Magnesium Sulfate Enhances Glucose Transporter-3 Expression in Gerbils Subjected to Short-term Forced Swimming

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Abstract

Although our previous studies demonstrated that magnesium sulfate increased extracellular glucose levels during short-term forced swimming in gerbils, a complete understanding of the effects of magnesium sulfate on glucose levels remains unclear. The transport of glucose into most mammalian cells is mediated by a family of glucose transporter proteins. The brain type glucose transporter glucose transporter-3 (GLUT-3) is the predominant neuronal glucose transporter and contributes to the transport of glucose, the main source of energy for survival, into neuronal cells. In this study, we further evaluated the effects of magnesium sulfate on the levels of GLUT-3 protein expression, glucose, and magnesium in brain in gerbils that had been subjected to short-term forced swimming. Gerbils were separated into four groups: gerbils in group 1 (control group, C group) were only pretreated with saline, gerbils in group 2 (Mg group) were only pretreated with magnesium sulfate (90 mg·kg⁻¹, i.p.), gerbils in group 3 (C+S group) were pretreated with saline before the 15 min forced swimming then rested for 30 min prior to sacrifice, gerbils in group 4 (Mg+S group) were pretreated with saline before the 15 min forced swimming then rested for 30 min prior to sacrifice. Data were obtained by western blotting assays, a microdialysis analyzer and a flame atomic absorption spectrometer. These results showed that pretreatment with magnesium sulfate (Mg group) significantly elevated GLUT-3 protein, glucose and magnesium levels in the cortex (p<0.01). Saline+short-term forced swimming (C+S group) had no significant effect on GLUT-3 protein, glucose and magnesium levels; however, the cortex GLUT-3 protein, glucose, and magnesium levels of the magnesium sulfate+swimming group (Mg+S) were higher than the C group (p<0.05). These results indicate that magnesium sulfate enhances glucose concentrations and GLUT-3 protein levels in the cortex of gerbils, irrespective of whether they were subjected to forced swimming or not. These preliminary data suggest that GLUT-3 protein may play an important role in the magnesium sulfate-stimulated increases in glucose levels in the brain.

Keywords: Exercise, Glucose, Magnesium

Introduction

Magnesium is ubiquitous in all living organisms, and it is the second most abundant intracellular cation [1]. It serves as a cofactor in more than 300 enzymatic reactions in which food is catabolized and in the biosynthesis of new products [2]. Magnesium is also involved in cellular energy production, glycogen breakdown, and modulating the activity of adenosine triphosphatase, thus providing energy for cells. Magnesium also serves as a physiological regulator of neuromuscular functions [2-4].

Magnesium depletion may lead to changes in neuromuscular function and reduce physical performance and the efficiency of energy metabolism [5] and there is a direct relationship between magnesium and physical performance. As a result, magnesium may be regarded as one of the important elements that affect physical performance and exercise. Long-term exercise or exercise until exhaustion result in hypomagnesaemia [6,7]. In contrast, plasma magnesium increases during short-term exercise, which is caused by short-term, high-intensity anaerobic exercise because of the reduction in plasma volume and a shift in cellular magnesium resulting from acidosis [8]. These data suggest that the duration and intensity of exercise play critical roles in the regulation of both peripheral and cerebral magnesium homeostasis.

Plasma glucose is an important source during exercise, and it supplies oxidative energy production during submaximal and prolonged exercise [9,10]. Prolonged exhausting exercise results in hypoglycaemia and hypomagnesaemia [7,10], whereas short-term, high-intensity exercise induces hypermagnesaemia and hyperglycemia [8,11,12]. We previously demonstrated that, in gerbils, short-term forced swimming decreased brain extracellular glucose levels, and that pretreatment with magnesium sulfate immediately increased...
glucose levels by microdialysis [13]. However, the mechanism regarding the effects of magnesium sulfate on glucose levels continues to remain unclear.

Glucose is an essential energy source for most cells and is the primary energy source for brain tissue. Since glucose is a hydrophilic molecule, its transport through cellular membranes requires the presence of specific transport proteins. 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].

In adult skeletal muscle, glucose transport is a facilitated diffusion process which is mediated by GLUT-1 and GLUT-4. GLUT-4 is an insulin-regulated glucose transporter found in adipose tissues and muscle and is responsible for insulin-regulated glucose disposal [18-22]. GLUT-3 and GLUT-5 are the predominant glucose transporter isoforms in the brain. GLUT-3 mRNA is detected in many tissues from human, rabbit, rat and mouse, especially in brain. GLUT-5 is a high-affinity isoform that is expressed in the microglia.

Multiple isoforms of glucose transporters are found in muscle, the tissue that normally account for 85% of insulin-stimulated glucose uptake. Glucose uptake into muscle cells in fasting state is mediated primarily by GLUT-1 and GLUT-3 protein, whereas exercise-related increment in muscle glucose uptake is mediated primarily by GLUT-4 [23]. A number of studies have reported that exercise results in an increase in GLUT-1 or GLUT-4 expression [24-28]. Kimura et al. reported that the degree of translocation of GLUT-4 to plasma membranes in adipocytes stimulated by insulin was reduced in magnesium deficient rats [29]. However, whether exercise results in increased or decreased cerebral GLUT-3 levels remains unclear. The aim of this study was to investigate the effects of short-term forced swimming on cortex GLUT-3 levels. In addition, the effects of magnesium sulfate on changes in cortex GLUT-3, glucose, and magnesium levels in gerbils that had been subjected to short-term forced swimming were evaluated.

**Methods**

**Animals**

Adult and naïve male gerbils (n=16), weighing between 70 and 80 g, were obtained from the Laboratory Animal Center of the Taichung Veterans General Hospital. All animals were housed in a temperature (25 °C) and light (12:12 h light-dark cycle)-controlled room with free access to rat chow and tap water. Animal care and experimental procedures (# L a-95275, 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.

**Experimental design**

The gerbils 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, 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.

**Forced swimming**

Each gerbil was placed on a polystyrene board floating in a Plexiglas cylinder that was 40 cm in diameter, 35 cm high, 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 performing the swimming exercise again). After the forced swimming, each gerbil was removed from the water, and then carefully and rapidly dried with a towel and a hair blower. Each gerbil was then re-placed on the polystyrene board, and allowed to rest for 30 min before sacrificing.

**Brain homogenization**

After the forced swimming period, the gerbils allowed to rest for 30 min and then were sacrificed and the brains removed. The cortex 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 its pH to about 7.0 for further glucose analysis. 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 (cortex) 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 resulting 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 the 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 for 1 h with horseradish peroxidase-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, CA) in TBS-T containing 5% skimmed milk. Finally, after four washes with TBS-T, the protein bands were visualized using an enhanced chemi-luminescence Western blots analysis system (Pierce, Rockford, IL, USA).

**Determination of glucose and magnesium concentrations**

A Perkin-Elmer Model 5100 Flame atomic absorption spectrometer (FAAS, Perkin-Elmer, Uberlingen, Germany) was used to quantitatively determine the content of magnesium. A 25 µl aliquot of supernatant was diluted with 975 µl of 0.2%
HNO3 for further analysis by FAAS. All reagents used were of analytical grade and were purchased from E. Merck. All containers were soaked in 20% nitric acid, rinsed with water and then dried in a clean room for later use.

A microdialysis analyzer (CMA/600, Carnegie Medicin) was employed for the determination of glucose. The supernatant was used in the analysis of glucose. Glucose concentrations were determined by the glucose oxidase method in the microdialysis autoanalyser.

Reactions involved:

\[ \text{D-glucose} + \text{O}_2 \rightarrow \text{gluconolactone} + \text{H}_2\text{O}_2 \]
\[ 2\text{H}_2\text{O}_2 + \text{phenol} + 4\text{-amino-antipyrine} \rightarrow \text{quinoneimine} + 4\text{H}_2\text{O} \]

Statistical analysis

All data are expressed as means±SEM. One-way ANOVA followed by the Student-Newman-Keuls’ multiple-range test was used to test the significance of difference among groups. Differences were considered to be statistically significant at p<0.05.

Results

The effects of magnesium sulfate, swimming, and magnesium sulfate plus swimming on GLUT-3 levels, glucose, and magnesium concentrations in the cortex are shown in figures 1-3, respectively. Swimming had no effect on GLUT-3 expression, glucose, and magnesium levels; however, in cases of pretreatment with magnesium sulfate and magnesium sulfate plus swimming, both groups showed significantly increased GLUT-3 expression, glucose, and magnesium concentrations (p<0.01 and p<0.05, respectively).

![Figure 1](image1.png)

**Figure 1.** The effect of magnesium sulfate, swimming, and magnesium sulfate+swimming on glucose levels in the cortex. Data are the mean±SEM of four gerbils. *p<0.05, **p<0.01 compared with the saline control.

![Figure 2](image2.png)

**Figure 2.** The effect of magnesium sulfate, swimming, and magnesium sulfate+swimming on glucose levels in the cortex. Data are the mean±SEM of four gerbils. *p<0.05, **p<0.01 compared with the saline control.

![Figure 3](image3.png)

**Figure 3.** Animals were treated with magnesium sulfate, swimming, and magnesium sulfate+swimming. GLUT-3 protein expression in the cortex was determined by Western blotting. The upper panel shows a representative blot and a lower panel densitometric comparison. Data are the mean±SEM of four gerbils. *p<0.05, **p<0.01 compared with the saline control.

Discussion

This preliminary data reported herein demonstrate that magnesium sulfate increased cortex GLUT-3 expression, and also increased glucose and magnesium levels in gerbils, irrespective of whether forced swimming was or was not involved. However, the short-term forced swimming had no effect on the levels of GLUT-3 protein expression in the cortex. Exercise has profound effects on numerous biological systems, including the peripheral and central nervous systems. In the peripheral system, long-term or exhausting exercise induced hypoglycaemia, hypomagnesaemia and lactate accumulation result in the reduced efficiency of muscular contraction and exercise. In contrast to the levels of glucose in the brain, exhausting swimming (150 min) or 6 min forced swimming test leads to a decrease in glucose levels [12,30]. Our previous studies demonstrated that 15 min short-term forced swimming decreased brain extracellular glucose levels, whereas it had no effect extracellular magnesium levels by using microdialysis analysis [13]. These data suggest that the intensity and duration of exercise play important roles in regulation of glucose and magnesium levels. Some studies have focused on the relation between the magnesium and glucose during exercise. Short-term, high-intensity exercise (10 min swimming stress or treadmill running unit exhaustion or 6 min forced swimming test) that causes hypermagnesaemia and hyperglycaemia [8,11,12]. However, the present study has indicated that the short-term forced swimming had no effect on the levels of glucose and magnesium in the cortex. These data suggest that even the same exercise model can have a different effect on glucose and magnesium levels of the different brain compartments.

On the other hand, previous studies indicated that physical exercise, cycle training (30min on 3 d/wk for 8weeks), or swimming (3hr) induce the upregulation of GLUT-4 or GLUT-1 in muscle tissue [25-28]. However, our data indicated that short-term forced swimming not changed the levels of GLUT-3 protein in cortex. These present findings suggest that the duration and intensity of exercise play critical roles in regulating GLUTs protein expression in the different tissues. Magnesium deficiency results in a reduced, whereas supplemental magnesium improves physical performance [5]. Our previously reported data demonstrated that magnesium...
sulfate and short-term forced swimming played a role in regulating cerebral glucose concentrations, and that magnesium sulfate led to an increase in glucose levels [13]. As a result, there was a balance between supply and utilization in the concentrations of extracellular glucose. The effects of supplemental magnesium on glucose levels may be via the regulation of both the oxidative phosphorylation and glycolysis pathways, and hypermagnesemia reduces the rate of glucose metabolism in neural tissue by inhibiting glycolysis [4]. Furthermore, for intracellular uptake of glucose by insulin-sensitive cells, the translocation of GLUTs into plasma membrane is a vital step, which insulin induces by autophosphorylating beta subunits of insulin receptor after its specific binding. Magnesium acts in a stimulatory manner in the step of insulin binding to its receptor and autophosphorylating of beta subunits. Intracellular magnesium stimulates intracellular glucose transport and its oxidation step [31,32].

In this study, it is interesting to note that the pretreatment with magnesium sulfate increased glucose and magnesium levels in the cortex, and also resulted in increased GLUT-3 protein expression. Therefore, it can be concluded that the magnesium sulfate increased cerebral glucose levels may be enhanced by GLUT-3 expression. However, the specific role of magnesium on GLUT-3 protein will require further clarification. In summary, the preliminary data reported herein demonstrate that magnesium sulfate causes an increased GLUT-3 expression in the cortex; moreover, GLUT-3 protein may play an important role in the magnesium sulfate-stimulated increase in glucose levels in the brain.

References


