Amino Acids (2005) 28: 305–308
DOI 10.1007/s00726-005-0186-9

Amino Acids
Printed in Austria

Effect of hyperprolinemia on acetylcholinesterase and butyrylcholinesterase activities in rat

D. Delwing, F. Chiarani, C. M. D. Wannmacher, M. Wajner, and **A. T. S. Wyse**

Departamento de Bioquímica, ICBS, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

Received June 29, 2004
Accepted January 26, 2005
Published online March 25, 2005; © Springer-Verlag 2005

Summary. We observed here that acute proline (Pro) administration provoked a decrease (32%) of acetylcholinesterase (AChE) activity in cerebral cortex and an increase (22%) of butyrylcholinesterase (BuChE) activity in the serum of 29-day-old rats. In contrast, chronic administration of Pro did not alter AChE or BuChE activities. Furthermore, pretreatment of rats with vitamins E and C combined or alone, N^{ω}-nitro-L-arginine methyl ester or melatonin prevented the reduction of AChE activity caused by acute Pro administration, suggesting the participation of oxidative stress in such effects.

Keywords: Hyperprolinemia type II – Proline – Acetylcholinesterase – Butyrylcholinesterase – Experimental model

Introduction

Hyperprolinemia type II is an inherited disorder caused by deficiency of Δ^1-pyrroline-5-carboxylic acid dehydrogenase activity, leading to tissue accumulation of proline (Pro). Most patients have seizures and a variable degree of mental retardation, although asymptomatic hyperprolinemic siblings have been identified (Phang et al., 2001).

Although neurological dysfunction is found in a considerable number of hyperprolinemic patients, the underlying mechanisms by which this occurs are poorly understood. In this respect, high cerebral Pro concentrations have been shown to provoke cognitive deficits in rats (Cherkin et al., 1976; Moreira et al., 1989).

Acetylcholinesterase (AChE) plays a key role in cholinergic transmission in the central nervous system (CNS) of mammals (Inestrosa and Perelman, 1990), terminating the synaptic action of acetylcholine (ACh) (Silver, 1974). AChE was shown to be critical for attention and memory and reduced cortical AChE is reported to be associated with dementia (Blokland, 1995; Petersen et al., 1999; O'Brien et al., 2003). AChE activity has been observed to be reduced in cerebrospinal fluid (Appleyard et al., 1983), erytrocytes (Chipperfield et al., 1981; Smith et al., 1982) and plasma (Yamamoto et al., 1990) of patients with Alzheimer's disease.

Butyrylcholinesterase (BuChE), also known as "pseudo" cholinesterase, is present in haematopoietic cells, serum, proliferating embryonic tissues, peripheral cholinergic synapses and CNS (Dubovy and Haninec, 1990; Mack and Robitzki, 2000). Recent studies have shown that BuChE is involved in the regulation of neuronal proliferation and differentiation (Mack and Robitzki, 2000). Furthermore, glial BuChE can hydrolyze released ACh producing choline that can be returned into cholinergic neurons (Mesulam et al., 2002).

Furthermore, there is some evidence showing that both AChE and BuChE, as well as other cholinesterases that can hydrolyze ACh (Mesulam et al., 2002), participating in the pathological processes of Alzheimer's disease, including β-amyloid formation or deposition (Benzi and Moretti, 1998).

This study extends a previous report of our laboratory showing that Pro (acute and *in vitro* effects) significantly decreases AChE activity in brain of very young (12-day-old) rats (Delwing et al., 2003). We investigated the *in vivo* acute and chronic effect of Pro on AChE and BuChE activities in homogenates from cerebral cortex of 29-day-old rats. We also evaluated the influence of chronic pretreatment of rats with vitamins E and C or acute administration of vitamin E, vitamin C, N^{ω}-nitro-L-arginine methyl ester (L-NAME) or melatonin on the effects elicited by Pro on AChE activity.

Materials and methods

Wistar rats were used in the experiments. All chemicals were purchased from the Sigma Chemical Co., St Louis, MO, USA.

Pro was dissolved in 0.9% NaCl and the pH adjusted to 7.2–7.4 with 0.1 N Na(OH). For acute treatment, 29-day-old-rats received one single injection of Pro (18.2 μmol/g of body weight) and control rats received an equivalent volume of saline. The animals were killed 1h after injection by decapitation and without anaesthesia.

For chronic treatment, Pro solution was administered subcutaneously twice a day at 10 h intervals from the 6th to the 28th days of age at increasing doses (12.8 to 18.2 μmol/g), as previously reported (Moreira et al., 1989). Rats subjected to this treatment achieved plasma Pro levels between 1.0 and 2.0 mM, similar to those found in plasma of hyperprolinemic type ll patients (Moreira et al., 1989; Phang et al., 2001). Control animals received saline injections in the same volumes as those applied to Pro-treated rats. The animals were killed 12 h after the last injection by decapitation without anesthesia.

Twenty-two-day-old rats were also pretreated for 1 week with daily i.p. administration of either saline (control), vitamins E (40 mg/kg) and C (100 mg/kg), or vitamin E alone (Wyse et al., 2002). Twelve hours after the last injection, animals received one injection of Pro (18.2 μmol/g body weight) or saline and were killed 1 h later by decapitation without anesthesia.

For acute antioxidant treatment, 29-day-old-rats received a single i.p. injection of vitamin E (40 mg/kg), vitamin C (100 mg/kg), L-NAME (2 mg/kg), melatonin (10 mg/kg) or saline (control) and 1 h later the animals received a s.c. injection of Pro (18.2 μmol of Pro/g body weight) or saline.

After decapitation, the brain and the blood were rapidly removed. The cerebral cortex was dissected, homogenized (1:10 w/v in 0.5 M potassium phosphate buffer, pH 7.5) and centrifuged at 1000 × g for 10 min. The supernatant was used for the enzymatic (AChE) analyses. Protein concentration was 0.5–0.7 mg/ml. After collection, blood was rapidly centrifuged at 1000 × g for 10 min and the serum was separated and used for the enzymatic (BuChE) analyses.

AChE and BuChE were measured by the colorimetric method of Ellman et al. (1961) with some modifications. Protein was measured by the method of Lowry et al. (1951).

Animal care followed the official guidelines in compliance with the Federation of Experimental Biology Societies Policy and was approved by the Ethics Committee of the Universidade Federal do Rio Grande do Sul, Brazil.

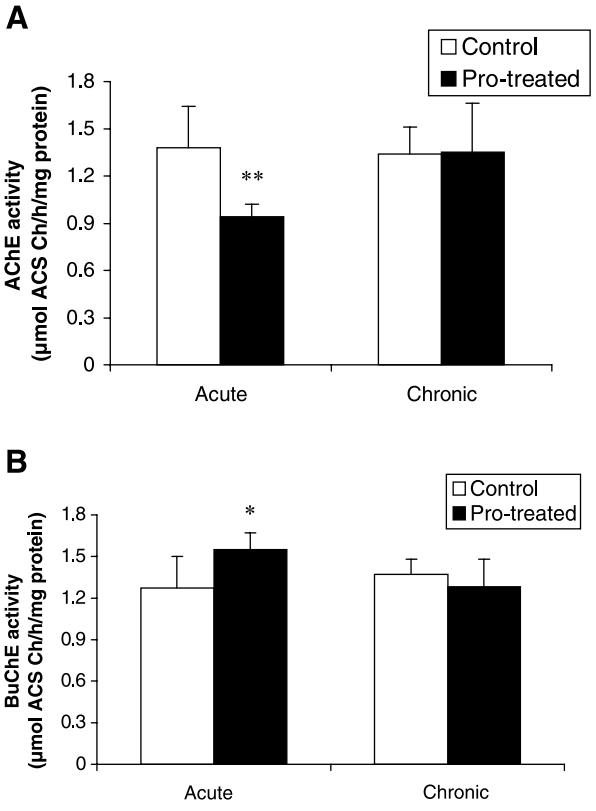

Fig. 1. Effect of acute and chronic administration of proline on cerebral cortex homogenates acetylcholinesterase (*AChE*) (**A**) and serum butyrylcholinesterase (*BuChE*) (**B**) activities of rats. Data are mean ± S.D., for 5–8 independent experiments performed (animals) in duplicate. Different from control, $^{*}p<0.05$, $^{**}p<0.01$ (Student's t-test)

Results and discussion

Figure 1A shows that rats subjected to acute administration of Pro significantly reduced (32%) AChE activity [$t(10)=5.30$; $p<0.01$], whereas chronic administration of Pro did not alter this parameter [$t(14)=0.72$; $p>0.05$]. Figure 1B shows that BuChE was significantly increased (22%) in acutely [$t(10)=2.51$; $p<0.05$], but not in chronically [$t(10)=0.96$; $p>0.05$] Pro-injected animals.

Considering that a similar decrease of AChE activity was demonstrated to provoke brain damage, as observed in cerebral ischemia (Perry et al., 1978; Wang et al., 2000; Saéz-Valero et al., 2003), it is feasible that our results may be of pathophysiological significance. These results are in agreement with other studies showing that in advanced Alzheimer's disease, AChE activity is reduced, in contrast to BuChE, whose activity is increased (Perry et al., 1978; Giacobini, 2003). It may be therefore presumed that the increase of BuChE activity after Pro administration may have occurred to compensate the reduction caused by Pro on AChE activity, since both enzymes are able to hydrolyze ACh, being critical to cholinergic transmission. On the other hand, considering that chronic administration of Pro caused no effect on these parameters, it is likely that the presence of Pro is necessary for its action since in the acute treatment the animals were sacrificed 1 h after injection when Pro levels were high, whereas in the chronic treatment they were killed 12 h after the last Pro injection when the levels of this amino acid returned to normal values. On the other hand, the results showing that chronic Pro administration did not alter AChE and BuChE activities indicate that long term Pro treatment does not alter the transcription (the number of enzymatic molecules) of the involved genes.

We also examined the effect of pretreatment for 7 days with antioxidants on the inhibitory effect of acute Pro administration on AChE activity. As can be observed in

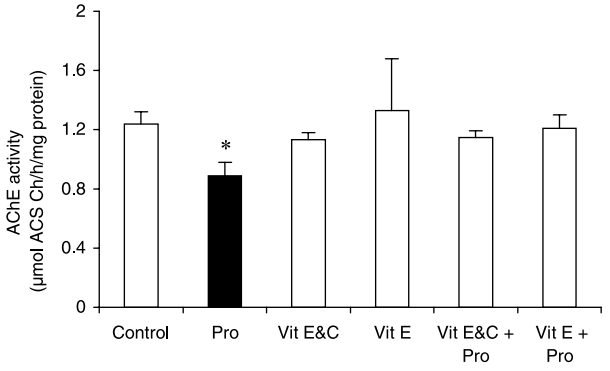

Fig. 2. Effect of acute administration of proline, vitamins E and C, vitamin E, proline plus vitamins E and C, and proline plus vitamin E on acetylcholinesterase activity in homogenates of rat cerebral cortex. Data are mean ± S.D., for five independent experiments (animals) performed in duplicate. Different from other groups, $^{*}p < 0.05$ (ANOVA). *Pro*, proline; *Vit*, vitamins

Fig. 2, vitamins E and C combined or vitamin E alone *per se* did not alter the enzyme activity, but prevented the reduction of AChE activity [$F(5,18) = 3.59$; $p < 0.05$] caused by Pro-treated rats. We also verified that acute pretreatment with vitamin E, vitamin C, L-NAME or melatonin did not alter AChE activity, but prevented the inhibition of this activity caused by Pro [$F(9, 30) = 3.82$; $p < 0.01$] (control: 1.54 ± 0.14 μmol ACS Ch/h/mg protein; Pro: $1.11 \pm 0.07^{**}$; vitamin E: 1.40 ± 0.08; vitamin C: 1.47 ± 0.09; L-NAME: 1.52 ± 0.09; melatonin 1.43 ± 0.11; vitamin E + Pro: 1.39 ± 0.09; vitamin C + Pro: 1.36 ± 0.09; L-NAME + Pro: 1.43 ± 0.04; melatonin + Pro: 1.40 ± 0.24).

These data are in agreement with previous findings reporting that this activity is decreased by free radicals and prevented by antioxidants (Tsakiris et al., 2000; Melo et al., 2003). Therefore, it is feasible that oxidative stress is elicited by reactive oxygen and nitrogen species and involved in the inhibitory effect of Pro on AChE.

Regarding the effect of ascorbic acid preventing the alteration of AChE induced by acute Pro administration, we cannot rule out that at least part of the effect occurred because Pro was unavailable to AChE, since ascorbate is a cofactor of Pro incorporation into polypeptide precursors of collagen. It is well known that, in the presence of ascorbate, Pro incorporation into collagen is increased several fold (Tajima and Pinnell, 1982). Furthermore, in the procollagen polypeptide (precursor to the mature collagen), proline is converted to hydroxyproline by the enzyme proline hydroxylase, which also requires ascorbic acid as a cofactor (Nietfeld and Kemp, 1981).

If these findings also occur in human hyperprolinemia, and considering that the cholinergic system is essential for normal brain functioning, it is feasible that reduction of AChE activity may contribute to the neurological symptoms characteristic of hyperprolinemia type II.

Acknowledgements

This work was supported in part by grants from CNPq and FAPERGS-Brazil).

References

Appleyard ME, Smith AD, Wilcock GK, Esiri MM (1983) Decreased CSF acetylcholinesterase activity in Alzheimer's disease. Lancet 2: 452

Benzi G, Moretti A (1998) Is there a rationale for the use of acetylcholinesterase inhibitors in the therapy of Alzheimer's disease? Eur J Pharmacol 346: 1–13

Blokland A (1995) Acetylcholine: a neurotransmitter for learning and memory? Brain Res 21: 285–300

Cherkin A, Eckardt MJ, Gerbrandt LK (1976) Memory: proline induces retrograde amnesia in chicks. Science 193: 242–244

Chipperfield B, Newman PM, Moyes ICA (1981) Decreased erythrocyte cholinesterase activity in dementia. Lancet 2: 199

Delwing D, Chiarani F, Delwing D, Bavaresco CS, Wannmacher CMD, Wajner M, Wyse ATS (2003) Proline reduces acetylcholinesterase activity in cerebral cortex of rats. Metab Brain Dis 18: 79–86

Dubovy P, Haninec P (1990) Non-specific cholinesterase activity of the developing peripheral nerves and its possible function in cells in intimate contact with growing axons of chick embryo. Ont J Dev Neurosci 8: 589–602

Ellman GL, Courtney KD, Andres V, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7: 88–95

Giacobini E (2003) Cholinesterases: new roles in brain function and in Alzheimer's Disease. Neurochem Res 28: 515–522

Inestrosa NC, Perelman A (1990) Association of acetylcholinesterase with the cell surface. J Membr Biol 118: 1–9

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–267

Mack A, Robitzki A (2000) The key role of butyrylcholinesterase during neurogenesis and neural disorders: an antisense-5′-butyrylcholinesterase – DNA study. Prog Neurobiol 60: 607–628

Melo JB, Agostinho P, Oliveira CR (2003) Involvement of oxidative stress in the enhancement of acetylcholinesterase activity induced by amyloid beta-peptide. Neurosci Res 45: 117–127

Mesulam M-M, Guillozet A, Shaw P, Levey A, Duysen EG, Lockridge O (2002) Acetylcholinesterase knockouts establish central cholinergic pathways and can use butyrylcholinesterase to hydrolyze acetylcholine. Neuroscience 170: 627–639

Moreira JCF, Wannmacher CMD, Costa SM, Wajner M (1989) Effect of proline administration on rat behavior in aversive and nonaversive tasks. Pharmacol Biochem Behav 32: 885–890

Nietfeld JJ, Kemp A (1981) The function of ascorbic with respect to propyl 4-hydroxylase activity. Biochim Biophys Acta 657: 159–167

O'Brien KK, Saxby BK, Ballard CG, Grace J, Harrington F, Ford GA, O'Brien JT, Swan AG, Fairbairn AF, Wesnes K, del Ser T, Edwardson JA, Morris CM, McKeith IG (2003) Regulation of attention and response to therapy in dementia by butyrylcholinesterase. Pharmacogenetics 13: 231–239

Perry EK, Tomlinson BE, Blessed G, Bergmann K, Gibson PH, Perry RH (1978) Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. Br Med J 2: 1457–1459

Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E (1999) Mild cognitive impairment: clinical characterization and outcome. Arch Neurol 56: 303–308

Phang JM, Hu CA, Valle D (2001) Disorders of proline and hydroxyproline metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease, 8th edn. McGraw-Hill, New York, pp 1821–1838

Saéz-Valero J, González-Garcia C, Ceña V (2003) Acetylcholinesterase activity and molecular isoform distribution are altered after focal cerebral ischemia. Mol Brain Res 117: 240–244

Silver A (1974) The biology of cholinesterases. American Elsevier Publishing, New York

Smith RC, Ho BT, Hsu L, Vroulis G, Claghorn J, Schoolar J (1982) Cholinesterase enzymes in the blood of patients with Alzheimer's disease. Life Sci 30: 543–546

Tajima S, Pinnell SR (1982) Regulation of collagen synthesis by ascorbic acid. Ascorbic acid increases type I procollagen mRNA. Biochem Biophys Res Commun 106: 632–637

Tsakiris S, Angelogianni P, Schulpis KH, Stavridis JC (2000) Protective effect of L-phenylalanine on rat brain acetylcholinesterase inhibiton induced by free radicals. Clin Biochem 33: 103–106

Wang LM, Han YF, Tang XC (2000) Huperzine A improves cognitive deficits caused by chronic cerebral hypoperfusion in rats. Eur J Pharmacol 398: 65–72

Wyse ATS, Zugno AI, Streck EL, Matte C, Calcagnotto T, Wannmacher CM, Wajner M (2002) Inhibition of Na^+, K^+-ATPase activity in hippocampus of rats subjected to acute administration of homocysteine is prevented by vitamins E and C treatment. Neurochem Res 27: 1685–1689

Yamamoto Y, Nakano S, Kawashina S, Nakamura S, Urakami K, Kato T, Kameyama M (1990) Plasma and serum G_4 isoenzyme of acetylcholinesterase in patients with Alzheimer-type dementia and vascular dementia. Ann Clin Biochem 27: 321–326

Authors' address: Dr. 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,
E-mail: wyse@ufrgs.br