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The Effect of Monosodium Glutamate Infusion on Time to Fatigue

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ABSTRACT. Seven horses were used to investigate the possibility of delaying fatigue by infusing monosodium glutamate (MSG) prior to near maximal exercise. Each horse was exercised to fatigue on a high speed treadmill once per week for up to 6 weeks at 13.5 m s^{-1}. Muscle biopsies were taken prior to and immediately after exercise for amino acid analyses. Blood pH was determined and the plasma analyzed for amino acid, ammonia and lactate at rest, fatigue and 5 min recovery. Resting plasma and muscle glutamate concentrations were increased after MSG infusion. Plasma ammonia concentrations were lower and plasma lactate tended to be lower at fatigue in infused horses than in controls. This could be due to a combination of possible increased urea cycle activity, and increased glutamine, aspartate and alanine formation. The results of this study indicate that MSG infusion can decrease accumulation of plasma ammonia during intense exercise, however a delay of fatigue was not detected.

Key words. Horses; exercise; amino acids; lactate.

INTRODUCTION

Ammonia has been shown to accumulate in the plasma after intense exercise in humans[1,3] and horses.[5,13,14] The major source of ammonia in the muscle during intense exercise is from the deamination of AMP to IMP.[10] An accumulation of ammonia in muscle may affect performance in several ways. Ammonia inhibits isocitrate dehydrogenase,[7] pyruvate carboxylation[2] and may decrease the flux of alpha-ketoglutarate into the TCA cycle by enhancing glutamate formation.[15] Therefore, actions of ammonia on metabolic reactions in muscles can result in accumulation of pyruvate which may result in lactate formation and decreased muscle pH.[15] Ammonia produced in the muscles enters into circulation, crosses the blood brain barrier and its neurophysiological effects may contribute to the ataxia and cerebral disorientation observed in long distance runners following a strenuous race.[1] It appears that ammonia is a by-product of metabolism in the muscle during strenuous exercise with no apparent immediate local benefit. Its known adverse effects on metabolic and physiological systems suggests that it may contribute to the onset of fatigue. Increasing the availability of glutamate may help in the detoxification of ammonia and, thereby lessen its adverse effects. The purpose of this experiment was to investigate the possibility of delaying fatigue in horses performing at near maximal speed, by infusing monosodium glutamate (MSG) prior to an exercise bout.

EXPERIMENTAL PROCEDURES

Experimental animals

Seven mature Quarter Horse and Thoroughbred geldings were used in the study. The horses were maintained in large outdoor dry lots throughout the trial. Each horse was al-

lowed to recover fully from having the left carotid artery surgically elevated to a subcutaneous position prior to receiving intense conditioning 5 days per week for 12 weeks on a high speed treadmill (Sato, Uppsala, Sweden).

Instrumentation

One 16 gauge ×8 cm intravenous catheter (Angiocath, Deseret Medical Inc.) was placed in the carotid artery. Two 7F catheter introducers (USCI Cardiology and Radiology Division, C. R. Bard Inc.) were inserted into the right jugular vein and sutured in place under local anesthesia (2% lidocaine HCl). Through one introducer a 7F Swan-Ganz balloon-tip catheter was passed into the pulmonary artery. The catheter's distal thermistor was connected to a cardiac output computer (Columbus Instruments). A microbore tubing was inserted through the second jugular vein introducer for sampling of mixed venous blood. The position of both catheters was verified by monitoring pressure changes using a chart recorder (Beckman Instruments, model R 611). The mixed venous blood samples were drawn anaerobically into 5 ml heparinized plastic syringes and stored on ice for up to 3 h before determination of pH on a pH/blood gas analyzer (Instrumentation Laboratories, model 813). The pH values were corrected to the pulmonary arterial temperature recorded when the samples were drawn.

Exercise test

Experiments were conducted in an air conditioned laboratory at temperatures of 18±2°C. During exercise, air was moved over the horses by two large box fans.

Each horse was exercise tested once per week for up to 6 weeks. Two horses developed lameness problems and did not complete the protocol. The exercise test consisted of a warm-up period followed by a run on the treadmill at 13.5 m s^{-1} to exhaustion. Preliminary trials indicated that this speed was the fastest that all horses could run. As a warm-up each horse was walked 2 m s^{-1} for 200 m, trotted 4 m s^{-1} for 300 m, then galloped 10 m s^{-1} for 500 m. Timing was started when the horses reached test speed (13.5 m s^{-1}). Carotid and pulmonary arterial blood samples were collected in heparinized syringes at rest, at time 0 of the exercise test and at the fatigued time. "Fatigued" was referred to as the time when a horse, despite urging, no longer maintained position near the front of the treadmill. Signs interpreted as evidence of fatigue (e.g. stumbling) were often observed then. Blood samples were collected, the time of the run recorded and the treadmill stopped. The horse remained stopped for 5 min, until recovery blood samples were taken. Carotid blood samples taken for lactate determination were placed in tubes containing potassium oxalate and sodium fluoride and immediately centrifuged. Within 3 h of collection plasma lactates were determined on a YSI 23L lactate analyzer (Yellow Springs Instrument Co.). Immediately after withdrawal, a portion of carotid blood sample was centrifuged, the plasma harvested and frozen in liquid nitrogen. Samples were stored at −70°C until analyzed for total protein, ammonia and amino acid concentrations. A muscle biopsy was taken from the gluteus medius prior to and immediately after each exercise test. The muscle biopsies were frozen in liquid nitrogen and stored at −70°C until analyzed for amino acid concentration.

To examine the effects of MSG on fatigue time, 385 g of MSG was dissolved in 10 l of sterile distilled water and autoclaved. Prior to insertion of the 7F Swan-Ganz catheter, the MSG solution was infused through a 7F catheter introducer. Infusion took approximately 0.5 h, and the time from post-infusion to the exercise test varied from 0.75 to 1.5 h. The exercise test followed the same protocol as described above. Each horse served as its own control, with the treatment and control runs conducted on alternate weeks. Hematocrit measurement during preliminary studies indicated that by the time the exercise test began, the horses had corrected the change in plasma volume and

Table 1. *Number of runs, average time to fatigue and resting total plasma protein for each horse*

C = control, T = MSG treatment

Horse	No of runs	trt	Time to fatigue[a]	Total plasma protein[b]
1	2	C	3:13±0:10	6.4
	2	T	3:18±0:39	6.4±0.2
2	3	C	1:20±0:19	7.3±0.1
	3	T	1:11±0:16	7.1±0.7
3	3	C	3:21±1:27	6.2±0.1
	3	T	2:38±1:11	6.0±0.2
4	3	C	4:11±1:11	6.6±0.1
	3	T	3:00±1:36	6.3±0.1
5	3	C	4:50±0:58	6.4±0.1
	3	T	5:17±1:13	6.2±0.3
6	3	C	3:02±1:08	6.7±0.4
	3	T	3:05±1:06	6.1±0.2
7	1	C	7:06	6.5
	1	T	5:33	6.4
Mean		C	3:55±1:44	6.6±0.4
		T	3:30±1:31	6.4±0.4

[a] Mean min: s ± SD.
[b] Means ± SEM expressed in g l^{-1}.

therefore control horses were not given a placebo. Microscopic examination did not reveal any abnormal changes in morphology of erythrocytes immediately post MSG infusion.

Biochemical analyses

Since not all horses ran the same number of exercise test runs, to prevent biases plasma samples of individual horses were pooled for amino acid and ammonia concentration determination. Plasma lactate, total protein and blood pH were measured for every run and the values averaged for each horse. Muscle amino acid concentrations were determined from only the longest fatigue times of the control and the infused test runs for each horse.

Total plasma protein was determined on a random access analyzer (Olympus Demand, Olympus). Plasma ammonia concentrations were determined by spectrophotometric methods (Sigma Chemical Co.). Plasma for amino acid analyses was deproteinized by addition of 0.25 ml plasma to 0.75 ml 5% sulfosalicylic acid (pH 1.9) containing 133 nmol ml^{-1} thienylalanine as internal standard. After the proteins were precipitated, the sample was centrifuged in a refrigerated centrifuge at 3400 G for 15 min and the supernatant was filtered and analyzed on a Beckman 121-MB Automated Amino Acid Analyzer. Muscle biopsies were homogenized using 3 times their weight in 5% sulfosalicylic acid (pH 1.9) containing 133 nmol ml^{-1} thienylalanine as internal standard.

Statistical analyses

Variable responses were analyzed using a commercial software (Proc GLM, SAS Institute Inc., 1985) according to a factorial arrangement of control and treatment and the sampling periods employing a completely randomized experimental design. Means were separated using Tukey's procedure following significant level of effect from the Analysis of Variance.

RESULTS

The number of runs, time to fatigue and resting total plasma protein for each horse are presented in Table 1. The time to fatigue and total plasma protein for all infused runs were not different ($p>0.05$) from that of control runs.

The changes in measured muscle amino acid concentrations from rest to fatigue did not differ ($p>0.05$) between the control and treatment runs (Table 2). There were no differences ($p>0.05$) in the runs with the longest times to fatigue for each horse between control and infused runs (mean min:s±SD; 4:22±1:48 vs 4:09±1:16, respectively).

Selected plasma amino acids, ammonia, lactate and blood pH are presented in Table 3. The very high concentrations of plasma glutamate from the infused runs resulted in HPLC peaks so large that amino acid peaks

Table 2. *Muscle amino acid concentrations in horses at rest and fatigue in control and infused test runs*

Values presented are means ±SEM expressed in μmol g⁻¹ wet wt. C = control, T = MSG treatment

	trt	Rest	Fatigue
Glutamate	C	$2.64 \pm 0.22^{a**}$	$1.25 \pm 0.05^{b**}$
	T	5.42 ± 0.34^{a}	4.64 ± 0.96^{a}
Glutamine	C	1.90 ± 0.20^{a}	1.86 ± 0.16^{a}
	T	1.89 ± 0.23^{a}	1.52 ± 0.17^{a}
Alanine	C	1.36 ± 0.21^{a}	2.49 ± 0.33^{b}
	T	1.37 ± 0.17^{a}	2.52 ± 0.10^{b}
Aspartate	C	0.95 ± 0.07^{a}	1.04 ± 0.06^{a}
	T	0.96 ± 0.04^{a}	0.95 ± 0.05^{a}

$^{a\ b}$ Values with different superscript within a row differ ($p<0.05$).
** C values are different ($p<0.01$) from T values.

recorded immediately following glutamate were obscured and could not be quantitated. Thus, there were no data for glutamine from the treatment runs. The changes in plasma ammonia and lactate concentration from rest to the 5 min recovery sampling tended to be different ($p<0.10$) between infused and control runs. The change in blood pH from rest to the 5 min recovery sampling was different ($p<0.05$) between infused and control runs.

DISCUSSION

The decrease in muscle glutamate concentrations observed at fatigue in the control runs appeared to be balanced by a similar increase in alanine (Table 2). This is desirable in heavily exercising muscle as there is a large increase in pyruvate during contraction[9] which transaminates with glutamate to form alanine and alpha-ketoglutarate. The alpha-ketoglutarate can enter the TCA cycle for increased aerobic metabolism and tend to decrease metabolic acidosis which would occur if all the pyruvate not entering the TCA cycle were converted to lactate.[16] The alanine enters the circulation, as evidenced by increase plasma alanine concentration (Table 3). The alanine response in this study is similar to that reported by Harris et al.[6] who found a doubling of muscle alanine concentration in Thoroughbreds after an 800 and 2000 m maximal gallop. The changes in muscle glutamate, glutamine and alanine in the control runs were consistent with changes observed in human muscle following dynamic[8] and isometric exercise to fatigue.[9]

Muscle aspartate was measured due to its roles in transamination reactions, and in reamination of IMP in the purine nucleotide cycle. However, the accumulation of IMP at fatigue in human[8,9,12] and horse muscle[6,17] suggests that reamination of IMP to AMP is negligible during intense exercise. Muscle aspartate concentrations in both control and treatment test runs did not change from rest to fatigue (Table 2) suggesting that aspartate consumption in the purine nucleotide cycle was minimal.

The large amount of glutamate in the muscle (Table 2) possibly could have tied up more ammonia by formation of glutamine and/or transamination forming alpha-ketoglutarate and alanine. However, the increase in alanine at fatigue was not different ($p>0.05$) between infused and control runs. Glutamate tended to be lower at fatigue compared to resting levels in the infused runs. The high concentration of glutamate prior to exercise in the infused runs may have masked the decrease in glutamate responsible for the increase in alanine. Nevertheless, the lack of differences in muscle and plasma alanine concentrations at fatigue between infused and control runs suggests that glutamate-pyruvate transamination during exercise in infused runs was not greater than in control runs. The reason for this is uncertain.

Since the muscle amino acid concentrations were determined on a wet weight basis, the increase in muscle glutamate following infusion could, in part, be attributed to high plasma (Table 3) and high interstitial fluid

Table 3. *Plasma amino acid, ammonia and lactate concentrations and blood pH in horses at rest, fatigue and 5 min recovery in control and infused test runs*

Values presented are means ± SE expressed in μmol l^{-1}, except lactate (mmol l^{-1}) and pH. C = control, T = MSG treatment

	trt	Rest	Fatigue	Recovery
Glutamate	C	23±2[a]**	33±7[ab]**	40±6[b]**
	T	10 733±1 355[a]	10 663±1 138[a]	9 101±1 050[a]
Aspartate	C	7±0.4[a]**	11±2[b]**	13±1[b]**
	T	192±12[a]	237±11[b]	222±14[ab]
Glutamine	C	307±16[a]	400±24[b]	342±22[a]
	T	-----	-----	-----
Alanine	C	251±14[a]	415±29[b]	416±21[b]**
	T	289±33[a]	504±86[b]	610±57[c]
Ammonia	C	46±7[a]	390±54[b]**	294±65[b]
	T	63±24[a]	219±46[b]	139±36[ab]
Lactate	C	1.0±0.1[a]	37.3±1.6[b]	33.9±1.5[b]
	T	1.0±0.1[a]	31.9±2.2[b]	30.6±1.6[b]
pH	C	7.4±0.01[a]	6.88±0.02[b]	6.97±0.03[c]*
	T	7.4±0.01[a]	6.94±0.03[b]	7.06±0.02[c]

[a, b, c] Values with different superscript within a row differ ($p<0.05$).
* C values are different ($p<0.05$) from T values. ** C values are different ($p<0.01$) from T values.

levels. However, the large concentration gradient of glutamate between plasma and muscle following infusion probably resulted in glutamate diffusion into the muscle. Further indication of the entry of glutamate into the muscle is that while the resting plasma aspartate concentrations post-infusion were increased 27-fold above control values (Table 3), muscle aspartate concentrations were not increased ($p>0.05$).

The increased plasma glutamine at fatigue in the control runs may have been due, in part, to increased release from muscle, although there was no significant change in muscle glutamine. Similarly, during dynamic exercise in man, a change in muscle glutamine was not observed, although there was an increase in the rate of glutamine release from the muscles.[8]

Plasma ammonia levels attained at fatigue in this study (Table 3) far exceed concentrations previously reported for horses.[5,6,13] The faster treadmill speed attained in the present study may account for the very high ammonia levels. The higher exercise intensity would result in the recruitment of proportionally more FT fibers which have a higher amount of AMP deaminase activity than ST fibers.[11] Furthermore, AMP deaminase activity is stimulated when estimated cellular pH is approximately 6.6 and below.[4] In this study, mixed venous pH of 6.9 were observed at fatigue (Table 3). Considering that the muscle pH was probably lower than the venous pH, AMP deaminase activity should have been greatly enhanced. In addition to intensity, duration of exercise may further elevate plasma ammonia levels. In Thoroughbred horses, plasma ammonia concentrations of 89 and 120 μmol l^{-1} immediately after maximal gallops of 800 (calculated 16.7 m s^{-1}) and 2 000 m (15.7 m s^{-1}), respectively are reported.[6] Although these horses ran faster, they did not run as long as those in the present study. Therefore, the high plasma ammonia concentration at fatigue seen here may be attributed to high intensity and long duration of exercise.

Several studies have shown a significant linear relationship between the blood concentrations of ammonia and lactate during exercise[1,3,5,13] and, likewise, studies demonstrating high concentrations of plasma lactate at muscular fatigue are not uncommon. Although many factors have been identified, a great deal of controversy still remains concerning the physiological causes of fatigue. Hence, there is difficulty in identifying a single factor which could be accurately measured to determine fatigue. The large within-horse variation in fatigue times for some horses in this study was unexpected and suggests that run time may be a poor repeatable measure of fatigue.

The lower concentrations of plasma ammonia observed in the infused runs is probably attributable to a combination of increased urea cycle activity, and increased glutamine, aspartate and alanine production. The results of this study support the hypothesis that MSG infusion can decrease the accumulation of ammonia during exercise, thereby possibly alleviating its negative effects on energy production. A delay of fatigue for 1 second could easily change the results of a race, however a delay this small could not have been detected in this study.

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