The Seoul Journal of Medicine
Vol. 30, No. 3:163-169, September 1989

Effect of Glutamate on Lactate-Induced Performance Decrease of Muscular Contraction

Myoung Ae Choe[1], Jun Kim[2], Sang Ah Seoh[2], Chang Keun Kim, Mee Sook Lee
Kwang Ho Lee[3], Chang Soon Koh[4] and Sang Chul Park[5]*

The Physical Culture Research Institute.
Departments of Nursing[1], Physiology[2], Anatomy[3], Nuclear Medicine[4] and Biochemistry[5], College of Medicine, Seoul National University, Seoul 110-460, Korea

= Abstract =The accumulation of lactate in exercising muscle causes a fatigue phenomenon with deterioration in muscle performance. In a relative anaerobic condition, it is a normal physiological response to generate lactate for the compensation of NAD/NADH balance of the tissues in order to continue ATP production. The alternative route of lactate metabolism in such a state would be opened via transamination reaction to alanine through pyruvate. Since LDH and GPT are near-equilibrium enzymes, whose kinetics are operated under the simple mass-action law of substrates, it is possible to modulate the metabolism of lactate through the supply of glutamate. In the present experiment, we analyzed the effect of lactate perfusion on muscular contractility and the preventive effect of glutamate on the lactate-induced decrease of muscle performance. The maximal twitch tension was decreased by lactate infusion in contrast to its increase by glutamate and its rapid decrease by alanine infusion. From these results, it can be concluded that the administration of glutamate may improve exercise efficiency through prevention of lactate accumulation in exercising muscle tissues.

Key works: ***Glutamate, Lactate, Fatigue, Muscle contraction***

INTRODUCTION

During intense muscular exercise, lactic acid is formed and accumulated within the muscle. Accompanying with lactic acid accumulation, there is a decrease in the ability of the muscle to perform work or sustain force. An inability to maintain the expected force or power output is defined as fatigue (Edwards 1981). One factor that has been postulated to cause fatigue during muscular work is the accumulation of lactic acid (Fletcher and Hopkins 1907; Hill and Kupalov 1929; Karlsson and Saltin 1970).

A close correlation between lactate content and decrease in contractile force in anaerobically stimulated frog muscle was reported by Fitts and Holloszy (1976). The production of lactate in the exercising muscle is a physiological response to overcome the metabolic demand of NAD/NADH balance and ATP production. However, at physiological pH, lactic acid is almost completely dissociated to form H^+ ions in an amount equivalent to lactate. Though most of the produced H^+ ions can be buffered within the tissue, its small fraction as free ions causes a decrease in pH. The lactate ion itself is not known to have any adverse effect on energy metabolism or on the contraction process. Rather, a decrease in muscle pH will influence

Received 30/6/89; revised 19/8/89; accepted 23/8/89
This study was partially supported by the grant from the Basic Research Promotion Fund of College of Medicine, Seoul National University.

*Corresponding author

many of the processes involved in the transformation of chemical energy into mechanical work, in the development of muscular fatigue. (Hill, 1955). Decline in tension during muscular activity was associated with decreased ATP content and increased lactate, hydrogen ions, and ADP (Sahlin *et al*, 1981). The high concentration of lactate and low level of creatine phosphate delayed the rate of ATP generation. When the rate of ATP production is insufficient to meet the demands, fatigue occurs.

It is obvious that the more energy is required for the greater performance capacity of muscle. The more energy generation is, the more lactate production follows, in this relative anerobic condition, which will further increase the fatigue intensity of exercising muscle tissues. This contradictory phenomenon is an essential problem in the improvement of exercise efficiency. Therefore, it would be very useful and necessary to develop a measure to prevent lactate accumulation in exercising muscle tissue without any deleterious effect on exercise efficiency. For the probable candidate, we assumed that lactate could be bypassed to alanine in muscle tissue by activation of glutamate pyruvate transaminase and lactate dehydrogenase step through the supply of glutamate.

The purpose of this study was to determine the effect of glutamate on the decreased muscular contraction induced by the accumulated lactate in exercising muscles of cats with a purpose to establish the measure to maximize exercise efficiency and to minimize fatigue of the muscles.

METHODS AND MATERIALS

Materials

Reagents and Animals: The sodium glutamate was donated by Mi-Won Co. (Seoul, Korea), lactic acid and sodium lactate were purchased from Sigma Co. (St. Louis, MO, USA). The experimental animals, cats, were supplied by Seoul National University breeding house. Other reagents or chemicals of analytical grade were obtained from the local commercial sources.

Preparation for stimulation: Cats were anesthesized with pentobarbital sodium (30 mg/kg, i.p.). Trachea and external jugular vein were cannulated. Common carotid artery was cannulated and arterial blood pressure was recorded through carotid cannula. Sural artery was cannulated. Achilles tendon was cut, tied to a silk, and connected to a force transducer. Medial gastrocnemius nerve was stimulated electrically by platinum electrode at a frequency of 2Hz with 0.1 ms duration (Fig. 1). Intensity of stimulation was tenfold that of the threshold of motor nerve activation.

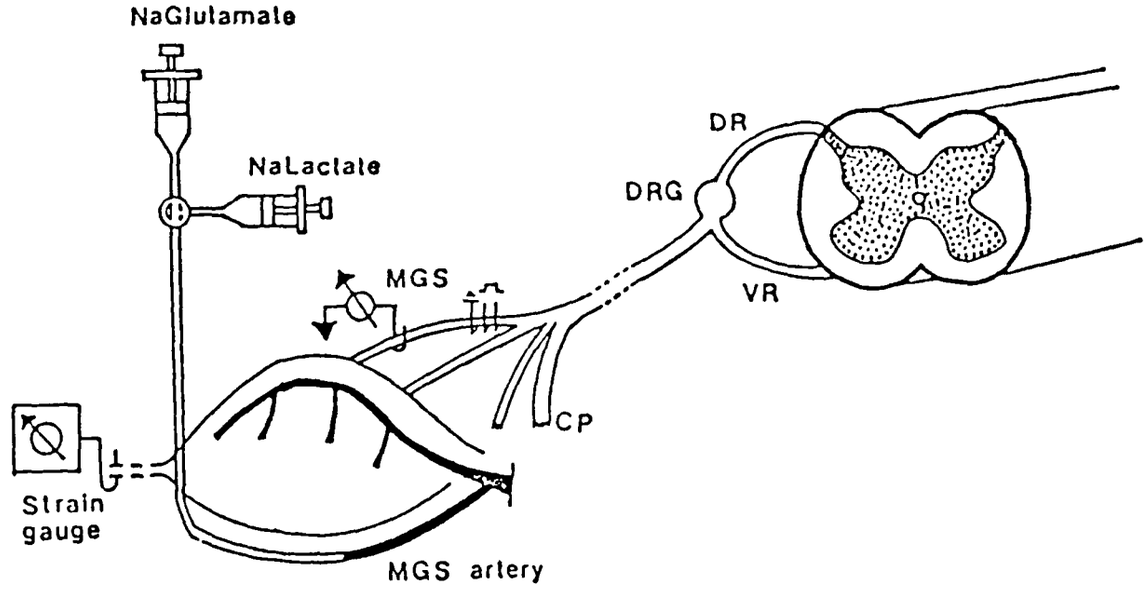

Fig. 1. A diagram of experimental set up. Medial gastrocnemius nerve (MGS) was stimulated electrically by platinum electrode. Achilles tendon was cut, tied to a silk and connected to force transducer. Various solutions were infused by sural artery.

Infusion of amino acids: Isometric twitch tension of medial gastrocnemius muscle was recorded on a recorder. To accumulate lactate in an exercising muscle, 50 mM sodium lactate was infused at the rate of 0.84 ml per minute by sural artery for an hour to one group. 50 mM sodium glutamate or 20 mM sodium glutamate mixed with 50 mM sodium lactate was infused at the rate of 0.84 ml per minute by sural artery to other two groups respectively. 20 mM alanine mixed with 50 mM sodium lactate was infused to the last group with same speed.

STATISTICAL ANALYSIS OF DATA

Ordinary statistical methods were applied for calculations of mean values and standard error, and ANOVA was used to determine statistical significance.

RESULTS

Effect of sodium lactate

Isometric twitch tension fell progressively by infusion of 50 mM sodium lactate. Maximal tension was shown to be 90.3 % at 60 min of the control level after infusion of that solution, and 89.6 % at 30 minutes, 74.2 % at 60 min, and 59.2 % at 90 min of the control level respectively, during recovery (Fig. 2).

Effect of sodium glutamate

An increase in twitch tension occurred by administration of 50 mM sodium glutamate. The tension increased to be 106.1 % at 30 min, 106.3 % at 60 min and 101.3 % at 90 min during recovery compared to the control, respectively. Tension of 50 mM sodium glutamate was significantly greater than that of 50 mM sodium lactate at 60 min and at 90 min during recovery. ($p < 0.01$) (Fig. 3).

Effect of sodium glutamate mixed with sodium lactate

There was little change of the tension by infusing 20 mM glutamate mixed with 50 mM sodium lactate. Maximal twitch tension was 95.6 % at 30 min, 92.7 % at 60 min and 98.7 % at 90 min compared to the control level during recovery, respectively. The tension of 20 mM sodium glutamate with 50 mM sodium lactate was significantly greater than that of 50 mM

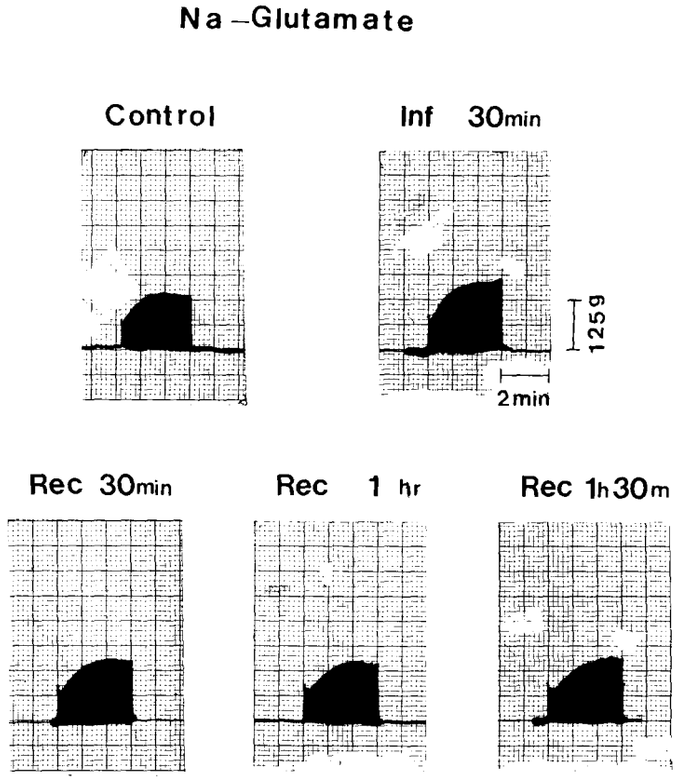

Fig. 2. Examples of isometric twitch tension during infusion of and recovery from 50 mM sodium lactate. Cont; control. Inf: infusion. R: recovery.

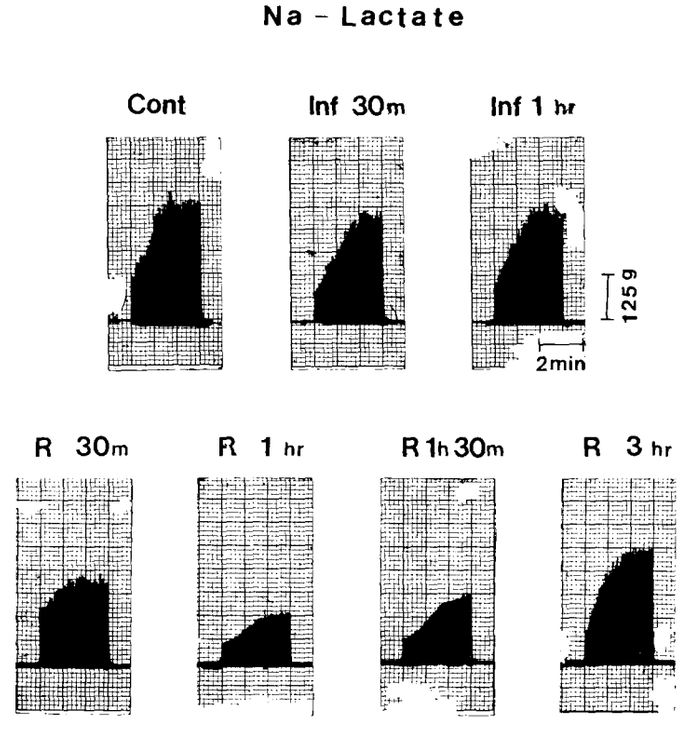

Fig. 3. Examples of isometric twitch tension during infusion of and recovery from 50 mM sodium glutamate. Inf: infusion. Rec: recovery.

sodium lactate alone at 90 min during recovery. ($p < 0.01$) (Fig. 4).

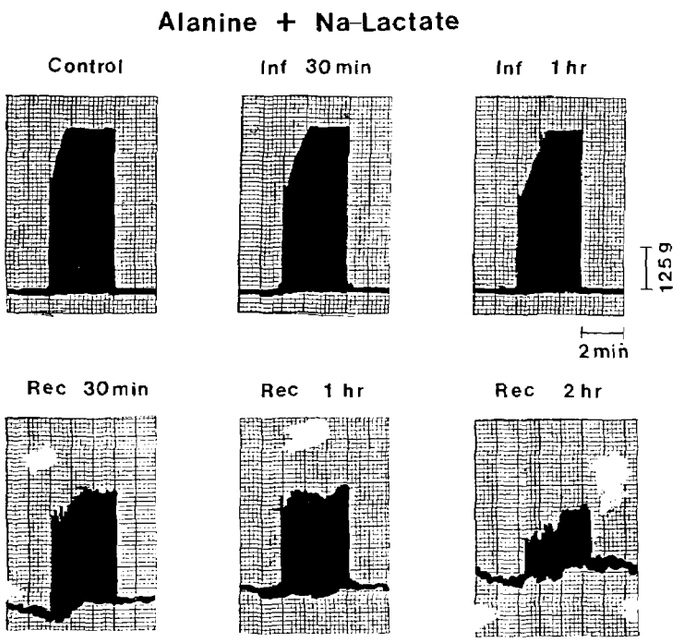

Fig. 4. Examples of isometric twitch tension during infusion of and recovery from 20 mM sodium glutamate mixed with 50 mM sodium lactate. Inf: infusion. Rec: recovery.

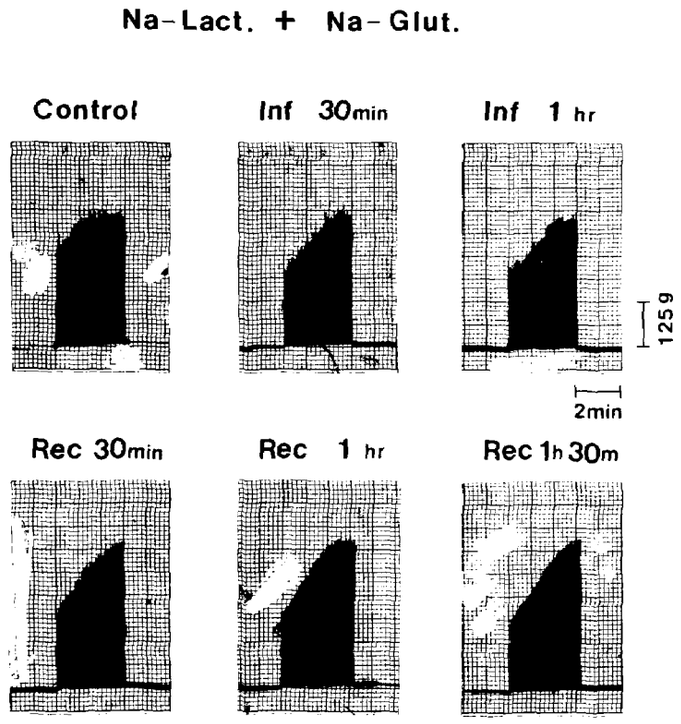

Fig. 5. Examples of isometric twitch tension during infusion of and recovery from 20 mM alanine mixed with 50 mM sodium lactate.

Effect of alanine mixed with sodium lactate

The tension was decreased by infusion of 20 mM alanine with 50 mM sodium lactate. Maximal twitch tension decreased to be 92.5 % at 30 min, 84.1 % at 60 min compared to the control after infusion, while the tension increased gradually to be 80.2 % at 30 min of the control level during recovery. Moreover, alanine treatment induced the muscular fibrillation (Fig. 5).

DISCUSSION

Lactic acid is generated and accumulated in the exercising muscle tissues under the conditions of high energy demand, rapid fluctuations of the energy requirement and insufficient supply of oxygen (Sahlin 1986). Although lactic acid formation is an inefficient way of utilizing carbohydrate for ATP production, it has several advantages over the aerobic ATP generation processes: (i) the maximal rate of ATP production from glycolysis is about 2-fold higher than from oxidative phosphorylation in the relative anerobic condition of the muscle tissues (McGilvery 1973), (ii) ATP generation from glycolysis can accelerate from a low resting level to the maximal power in a very short time (< 5s), in contrast to aerobic ATP production requiring a longer time (2-3 min) due to the necessity of adjusting the O_2 transporting system (circulation and respiration), and (iii) glycolytic ATP production can occur in the absence of oxygen (Sahlin 1986). Despite those metabolic advantages of lactate generation in the exercising muscle tissues, the lactic acid contributes more than 85 % of the released H^+, which causes the lowering of tissue pH in those tissues and the eventually muscle fatigue during intense exercise (Sahlin 1978). Recent studies of skinned muscle fibers have shown that the activation of muscular contraction is inhibited at high concentrations of H^+ ions (Donaldson *et al*, 1978; Fitts and Holloszy 1976). Tension decline is closely related to decrease in muscle pH and increase in ADP (Sahlin 1981).

Fuchs *et al.* (1970) have found that an increase in H^+ concentration interferes with Ca^{++} binding to troponin by lowering the apparent binding constant. Nakamura and Schwartz (1972) found that a decrease in pH increases the Ca^{++} binding capacity of the sarcoplasmic reticulum.

Both mechanisms would function to decrease the number of calcium ions bound to troponin during excitation-contraction coupling. This would reduce the number of active interactions between actin and myosin and thus could decrease contractile force. Phosphofructokinase activity is inhibited by a decrease in pH (Trivedi and Danforth 1966) and accumulation of lactic acid could slow down glycolysis during intense muscular work. The decreased muscle pH caused the increased requirement for Ca^{++} and the decreased maximal tension (Donaldson *et al*, 1978), decreased myosin ATPase activity (Schadler 1967) and increased protein binding of Ca^{++} in sarcoplasmic reticulum (Nakamura and Schwartz 1972) resulting in the decrease of muscle contraction process, the muscle fatigue.

Therefore, it would be desirable to develop a method to prevent lactate accumulation without disturbing glycolysis in the exercising muscle tissues. Lactate is generated from glucose though pyruvate in case of oxygen-short state, such as the strenuous muscle exercise, and the accumulated lactate can be cleared biochemically through pyruvate. But the inadequacy of Krebs cycle enzymes pyruvate carboxylase and malic enzyme, in muscle tissues, limits the metabolic clearance of pyruvate only either to lactate through lactate dehydrogenase reaction or alanine through transamination reaction. In the aspect of the immediate energy generation, the former reaction is physiologically favored, because that reaction would regenerate NAD to activate the glycerol 3-phosphate shuttle. Moreover, lactate dehydrogenase (LDH) and glutamate pyruvate transaminase (GPT) are the near-equilibrium enzymes, which could be kinetically controlled by simple mass-action law, which means that the determinants for the activity control are the concentration of the substrates and products. Therefore, it could be assumed that the turnover of pyruvate to alanine would result in the metabolic change of lactate to pyruvate with the consequent decrease of lactate content in the tissues. Under this assumption, we designed an experiment to stimulate the turnover of pyruvate to alanine by supplying the additional glutamate to the tissues in order to shift the GPT reaction in the direction of alanine formation, thereby to decrease the lactate accumulation for the purpose of increasing the exercise efficiency. In support of our assumption, the amino acid analysis showed that the contents of alanine and glutamine in the muscle veins exceeded 50 % of the total amino acids in spite of the low content of those amino acids in the tissues, which suggested the active metabolic role of alanine in the exercise.

In this study, our assumption was tested through the experiment on the cats. From this experiment we monitored the effect of lactate, glutamate, glutamate mixed with lactate, and alanine mixed with lactate on the isometric maximal twitch tension. The tension fell progressively by infusion of lactate, while administration of glutamate did not affect the tension significantly. There was little change of tension by infusing glutamate mixed with lactate. The tension was decreased after combined infusion of alanine with lactate, while the tension increased gradually during recovery. The tension decreased by lactate infusion supports Hill's hypothesis that lactic acid accumulation results in development of muscle fatigue. These suggest that infusion of glutamate can improve the performace capacity of muscle in contrast that of alanine worsens it (Fig. 6). Glutamate and aspartate are candidates to have the lactate scavenging role. Since most of the amino acids are turned over to glutamate,

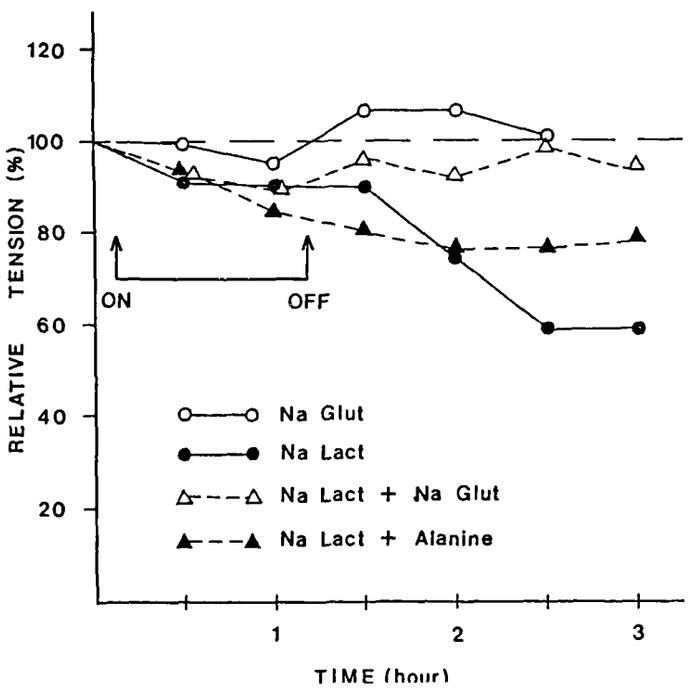

Fig. 6. Changes in contractile force during infusion of and recovery from lactate, glutamate and alanine.

which pushes the mass action law to work in the direction of clearing lactate, the supply of other amino acids except alanine would be advantageous to muscle exercise. Mole *et al.* (1973) reported that glutamate was decreased significantly during contraction in the muscle tissues but its decrease was for the most part balanced by the increase in alanine. Therefore, the turnover of pyruvate to alanine via glutamate-pyruvate transamiation was stimulated by the additional infusion of glutamate, which resulted in the improvement of exercise performance of the muscle tissues. The detailed mechanism of glutamate effect on lactate-induced muscle fatigue is still under investigation in our laboratory. The biochemical changes in relation with the muscle exercise will be described in an accompanying paper (Park *et al.* 1989).

REFERENCES

Choe MA, Kim J, Park SC, Lee KH, Koh C-S. Effect of arterial blood lactate on the contractile force of cat hindlimb, Seoul Olympic Scientific Congress, 1988

Danaldson SKB, Hermansen L, Bolles L. Differential, direct effects of H^+ on CA2-activated force of skinned fibers from the soleus, cardiac and adductor magnus muscles of rabbits, Pfluegers Arch. 1978, 276: 55-65

Edwards RHT. Human muscle function and fatigue, In: Porter R, Whelon J (Ed) Human muscle fatigue: physiological mechanisms, Pitman, Medical, London, 1981, pp 1-18

Fitts RH, Holloszy JO. Lactate and contractile force in frog muscle during develpment of fatigue and recovery, Am. J. Physiol. 1976, 231 (2): 430-433

Fletcher WW, Hopkins HG. Lactic acid in mammalian muscle, J. Physiol, London, 1907, 35: 247-303

Fuchs FO, Reddy V, Briggs FN. The interaction of cations with the calcium-binding site of troponin, Biochim. Biohys, Acta. 1970, 221: 407-409

Hajek I, Perry SV, Enzyme adaptation in isolated muscle. Biochem. J. 1967, 105:45-46

Hill AV. The influence of the external medium on the internal pH of muscle, Proc Royal Soc London Biol Sci., 1955, 144: 1-22

Hill AV, Kupalov P. Anaerobic and aerobic activity in isolated muscles, Proc. Roy. Soc., London, Ser. B. 1929, 105: 313-328

Karlsson J Saltin B. Lactate, ATP and CP in working muscles during exhaustive exercise in man, J. Appl. Physiol. 1970, 29: 598-602

McGlivery, RW. The use of fuels for muscular work. In: Howald H, Poortsman JR (ed) Metabolic adaptation to prolonged physical exercise, Brauheuser Verlag, Basel, pp 12-30

Mole PA, Baldwin KM, Terjung RL, Holloszy JO. Enzymatic pathways of pyruvate metabolism in skeletal muscle: adaptations to exercise, Am. J. Physiol. 1973, 224: 50-54

Nakamura Y, Schwartz S. The influence of hydrogen ion concentration on calcium binding and release by skeletal muscle sarcoplasmic reticulum. J. Gen. Pyysiol. 1972, 59: 22-32

Park SC, Kim J, Choe MA, Kim CK, Lee MS, Lee KH, Koh CS. Biochemical changes of muscle tissues in exercise-effect of training and glutamate uptake on lactate metabolism. Seoul J. Med. 1989, 30, 329-331

Sahlin K. Intracellular pH and energy metabolism in skeletal muscle. Acta Physiol. Scand. Suppl 1978, 455: 1-56

Sahlin K. Muscle fatigue and lactic acid accumulation, Acta Physiol. Scand. 1986, 128 (Suppl 556), 83-91

Schadler M. Propertionale aktiverung von ATP ase-aktivitat und Kontraktionspannung durch calciumionen in isolierten contractilen strukturen verschiedener muskelarten. Pfluegers Arch. 296: 70-90.

Trivedi B, Danforth WH. Effect of pH on kinetics of frog muscle phosphofructokinase, J. Biol. Chem. 1966. 241: 4110-4112

= 국문초록 =

Glutamate의 락트산유도 근육수축 저하 방지효과

서울대학교 의과대학, 국민체력과학 연구소, 간호학과[1], 생리학교실[2], 해부학교실[3], 핵의학교실[4], 생화학교실[5]

최명애[1] · 김 전[2] · 서상아[2] · 김창근 · 이미숙 · 이광호[3] · 고창순[4] · 박상철[5]

근육운동시 락트산 축적으로 인한 근피로는 근육운동의 효율을 저하시킨다. 이러한 상황에서 생성되는 락트산은 조직의 NAD/NADH 균형과 ATP 생성을 위하여 불가피하다. 그러나 락트산 대사의 다른경로로 transamination 반응에 의한 피루브산을 통한 alanine으로의 전환이 가능하며, 이 대사에 관여하는 LDH 및 GPT 효소가 near-equilibrium 효소로서 기질의 농도 변화로 쉽게 활성이 제어된다. 본 연구에서는 이러한 성상을 이용하여 락트산의 perfusion에 의한 고양이 비근의 수축에 미치는 영향을 검토하고, 동과정에 추가한 glutamate가 미치는 영향을 검토하여 glutamate 투여는 락트산으로 유도되는 근육수축의 저하를 방지해주는 효과가 있음을 규명하였다. 즉 glutamate 투여는 조직의 락트산 축적을 억제하여 근육의 피로를 방지하며, 따라서 운동의 효율을 증가시킬 수 있음을 밝혔다.