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Effects of Exercise and Metabolic Alkalosis on Selected Plasma Amino Acid Concentrations in Thoroughbred Racehorses

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ABSTRACT. The present study was undertaken to investigate: 1) changes in selected plasma amino acid concentrations during 1 000 m of high intensity exercise, 2) the influence of pre-exercise metabolic alkalosis on these changes. Twenty-four Thoroughbred horses in training raced over a distance of 1 000 m on two occasions separated by 6–7 days. Four hours prior to exercise each horse was administered in randomised manner, a 2 l solution of sodium bicarbonate $NaHCO_3$, (0.6 g kg^{-1} body weight, test) or water (control) by nasogastric intubation. Venous blood samples were taken prior to exercise and 5 min after exercise. The concentration of 22 plasma amino acids were determined using an automatic amino acid analyser. At rest considerable variation was found between horses in individual amino acid concentrations. Exercise produced changes in plasma amino acid concentrations; some of them could be accounted for by the decrease of plasma volume. Marked increases (mean ± SE) were found in alanine (352.3 ± 24.7 to 509.7 ± 36.3 μmol l^{-1}, $p<0.001$) and glutamine (263.7 ± 16.0 to 304.2 ± 22.7 μmol l^{-1}, $p<0.05$). Significant increases were also recorded for leucine ($p<0.001$) and the total amino acid content ($p<0.01$). Tryptophan decreased from 75.1 ± 3.7 to 63.9 ± 3.8 μmol l^{-1} ($p<0.01$). Metabolic alkalosis, whilst affecting acid–base status, produced only minor changes in some of the amino acid concentrations between control and test group before and after exercise.

Key words: Horses; plasma amino acids; exercise; metabolic alkalosis; alanine; glutamine.

INTRODUCTION

Despite considerable recent research in the field of equine exercise physiology, there have been few investigations of changes in amino acids during high intensity exercise. Some studies have been done examining the alterations in alanine,[15,19,21] glutamate and glutamine[15,21] during exercise, which are correlated with their role as a transporter of ammonia produced by working muscles. However, there has been less interest in the role of branched chain amino acids—leucine, isoleucine and valine[15,21] and other free plasma amino acids during exercise.

The aim of the present study was to investigate: 1) the changes in selected plasma amino acids during 1 000 m of high intensity exercise, and 2) the influence of metabolic alkalosis on the plasma amino acid profile following submaximal exercise. One might expect a change to occur as a result of a pH induced alteration in amino acid efflux and/or production.

MATERIAL AND METHODS

Twenty 2 year old Thoroughbred racehorses in training, but novices to competition, and

four 3 year old horses, in training and in competition, took part in the present experiment. Horses used were from 4 training stables. All horses were fed on the same diet (oats and grass hay), and the feeds were obtained from the same source.

Four hours prior to racing each horse was administered either a 2 l solution of sodium bicarbonate (0.6 g kg^{-1} body weight, test) or water (control) by nasogastric intubation. $NaHCO_3$ administration resulted in major changes in pre-exercise acid–base status (pH 7.43±0.02, pCO_2 6.98±0.37 kPa, HCO_3^- 34.5±1.7 mmol l^{-1}, BE 8.3±1.4 mmol l^{-1}) when compared with the control treatment (pH 7.35±0.02, $p<0.001$; pCO_2 6.61±0.4 kPa, $p<0.001$; HCO_3^- 27.1±1.8 mmol l^{-1}, $p<0.001$; BE 0.0±1.6 mmol l^{-1}, $p<0.001$)—see in Greenhaff et al.[4] In the 4 hours between intubation and racing, all horses were allowed free access to hay and fresh water. After 25 min of light warm-up exercise (20 min walking and 5 min walk and trot to the race starting point), each horse raced over a distance of 1 000 m (flying start) on two occasions separated by 6–7 days. Each race involved one test and control horse of matched training ability. Two jockeys were used for all 24 races, each rode the same horse for both of the experimental treatments. None of the horses performed strenuous exercise the day prior to racing.

Venous blood samples were taken 5 min prior to exercise (after 20 min walking warm-up) and 5 min after racing (following a walk recovery). Samples were collected into heparinised syringes and immediately centrifuged. Plasma was frozen in liquid nitrogen and stored at −20°C until analysis. After deproteinisation, by the addition of 3% sulfosalicylic acid, concentrations of 22 amino acids (Table 1) were determined using an automatic amino acid analyser Chromaspek (Hilger Analytical, UK).

Differences between test and control treatments, were determined using *t*-test for paired data. Values in the text and tables represent mean ± SE. They have not been corrected for the plasma volume changes.

RESULTS

The plasma amino acid changes that occurred during exercise on the test and control treatments are shown in Table 1. Marked increases were observed in the total plasma amino acids content ($p<0.01$ on both treatments) and in the levels of alanine ($p<0.001$ on both treatments), glutamine ($p<0.001$ test, $p<0.05$ control) and leucine ($p<0.001$ on both treatments). Increases also occurred in the concentrations of taurine ($p<0.001$ test), threonine ($p<0.05$ control) and lysine ($p<0.05$ control). A significant decrease in tryptophan was observed ($p<0.001$ test, $p<0.01$ control).

Concentrations of the majority of amino acids were lower in the test group when compared with the control group before and after exercise. Before exercise significant differences between groups were observed in serine, alanine, histidine ($p<0.01$ in each case), glutamine, arginine and the total plasma amino acid content ($p<0.05$ in each case). After exercise significant differences between treatments were in histidine ($p<0.01$), threonine, serine, glutamic acid and proline ($p<0.05$ in each case).

DISCUSSION

Considerable variation exists in the reported concentrations of the individual plasma amino acids in the horse at rest. Many factors can influence resting concentrations such as time of feeding prior to sampling,[10,12,22] composition of diet, age of horses,[20] as well as the health[6] and fitness[14,21] level. These concentrations are not influenced by sampling location.[21]

To determine the effect of high intensity exercise on the concentration of selected plasma amino acid, we compared samples taken 5 min prior to exercise with those obtained 5 min after exercise. The changes observed could be partly accounted for by the change in plasma volume during exercise, which can achieve values more than 8%.[7] The greatest increase occurred in plasma ala-

Table 1. *Concentrations of plasma amino acids in horses before and after exercise (μmol l^{-1})*

	Test group		Control group	
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Alanine	310.3±22.0***	484.1±27.6	352.3±24.7***	509.7±36.3
AAAA[a]	15.1±1.7	14.3±1.9	11.6±1.0	13.5±1.3
Arginine	147.6±11.6	161.2±13.8	202.9±19.9	198.0±22.5
Aspartate	22.1±3.8	23.7±4.4	24.4±4.2	21.5±3.6
Glutamate	80.1±9.0	76.2±2.9	75.9±8.1	84.1±5.1
Glutamine	226.6±13.5***	261.3±13.3	263.7±16.0*	304.2±22.7
Glycine	508.7±23.2	510.8±22.7	522.6±26.0	524.9±24.5
Histidine	79.1±3.1	77.9±3.0	89.8±4.5	88.7±4.0
Isoleucine	60.2±4.4	63.0±3.0	61.3±4.7	62.8±3.2
Leucine	108.8±4.7***	127.4±5.2	107.9±5.2***	128.0±5.4
Lysine	97.4±9.4	107.4±6.0	99.1±7.7*	117.2±8.1
Methionine	36.8±1.7	37.5±1.9	32.4±1.5	36.6±2.9
3 Mhis[b]	32.8±2.1	34.4±2.9	36.6±3.0	34.9±3.1
Ornithine	62.8±5.6	73.1±7.0	66.5±6.7	76.9±6.1
Phenylalan.	73.0±3.4	72.1±2.8	70.7±2.9	73.3±2.5
Proline	104.3±5.3	107.9±5.4	109.1±7.1	118.5±7.0
Serine	247.5±13.1	238.8±11.4	281.3±15.7	282.7±21.0
Taurine	77.1±14.6***	102.5±14.0	78.6±14.6	97.7±20.0
Threonine	116.9±6.5	119.4±5.6	121.6±8.8*	145.8±12.0
Tryptophan	76.2±4.1***	60.2±3.9	75.1±3.7**	63.9±3.8
Tyrosine	69.3±2.0	73.1±2.4	68.4±2.6*	75.1±3.4
Valine	187.1±8.1	191.9±8.0	184.8±8.7	194.2±8.8
ΣAA	2 748.9±104.4**	3 028.3±98.3	2 969.8±127.7**	3 270.5±143.0

[a] α-aminoadipic acid
[b] 3-methylhistidine
*$p<0.05$, **$p<0.01$, ***$p<0.001$

nine. This increase has previously been described in the exercising human[3] and the horse[19,15] and is explained by the existence of the glucose-alanine cycle and the necessity of gluconeogenesis and detoxication of ammonia formed in muscle during exercise.[3,19,23] Further large increases were noticed in the case of glutamine. This amino acid serves as a carrier of ammonia from the exercising muscle and from the brain.[1,3,17] According to Plumley et al.[18] and Welbourne,[24] glutamine is also synthesized by the lungs. Glutamine is consumed by the kidneys, where it supports renal urogenesis,[24] while alanine is taken up by the liver to support hepatic gluconeogenesis.[23] According to Felig,[3] alanine is released from muscle tissue in proportion to severity of the exercise and to the availability of glucose-derived pyruvate. Uptake of alanine by the liver during short term exercise remains at resting levels, resulting in an accumulation of this amino acid in the blood.

The significant increase in leucine during the present experiment has been described previously during mild exercise.[15] This amino acid together with valine and isoleucine can be utilised by contracting muscle during prolonged exercise when the availability of carbohydrate becomes limited.[1,3,9] Its increase in plasma during high intensity short term exercise could be caused by its release from the splanchnic bed as a fuel for a possible prolongation of exercise. Similarly, in-

creased concentration of lysine, also observed by McLean et al.,[15] is probably due to its liberation from proteins since mammalian organisms are not able to synthesize it. The increase observed in taurine is in agreement with the results of McLean et al.,[15] who suggested that an increase in the circulatory level of this amino acid may occur because of a redistribution of the free taurine level in excitable tissues.

The only amino acid to decrease in concentration was tryptophan. Similar changes were reported by McLean[15] after mild exercise in horses and by Kron[11] after endurance exercise in human. This may be due to the role played by tryptophan as a precursor of 5-hydroxytryptamine (serotonine),[13] levels of which are increased in the brain during exercise.[1] Elevated brain 5-hydroxytryptamine resulting from exercise could trigger fatigue-related symptoms as suggested by Parry-Billings.[17] A recent report, however, seems to challenge this idea, suggesting that tryptophan ingestion prior to exercise enhanced endurance performance. Increased 5-hydroxytryptamine concentration was postulated to decrease sensitivity to pain, thus allowing intense exercise to continue significantly longer.[1]

We have found little information concerning the effect of altered acid–base status on plasma amino acids in the literature. One might have expected the appearance of plasma glutamine and alanine to be lower in test treatment, because of the reported lower adenine nucleotide loss and NH_3 production during exercise after $NaHCO_3$ ingestion.[5] Increased production of glutamine in acidotic organism via stimulation of its synthesis has been reported in rat and human.[2,24] However, because the exercise duration/intensity of the present study was not sufficient enough to measurably affect muscle nucleotide loss and ammonia production,[4] the similar change in plasma alanine and glutamine levels on each treatment is perhaps not surprising. The lower concentrations of the majority of the amino acids seen in the pre-exercise bicarbonate treated group are similar to the decrease in total plasma protein concentration. It is likely that these decreases result from the high osmolality of sodium bicarbonate causing an uptake of water into the intravascular compartment. A larger difference between treatments was found only in the case of threonine. Because this amino acid is essential, we suggest that threonine concentration increased in the control group due to its redistribution in plasma.

The following conclusions were drawn from this investigation.

1. Considerable variations exist in the resting concentrations of plasma amino acids in Thoroughbred racehorses.

2. High intensity exercise led to an increase of total amino acids from 2969.8 ± 127.7 to 3270.6 ± 143.0 $\mu mol\ l^{-1}$ ($p < 0.01$). Among 22 amino acids studied, the largest increases were observed in alanine, glutamine and leucine and a decrease was observed in tryptophan. Small changes were observed in the case of tyrosine and lysine. The changes observed in the remainder of amino acids studied were minor or non-existent.

3. Sodium bicarbonate administration produced small but statistically significant differences between the test and control group (before exercise in serine, glutamine, alanine, histidine, arginine and total plasma amino acids content; after exercise in threonine, serine, glutamic acid, proline and histidine). The most likely reason for these differences is the removal of the water resulting from changes in osmolality.

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