Original Research

Microdialysis Analyzer and Flame Atomic Absorption Spectrometry in the Determination of Blood Glucose, Lactate and Magnesium in Gerbils Subjected to Cerebral Ischemia/Reperfusion

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Objective: Flame atomic absorption spectrometry (FAAS) and a microdialysis analyzer were employed for dynamic monitoring of magnesium (Mg), glucose and lactate levels in blood samples of gerbils subjected to cerebral ischemia.

Methods: Focal cerebral ischemia was induced by occlusion of the unilateral common carotid artery and the middle cerebral artery for 60 minutes followed by 180 minutes of reperfusion. Whole blood samples were continuously collected from the jugular vein via an auto-blood sampling system. The dynamic profiles of Mg, glucose and lactate before, during and after ischemia were determined.

Results: During cerebral ischemia, blood Mg levels gradually rose to 130% of the baseline and returned to the basal levels within 30 minutes after reperfusion. Lactate concentrations decreased to approximately 50% of the basal levels during cerebral ischemia and returned to basal levels immediately after reperfusion. Glucose levels remained the same during cerebral ischemia and gradually fell to 50% of basal levels at the end of reperfusion. The linearity ranges of glucose, lactate and Mg were 0.1–25 mM, 0.02–2.5 mM and 5–1500 μg/L, respectively. The required volume of each blood sample is less than 30 μL. The intra- and inter-assay variation was less than 3%. Since blood loss is minimal from repeated blood sampling, it is suitable for small animals.

Conclusions: Mg may be accumulated in blood cells, which are helpful for reducing glucose utilization. As a result, less lactate was produced during the acute phase of cerebral ischemia. Preservation of glucose is advantageous for brain cells' restoration after ischemia.

INTRODUCTION

The essential mineral, Mg, is important in the maintenance of normal cellular and body functions. Mg is the second most abundant intracellular cation, and its many biological values for living organisms have been well reported [1]. Among the biological functions of Mg are: acting as a cofactor in many enzymatic catalyzations, modulating the activity of adenotriphosphatases (ATPases), which have central importance in energy metabolism, enhancing cerebral blood flow to ischemic areas, inhibiting calcium (Ca) flow into cells, and reducing the size of brain infarcts [2–6]. Loss of ionic homeostasis during cerebral ischemia is recognized as important [7], but derangement of Ca, potassium (K), and sodium (Na) has received more

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attention than that of Mg, despite the fact that brain Mg seems to be associated with a variety of diseases and its loss is important in stroke [8–13]. In addition, the function of Mg in regulating cerebral vascular tone and blood flow has been reported [14,15]. However, the role or function of Mg in blood vessels during cerebral ischemia remains an unsettled question and awaits further elucidation. To ascertain the role of Mg in blood vessels, dynamic changes of Mg were investigated by flame atomic absorption spectrometry (FAAS) during cerebral ischemia/reperfusion.

Glucose is normally a major and important energy source for all cells. Appropriate glucose concentration is helpful for cells in maintenance of normal physiological functions. Erythrocytes, being non-nucleated and devoid of organelles, are one of the simplest cells in blood. Through the glycolysis pathway, glucose is catabolized in producing adenosine triphosphate (ATP) and pyruvate in cells, thus, their supply of ATP is not limited. However, pyruvate is limited, and production of lactate is increased under anaerobic conditions such as severe exercise or ischemia. Under these conditions, lactate is accumulated and is harmful to the cells.

Microdialysis analysis is a powerful tool in the determination of glucose and lactate in a sample volume as small as less than 1 μL. This analyzing system is excellent in providing advanced information about energy metabolites in a small sample volume. For small animals, such as gerbils (approximately 65–85 g), it is difficult for investigators to collect enough blood samples throughout the experimental processes for dynamic investigation, therefore, it is important to develop an appropriate tool to overcome the above problems, which adapt to our experimental needs in understanding dynamic changes during cerebral ischemia. In the present study, blood samples from the jugular vein were collected through an auto-blood sampling system. This advanced system allows automatic blood sampling via an implanted catheter in a small laboratory animal. This system is advantageous, not only in eliminating the intensive labor of researchers, but also in reducing the stress on animals caused by a human approach.

MATERIALS AND METHODS

Male gerbils (n = 3, 65–85 g) were obtained from the Laboratory Animal Center at the Taichung Veterans General Hospital (Taichung, Taiwan). They were allowed to become acclimated to their environmentally controlled quarters (25°C and 12:12 h light-dark cycle) before the experiments. The gerbils were then anesthetized with chloral hydrate (360 mg/kg body weight, i.p.), with additional chloral hydrate (200 mg/kg) when needed throughout the experimental process. The body temperature was maintained at 37°C with a heating pad (CMA/150). The right common carotid artery (CCA), exposed through a ventral midline incision in the neck, was carefully separated from the vago-sympathetic trunks and loosely encircled with 3-0 sutures for later occlusion. The gerbil's head was mounted on a stereotaxic apparatus (Stoelting, IL, USA) with the nose bar positioned 4.0 mm below the horizontal line. Following a midline incision, the skull was craniectomised to expose the right middle cerebral artery (MCA). An 8-0 suture (blue monofilament polypropylene, DG, Davis-GECK, Wayne, N.J.) was positioned so that it encircled the middle cerebral artery for later ligation. A transient focal ischemic lesion was induced by simultaneous occlusion of the right CCA and the right MCA for 60 minutes followed by 180 minutes of reperfusion.

Blood samples were automatically collected via an implanted catheter in the jugular vein by the auto-blood sampling system (DR-II, EICOM, Kyoto, Japan). Thirty μL of whole blood samples were collected every 15 minutes. In order to preserve glucose, 15 μL of NaF solution was added to the whole blood sample and then centrifuged at 1500 g for 10 minutes at 4°C. The supernatant was removed and mixed with 90 μL of the $HClO_4$ (1 N), and then centrifuged again at 1500 g for 10 minutes at 4°C. A Perkin-Elmer Model 5100 Flame atomic absorption spectrometer (FAAS, Perkin-Elmer, Uberlingen, Germany) was used for analyzing Mg. Twenty-five μL of supernatant was taken and diluted with 975 μL of 0.2% HNO_3 for further analysis by FAAS. All reagents used were of analytical grade and were purchased from E. Merck. All containers were soaked with 20% of nitric acid, rinsed with water and then dried in a clean room for later use.

The microdialysis autoanalyser was employed for determination of glucose and lactate. Two μL of Tris buffer was added to 8 μL of the supernatant to adjust its pH value to about 7.0 for further glucose and lactate analysis. The glucose concentration was determined by glucose oxidase and the lactate level was determined by lactate oxidase in the microdialysis autoanalyser.

RESULTS

Blood Mg levels rose to approximately 130% of the baseline levels during cerebral ischemia and returned to baseline levels within 3 hours of reperfusion (Fig. 1).

Lactate decreased to approximately 50% of the baseline levels during cerebral ischemia and returned to the baseline levels right after reperfusion (Fig. 2).

Slightly decreased glucose levels were observed during cerebral ischemia/reperfusion (Fig. 3).

The linearity ranges of glucose, lactate and Mg were 0.1–25 mM, 0.02–2.5 mM and 5–1500 μg/L (correlation coefficient value $>$ 0.9992), respectively. The detection limits of glucose, lactate and Mg were 20 μM, 10 μM and 1 μg/L, respectively. The required volume of each blood sample is less than 30 μL. The intra- and inter-assays variation of glucose, lactate and Mg were less than 3%.

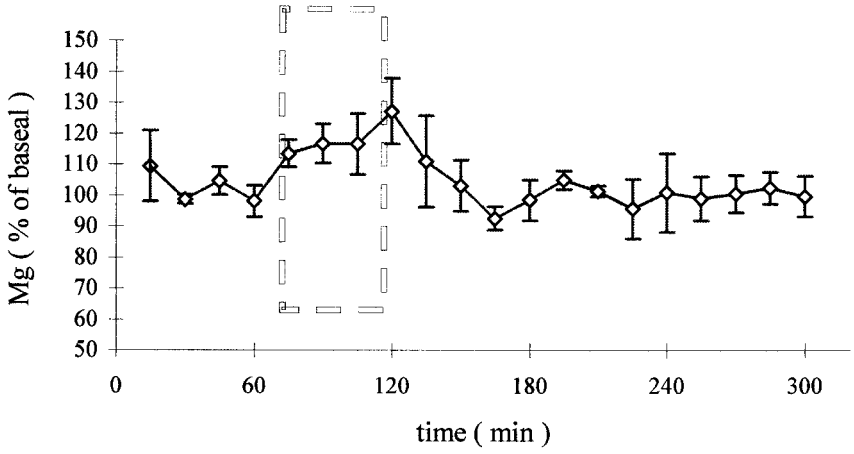

Fig. 1. Dynamic changes of Mg levels in blood of gerbils during 60 minutes CCA + MCA occlusion and 3 hours reperfusion. Data are presented as mean ± SEM (n = 3).

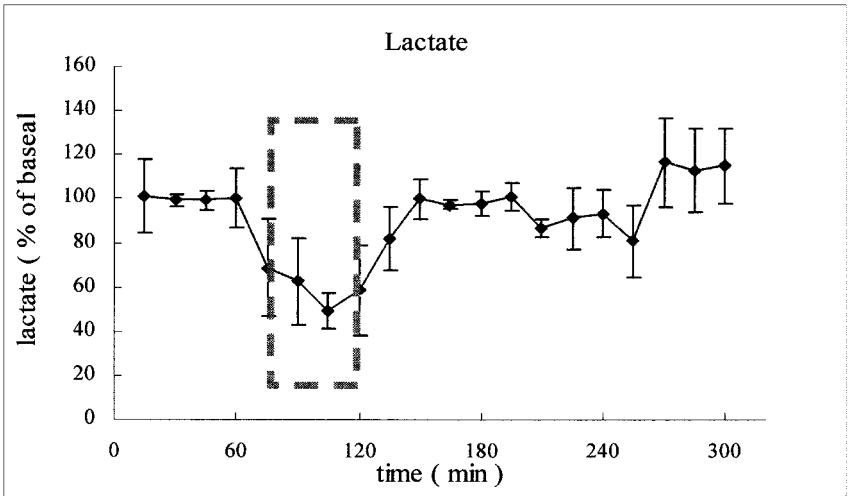

Fig. 2. Dynamic changes of lactate levels in blood of gerbils during 60 minutes CCA + MCA occlusion and 3 hours reperfusion. Data are presented as mean ± SEM (n = 3).

DISCUSSION

In the past, studies have been largely concerned with the relation between the disturbance of Ca levels and various diseases. Accruing evidence has focused on the importance of Mg [16]. Mg possesses an important role in oxidative phosphorylation and glycolysis in the cells [17]. In general, 54% of total body Mg exists in skeleton, 45% is stored in soft tissue, and only 1% is in extracellular fluid, which is drawn upon for maintenance of the intracellular Mg. Cerebral ischemia results in a reduced glucose and oxygen supply and decreases ATP formation in ischemic area [18]. We have previously demonstrated Mg pre-treatment significantly preserved brain glucose levels during cerebral ischemia [19]. Also, previous study showed that serum Mg level was lower than normal in patients shortly after a stroke [20]. Similarly, hypomagnesemia has been reported to correlate with a variety of diseases such as ventricular arrhythmias, myocardial infarction, and head injury [9–11]. Accordingly, these results suggest that higher Mg levels may attenuate the severity of cerebral energy failure during ischemia. Conversely, any decline in serum Mg levels may adversely influence these critical processes.

Cellular Mg determinations provide a more accurate assessment of Mg homeostasis than do serum-based determinations in diagnosing Mg deficiency [21]. Our data showed that the blood Mg concentration increased to 130% of the baseline levels during cerebral ischemia and gradually returned to the baseline levels within 3 hours of reperfusion. It has been proposed that hypermagnesemia substantially reduces the rate of glucose metabolism in neural tissue by directly inhibiting glycolysis [22]. It has also been reported that high Mg concentrations decrease human leukocyte activation [23]. Our data also support the trend in reduction of blood glucose observed during cerebral ischemia. By reducing the rate of glucose

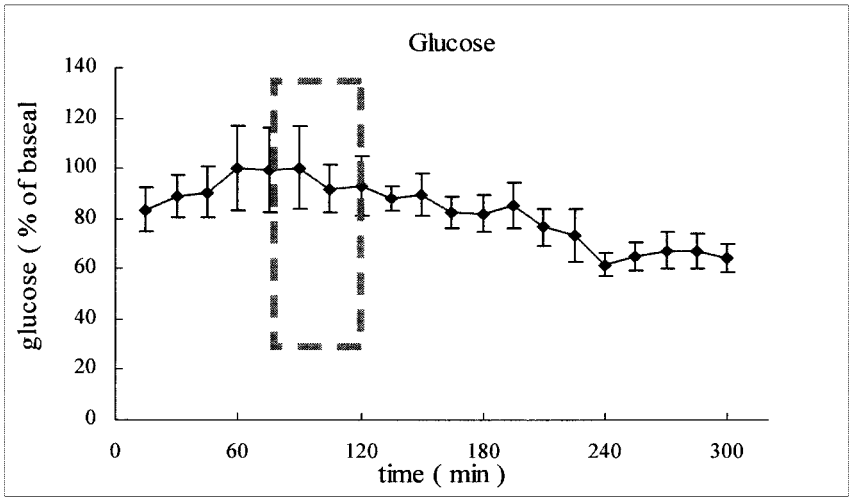

Fig. 3. Dynamic changes of glucose levels in blood of gerbils during 60 minutes CCA + MCA occlusion and 3 hours reperfusion. Data are presented as mean ± SEM (n = 3).

metabolism resulting from higher blood Mg levels, lactate levels decreased and returned to baseline values right after reperfusion.

CONCLUSION

Our data support speculation that Mg is accumulated in blood vessels during the acute phase of cerebral ischemia in gerbils. This phenomenon may be important for blood dynamics, not only in reducing the glucose utilization rate but also in decreasing blood cell activities. As a result, less lactate and preserved glucose are advantages for supporting brain cells during cerebral ischemia/reperfusion.

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