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Proline promotes decrease in glutamate uptake in slices of cerebral cortex and hippocampus of rats

Daniela Delwing, Débora Delwing, Renan J. Sanna, Susana Wofchuk, Angela T.S. Wyse*

Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2600-Anexo, CEP 90035-003, Porto Alegre, RS, Brazil

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Abstract

In the present study we first investigated the in vitro and in vivo effects of proline on glutamate uptake in the cerebral cortex and hippocampus slices of rats. The action of α-tocopherol and/or ascorbic acid on the effects elicited by administration of proline was also evaluated. For in vitro studies, proline (30.0 μM and 1.0 mM) was added to the incubation medium. For acute administration, 29-day-old rats received one subcutaneous injection of proline (18.2 μmol/g body weight) or saline (control) and were sacrificed 1 h later. Results showed that addition of proline in the assay (in vitro studies) reduces glutamate uptake in both cerebral structures. Administration of proline (in vivo studies) reduces glutamate uptake in the cerebral cortex, but not in the hippocampal slices of rats. In another set of experiments, 22-day-old rats were pretreated for one week with daily administration of α-tocopherol (40 mg/kg) or ascorbic acid (100 mg/kg) or with both vitamins. Twelve hours after the last vitamins injection, rats received a single injection of proline or saline and were killed 1 h later. Pretreatment with α-tocopherol and/or ascorbic acid did not prevent the effect of proline administration on glutamate uptake. α-Tocopherol plus ascorbic acid prevented the inhibitory effect of acute hyperprolinemia on Na^+,K^+-ATPase activity in the cerebral cortex of 29-day-old rats. The data indicate that the effect of proline on reduction of glutamate uptake and Na^+,K^+-ATPase activity may be, at least in part, involved in the brain dysfunction observed in hyperprolinemic patients.

Keywords: Hyperprolinemia; Glutamate uptake; Na^+,K^+-ATPase; α-Tocopherol; Ascorbic acid

Introduction

Type II hyperprolinemia (HPII) is a rare inherited autosomal recessive disorder of amino acid metabolism characterized by the hepatic deficiency of Δ-1-pyrroline-5-carboxylic acid dehydrogenase activity, which leads to tissue accumulation of proline (Pro) (Phang et al., 2001). Although asymptomatic hyperprolinemic siblings have been identified in various pedigrees (Pavone et al., 1975), a considerable number of hyperprolinemic patients so far detected show neurological manifestations including seizures and mental retardation. In this context, a relationship between high concentration of Pro and neurological symptoms has been demonstrated in patients with HPII (Flynn et al., 1989). However, the exact mechanism(s) underlying these symptoms are far from being understood.

Excitotoxic properties have been also demonstrated for Pro, which at higher concentrations activates NMDA and AMPA receptors, being therefore proposed that Pro might potentiates glutamate transmission (Nadler, 1987; Nadler et al., 1992; Fremeau et al., 1992; Cohen and Nadler, 1997). The presence of high levels of Pro and glutamate in the cerebrospinal fluid (CSF) of patients with HPII is in agreement with this hypothesis (Van Harreveld and Fifková, 1973; Rhoads et al., 1983; Phang et al., 2001). In addition, studies carried out in our laboratory have shown that Pro decreases Na^+,K^+-ATPase in synaptic plasma membrane and induces oxidative stress in brain of rats (Pontes et al., 2001; Delwing et al., 2003b). Therefore, it seems that high Pro levels may be neurotoxic or at least may predispose to brain damage.

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (CNS), participating in plastic processes, which underlie memory and learning (Izquierdo and Medina, 1997), development and aging (Segovia

* Corresponding author. Tel.: +55 51 3316 5573; fax: +55 51 3316 5535.
E-mail address: wyse@ufrgs.br (A.T.S. Wyse).

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et al., 2001) and environmental adaptation (Ozawa et al., 1998). However, glutamate may also be a potent neurotoxin and the equilibrium between the physiological/pathological glutamatergic tonus is essential for brain function. When glutamate is present in high concentrations in the synaptic cleft, may lead to excitotoxicity, a process corresponding to glutamate receptor over-stimulation that subsequently leads to neuronal damage (Furuta et al., 1997; Danbolt, 2001; Mattson et al., 2002).

Therefore, considering it has been proposed that Pro might potentiates glutamate transmission, the aim of the present study was to investigate the in vitro and in vivo (acute) effects of Pro on glutamate uptake by cerebral cortex and hippocampal slices of rats subject to HPII. We also verified the effect of acute administration of Pro on Na^+,K^+-ATPase activity in cerebral cortex of rats. The influence of antioxidants α-tocopherol and/or ascorbic acid on the effects elicited by acute administration of Pro on glutamate uptake and Na^+,K^+-ATPase activity was also investigated.

Materials and methods

Animals and reagents

Wistar rats obtained from the Central Animal House of the Department of Biochemistry of the Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil, were housed in groups of eight with their mothers on the day of birth. They were maintained on a 12 h light/12 h dark cycle (lights on between 7:00 a.m. and 7:00 p.m.) in an air-conditioned constant temperature (22 ± 1 °C) colony room. The 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.

Proline was purchased from Sigma Chemical Co. (St. Louis, MO, USA). L-[^{3}H]glutamate (specific activity 30 Ci mmol^{-1}) was purchased from Amersham International, UK. All other reagents were of analytical grade.

Proline and vitamins administration

We performed three sets of experiments. First, for in vitro studies, slices from cerebral cortex and hippocampus of 29-day-old untreated rats were used. Pro was added to the assay at different concentrations (30.0 μM and 1.0 mM). The control samples were performed without Pro addition. The in vitro concentrations 30 μM and 1 mM were chosen according to CSF and plasma Pro levels, respectively, found in hyperprolinemic patients (Phang et al., 2001).

For the second set of experiments (in vivo acute treatment), 29-day-old Wistar rats received a single subcutaneous injection of Pro correspondent to 18.2 μmol/g of body weight and control rats received an equivalent volume of saline. The animals were killed 1 h after injection by decapitation without anaesthesia. Pro was dissolved in 0.9% NaCl and the pH adjusted to 7.2–7.4 with 0.1 N NaOH. Plasma Pro levels achieved by rats subjected to this treatment were between 1.2 and 1.5 mM, similar to those found in plasma of hyperprolinemic type II patients (Moreira et al., 1989).

The third set of experiments was performed in two phases: a) 22-day-old rats were pretreated for 1 week with daily intraperitoneal administration of saline (control) or α-tocopherol (40 mg/kg) plus ascorbic acid (100 mg/kg); b) 22-day-old rats were pretreated for 1 week with daily intraperitoneal administration of saline (control) or α-tocopherol (40 mg/kg) or ascorbic acid (100 mg/kg). α-Tocopherol and ascorbic acid doses were chosen according to the protocols described by Wyse et al. (2002). Twelve hours after the last injection, animals received one single injection of Pro (18.2 μmol/g body weight) or saline and were killed 1 h after the injection by decapitation without anaesthesia.

Tissue preparation

For glutamate uptake, the animals were decapitated, their brains immediately removed and humidified with Hank's balanced salt solution (HBSS) containing (in mM): 137 NaCl; 0.63 Na_2HPO_4; 4.17 $NaHCO_3$; 5.36 KCl; 0.44 KH_2PO_4; 1.26 $CaCl_2$; 0.41 $MgSO_4$; 0.49 $MgCl_2$ and 1.11 glucose, in pH 7.2. Cerebral cortex and hippocampus were dissected onto Petri dishes with HBSS and slices (0.4 mm) were obtained using a McIlwain tissue chopper. Slices of cerebral cortex and hippocampus were separated with the help of a magnifying glass and transferred to 24-well culture plates: one plate was maintained at 35 °C and the other on ice. The slices from the first plate were washed once with 1 mL of 35 °C HBSS and the second with 1 mL of 4 °C sodium-free HBSS for the analysis of non-specific uptake (see below).

For Na^+,K^+-ATPase activity determination, the animals were sacrificed by decapitation 1 h after Pro injection, the brain was rapidly removed, the cerebral cortex was isolated on ice and homogenized in 10 volumes of 0.32 M sucrose solution containing 5.0 mM HEPES and 0.1 mM EDTA. Membranes were prepared according to the method of Jones and Matus (1974) with some modifications (Wyse et al., 1995) using a discontinuous sucrose density gradient consisting of successive layers of 0.3, 0.8 and 1.0 mM. After centrifugation at 69,000 ×g for 2 h, the fraction at 0.8–1.0 mM sucrose interface was taken as the membrane enzyme preparation.

Glutamate uptake

Total uptake

Glutamate uptake was performed according to Frizzo et al. (2002) and Thomazi et al. (2004). Slices were pre-incubated at 35 °C for 30 min, followed by the addition of 0.33 and 0.66 μCi mL^{-1} L-[^{3}H]glutamate for cerebral cortex and hippocampus, respectively, and 100 μM (final concentration) of glutamate. Incubation was stopped after 7 or 5 min for cerebral cortex and hippocampus, respectively, with two ice-cold washes of 1 mL HBSS, immediately followed by the addition of 0.5 N NaOH, which were then kept overnight.