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The role of skeletal muscle and liver on lactate metabolism during hypoxia in rats

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Abstract

Purpose. This study was planned to investigate whether the skeletal muscle and liver produce or consume lactate under hypoxic conditions.

Methods. Wister rats were anesthetized and mechanically ventilated. Microdialysis probes were inserted into the rat skeletal muscle and liver, and arterial cannulation was performed. Hypoxia was induced for 30 min by the inhalation of 10% oxygen in nitrogen. Interstitial lactate concentrations in the skeletal muscle and liver were measured using an in vivo microdialysis method before, during, and after hypoxic hypoxia. The blood lactate concentration, mean arterial blood pressure, and blood gas were also measured.

Results. Before hypoxia, there was no significant difference among the blood lactate concentration and interstitial lactate concentrations of the skeletal muscle and liver. During hypoxia, arterial oxygen tension decreased to 34.2 ± 1.3 mmHg, and the lactate concentrations in these tissues increased significantly in comparison to the control values. However, the lactate concentrations in the skeletal muscle and liver interstitium were significantly lower than that in the blood, with the peak lactate concentration in the skeletal muscle interstitium being only one-third of that in the blood. After correction of hypoxia, the blood lactate concentration decreased to levels comparable to the skeletal muscle and liver interstitial lactate concentrations.

Conclusion. It is suggested that the skeletal muscle as well as the liver may consume lactate under hypoxic hypoxia.

Key words: Lactic acid, Hypoxia, Microdialysis, Skeletal muscle, Liver

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Introduction

It is generally accepted that the skeletal muscle generates lactate during tissue hypoxia. However, we have recently demonstrated, using a microdialysis method, that the lactate concentration in the skeletal muscle interstitium is significantly lower than that in the blood after acute hemorrhagic and endotoxemic conditions. We accordingly suggested that the skeletal muscle functions as a lactate consumer during such circulatory failure [1–3].

Recently, Gutierrez et al. [4], using the conventional Fick's method, found evidence suggesting that lactate was utilized in the rabbit skeletal muscle during hypoxic hypoxia. Few other investigations have examined lactate metabolism of skeletal muscles during hypoxia. We investigated whether or not the skeletal muscle consumes lactate during hypoxic hypoxia using an in vivo microdialysis method. In addition, the changes in liver lactate concentration during hypoxia were investigated.

Materials and methods

Surgery

This study was approved by the Animal Research Committee of our university. Eight male Wister rats (220–280 g) were used. Laboratory chow and tap water were given ad libitum until just before the experiment. Each rat was then anesthetized by intraperitonial injection of pentobarbital (50 mg·kg^{-1}) and tracheostomized. After intraperitoneal administration of pancuronium bromide (1 mg·kg^{-1}), the rats were mechanically ventilated with 100% oxygen, and the right femoral artery was then cannulated. The arterial cannula was connected to a pressure transducer to measure the mean arterial pressure. This cannula was used to obtain samples for blood gas and lactate analysis. Parts of the muscle of the left

hindlimb and liver were exposed to insert microdialysis probes. Body temperature was maintained at 37°C throughout the experiment using an electric blanket.

Microdialysis

A CMA/10 microdialysis probe (10 mm, Carnegie Medicine, Stockholm, Sweden) was used to measure the interstitial muscle and liver lactate concentrations. The microdialysis probe was placed at a depth of about 1 mm in the medial region of the left thigh, which consists of musculus gracilis anticus and posticus, musculus adductor longus, magnus, and brevis, and musculus pectineus. The other probe was placed at about 1 mm depth in the medial region of the liver. Then normal saline was perfused through each probe at 10 μl·min^{-1} using a CMA/100 microinjection pump (Carnegie Medicine). The absolute interstitial lactate concentrations in the skeletal muscle and the liver were measured using the in vivo calibration technique in the control state, and the recovery rate of each probe was calculated [5–7]. In brief, the muscle or liver was perfused with normal saline containing different concentrations of lactate. The delta lactate concentration (dialysate lactate concentration − perfusate lactate concentration) was plotted against each perfusate lactate concentration. Using regression analysis, the perfusate lactate concentration against which the delta lactate concentration was zero was calculated as the absolute interstitial lactate concentration, and the slope of the regression line was calculated as the recovery rate for the probe. The recovery rates of the microdialysis probes ranged from 12.7 to 22.3%. The interstitial lactate concentrations in the skeletal muscle and the liver were calculated by dividing the concentrations in the samples by the respective in vivo recovery rates.

Hypoxia

Hypoxia was induced by the inhalation of 10% oxygen gas mixed with 90% nitrogen for 30 min, followed by a recovery period with inhalation of 100% oxygen for 60 min. Six blood specimens were obtained (in the control state and 10, 20, 30, 60, and 90 min after the beginning of hypoxia) to measure lactate and blood gases. Microdialysis samples (30 μ l) were collected in the muscle and liver just before each blood sample was obtained. Lactate concentrations were measured with an analyzer equipped with a polarographic enzyme electrode (model 23L; Yellow Springs Instruments, Yellow Springs, OH, USA).

Statistics

The data were analyzed statistically using analysis of variance with repeated measures followed by the Bonferroni test [8].

Results

Table 1 shows arterial blood gas data acquired before, during, and after hypoxia. PaO_2 decreased from 376.4 ± 29.2 mmHg (mean ± SEM) to 32.7 ± 1.4 mmHg 10 min after the beginning of hypoxia, and it remained in the range of around 30–40 mmHg during the hypoxic period. $PaCO_2$ did not change significantly before, during, or after hypoxia, although the pH and base excess decreased significantly during hypoxia.

As shown in Fig. 1, the blood pressure decreased to around 85 mmHg during the hypoxic state, but recovered to the previous value after hypoxia.

Figure 2 shows the changes in lactate concentrations of the blood and skeletal muscle and liver interstitium. That of the blood increased from 0.57 ± 0.07 mM to 9.93 ± 1.31 mM at 30 min after the beginning of hypoxia. The lactate concentrations in the skeletal muscle and in the liver interstitium also increased, from 0.53 ± 0.09 mM to 3.25 ± 0.35 mM and from 1.05 ± 0.15 mM to 6.56 ± 0.98 mM, respectively, but both were significantly lower than that of the blood. The muscle and liver lactate concentrations then decreased gradually to the basal level 60 min after the end of the hypoxic period. In contrast, the blood lactate concentration decreased

Table 1. Blood gas measurements before (0 min), during (10, 20, 30 min), and after (60, 90 min) hypoxic hypoxia

Time (min)	pH (unit)	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	BE (mEq·l^{-1})
0	7.330 ± 0.028	41.5 ± 2.5	376.4 ± 29.2	−3.4 ± 1.8
10	7.245 ± 0.021*	38.0 ± 2.8	32.7 ± 1.4*	−10.2 ± 1.4*
20	7.162 ± 0.020*	36.9 ± 2.6	33.3 ± 1.0*	−15.2 ± 1.6*
30	7.077 ± 0.030*	36.0 ± 2.6	36.5 ± 1.0*	−19.6 ± 1.9*
60	7.164 ± 0.032*	44.9 ± 2.0	284.2 ± 24.9*	−13.1 ± 1.6*
90	7.285 ± 0.016	41.8 ± 2.4	273.2 ± 23.4*	−6.1 ± 1.3

Values are mean ± SEM.
* $P < 0.05$ versus control values.

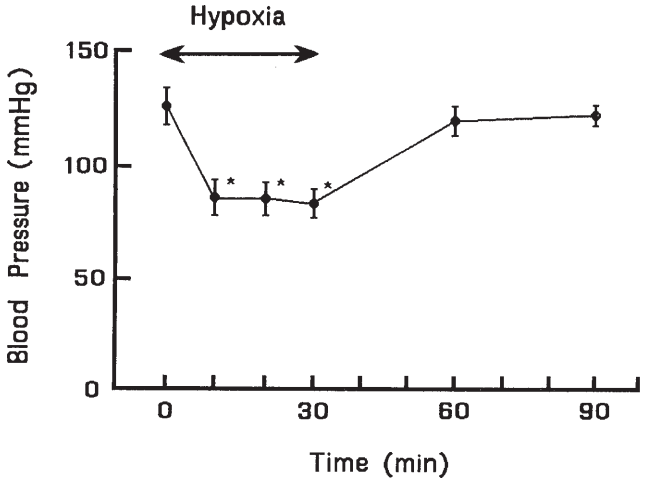

Fig. 1. Change in mean arterial pressure before (0 min), during (10, 20, 30 min), and after (60, 90 min) hypoxic hypoxia. All values are mean ± SEM. $^{\star}P < 0.05$ versus control value

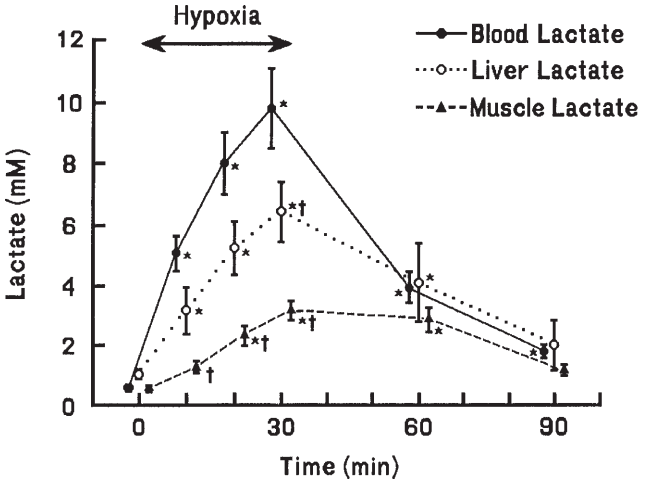

Fig. 2. Changes in blood, skeletal muscle, and liver interstitium lactate concentrations before (0 min), during (10, 20, 30 min), and after (60, 90 min) hypoxic hypoxia. All values are mean ± SEM. $^{\star}P < 0.05$ versus control value in each tissue. $^{\dagger}P < 0.05$ versus blood lactate concentration

rapidly after hypoxia, but it was significantly higher than the basal value even at the end of the experiment.

Discussion

These findings showed that absolute interstitial lactate concentrations in the skeletal muscle and liver were significantly lower than the blood lactate concentration throughout the 30-min period of hypoxic hypoxia. Lactate movement between blood and the intercellular space has been explained by the combination of passive diffusion and active transportation by the transporter, which exsits in the plasma membrane of the skeletal muscle and liver [9–12]. However, it has not been demonstrated that his transporter carries lactate against the concentration gradient. Therefore, we consider that lactate moves between the blood and the intercellular space due to a concentration gradient caused by a certain state, and the present results indicate that lactate moved from the blood to the skeletal muscle and liver cells, and that these organs may consume lactate in hypoxic conditions.

We previously demonstrated that the absolute concentrations of lactate in the skeletal muscle and liver interstitium in acute hemorrhagic and endotoxic states could be measured using the in vivo calibration technique. We further suggested that these structures function as lactate consumers rather than lactate producers under such conditions, since the absolute interstitial lactate concentrations in the skeletal muscle and liver were significantly lower than that in the blood [1–3]. The measurement of the absolute concentrations of lactate in the skeletal muscle and liver interstitium using the in vivo calibration technique could also be applied to the hypoxic model in the present study. The present finding that the skeletal muscle and liver interstitium showed significantly lower lactate concentrations than that of the blood during hypoxia suggests that these structures act as lactate consumers even in the hypoxic state.

Lactate consumption by the skeletal muscle, both at rest and during contraction, has been demonstrated [13,14]. However, the role of the skeletal muscle in lactate kinetics in the hypoxic state has not been fully investigated. Recently, Gutierrez et al. [4] demonstrated lactate uptake by the rabbit hindlimb during hypoxic hypoxia using the conventional Fick's principle, and suggested that skeletal muscle consumes lactate even in a hypoxic state. However, they did not distinguish the muscle from the skin and bone in the bindlimb, and we must ask whether Fick's principle can be used in such an unsteady state that lactate concentration increased rapidly. A microdialysis method allows us to demonstrate directly the changes of lactate in the skeletal muscle, and could be applied in an unsteady state [1].

Rat skeletal muscle contains three types of fibers. The muscles examined in the present study consisted of a mixture of 25% oxidative fibers (I and IIa) and 75% glycolytic fibers (IIb) [15]. With isolated resting skeletal muscles in the rabbit, Pagliassotti and Donovan [16] demonstrated that oxidative fibers consume lactate to a greater extent than do glycolytic fibers during hyperlactemia. Other investigators have also reported that lactate is oxidized in resting and exercising skeletal muscles [14,17]. Therefore, although we could not

clarify the differences among the three types of fibers in lactate metabolism during hypoxia, we speculate that such differences may exist during hypoxia.

Gutierrez et al. [4] suggested that there may be three potential fates of the lactate taken up by the skeletal muscle: oxidation, glycogen synthesis, and intercellular accumulation. They demonstrated that oxidation was the least probable, because oxygen consumption in the rabbit hindlimb during hypoxia was so low that it was not correlated with oxidation of the lactate taken up by the skeletal muscle. Intercellular accumulation also seems unlikely, because the interstitial lactate concentration in the skeletal muscle is much lower than the blood lactate concentration, as was demonstrated in the present study. Thus glycogen synthesis may be the most probable explanation, although we have no supporting data at present. We must measure other metabolites, glucose, pyruvate, and glycogen, to solve this problem.

The liver is thought to be one of the most important lactate-utilizing organs. However, it was demonstrated that the lactate uptake by the liver was impaired during hypoxia, and even the liver showed a transition to lactate release under severe hypoxia [18,19]. We believe that the liver acted as a lactate consumer under hypoxic conditions in this experiment, even though the lactate concentration in the liver interstitium was much higher than that in the skeletal muscle interstitium. This relative proportion may not necessarily suggest that the consumption of lactate by the liver was less marked than that by the skeletal muscle, because the liver receives blood from the portal vein in addition to arterial blood flow. Considering that the splanchnic region has been reported to function as a lactate-releasing site during hypoxia [20], the liver may prevent the release of lactate from the splanchnic region into the systemic circulation.

A rise in the arterial blood lactate concentration during acute hypoxia has been reported to be due to both an increase in lactate entry to the circulation and diminished removal of lactate from the blood [20,21]. Although the present results demonstrated that the skeletal muscle and liver consume lactate during hypoxia, the consumption of lactate by both may be decreased because the metabolism of lactate requires either ATP or oxygen. The release of lactate into the systemic circulation from some organs other than the skeletal muscle and liver may be accelerated. Our previous study also demonstrated that the brain may not be involved in the rise in blood lactate level during hypoxia [22]. As to the tissues that produce lactate during hypoxia, adipose tissue was recently reported to release lactate into the blood circulation during hyperglycemia in rats and humans [5,23,24]. Further studies may be needed to identify the lactate-releasing tissue supplying the blood during hypoxia.

In conclusion, we found that skeletal muscle and liver interstitial lactate concentrations measured using in vivo microdialysis methods were significantly lower than the blood lactate concentration during hypoxica hypoxia in anesthetized and muscle-relaxed rats. Therefore, we considered that the skeletal muscle as well as the liver function as lactate consumers rather than lactate producers under hypoxic conditions.

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References

1. Okuda C, Sawa T, Harada M, Murakami T, Matsuda T, Tanaka Y (1992) Lactate in rat skeletal muscle after hemorrhage measured by microdialysis probe calibrated in situ. Am J Physiol 263:E1035–E1039
2. Okuda C, Sawa T, Harada M, Murakami T, Tanaka Y (1992) Continuous measurement of lactate concentration in skeletal muscle and liver interstitium using a microdialysis method after acute hemorrhage in anesthetized rats. Circ Shock 37:230–235
3. Harada M, Okuda C, Sawa T, Fuse A, Imai H, Tanaka Y (1994) Changes in muscle and liver lactate concentrations after endotoxin infusion in rats. Circ Shock 43:166–170
4. Gutierrez G, Hurtado FJ, Gutierrez AM, Fernandez E (1993) New uptake of lactate by rabbit hindlimb during hypoxia. Am Rev Respir Dis 148:1204–1209
5. Hagström E, Arner P, Ungerstedt U, Bolinder J (1990) Subcutaneous adipose tissue: a source of lactate production after glucose ingestion in humans. Am J Physiol 258:E888–E893
6. Lönnroth P, Jansson PA, Smith U (1987) A microdialysis method allowing characterization of intercellular water space in humans. Am J Physiol 253:E228–E231
7. Lönnorth P, Jansson PA, Fredholm BB, Smith U (1989) Microdialysis of intercellular adenosine concentration in subcutaneous tissue in humans. Am J Physiol 256:E250–E255
8. Winer BJ (1971) Multifactor experiments having repeated measures on the same elements. In: Statistical principles in experimental design, 2nd edn. MacGraw-Hill, New York, pp 514–603
9. Roth DA, Brooks GA (1990) Lactate transport is mediated by a membrane-bound carrier in rat skeletal muscle sarcolemmal vesicles. Arch Biochem Biophys 279:377–385
10. Watt PW, MacLennan PA, Hundal HS, Kuret CM, Rennie MJ (1988) L(+)-lactate transport in perfused rat skeletal muscle: kinetic characteristics and sensitivity to pH and transport inhibitors. Biochim Biophys Acta 944:213–222
11. Fafournoux P, Demigné C, Rémésy C (1985) Carrier-mediated uptake of lactate in rat hepatocytes. Effects of pH and possible mechanisms for L-lactate transport. J Biol Chem 260:292–299
12. Monson JP, Smith JA, Cohen RD, Iles PA (1982) Evidence for a lactate transporter in the plasma membrane of the rat hepatocyte. Clin Sci 62:411–420
13. Chin ER, Lindinger MI, Heigenhauser GJF (1991) Lactate metabolism in inactive skeletal muscle during lactacidosis. Am J Physiol 261:R98–R105

14. Gladden LB (1991) Net lactate uptake during progressive steady-level contractions in canine skeletal muscle. J Appl Physiol 71:514–520
15. Armstrong RB, Phelps RO (1984) Muscle fiber type composition of the rat hindlimb. Am J Anat 171:259–272
16. Pagliassotti MJ, Donovan CM (1990) Role of cell type in net lactate removal by skeletal muscle. Am J Physiol 258:E635–E642
17. Lindinger MI, Heigenhauser GJF, McKelvie RS, Jones NL (1990) Role of nonworking muscle on blood metabolites and ions with intense intermittent exercise. Am J Physiol 258:R1486–R1494
18. Tashkin DP, Goldstein PJ, Simmons DH (1972) Hepatic lactate uptake during decreased liver perfusion and hypoxemia. Am J Physiol 223:968–974
19. Samsel RW, Cherqui D, Pietrabissa A, Sanders WM, Roncella M, Emond JC, Schumacker PT (1991) Hepatic oxygen and lactate extraction during stagnant hypoxia. J Appl Physiol 70:186–193
20. Arieff AI, Graf H (1987) Pathophysiology of type A hypoxic lactic acidosis in dogs. Am J Physiol 253:E271–E276
21. Stanley WC, Mazer CD, Neese RA, Wisneski JA, Cason BA, Demas KA, Hickey RF, Gertz EW (1990) Increased lactate appearance and reduced clearance during hypoxia in dogs. Horm Metab Res 22:478–484
22. Harada M, Okuda C, Sawa T, Murakami T (1992) Cerebral extracellular glucose and lactate concentrations during and after moderate hypoxia in glucose- and saline-infused rats. Anesthesiology 77:728–734
23. Crandall DL, Fried SK, Francendese AA, Nickel M, DiGirolamo M (1983) Lactate release from isolated rat adipocytes: influence of cell size, glucose concentration, insulin, and epinephrine. Horm Metab Res 15:326–329
24. Jansson PA, Smith U, Lönnroth P (1990) Evidence for lactate production by human adipose tissue in vivo. Diabectologia 33:253–256