Activity of phospholipases A and some lysosomal enzymes in rat testes at different stages of hypervitaminosis A

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Summary. Excess of vitamin A induces decrease of neutral phospholipase A_1 and A_2 activity in rat testes homogenates on the 4th day, and increase of β-galactosidase activity on the 8th day of treatment. It is suggested that phospholipase A activity decrease is of great importance in development of testicular disorders, caused by disbalance of vitamin A.

Our previous investigations have shown that retinol deficiency resulted in the impairment of spermatogenesis which was accompanied by a significant decrease in the activity of phospholipases A_1 (PLA_1) and A_2 (PLA_2) with optimum pH 7.4-key enzymes of phospholipid metabolism as well as by alterations in the activity of lysosomal enzymes of rat testis[2,3]. Recently Lopes et al. demonstrated that the other form of severe vitamin A-imbalance, hypervitaminosis A, also caused testicular degeneration and delay in spermatogenesis[4].

Therefore the present investigation was undertaken to study the effect of hypervitaminosis A on the activities of neutral phospholipases A (PLA), and also of some enzymes of lysosomes, cell organells which are considered to be important in spermatogenesis[5].

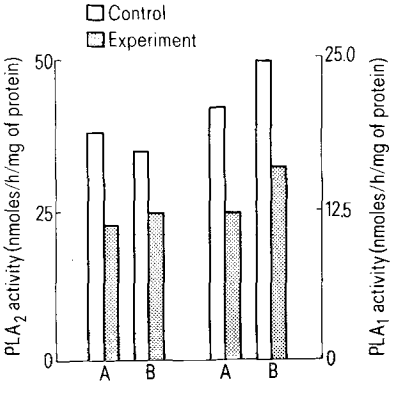
Materials and methods. Male albino Wistar rats (80–90 g) were used in our experiments. Hypervitaminosis A was induced by feeding animals 50,000 IU of retinyl-acetate p.o. daily per rat in groundnut oil. Control rats recieved equivalent amount of groundnut oil. Rats were killed on 4th and 8th day of experiment by decapitation. The testes were removed, decapsulated and homogenized in 0.25 M sucrose, contained 0.001 M EDTA (pH 7.4). PLA activity was assayed in prepared homogenates as previously described[2,3] using 1-acyl-2-[1(C^{14}]-oleoyl-sn-glycero-3-phosphocholine as substrate. The radioactivity was determined in liquid scintillation counter Nuclear Chicago (Mark II). The activities of PLA_1 and PLA_2 were expressed as nmoles of lysolecitine or fatty acids, respectively, produced in 1 h per mg of protein. Activities of acid phosphatase and β-galactosidase in homogenates were assayed using p-nitrophenylphosphate and o-nitrophenyl-β-D-galactopyranoside, respectively, as substrates accordingly to procedures described by Males and Turkington[6] and Majumder et al.[7] and expressed as nmoles of substrate transformed in min per mg of protein.

Results and discussion. Administration of high doses of vitamin A did not change the general condition of animals on the 4th day of experiment. However on the 8th day, the rats showed typical signs of hypervitaminosis A. Body and testicular weight was significantly decreased in experimental animals as compared to controls.

In spite of the absence of clinical symptoms of hypervitaminosis A, the activity of PLA_1 and PLA_2 was significantly decreased on the 4th day of the experiment, as compared to the control rats (figure). Further administration of vitamin A did not induce any more decrease in the activity of PLA_1 and PLA_2. No alterations in the activity of acid phosphatase were observed on day 4 and 8 of the experiment. However, β-galactosidase activity was significantly increased on the 8th day.

Thus our results showed that the excess of vitamin A caused significant changes in the activity of neutral PLA_1 and PLA_2, as well as in the activity of lysosomal enzyme-β-galactosidase. It seems to be the first investigation showing that the excess of vitamin A causes pronounced biochemical changes in rat testes. It is worth noting that the decrease of PLA_1 and PLA_2 activity was found at early stage of intoxication when the general condition of animals practically did not change.

In our previous work, it was shown that the impairment of spermatogenesis in rat testes, induced by retinol deficiency, was accompanied by decrease of neutral PLA_1 and PLA_2 activity. The results of this work demonstrate that the excess of vitamin A also causes considerable decrease in the activity of these enzymes. According to the data of Lopes et al.[4], there are pronounced disturbances of spermatogenesis in rat testis in hypervitaminosis A. Taken together, these facts indicate that the impairment of spermatogenesis induced by either deficiency or excess of vitamin A is accompanied by great decrease in neutral PLA_1 and PLA_2 activity. It may be suggested, therefore, that decrease of activity of these enzymes may be of great importance in development of testicular degeneration caused by vitamin A-imbalance. Well-known data about the key role of PLA in degradation of phospholipids, which in their turn are of great importance for building and functioning of biological membranes, and the fact of active metabolism of cell and subcellular membranes in testes during process of spermatogenesis make this suggestion especially attractive.



Activity of neutral phospholipases A_1 and A_2 in rat testes at different stages of hypervitaminosis A. *A* 4th day of experiment (150,000 IU of vitamin A); *B* 8th day of experiment (350,000 IU of vitamin A).

Activity of lysosome enzymes in rat testes homogenates at different stages of hypervitaminosis A

Enzyme	Day 4 of experiment (150,000 IU of vitamin A)		Day 8 of experiment (350,000 IU of vitamin A)	
	Control	Hypervitaminosis A	Control	Hypervitaminosis A
Acid phosphatase	17.70 ± 0.76*	15.20 ± 0.65**	24.80 ± 0.69	26.60 ± 1.08**
β-galactosidase	1.95 ± 0.04	1.84 ± 0.12**	1.50 ± 0.14	2.25 ± 0.19***

* Each figure is the mean ± SD from 6–7 experiments; ** $p > 0.05$; *** $p < 0.02$.

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Comparative study of the sensitivity of acetylcholinesterases and cholinesterases from animal and bacterial sources to inhibition by serotonin and its derivatives

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Summary. Serotonin was found to inhibit human erythrocyte and electric-eel acetylcholinesterase activities. The serotonin amino group, free of negative charges in its vicinity and its hydroxyl group, were important for the inhibition. Serotonin precursors and several related compounds had little or no effect. Human plasma cholinesterase was also inhibited by serotonin and tryptamine. In contrast to these animal enzymes, the cholinesterase of *Pseudomonas aeruginosa* was refractory to serotonin and its derivatives under the same experimental conditions.

Serotonin and acetylcholine (ACh) are closely related in their diverse important biological and pharmacological activities, such as neurohumoral transmission, constrictory and dilatory effects on muscle and blood vessels, etc.[1-3]. Both of them are of wide occurrence in nature, frequently present in the same tissues as they are in brain. The observation that serotonin inhibits the activity of brain and erythrocyte acetylcholinesterases[4] was therefore of interest.

In the present investigation, the effect of serotonin derivatives and related compounds on acetylcholinesterases (AChE) and cholinesterases (ChE) from different sources was compared. The aim of this comparison was to evaluate the contribution of the serotonin structural components to its effect and to determine the enzymes sensitivity to inhibition by it. The enzymes employed were: human erythrocyte[5] and electric-eel[6] acetylcholinesterases and human plasma and *Pseudomonas aeruginosa*[7,8] cholinesterases.

Materials and methods. Human erythrocytes were separated from heparinized venous blood from healthy donors, by centrifugation at 1000×g for 10 min (4 °C) and washed 3 times in 0.9% NaCl solution. 1 vol. of the thrice washed cells was suspended in 9 vol. of either 0.9% NaCl solution ('intact erythrocytes') or distilled water with sonication (80 sec in MSE 150 W ultrasonic disintegrator Mk2) and after centrifugation at 30,000×g for 10 min, the supernatant fluid was separated ('hemolysate'). Both the 'intact erythrocytes' suspension and the 'hemolysate' were diluted 1:10 before use for AChE determination.

Electric-eel *(Electrophorus electricus)* AChE purified preparation was purchased from Sigma and used at a concentration of 0.003 ng/ml for the enzyme activity determination. Human plasma was separated from heparinized venous

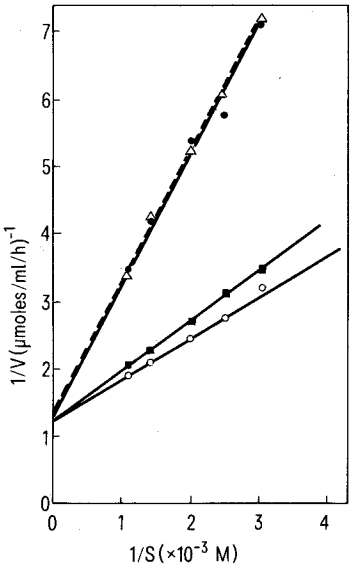

Fig. 1. Double reciprocal plot indicating the kinetics of the reaction of human erythrocyte AChE with varying concentrations of ATCh ○——○ and in the presence of serotonin-creatinine sulfate ●——●, serotonin oxalate △– – –△ and creatinine sulfate ■——■ employing intact erythrocytes or hemolysate and Ellman's reaction[9]. The plots represent mean values of 6 experimental results.

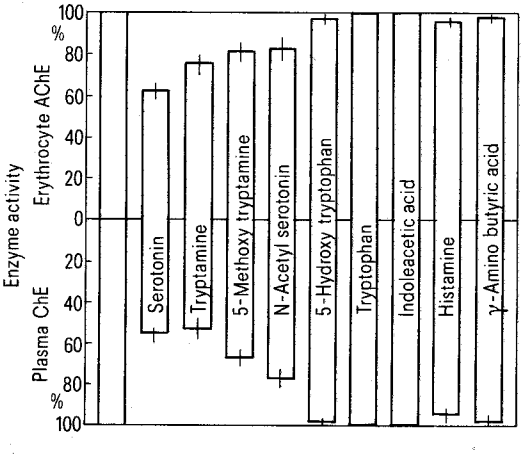

Fig. 2. Effect of serotonin, its derivatives and related compounds on the activities of human erythrocyte AChE and plasma ChE. Each compound was included at a final concentration of 2×10^{-3} M. Acetylcholine concentration was 7 μmoles/ml and 100% enzyme activity catalyzed decomposition of 3 μmoles of it during 30 min at 37 °C (determined by the hydroxamate reaction according to Hestrin[10] and confirmed by Ellman's reaction). The columns represent mean values of 6 experimental results. SEM are represented by vertical bars.