Effects of Magnesium Sulfate on Dynamic Changes in Blood Glucose Levels and Glucose Transporter-3 Expression in the Striatum during Short-term Forced Swimming in Gerbils

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Abstract

In a previous study, we reported that when gerbils were subjected to a period of forced swimming, glucose levels and magnesium levels in the plasma became elevated. In addition, a pretreatment with magnesium sulfate increased the expression of glucose transporter-3 (GLUT-3) in the cortex. The goal of the present study was to evaluate dynamic changes in blood glucose, lactate and magnesium levels in gerbils that had been subjected to short-term forced swimming using an auto-blood-sampling system. A second goal was to investigate the effects of forced swimming and magnesium sulfate on GLUT-3 expression, glucose and magnesium levels in the striatum, which is involved in energy delivery and motor function. Data collection involved the use of a microdialysis analyzer, a flame atomic absorption spectrometer and western blotting assays to detect glucose, lactate, magnesium and GLUT-3 levels, respectively. Gerbils were subjected to a blood sampling assay and pretreated with saline (n=6) or magnesium sulfate (n=6, 90 mg·kg⁻¹, ip) 30 min before the period of forced swimming. Whole blood glucose, lactate, and magnesium levels increased to 175% and 235%, 305% and 190%, and 102% and 128% of basal levels during swimming in both the control and magnesium sulfate-treated groups (p<0.05). The glucose and magnesium levels during swimming were 60% and 26% greater in the magnesium sulfate-treated group (p<0.05), respectively, whereas the lactate levels were attenuated at 115% (p<0.05). Another group of gerbils was subjected to a brain sampling assay. The gerbils were separated into four groups (n=4, each group): control group (C group) pretreated only with saline; magnesium sulfate group (Mg group) pretreated only with magnesium sulfate; saline control and forced swimming group (C+S group) pretreated with saline 15 min prior to the forced swimming period then rested for 30 min; the magnesium sulfate plus the forced swimming group (Mg+S group) was pretreated with magnesium sulfate 15 min prior to the forced swimming period then rested for 30 min. The results indicated that GLUT-3 and magnesium levels were slightly, but not significantly higher in the Mg group, the C+S group and the Mg+S group. These data suggest that magnesium increased whole blood glucose and magnesium levels during forced swimming; however, the effects of magnesium and swimming on GLUT-3 protein expression varied in different regions of the brain.

Keywords: Forced swimming, Glucose transporter, Magnesium, Striatum

Introduction

Magnesium (Mg), the second most abundant intracellular cation, functions as a cofactor in more than 300 enzymatic reactions. Magnesium is also involved in cellular energy production, neuromuscular functions, glycolysis breakdown, and modulating the activity of adenosine triphosphatase, providing energy for cells [1-3]. Thus, the relationship between magnesium status and exercise has received significant research attention. Magnesium depletion may lead to changes in neuromuscular function and reductions in physical performance and the efficiency of energy metabolism [4].

Accumulating evidence indicates the existence of a direct relationship between the redistribution of magnesium in the body and the type of exercise and that the status of magnesium influences the nature of this redistribution [5,6].

In general, long-term exercise or exercise until exhaustion results in hypomagnesaemia and hypoglycaemia [7,8], whereas short-term and high-intensity exercise induces hypermagnesaemia and hyperglycaemia [9-11]. Conflicting findings regarding on the effect of exercise on erythrocyte concentration have been reported. A mechanism for the observed decrease in plasma magnesium concentration after long term physical exercise could be a shift of Mg into erythrocytes. However, in several studies a decrease in plasma Mg was not accompanied by an increase in erythrocyte Mg [9,12]. In our previous study, we reported that short-term forced swimming results in an increase in plasma glucose and magnesium levels in gerbils [13]. Short-term, high intensity...
exercise transiently increased plasma magnesium concentrations as the consequence of a decrease in plasma volume [6,9]. The two major fractions of whole blood are plasma and erythrocytes. Therefore, the aim of this study was to measure the dynamic levels of whole blood magnesium, glucose and lactate levels in gerbil subjected to forced swimming using an auto-blood-sampling system coupled to a microdialysis analyzer.

Glucose is an essential energy source for most cells and is the primary energy source for brain tissue during exercise. As glucose is a hydrophilic molecule, the facilitated diffusion of glucose across the plasma membrane is mediated by a family of specific glucose transporters (GLUTs). To date, 13 members of this family of transporters have been identified [14-17]. GLUT-3 and GLUT-5 are major glucose transporter isoforms in the brain. GLUT-3 (a 496-amino-acid isoform) is a high-affinity isoform largely expressed in neurons where it is believed to be the predominant glucose transporter isoform; GLUT-5 is expressed in microglia. On the other hand, magnesium regulates glycolysis [1], and we have reported that magnesium supplementation enhanced glucose and GLUT-3 levels in the cortex of gerbil [18]. Moreover, using a microdialysis technique, we found that magnesium sulfate increased extracellular glucose levels in the striatum of gerbils that had been subjected a short-term period of forced swimming [19]. As the striatum is involved in the control of motor function and energy delivery [20], magnesium sulfate supplementation was also employed to evaluate changes in the striatum levels of GLUT-3, glucose, and magnesium during short-term forced swimming in this study.

Materials and Methods

Animals

Adult and naïve male gerbils (n=28), weighing between 70 and 80 g, were obtained from the Laboratory Animal Center of the Taichung Veterans General Hospital. All animals were housed at a temperature of 25 °C in a light (12:12 h light-dark cycle)-controlled room with free access to rat chow and tap water. Animal care and experimental procedures (# La-95225, TCVGH, Taiwan) were in accordance with the Guide for the Care and Use of Laboratory Animals by the U. S. Department of Health and Human Services and with the policy statement regarding the care and use of experimental animals by the American College of Sports Medicine. The gerbils were randomly separated into two groups and subjected to two experiments. In the first experiment, gerbils (n=12) were subjected to blood sampling during the forced swimming, and in the second experiment, the gerbils (n=16) were subjected to brain sampling. For each gerbil in the blood-sampling group, a blunt dissection to clear the fat and connective tissue beside the jugular vein was performed. A length of PUC-40 tube was then connected to the jugular vein of each gerbil and blood was withdrawn according to the method described by Harms and Ojeda [21]. The PUC-40 was connected to the DR-II, the auto-blood-sampling device through a subcutaneous vein in the neck from the back.

Forced swimming

The gerbils were randomly divided into a control (saline) group or a magnesium sulfate supplement group. Saline or magnesium sulfate (90 mg·kg⁻¹, intraperitoneal injection) was injected 30 min prior to the forced swimming task. Each gerbil was placed on a polystyrene board floating in a Plexiglas cylinder that was 40 cm in diameter, 35 cm in height, and filled with warm water (about 35 °C, no heat loss or gain occurred) to a height of 18 cm [13]. The polystyrene board was then removed gently, and each gerbil was forced to swim for 15 min (if a gerbil was judged to be immobile, i.e., when it floated passively with the head above water, it was allowed to rest for 10 sec, and then forced to continue with the swimming exercise). After the forced swimming period, each gerbil was removed from the water, and then carefully and rapidly dried with a towel and a hair blower. In the first experiment, each gerbil was then placed on the polystyrene board again, and blood samples were collected during the 3 h recovery period. In the second experiment, each gerbil was placed on the polystyrene board again, and allowed to rest for 30 min before sacrificing. The gerbils were quickly decapitated and their brains were removed and frozen (a diagram is shown in Fig. 1).

Auto-blood-sampling

In the first experiment, blood samples were automatically collected via a catheter implanted in the jugular vein by a computer aided auto-blood sampling system (DR-II, Eicom, Kyoto, Japan) in conscious, freely moving gerbils. Thirty µl samples of whole blood were collected at 15 min intervals. In order to preserve glucose, 15 µl of a NaF solution and 90µl of 1N HClO₄ were added to the whole blood sample, which was then centrifuged at 1,000 g for 10 min at 4°C. 10µl of Tris buffer was added to the aliquots of the supernatant (40 µl) and directly injected into the microdialysis analyzer (CMA/600, Carnegie Medicin), equipped with a flame atomic absorption spectrometer for detecting the concentrations of glucose, lactate and magnesium, respectively.

Brain homogenization

In the second experiment, the gerbils (n=16) were randomly separated into four groups: saline control (C group, n=4), magnesium sulfate (Mg group, n=4), saline control plus short-term forced swimming (C+S group, n=4), and
magnesium sulfate plus short-term forced swimming (Mg+S group, n=4) groups. The saline control or magnesium sulfate groups (90 mg·kg⁻¹, intraperitoneal injection) were injected 30 min before forced swimming. The C+S group was injected with saline, rested for 30 min, forced to swim for 15 min, and then allowed to rest for 30 min prior to sacrifice. The Mg+S group was injected with magnesium sulfate, rested for 30 min, forced to swim for 15 min, and then rested for 30 min prior to sacrifice.

After the forced swimming period, the gerbils were allowed to rest for 30 min and were then sacrificed and the brains removed. The striatum tissues were rapidly frozen on dry ice, weighed, homogenized in ice-cold 1 N HCl, and the homogenate centrifuged twice (4°C, 10,000 rpm for 10 minutes). A 2 µl portion of Tris-buffer was added to 8 µl of the supernatant to adjust the pH to about 7.0 for further glucose analysis by the microdialysis analyzer. A 25 µl aliquot of the supernatant was diluted with 975 µl of 0.2% HNO₃ for analysis for magnesium by Flame atomic absorption spectrometry.

Western blot analysis

Brain tissue (striatum) was placed in 2 ml of ice-cold lysis buffer (0.32 M Sucrose, 1 mM EDTA, 50 mM Tris-Cl pH 7.4, and a protease inhibitor cocktail from Boehringer Mannheim (Mannheim, Germany), and homogenized with a motor-driven glass homogenizer equipped with a Teflon pestle. The resulting brain homogenate was then centrifuged at 1,400g for 10 min. The supernatant was centrifuged at 45,700g for 30 min. The pellet, containing the plasma membrane fraction, was collected and washed twice with lysis buffer by repeating the above centrifugation. The final pellet was suspended in 1 ml of lysis buffer and stored at -70°C.

The entire procedure was carried out at 4°C. Each lysate was applied to SDS-polyacrylamide gels and electrophoreses conducted at less than 10 % reducing gel conditions. The separated proteins were electroblotted onto Hybond-P: PVDF membranes (Amersham Biosciences, Piscataway, NJ, USA) using a Mini Trans-Blot Cell (Bio-Rad Laboratories, Hercules, CA) according to the manufacture’s instructions. The membranes were then washed once with Tris-buffered saline (TBS, Bio-Rad Laboratories, Inc, USA), pH 7.2, containing 0.1% Tween-20 (TBS-T, Bio-Rad Laboratories, Inc, USA) and blocked at room temperature (about 26°C) for 1 h with TBS-T containing 5% skimmed milk. Antibodies against GLUT-3 (Chemicon International, Inc, USA) were then added to give a final dilution of 1/1500, respectively, in TBS-T containing 5% skimmed milk. The membranes were incubated at room temperature for 2 h, washed three times with TBS-T, and treated with horseradish peroxidase-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, CA) in TBS-T containing 5% skimmed milk for 1 h. Finally, after four washes with TBS-T, the protein bands were visualized using an enhanced chemiluminescence Western blot analysis system (Pierce, Rockford, IL, USA).

Statistical analysis

All data are expressed as means±SEM. The first experiment used a repeated-measures two-way ANOVA and Fisher’s least significant difference (FLSD) test were used to analyze statistically significant differences between the saline control and the magnesium sulfate-treated groups. The second experiment used one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls’ multiple-range test to test the significance of difference among groups. Differences were considered to be statistically significant at p<0.05.

Results

Figure 2. The changes in blood levels of glucose. Data are presented as the mean±SEM (n=6). *p<0.05 and **p<0.01 compared with basal levels; #p<0.05 compared with the control group.

The basal levels of whole blood glucose in the control and magnesium sulfate-treated groups during forced swimming are shown in Figure 2. The glucose levels of the control group increased to about 175% of the basal level (p<0.01) during the period of forced swimming, then returned to the basal level after a 30 min recovery period. In the magnesium sulfate-treated group, the glucose levels immediately increased to 160%-180% of the basal levels (p<0.05) after magnesium sulfate treatment, gradually increased to 235% of the basal levels (p<0.01) at post-exercise, and returned to the basal level at 60 min of recovery. The glucose levels for the magnesium
sulfate-treated group after swimming was about 60% greater than those for the control group (p<0.05).

Figure 3. The changes in blood levels of lactate. Data are presented as the mean±SEM (n=6). *p<0.05 and **p<0.01 compared with basal levels; #p<0.05 compared with the control group.

The basal levels of whole blood lactate in the control and magnesium sulfate-treated groups during forced swimming were shown in Figure 3. After swimming, the lactate levels increased to 190% (p<0.05) and 305% (p<0.01) of the basal level in the magnesium sulfate-treated group and control group, respectively, and returned to the basal level at 30 min of recovery. The lactate levels after swimming were attenuated at 115% in the magnesium sulfate-treated group (p<0.05) compared to those in the control group.

The basal levels of whole blood magnesium in the control and magnesium sulfate-treated groups were shown in Figure 4. In the magnesium sulfate-treated group, the magnesium levels increased to 128% after the swimming period (p<0.05) and then diminished to the basal levels within 30 min of recovery. The magnesium levels in the magnesium sulfate-treated group was 26% greater than those in the control group before swimming begun (p<0.05).

Figure 4. The changes in blood levels of magnesium. Data are presented as the mean±SEM (n=6). *p<0.05 compared with basal levels; #p<0.05 compared with the control group.

Table 1. Effects of magnesium sulfate, swimming, and magnesium sulfate+swimming on glucose, magnesium and GLUT-3 protein levels in the striatum. Data are the mean±SEM of four gerbils. C: saline control group; Mg: magnesium sulfate group; C+S: saline control plus short-term forced swimming group; Mg+S: magnesium sulfate plus short-term forced swimming group.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>Mg</th>
<th>C+S</th>
<th>Mg+S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (% of control)</td>
<td>100.00±0.00</td>
<td>94.17±3.52</td>
<td>74.96±7.25</td>
<td>86.80±12.20</td>
</tr>
<tr>
<td>Mg (% of control)</td>
<td>100.00±0.00</td>
<td>105.71±0.42</td>
<td>105.96±2.22</td>
<td>104.51±3.72</td>
</tr>
<tr>
<td>GLUT-3 relative expression level (%)</td>
<td>1.00±0.00</td>
<td>1.18±0.08</td>
<td>1.18±0.07</td>
<td>1.25±0.14</td>
</tr>
</tbody>
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The results of this study indicate that forced swimming or magnesium sulfate supplementation has no effect on the levels of GLUT-3 protein, glucose and magnesium in the striatum, as shown in Table 1. Swimming, pretreatment with magnesium sulfate and magnesium sulfate plus swimming had no effect on GLUT-3 expression, or the glucose, and magnesium levels, respectively.

Discussion

The results of this study indicate that forced swimming results in an increase in both whole blood glucose and lactate levels, whereas pretreatment with magnesium sulfate increased glucose levels and attenuated lactate levels during forced swimming. Pretreatment with magnesium resulted in a significant and immediate increase in whole blood glucose levels prior to exercise. This phenomenon was also observed in our previous study [19]. The findings reported in that study showed that pretreatment with magnesium sulfate resulted in an immediate increase in brain glucose concentrations. Therefore, the peripheral and central glucose concentrations may be elevated via regulation of the oxidation phosphorylation, glycolysis pathways, and possibly other mechanisms as well. Lactate production is generally considered to be the major cause of muscle fatigue and to have a positive influence on muscle contraction during exercise. Numerous studies have indicated that glucose and lactate levels are closely correlated with exercise intensity [22]. During a short term light- or moderate-intensity exercise, blood lactate remains at or very close to the basal levels. As the intensity of the exercise increases, a threshold is reached, beyond which blood lactate levels rise sharply [23,24]. It was also found that lactate accumulation in muscle cells until transporting out of the cell equals net production. This accumulation reflects a mismatch between the inefficient rapid production of ATP by glycolgenolysis and the oxidative processes that provide most of the net energy production over
a contraction cycle [25]. This may alter glycolytic processes and regulate glucose metabolism [22]. A number of reports have suggested that lactate is involved in the significant shift from predominantly aerobic metabolism to predominantly anaerobic energy production [23]. Supplementation with magnesium may result in the attenuation of lactate levels under anaerobic energy production [23]. Supplementation with magnesium may result in the attenuation of lactate levels under anaerobic energy production [23].

In general, long-term or exhausting exercise induced hypoglycaemia, hypomagnesaemia, while short-term and high-intensity exercise induced hypermagnesaemia and hyperglycaemia [9,10,26]. Our previous study demonstrated that short-term forced swimming resulted in an increase in both plasma glucose and magnesium levels [13]. However, the findings reported herein indicate that short-term forced swimming increased whole blood glucose levels, but no significant increase in whole blood magnesium levels was found, as shown in Figs. 2 and 4. With respect to blood extracellular magnesium, various studies have indicated that short-term and high-intensity anaerobic exercise led to hypermagnesaemia as a consequence of a reduction in plasma volume and a shift in cellular magnesium concentration [9,27].

Our previous data shown that in the short-term forced swimming resulted in an increase in plasma magnesium [13]; however, whole blood magnesium levels remained unchanged in gerbil subjected the same short-term forced swimming model in this study. A possible explanation for increased plasma magnesium concentration during forced swimming events may elicit magnesium from red blood cells into plasma compartments.

On the other hand, our previous data indicated that magnesium sulfate and short-term forced swimming played a role in regulating cerebral glucose concentrations and magnesium sulfate led to an increase in glucose levels [19]. Moreover, for the intracellular uptake of glucose by insulin-sensitive cells, the translocation of GLUTs across the plasma membrane is a vital step, in which insulin is induced by autophosphorylation of beta subunits of insulin receptors after its specific binding. Magnesium acts in a stimulative manner in the step where insulin binds to its receptors and the autophosphorylation of beta subunits. Intracellular magnesium stimulates intracellular glucose transport and its oxidation [31,32]. We previously reported that short-term forced swimming had no effect on GLUT-3 protein expression, glucose and magnesium levels in the cortex, whereas pretreatment with magnesium sulfate increased glucose and magnesium levels in the cortex, and also resulted in an increased expression of GLUT-3 protein. The results of this study indicate that forced swimming or magnesium sulfate supplementation has no effect on the levels of GLUT-3 protein, glucose and magnesium in the striatum, as shown in Table 1. These findings suggested that even the same exercise model and treatment with same dosage of magnesium may have different effects on GLUT-3 protein expression, extracellular glucose and magnesium levels in the different regions of the brain.

In summary, a repeated blood-sampling assay was developed for determining dynamic changes in whole blood glucose, lactate and magnesium concentrations in gerbils that had been subjected to short-term forced swimming. Forced swimming resulted in an increase in blood glucose and lactate levels and magnesium sulfate supplementation resulted in enhanced glucose levels, but an attenuation effect on lactate levels. In addition, the possibility that short-term forced swimming exercise may be have given rise to shifts in magnesium to plasma compartments cannot be excluded, based on the findings reported herein. Moreover, magnesium supplementation had no effect on striatum GLUT-3 and glucose levels in the forced swimming model. Further studies will be required to investigate the effects of magnesium on variations in GLUTs expression in different regions of the brain.

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