resuspension of the residue. The acid-insoluble fraction of the homogenate was subjected to mild alkaline hydrolysis with 0.1 N NaOH at 37°C for 2 h. From each sample 100 μl was transferred into a flask with 10 ml of scintillation fluid. The flasks were kept for 24 h to reduce chemiluminescence, after which their radioactivity was counted on a "Beta-2" counter, with efficiency of 30%. The residual digest was neutralized with 0.1 N HCl, the residue separated by centrifugation, and its DNA concentration determined by Spirin's method. In order to count the cells, the thymocytes were separated by mild homogenization of the thymus followed by washing twice with centrifugation, and counting in a Goryaev's chamber. Incorporation of ^{3}H-thymidine into thymocytes in vitro was studied as follows. A cell suspension was prepared in medium 199 containing 20% fetal calf serum and 20 mM HEPES. The viability of the thymocytes was determined by the trypan blue test: at the beginning of the experiment it was over 90%. The final concentration

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TABLE 1. Effect of Testosterone, Methandrostenolone, and Ecdystene on Weight of Thymus, Number of Thymocytes, DNA Content, and Incorporation of ^{3}H-Thymidine into Mouse Thymus

Preparation	Weight of thymus, mg	Number of thymocytes, $\times 10^9$	DNA content in thymocytes	Incorporation of ^{3}H-thymidine into thymus, $\times 10^3$ cpm
Control	45,9±6,0	0,15±0,02	1,9±0,20	4,1±0,6
	Daily dose of 50 mg/kg for 10 days			
Testosterone	27,7±2,5*	0,07±0,03*	1,28±0,10*	1,9±0,3*
Methandrostenolone	20,8±4,7*	0,08±0,03*	0,94±0,16*	1,7±0,1*
Ecdystene	52,3±4,0	0,14±0,03	2,5±0,1	3,9±0,4
	Daily dose of 5 mg/kg for 10 days			
Testosterone	47,1±3,2	—	2,1±0,23	3,8±0,3
Methandrostenolone	40,1±1,7	—	1,71±0,28	4,2±0,4
	Single injection in a dose of 50 mg/kg			
Testosterone	44,0±7,0	0,13±0,01	2,02±0,25	4,3±0,4
Methandrostenolone	41,0±5,0	0,12±0,02	2,34±0,38	3,7±0,7
Ecdystene	48,0±3,5	0,15±0,04	2,18±0,32	4,0±0,7

Legend. Asterisk indicates significant differences from control at $p < 0.05$ level; n = 10) number of animals in group.

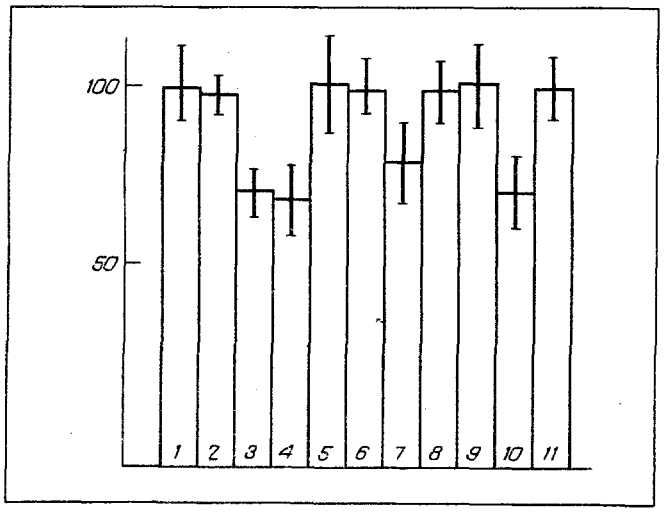

Fig. 1. Effect of testosterone, methandrostenolone, ecdystene, and dexamethasone on incorporation of ^{3}H-thymidine into thymocytes in vitro. Ordinate, incorporation of ^{3}H-thymidine (in per cent of control). 1) Control; 2-4) Dexamethasone in concentrations of 10^{-7}, 10^{-6}, and 10^{-5} M respectively; 5-7) Testosterone (in the same concentrations); 8-10) Methandrostenolone (in the same concentrations); 11) Ecdystene in a concentration of 10^{-5} M.

of cells in the sample was 10^7/ml. Solutions of the preparations in medium 199 were introduced into the tubes containing thymocytes up to final concentrations of 10^{-7}, 10^{-6}, and 10^{-5} M. The samples were incubated for 1 h at 37°C, after which ^{3}H-thymidine was added in a dose of 37 kBq/ml. After 4 h, 100-μl samples of the suspension were taken and applied to filters (2 × 2 cm). The filters were washed twice at 0°C with 5% TCA and distilled water, dried, and placed in flasks for their radioactivity to be counted. The results were subjected to statistical analysis by Student's test with a level of significance of $p = 0.05$.

EXPERIMENTAL RESULTS

Testosterone and methandrostenolone, administered for ten days in a dose of 50 mg/kg daily, caused a significant decrease in weight of the thymus, its DNA content, and incorporation of ^{3}H-thymidine into the thymic tissue. Administration of ecdystene in the same dose caused no significant differences from the control (Table 1). Testosterone, methandrostenolone, and ecdystene, given daily for ten days in a dose of 5 mg/kg or in a single dose of 50 mg/kg, had no thymolytic effect.

The number of cells in the thymus glands of animals receiving 50 mg/kg testosterone and methandrostenolone over a period of 10 days fells on average by 50%. Ecdystene had no effect on the number of thymocytes.

In the next series of experiments the effect of anabolic steroids on proliferation of thymic lymphocytes in vitro, as reflected in the incorporation of ^{3}H-thymidine into thymocyte suspensions in the presence of different concentrations of these substances (Fig. 1). Dexamethasone was used for comparison [2, 6]. The experiment showed that proliferative activity of the thymocytes was unchanged in the presence of the steroids in concentrations of 10^{-7} and 10^{-6} M. Testosterone and methandrostenolone in a concentration of 10^{-5} M significantly reduced incorporation of ^{3}H-thymidine by 30% compared with the control. Dexamethasone caused a similar effect in a concentration of 10^{-6} M. Many lipophylic steroid compounds, including some without hormonal activity [6], are known to inhibit the proliferative activity of lymphocytes in a concentration of 10^{-5} M. Ecdystene is a hydrophilic substance, and that may be the reason why it does not affect thymocyte proliferation.

It was shown previously that androgens, unlike glucocorticoids, do not possess lympholytic activity in vitro [5, 6]. It can be tentatively suggested that one possible mechanism of the thymolytic action of androgen-anabolic steroids is inhibition of proliferation of thymic lymphocytes.

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