Reduced Mg\(^{2+}\) -ATPase Activity in the Hypoglycemic Adult Rat Brain
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The effects of different α-D-Glucose (Glu) concentrations (0–16 mM) on Na\(^+\), K\(^+\) -ATPase and Mg\(^{2+}\) -ATPase activities were investigated in homogenates of adult male rat whole brain at 37 °C. The enzyme activities were determined after 1h preincubation with Glu. Brain Na\(^+\), K\(^+\) -ATPase was not affected by Glu different concentrations. On the contrary, Mg\(^{2+}\) -ATPase activity was considerably reduced with Glu concentrations lower than 4 mM. The enzyme was inhibited 40%, 50% or 80% with 3, 2 or 1 mM of Glu, respectively. The above results suggest: a) The various concentrations of Glu have no effect on brain Na\(^+\), K\(^+\) -ATPase activity. b) The inhibited brain Mg\(^{2+}\) -ATPase in hypoglycemia produces low intracellular Mg\(^{2+}\), which could modulate the activity of Mg\(^{2+}\) -dependent enzymes and the rates of protein synthesis and growth of the cell.

Introduction

Hypoglycemia represents a medical emergency because of the almost complete reliance of the brain on glucose (Glu) as substrate for its enormous energy requirements. In contrast, most tissues save energy for brain, whereas red cells, kidney and adrenal medulla can employ a number of substrates to supply their energy needs, depending on their availability at any moment. Except for the situation in prolonged starvation, in which ketones may supply much of the brain's energetic requirements, the brain does not manifest this type of flexibility (Owen et al., 1967; Cornblath and Schwartz, 1976). Indeed, diminution of blood glucose to below 0.5 mg/ml (2.5 mm) in adults or a precipitous fall from elevated levels to normal (1.2 mg/ml or 6.7 mm), but momentarily insufficient levels can bring about symptoms of cerebral dysfunction, while calling forth secretion of noradrenergic to counteract the fall in glucose (Brunjes, 1963; Cohn and Roth, 1983).

The combustion of glucose within the cells begins with the process termed "glycolysis" in which glucose is anaerobically converted through a number of enzyme mediated steps into pyruvate or lactate, with the generation of ATP in this anaerobic process. Further, complex combustion of glucose requires oxygen, generates additional ATP and requires the interaction of several mitochondrial systems (Owen et al., 1967; Cornblath and Schwartz, 1976). There are three Mg\(^{2+}\) and/or Mg\(^{2+}\) K-dependent enzyme-mediated steps in the glycolytic sequence that are irreversible (e.g. hexokinase, phosphofructokinase, pyruvate-kinase) and take part in the glucose homeostasis (Cohn and Roth, 1983; Illingworth, 1985). Additionally, hepatic glucogen reserves directly supply the central nervous system, while the mobilization of fatty acids from triglycerides depot provides energy for the large mass of skeletal and cardiac muscles, renal cortex and other tissues that utilize fatty acids as their basic fuel, thus sparing glucose for the brain (Young and Karam, 1991). The aim of this study was to investigate the effect of different Glu concentrations (0–16 mM) on the activity of two enzymes in adult rat brain: a) Na\(^+\), K\(^+\) -ATPase (EC 3.6.1.3), an integral membranous enzyme implicated in univalent cation transport (Swadeaner and Goldin, 1980), neural excitability (Sastry and Phillips, 1977) and metabolic energy production (Mata et al., 1980) and b) Mg\(^{2+}\) -ATPase, the role of which is to maintain high brain intracellular Mg\(^{2+}\), changes of which can modulate the activity of Mg\(^{2+}\) -dependent enzymes and control rates of protein synthesis and growth of the cell (Sanui and Rubin, 1982).

Material and Methods

Animals

Albino adult (4 mo) Wistar male rats (Saint Savvas Hospital, Athens, Greece) were used in all experiments. Boby weight was 225 ± 10 g (mean ± SD). The rats were housed four in a cage, at a constant room temperature (22 ± 1 °C) under a 12 hL: 12 hD (light 08.00–20.00 h) cycle and accli-
mated 1 week before use. Food and water were provided ad lib. Animals were cared for in accordance with the principles of the "Guide to the Care and Use of Experimental Animals" (Committee on Care and Use of Laboratory Animals, 1985).

**Tissue preparation**

Animals were sacrificed by decapitation. Whole brains from five rats were rapidly removed, weighed and thoroughly washed with isotonic saline. Tissues were homogenized in 10 vol. ice-cold (0–4 °C) medium containing 50 mM Tris (hydroxy-methyl) aminomethane-HCl (Tris-HCl), pH 7.4 and 300 mM sucrose using an ice-chilled glass homogenizing vessel at 900 rpm (4–5 strokes). Then, the homogenate was centrifuged at 1000 x g for 10 min to remove nuclei and debris. In the resulting supernatant, the protein content was determined according to the method of Lowry et al. (1951) and then the enzyme activities were measured.

**Glu preincubation**

The enzymatic activity measurements were carried out on homogenized rat whole brain. The activity was determined after a 1 h preincubation of the homogenate with 1, 2, 3, 4, 6.7, 8 or 16 mM of Glu at 37 °C. The brain intracellular concentrations of Glu were similar with those of blood for hyperglycemic (8–16 mM), normal (about 6.7 mM) or hypoglycemic conditions (e.g. 1.7–2.5 mM or 0.3–0.5 mg/ml).

**Determination of Na⁺, K⁺-ATPase and Mg²⁺-ATPase activities.**

Na⁺, K⁺-ATPase activity was calculated as the difference between total ATPase activity (Na⁺, K⁺, Mg²⁺-dependent) and Mg²⁺-dependent ATPase activity. Total ATPase activity was assayed at 37 °C in an incubation medium consisting of 50 mM Tris-HCl, pH 7.4, 120 mM NaCl, 20 mM KCl, 4 mM MgCl₂, 240 mM sucrose, 1 mM ethylenediaminetetraacetic acid K₂-salt (K ± EDTA), 3 mM disodium ATP and 80–100 μg protein of the homogenate, in a final volume of 1 ml. Ouabain (1 mM) was added to determine the activity of the Mg²⁺-ATPase. The values of Mg²⁺-dependent ATPase were similar in the presence of ouabain in the reaction mixture, as also in the absence of ouabain and without NaCl and KCl (in the presence of MgCl₂ only). The reaction was started by adding ATP and stopped after a 20 min incubation period by addition of 2 ml of a mixture of 1% lubrol and 1% ammonium molybdate in 0.9 M H₂SO₄ (Atkinson et al., 1971; Bowler and Tirri, 1974). The yellow colour which developed was read at 390 nm. Lubrol is a detergent from ICN Biochemicals Costa Mesa, CA, USA.

**Statistical analysis**

The data were analysed by two-tailed Student's t-test. P values < 0.05 were considered statistically significant.

**Results and Discussion**

The effect of various Glu concentrations (16–0 mM) on brain Na⁺, K⁺-ATPase of Mg²⁺-ATPase activity is presented in Fig. 1, as changes in the enzyme activity% of control (Glu 6.7 mM or...
1.2 mg/ml). Na\(^+\), K\(^+-\)ATPase activity was not found affected when incubated with the above mentioned Glu concentrations. On the contrary, Yorek et al. (1991) and Kern et al. (1994) have reported reduced Na\(^+\), K\(^+-\)ATPase and (Ca\(^2+\), Mg\(^2+\))-ATPase activities in the brain in some hyperglycemic conditions such as classical galactosemia. The absence of Na\(^+\), K\(^+-\)ATPase inhibition under our experimental conditions may be due to the presence of 240 mM sucrose in the enzyme reaction mixture. However, a such sucrose concentration is needed for the osmolarity of the mixture.

Mg\(^2+\) -ATPase activity was slightly decreased (non statistically, p > 0.05) in hyperglycemia (Glu 8–16 mM). On the contrary, the enzyme activity was considerably reduced in concentrations of Glu below 4 mM. For example, it was lowered 40% (p<0.01), 50% (p<0.001) or 80% (p<0.001) in Glu concentrations 3, 2 or 1 mM, respectively. Mg\(^2+\) -ATPase was inhibited about 40% or 60% in hyperglycemic conditions (Fig. 1, H, H'), e.g. Glu 2.5 mM (0.5 mg/ml) or 1.7 mM (0.3 mg/ml), respectively.

Since brain Mg\(^{2+}\) -ATPase has been inhibited, in hypoglycemia, low intracellular Mg\(^{2+}\) would be produced. Consequently, brain Na\(^+\), K\(^+-\)ATPase activity may be decreased (Carrahan et al. 1982).

The reduced brain Mg\(^{2+}\) -ATPase activity in our experimental hypoglycemia produces low intracellular Mg\(^{2+}\), which could modulate the activity of Mg\(^{2+}\) -dependent enzymes as well as the rates of protein synthesis and growth of the cell (Sanui and Rubin, 1982). Moreover, the inhibited Mg\(^{2+}\) -ATPase (as an ectoenzyme) may also modulate the ATP availability and activity of other extracellular ATP-dependent enzymes (e.g. protein kinases). It is also proposed that the enzyme may modulate the neurotransmitter role of ATP (Cunningham et al., 1993).

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