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Antioxidant status of the brain in a glutamate excitotoxicity model and the effect of N-acetyl-cysteine

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Abstract

The aims were to evaluate changes in antioxidant status in brain of rats subjected to a model of glutamate excitotoxicity and to identify modifications when an antioxidant therapy was given. Four groups were performed: Glutamate group (GG) was injected with 1g/kg of monosodium glutamate, Control Group (CG) was injected with saline solution, Treated Glutamate Group (TG) was supplemented with 150 mg/kg of N-acetyl cysteine and with 1 g/kg of monosodium glutamate and Treated Control Group (TC) was supplemented with 150 mg/kg of N-acetyl cysteine. Levels of antioxidants, the activities of antioxidant enzymes, and protein damage were measured. Glutamate increased superoxide dismutase (28% $p< 0.05$), catalase (95% $p< 0.05$), NADPH oxidase (29% $p< 0.001$), protein oxidation (22% $p< 0.05$), and decreased glutathione (30% $p< 0.05$) N-acetyl cysteine decreased superoxide dismutase (19% $p< 0.05$), NADPH oxidase (45% $p< 0.001$), glutathione reductase (25% $p< 0.05$) activities and increased glutathione (30% $p< 0.05$). No changes were found in protein damage. Decrease in non-enzymatic antioxidants and compensatory up-regulation of antioxidant enzymes activities may be consequence of an increase in oxidative process in glutamate excitotoxicity. N-acetyl cysteine could be useful as a donor of sulfhydryl groups, increasing glutathione, and producing a decay in enzymes related with reactive oxygen species production.

Introduction

The central nervous system is particularly vulnerable to oxidative stress because of the high lipid content and the high aerobic metabolism, and treatment strategies aimed at preventing neuronal death is called neuroprotection (Caprioli, 1997; Osborne, 1999). Glutamate is an important excitatory neurotransmitter in central nervous system and glutamate excitotoxicity is thought to play an important role in neuronal damage. Cells exposed to oxidative stress modified its redox homeostasis. Oxidative stress can be defined as an increase over physiological values in the intracellular concentrations of reactive oxygen species and /or a decrease of antioxidants. This situation could be produced by an increase in the production of oxidant species or a decrease in the antioxidants levels, or a combination of both (Sies, 1985). The balance between the oxidized and reduced compounds is essential for cellular survival. Glutamate produces oxidative stress in glia cells and its toxicity could be decreased with the use of an antioxidant rich in thiols like glutathione

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(Han, 1997). N-acetyl cysteine (NAC) could be useful as a donor of sulfhydryl groups, increasing glutathione levels. The aims of this study were to evaluate changes in antioxidant status in brain of rats subjected to a glutamate excitotoxicity model and to identify modifications when an antioxidant therapy with N-acetyl-cysteine was given.

In order to assess the occurrence of oxidative stress and the protection of an antioxidant therapy the following markers were evaluated in brain homogenates: activities of antioxidant enzymes, levels of non-enzymatic antioxidants and markers of oxidative damage.

Materials & methods

Experimental model: The experimental model consisted of female wistar rats of 120 g weight (4 weeks), divided into four groups: Glutamate Group which was injected (i.p) with 1g/kg weight of monosodium glutamate, Control Group was injected (i.p) with saline solution, Treated Glutamate Group was supplemented with 150 mg/kg weight of NAC and with 1 g/kg weight of monosodium glutamate and Treated Control Group was supplemented with 150 mg/kg weight of NAC. The supplementation was given every day while the glutamate was injected at days 1, 5 and 9. At day 13 rats were sacrificed with ketamine/xylazine and brains were separated. The Committee for Care and Use of Laboratory Animals (CICUAL) of the School of Pharmacy and Biochemistry University of Buenos Aires approved every experimental procedure. The following markers were evaluated in brain homogenates:

Catalase (CAT): was determined by measuring the decrease in absorption of hydrogen peroxide at 240 nm. The results were expressed as CAT content in pmol/mg of protein. (Ferreira *et al.*, 2013).

Superoxide dismutase activity (SOD): was determined by following the inhibition of the rate of autocatalytic adenochrome formation at 480 nm. The enzyme activity was expressed as U SOD/mg of protein. One unit was defined as the amount of enzyme that inhibited the rate of adrenochrome formation by 50% (Ferreira *et al*., 2013).

Thioredoxin reductase activity (TRxR): was evaluated spectrophotometrically with di tio nitro benzene (DTNB) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) a 412 nm. Results were expressed as nmol/ min.mg protein (Holmgren & Bjornstedt, 1995).

Glutathione peroxidase activity (GPx): was determined by following NADPH oxidation at 340 nm. Results were expressed as nmol/ min mg of protein (Ferreira *et al.*, 2013).

Glutathione Reductase activity (GR): was determined by following NADPH oxidation at 340 nm. Results were expressed as nmol/ min mg of protein (Ferreira *et al*., 2013).

Glutathione S-transferase (GST): the reaction medium consisted of 1-Chloro-2,4-dinitrobenzene (CDNB) and glutathione. In presence of the enzyme it formed an adduct which could be followed spectrophotometrically at 340 nm. Results were expressed as U/ mg of protein (Habig, 1974).

Glucose-6-phosfate dehydrogenase activity (G6PDH): was determined by following NADPH reduction at 340 nm in presence of 20 mM glucose-6-phosphate and 10 mM $NADP^+$. Results were expressed as U/ min mg of protein (Leong, 1984).

NADPH oxidase (NOX): was measured by chemiluminescence in presence of buffer phosphate, EGTA, sucrose, lucigenin and NADPH. Results were expressed as U/ mg protein (Wei, 2006).

Glutathione levels (GSH): the reaction medium consisted of phosphate buffer pH 7.00, 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), NADPH and glutathione reductase in a kinetic assay at 412 nm. Results were expressed in μmol/ g organ (Ferreira *et al.*, 2013).

Ascorbic acid levels (AA): samples were treated with phosphotungstic acid and incubated at room temperature. The absorbance of the supernatant was measured at 700 nm, and a standard curve was established with a set of serial dilutions of AA. Results were expressed as μM (Ferreira *et al.*, 2013).

Thiobarbituric acid reactive substances (TBARS): were determined using a spectrophotometric method based on the 2-thiobarbituric acid reaction. TBARS was detected at 535 nm. Results were expressed in pmol/mg protein (Ferreira *et al.*, 2013).

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Protein carbonylation (PC): was detected with 2,4-di nitro-phenylhydrazine (DNPH), which leads to the formation of a stable product that is soluble in 6 M guanidine and quantified by a spectrophotometric assay at 370 nm. Results were expressed as nmol/mg of protein (Ferreira *et al.*, 2013).

Data and statistical analysis: Statistical calculations were performed with the GraphPad InStat statistical package for Windows. Data are expressed as mean value ± SEM (standard error of the mean). Statistical significance of the differences between the groups was calculated by ANOVA and a probability value of p smaller than 0.05 indicated a statistically significant difference.

Results

SOD activity was 5.90 ± 0.10 U/mg protein in Glutamate Group (Control Group 4.63 ± 0.17 U/mg protein *p<0.05*), CAT levels were 0.35 ± 0.06 pmol/mg protein in Glutamate Group (Control Group 0.18 ± 0.02 pmol/mg prot *p<0.05*), TRxR was 9.4 ± 1.5 nmol/min. mg protein in Glutamate Group (Control Group 7.1 ± 0.4 nmol/ min.mg protein *p<0.05*).

Table 1: Activities of different enzymes

	CONTROL	GLUTAMATE	EFFECT
CAT (pmol/mg protein)	0.18 ± 0.02	0.35 ± 0.06*	94% ↑
TRxR (nmol/min mg protein)	7.1 ± 0.4	9.4 ± 1.5*	32% ↑
GST (U/mg protein)	0.028 ± 0.001	0.038 ± 0.004*	36% ↑
G6PDH (U/min mg protein)	0.014 ± 0.001	0.017 ± 0.001*	21% ↑

p<0.05

GT in Glutamate Group was 0.038 ± 0.004 U/mg protein (Control Group 0.028 ± 0.001 U/mg protein *p<0.05*). GR in Glutamate Group was 22.6 ± 1.0 nmol/ min.mg protein (Control Group 19.6 ± 1.2 nmol/min.mg protein *p<0.05*). G6PDH was 0.017 ± 0.001 U/min.mg protein for Glutamate Group (Control Group 0.014 ± 0.001 U/min. mg protein *p<0.05*) (Table 1).

PC was 6.30 ± 0.02 nmol/ mg protein for Glutamate Group (Control Group 5.07 ± 0.05 nmol/ mg protein *p<0.05*). AA was 64 ± 18 mM for Glutamate Group (Control Group 160 ± 3 mM *p<0.01*). GSH concentration was 0.32 ± 0.04 mmol/g organ for Glutamate Group (Control Group 0.43 ± 0.04 mmol/g organ *p<0.05*). NOX activity was 0.067 ± 0.001 U/mg protein for Glutamate Group (Control Group 0.051 ± 0.0020 U/mg protein *p<0.01*) No significant differences were found in TBARS levels and GPx activity. (Table 2)

Table 2 : Markers of oxidative damage and levels of non-enzymatic antioxidants

	CONTROL	GLUTAMATE	EFFECT
TBARS (pmol/mg protein)	837 ± 68	735 ± 90	=
PC (nmol/mg protein)	5.07 ± 0.05	6.30 ± 0.02*	24% ↑
GSH (µmol/g)	0.43 ± 0,04	0.33 ± 0,04*	26% ↓
AA (µmol)	160 ± 3	64 ± 18**	60% ↓

p<0.05 and **p<0.001

The supplementation with NAC decreased SOD activity 19% (*p<0.05*), GR 25% *(p<0.05*), NADPH oxidase 48% *(p<0.01*), and increased glutathione 28% *(p<0.05*) compared to glutamate group. NAC also increased glutathione 30% (*p<0.05*) respect control group. No changes were found in protein carbonylation, which indicates that this type of protein damage was not observed in this model (Table 3).

Table 3: Effect of the suplementation with N-acteyl –cysteine in glutamate excitotoxicity model

	CONTROL	TREATED CONTROL	GLUTAMATE	TREATED GLUTAMATE
SOD (U/ mg protein)	4.63±0.17	4.59 ± 0.20	5.90 ± 0.10*	4.78 ± 0.10†
GR (nmol/ min mg protein)	19.6 ± 1.2	21.0 ± 1.0	22.6 ± 1.0**	16.95 ± 1.0†
NOX (mUA/ mg protein)	50 ±2	47 ± 3	67 ± 1**	35 ±2††
GSH (µmol/ g)	0.43±0.04	0.56±0.04*	0.33±0.04*	0.42± .02†

p<0.05 vs CG, **p<0.01 vs CG, †p<0.05 vs GG, ††p<0.01 vs GG

Conclusion

In this study we evaluated the changes in antioxidant status in brain of rats subjected to a glutamate excitotoxicity model and the modifications when N-acetyl-cysteine was given since it was a donor of sulfhydryl groups.

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The increase of reactive oxygen species in this model could be evidenced by the enhanced in NADPH oxidase activity, which led to an elevated concentration of superoxide, and an increase of protein oxidation as a consequence of the generated damage by free radicals. The decrease in non-enzymatic antioxidants, such as ascorbic acid and glutathione, as well as the compensatory up-regulation of antioxidant enzymes activities could be consequence of an increase in oxidative process. Ascorbic acid is important to prevent lipid peroxidation because it participates in the regeneration of vitamin E which protects the cells membranes against oxidative damage by scavenging peroxyl radicals. A reduction in the levels of this lipid-soluble antioxidant in the brain could be related to its reaction with the free radicals generated. Moreover, the activities of enzymes associated with glutathione metabolism, like glutathione reductase and glutathione s-transferase, were increased suggesting a response to GSH decay which is a water-soluble antioxidant and its decrease in the brain may be due to an increase in oxidative processes. The activity of glucose-6-P dehydrogenase was increased suggesting the increment of NADPH which is an important cofactor for antioxidants enzymes. In such conditions of deficient antioxidant non-enzymatic defenses, an increase in the activities of the antioxidant enzymes could be expected as a tissue adaptative response.

SOD activity was increased suggesting an adaptative response to oxidative damage. SOD catalyzes the dismutation of superoxide anion to hydrogen peroxide, the increase in SOD activity in glutamate excitotoxicity model would lead to an increase in the concentration of hydrogen peroxide which would exacerbate this situation. This hydroperoxide is metabolized by two enzymes: glutathione peroxidase and catalase; the first enzyme needs glutathione as a cofactor for hydrogen peroxide removal. As glutathione levels decreased, catalase is the main enzyme to detoxificate hydrogen peroxide to avoid the formation of hydroxyl radical. The increase of TRxR and GR activities would lead to enhace the glutathione and thioredoxin by recycling their oxidized forms. The thioredoxin and glutathione systems are essential to maintain redox homeostasis in the cell so alterations in both systems could lead to an oxidative imbalance. Glutathione transferase is involved in the detoxification of compounds, using GSH as a substrate and the detoxification is essential to cell survival. In this excitotoxicity model glutathione transferase activity was increased as a response to glutamate toxicity.

The supplementation with NAC in this model improved the redox status because it produced a decrease in the enzymes related to reactive oxygen species production, as NADPH oxidase and superoxide dismutase, and an increase of glutathione levels. Therefore, the use of antioxidants rich in sulphydryl groups as a preventive therapy could be useful in glutamate excitotoxicity model.

After this study we concluded that the brain is vulnerable to oxidative stress in glutamate excitotoxicity model and N-acetyl-cysteine could be useful to maintain the redox status.

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References

Caprioli J. neuroprotection of the optic nerve in glaucoma. Acta Opthalmol.1997; 75: 364-367

Ferreira S, Lerner F, Reides C, Brunzini R, Llesuy S.Acta Ophthalmol. Scand. 2013, 91: 64-70.

Habig, W; Pabst, M; Jakoby, W (1974) J. Biol. Chem. 249: 7130-7139

Han D, Sen C, Roy S, Kovayaski M, Tritschler H, Packer L. Am J Physiol. 1997; 1771-1778.

Holmgren A., Björnstedt M. (1995) Thioredoxin and thioredoxin reductase.Methods Enzymol. 252:199–208.

Leong y Clark (1984) J. Biol. Chem. 218(1);131-8.

Sies H. Oxidative stress. San Diego, Academic Press. 1985; 1-7.

Osborne M Surv Ophthal 1999; 43: 102-126.

Wei Y, Sowers JR, Nistala R, Gong H, Uptergrove GM, Clark SE, Morris EM, Szary N, Manrique C, Stump C. J Biol Chem. 2006; 281: 35137-35146.