EFFECT OF VARIOUS DOSES OF WATER-SOLUBLE RETINOIC ACID ON LIPID CONCENTRATION AND 3-β-OL-STEROID DEHYDROGENASE ACTIVITY IN INTERSTITIAL TISSUE AND SUSTENTACULAR CELLS OF THE MOUSE TESTIS

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To guarantee the normal generative function of the male, sex steroids synthesized in the testes by interstitial tissue cells (Leydig's cells) and also, possibly, by the sustentacular cells (SC) of the seminiferous tubules (the follicular cells of Sertoli) are essential. The view is held that SC, by producing androgens, regulate the development of male sex cells locally in the seminiferous tubules. This is confirmed to some extent by the asynchronous character of spermatogenesis along the length of the seminiferous tubule [6, 7, 9-11]. Vitamin A has a strong influence on testicular function [2, 8, 12, 13]. It is used in medicine for prophylactic and therapeutic purposes in the form of several different preparations [3]. Among the new vitamin A preparations, with a powerful action on the body, water-soluble retinoic acid (WSRA) has attracted attention. However, its effect on several organs, including the male gonads, has been inadequately studied.

The aim of this investigation was to study the effect of WSRA on synthesis of male sex hormones in the mouse testis.

EXPERIMENTAL METHOD

Experiments were carried out on 20 male CBA/C57BL mice with an average weight of 18-20 g. WSRA was synthesized in the Laboratory of Chemistry of Polyene Compounds (Head, Professor G. I. Samokhvalov, "Vitaminy" Research and Production Division, Ministry of the Medical Industry of the USSR.) WSRA was injected intraperitoneally into animals of three groups in the form of a 1% solution in total doses of 0.1, 0.2, and 0.3 ml respectively. Animals receiving 0.1 ml of physiological saline, which was the solvent for the retinoic acid, served as the control. Material was taken and fixed and histochemical reactions were carried out in accordance with the requirements for quantitative methods of investigation used in morphology [1]. Steroid-producing capacity and its activity were judged by the demonstration of lipid-like substances and a positive reaction for the enzyme 3-β-ol-steroid dehydrogenase (3-β-ol-SD), and also by determination of the optical density of the above-mentioned substances in the cells. Frozen sections 9 μ thick were stained with oil to demonstrate 3-β-ol-SD [3-5]. The substrate for this reaction was dehydroepiandrosterone. The optical density of the substances in the cells was determined on a cytospectrophotometer (from Reichert, Austria) by a single-wave multiple point method, with a probe 1 μ in diameter, and at the following wavelengths: 550 nm to detect lipids, 550 nm for 3-β-ol-SD. Concentrations of lipids and 3-β-ol-SD were expressed in absorbance units.

EXPERIMENTAL RESULTS

Lipids were discovered in the interstitial cells (IC) and SC of the testes. Their concentration in IC of the control animals was significantly higher than in SC – 3.0±0.38 and 2.0±0.26 respectively. Comparative analysis of the lipid content in the control and experimental animals showed an increase in the concentration of these substances in the cells after injection of WSRA. The degree of increase in the concentration of the

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TABLE 1. Changes in Concentrations of Lipids and 3-β-ol-SD in IC and SC of Testes after Injection of WSRA (M±m, $P < 0.05$)

Substance	Type of cells	Control	Injection of WSRA		
			0,1 ml	0,2 ml	0,3 ml
Lipids	IC	3,0±0,38	8,0±0,9	9,1±0,94	10,0±0,2
	SC	2,0±0,26	3,76±0,4	4,0±0,14	3,0±0,08
3-β-ol-SD	IC	6,8±0,18	9,6±0,2	13,9±0,22	8,5±0,11
	SC	4,0±0,12	5,6±0,18	6,5±0,14	2,9±0,12

TABLE 2. Concentration of Lipids and 3-β-ol-SD in IC and SC of Testes after Injection of WSRA (in % of control)

Experimental conditions	Lipids		3-β-ol-SD	
	IC	SC	IC	SC
	100	100	100	100
Injection of WSRA				
0,1 ml	267	188	141	140
0,2 ml	303	200	204	162,5
0,3 ml	333	150	125	72,5

substances depended on the type of cells and dose of the compound. The highest lipid level was found in IC of the testes, and it rose with an increase in the dose of the compound. For instance, after injection of 0.1 ml of the solution the lipid content in IC was increased by 167% of the control level, and after injection of 0.3 ml the increase was 233%. The increase in the lipid content in SC under the influence of WSRA was less marked. Injection of WSRA in a dose of 0.1 ml caused an increase of only 88% in the lipid concentration in SC, an increase of 100% was observed after injection of 0.2 ml, and after injection of 0.3 ml of WSRA the lipid content actually fell a little below that observed when smaller doses of the compound were used, and it exceeded the control level only by 50%.

Activity of 3-β-ol-SD (a key enzyme of steroid production) was found in IC and SC of the testis. In the control the concentration of this enzyme in cells of both types differed: It was higher in IC (6.8±0.18) and lower in SC (4.0±0.12) WSRA caused changes in the 3-β-ol-SD concentration in cells of the testis. Injection of 0.1 ml of WSRA increased the enzyme concentration in IC by 141% compared with the control. WSRA in a dose of 0.2 ml had a more significant effect: 3-β-ol-SD activity in the cells of the tetis rose by 204%. After injection of 0.3 ml of WSRA activity of the enzyme exceeded that in control by 125%, which was appreciably lower than when smaller doses of the compound were given (Tables 1 and 2).

The study of the action of WSRA on the 3-β-ol-SD concentration in SC revealed rather different changes. To begin with, their amplitude was lower than in IC. In a dose of 0.1 ml WSRA caused an increase in concentration of the enzyme in SC by about the same degree as in IC, but in a dose of 0.2 ml it increased activity of the enzyme in SC by only 62.5% compared with the control, i.e., its effect was several times weaker than in IC. After injection of WSRA in a dose of 0.3 ml a quite substantial fall in the 3-β-ol-SD concentration was found in SC (72.5% of the control).

The steroid-producing structures in the testes are thus IC and SC. They can accumulate lipids, which are the original material for steroids, and they possess activity of 3-β-ol-SD, a key enzyme for steroid synthesis. Under these circumstances activity of 3-β-ol-SD is significantly higher in IC than in SC. Under the influence of WSRA in a dose of 0.1-0.2 ml the lipid concentration and enzyme activity in the cells were increased by a greater degree in IC and by a lesser degree in SC. Injection of a larger dose of WSRA (0.3 ml) had the opposite effect: The lipid concentration and enzyme activity fell in both IC and SC, but below the control level in SC. This is probably associated with the state of hypervitaminosis A, the clinical manifestations of which were visible in the animals of this group.

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