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Methionine alters Na^+,K^+-ATPase activity, lipid peroxidation and nonenzymatic antioxidant defenses in rat hippocampus

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Abstract

In the present study we investigated the effect of methionine exposure of hippocampus homogenates on Na^+,K^+-ATPase activity from synaptic plasma membrane of rats. Results showed that methionine significantly decreased this enzyme activity. We also evaluated the effect of incubating glutathione (GSH) and trolox (α-tocopherol) alone or combined with methionine on Na^+,K^+-ATPase activity. The tested antioxidants per se did not alter the enzymatic activity, but prevented the inhibitory action of methionine on Na^+,K^+-ATPase activity, indicating that Met inhibitory effect was probably mediated by free radical formation. Besides, we tested the in vitro effect of methionine on some parameters of oxidative stress, namely chemiluminescence, thiobarbituric acid reactive substances (TBARS), total radical-trapping antioxidant potential (TRAP), as well as on the antioxidant enzyme activities catalase, glutathione peroxidase and superoxide dismutase in rat hippocampus. We observed that methionine significantly increased chemiluminescence and TBARS, decreased TRAP, but did not change the activity of the antioxidant enzymes. These findings suggest that reduction of Na^+,K^+-ATPase activity and induction of oxidative stress may be involved in the brain damage observed in human hypermethioninemia.

Keywords: Methionine; Na^+,K^+-ATPase; Oxidative stress; Antioxidants; Hippocampus; Rat

1. Introduction

Hypermethioninemia is the biochemical hallmark of methionine adenosyltransferase (MAT, EC 2.5.1.6) activity deficiency, as well as of homocystinuria. Affected patients with these disorders present neurological manifestations, including cognitive deficit, mental retardation, cerebral edema and demyelination, whose underlying mechanisms are not yet fully established (Chamberlin et al., 1996; Mudd et al., 2000; Mudd et al., 2001).

Na^+,K^+-ATPase (EC 3.6.1.37) is a crucial enzyme responsible for maintaining the ionic gradient necessary for neuronal excitability. It is present at high concentrations in brain cellular membranes, consuming about 40–50% of the ATP generated in this tissue (Erecinska and Silver, 1994). It has been demonstrated that this enzyme is susceptible to free radical attack (Lees, 1993). Besides, there are some reports showing that Na^+,K^+-ATPase activity is decreased in cerebral ischemia (Wyse et al., 2000), in epilepsy (Grisar et al., 1992) and in various chronic neurodegenerative disorders (Lees, 1993).

On the other hand, there is considerable evidence showing that oxidative stress is an important event occurring in various common acute and chronic neurodegenerative pathologies, such as seizures, cerebral ischemia, demyelination, dementia and Alzheimer's disease (Halliwell and Gutteridge, 1985; Reznick and Packer, 1993; Karelson et al., 2001; Méndez-Álvarez et al., 2001). This is understandable since the central nervous system is potentially sensitive to oxidative damage due to its great oxygen consumption, high lipid content and poor antioxidant defenses (Halliwell, 1996).

We have recently shown that methionine (Met) added to the enzymatic assay, at concentrations usually found in homocystinuria, inhibits Na^+,K^+-ATPase in purified

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synaptic plasma membrane preparations from hippocampus of rats, suggesting a direct effect of this amino acid on this enzyme activity (Streck et al., 2002).

In the present study we investigated the effect of exposing hippocampus homogenates to Met on Na^+,K^+-ATPase activity from rat synaptic plasma membranes. We also evaluated the role of the antioxidants glutathione (GSH) and trolox (α-tocopherol) on the inhibition of this enzyme activity caused by Met. We finally assessed whether Met could induce oxidative stress by measuring some parameters of oxidative stress, namely chemiluminescence, thiobarbituric acid reactive substances (TBARS), total radical-trapping antioxidant potential (TRAP), and the activities of the antioxidant enzymes catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD).

2. Experimental procedure

2.1. Subjects and reagents

Thirty-seven Wistar rats with 29-day-old were obtained from the Central Animal House of the Department of Biochemistry, Institute of Basic Science of Health, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil. Animals were maintained on a 12/12 h light/dark cycle in an air-conditioned constant temperature (22 ± 1 °C) colony room. Rats had free access to a 20% (w/w) protein commercial chow and water. Animal care followed the official governmental guidelines in compliance with the Federation of Brazilian Societies for Experimental Biology and was approved by the Ethics Committee of the Federal University of Rio Grande do Sul, Brazil. The chemicals were purchased from Sigma Chemical Co., St. Louis, MO, USA.

2.2. Tissue and homogenate preparation

Animals were killed by decapitation without anaesthesia, the brain was quickly removed and the hippocampus was dissected and homogenized in 10 volumes of 0.32 mM sucrose solution containing 5.0 mM HEPES and 1.0 mM EDTA, pH 7.4. The homogenates were separately incubated at 37 °C for 1 h with Met (0.02–5.0 mM), 1.0 mM GSH, 3.0 mM trolox, and with 5.0 mM Met combined with 1.0 mM GSH or 3.0 mM trolox. All substances were dissolved in Tris–HCl buffer, pH 7.4. After incubation, synaptic plasma membranes were prepared as described below and the activity of Na^+,K^+-ATPase was determined.

For chemiluminescence and TBARS assays, hippocampus was homogenized in 5 volumes (1:5 w/v) of 20 mM sodium phosphate, pH 7.4, containing 140 mM KCl. For TRAP measurement, the same structure was homogenized (1:5 w/v) in 0.1 M glycine buffer, pH 8.6. For CAT and GSH-Px assays, hippocampus was homogenized (1:10, w/v) in 10 mM potassium phosphate buffer, pH 7.6 and, for SOD activity, hippocampus was homogenized (1:10, w/v) in 50 mM Tris–HCl buffer with 1.0 mM EDTA, pH 8.2.

Met was dissolved in Tris–HCl buffer, pH 7.4 and added to the incubation medium in concentrations ranging from 0.02 to 5.0 mM.

2.3. Preparation of synaptic plasma membrane

Synaptic plasma membranes from hippocampus were prepared according to the method of Jones and Matus (1974) with some modifications (Wyse et al., 1995). These membranes were isolated using a discontinuous sucrose density gradient consisting of successive layers of 0.3, 0.8 and 1.0 M. After centrifugation at $69{,}000 \times g$ for 2 h, the fraction between 0.8 and 1.0 M sucrose interface was taken as the membrane enzyme preparation.

2.4. Na^+,K^+-ATPase activity assay

The reaction mixture for Na^+,K^+-ATPase activity assay contained 5.0 mM $MgCl_2$, 80.0 mM NaCl, 20.0 mM KCl and 40.0 mM Tris–HCl, pH 7.4, in final volume of 200 μL. The reaction was initiated by addition of ATP to a final concentration of 3.0 mM. Controls were carried out under the same conditions with the addition of 1.0 mM ouabain. Na^+,K^+-ATPase activity was calculated by the difference between the two assays, as described by Wyse et al. (2000). Released inorganic phosphate (Pi) was measured by the method of Chan et al. (1986). Specific activity of the enzyme was expressed as nmol Pi released per min per mg of protein.

2.5. Chemiluminescence

Chemiluminescence, representing the spontaneous light emission mainly from peroxidizing lipids, was assayed in a dark room by the method of González-Flecha et al. (1991). Incubation flasks contained 3.5 mL of medium consisting of 20 mM sodium phosphate, pH 7.4 and 140 mM KCl. The background chemiluminescence was measured for 5 min. An aliquot of 0.5 mL of hippocampus homogenate was added and chemiluminescence was measured for 10 min at room temperature. The background chemiluminescence was subtracted from the total value. Chemiluminescence was expressed as cpm/mg protein.

2.6. Thiobarbituric acid reactive substances (TBARS)

TBARS, a measure of lipid peroxidation, was determined according to Esterbauer and Cheeseman (1990). Briefly, homogenates were mixed with trichloroacetic acid 10% and thiobarbituric acid 0.67% and heated in a boiling water bath for 25 min. TBARS was determined by the absorbance at 535 nm. Results were reported as nmol of malonaldehyde per mg protein.