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Magnesium sulfate effect on erythrocyte membranes of asphyxiated newborns

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Abstract

Objectives: Magnesium sulfate has been recognized as a neuroprotective agent against hypoxia–ischemia, mainly by the protection from the excitotoxicity associated with increased glutamate concentration. However, the mechanism of $MgSO_4$ action is not fully understood and is considerably controversial.

Design and methods: During the 2 first hours of life, the asphyxiated full-term newborns were treated intravenously with one dose of $MgSO_4$ 250 mg/kg body weight. At birth, after 6 and 48 h of life the activity of ATP-dependent enzymes in erythrocyte membranes: Mg^{2+}-ATPase, Ca^{2+}-ATPase, protein kinases A and C, were determined. Using monoclonal antibodies, the band 3 and its phosphotyrosine level were also assayed.

Results: The time-dependent decrease of Ca^{2+}-ATPase activity was detected in untreated newborns, whereas $MgSO_4$ prevented this reduction. After 48 h, protein kinases activities differed in $MgSO_4$-treated and untreated groups. Magnesium therapy increased the amount of band 3 and diminished proteolytic degradation of this protein.

Conclusion: Our results demonstrated, for the first time, that magnesium sulfate treatment significantly altered the activities of some important enzymes in erythrocyte membrane from asphyxiated newborns. It also reduced the post-asphyxial damages of membrane compounds. These data may partly explain the molecular mechanisms of $MgSO_4$ action in asphyxiated newborns.

Keywords: Magnesium sulfate; Plasma membrane Ca^{2+}-ATPase; Band 3; Protein kinases; Neonate; Asphyxia

Introduction

Magnesium sulfate has been recognized as a neuroprotective agent against hypoxia–ischemia brain damage. For that reason, it is also used for treatment of perinatal asphyxia, one of the most devastating neurological processes [1,2]. Intravenously administered magnesium sulfate enters the CNS system, even in the presence of an intact blood–brain barrier, and it occurs within 20 min after injection [3]. However, the mechanism of $MgSO_4$ action is not fully understood and is considerably controversial. Treatment of asphyxiated newborns with magnesium sulfate has been studied using different models, and both protective and nonprotective effects have been demonstrated [4–7]. During prolonged asphyxia, the overstimulation by neurotransmitters has been shown, and one of the receptors for these neurotransmitters is the *N*-methyl-D-asparagine (NMDA) receptor, associated with ion channels. An important consequence of its excessive activation is the increased Ca^{2+} influx into the cells [8,9]. Ca^{2+} overloading and cellular energy depletion are the well-defined damaging effect during asphyxia which, due to disturbance of ions homeostasis and free radical release, could potentially intensify the cell mortality [10]. The most documented mechanism suggests that magnesium sulfate action could be exerted mainly by the competition of Mg^{2+} ions with calcium [11]. As a result, it could reduce Ca^{2+} entry into the cells and inhibits the release of excitatory amino acids,

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glutamate and glycine. In young brain, the NMDA receptors revealed diminished sensitivity to Mg^{2+} block; however, this potency was shown to be region-dependent and altered during development [9]. Blockade by Mg^{2+} of NMDA channels could be also modified by regional heterogeneity of NMDA subunits [12].

We have previously shown that under asphyxia condition, some erythrocyte membrane components significantly differed from healthy newborns [13,14]. We observed the enhanced lipid peroxidation, the degradation of plasma membrane calcium pump with concomitant suppression of its activity, as well as changed activities of protein tyrosine kinases and serine/threonine kinases: protein kinase A (PKA) and protein kinase C (PKC). Moreover, under asphyxia, an integral erythrocyte membrane protein–anion exchanger (known also as a band 3) which catalyses the electroneural exchange of Cl^- and HCO_3^- was overphosphorylated, however, its amount decreased.

The aim of the present study was to examine the effect of magnesium sulfate treatment on the selected erythrocyte membrane enzymes of asphyxiated neonates, including of Mg^{2+}- and Ca^{2+}-dependent ATPases activities, protein kinase A, and protein kinase C. We have also characterized the effect of magnesium therapy on band 3, the important membrane component responsible for the proper erythrocyte function.

Methods

$MgSO_4$ treatment

The parents give informed consent to $MgSO_4$ administration, and the institutional review boards of the participating hospital approved the study. The asphyxiated newborns were qualified to the study if they fulfill the following criteria: more than 36 weeks of gestation age, absence of major congenital malformations, Apgar score below 6 in 10th min or below 6 in 5th min and above 6 in 10th min if the following were present: fetal bradycardia < 100 per minute, green amniotic fluid, umbilical artery blood pH < 7.11, umbilical artery blood base excess (BE) < −10 mmol/L. Magnesium sulfate (Polpharma, Poland) was given in the first 2 h of life in a group of 18 full-term infants who were born in perinatal asphyxia. The newborn received one dose of $MgSO_4$ 250 mg/kg body weight, during 30 min intravenously infusion. In every child, concentration of magnesium in serum was measured at birth, after 6 and 48 h. The heart rate, respiratory rate, saturation and arterial blood pressure were monitoring continuously, but the side effects of $MgSO_4$ administration were not observed during the study. The control groups were healthy (Apgar ≥ 8, $n = 20$), with similar gestational age. For some experiments, the asphyxiated infants, who did not received magnesium sulfate ($n = 7$) were included.

Determination of Mg total in serum

The serum Mg level was determined colorimetrically (COBAS INTEGRA Roche Company) using chlorophosphonazo III [15].

Erythrocyte membrane preparation

Blood from umbilical arterial cord of healthy and born with asphyxia infants was collected with citrate in the first hour of life (time 0). The volume of whole blood drawn from peripheral venous after 6 and 48 h of life was 0.5 ml. Erythrocytes were isolated by centrifugation at 4°C at 2000 × *g* and purified by washing with 20 volumes of phosphate-buffered saline, as described previously [13]. In brief, residual leukocytes were removed by passing through a column of HBS cellulose (Serva, Germany). The purified erythrocytes were hemolyzed on ice for 1 h with 20 volumes of 5 mM Tris–HCl buffer, pH = 7.4, containing 1 mM EGTA, 1 mM EDTA, 1 mM PMSF, and next centrifuged for 15 min at 4°C at 20,000 × *g*. The ghosts were washed 4 times with the same buffer and, finally, resuspended in 10 mM Tris–HCl, pH = 7.4. The concentration of protein was measured using Protein Assay kit (BioRad, USA).

Determination of the ATPase activities in erythrocyte membranes

10 μg of erythrocyte ghosts were incubated for 15 min at 37°C in the reaction mixture containing: 50 mM Tris–HCl, pH = 7.4, 100 mM KCl, 3 mM $MgCl_2$, 3 mM ATP, 1 mM EGTA, 0.1 mM ouabain, in a total volume 200 μl. Mg^{2+}-ATPase activity was determined in the absence of Ca^{2+}. The Mg^{2+}-dependent Ca^{2+}-ATPase activity was determined in the presence of 1.020 mM $CaCl_2$ (10 μM Ca^{2+} free) and corrected for Mg^{2+}-independent ATPase activity. Calmodulin-stimulated activity of Mg^{2+}-dependent Ca^{2+}-ATPase was measured in the presence of 72 nM CaM (Calbiochem, Switzerland). The activities were measured by colorimetric determination of Pi hydrolyzed from ATP.

Determination of protein kinase A and C activities

The phosphorylation reaction was carried out in a standard reaction mixture with 1.5 mM $CaCl_2$ and 1 μM PMA (phorbol 12-myristate, 13-acetate) (Sigma, Germany) as activators of protein kinase C, and 5 μM cAMP (Sigma, Germany) as an activator of protein kinase A, as described previously (14). The reaction was started by the addition of 90 μM [γ-^{32}P]ATP (200–300 cpm/pmol) (Amersham Corp., England), and terminated by the addition of a mixture of 5 mM ATP and 0.25 mM EDTA. The samples containing 10 μg of erythrocyte membranes (in triplicate) were spotted onto Whatman 3MM filter and washed with cold 10% TCA and 10 mM sodium pyrophosphate. The level of ^{32}P incorporation was measured by the counting