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Effect of Ca^{2+}-entry blocker on the stimulation of aerobic metabolism in rats acclimatized to high altitude hypoxia

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Prolonged and repeated exposure to high altitude causes a metabolic acclimatization to hypoxia: aerobic metabolism is enhanced, but anaerobic glycolysis is reduced in rats acclimatized by repeated exposure to a simulated altitude above 5000 m [1, 2]. A higher activity of the mitochondrial oxidative enzyme in the acclimatized rats appears to contribute to the elevated aerobic metabolism [2]. Mitochondrial metabolism can be controlled by Ca^{2+}: Ca^{2+} incorporated into mitochondria activates some dehydrogenases and then enhances oxidative metabolism in mitochondria [3, 4]. In this paper, we analyzed the role of Ca^{2+} in the stimulation of aerobic metabolism of acclimatized rats using a Ca^{2+} entry blocker: Ca^{2+} influx is essential for the stimulation of aerobic metabolism under highly hypoxic conditions.

Materials and Methods

Chemicals and kits for determination of metabolites were purchased as described previously [1, 2]. Diltiazem hydrochloride was a product of the Tanabe Pharmaceutical Co. (Osaka, Japan).

Male Sprague–Dawley rats weighing 120–150 g were acclimatized to hypoxia by repeated exposure to a simulated altitude of 6000 m for 2 hr/day throughout 11 days as described previously [1]. Control groups were not pre-exposed to high altitude. Plasma ketone bodies are markedly lower under fed conditions; hence, both groups were starved overnight before an exposure to a simulated altitude of 8000 m. They were permitted free access to water. Prior to an exposure to the 8000 m altitude, diltiazem solution (2 mg/mL in saline) was injected intraperitoneally into the rats at a dose of 5 mg/kg. An equivalent volume of saline was injected into control animals. Experiments were performed between 1:00 and 4:00 p.m. The decompression rate was 120–150 m/min. The rats remained at the 8000 m-simulated altitude for 1 hr, 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, uric acid, and ketone bodies were determined as described previously [1]. Data are expressed as means ± SD.

Results

3-Hydroxybutyrate, a major ketone body in plasma, did not increase when the control rats were exposed to a simulated altitude of 8000 m. However, rats acclimatized by repeated exposure to 6000 m altitude for 11 days showed a marked increase in 3-hydroxybutyrate when exposed to an 8000 m altitude (Fig. 1). Administration of a Ca^{2+}-entry blocker, diltiazem, prior to an exposure to the 8000 m altitude completely blocked the increase in 3-hydroxybutyrate (Fig. 1), and no significant increase in the ketone body was observed in the control and the diltiazem-administered rats. Diltiazem did not show any effect on the level of the ketone body of the control group (data not shown). The ratio of 3-hydroxybutyrate to acetoacetate was 10 and over under the experimental conditions, and, thus, changes in total ketone bodies including 3-hydroxybutyrate plus acetoacetate were essentially identical to those of 3-hydroxybutyrate (data not shown).

Plasma lactate, a marker of glycolytic activity, increased markedly during an exposure of the control rats to an 8000 m simulated altitude. Acclimatized rats showed a

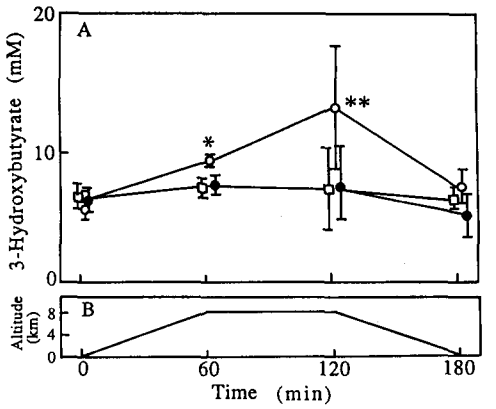

Fig. 1. Effect of diltiazem on the change in plasma concentrations of 3-hydroxybutyrate in rats acclimatized to high altitude hypoxia under conditions of an exposure to an 8000 m simulated altitude. Rats were acclimatized as described in the text. Diltiazem (2 mg/mL) was injected intraperitoneally into the acclimatized rats at a dose of 5 mg/kg prior to submission to an altitude chamber. Blood was collected by decapitation, and plasma ketone bodies were determined. The barometric pressure is represented as the equivalent altitude in panel B. Each point is the mean ± SD; sample size was 4 or 5. Key: (□) control; (○) acclimatized; and (●) diltiazem administered to the acclimatized rats. Asterisks indicate a significant increase in 3-hydroxybutyrate of the acclimatized rats: *$P < 0.001$, and **$P < 0.05$ (Student's *t*-test).

decreased enhancement of lactate production. Administration of diltiazem to the acclimatized group significantly enhanced the lactate formation: increase in plasma lactate during an exposure to 8000 m altitude was comparable to the level of the control group (Fig. 2A).

Plasma uric acid also increased when control rats were exposed to an 8000 m simulated altitude, but the acclimatized group showed only a slight increase in plasma uric acid under highly hypoxic conditions. Administration of diltiazem tended to stimulate the rise of uric acid formation when the rats were exposed to the 8000 m altitude (Fig. 2B).

Discussion

Acclimatization to hypoxia, which can be acquired by repeated exposure to a simulated high altitude [1], involves an enhancement of aerobic metabolism and a reduction of anaerobic glycolysis: we demonstrated that one of the principal mechanisms of the stimulation of aerobic metabolism as a result of acclimatization to hypoxia is the induction of the mitochondrial enzyme, that is glutamate dehydrogenase [2].

Mitochondrial aerobic metabolism is closely related to the Ca^{2+} concentration: Ca^{2+} can activate three dehydrogenase steps in the citric acid cycle, that is, pyruvate dehydrogenase phosphate phosphatase (EC 3.1.3.43), NAD-isocitrate dehydrogenase (EC 1.1.1.41) and 2-oxoglutarate dehydrogenase (EC 1.2.4.2) in mitochondria [3]. Several lines of evidence have indicated that Ca^{2+} incorporated into mitochondria stimulates the citric acid cycle and aerobic metabolism under a variety of conditions [4].

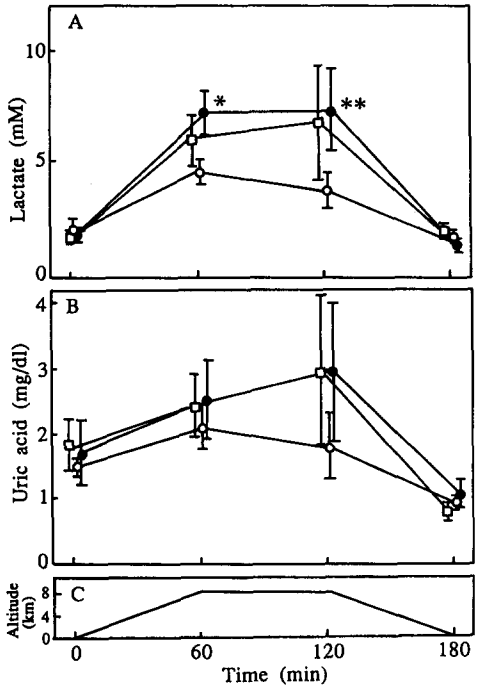

Fig. 2. Effect of diltiazem on the change in plasma concentrations of lactate (A) and uric acid (B) of rats acclimatized to high altitude hypoxia under conditions of an exposure to an 8000 m simulated altitude. The barometric pressure is represented as the equivalent altitude in panel C. Experimental conditions and symbols are similar to those of Fig. 1. Each point shows the mean ± SD; sample size was 4 or 5. Asterisks indicate a significant difference in the lactate levels between the acclimatized group and the diltiazem-administered rats: $^*P < 0.01$, and $^{**}P < 0.05$.

Thus, the role of Ca^{2+} in the control of aerobic metabolism can be analyzed by application of Ca^{2+}-entry blockers [5, 6].

The Ca^{2+}-entry blocker diltiazem can completely block the metabolic changes induced by acclimatization to hypoxia under highly hypoxic conditions. Diltiazem suppressed the aerobic metabolism and enhanced anaerobic energy production in the acclimatized rats, suggesting that mitochondrial aerobic metabolism was reduced by lowering the Ca^{2+} level through the inhibition by diltiazem of Ca^{2+} uptake. Thus, the shift of anaerobic glycolysis to aerobic energy metabolism in the acclimatized rats appears to be due to the Ca^{2+} influx into mitochondria causing activation of mitochondrial dehydrogenase steps. We conclude that the mechanism of the acclimatization to hypoxia can be accounted for by the direct activation of some dehydrogenase steps by Ca^{2+} incorporated into mitochondria where synthesis of aerobic enzymes has been completed.

Department of Genetics
Institute for Developmental Research
Aichi Prefectural Colony
Aichi 480-03, Japan
MASATAKA YOSHINO*

Nagoya Gakuin University
Aichi, Japan
CHIKASHI YAMAMOTO

Inazawa Junior College
Aichi, Japan
KEIKO MURAKAMI

Department of Legal Medicine
Nagoya University School of Medicine
Nagoya 466, Japan
YOSHINAO KATSUMATA

Research Institute for Environmental Medicine
Nagoya University
Nagoya, Japan
SHIGEO MORI

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* Correspondence: Masataka Yoshino, Department of Genetics, Institute for Developmental Research, Aichi Prefectural Colony, Kamiya-cho 713-8, Kasugai, Aichi 480-03, Japan.