FREE ADENINE NUCLEOTIDES IN HUMAN LEUKOCYTES

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The following content of ATP, ADP, and AMP was determined by specific enzymic methods in human white blood cells in micromoles per 10^9 cells: in a donor's granulocytes, 1.22, 0.46, and 0.14; in a donor's lymphocytes, 1.4, 0.53, and 0.15; in the leukocytes of patients with chronic myeloid leukemia, 1.55, 0.38, and 0.20; in the lymphocytes of patients with chronic lymphatic leukemia, 0.76, 0.23, and 0.12; in the leukocytes of patients with acute myeloblastic leukemia, 1.94, 0.45, and 0.20; and in the lymphocytes of patients with acute lymphoblastic leukemia, 2.02, 0.41, and 0.18. Under anaerobic conditions there is virtually no decrease in the ATP level in the leukocytes.

Information on the content of free adenine nucleotides in the leukocytes is limited either to the combined determination of ATP and ADP or the content of ATP in leukemic cells [8, 9]. Separate figures for the content of ATP and ADP are available only for normal granulocytes [7].

This paper describes the results of a study of the content of ATP, ADP, and AMP in healthy human granulocytes and lymphocytes and also in the white blood cells of patients with various forms of leukemia.

EXPERIMENTAL METHOD

Leukocytes isolated by methods described previously [2, 3] from the blood of healthy donors and patients with leukemia were studied. Suspensions of granulocytes obtained from normal blood contain 85-90% of these cells, while suspensions of isolated lymphocytes contained 95-90% of principally small lymphocytes. Suspensions containing up to 96% of cells of the myeloid series, of different degrees of maturity (not more than 3% of myeloblasts) were obtained from the blood of patients with chronic myeloid leukemia; in the case of chronic lymphatic leukemia, 96-98% of the cells in the suspension consisted of lymphocytes. In acute leukemia the number of undifferentiated cells varied from 75 to 98%.

The content of ATP, ADP, and AMP was determined in perchloric acid extracts of cells neutralized with K_2CO_3 to pH 7.4 by a combined enzymic and spectrophotometric method, the absorption of light ultimately being measured at 340 nm.

The composition of the samples for determination of ATP (total volume 3 ml) was as follows: 1 ml neutralized perchloric acid extract of the cells, 150 μmoles tris-buffer, pH 7.4; 20 μmoles $MgCl_2$; 0.5 μmoles NAD · H_2, 2 μmoles phosphenolypyruvate (PEP). After stabilization of the readings, lactate dehydrogenase containing PEP-kinase was added (150 μg protein). After the decrease in extinction had ceased, and determination of ADP was thus complete, myokinase (100 μg protein) was added to the same system and the readings were continued to determine AMP.

In the determination of ATP an increase of 2.073 in optical density (at 340 nm) corresponded to 1 μmole ATP (in a volume of 3 ml).

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TABLE 1. Content of Adeninenucleotides (in μmoles/10^9 cells) in Healthy Human Leukocytes and Leukocytes of Patients with Leukemias

Persons from whom cells were obtained	ATP	ADP	AMP	ATP + ADP + AMP
Leukocytes				
Donor	1,22±0,11 (13)	0,46±0,04 (11)	0,14±0,02 (10)	1,82±0,17
Patients with chronic myeloid leukemia	1,55±0,11 (10)	0,38±0,03 (10)	0,20±0,03 (10)	2,13±0,17
P	<0,05	>0,1	>0,1	
With acute myeloblastic leukemia	1,94±0,14 (7)	0,45±0,11 (7)	0,20±0,06 (7)	2,59±0,31
P	<0,02	>0.1	>0,1	
Lymphocytes				
Donor	1,40±0,09 (7)	0,53±0,05 (7)	0,15±0,02 (7)	2,08±0,16
Patients with chronic lymphatic leukemia	0,76±0,04 (12)	0,23±0,02 (12)	0,12±0,02 (12)	1,11±0,08
P	<0,01	<0,01	>0,2	
With acute lymphoblastic leukemia	2,02 (2)	0,41 (2)	0,18 (2)	2,61

In the determination of ADP, a decrease of 2.073 in optical density corresponded to 1 μmole ADP. The determination of AMP was carried out in the same samples as ADP, but after completion of determination of the latter. In the calculations it was taken that 2 μmoles ADP is formed per mole of existing AMP.

The following preparations were used: crystalline hexokinase (Gee Lawson Chemicals Ltd, England); glucose-6-phosphate dehydrogenase in NADP (Fluka A. G., Buchs G., Switzerland); lactate dehydrogenase containing PEP-kinase (Schnuchardt T., West Germany); myokinase and NAD · H_2 (Biochemica "Boehringer," West Germany); PEP and ATP (Sigma Chemical Co., USA).

EXPERIMENTAL RESULTS

The results are given in Table 1 show that in cells of the myeloid series (normal granulocytes – leukocytes of patients with chronic myeloid leukemia – blast cells from patients with acute myeloid leukemia), rejuvenation of the cell composition is accompanied by an increase in energy potential.

It is also clear that undifferentiated blood cells from patients with acute lymphatic leukemia contain the same quantity of adenine nucleotides as cells of myeloid origin. Hence, regardless of their tissue origin, blast cells contain significantly larger quantities of the components of the adenylic system than more mature cells.

The writers have previously shown that all morphological varieties of white blood cells resynthesize ATP completely regardless of the conditions of aeration. However, in investigations during previous years the method of acid hydrolysis of nucleotides after their absorption with charcoal was used, and in this way it is impossible to determine the proportions of diphosphates and triphosphates separately. In the present investigation the concentrations of ATP, ADP, and AMP were determined in enzymic reactions in cells incubated under both aerobic and anaerobic conditions. These experiments showed that in the absence of oxygen, but in the presence of glucose in the medium, the general level of adenine nucleotides and their relative proportions remained virtually the same as when a free access of air was allowed. For example, in healthy human granulocytes during incubation under aerobic conditions the level (in μmoles/10^9 cells) of ATP was 1.36, of ADP 0.59, and of AMP 0.15, while under anaerobic conditions the values were 1.27, 0.47, and 0.15, respectively, in leukocytes of patients with chronic myeloid leukemia the figures were 1.63, 0.38, 0.21, 1.49, 0.41, and 0.19, respectively, and in the lymphocytes of patients with chronic lymphatic leukemia 0.74, 0.14, 0.07, 0.68, 0.16, and 0.07. In other words, human leukocytes can maintain their inherent energy potential at the normal level not only under aerobic conditions, but also on account of the glycolytic mechanism only in a strictly anaerobic medium.

Hence, leukemia transformation of white blood cells of the myeloid series leads to an increase in their content of free adenine nucleotides, principally of ATP: moderate in the case of chronic myeloid leukemia and considerable in the case of acute myeloid leukemia.

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