Decreased taurine concentration in skeletal muscles after exercise for various durations

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ABSTRACT


Purpose: To examine the changes of taurine concentrations in blood and skeletal muscles after transient exercise. Methods: Rats were placed on a treadmill set at 25 m·min⁻¹. The animals were divided into four groups: control (no exercise) and exercise groups 1, 2, and 3. The exercise duration for groups 1, 2, and 3 was 30, 60, and 100 ± 12.5 min (to exhaustion: mean ± SD), respectively. We examined the plasma concentrations of taurine and lactate, the serum concentrations of sodium and chloride ions, as well as the skeletal muscle taurine content in the soleus (slow-twitch fiber dominant type), gastrocnemius (slow- and fast-twitch fiber mix type), and plantaris and extensor digitorum longus (fast-twitch fiber dominant type) muscles. Results: Although the plasma taurine concentration was not affected by the increased exercise duration, that in skeletal muscles was significantly decreased. The gastrocnemius and plantaris muscles from the exercise group 3 had a significantly lower concentration of taurine than those of the control group. The extensor digitorum longus taurine concentration from the different exercise groups was significantly decreased compared with that from the control group. However, there was no significant difference among the exercise groups. Conclusion: Taurine concentration was decreased in all skeletal muscles after exercise, regardless of the duration. Moreover, this decrease was specific to fast-twitch dominant fibers. However, under these conditions, the plasma taurine concentration remained unchanged. Key Words: TAURINE, AMINO ACID, SKELETAL MUSCLE, EXERCISE DURATION

Taurine, a sulfur-containing amino acid, is the most abundant free amino acid found in myocardium (4), skeletal muscles (16), nerve, brain, and other organs (12,16). In skeletal muscles, the steady state levels of the content and transport of taurine are much higher in slow-twitch fiber than in fast-twitch fiber type muscles (1,2,13,15). In this tissue, taurine affects various electrophysiological and biochemical parameters (2,13,15). It has been reported that taurine stabilizes the intracellular membrane (11) and modulates nerve excitement (7,24). Furthermore, the effects of taurine on muscle cramps have been reported based on the results of oral treatment of taurine in patients with myotonic dystrophy (8,9,10). Oral taurine administration also improves painful muscle cramps in patients with liver cirrhosis (21,22).

Skeletal muscle cramps generally occur during and immediately after exercise (5,28). It has been speculated that these muscle symptoms occur as a result of a breakdown in electrolyte balance due to a loss of NaCl during excessive sweating and a possible reduced muscular cell membrane potential due to a decreased serum calcium ion concentration (23).

Therefore, it is suggested that oral taurine administration may help to alleviate muscle cramps occurring during and after exercise (5,23,28). However, the role of taurine in relation to exercise remains unclear. The aim of this study was to examine the changes in taurine concentrations in blood and skeletal muscles after exercise.

MATERIALS AND METHODS

Animals and exercise protocols. Male Sprague-Dawley rats (Japan SLC, Shizuoka, Japan; N = 28) of 8–10 wk of age were used. The rats were divided into four groups: control group (N = 7), exercise group 1 (N = 7), exercise group 2 (N = 7), and exercise group 3 (N = 7). The rats in the exercise groups were placed on a treadmill (KN-73, Nazme, Tokyo, Japan) set at 25 m·min⁻¹. The rats in groups 1–3 ran continuously for 30 min, 60 min, and to complete exhaustion (100 ± 12.5 min), respectively. The rats were considered to be completely exhausted when it was impossible for them to continue running, even after being electrically stimulated, with a system attached to the treadmill. All animals received humane care in accordance with the guidelines of the University of Tsukuba for the care of laboratory animals and in agreement with the policy statement of the American College of Sports Medicine.

Blood and muscle preparation. Immediately after finishing the exercise, the rats were anesthetized with ether. Five mL of blood was quickly collected from the left
ventricle while under anesthesia. The rats were then euthanized by ether overdose. The soleus muscle, i.e., slow-twitch fiber dominant type; gastrocnemius muscle, i.e., slow- and fast-twitch fiber mix type; and plantaris muscle and extensor digitorum longus (EDL) muscle, i.e., fast-twitch fiber dominant type, were removed from the calf and rear-thigh muscles, washed in physiological salt solution, cleared of adipose, nerve, and connective tissue, and weighed. Each muscle sample was frozen in liquid nitrogen and kept at −80°C until the biochemical analyses were carried out.

Biochemical analyses. We determined both the plasma lactate and serum sodium and chloride ion concentrations. To determine the plasma lactate concentration, 1 mL of blood was mixed with an equal volume of 0.8N perchloric acid, and centrifuged at 2000 rpm for 20 min. The supernatant was then assayed for lactate by using a commercially available kit (Determiner® LA, Kyowa Medex, Tokyo, Japan) and an automatic analyzer (Hitachi-7170, Hitachi, Tokyo, Japan) by the lactate oxidase-pyruvate oxidase method (3). Intra- and inter-assay coefficients of variance of the lactate oxidase-pyruvate oxidase method were 0.01 ± 0.01% and 4.82 ± 4.84%, respectively. Serum sodium and chloride ion concentrations were measured by the selective ion electrode method (30). Specifically, 40 μL of serum was diluted to 600 μL with the specific reagent for this assay (Hitachi-7551), and the ion concentrations were measured at 37°C using an automatic analyzer (Hitachi-736–60E). The intra-assay coefficients of variance for sodium ion and chloride ion were 1.17 ± 0.50% and 1.32 ± 0.71%, respectively; the inter-assay coefficients of variance were 0.54 ± 0.41% and 0.61 ± 0.43%, respectively.

Taurine concentration in both plasma and skeletal muscles. The plasma and muscle taurine content was determined as follows. Plasma was mixed with 10 volumes of 5% trichloroacetic acid (TCA) while muscles were homogenized with 20 volumes of 5% TCA (Homogenizer PT 10/35, Generator PTA-10S, Brinkmann/KINEMATICA POLYTRON®, Kinematica AG, Lucerne, Switzerland). Each sample was then centrifuged at 6200 rpm for 20 min, and the supernatant was rinsed four times with four volumes of ether (14). The supernatant (50 μL) was applied directly to an automatic amino acid analyzer (JLC-300V, JEOL, Tokyo, Japan). The analyses were carried out using the one column–five step method with lithium citrate buffers (G-JX 11 M pH 2.93, G-JX 12 M pH 3.28, G-JX 13 M pH 3.46, G-JX 14 M pH 2.85, and G-JX 15 M pH 3.65; JEOL). The column (6.0 × 90 mm) was packed with JEOL Resin LCR-6 (lithium-form ion exchange resin; JEOL). The intra- and inter-assay coefficients of variance of this assay were 1.55 ± 1.36% and 9.85 ± 7.06%, respectively.

Statistical analysis. Plasma taurine concentration was expressed as nanomoles per milliliter (nmol·mL⁻¹), plasma lactate concentration as milligrams per dL (mg·dL⁻¹), serum sodium and chloride ion concentrations as milliequivalents per liter (mEq·L⁻¹), and skeletal taurine concentration as μmol per gram wet weight (μmol·g⁻¹). All data were presented as the mean value ± SD. Significant differences were determined by one-way ANOVA and the Scheffé’s post hoc analysis. Correlations among data were expressed by Pearson’s correlation coefficient. Multiple regression analyses were made using the stepwise method. Statistical significance levels were set at P < 0.05 and P < 0.01. The statistical analyses were performed using Stat View (SAS Institute, Cary, NC).

RESULTS

Plasma taurine and lactate concentrations and serum sodium and chloride ion concentrations. Table 1 shows plasma lactate and taurine concentrations, as well as serum sodium and chloride ion concentrations in each group. The plasma lactate concentration in all exercise groups was significantly higher than that in the control group. The plasma lactate concentration increased relative to exercise duration. However, there were no significant differences among the exercise groups. There were no significant differences in plasma taurine concentration between the control and any of the exercise groups. These results show that the plasma taurine concentration was not influenced by exercise duration. Serum sodium ion concentration decreased in relation to exercise duration; particularly, that of group 3, which was significantly lower than that of the control group. Among the exercise groups, that of group 3 was also significantly lower than that of either group 1 or group 2. Serum chloride ion concentration was not significantly affected by exercise duration.

Skeletal muscle taurine concentration. Figure 1 illustrates skeletal muscle taurine concentration. In the soleus muscle, the taurine concentration was not significantly affected by exercise duration. The gastrocnemius and plantaris muscles in group 3 had a significantly lower taurine concentration than the control group. The taurine concent-

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<th>Table 1. Plasma taurine and lactate concentrations, as well as serum sodium and chloride ion concentrations in the control and exercise groups 1, 2, and 3.</th>
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<td>Plasma Concentration</td>
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In the groups 1, 2, and 3, the rats were exercised on a treadmill (25 m/min). The duration of the exercise was 30 min (group 1), 60 min (group 2), and 100 ± 12.5 min, exhaustion, (group 3), respectively. Data are the mean ± SD; ANOVA, one-way ANOVA P value. * P < 0.05; ** P < 0.01 vs the control group. § P < 0.05 vs the Group 3.
Gastrocnemius muscle; EDL, extensor digitorum longus muscle.

TABLE 2. Correlation coefficients between taurine concentration of the different skeletal muscles and the respective taurine, lactate, sodium, and chloride ion concentration in plasma and blood data.

Table 2 shows the correlation coefficients between the muscle taurine concentration as nmole per gram wet weight and the plasma concentrations of sodium and chloride ions expressed as nmol per mL. There was a negative correlation between the taurine concentration in the soleus and EDL muscle and plasma lactate concentration, whereas a significant positive correlation existed between the plantaris and EDL muscles taurine concentration and the serum sodium ion concentration. Moreover, there was a positive correlation between the taurine concentration in all skeletal muscles and the serum sodium ion concentration.

Comparative study of the taurine concentration and sodium and chloride ions as well as lactate blood level by multiple regression analysis. Table 3 presents the comparative study of the muscle taurine content per gram wet weight and the blood sodium level determined by multiple regression analysis (using the step-wise method). The decreased taurine concentration in the soleus muscle was associated with a significant increased plasma lactate concentration. In the plantaris and EDL, the respective taurine concentration was associated with a significant increase in serum sodium ion concentration.

FIGURE 1—Determination of the soleus, gastrocnemius (Gastro), plantaris, and extensor digitorum longus (EDL) muscle taurine concentration in the control and exercise groups (groups 1, 2, and 3). Data are the mean ± SD. One-way ANOVA P-value: soleus, P = 0.1617; gastrocnemius, P = 0.0299; plantaris, P = 0.0161; EDL, P = 0.0128. *P < 0.05; **P < 0.01 vs control group (Scheffe’s post hoc analysis).

TABLE 3. Comparative study of the muscle taurine concentration and the respective blood sodium and lactate concentration by multiple regression analysis.

The multiple regression analysis was performed as stepwise method.

DISCUSSION

The present data indicate that the taurine concentration in the fast-twitch fiber dominant type muscle (plantaris and EDL) significantly decreased during prolonged exercise. In contrast, Kim et al. (18) reported that under chronic sciatic nerve stimulation in rats, the taurine concentration increased in the fast-twitch fiber but not in the slow-twitch fiber dominant skeletal muscle. The apparent discrepancy between the results in this study and ours may be due to a chronic nerve stimulatory condition in the former study (18), whereas the exercise in the present study was rather transient. Furthermore, the increased taurine concentration in the fast-twitch fiber could be attributed to the fact that muscle fiber type may have changed from fast-twitch to slow-twitch after sustained muscle activity under chronic nerve stimulation. On the other hand, skeletal muscle taurine concentration might be temporarily decreased under transient nerve stimulation, resulting in an increased taurine concentration only in the fast-twitch fiber dominant type muscle during chronic exercise, similar to the observations made by Kim et al. (18). Considering the available data, one may suggest that taurine in the fast-twitch dominant fiber was more sensitive to stimulation than that in the slow-twitch dominant fiber.

The decreased taurine concentration was only significant in the fast-twitch fiber dominant type muscle. It has been reported that taurine administration enhanced the activity of skeletal muscle glycolytic and oxidative enzymes (creatine kinase, lactate dehydrogenase, and phosphofructokinase),
which catalyze the required energy for muscle contraction (31). Furthermore, it has been reported that the synthesis of cyclic AMP (cAMP), a stimulator of glycolytic enzyme (phosphorylase) activity, is facilitated by the secretion of catecholamine, which occurs during and as a function of exercise intensity (6). Finally, cAMP production can be directly stimulated by taurine, through adenyl cyclase activation (25). Taken together these data suggest that the synergistic effect of taurine and cAMP during skeletal muscle contraction contribute to the enhanced overall glycolytic enzyme activity through a probable increased secretion of catecholamine (31). This would explain, at least partially, the increased glycolytic enzyme activity associated with a parallel decreased taurine concentration reported in the present study. Furthermore, although the fast-twitch fiber is particularly susceptible to fatigue, the plasma lactate concentration data presented in this study support a glycolytic promoting role for taurine rather than being the direct cause for muscle fatigue. Another hypothesis to explain the decrease in taurine concentration in plasma and muscle in response to exercise and exercise intensity (6). Finally, cAMP production can be cyclically stimulated fast- and slow-twitch muscles of the rat. Proc. Natl. Acad. Sci. USA 89:1466–7, 1993.


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This study was supported by an educational grant from Taisho Pharmaceutical Co., LTD. (Y.M.) and NIH DK 46954 (B.B.).

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