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Ammonium Salts Increase Physical Performance and Reduce Blood Lactate Level in Rats in a Model of Forced Swimming

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We compared the effects of two doses of ammonium chloride and ammonium carbonate (10 and 20 mg/kg) on the duration of swimming and blood lactate level. Ammonium chloride in a dose of 20 mg/kg was more efficient than in a dose of 10 mg/kg. The efficiency of ammonium carbonate in a dose of 10 mg/kg was similar to that of ammonium chloride in a dose of 20 mg/kg. Increasing the dose of ammonium carbonate to 20 mg/kg led to a decrease in the duration of swimming. On the last day of the experiment, lactate level in 5 min after exhausting load was maximum in control rats, while in rats treated with 10 mg/kg ammonium carbonate and 20 mg/kg ammonium chloride it was lower by 27 and 33%, respectively. In the control group, the amplitude of the decrease in lactate concentration in 1 h after load was 2-fold greater than in the group receiving ammonium chloride in a dose of 20 mg/kg and 1.6-fold greater that in groups treated with ammonium carbonate in a dose of 10 mg/kg and ammonium chloride in a dose of 20 mg/kg.

Key Words: *ammonium; lactate; rats; forced swimming*

Nutraceuticals (dietary supplements and compounds of nutrition, minerals and metabolites of natural origin not included in the lists of banned drugs) can enhance physical performance by positively affecting the balance of signal and metabolic processes in cells and tissues of organism [8]. For instance, it was demonstrated that green tea extract enhances physical performance of rats in the forced swimming test due to its effect on slow muscles whose adaptation to extreme load is associated with enhanced expression of genes responsible for Ca^{2+} balance regulation [2].

Among the biochemical mechanisms of adaptation reciprocally related to Ca^{2+} metabolism, processes of ATP generation and utilization under conditions of aerobic and anaerobic physical exercise are of special importance. It was previously thought that lactate and ammonium are waste products of metabolism and their transfer and neutralization are performed exclusively in the liver in the Cori and alanine cycles. The role of lactate has been long revised and now it is considered as an important intracellular energy transporter and signal agent [7]. The expression of type 4 monocarboxylate transporter (MCT4) is enhanced in phasic muscles to increase export of lactate that is transported into erythrocytes and postural muscles via MCT1 [11]. Erythrocytes participate in lactate transport from producing cells to consuming cells and the role of erythrocytes in this process in athletes increases [14]. Ammonium is still considered as a toxic agent. However, its production in muscles should be considered not only as a result of amino acid catabolism for energy production, but also as a compensatory mechanism for binding protons and neutralization of organic acids (primarily lactate) that are accumulated during physical exercise which disturbs ion balance. As distinct from non-trained volunteers, enhanced physical performance in trained athletes is related not to an increase in maximum oxygen consumption (VO_2max) and oxidative capacity of muscles, but to adaptive changes in the system of regulation of balance of Ca^{2+},

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K^+, Na^+, Cl^-, H^+, and lactate ions [9]. The role of ammonium in this system of adaptive changes is poorly studied, while the effects of low doses of ammonium on physical performance have not been investigated yet. Stimulating properties of ammonium chloride, which were more pronounced than superior to the effects of green tea extract were shown on the model of forced swimming [1].

Here we compared the effects of ammonium chloride (ACh) and ammonium carbonate (ACa) in low doses on the duration of swimming and lactate level in the blood of rats on the model of forced swimming.

MATERIALS AND METHODS

The experiments were performed in accordance with the Rules of Animal Experiments approved by the Ethical Committee of the I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry.

Outbred male Wistar rats weighing 210±20 g were kept under standard vivarium conditions. The animals were preliminary adapted to water (32°C) for 5 days. On day 6 they were tested with a load (7% of body weight): swimming in water of 28°C for 3 min with 1 min interval to complete exhaustion. Five groups were formed in accordance to the results of testing: group 1 (control; n=11) received NaCl solution in a dose of 10 mg/kg; groups 2 (n=10) and 3 (n=11) received ACh in doses of 10 and 20 mg/kg, respectively; groups 4 (n=15) and 5 (n=8) received ACa in doses of 10 and 20 mg/kg, respectively. All substances were administered orally 5 min before swimming starting from Monday of week 2 of the experiment. The volume of administered solutions did not exceed 0.3 ml.

During weeks 2 and 3, the animals swam for 5 days per week with a load (7% body weight; water temperature 28°C) for 3 min with 1 min interval. The total duration of swimming was 50-60% of values recorded during testing. On day 6 of weeks 2 and 3, intermediate testing (swimming in water of 28°C for 3 min with 1 min interval up to complete exhaustion) was performed. During week 4, the rats were subjected to exhausting load for 4 days as described before. On day 4, the blood was taken from the caudal vein 5 min after swimming. Blood lactate level was measured photometrically using portable Accutrend Plus biochemical analyzer (Roche Diagnostics GmbH).

The data were statistically processed using Microsoft Excel 2016 and Past 3.24 software; the median was calculated. Significance of differences between the groups was estimated using Mann—Whitney test. The differences between dependent samples were evaluated using Wilcoxon's T test. Correlation was calculated using the Spearmen's test. The differences were considered significant at 95% significance level (p<0.05) and as a trend at 90-95% significance level (p<0.1).

RESULTS

Similar to our previous experiments, the duration of swimming in control group on the last day of the experiment and the mean duration of swimming over 4 days of exhausting load did not significantly differ from the initial levels. No significant increase in physical performance was also observed in rats receiving 10 mg/kg ACh. In the group receiving 20 mg/kg ACh, the mean duration of swimming increased by 75-110% in comparison with the control (Fig. 1). However, in groups receiving ACa, increasing the dose increase did not change swimming duration. Indeed, in rats receiving ACa in a dose of 10 mg/kg, the mean swimming duration was by 50-80% higher than in the control (p<0.01), but after administration of ACa in a dose of 20 mg/kg, a minor increase in this parameter was observed.

On the last day of the experiment, the mean level of lactate measured in 5 min after swimming was 7.8 mM in the control group and was lower in all experimental groups (Fig. 2). The lowest lactate concentration as well as swimming duration were observed in the group treated with ACh in a dose of 20 mg/kg (by 33%, p<0.05). Lactate level in the group treated with ACh in a dose of 10 mg/kg was by 27% lower than in the control (p<0.05). In other groups the concentration of lactate did not significantly differ from the control level. Thus, lengthening of the swimming duration on the last day of exhausting load in comparison with the initial level correlated with the lactate level measured 5 min after swimming. The most pronounced negative correlation was observed in the groups receiving

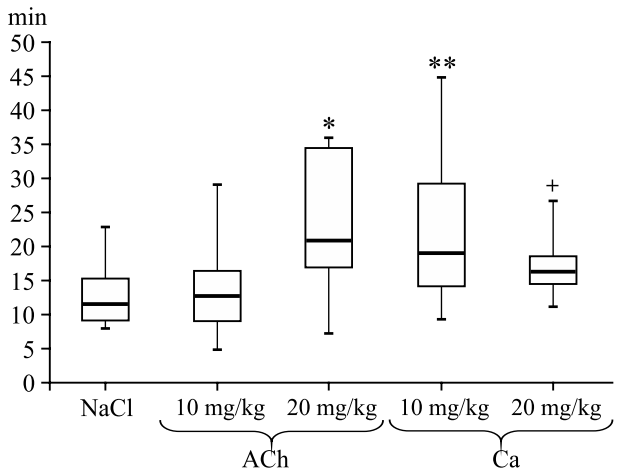

Fig. 1. Mean duration of swimming during 4 days of exhausting load. *p<0.05, **p<0.01 in comparison with NaCl administration. ^+p<0.1 in comparison with NaCl administration (tendency).

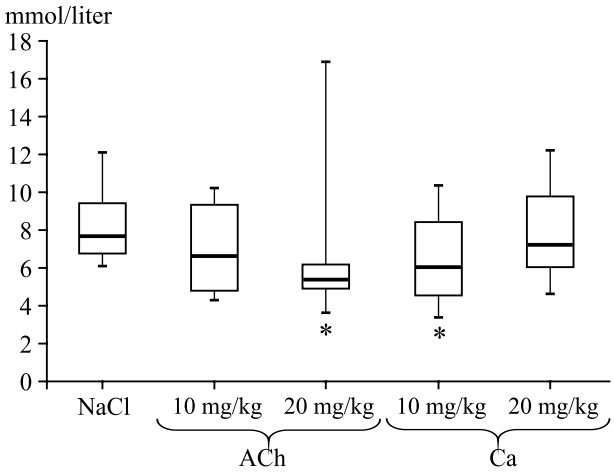

Fig. 2. Lactate level in rat blood 5 min after the termination of swimming on the last day of the cycle of forced swimming. *$p<0.05$ in comparison with NaCl administration.

ACa in doses of 10 and 20 mg/kg (-0.74 and -0.73, respectively; $p<0.05$). In the groups receiving ACh in doses of 10 and 20 mg/kg, the correlation did not reach significance level: -0.45 and -0.38, respectively ($p<0.1$). In 1 h after swimming, blood lactate concentration returned to the control level, *i.e.* no significant between-group differences were observed.

Comparison of the amplitude of changes, *i.e.* differences between the maximum and minimum lactate concentrations in 5 min and 1 h, respectively, did not revealed significant differences between the groups. The maximum amplitude of a decrease in lactate concentration was found in the control group. This parameter was by 2 times higher in the group receiving ACh in a dose of 20 mg/kg ($p<0.05$), and by 1.6 times in the groups treated with ACh and ACa in a dose of 10 mg/kg ($p<0.1$). The amplitude of a decrease in lactate level was similar in the group receiving ACh in a dose of 20 mg/kg and the control group. This difference was statistically insignificant. Moderate decrease in blood lactate concentration in rats of these groups might indicate higher lactate capacity of erythrocytes, which serves as an alternative to the well-known mechanisms of increase in lactate level due to oxygen deficiency.

It is known that the excess of ammonium is an adverse factor, and thus conversion of ammonium by arginine or citrulline allows improving endurance of long-distance runners [4]. However, end-products of catabolism, such as ammonium and carbon dioxide in the form of ammonium and bicarbonate ions promote the adaptive transformation of metabolic pathways on the model of forced swimming. These ions prevent lactate accumulation in the blood during physical activity and enhance endurance of animals. Prevention of lactate accumulation does not mean prevention of its production, even though adaptation to physical exercise is associated with increased lactate threshold, reduction of lactate generation, and right shift of lactate minimum in the corresponding tests [12]. Arterial-venous difference in lactate level due to its slow increase in the venous blood during physical exercise of various intensity is normally analyzed within relatively short time intervals (5-10 min), when this difference is appreciable [6]. As for mechanisms of delayed increase in lactate concentration, they are discussed in terms of changes in peripheral vessel resistance and lactate redistribution between intensively working and not active muscles [6]. From this position, the delayed decrease in lactate level in the venous blood of rats reaching the minimum threshold in 5 min after the termination of exhausting exercise cannot be explained. It is not clear why both phasic and postural muscles release captured lactate if mitochondria are not in hypoxia and can process lactate in the Krebs cycle. Erythrocytes best suit to the role of lactate depot due to their availability in blood plasma and in view of the role played by lactate entering erythrocytes via MCT1. The presence of the "cardiac" LDH-1 isoenzyme in erythrocytes indicate the possibility of rapid conversion of lactate to pyruvate accompanied by an increase in the level of NADH that is required for methemoglobin reductase [5], especially under conditions of glyceraldehyde 3-phosphate dehydrogenase inhibition (main source of NADH) in oxidative stress or type 2 diabetes mellitus [15]. Pyruvate generated from lactate upon glycolysis reactivation in erythrocytes or NADH generation can be easily converted into lactate, because all LDH isoforms work under near-equilibrium conditions [3]. If erythrocytes can be considered as lactate transporters, the possible mechanism of preconditioning to physical load by ammonium ions can be easily explained. Administered in fasting state before exercise, ammonium ions pass to the blood flow mainly from the stomach and enter erythrocytes by an electrically neutral mechanism; they do not accumulate in the liver, because this process requires significantly higher concentrations of ammonium salts. Trigger or enhancing role of ammonium ions in erythrocytes can be related to activation of carbonic anhydrase and Cl^-/HCO_3^- exchanger (AE1) due to pH_i increase and particularly due to acception of H^+ ion near the ion exchanger AE1 by neutral ammonium molecules passing through the RgAG-channel [10]. Local increase of intracellular pH reduces activity of the Rapoport—Luebering shunt, thus increasing the intensity of ATP-producing reactions coupled with pyruvate generation and its catalytic conversion into lactate. High concentration of carbon dioxide has stronger effects on lactate binding to hemoglobin than high lactate levels on hemoglobin carbamylation [13]. This determines export of lactate from erythrocytes

in most actively working postural muscles generating significant amount of carbon dioxide. The intracellular and tissue mechanisms of adaptation are primarily coupled via the transport function of erythrocytes in broad sense of this word, *i.e.* transport of not only oxygen and CO_2, but also lactate and ammonium. These molecules are not just passively transported by erythrocytes, but significantly contribute to the intensification of their functioning due to induction or potentiation of Bohr and/or Haldane effects. Under normal conditions, this function of ammonium is activated at relatively late stages of physical load, when ATP is supplied from reactions with participation of amino acids and adenine nucleotides with parallel generation of ammonium (glutaminase and AMP-deaminase) serve as the source of. Exogenous ammonium ions imitate the terminal stage of metabolic acidosis of skeletal muscles and activate the glycolytic pathway of glucose oxidation in erythrocytes. Additional investigations are needed for understanding of physiological and biochemical mechanisms of ammonium-induced preconditioning.

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REFERENCES

1. Goncharov NV, Mindukshev IV, Novozhilov AV, Korf EA, Tavrovskaya TV, Terpilovsky MA, Khmelevskoy DA, Skverchinskaya EA, Krivchenko AI. Adaptive biochemical changes of rat erythrocytes after the cycle of forced swimming with the use of green tea extract and ammonium preconditioning. Ross. Fiziol. Zh. 2018;104(12):1428-1441. Russian.
2. Korf EA, Kubasov IV, Vonsky MS, Novozhilov AV, Runov AL, Kurchakova EV, Matrosova EV, Tavrovskaya TV, Goncharov NV. Green Tea Extract Increases the Expression of Genes Responsible for Regulation of Calcium Balance in Rat Slow-Twitch Muscles under Conditions of Exhausting Exercise. Bull. Exp. Biol. Med. 2017;164(1):6-9. doi: 10.1007/s10517-017-3913-9
3. Bak LK, Schousboe A. Misconceptions regarding basic thermodynamics and enzyme kinetics have led to erroneous conclusions regarding the metabolic importance of lactate dehydrogenase isoenzyme expression. J. Neurosci. Res. 2017;95(11):2098-2102.
4. Cheng IS, Wang YW, Chen IF, Hsu GS, Hsueh CF, Chang CK. The Supplementation of Branched-Chain Amino Acids, Arginine, and Citrulline Improves Endurance Exercise Performance in Two Consecutive Days. J. Sports Sci. Med. 2016;15(3):509-515.
5. Elahian F, Sepehrizadeh Z, Moghimi B, Mirzaei S.A. Human cytochrome b5 reductase: structure, function, and potential applications. Crit. Rev. Biotechnol. 2014;34(2):134-143.
6. Felippe LC, Ferreira GA, De-Oliveira F, Pires FO, Lima-Silva AE. Arterialized and venous blood lactate concentration difference during different exercise intensities. J. Exerc. Sci. Fit. 2017;15(1):22-26.
7. Ferguson BS, Rogatzki MJ, Goodwin ML, Kane DA, Rightmire Z, Gladden LB. Lactate metabolism: historical context, prior misinterpretations, and current understanding. Eur. J. Appl. Physiol. 2018;118(4):691-728.
8. Goncharov N, Maevsky E, Voitenko N, Novozhilov A, Kubasov I, Jenkins R, Avdonin P. Nutraceuticals in sports activities and exhaustion. Nutraceuticals: Efficacy, Safety and Toxicity. Gupta RC, ed. Amsterdam, 2016. P. 177-188.
9. Hostrup M, Bangsbo J. Limitations in intense exercise performance of athletes - effect of speed endurance training on ion handling and exhaustion development. J. Physiol. 2017;595(9):2897-2913. doi: 10.1113/JP273218
10. Johnson DE, Casey JR. Cytosolic H+ microdomain developed around AE1 during AE1-mediated Cl-/HCO3- exchange. J. Physiol. 2011;589(Pt 7):1551-1569.
11. Maciejewski H, Bourdin M, Féasson L, Dubouchaud H, Denis C, Freund H, Messonnier LA. Muscle MCT4 Content Is Correlated with the Lactate Removal Ability during Recovery Following All-Out Supramaximal Exercise in Highly-Trained Rowers. Front. Physiol. 2016;7:223. doi: 10.3389/fphys.2016.00223
12. Messias LHD, Gobatto CA, Beck WR, Manchado-Gobatto FB. The Lactate Minimum Test: Concept, Methodological Aspects and Insights for Future Investigations in Human and Animal Models. Front. Physiol. 2017;8:389. doi: 10.3389/fphys.2017.00389
13. Nielsen MS, Weber RE. Antagonistic interaction between oxygenation-linked lactate and CO_2 binding to human hemoglobin. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 2007;146(3):429-434.
14. Opitz D, Lenzen E, Opiolka A, Redmann M, Hellmich M, Bloch W, Brixius K, Brinkmann C. Endurance training alters basal erythrocyte MCT-1 contents and affects the lactate distribution between plasma and red blood cells in T2DM men following maximal exercise. Can. J. Physiol. Pharmacol. 2015;93(6):413-419. doi: 10.1139/cjpp-2014-0467
15. Senhaji N, Elkhalfi B, Soukri A. Contribution to the study of glyceraldehyde-3-phosphate dehydrogenase in patients with type 2 diabetes. Pathol. Biol. (Paris). 2015;63(2):74-79.