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Biochemical Changes of Muscle Tissues in Exercise: Effect of Training and Glutamate Infusion on Lactate Metabolism

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= Abstract =In exercise physiology, it is inevitable for the skeletal muscle tissues to generate and accumulate lactate in the tissues, because lactate is the final oxidized product of glycolysis in the relative anerobic condition. The resulting accumulated lactate is supposed to be the main cause of fatigue generation in the exercising muscle tissues. To endure severe exercise, it is known that training can modify lactate metabolism via increase of anerobic threshold. In order to prevent the accumulation of lactate and to activate its clearance in the exercising muscles, it was assumed that the muscle tissue should adapt itself through training to tolerate the high dose of lactate by induction of the related enzymes. The high dose of glutamate infusion prior to exercise would shift GPT and LDH reactions in the direction of lactate elimination. In the present experiment, we determined the changes of enzymic activities such as LDH, GPT and GGTP in muscle tissues of rats after one month swimming training and lactate clearance after strenuous exerise. We compared the glutamate effect on prolonging the survival time. From these results, it was concluded that the enzymes of lactate metabolism are induced in the muscle tissues and lactate clearance is stimulated by training. Moreover, the additional supply of glutamate may improve exercise efficiency via preventing lactate accumulation in exercising muscle tissues.

Key words: ***Exercise efficiency, Lactate accumulation, Training, Glutamate supply***

INTRODUCTION

Strenuous exercise results in the accumulation of lactate in muscle tissues with the concomittant decrease in the ability of muscular performance, one of the fatigue phenomena (Edwards 1981). Since the basic energy for muscular activities is supplied by the oxidation of carbohydrate, the generation of lactate and its consequent accummulation is inevitable from the view point of metabolic control in exercise. The relative shortness of oxygen supply to exercising muscle tissues causes the relative anerobic condition to the tissues and consequently, hampers the entrance of pyruvate into Krebs cycle and the consequent oxidation in the metabolic cycle (Sahlin 1986).

The fate of pyruvate, the final oxidized product

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of glycolysis, is mostly bypassed to lactate under the anerobic situation with the beneficial generation of NAD for shuttling the glycolytic pathway through compensation of NADH at glycerol 3 phosphate dehydrogenase step. In other words, the generation of lactate in strenuous muscle exercise is a physiological response to overcome the metabolic demand of NAD/NADH balance and ATP generation.

The resulting lactate is almost completely dissociated at physiological pH and H^+ ions are formed equivalently to lactate. Although most of the produced H^+ ions are buffered within the tissue, the small fraction appear as free ions and causes a decrease in pH of the tissue. The lactate ion, itself, is not known to have any adverse effect on energy metabolism or on the muscular contraction process. The decrease in muscle pH, however, influences many of the processes involved in the transformation of chemical energy into mechanical work, resulting in muscle fatigue (Fletcher & Hopkins 1907, Karlsson & Saltin 1970, Hill 1955, Sahlin 1978). It is apparent that for better strength of muscle activity, the more energy is required. And the higher the energy generation is, the higher the lactate production is followed, which will further increase the fatigue intensity of the exercising muscle tissues. This contradictory phenomenon is the very essential problem in the improvement of muscle exercise efficiency.

The biochemical mechanism of the muscle tissues to overcome the above problem can be summarized into the increase of the lactate threshold and the acceleration of the lactate clearance. The increase of lactate threshold can be achieved through the induction of high buffering capacity of the tissues, while the acceleration of lactate clearance can be fulfilled by speeding up the metabolic turnover of lactate via activation of oxygen utilization and increase of vascular supply. Both of these mechanisms are supposed to be induced by endurance training (Hajek & Perry 1967).

Therefore, it would be very useful and necessary in the field of exercise physiology to develop a strategy to prevent lactate accumulation in the exercising muscle tissues without any deleterious effect on its efficiency. In the present experiment, we tried to determine the effect of training on lactate metabolism of muscle tissues and to develop a measure to modify the lactate clearance of the tissues. Moreover, it is assumed that lactate would be bypassed into alanine in the muscle tissues by the consecutive activation of lactate dehydrogenase and glutamate pyruvate transaminase. Therefore, as a candidate to accelerate lactate elimination, the effect of glutamate supply on lactate clearance and performance was tested.

MATERIALS AND METHODS

1. Materials and reagents

The diagnostic kits for lactate, lactate dehydrogenase (LDH), glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) were purchased from Sigma Co. (St. Louis, MO. USA). The sodium glutamate and sodium aspartate were donated by Mi-Won Co. (Seoul, Korea) and other reagents and chemicals of the analytical grade were obtained from the local commercial sources. The experimental animals, Sprague-Dawley male rats, were supplied by Seoul National University breeding house after weaning.

2. Training of animals

The animals of approximately 200 g weight were loaded with swimming exercise in warm (about 30°C) and wavy water with 10 g weight on the tail for five minutes everyday (Baker & Horvath 1964). After four weeks of training, the rats were subjected to the survival test with or without the aminoacid infusion.

3. Conditions for swimming test

The experimental rats, trained for four weeks, were subjected to swimming test on the pool of warm (about 30°C) and wavy water with 10 g weight loading on tail to prevent the floating effect of the long tail. The rats of varying conditions of aminoacid infusion were sacrificed in the time schedule after the swimming test for 10 minutes. And the survival time of the rats in the water was determined as the time in minutes until ten seconds submergence.

4. Biochemical analysis

The content of lactate and the activities of lactate dehydrogenase (LDH), glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate

transaminase (GOT) in the samples were determined with the respective diagnostics kits, supplied by Sigma Co. as the instruction manual. And the activity of gamma-glutamyltranspeptidase was analysed after Szasz (1974).

RESULTS

1. Effect of training on survival time

When the animals were subjected to the survival test on swimming, the trained animals showed a very significant increase of its survival time. As shown in Table 1, the survival time of the trained group was almost two fold lengthened than that of the age-matched non-trained control group.

Table 1. Effect of tranining on survival time in swimming test of rats

Groups	Survival Time (min)
Non-trained group (n = 8)	8.49 ± 1.11
Tranined group (n = 8)	15.42 ± 2.35*

All the data are means ± S.D.
*P < 0.01 by t-test

2. Effect of training on lactate clearance

Both groups of animals, trained and non-trained, were subjected to strenuous exercise for 10 minutes swimming. Immediately after exercise, the survived animals were sacrificed and the contents of blood lactate were determined. As summarized in Table 2, the trained rats had much lower amount of blood lactate: that is, 8.3 mmol/l in non-trained animals, and 5.5 mmol/l in trained group. The trained group showed only 67 % of blood lactate level to that of non-trained group. When the lactate clearance was compared between the trained and the non-trained, it was clearly revealed that the trained group had the lower level of blood lactate immediately after the exercise load, which indicated the more rapid return to normal level than the control group (Table 3).

Table 2. Effect of training on the level of blood lactate in rat after strenuous exercise

Groups	Blood Lactate
Non-trained (n = 5)	8.261 ± 2.243
Trained (n = 5)	5.490 ± 0.439*

All the data are means ± S.D. in m mol/L
*P < 0.01 by t-test

Table 3. Comparison of changes in blood lactate level of rats in recovery period following exercise

Time after Exercise	Non-trained (n = 4)	Trained (n = 4)
Resting	2.104 ± 0.476	2.895 ± 1.502
0 min.	10.188 ± 1.018*	6.414 ± 1.275
3 min.	6.736 ± 1.006	5.190 ± 1.180
30 min.	3.211 ± 1.792	3.489 ± 1.147

All the data are means ± S.D. in mmol/L
*P < 0.05 by t-test

Table 4. Effect of training on enzymic activities in rat skeletal muscles

Enzyme	Nontrained (n = 13)	Trained (n = 14)
LDH	1.756 ± 0.48	1.922 ± 0.576
GPT	0.0782 ± 0.0272	0.0932 ± 0.039
GOT	0.490 ± 0.099	0.666 ± 0.274*
GGTP	1.683 ± 0.877	1.463 ± 0.617

All the data are means ± S.D. in I.U./mg protein
*P < 0.05 by t-test

3. Effect of training on enzymic activities

The comparison study of enzymic activities in the skeletal muscles of the trained and the non-trained groups showed significant differences. As shown in Table 4, through endurance training, LDH was increased 10 % from 1.756 to 1.922 I.U./mg protein, while GPT, 20 % from 0.0782 to 0.0932 I.U./mg protien, and GOT, approximately 40 % from 0.48 to 0.666 I.U./mg

protein, in the muscle tissues of the trained group relative to those in the non-trained group. However, the GGTP activity was not increased by endurance training.

4. Effect of glutamate on survival time

To improve the lactate clearance, we infused the glutamate solution in the respective amount to the trained animals. Thirty minutes after the infusion, the rats were subjected to the survival test for swimming. The glutamate infusion prolonged the survival time: 40 mg of glutamate prolonged 18 %, while 80 mg of glutamate, 34 % longer than the control group.

DISCUSSION

The accumulation of lactate in muscles tissues during exercise is one of the most probable direct cause of muscular dysfuncion, leading to fatigue. On the other hand, lactate generation in the anerobic state of sustained muscle contraction has several metabolic advantages over aerobic respiration such as rapid acceleration of ATP generation, energy production in the oxygen debt state and compensation of NAD/NADH balance. However, the generated lactate contributes more than 85 % of the released H^+ in the exercising muscle tissues, which causes the lowering of tissue pH (Sahlin 1978). The decreased muscle pH causes the increased Ca^{++} requirement, the decreased maximal tension (Donaldson *et al,* 1978), decreased myosin ATPase activity (Schadler 1967), and increased protein binding of Ca^{++} in sarcoplasmic reticulum (Nakamura & Schwartz 1972), resulting in dysfunction of muscle contraction, or muscle fatigue. Since the metabolic fate of lactate is dependent on its turnover into pyruvate, the control of pyruvate metabolism limits the lactate metabolism. In exercising muscle tissues, the glycolysis should be continued to maintain ATP supply, which results in the accumulation of pyruvate. However, the inadequacy of Krebs cycle enzymes due to oxygen-debt, as well as paucity of pyruvate carboxylase and malic enzyme, restricts pyruvate metabolism either to lactate or to alanine (Mole et al 1973). The lactate generation is the normal physiological process to compensate the NAD/NADH balance via the glycerol phosphate shuttle. However, the continuing accumulation of lactate would cause the decrease of muscle pH, leading to fatigue, functional loss as described above.

Therefore, muscle tissue should overcome this difficulty through induction of systems to eliminate lactate from the tissues such as increase of buffering capacity of the tissues, transport of lactate from the muscle tissues and biochemical turnover of lactate. In other words, muscle tissues will adapt themselves to the high level of lactate generation by increase of lactate threshold and acceleration of lactate clearance.

Actually muscle tissue can accommodate this purpose through the induction of enzymes of lactate dehydrogenase and varying transaminases by endurance training. As shown in Table 4, the induced level of LDH in the trained group would facilitate the turnover of lactate into pyruvate and *vice versa*. And the higher level of GPT in the training group would also accelerate the metabolic turnover of pyruvate into alanine and vice versa, compared to that in the control group. The significant induction of GOT in the trained group also activates the turnover of glutamate into aspartate and *vice versa*. These induced levels of LDH, GPT and GOT in the trained group would suggest that the aminoacids can be more readily interconverted and the lactate can be more easily turned over into alanine via pyruvate. In other words, endurance training can induce those enzymes, related in lactate metabolism, which leads to the acceleration of lactate clearance. Actually as shown in Table 2 and 3, the trained group showed the lower level of blood lactate level after strenuous exercise than the control non-trained group. The lower level of blood lactate in the trained group after the same load of exercise than that in the non-trained group would suggest that those tissues of trained group can endure more heavier exercise, which means better exercise efficiency. The lowering of blood lactate after the exercise load in the training group matches the data of enzyme induction on lactate metabolism, which clearly explains that the higher level of LDH, GPT and GOT would accelerate the lactate turnover. Moreover, the training had prolonged the survival time in the swimming test; almost doubled its survival time compared to that of the non-trained group (Table 1). Since the survival time of any organism depends on variety of factors, it cannot be traced only to the effect of

lactate clearance acceleration. But at least, it is very plausible that the training can modify the lactate metabolism, which leads to acceleration of its clearance and, consequently, helps in prolonging the survival time of the whole organism.

From the biochemical aspect of lactate metabolism, it is very interesting that the enzymes, LDH, GPT and GOT are near-equilibrium enzymes, whose kinetics are simply modulated by the simple mass-action low. In other words, the determinants for the activities of these enzymes are the amounts of substrates and products, which means that the change of the concentration of either substrates or products can modify the direction of metabolic turnover. Therefore, it is reasonable to assume that the supply of glutamate, the substrate for GPT reaction toward pyruvate elimination, would shift the turnover of pyruvate to alanine rather than lactate. If so, then the lactate level in the tissues would be decreased, through which exercise efficiency of the muscle tissues can be improved. The second experiment was designed to determine the effect of glutamate on exercise by swimming test. As illustrated in Table 5, the infusion of glutamate prolonged the survival time in the trained groups of rats, which showed the dose-response; the higher the glutamate supply was, the longer the survival time prolonged. Actually, the effects of amino acid infusion including glutamate, alanine and aspartate on muscle exercise are being tested in our laboratory by monitoring the survival time, lactate clearance, enzyme activities, and muscle performances. The effect of glutamate infusion on lactate-induced performance decrease of muscle contraction will be described in the accompanying paper (Choe *et al.*, 1989). And the effect of glutamate and other amino acids on strenuous exercise was briefly introduced elsewhere (Park 1989). These results suggest that the glutamate, as well as other amino acids, can affect lactate metabolism and can improve exercise efficiency. These effects of amino acids can be enhanced by training, since training would induce the enzymes in relation with lactate clearance of the tissues. Therefore, it can be concluded that endurance training would accelerate lactate clearance by induction of enzymes, related with lactate metabolism, and the supply of glutamate and other amino acids might modulate it. And it is apparent that through the supply of glutamate and other amino acids, the improvement of exercise efficiency can be enhanced.

Table 5. Dose effect of glutamate on survival time in swimming test of the trained rats

Goups	Survival Time (min)
Control saline group (n = 5)	14.6 ± 4.88
Glutamate group I (40 mg/head) (n = 4)	17.3 ± 2.85
Glutamate group II (80 mg/head) (n = 4)	19.6 ± 2.84

All the data are means ± S.D.

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= 국문초록 =

운동중 근육조직의 생화학적 변화 : 훈련과 glutamate투여가 lactate대사에 미치는 영향

서울대학교 의과대학 국민체력과학연구소, 생화학교실, 생리학교실[1], 간호학과[2], 해부학교실[3], 핵의학교실[4], 한국체육대학 생리학교실*, 한남대학교 이공대학 식품영양학과#

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운동으로 근육조직내 락트산이 생성되고 축적되는 현상은 상대적 산소부재 상황에서는 해당대사의 종산물이 락트산이므로 불가피하며, 이러한 결과는 근육조직의 피로 및 운동효율저하의 주요인으로 인정되고 있다. 운동조직이 이러한 현상을 극복하고 적응하기 위하여서는 락트산 대사 유관효소계를 증진시키거나, 또는 조직의 buffering capacity를 증가시켜야 한다. 따라서 본 연구에서는 훈련과정을 통하여 보다 운동효율이 증가되고, 이러한 효율증가 요인으로서 효소계가 증진되는 현상을 관찰하여 이러한 기능을 인위적으로 조절할 수 있는 방안을 개발하고자는 목적으로 실험하였다. 흰쥐를 대상으로 1개월간 수영 적응 훈련을 시킨 군과 대조군을 비교해 본 결과, 훈련군에서 수영 생존기간이 2배 증가되고, 근육 조직중 LDH, GPT, GOT 효소활성이 유의하게 증가되었음을 구명하였다. 또한 lactate clearance를 촉진시키고자는 목적으로 투여한 glutamate가 흰쥐의 수영생존시간을 유의하게 증가시킴을 관찰하여, 인위적으로 근육의 운동효율을 증진시킬 수 있는 방안이 가능함을 규명하였다.