METABOLISM OF THE FREE NUCLEOTIDES OF THE LIVER IN EXPERIMENTAL ATHEROSCLEROSIS

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UDC 616.13-004.6-092.9-07:616.36-008.939.633

In atherosclerosis the lipid metabolism is disturbed, protein synthesis is slowed [3], the activity of enzymes is lowered [4, 5, 9, 10, 12, 15, 22, 26, 27], and tissue respiration is inhibited in several organs [6, 7]. All these changes may be associated with a disturbance of the metabolism of the free nucleotides.

The free nucleotides perform many different functions in metabolism. Adenosinetriphosphate (ATP) activates coenzyme A (CoA) in the reaction of formation of acetyl-CoA [13, 17] and amino acids with the formation of aminoacyladenylates. Cytosine triphosphate (CTP) and ATP take part in the biosynthesis of lipids [21]. Besides ATP, guanosinetriphosphate (GTP) or guanosinediphosphate (GDP) are essential for the introduction of amino acids into proteins [18]. The nucleotides are the precursors of the nucleic acids [23, 24].

Investigations have shown a decrease in the content of free nucleotides in various organs with age [19, 20, 25]. One of the nucleotides (ATP) has been used for the treatment of atherosclerosis. When given by subcutaneous injection it inhibited the development of this disease [14].

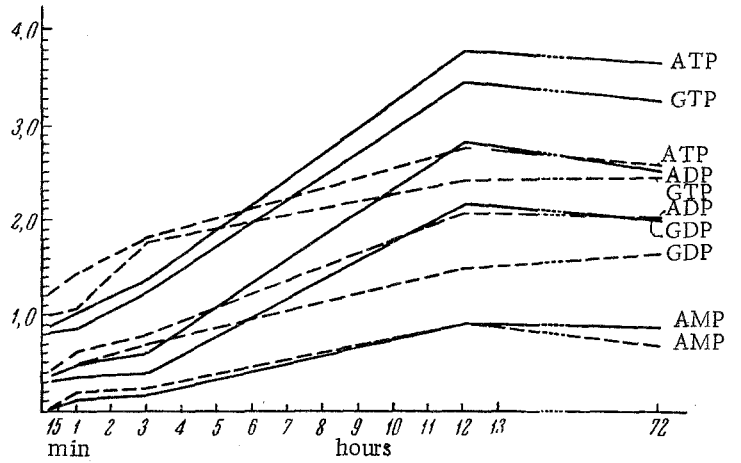
There is no information in the literature on the intensity of metabolism of the free nucleotides in atherosclerosis. The object of the present investigation was to study the rate of metabolism of the free nucleotides in the liver in rabbits with experimental atherosclerosis.

EXPERIMENTAL METHOD

Experiments were carried out on rabbits weighing 3.0-3.5 kg. Atherosclerosis was produced in the rabbits by the addition of cholesterol to the diet at the rate of 0.5 g/kg body weight daily for 3-3½ months. The cholesterol was given with shredded carrot or beetroot. Radioactive phosphate (P^{32}), in the form of disubstituted sodium phosphate, was injected intraperitoneally in a dose of 0.2 μCi/g body weight. The rabbits were decapitated 15 min and 1, 3, 12, and 72 h after injection of the isotope. A piece of liver was then immediately frozen under a jet of carbon dioxide. At the same time blood was taken from the animals' aorta to detect the presence of atherosclerosis. The nucleotides were isolated by the method described by T. N. Ivanova, N. I. Pravdina, and L. N. Rubel' [2]. Samples (weighing 5 g) of the frozen liver tissue were homogenized with 50 ml of 10% trichloroacetic acid (TCA), cooled to 0°. The nucleotides were extracted at 0° for 20 min and the homogenate was centrifuged in a refrigeration centrifuge. The nucleotides were precipitated from the TCA extract in the form of mercury salts by the addition of 0.1 volume of a 20% solution of mercury acetate at pH 2.5-3.0. The precipitate of nucleotides was dissolved in 1.5 ml of 0.5 N HCl. The mercury was removed in the cold by a current of hydrogen sulfide. After centrifugation the transparent supernatent was investigated by distributive chromatography on Leningrad No. 2 Factory paper and washed with a 1 N solution of HCl and Trilon B. Chromatography was carried out successively in two solvents: an acid solvent N-propanol – N butanol – acetone – 30% TCA – 80% HCOOH (20:40:26:12:26, ascending, 24 h) and an alkaline solvent N propanol – 22% ammonia – H_2O (60:46:4, descending, double distillation, 48 h each time). To determine the distribution of inorganic phosphorus, strips of individual chromatograms were developed by the method of Bandurski and Axelrod [11], using the developer recommended by Hanes and Isherwood [16]. The localization of the nucleotide stains on the chromatograms was determined in the UI-1 ultrachemiscope at 255 mμ. Stains absorbing in the ultraviolet were cut out and the radioactivity in them determined by a T-25-BFL end-type counter together with a type PP-8 apparatus. The nucleotides were extracted with 0.1 N HCl solution for 1 h with constant agitation on a shaker.

The nucleotides were identified from the ratio between ribose and phosphorus (total and labile), by "witnesses," by the ratio between the absorptions E_{250}/E_{260}, E_{280}/E_{260}, and E_{290}/E_{260} [2], and the arrangement of the guanyl nucleotides on the chromatograms was further determined from their fluorescence in ultraviolet light in an acid medium [8].

Department of Biochemistry, Vitebsk Medical Institute (Presented by Active Member of the Academy of Medical Sciences of the USSR S. E. Severin). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 63, No. 4, pp. 53-55, April, 1967. Original article submitted July 24, 1965.



Relative specific activity of free nucleotides in rabbits' liver. Explanation in text. Continuous lines – experiment; broken lines – control.

The phosphorus of the nucleotides was estimated quantitatively by the Fiske – Subbarow method as modified by Braunshtein [1]. The inorganic phosphorus was extracted from the TCA extract by isobutanol, and its content was determined colorimetrically and the radioactivity determined after the isobutanol extract had been placed on a target and evaporated.

The intensity of metabolism of the free nucleotides was determined by calculating their relative specific activity (RSA), i.e., the ratio between the activity of 1 μmole of nucleotide and the specific activity of 1 μmole inorganic phosphate of the acid-soluble fraction of the liver.

The lipids of the blood serum were investigated. They were extracted with 20 volumes of a 3:1 mixture of alcohol and ether for 30 min during boiling with a reflux condenser. The phospholipids were estimated as phosphorus after mineralization, and the cholesterol by the reaction with acetic anhydride and sulfuric acid. The serum protein and lipoprotein fractions were investigated by electrophoresis on paper.

EXPERIMENTAL RESULTS

The cholesterol concentration in the blood serum of the experimental rabbits was increased on the average to 420 mg%, the phospholipids to 382 mg%, and the cholesterol/phospholipids ratio to 1.099 compared with 57/175 mg% and 0.326 respectively in the control. Proportion of β-lipoproteins was also increased to 86.2% and the atherogenic index to 6.36 compared with 56.4% and 1.31 in the controls. The changes in the protein fractions were not statistically significant. Typical atherosclerotic changes were observed in the aorta of the experimental group of rabbits.

In all the chromatograms five distinct stains were visible in ultraviolet light, and identified as GTP, GDP, ATP, ADP, and AMP. The RSA of these nucleotides 15 min and 1, 3, 12, and 72 h after injection of the isotope are shown in the figure. The values of the RSA of the ATP, ADP, GTP, and GDP in the experimental animals 15 min and 1 and 3 after injection were much lower than in the controls. In the case of AMP, the decrease was significant only 1 and 3 h after injection. It may be concluded from these results that the incorporation of P^{32} into the free nucleotides of the liver was slowed in the rabbits with experimental atherosclerosis, i.e., that the synthesis of these compounds was retarded. After 12 and 72 h, the RSA of the free nucleotides was higher in the experimental animals than in the control. The exception was AMP, the RSA of which was higher after 72 h only. This indicates slowing of the elimination of the label from the free nucleotides.

The absolute values of the RSA 12 and 72 h after injection of the isotope were higher than 3 h after injection. This was connected with the more rapid decrease in radioactivity of the inorganic phosphate of the liver compared with the decrease in radioactivity of the free nucleotides.

It is concluded that the slowing of metabolism of the free nucleotides discovered in these experiments may play a role in the disturbance of lipid and protein metabolism and the lowering of the activity of the enzymes in atherosclerosis.

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