STIMULATION OF ANAEROBIC METABOLISM BY α_1 -ADRENERGIC BLOCKADE IN RATS AT HIGH ALTITUDE HYPOXIA

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ABSTRACT

The effect of α_1 -adrenergic blockade on hypoxia-induced stimulation of anaerobic metabolism in rats was investigated. Exposure to 8,000 m-simulated altitude rapidly increased lactate and uric acid in plasma: administration of an α_1 -adrenergic antagonist, prazosin, enhanced a marked increase in these metabolites, and resulted in a decreased formation of acetoacetate and some elevation of blood glucose. However, α_1 -blockade showed no effect on the anaerobic metabolism in rats exposed to a moderate hypoxia below 6,000 m-simulated altitude. The effect of prazosin on rats exposed to highly hypoxic environments can be explained by α_1 -blockade-induced 1) inhibition of respiration, which in turn stimulates the glycolytic flux coupled to adenylate degradation, 2) decrease in gluconeogenic flux due to an inhibition of fatty acid oxidation in liver, and 3) stimulation of β -adrenergic receptor in muscle, which can supply the substrate for glycolysis through the degradation of glycogen.

Exposure to hypoxia causes a series of physiological and biochemical changes which induce a metabolic adaptation to lower oxygen availability (6). Mechanism of anaerobic stimulation of glycolysis with adenylate degradation (7, 15, 22-24) is operative in rats exposed to an altitude above 6,000 m, and anaerobic glycolysis is the only mechanism available for tissues to generate ATP under these conditions (25). The concentrations of uric acid and lactate in plasma can be regarded as good indicators of the metabolic adaptation to hypoxia in tissues (4, 13). Recently, stimulative effect of α_1 -adrenergic agonist on tissue respiration and ATP production has been demonstrated: α_1 -agonist can stimulate the tricarboxylic acid cycle and respiration through the influx of Ca^{2+} into mitochondria (17). In this work, we examined the role of α_1 -adrenergic receptors in the stimu-

lation of anaerobic metabolism in rats submitted to high altitude. α_1 -Blockade caused a marked stimulation of glycolysis with the degradation of adenine nucleotides, and an inhibition of fatty acid oxidation during an exposure of rats to an 8,000 m altitude. α_1 -Adrenergic receptor is suggested to participate in the shift of aerobic to anaerobic metabolism under the *in vivo* hypoxic conditions.

MATERIALS AND METHODS

The sources of materials in this work were as follows: Lactate-UV-Test and Pyruvate-UV-Test from Boehringer-Mannheim, and TZ-UA and Glucose Kit TZ from Tokyo Zoki Chemical Co. (Tokyo, Japan).

Male Sprague-Dawley rats weighing 120-150 g were starved overnight. They were per-

Fig. 1 Effect of prazosin on the change in plasma lactate concentrations of rats exposed to 8,000 m-(A) and 6,000 m-(B) simulated altitudes. Rats were starved overnight. Prazosin was injected to the rats intraperitoneally prior to submission to an altitude chamber. Blood was collected by decapitation and plasma was immediately separated by centrifugation of the heparinized blood. The barometric pressure is represented as the equivalent altitude in Panel C. Each point shows the mean $\pm$ SD with four rats. $\circ$, control (saline injected); $\bullet$, prazosin injected

mitted free access to water. Groups of 4 or 5 rats were submitted to an altitude chamber at the Research Institute of Environmental Medicine (Nagoya University). Prior to an exposure to high altitude, prazosin (Pfizer) solution (1 mg/ml in saline) was injected intraperitoneally into the rat at a dose of 1 mg/kg. An equivalent volume of saline was injected and used as the control. Experiments were made between 1300 and 1600. The decompression rate was 120–150 m/min. The rats remained at the simulated altitude for 1 h, and returned to sea-level with the same rate. Rats were killed by cervical dislocation at appropriate intervals and blood was collected in heparinized test tubes. Plasma was immediately separated by centrifugation, and was

frozen until analysis.

Plasma lactate, pyruvate and uric acid were determined by the methods described previously (25). Blood glucose was determined enzymatically using a commercial kit (Glucose Kit TZ) based on glucose oxidase-mutarotase-peroxidase system (9). Acetoacetate was determined by the diazo-coupling method (20). Data were expressed as means $\pm$ SD.

RESULTS

Plasma lactate increased during an exposure of rats to 8,000 m-simulated altitude, and was reversed rapidly after the rats were returned to sea-level. Administration of prazosin prior to

Fig. 2 Effect of prazosin on the change in plasma pyruvate concentrations of rats exposed to 8,000 m-(A) and 6,000 m-(B) simulated altitudes. Experimental conditions were similar to those in Fig. 1. ○, control; ●, prazosin injected

an exposure to high altitude enhanced the increase in plasma lactate markedly (Fig. 1A). On the other hand, little or no increase in lactate was observed in rats submitted to 6,000 m-simulated altitude, and α_1 -blockade showed no effect on the lactate level in plasma (Fig. 1B).

Pyruvate also increased during an exposure to 8,000 m altitude, and α_1 -blockade enhanced the increase in pyruvate (Fig. 2A). However, prazosin had no effect on the level of plasma pyruvate in rats exposed to 6,000 m altitude (Fig. 2B). Glycolytic flux is suggested to be enhanced markedly by α_1 -adrenergic blockade in rats exposed to the highly hypoxic conditions.

Fig. 3 shows the effect of prazosin on change in plasma uric acid level. Administration of prazosin strongly enhanced the increase in plasma uric acid during an exposure to 8,000 m altitude (Fig. 3A), but showed no effect on the formation of uric acid in rats

submitted to 6,000 m altitude (Fig. 3B).

Exposure of rats to 8,000 m altitude showed little change in blood glucose, but the level increased after the rats were returned to sea-level. Prazosin tended to rise blood glucose level slightly 2 h after its intraperitoneal injection (Fig. 4A).

Plasma concentration of acetoacetate was scarcely varied when rats were submitted to 8,000 m altitude. Treatment of rats with prazosin caused a considerable decrease in acetoacetate level during a stay at 8,000 m altitude (Fig. 4B), suggesting an inhibition of fatty acid oxidation by α_1 -blockade under the hypoxic conditions.

DISCUSSION

Stimulation of anaerobic glycolysis with inhibition of respiration, which is known as the Pasteur effect (10, 19), is an important metabolic adaptation to a deficient oxygen supply

Fig. 3 Effect of prazosin on the change in plasma uric acid concentrations of rats exposed to 8,000 m-(A) and 6,000 m-(B) simulated altitudes. Experimental conditions were similar to those in Fig. 1. ○, control; ●, prazosin injected

(6). Exposure of rats to hypoxia above 6,000 m-simulated altitude causes a concomitant increase in plasma lactate and uric acid (25), which can be explained by the glycolytic stimulation coupled to the degradation of adenine nucleotides in hypoxic tissues (7, 15, 22-24). Tissue hypoxia due to an exposure to the altitude above 6,000 m causes a decrease in ATP, which results in the activation of AMP deaminase (EC 3.5.4.6) to stabilize the adenylate energy charge (5, 21). Activation of AMP deaminase causes the stimulation of phosphofructokinase (EC 2.7.1.11) and pyruvate kinase (EC 2.7.1.40), and the glycolytic flux through the rise in ammonium ions (15, 22). Since the product of AMP deamination, IMP, is dephosphorylated to inosine, and then converted to hypoxanthine, xanthine, uric acid and allantoin, the increase in oxypurines can correlate with the glycolytic stimulation (25). This mechanism can explain a variety of metabolic changes observed under some

hypoxic and anoxic conditions (3, 8, 25). Glycolytic stimulation coupled to hyperuricemia may be a general phenomenon observed in rats exposed to hypoxic conditions.

The role of adrenergic receptors in regulation of respiration has been demonstrated by many workers. Catecholamine receptors are divided into α_1 , α_2 , β_1 and β_2 receptors on the basis of ligand binding studies (16, 17). α_1 -Adrenergic effects appear to be mediated through a receptor-linked Ca^{2+} -dependent mechanism, whereas α_2 - and β -adrenergic effects are mediated through modulation of the cAMP concentration (12, 17). α_1 -Adrenergic agonists increase the rate of tricarboxylic acid cycle and respiration in perfused livers (11, 16, 18), and isolated cells including hepatocytes (2) and adipocytes (14). Hepatic glycogenolysis in young rats is mediated by both α_1 - and β -adrenergic stimulation (11), but β -adrenergic effect of epinephrine participates in glycogen degradation in

Fig. 4 Effect of prazosin on the change in plasma glucose (A) and acetoacetate (B) concentrations of rats exposed to 8,000 m-simulated altitudes. Experimental conditions were similar to those in Fig. 1. ○, control; ●, prazosin injected

human liver (1). Prazosin acts as a specific antagonist for α_1 -receptors and can inhibit α_1 -agonist-induced glucose release and respiration in perfused liver and isolated cells (2, 11). Our results show that α_1 -blockade by prazosin can enhance the hypoxia-induced glycolytic flux coupled to adenylate degradation, resulting in an increase in plasma lactate and uric acid. Since α_1 -adrenergic activation of gluconeogenesis is ascribed to an increase in fatty acid oxidation and in tricarboxylic acid cycle flux (16), the decreased rate of respiration and fatty acid oxidation due to hypoxia and α_1 -blockade causes an inhibition of hepatic gluconeogenesis, which results in further elevation of plasma lactate. Moreover, α_1 -blockade may induce a relative β -stimulant effect in some organs including skeletal muscles: β -adrenergic stimulation results in muscle glycogenolysis, which can elevate the concentration of plasma lactate through the increased supply of glycolytic substrate. A slight in-

crease in blood glucose level in rats to which prazosin was administered may imply an enhanced glycogenolysis due to the β -adrenergic effect in liver.

In conclusion α_1 -blockade can enhance the glycolytic flux with adenylate degradation under the anaerobic conditions. α_1 -Adrenergic receptors may participate in the energy metabolism, that is, the conversion of respiration to anaerobic glycolysis in rats exposed to highly hypoxic conditions.

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