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Maturation-dependent changes of the rabbit reticulocyte energy metabolism

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Rabbit reticulocytes were separated into four fractions of different maturity in order to investigate the changes of cellular respiration and glycolysis, adenine nucleotides, 2,3-biphosphoglycerate (2,3-BPG) as well as cyclic AMP level during the transition from the youngest to the most mature reticulocytes. A significant reduction of total oxygen consumption, mainly due to depression of coupled respiration was found. The decline of respiration was accompanied by a 2-fold increase of the rate of aerobic glycolysis indicating a reduced Pasteur effect during maturation. A decline of ATP and an increase of ADP concentration was found. The oxygen-delivery capacity of the red cells increased by about 26% caused by an increase of the 2,3-BPG level of about 2 mmol/l cells. Cyclic AMP level in the fraction of youngest reticulocytes was about 60-fold higher than that in mature rabbit erythrocytes. The biggest decline of cyclic AMP was registered during the transition from youngest to the intermediate stage of maturity.

Respiration; Glycolysis; Adenine nucleotide; Biphosphoglycerate, 2,3-; cyclic AMP; (Reticulocyte)

1. INTRODUCTION

The reticulocyte represents a stage of maturation of erythroid cells well defined by morphological and biochemical criteria. It is characterized by the elimination of the nucleus on the one hand, and on the other by the presence of functional mitochondria and ribosomes. Its energy production, mainly by way of oxidative phosphorylation, is nearly 2 orders of magnitude higher than that of the erythrocyte [1] which contains only glycolysis as an ATP-producing system. The transition of the reticulocyte to the erythrocyte, requiring about 3 days, involves the complete degradation of mitochondria and ribosomes. Therefore one may assume that the general term reticulocyte represents a heterogeneous population of more or less mature cells. We have developed a standardized system to fractionate rabbit reticulocytes according to density, which yields populations of different maturity, which differ in their properties with respect to the proteolytic breakdown of mitochondria [2]. It appeared reasonable to assume that a specific sequence of events exists also with respect to the energy metabolism.

The aim of the present investigation was the characterization of the maturation-dependent changes of rabbit reticulocyte energy metabolism. For this purpose changes of cellular respiration, glycolysis, adenine nucleotides, 2,3-BPG, as well as cyclic AMP were followed.

Dedicated to Professor R.V. Živković (Institute of Biochemistry, Faculty of Medicine, Belgrade, Yugoslavia) on the occasion of his 60th birthday

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The experimental part of this work was performed in the Institute of Physiology, Faculty of Medicine, Kragujevac, Yugoslavia

2. MATERIALS AND METHODS

Reticulocytosis of 30–40% was induced by daily bleeding of

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rabbits for 7 days. Reticulocyte-rich red blood cell suspensions were prepared as described [3]. Fractionation of reticulocytes according to density was performed by means of the technique developed earlier [2]. Fraction I, with 72 ± 4% of reticulocytes, contains the cells of the lowest density on the 7th day of bleeding. In fraction II, containing reticulocytes of intermediate maturity, reticulocytosis amounted to 58 ± 2%. The third fraction, with 40 ± 2% of reticulocytes, represents the population of mature reticulocytes. The fourth fraction, containing 18 ± 3% of reticulocytes, constitutes the most mature reticulocytes.

Oxygen consumption was measured by the Warburg technique. Coupled oxygen consumption was calculated as difference between total and 5 μmol/l oligomycin-resistant oxygen consumption obtained with the same batch of cell suspension. Lactate accumulation was measured enzymatically [4] after 30, 60 and 120 min of aerobic incubation. ATP, ADP and 2,3-BPG were determined enzymatically [5–7] in neutralized perchloric acid extracts. Cyclic AMP was determined in neutralized perchloric acid extracts of reticulocytes by a protein-binding assay [8], but with an immunoassay in extracts of rabbit erythrocytes [9].

3. RESULTS AND DISCUSSION

The data summarized in table 1 demonstrate a decline of respiration and energy producing capacity during the maturation of reticulocytes. Decreases of 40% of total and of 60% of coupled oxygen consumption were observed. The largest change of coupled respiration, amounting to 3.76 ± 0.15 mmol O_2/h per l reticulocytes, or 53% of the whole decline of coupled oxygen consumption, occurred during the transition of reticulocytes from fraction I to fraction II. It coincides with a significant increase of uncoupled oxygen consumption by 0.75 mmol O_2/l reticulocytes ($P < 0.01$) which represents 19% of the uncoupled and 6% of the total oxygen consumption measured in reticulocytes of fraction II. The values are almost the same as those previously estimated for lipoxygenase-mediated share of uncoupled oxygen consumption [10]. Transition of reticulocytes from fraction II to fraction IV was not accompanied by changes of uncoupled oxygen consumption, indicating that the observed decay of total respiration is caused mainly by the decline of coupled respiration. Changes of respiration may be connected with the combined activity of lipoxygenase and the mitochondrial susceptibility factor (MSF), which together trigger the breakdown of mitochondria by ATP-dependent proteolysis during the intermediate stage of maturity of reticulocytes, i.e. in fraction II [2]. Furthermore, it is already well known that lipoxygenase exerts a strong inhibitory effect on the respiratory chain [1]. The same is true for the MSF at a different site of the respiratory chain (unpublished). Hand in hand with the maturational decline of oxidative phosphorylation and respiration in reticulocytes goes an increase of aerobic lactate formation (fig.1). The lowest lactate formation (1.18 ± 0.06 mmol/h per 1 reticulocytes) was found in the reticulocytes of fraction I, i.e. in the cells with the most intensive respiration. During maturation lactate formation continuously increased reaching in fraction IV 2-fold higher values than in fraction I. The observed increase of aerobic lactate formation accompanied by a simultaneous decline of cellular respiration represents a lower Pasteur effect. Although changes of the Pasteur effect during reticulocyte maturation were predicted in the earlier works [11,12], it was not proved before. Oxidative phosphorylation is the main energy producing system in reticulocytes, whereas the share of aerobic glycolysis represents 5–10% of overall ATP production in rabbit reticulocytes [13]. On

Table 1

Cellular respiration and ATP production during maturation of rabbit reticulocytes

Fraction of reticulocyte-rich blood	Reticulocytes (%)	Oxygen consumption			ATP production
		Total	Coupled	Uncoupled	
I	72 ± 4	14.86 ± 1.28	11.88 ± 0.22	3.17 ± 0.32	71.28 ± 1.32
II	58 ± 2	12.38 ± 0.59	8.12 ± 0.07	3.92 ± 0.12	48.72 ± 0.42
III	40 ± 2	11.40 ± 1.28	6.84 ± 0.15	4.07 ± 0.04	41.04 ± 0.90
IV	18 ± 3	8.83 ± 1.06	4.85 ± 0.08	3.98 ± 0.35	29.10 ± 0.48

The results are expressed in mmol/h per 1 reticulocytes. All values represent mean ± SE of 4 experiments

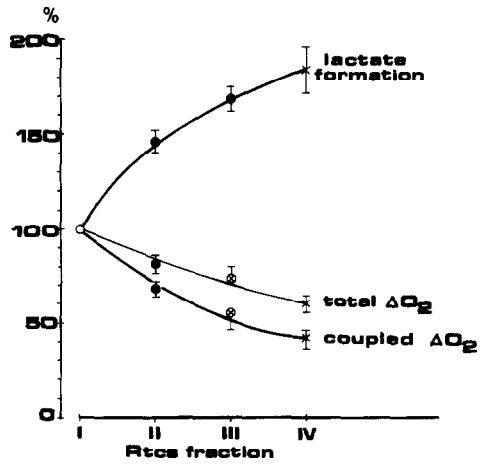

Fig.1. Aerobic lactate formation, total and coupled oxygen consumption of maturity fractionated reticulocytes expressed as percentage of those in reticulocytes of fraction I which are denoted as 100%. The values were calculated per 100% reticulocytes and per hour from the measurements after varying periods. Each value with a bar represents the mean ± SE of 4 experiments. Rtcs denotes reticulocytes.

the basis of ATP production calculated from coupled oxygen consumption and lactate formation the contribution of aerobic glycolysis to overall ATP production amounts to 1.6% in reticulocytes of fraction I, 3.6% in fraction II, 4.9% and 7.7% in fraction III and IV, respectively. However, it is clear that despite a doubled rate of glycolysis a remarkable decline of reticulocyte energy production occurs owing to the decrease of respiration. Since energy production is geared to the energy consuming processes, the results indicate also a significant decay of energy consuming processes during reticulocyte maturation as described before [1]. The data presented in table 2 show a continuous decline of the ADP concentration which is accompanied by an increase of ATP by about 25%. A sharp decline of the ATP/ADP ratio also occurs during maturation of reticulocytes. The higher ATP/ADP ratio in less mature reticulocytes is in accordance with the assumption that the mitochondrial respiration is mainly regulated by the ATP/ADP ratio. In the rabbit erythrocytes the ATP/ADP ratio does not play a role in the regulation of its energy metabolism. The changes of the ATP/ADP ratio may be presumed to affect the lower ATP production by the mitochondria. The obvious decline of total nucleotides, primarily consisting of ATP, indicates the predominance of the breakdown via AMP [14].

Increases of ADP, and probably AMP, positive effectors of phosphofructokinase (PFK), combined with a decrease of the concentration of the negative effector ATP, offer an acceptable explanation for the increase of aerobic glycolysis, i.e. a lower Pasteur effect.

2,3-BPG plays a role both for the energy metabolism and for the respiratory function of red blood cells. An increase of 2,3-BPG of about 2 mmol/l cells was found during maturation of reticulocytes (table 2). It may be explained by the maturational decline of pyruvate-kinase activity [15], the main regulator of the concentration of substrates in red blood cells below the PFK reaction, rather than by changes of activity of the BPG-mutase/phosphatase system, since its definite relationship to the 2,3-BPG concentration could not be established [1]. The physiological

Table 2

ATP, ADP, 2,3-BPG concentrations and ATP/ADP ratio during rabbit reticulocyte maturation

Fraction of reticulocyte-rich blood	ATP	ADP	ATP/ADP	2,3-BPG
I	3.21 ± 0.45	166 ± 13.4	20.08 ± 0.76	6.01 ± 0.15
II	2.47 ± 0.03	179 ± 23.2	13.65 ± 0.23	6.52 ± 0.19
III	1.91 ± 0.09	189 ± 23.1	8.62 ± 0.61	7.80 ± 0.22
IV	1.53 ± 0.16	208 ± 26.7	6.37 ± 0.32	8.15 ± 0.11

The results are expressed in mmol/l packed cells (ATP; 2,3-BPG) or in μmol/l packed cells (ADP). All values represent mean ± SE of 4 experiments

significance of this change may be estimated: knowing that an increase of 2,3-BPG level by 0.4 mmol/l shifts the p_{50} value by 0.13 kPa to the right [16], one may calculate that the maturation-dependent elevation of 2,3-BPG in erythroid cells increases the O_2 delivery of the blood by 0.58 mmol O_2/l blood, i.e. by 26%. Furthermore, simultaneously with the 2,3-BPG increase an almost 2-fold increase of the hemoglobin content was found (not shown). Reticulocyte maturation is accompanied also by partial or complete loss of various receptors and decay of hormonal responsiveness. Recently the strong maturation-dependent decline of the turnover of phosphoinositides in rabbit red blood cells was reported [3]. The changes of the adenylate cyclase system during erythropoiesis have also been studied [17,18]. Interestingly, despite the continuous decline of adenylate cyclase activity of rabbit bone marrow erythroid cells, no changes of the cyclic AMP level were observed after the final cell division [17]. However, our results (fig.2) clearly show a large decline of cyclic AMP by as much as 90% during transition from the youngest to the most mature reticulocytes. Maturation of reticulocytes of fraction IV to erythrocytes is accompanied with a further decline of cyclic AMP level. In rabbit erythrocytes it represents only 1.6% of the cyclic AMP level found in fraction I containing the most immature reticulocytes. These changes of cyclic AMP level are probably due to the decrease of activity of the complete adenylate cyclase system, since the cyclic AMP-phosphodiesterase activity is extremely low and remains constant during maturation of reticulocytes [18].

The observed changes of cyclic AMP during the maturation of reticulocytes may be of importance for the regulation of energy metabolism in reticulocytes, especially concerning the rate of glycolysis, Na^+,K^+-ATPase activity and the polyphosphoinositide cycle [19,20].

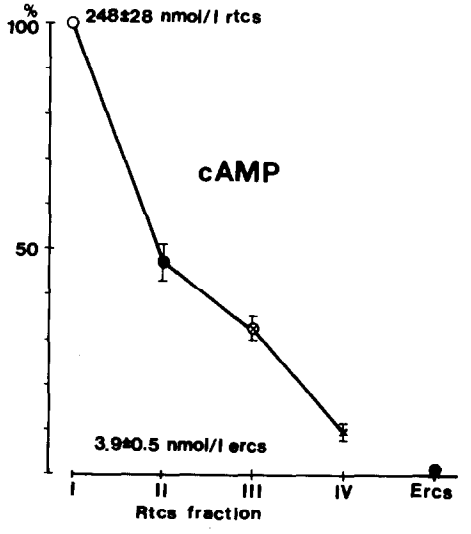

Fig.2. Decline of basic cyclic AMP level during maturation of rabbit reticulocytes. The content of cyclic AMP in fraction I (the youngest reticulocytes) is denoted as 100%. Mean ± SE; n = 4 (for reticulocytes) or n = 7 (for erythrocytes). Rtcs, reticulocytes.

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