Adaptation to hypoxia: Changes in the level of erythrocyte 2,3-diphosphoglycerate and liver adenylates

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ABSTRACT

The effects of acute hypoxia (9% O₂) on the levels of erythrocyte 2,3-diphosphoglycerate (2,3-DPG) and hepatic adenine nucleotides were investigated. Measurements were made from both fed and starved mice. 1) Exposure of fed mice to 9% O₂ rapidly increased erythrocyte 2,3-DPG, which is considered to be a reflection of metabolic adaptation to hypoxia. The liver adenylate pool remained unchanged for one day under hypoxic states, and a marked reduction in the hepatic adenylates occurred after 3 days. 2) When mice were starved, little or no increase in erythrocyte 2,3-DPG was observed, and hepatic adenylates decreased rapidly within 1 day.

Thus, a decrease in the liver adenylate pool and an increase in erythrocyte 2,3-DPG, the most important factor favoring oxygen release at tissue levels, were demonstrated, and this suggests the importance of the adenylate level in liver for metabolic adaptation to hypoxia.

KEY WORDS diphosphoglycerate / hypoxia / adaptation / liver adenylate pool

Hypoxia results in a series of physiological and biochemical changes that can maintain normal oxygen delivery and also induce a metabolic adaptation to an anaerobic environment (11). Some metabolic changes to a deficient oxygen supply may act in most tissues: an elevation of erythrocyte 2,3-diphosphoglycerate (2,3-DPG) (10), an increased rate of glycolysis (13), and adenine nucleotide degradation (7, 14-16) under the conditions causing tissue hypoxia. The increase in erythrocyte 2,3-DPG during hypoxia, which is considered to be the most important factor favoring oxygen release at tissue level (10), is explained by the activation of erythrocyte phosphofructokinase (EC 2.7.1.11) due to respiratory alkalosis in hypoxia (8). Our recent studies on the relationship between the increase in 2,3-DPG and the oxygen saturation in arterial blood at high altitude suggest that

the mechanism of rapid adaptation to hypoxia for facilitating the supply of oxygen to tissues is not operative above an altitude of 6,000 m (20). This paper concerns metabolic adaptation to hypoxia, that is, the changes in erythrocyte 2,3-DPG and hepatic adenylate contents which occurred in mice exposed to 9% O₂ (6,000 m simulated altitude). An increase in erythrocyte 2,3-DPG and a decrease in hepatic adenylates were demonstrated: the importance of the hepatic adenylate level is discussed in relation to liver gluconeogenesis, which can supply glucose, a precursor of 2,3-DPG in erythrocytes.

Male *ddy*-mice, aged 4 weeks, were separated into control and fasted groups. The control groups were fed standard laboratory chow and water *ad libitum*. Five mice per group were exposed to a gas mixture of 9% O₂ and 91% N₂ in chambers through which the gas mixture was

passed at 700 ml per min. The composition of the gas mixture was controlled and monitored by regulating the flow of each component into a mixing vessel with calibrated flowmeters. The compositions of all gas mixtures were expressed on a v/v basis. In the fasted group the mice were fasted for 24 hr prior to exposure to hypoxia, and starvation was continued under the hypoxic conditions.

Blood samples were collected by decapitation, and erythrocytes were separated by centrifugation of heparinized blood. The livers were separated and immediately frozen in liquid nitrogen. The frozen livers were then broken, rapidly weighed, and ground in frozen form with a mortar and pestle. The powder was homogenized in 5 parts (w/v) of 6% perchloric acid. The precipitate was removed by centrifugation and the supernatant fluid was neutralized with 0.6 M K_2CO_3 . The supernatants were used for the determination of adenylates. Adenylates were measured after enzymatic conversion to ATP, which was analyzed by luciferase reaction (5). 2,3-DPG was determined according to the method of Keitt (9). Hemoglobin was measured as cyanomethemoglobin. Data are given as means $\pm$ SD. Standard statistical methods were employed.

Fig. 1 shows the increase in the concentrations of erythrocyte 2,3-DPG in mice occurring with exposure to a gas mixture of 9% O_2 (corresponding to an altitude of 6,000 m). A remarkable and rapid increase in 2,3-DPG was observed. During the following 7 days of O_2 deficiency, 2,3-DPG concentrations still remained elevated (Fig. 1). When the animals were exposed to normal air, 2,3-DPG levels returned to normal value within one day. It should be noted that 2,3-DPG did not tend to increase when the animals were starved: the concentration of 2,3-DPG remained at normal levels (Fig. 1).

We also determined the concentrations of liver adenine nucleotides during exposure to hypoxic conditions. The adenylate pool in liver remained unchanged for a day after the exposure to hypoxia. The concentration drastically decreased on the third day, and the decreased level of liver adenylates was further maintained under the hypoxic conditions. The level returned to normal value when the mice were returned to normal air (Fig. 2). Animals which had been starved for 24 hr showed a significant decrease in liver adenylates on the first day after exposure to hypoxia (Fig. 2).

Exposure to hypoxia initiates a series of

Fig. 1 Changes in erythrocyte 2,3-diphosphoglycerate (2,3-DPG) concentrations in mice during exposure to 9% O_2 (corresponding to 6,000 m simulated altitude). Blood was collected by decapitation, and erythrocytes were separated by centrifugation of the heparinized blood. 2,3-DPG was determined by the method of Keitt (9). $\circ$, fed; $\triangle$, starved for 24 hr prior to exposure to 9% O_2 and further fasted for 24 hr during exposure to hypoxia

Fig. 2 Changes in mouse liver adenylate pool during exposure to 9% O₂. Conditions and symbols are the same as in Fig. 1. Sample preparation and determination of adenylates are described in the text. Data are given as means $\pm$ SD.

physiological and biochemical changes that tend to maintain oxygen delivery and also induce a metabolic adaptation to lower oxygen delivery (11). Our previous paper has shown that a rapid increase in the plasma oxypurines and erythrocyte 2,3-DPG occurs during hypoxia (21). Erythrocyte 2,3-DPG, which is a powerful modifier of the oxygen affinity of hemoglobin (1, 4), is considered to be the most important factor favoring oxygen release at tissue levels (10). A close correlation of oxypurines and 2,3-DPG with the oxygen pressure in humans suggests that the levels of these metabolites may be indicative of tissue hypoxia (21). Furthermore, a relation between the increase in erythrocyte 2,3-DPG and the recovery of oxygen saturation in arterial blood during a stay at high altitude suggests that the mechanism whereby oxygen is more effectively transported to the tissues has a central role in the rapid acclimatization toward hypoxia below 6,000 m altitude (20).

We have demonstrated a decrease in liver adenylate pool and an increase in erythrocyte 2,3-DPG under hypoxic conditions. Our data do not provide a detailed explanation of the mechanism, but the following conclusions can be drawn. First, hypoxic stress results in marked increase in plasma glucose (2), lactate (2), fatty acids (3), cortisol (2), and catecholamines (2), which are known to induce the degradation of adenine nucleotides (19). The increased level of catecholamines during

hypoxia (2, 6) could be responsible for the degradation of adenine nucleotides. Second, liver adenylate content decreased more rapidly under starved-hypoxic conditions than under fed-hypoxic conditions. Stimulation of gluconeogenesis, which is an energy-requiring reaction, can result in a slight decrease in the liver adenylate pool under starved conditions (18). Hypoxia-induced increase in catecholamines and cortisol would favor an increased glycolysis and hepatic gluconeogenesis (2, 3, 6, 17), which can provide blood glucose, a good substrate for anaerobic metabolism in many tissues under hypoxic conditions, and for the synthesis of erythrocyte 2,3-DPG. When fed *ad libitum* before and during hypoxia, little or no depletion of liver glycogen and no stimulation of hepatic gluconeogenesis may occur at the first stage; thus, the liver adenylate pool remains unchanged for 3 days. Prolonged exposure to hypoxia can result in the exhaustion of liver glycogen (12, 17); liver gluconeogenesis has a central role in the production of blood glucose, and a decrease in the adenylate pool can occur. On the other hand, if the organism is starved prior to hypoxic exposure, blood glucose must be supplied mainly by hepatic gluconeogenesis because of depletion of the glycogen stores in liver; thus, a rapid decrease in liver adenylates can be observed.

A decrease in the hepatic adenylate pool, which may be the result of both the direct effect of catecholamines and the stimula-

tion of gluconeogenesis, and an increase in erythrocyte 2,3-DPG were clearly demonstrated during hypoxia: the liver adenylate level and energy states seem to be important factors in metabolic adaptation to hypoxia.

We would like to express our gratitude to Professor K. Tsushima in the Department of Biochemistry, Yokohama City University School of Medicine for his valuable discussions and criticisms concerning this work.

Received for publication 17 December 1980

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