ATP CONTENT IN PLATELETS AT VARIOUS STAGES OF ONTOGENY

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The intracellular content of ATP was determined by a bioluminescence method in the platelets of rabbits of two age groups. The results showed that platelets of rabbits aged 7-8 days contain 5.8 ± 0.7 μmoles and platelets of adult rabbits 8.9 ± 0.9 μmoles ATP per 10^{11} cells. The difference discovered is not due to the procedure used to isolate platelets from the blood.

KEY WORDS: ATP; platelets; ontogeny.

One of the most important indices of the metabolic activity of the cell is the intracellular ATP level [1, 3]. There have been several recent studies of the ATP content in the platelets of man and laboratory animals [4, 7, 8, 10]. However, no such investigations have been undertaken from the age aspect.

The ATP content in the platelets of rabbits of different age groups was determined.

EXPERIMENTAL METHOD

The intracellular ATP content was determined by a bioluminescence method [5, 9] using unpurified extracts of the phosphorescent organs ("lamps") of the glowworm Luciola. The dried "lamps" were kept in sealed ampules at −20°C. To obtain a luciferin–luciferase system about 30 mg of "lamps" was ground with sand on ice and extracted with 15 ml of cold isotonic tris-acetate buffer (pH 7.4). The extract was centrifuged at 630 g for 5 min. The supernatant was treated with 100 mg $MgSO_4$ and a sample of 0.2 ml was placed in a constant-temperature cell at 24°C, into which 0.15 ml of the test solution was injected from a semiautomatic syringe. Emission of light thereupon took place and was recorded with the FÉU-29 instrument. The signal from the photoelectronic multiplier was amplified by the ÉD-05 electrometer and recorded on a KSP-4 self-writing potentiometer. The signal intensity was a linear function of ATP concentration in the test solution over a wide range of concentrations. The mean error of measurement of the ATP concentration under these conditions was 10%.

Samples for determination of the ATP content were prepared as follows. Platelet-rich plasma was obtained from citrated rabbits' blood in the usual way and poured into graduated centrifuge tubes in doses of 0.7 ml. To each tube 0.14 ml of 78 mM EDTA in physiological saline was added and the contents were shaken and centrifuged at 630 g for 5 min to sediment the platelets. The plasma was removed and the sedimented cells carefully resuspended in 0.7 ml tris-acetate buffer. The cells were counted in a Goryaev's chamber by phase-contrast microscopy. Next, 2.3 ml of boiling hypotonic tris-acetate buffer was added to the suspension, which was kept for 20 min on a boiling water bath. The samples were cooled, centrifuged at 1100 g for 10 min, and the ATP concentration was determined in the supernatant. The content of extracellular ATP in native plasma was determined in control experiments. By means of a micropipet, 0.2 ml of platelet-rich or platelet-poor plasma was added to the cell containing the extract. Measurements were made 30 sec after addition.

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For the quantitative estimation of ATP internal standards were used: The intensity of the signal from the test sample was compared with the intensity of the signal produced after addition of a known quantity of ATP to the same sample.

EXPERIMENTAL RESULTS

The intracellular ATP content in the platelets of adult rabbits (9 experiments) was 8.9 ± 0.9 μmoles/10^{11} cells, but in platelets of young rabbits aged 1 week (12 experiments) it was only 5.8 ± 0.7 μmoles/10^{11} cells ($P < 0.01$).

The lower intracellular ATP content in the platelets of animals in the early stages of ontogeny is not the result of loss of ATP in the course of their separation from the blood. In fact, in platelet-rich plasma containing 500,000 cells/mm^3 the loss of one-third of the intracellular ATP would have led to the appearance of extracellular ATP in a concentration higher than 10^{-5} M. If exogenous ATP was added to whole blood in that concentration, after 5 min its concentration fell to $0.4 \cdot 10^{-5}$ M, and after 10 min to $0.2 \cdot 10^{-5}$ M. In native plasma immediately after sedimentation of the red cells, i.e., 4-5 min after stopping the centrifuge, and also after sedimentation of the platelets which was always carried out in the presence of EDTA (0.015 M) which inhibits the hydrolysis of extracellular ATP in the plasma, the concentration of extracellular ATP did not exceed $2 \cdot 10^{-7}$ M. Consequently, the substantial differences discovered in these experiments in the intracellular content of ATP in the platelets of rabbits of different ages were not attributable to the isolation procedure.

The method used in this investigation is capable of determining only the total ATP content in platelets. It is quite possible, however, that in the blood platelets of animals in the early stages of ontogeny the reserves of metabolically active ATP are lowered as well as those of granular ATP.

The existence of a close connection between the metabolism of a cell and its functional activity is now not in dispute. There is abundant experimental evidence to show that an increase in the level of metabolically active ATP leads to improved functioning of energy-dependent systems of the cell and to their greater resistance to various harmful factors [2, 6]. Performance of their basic function of hemostasis by platelets is connected with their ability to respond to various (chemical, optical, mechanical) stimuli by a specific assortment of biochemical and structural transformations, leading to adhesion and aggregation. Very probably increased ability of the platelets to undergo adhesion and aggregation corresponds to some decrease in the level of intracellular ATP and reduced resistance of the cell systems to external factors linked with it. In this connection it can be expected that the platelets of animals in the early stages of ontogeny, characterized by a lower ATP level, would be more sensitive to changes in external conditions and, possibly, to the action of certain aggregating agents than the platelets of adult animals.

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