MECHANISM OF ACTION OF ERYTHROPOIETIN ON ENERGY METABOLISM IN THE BONE MARROW

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The effect of erythropoietin on the intensity of oxidative phosphorylation in the bone marrow mitochondria was studied in experiments on 35 rabbits. An increase in the erythropoietic activity of rabbits rendered anemic by bleeding led to an increase in the content of mitochondrial protein in the hematopoietic tissue and stimulated oxygen utilization by the mitochondria of myelokaryocytes. As erythropoiesis is reduced in animals with posttransfusion polycythemia, the decrease in content of mitochondrial protein in the bone marrow was accompanied by a decrease in the rate of oxidation. Administration of erythropoietic serum to the animals with polycythemia stimulated the oxidative systems of the mitochondria and increased the activity of structural processes in the hematopoietic tissue.

Erythropoietin stimulates the development of differentiation of the stem cells of hematopoietic tissue toward the erythroid series [14, 17, 20]. Proliferation and differentiation of erythroblastic cells are under the control of nucleic acids [3, 15, 19] and require definite expenditure of energy [4, 5, 13]. The level of erythropoietic activity rises during oxygen lack whether due to a deficiency of oxygen in the inspired air or to a decrease in the oxygen capacity of the blood in anemia [1, 2, 6-8, 11, 18]. Under these conditions, despite the deficient oxygen supply to the body, structural processes in the bone marrow are stimulated. Besides its other action, erythropoietin may also perhaps influence the energy metabolism of hematopoietic tissue. The effect of erythropoietin on energy metabolism of the bone marrow is not discussed in the literature.

The object of this investigation was to study the mechanism of action of serum with erythropoietic activity on the intensity of respiration and oxidative phosphorylation in myelokaryocytes.

EXPERIMENTAL METHOD

Experiments were carried out on 35 male rabbits weighing 1.5-2.0 kg; 20 rabbits were rendered anemic by a single blood loss to the extent of 20 ml/kg body weight. On the 4th-5th day after bleeding, blood was taken from the rabbits by cardiac puncture in order to obtain erythropoietically active serum, which was injected in a dose of 6 ml intravenously into 15 animals with posttransfusion polycythemia, induced as a preliminary procedure in order to reduce erythropoiesis [16]. The erythropoietic effect was assessed from the value of the statokinetic index during cultivation of a suspension of bone-marrow cells in vitro [12], and also from the results of examination of the myelogram and differential counting of the erythroblasts in the experimental animals.

Bone marrow for investigation was obtained by puncture of the lower femoral epiphysis. Mitochondria of the myolokaryocytes were isolated in 0.25 M sucrose solution at 0-2°C. Oxygen absorption was measured manometrically in a Warburg apparatus for 20 min at 37°C in an atmosphere of air. The incubation medium, in a volume of 2 ml, contained the following (in micromoles): potassium phosphate (pH 7.4) 40, potassium chloride 30, sucrose 200, magnesium cloride 50, EDTA 1, succinate 10, ATP 3, glucose 150,

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TABLE 1. Relationship between Oxidative Phosphorylation of Bone Marrow Mitochondria in Rabbits and Level of Erythropoietic Activity (M ± m)

Experimental conditions	No. of animals	Erythropoietic activity of serum	Oxygen consump. and phosphorylation of mitochondria during incub. for 20 min (μatoms/mg mitochondrial protein)		P/O	Respiratory control
Control (original state)	35	62±9	2,81±0,21	5,02±0,46	1,82±0,12	2,91±0,21
Five days after bleeding	20	186±18	4,75±0,33	3,81±0,29	0,81±0,18	3,80±0,30
P		<0,001	<0,001	<0,05	<0,001	<0,05
Posttransfusion polycythemia	15	19±7	1,82±0,17	3,83±0,21	2,11±0,13	2,80±0,20
P		<0,001	<0,001	<0,05	>0,5	>0,5
One day after injection of erythropoietic serum into animals with polycythemia	15	186±18 Activity of serum injected	4,52±0,28	5,41±0,47	1,20±0,19	3,71±0,28
P		<0,001	<0,001	>0,5	<0,05	<0,05

Note: P calculated relative to control. Erythropoietic activity assessed from statokinetic index during cultivation of a bone marrow cell suspension by Shekhter's method [12].

together with hexokinase 0.5 mg. The suspension of mitochondria was added in an amount equivalent to 1.0-1.2 mg protein. In the respiratory control a mixture with or without a phosphate accepting system (hexokinase + glucose) was used. The intensity of phosphorylation was estimated from the decrease in the content of inorganic phosphate, determined by the method of Fiske and Subbarow. The quantity of oxygen absorbed and of esterified phosphorus was calculated in μatoms/mg mitochondrial protein.

EXPERIMENTAL RESULTS

The number of erythroblasts and normoblasts in rabbits with posttransfusion polycythemia fell to 71 ± 5% ($P < 0.01$) of the original level. Addition of the serum of these animals to a cultivated bone marrow cell suspension led to a decrease in the statokinetic index to 19 ± 7 (control 62 ± 9), confirming the absence of endogenous erythropoietin. Inhibition of structural processes in the bone marrow in animals with experimental polycythemia correlated with a decrease in the content of mitochondrial protein to 0.83 ± 0.12 mg/g myelokaryocytes (1.21 ± 0.20 mg/g myelokaryocytes in the control group).

In the anemic rabbits on the 4th-5th day after bleeding the numbers of erythroblasts and normoblasts rose sharply (198 ± 21%; $P < 0.001$), and there was a corresponding increase in the content of mitochondrial protein (2.71 ± 0.41; $P < 0.001$). The serum of these animals raised the statokinetic index in the bone marrow culture (186 ± 18; $P < 0.001$), indicating its high erythropoietic activity. Injection of serum of the anemic animals into rabbits with posttransfusion polycythemia led to an increase in the number of nucleated cells of the erythroblastic series (162 ± 14; $P < 0.05$) and stimulated the formation of mitochondrial protein (2.30 ± 0.32; $P < 0.05$).

The effect of erythropoietin on the formation of erythroblasts and normoblasts was thus directly connected with synthesis of mitochondria of the myelokaryocytes.

The results given in Table 1 show that oxygen consumption by the bone marrow mitochondria increased both during acute blood loss (against the background of increased endogenous erythropoietic activity) and also after administration of the serum of anemic rabbits, with high erythropoietic activity, into the animals with reduced erythropoiesis.

The decrease in the P/O ratio observed at the same time indicates some weakening of the coupling between oxidation and phosphorylation, possibly due to activation of free oxidation or to structural changes in the mitochondrial membranes. The combination of increased oxygen consumption and the increased respiratory control in these experiments rules out the possibility of a disturbance of the phosphorylating ability of the mitochondria [10]. Characteristically the trend of energy metabolism corresponded to an increase in the proliferative activity of the erythroblastic cells in the hematopoietic tissue.

In the group of rabbits with posttransfusion polycythemia the decrease in polyphorylating ability of the mitochondria was combined with a reduced oxygen consumption, simulating an increase in the coupling effect. The decrease in the respiratory control under these conditions is evidence of a relative decrease in the respiratory activity of the mitochondria associated with the reduction of erythropoiesis.

Erythropoietin, which stimulates energy metabolism of hematopoietic tissue, thus brings about a marked increase in oxygen consumption by the mitochondria of myelokaryocytes. Considering that the intensity of structural metabolism is proportional to the activation of free oxidation [9], the correlation between these important mechanisms of action of erythropoietin will be clear.

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