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A Novel Therapeutic Measure for Metabolic Acidosis with Amino Acids

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= ABSTRACT =

In hypoxic tissue conditions, pyruvate can not enter the Krebs cycle and lactic acid, produced from pyruvate, accumulates to induce lactic acidosis. Pyruvate, however, can also be converted to alanine by glutamate-pyruvate transaminase, that could be enhanced by glutamate. Therefore, it would be a fundamental measure to treat the lactic acidosis in tissue hypoxic conditions when one can convert the accumulated lactic acid, through pyruvate, to alanine. To test the above hypothesis, we induced a lactic acidosis in cats and the effect of glutamate on recovery of acid-base state and removal of the lactic acid from blood were assessed and the results were compared with those of bicarbonate administration, which is one of the most frequently used conventional measure for correction of the acid-base state during lactic acidosis. The results were that glutamate and combined glutamate-bicarbonate solutions not only restored the acid-base status completely from the lactic acidosis in an hour or two, but also restored the blood level of lactate partially. We concluded that administration of glutamate solution to convert pyruvate into alanine is effective in preventing lactic acid accumulation and treating lactic acidosis.

Key Words: Lactic acidosis, Pyruvate, Alanine, Glutamate, Glutamate-pyruvate transamination.

INTRODUCTION

Our body has regulatory measures for the acid-base balance to hold the concentration of hydrogen ion in the body fluid within narrow limits under various conditions. Derangements of the balance may be brought about either from metabolic or respiratory origin. Clinically lactic acidosis is one of the most frequent causes of metabolic acidbase disturbances. For example hemorrhagic shock, diabetes mellitus, grand mal seizure, congestive heart failure, severe exercise, over dose of drugs such as ethanol, phenformin and salicylate and cancerous changes in various tissues can result in lactic acidosis (Williams & Palmer, 1975; Spechler et al, 1978; Newsholme & Leech, 1983; Goldberger, 1986). The common pathophysiological mechanism of various forms of lactic acidosis is thought to be the lactic acid accumulation under various conditions of tissue hypoxia to elevate the hydrogen ion concentration.

The best measure for the treatment of lactic acidosis should involve the elimination of factors resulting in lactic acid accumulation but considering the various causes of lactic acidosis it is not easy to find such a measure. The second best measure may be either to take out intracellular lactic acid and eliminate it or to convert lactic acid into other substances

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metabolically. The commonly used measure for lactic acidosis, however, set the goal at correcting the acid-base status of blood by intravenous administration of sodium lactate solution and not at the elimination of tissue lactic acid accumulated. Furthermore acute correction of blood pH would result in aggravation of tissue hypoxia due to the so called "Bohr effect", by which the elevated hydrogen ion concentration reduces the affinity of hemoglobin with oxygen. Considering the clinical significance of lactic acidosis, therefore, it is urgent to develop a measure, not a simple correction of blood pH, to prevent the lactic acid accumulation and elimination of accumulated lactic acid, for the fundamental treatment of lactic acidosis.

The source of tissue lactic acid is pyruvate. Pyruvate is converted to lactic acid under tissue hypoxic conditions and lactic acid enters the metabolic processes only through the conversion into pyruvate. When the tissue oxygen tension decreases, activity of the pyruvate dehydrogenase complex is inhibited and pyruvate can not enter the Krebs cycle, resulting in the conversion of pyruvate into lactic acid by lactic dehydrogenase (LDH) or into alanine by transamination reaction. During conversion reaction into lactic acid, NADH is oxidized to NAD by LDH and this NAD is used in oxidizing glycerol-3-phosphate (glycerol-3-phosphate shuttle). Most researchers take it for granted that lactic acid be accumulated under hypoxic condition and hence have not argued for other possible measure to eliminate the accumulated lactic acid (Sahlin, 1978; 1986).

Conversion of the pyruvate into alanine under tissue hypoxic condition, however, would be as important as conversion of pyruvate into lactic acid. The process occurs easily by transamination reaction. Glutamate-pyruvate transaminase (GPT), the enzyme catalyzing the process, is abundant in tissues. Both LDH and GPT are near-equilibrium enzymes and their action complies with mass action law. When one can direct the metabolic pathway of pyruvate which is produced massively under hypoxic condition to alanine formation, therefore, lactic acid production would be prevented and already accumulated lactic acid would be eliminated via pyruvate-alanine pathway. As far as we know few studies have been concerned with lactic acidosis regarding on the above contention.

The aim of present study is to test the above hypothesis. For this, we used glutamate to direct the metabolic pathway of lactic acid to alanine formation and observed whether glutamate reduced the experimentally elevated level of blood lactic acid and compared the effect with that of bicarbonate solution.

METHOD

Preparation of animal

Adult cats (body weight: 2-3 kg) of either sex were used. Animals were sedated by ketamine (Ketalar, 20mg/kg, i.m), followed by anesthesia with a-chloralose (60mg/kg, i.p.). Trachea was intubated for artificial respiration and femoral artery and vein were cannulated for arterial blood pressure monitoring and drug administration. End-tidal PCO_2 was maintained within 3-4% and rectal temperature was maintained at $37 + 1°C$

Animals were rested for at least an hour after surgery and then lactic acidosis was induced by infusing 0.3 M lactic acid solution for one to two hours (15-30 ml/hour). Animals were divided into two groups: spontaneous respiration group and artificial respiration group. Arterial blood samples were taken out every thirty minutes and acid-base parameters (pH, PCO_2, PO_2 and HCO_3-) and blood lactate level were determined.

To see the recovery processes from the induced lactic acidosis, only the animals with artificial respiration were used to exclude the respiratory compensation. Animals were subdivided into four groups: spontaneous recovery group, bicarbonate group, glutamate group and combined bicarbonate and glutamate group. Acid-base parameters of the arterial blood were determined every thirty minutes during recovery process from lactic acidosis.

Determination of acid-base parameters

Acid-base parameters of the arterial blood were analyzed using blood gas analyzer (Stat Pro 3, Nova), immediately after sampling. The remaining blood samples were centrifuged and separated the plasma. 1 ml of methanol or 100 μl of perchloric acid were added to each 1 ml of separated plasma and these were centrifuged again. Supernatants were taken out and stored in freezer for lactate measurement.

Treatment of experimental animal and statistics

At the end of the experiment animals were sacrificed by overdose of a-chloralose. All the parameters were tested for the statistical significance using paired or non-paired t-test ($p<0.05$).

RESULT

Induction of lactic acidosis

The first step of the experiment was induction of lactic acidosis. For this we infused 0.3 M lactic acid solution at the rate of 30 ml/hour and arterial blood gases were sampled before, 30 and 60 minutes after injection. The acid-base parameters of arterial blood at the end of one hour infusion are shown in Table 1. In spontaneous respiration group, the control pH, PCO_2 and $[HCO_3^-]$ were 7.37 ± 0.015, 30.7 ± 1.77 mmHg and 17.1 mM/1, respectively. Lactic acidosis was induced in an hour by infusion of lactic acid solution. pH was lowered to 7.27 ± 0.018 and PCO_2 decreased to 25.0 ± 2.30 mmHg, indicating that the respiratory compensatory process was activated. In artificial respiration group, the control values of arterial pH, PCO_2 and $[HCO_3^-]$ were 7.35 ± 0.015, 26.7 ± 1.00 mmHg and 14.2 ± 0.49 mM/l, which were changed to 7.20 ± 0.028, 31.8 ± 1.57 mmHg and 12.1 ± 0.78 mM/1, respectively, by lactic acid infusion. The rather increase of PCO_2 and marked decrease in $[HCO_3^-]$ indicate that there was no respiratory compensation to lactic acidosis in this group.

The acid-base status of the arterial blood during lactic acid infusion was plotted on a pH-HCO_3^- diagram as shown in Fugure 1. Dots indicate all the data determined during experiment and the CO_2 isobar line of mean control PCO_2 of each group were also drawn on the figure. Figure 1 clearly shows that infusion of lactic acid induced the metabolic acidosis. As shown in A, the majority of dots were either on the isobar line or below the line in spontaneous respiration group, while most values were above the line in artificial respiration group, also indicating no respiratory compensation in artificial respiration group.

In Figure 2, relations between arterial blood pH and lactate were shown. The control arterial blood lactate was 1.9 ± 0.19 mM/1 ($n = 15$). As shown in the figure, arterial pH decreased in along with the increase of blood lactate by lactic acid infusion. Since the blood lactate could increase in hypoxic and other conditions than lactic acid infusion and the increased lactate

Table 1. Acid-base parameters of arterial blood in cats during lactic acidosis induced by intravenous infusion of 0.3M lactic acid solution

	Self respiration (n = 11)			Artificial respiration (n = 15)		
	pH	PCO_2	$[HCO_3^-]$	pH	PCO_2	$[HCO_3^-]$
Control	7.37 ±0.015	30.7 ±1.77	17.1 ±0.81	7.35 ±0.015	26.7 ±1.00	14.2 ±0.49
LA infusion (0.3M Lactic acid)	7.27 ±0.018	25.0 ±2.30	11.2 ±1.03	7.20 ±0.028	31.8 ±1.57	12.1 ±0.78

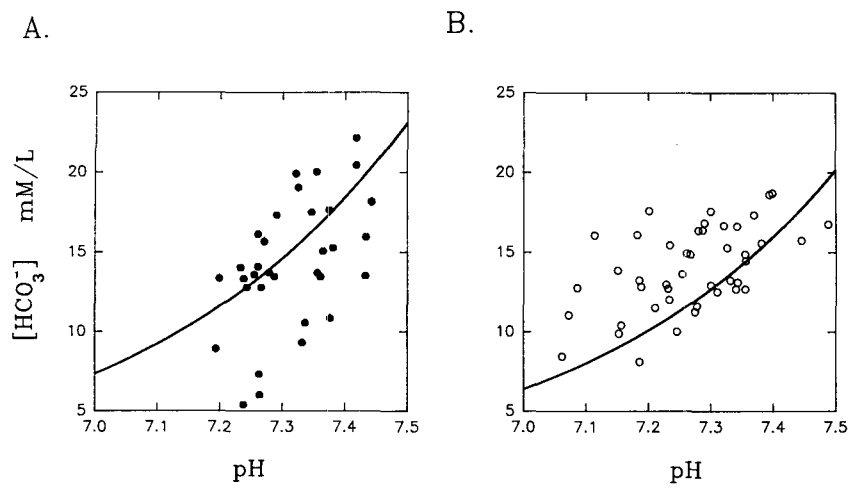

Fig. 1. The acid-base pathways of experimentally induced acute lactic acidosis in cats. Each point indicates the acid-base status of arterial blood sampled before and during intravenous infusion or lactic acid solutions. The curves in diagrams indicate the isobar lines of mean arterial carbon dioxide partial pressure of control blood in each group. A: self respiration group(n = 11), B: artificial respiration group(n = 15)

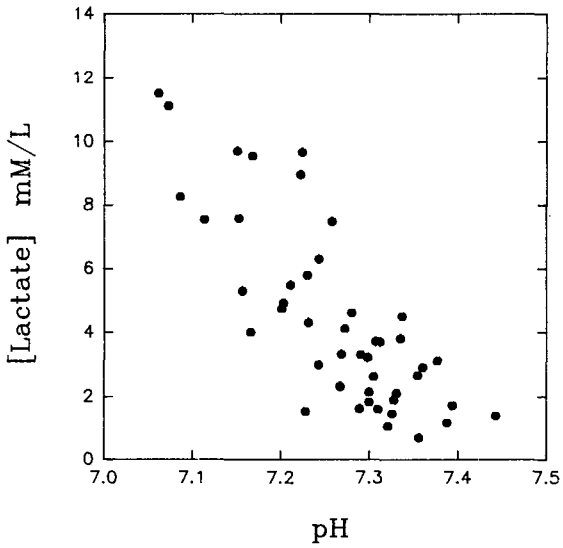

Fig. 2. Relations between arterial blood pH and arterial concentration of lactate in acute lactic acidosis in cats. Lactic acidoses were induced by infusion of lactic acid solutions.

would be handled through Krebs cycle by sufficient oxygen supply, we elevated the partial pressure of oxygen in inspired air in some animals. The results were shown in Figure 3. During respiration with normal oxygen pressure ($FIO_2 = 0.2$), arterial blood pH decreased easily by lactic acid infusion, while respiration with high-oxygen air resulted in less severe acidosis and bicarbonate level decreased less than that during normal oxygen respiration.

Recovery from lactic acidosis

Since the spotaneous respiration group showed respiratory compensation for induced lactic acidosis, we chose the artificial respiration group for the next step to see the recovery process from lactic acidosis. Animals were subdivided into spontaneous recovery group, bicarbonate group, glutamate group and bicarbonate-glutamate combined groups according to the measures applied during recovery period. The acid-base status of each group determined for control and at one hour after recovery were tabulated in Table 2. Control values of pH were not different significantly between experimental groups (pH range: 7.34-7.37). Administration of combined bicarbonate-glutamate

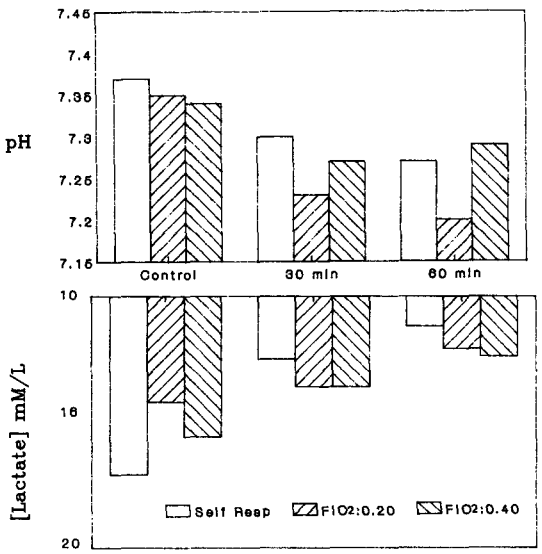

Fig. 3. effect of oxygen partial pressure in inspired air on the development of lactic acidosis. Arterial pH and bicarbonate concentration were determined before(control), 30 and 60 minutes during infusion of lactic acid into animals. Self Resp: self respiration group, FIO_2: fraction of oxygen gas in inspired air.

solution recovered the arterial blood pH from lactic acidosis in an hour to control level. In glutamate and bicarbonate groups, arterial pH recovered 0.04 and 0.09 to control arterial pH in an hour, respectively. On the contrary, in spontaneous recovery group arterial blood pH was 7.21 ± 0.016 at the end of one hour of recovery period indicationg that the animals were still in acidotic condition. The arterial levels of HCO_3^- in glutamate and combined groups were recovered from 14.1 ± 0.97 and 16.5 ± 0.53 mM/l of control level to 14.6 ± 1.49 and 13.4 ± 0.59 mM/l of lactic acidosis condition to 10.5 ± 0.88 and 12.8 ± 0.80 mM/l at the end of one hour recovery period, respectively.

In Figure 3, the recovery pathways from lactic acidosis for each group were plotted on the pH-HCO_3- diagrams. Numbers indicate: control (0), lactic acidosis (1), 30 minutes recovery (2), 60 minutes recovery (3) and 120 minutes recovery (4), respectively. As shown in the figure, in spontaneous recovery group the acid-base status was rather aggravated during two hours of recovery period. On the contrary in glutamate and combined groups acid-base status were recovered from acidosis after 120

Table 2. Changes in acid-base parameters of arterial blood in cats in control, experimental lactic acidosis and 60 minutes recovery from acidosis

	Control			Lactic acid			Recovery		
	pH	PCO_2	$[HCO_3^-]$	pH	PCO_2	$[HCO_3^-]$	pH	PCO_2	$[HCO_3^-]$
Glutamate (n=5)	7.34 ±0.014	26.9 ±1.33	14.1 ±0.97	7.24 ±0.013	32.1 ±3.81	11.2 ±2.76	7.30 ±0.015	30.6 ±2.23	14.6 ±0.79
Bicarbonate (n=5)	7.37 ±0.022	23.9 ±0.58	13.4 ±0.59	7.19 ±0.033	31.4 ±1.54	11.6 ±0.56	7.28 ±0.050	28.2 ±2.2	12.8 ±0.80
Glutamate+ Bicarbonate (n=5)	7.35 ±0.012	30.8 ±1.09	16.5 ±0.53	7.20 ±0.020	37.2 ±4.09	13.7 ±0.96	7.35 ±0.041	36.0 ±6.41	18.8 ±2.11
Spon. Recovery (n=3)	7.34 ±0.044	28.5 ±2.17	14.9 ±1.49	7.17 ±0.059	29.6 ±2.17	10.6 ±1.28	7.21 ±0.016	27.5 ±2.23	10.5 ±0.86

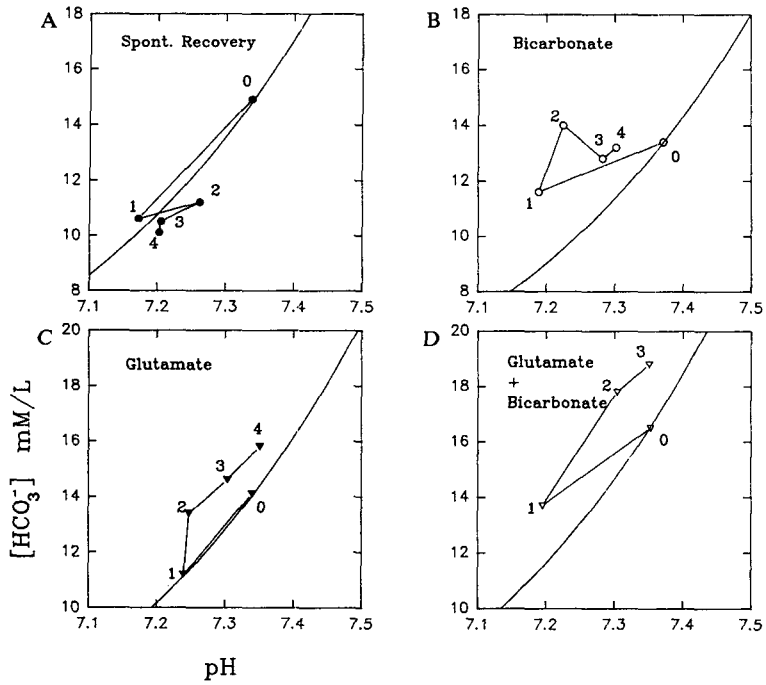

Fig. 4. Restoration of acid-base status from acute lactic acidosis in cats. Numbers in each firgure indicate: control (0), lactic acidosis. (1), 30(2), 60(3) and 120 minutes (4) recovery from lactic acidosis A: spontaneous recovery group. In B, C and D, bicarbonate, glutamate and combined glutamate-bicarbonate soultions were infused during recovery.

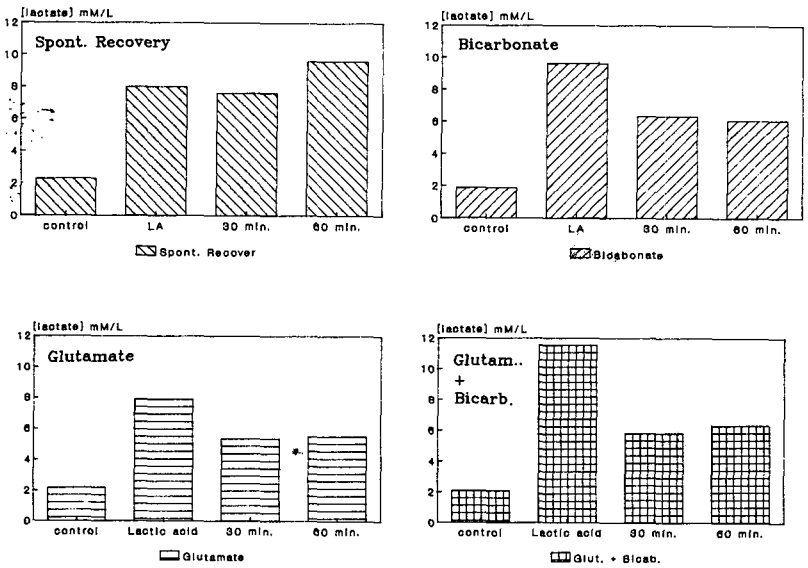

Fig. 5. Changes in the level of arterial lactate during recovery from lactic acidosis. Arterial blood was sampled in control, lactic acidosis(LA), 30 and 60 minutes during recovery from lactic acidosis.

and 60 minutes of recovery periods, respectively. Bicarbonate restored the acid-base parameters partially from acidotic condition.

Since complete recovery from lactic acidosis means removal of administered lactic acid from blood, the changes in blood lactate level for each group were shown in Figure 4. In spontaneous recovery group, lactate level increased from the control of 2.3 ± 0.09 to 8.0 ± 2.18 mM/l by lactic acid infusion and further aggravated to 7.6 ± 2.66, 9.6 ± 1.96 and 11.6 ± 1.87 mM/l at 30, 60 and 120 minutes after recovery periods, respectively. In bicarbonate group, lactate level increased from control of 1.9 ± 0.45 to 9.6 ± 3.2mM/l during lactic acidosis and changed to 6.3 ± 2.57, 6.1 ± 3.13 and 9.2 ± 4.82 at 30, 60 and 120 minutes of recovery. These results mean that bicarbonate administration alone can not eliminate accumulated lactic acid within an hour or two. In glutamate group, lactate level changed from control of 2.2 ± 0.44 mM/l to 7.9 ± 0.96 during lactic acidosis and recovered to 5.3 ± 0.78, 5.5 ± 0.81 and 4.9 ± 0.77 mM/l, respectively. In combined glutamate-bicarbonate group, lactate level changed from the control of 2.1 ± 0.27 to 11.6 ± 1.96 during lactic acidosis and recovered to 5.8 ± 1.8, 6.3 ± 1.5 mM/l, at the end of 30 and 60 minutes recovery periods.

DISCUSSION

The regulation of hydrogen ion concentration in body fluid is one of the typical examples of maintaining body homeostasis. The hydrogen ion concentration is maintained at around 40 nM/l despite daily production of 12,000-20,000 mEq. For this our body has both respiratory and metabolic compensation mechanisms, in addition to various body buffers. Derangement in these regulatory mechanisms would result in distrubances in the body acid-base status, and the acid-base disturbances could be grouped into metabolic and respiratory distrubances. Elimination of the cause is, of course, the fundamental measure for the restoration from acid-base disturbances.

Lactic acidosis, which results from accumulation of lactic acid, is one of the most frequent forms of metabolic acidosis in clinics. It could be caused by hemorrhagic shock, diabetes mellitus, congestive heart failure, grand mal seizure, severe anemia, renal failure, and heavy exercise, which result in tissue hypoxia. In conditions such as drug intoxication with ethanol, phenformin and salicylates or tissue cancerogenesis which decrease the tissue utilization of oxygen or oxygen supply (Williams & Palmer, 975; Spechler et al, 1978; Newsholme & Leech, 1983; Goldberger, 1986).

Accumulated lactic acid originates from pyruvate, which can not enter the Krebs cycle in tissue hypoxic conditions and converts to lactic acid. The only way of handling the accumulate lactic acid is through conversion again to pyruvate. Then the most critical factors are: relative ratio of pyruvate/lactate, lactate dehydrogenase (LDH), and the ratio of NAD/NADH. The pyruvate metabolism is under close control mechanisms. In hypoxic conditions, pyruvate dehydrogenase (PDH) complex was inhibited to prevent the entry of pyruvate into Krebs cycle, resulting in the conversion of pyruvate into lactic acid by LDH. This process has been taken for granted and there has been actually no measure for the elimination of accumulated lactic acid except oxygen supply (Sahlin, 1978; 1986).

Conversion of pyruvate to alanine is, however, as important as conversion of pyruvate to lactic acid in hypoxic conditions. This transamination process is mediated by glutamate-pyruvate transaminase (GPT), which exists abundantly in most tissues. Both LDH and GPT are kinetically equilibrium enzymes which act according to the mass-action law and, therefore, accumulation of pyruvate under hypoxic conditions leads not only to produce lactic acid but also to alanine formation. And also the accumulation of lactic acid would lead, through pyruvate, to alanine formation. After all, there are two possible ways of lactic acid removal: either entry into the Krebs cycle or conversion to alanine. Hitherto the main interest in treatment for the lactic acidosis has

been in removal of lactic acid through the Krebs cycle and no measure using pyruvate to alanine conversion for lactic acid treatment was developed. But in many cases of lactic acidosis, the use of Krebs cycle to metabolize the lactic acid is not available because of limit in oxygen utilization and hence only the simple correction of arterial acid-base parameters by administration of bicarbonate solution is widely used (Williams & Palmer, 1975; Spechler et al, 1978; Newsholme & Leech, 1983). Above method, however, has some problems. For example, acute correction of arterial blood pH leads to increase in the affinity of hemoglobin to oxygen molecules, which would result in aggravation of tissue hypoxia and lactic acid accumulation.

From this point of view we had tested the possibility of using glutamate to remove the tissue lactic acid during severe exercise. The hypothesis was that glutamate converts pyruvate to alanine using GPT while glutamate itself is converted to α-ketoglutarate and administration of glutamate would convert most of the pyruvate to alanine, decrease the lactic acid and hence increase the efficiency of muscle contraction. Intraarterial injection of lactic acid to triceps surae muscle in cats decreased the contractile force dramatically, which was reversed by simultaneous injection of glutamate (Choe et al, 1989). Glutamate also lengthened the swimming survival time in rat (Park et al, 1989) and decreased the blood lactate accumulatd during treadmill test in human subjects (Koh et al, 1989). These results suggested that it would be possible to develop measures to reduce the production and to eliminate the accumulated lactic acid.

In present study, we tested whether glutamate administration would be an effective measure to treat the lactic acidosis. A typical lactic acidosis was induced in animals by intravenous infusion of lactic acid solution. According to clinical criteria by blood lactate level (Goldberger, 1986), Infusion of 30-60 ml of 0.3 M lactic acid solution resulted in severe lactic acidosis. Elevation of inspiratory oxygen partial pressure had som effect of the prevention of lactic acidosis and sparing of blood buffers. There was no sign of spontaneous recovery from induced lactic acidosis within two hours after cessation of lactic acid infusion. And administration of glutamate or combined glutamate-bicarbonate solution were much more effective in restoration of acid-base status from lactic acidosis than administration of bicarbonate simply to correct the acid-base parameters. Restoration of acid-base parameters was faster than that of blood lactate. These results suggest that administration of glutamate to enhance the conversion of pyruvate to alanine is effective in the recovery from acute lactic acidosis.

In conclusion, the fundamental treatment for symptoms such as lactic acidosis and muscle fatigue which result from tissue lactic acid accumulation should include measures for the elimination of lactic acid and for this, administration of glutamate to enhance the conversion of pyruvate to alanine would be an effective measure.

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