Life Sciences 89 (2011) 276–281

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Glycine intrastriatal administration induces lipid and protein oxidative damage and alters the enzymatic antioxidant defenses in rat brain

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ARTICLE INFO

Article history:
Received 4 January 2011
Accepted 13 June 2011

Keywords:
Glycine
Nonketotic hyperglycinemia
Oxidative stress
Rat striatum

ABSTRACT

Aims: We investigated the effects of in vivo intrastriatal administration of glycine (Gly), which is found at high concentrations in the brain of patients affected by nonketotic hyperglycinemia (NKH), on important parameters of oxidative stress.
Main methods: Thiobarbituric acid-reactive substances values (TBA-RS, lipid peroxidation), carbonyl formation (protein oxidative damage), sulfhydryl content, reduced glutathione concentrations, nitric oxide production and the activities of the antioxidant enzymes glutathione peroxidase, glutathione reductase, catalase, superoxide dismutase and glucose-6-phosphate dehydrogenase (antioxidant defenses) were measured in striatum from 30-day-old rats after Gly injection.
Key findings: Gly administration significantly increased TBA-RS values, implying lipid oxidative damage. Furthermore, Gly-induced increase of TBA-RS was fully prevented by the NMDA receptor antagonist MK-801, indicating the involvement of the NMDA glutamate receptor in this effect. Gly injection also induced protein carbonyl formation, as well as elevation of the activities of glutathione peroxidase, glutathione reductase, catalase and superoxide dismutase. In contrast, glutathione levels, sulfhydryl content, nitric oxide production and the activity of glucose-6-phosphate dehydrogenase were not modified by Gly.
Significance: The data shows that Gly in vivo administration causes lipid peroxidation, probably secondary to NMDA stimulation, induces protein oxidation and modulates the activities of important antioxidant enzymes in the striatum. In case these findings can be extrapolated to the human NKH, it is feasible that oxidative stress may be involved in the pathophysiology of the brain injury observed in patients with this neurometabolic disease.

Introduction

Nonketotic hyperglycinemia (NKH) is an inborn error of glycine (Gly) metabolism caused by a defect in Gly cleavage. Patients affected by this disease have severe neurological symptoms with brain abnormalities, as well as hypotonia and crises with apnea (Hamosh and Johnston, 2001). Cerebral MRI findings include progressive cortical atrophy, abnormalities in the putamen and decreased or absent myelination in the white matter (Press et al., 1989; Wintermark et al., 2004; Shan, 2005; Cherian et al., 2009). The disorder presents a prevalence estimated in 1:60,000 newborns (Applegarth et al., 2000). The diagnosis of NKH is based on the finding of a raised cerebrospinal fluid-to-plasma-Gly ratio. Proton magnetic resonance spectroscopy has revealed that brain Gly concentrations may be as high as 7.3 mM with an average of 4.0 to 4.8 mM in NKH patients (Hamosh and Johnston, 2001). Such increases are harmless in blood, but can cause extensive neuronal damage in the neonatal brain (Applegarth and Toone, 2001). NKH variants are the classical neonatal form and the late onset type according to the period of onset of the symptoms. The neonatal form of the disease is mostly lethal, and patients that survive in the neonate period exhibit severe mental retardation and intractable seizures. Although milder, patients affected by the late onset form of the disorder also present neurological abnormalities. Early treatment consisting of sodium benzoate and dextromethorphan administration may improve the neurological sequel of these patients, but is usually not very successful (Korman et al., 2006).

It is long recognized that catabolic stress in NKH results in increased tissue Gly concentrations, leading to worsening of signs. Furthermore, the higher the cerebrospinal fluid (CSF)/plasma ratio, the more severe is the clinical phenotype of the disease (Hamosh and

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doi:10.1016/j.lfs.2011.06.013

Johnston, 2001). Therefore, it is likely that high Gly concentrations are toxic to the brain and are possibly involved in the pathophysiology of the characteristic leukoencephalopathy present in this disease, but this is so far not yet proven.

It has been postulated that excitotoxicity may be involved in the neurological damage found in patients affected by NKH, since Gly acts as a co-agonist on N-methyl-D-aspartate glutamate (NMDA) receptor, enhancing excitotoxic events related to glutamate neurotransmission (McNamara and Dingledine, 1990; Patel et al., 1990; Hara et al., 1993; Kure et al., 1997; Katsuki et al., 2007; Kono et al., 2007). In addition, it was recently demonstrated that Gly induces oxidative damage, reduces the antioxidant defenses (Leipnitz et al., 2009) and compromises bioenergetics in brain of rats (Busanello et al., 2010) in vitro, but Gly deleterious effects in vivo and the exact pathomechanisms involved are still not established. Therefore, in the present study we investigated the influence of in vivo Gly intrastriatal administration on important parameters of oxidative stress, namely thiobarbituric acid-reactive substances (TBA-RS), sulfhydryl oxidation, reduced glutathione (GSH) levels, protein carbonyl content, nitric oxide production and the activities of the antioxidant enzymes glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD) and glucose-6-phosphate dehydrogenase (G6PD) in striatum of young rats. We also examined the role of a NMDA-receptor antagonist to clarify the mechanisms involved in the oxidative damage induced by intrastriatal Gly injection.

Experimental procedures

Animals and reagents

Thirty-day-old Wistar rats, obtained from the Central Animal House of the Department of Biochemistry, ICBS, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil, were used. The animals were maintained on a 12:12 h light/dark cycle (lights on 07.00–19.00 h) in air conditioned constant temperature (22 ± 1 °C) colony room, with free access to water and 20% (w/w) protein commercial chow (SUPRA, Porto Alegre, RS, Brazil). All reagents used were of analytical grade and purchased from Sigma Co. (St Louis, MO, USA), except for dizocilpine maleate (MK-801), which was purchased from Tocris (Ballwin, MO, USA).

Glycine (Gly) administration

The rats were deeply anesthetized with equitesine (3.33 mL/Kg i.p.), which is a mixture of 0.25 M chloral hydrate, 88 mM magnesium sulfate heptahydrate, 10 mg/mL sodium tiopenthal, 5.8 M propylene glycol and 1.97 M ethanol, and thereafter placed in a stereotaxic apparatus. Two small holes were drilled in the skull and 1 μL of a 4 M Gly solution (4 μmol) or NaCl (controls) at the same volume and concentration (each solution was prepared in water and pH was adjusted to 7.4 with NaOH) was slowly injected bilaterally into each striatum over 3 min via a needle connected by a polyethylene tube to a 10 μL Hamilton syringe. The needle was left in place for another 1 min before being gently removed, so that the total procedure lasted 4 min. The coordinates for injections were as follows: 0.6 mm posterior to the bregma, 2.6 mm lateral to the midline and 4.5 mm ventral from dura (Paxinos and Watson, 1986). The correct position of the needle was tested by injecting 0.5 μL of methylene blue injection (4% in saline solution) and carrying out histological analysis. The experimental protocol was approved by the Ethics Committee for animal research of the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil and followed the NIH Guide for the Care and Use of Laboratory Animals (NIH publication 85–23, revised 1985). All efforts were made to minimize the number of animals used and their suffering.

In the experiments designed to evaluate the participation of NMDA-glutamate receptors, the animals received MK-801 (0.25 mg/kg, i.p.) or NaCl (0.9%, 10 mL/kg, i.p.) 30 min before Gly injection (Ribeiro et al., 2006).

Striatum preparation

The rats were sacrificed by decapitation without anesthesia 2 or 12 h after intrastriatal injection of either Gly or NaCl. The brain was rapidly excised on a Petri dish placed on ice. The olfactory bulb, pons, medulla, cortex, and cerebellum were discarded, and the striatum was dissected, weighed, and homogenized in 10 volumes (1:10, w/v) of 20 mM sodium phosphate buffer, pH 7.4 containing 140 mM KCl. Homogenates were centrifuged at 750 ×*g* for 10 min at 4 °C to discard nuclei and cell debris (Evelson et al., 2001). The pellet was discarded and the supernatant, a suspension of mixed and preserved organelles, including mitochondria, was separated and used to measure oxidative stress parameters.

Thiobarbituric acid-reactive substances (TBA-RS)

TBA-RS levels were measured according to the method described by Yagi (1998) with slight modifications. Briefly, 200 μL of 10% trichloroacetic acid and 300 μL of 0.67% TBA in 7.1% sodium sulfate were added to 100 μL of tissue supernatants containing 0.3 mg of protein and incubated for 2 h in a boiling water bath. The mixture was allowed to cool on running tap water for 5 min. The resulting pink-stained complex was extracted with 400 μL of butanol. Fluorescence of the organic phase was read at 515 nm and 553 nm as excitation and emission wavelengths, respectively. Calibration curve was performed using 1,1,3,3-tetramethoxypropane and subjected to the same treatment as supernatants. TBA-RS levels were calculated as nmol TBA-RS/mg protein.

Protein carbonyl formation

Protein carbonyl formation, a marker of protein oxidative damage, was measured spectrophotometrically according to Reznick and Packer (1994). Two hundred microliters of striatum supernatants containing 0.3 mg of protein were treated with 400 μL of 10 mM 2,4-dinitrophenylhidrazine (DNPH) dissolved in 2.5 N HCl or with 2.5 N HCl (blank) and left in the dark for 1 h. Samples were then precipitated with 600 μL 20% TCA and centrifuged for 5 min at 10,000 *g*. The pellet was then washed with 1 mL ethanol: ethyl acetate (1:1, V/V) and suspended in 550 μL 6 M guanidine prepared in 2.5 N HCl at 37 °C for 5 min. The difference between the DNPH-treated and HCl-treated samples (blank) was used to calculate the carbonyl content determined at 365 nm. The results were calculated as nmol of carbonyl groups/mg of protein, using the extinction coefficient of 22,000 × 106 nmol/mL for aliphatic hydrazones.

Nitrate and nitrite formation

Nitrite oxide production was determined by measuring nitrate (NO^{3-}) and nitrite (NO^{2-}) according to Miranda et al. (2001). Two hundred microliters of vanadium chloride was added to the tube containing 200 μL of striatal supernatants (0.3 mg of protein) for complete reduction of nitrate to nitrite. Then, 200 μL of Griess reagent (a mixture of N-1-naphtylethylenediamine dihydrochloride and sulfanilamide) was added, and the tube was incubated for 30 min at 37 °C in a water bath in a dark room. The resulting pink-stained pigment was determined in a spectrophotometer at 540 nm. A calibration curve was performed using sodium nitrate (2.5, 5, 10, 15, 25, 50 and 100 μM), and each curve point was subjected to the same treatment as supernatants. Nitric oxide production values were calculated as nmol/mg protein and represented as percentage of control.

Sulfhydryl content

This assay is based on the reduction of 5-5-dithio-bis (2-nitrobenzoic acid) (DTNB) by thiols, generating a yellow derivative (TNB) whose absorption is measured spectrophotometrically at 412 nm (Aksenov and Markesbery, 2001). Briefly, 30 μL of 10 mM DTNB and 980 μL of PBS were added to 50 μL of striatum supernatants containing 0.3 mg of protein. This was followed by 30 min incubation at room temperature in a dark room. Absorption was measured at 412 nm. Sulfhydryl content is inversely correlated to oxidative damage to proteins. Results were reported as nmol/mg protein.

Reduced glutathione (GSH) levels

GSH levels were measured according to Browne and Armstrong (1998). Tissue supernatants with approximately 0.3 mg of protein were diluted in 20 volumes (1:20, v/v) of 100 mM sodium phosphate buffer pH 8.0, containing 5 mM EDTA. One hundred microliters of this preparation was incubated with an equal volume of o-phthaldialdehyde (1 mg/mL methanol) at room temperature for 15 min. Fluorescence was measured using excitation and emission wavelengths of 350 nm and 420 nm, respectively. Calibration curve was performed with standard GSH (0.001–0.1 mM), and GSH concentrations were calculated as nmol/mg protein.

Glutathione peroxidase (GPx) activity

GPx activity was measured according to Wendel (1981) using tert-butylhydroperoxide as substrate. The enzyme activity was determined by monitoring the NADPH disappearance at 340 nm in a medium containing 100 mM potassium phosphate buffer/1 mM ethylenediaminetetraacetic acid, pH 7.7, 2 mM GSH, 0.1 U/mL glutathione reductase, 0.4 mM azide, 0.5 mM tert-butyl-hydroperoxide, 0.1 mM NADPH and approximately 3 μg of protein. One GPx unit (U) is defined as 1 μmol of NADPH consumed per minute. The specific activity was calculated as U/mg protein.

Glutathione reductase (GR) activity

GR activity was measured according to Calberg and Mannervik (1985) using oxidized glutathione (GSSG) and NADPH as substrates. The enzyme activity was determined by monitoring the NADPH disappearance at 340 nm in a medium with 200 mM sodium phosphate buffer, pH 7.5, containing 6.3 mM ethylenediaminetetraacetic acid, 1 mM GSSG, 0.1 mM NADPH and approximately 3 μg of protein. One GR unit (U) is defined as 1 μmol of GSSG reduced per minute. The specific activity was calculated as U/mg protein.

Catalase (CAT) activity

CAT activity was assayed according to Aebi (1984) by measuring the absorbance decrease at 240 nm in a reaction medium containing 20 mM H_2O_2, 0.1% Triton X-100, 10 mM potassium phosphate buffer, pH 7.0, and approximately 1 μg of protein. One unit (U) of the enzyme is defined as 1 μmol of H_2O_2 consumed per minute. The specific activity was calculated as U/mg protein.

Superoxide dismutase (SOD) activity

SOD activity was assayed according to Marklund (1985) and is based on the capacity of pyrogallol to autooxidize, a process highly dependent on $O_2^{\bullet -}$, which is a substrate for SOD. The inhibition of autoxidation of this compound occurs in the presence of SOD, whose activity can be then indirectly assayed spectrophotometrically at 420 nm. The reaction medium contained 50 mM Tris buffer/1 mM ethylenediaminetetraacetic acid, pH 8.2, 80 U/mL catalase, 0.38 mM pyrogallol and approximately 1 μg of protein. A calibration curve was performed with purified SOD as a standard to calculate the activity of SOD present in the samples. The results are reported as U/mg protein.

Glucose-6-phosphate dehydrogenase (G6PD) activity

G6PD activity was measured by the method of Leon and Clark (1984), in a reaction mixture (1 mL) containing 100 mM Tris–HCl pH 7.5, 10 mM MgCl2, 0.5 mM NADP+ and approximately 3 μg of protein. The reaction was started by the addition of 1 mM glucose-6-phosphate and was followed in a spectrophotometer at 340 nm. One G6PD unit corresponds to 1 mmol of substrate transformed per minute and the specific activity is represented as units per mg protein.

Protein determination

Protein levels were measured by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Statistical analysis

Results are presented as mean ± standard deviation. Assays were performed in triplicate and the mean was used for statistical calculations. Data were analyzed using the Student's *t* test for unpaired samples or one-way analysis of variance (ANOVA) followed by the Duncan multiple range test when the F value was significant. Only significant values are shown in the text. Differences between groups were rated significant at $P<0.05$. All analyses were carried out in an IBM-compatible PC computer using the Statistical Package for the Social Sciences (SPSS) software.

Results

Gly intrastriatal administration induces lipid peroxidation

Initially we studied the effect of intrastriatal injection of 4 μmol Gly on TBA-RS levels 2 h after drug infusion. Fig. 1 shows that Gly administration induced lipid peroxidation (TBA-RS increase) in striatum [$t_{(8)} = 6.334$; $P<0.001$] as compared to rats that received NaCl. We also observed that intraperitoneal injection of the NMDA antagonist MK-801 (0.25 mg/kg) 30 min prior to Gly administration fully prevented the increase of TBA-RS levels caused by this amino acid [$F_{(3,19)} = 22.14$; $P<0.001$] (Fig. 2). These results indicate that the lipid peroxidation caused by Gly administration was probably mediated by NMDA overstimulation.

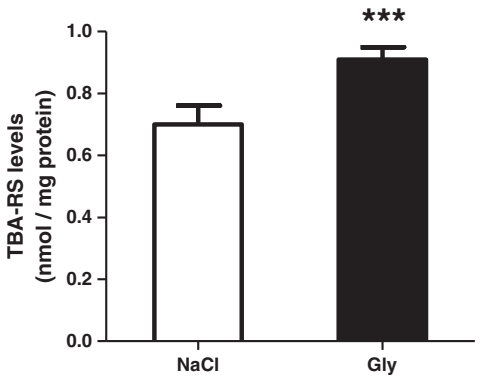

Fig. 1. Effect of intrastriatal administration of glycine (Gly, 4 μmol) on thiobarbituric acid-reactive substances (TBA-RS) levels in rat striatum 2 h after injection. Data are expressed as mean ± SD for 5–6 independent experiments (animals) performed in triplicate. ***$P<0.001$, compared to rats that received intrastriatal NaCl injection (Student's *t*-test for unpaired samples).

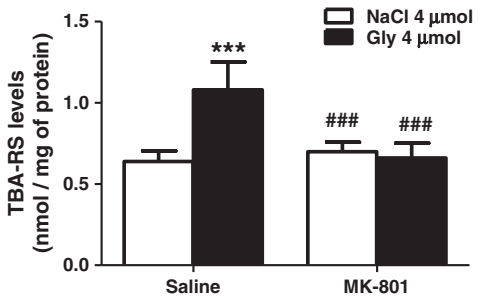

Fig. 2. Effect of MK-801 on the increased thiobarbituric acid reactive substances (TBA-RS) levels caused by glycine (Gly, 4 µmol) intrastriatal injection. Animals were pre-treated with saline or MK-801 i.p. 30 min prior to glycine (Gly, 4 µmol) injection and sacrificed 2 h later. Data are represented as mean ± SD for 5–6 independent experiments (animals) performed in triplicate. $^{***}P<0.001$ compared to rats pre-treated with saline and treated with NaCl; $^{###}P<0.001$ compared to rats pre-treated with saline and treated with Gly (ANOVA followed by Duncan's multiple range test).

Gly intrastriatal administration increases protein carbonyl formation

Fig. 3 shows that Gly provoked a marked increase of carbonyl formation 2 h after Gly injection [$t_{(8)} = 3.052$; $P<0.05$], implying that protein oxidative damage was caused by this amino acid.

Gly intrastriatal administration does not alter nitrate and nitrite formation, sulfhydryl content and glutathione (GSH) levels

We also investigated the effect of Gly administration on nitrate and nitrite production, sulfhydryl content and GSH concentrations in rat striatum. It can be observed in Table 1 that Gly injection did not modify these parameters 2 h after its infusion.

Gly intrastriatal administration increases glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD) activities

We investigated the effect of Gly intrastriatal administration on the activities of GPx, GR, CAT, SOD and G6PD in striatum of young rats. Our results demonstrate that a single Gly injection significantly increased GPx [$t_{(7)} = 6.663$; $P<0.001$], GR [$t_{(10)} = 12.44$; $P<0.001$], CAT [$t_{(7)} = 2.556$; $P<0.05$] and SOD [$t_{(7)} = 2.526$; $P<0.05$] 12 h after the administration, whereas G6PD activity was not changed (Fig. 4).

Discussion

The pathogenetic mechanisms involved in the severe cerebral damage of patients with NKH are insufficiently understood. However,

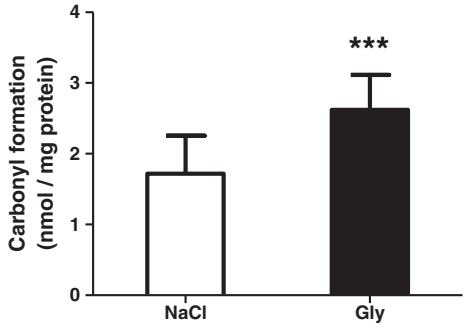

Fig. 3. Effect of intrastriatal administration of glycine (Gly, 4 µmol) on protein carbonyl formation in rat striatum at 2 h after injection. Data are represented as mean ± SD for 5 independent experiments (animals). $^{***}P<0.001$, compared to rats that received intrastriatal NaCl injection (Student's *t*-test for unpaired samples).

Table 1
Effect of glycine intrastriatal administration (Gly, 4 µmol) on nitrate and nitrite production, sulfhydryl content and reduced glutathione (GSH) levels in rat striatum 2 h after injection.

	Treatment	
	NaCl	Gly
Nitrates and nitrites	4.90 ± 0.20	4.70 ± 0.17
Sulfhydryl	89.8 ± 6.93	91.2 ± 4.30
GSH	13.1 ± 0.77	12.6 ± 0.88

Data are represented as mean ± SD for 5 independent experiments (animals). No significant differences were found between groups (Student's *t*-test for unpaired samples). Results are expressed as nmol/mg protein.

it is likely that excitotoxicity is involved at least in part in the neuropathology of this disorder since Gly is a co-agonist of NMDA receptors (McNamara and Dingledine, 1990; Patel et al., 1990; Hara et al., 1993; Kure et al., 1997; Katsuki et al., 2007; Kono et al., 2007; Kikuchi et al., 2008). On the other hand, considering that normal Gly values in human body fluids are sufficiently high to saturate the binding site for Gly on the NMDA-type glutamate receptors, it is feasible that higher concentrations of Gly may not cause overstimulation of these receptors (Obrenovitcha et al., 1997). Thus, other mechanisms may underlie brain damage in NKH. In this scenario, it has been recently demonstrated that Gly induces oxidative stress and compromises energy metabolism in vitro in rat brain, although the mechanisms underlying these effects were not elucidated (Leipnitz et al., 2009; Busanello et al., 2010). Therefore, it seems that more research, particularly in vivo studies, is required to clarify the mechanisms causing neuronal death in patients with NKH.

In the present report we investigated whether an acute intrastriatal injection of Gly could induce oxidative damage and/or alter the main antioxidant defenses in striatum of young rats. We observed that Gly in vivo administration significantly increased TBA-RS levels in the striatum, corroborating our previous in vitro findings (Leipnitz et al., 2009). Since TBA-RS measurement reflects the amount of malondialdehyde formation, an end product of membrane fatty acid peroxidation (Halliwell and Gutteridge, 2007), our present findings indicate that Gly provoked lipid oxidative damage in vivo. We also showed that the non-competitive NMDA receptor antagonist MK-801 prevented the increase of TBA-RS levels elicited by Gly, implying the involvement of the NMDA receptor in this effect. Our present findings are in accordance with previous studies demonstrating a prevention of Gly-induced degeneration in rat striatum caused by MK-801 and suggest that oxidative damage may underlie this neurodegeneration (Santamaría and Ríos, 1993; Jara-Prado et al., 2003).

Gly also markedly increased carbonyl formation after intrastriatal administration. Since carbonyl group generation is currently used as a marker of free radical-mediated protein oxidation (Levine et al., 1994), especially on amino acid side chain residues (Pro, Arg, Lys, and Thr) (Dalle-Donne et al., 2003), it is presumed that Gly induced protein oxidative damage in striatum.

In contrast, Gly did not alter GSH levels and sulfhydryl content in the striatum Endogenous GSH is the main naturally-occurring antioxidant in the brain and is used to evaluate the non-enzymatic antioxidant capacity of a tissue to prevent the damage associated to free radical processes (Halliwell and Gutteridge, 2007). On the other hand, approximately two thirds of sulfhydryl groups are bound to proteins, whereas one third is a component of small molecules such as GSH (Requejo et al., 2010). Furthermore, since protein-bound sulfhydryl groups (PBSG) can be reversibly oxidized by reactive species, it has been suggested that PBSG represent a potential active redox antioxidant pool in the cellular defense against oxidative stress (Thomas et al., 1995; Hansen et al., 2009). Taken together, our present data indicate that rat striatal non-enzymatic antioxidant defenses were not modified by Gly (Lissi et al., 1995; Halliwell and Gutteridge, 2007).

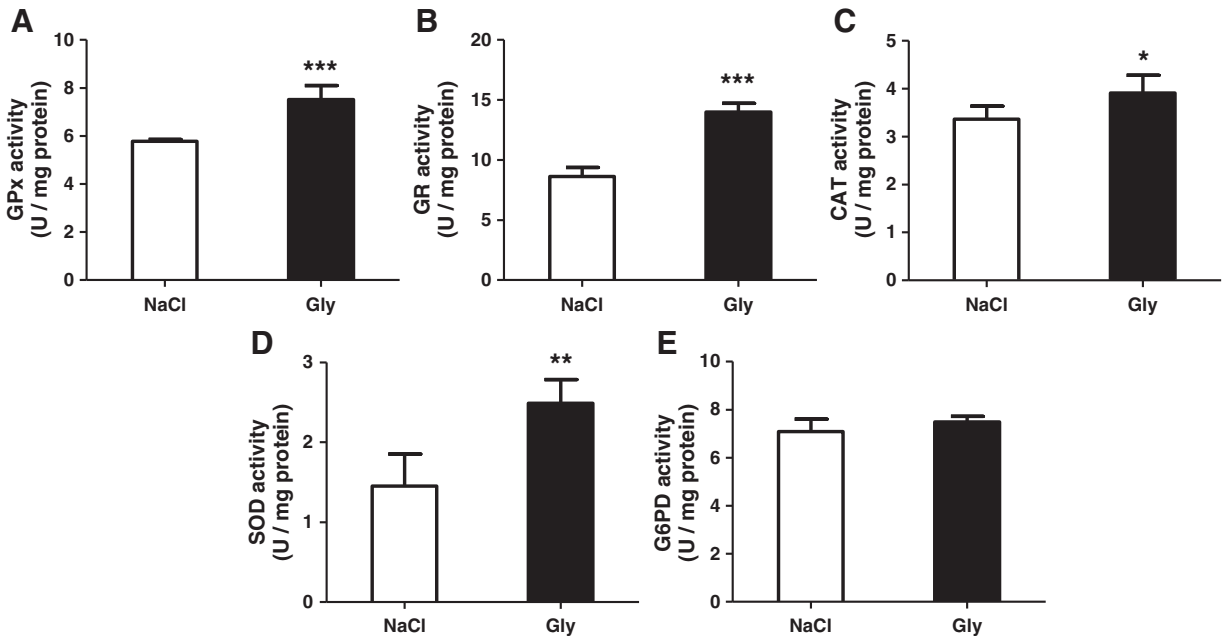

Fig. 4. Effect of intrastriatal administration of glycine (Gly, 4 μmol) on the activity of the enzymes glutathione peroxidase (GPx; A), glutathione reductase (GR; B), catalase (CAT; C), superoxide dismutase (SOD; D) and glucose-6-phosphate dehydrogenase (G6PD; E) in rat striatum 12 h after injection. Data are represented as mean ± SD for 5 independent experiments (animals). $^{*}P<0.05$; $^{**}P<0.01$ $^{***}P<0.001$, compared to rats that received intrastriatal NaCl injection (Student's *t*-test for unpaired samples).

Regarding the enzymatic antioxidant system, we found that GPx, GR, CAT and SOD activities were significantly increased after 12 h of Gly intrastriatal administration. In this context, it is widely known that reactive species may regulate the expression of numerous genes via signaling mechanisms (Rushmore et al., 1991; Lakshminarayanan et al., 1998; Halliwell and Gutteridge, 2007). It is therefore conceivable that induction of the expression of these endogenous antioxidant enzymes (GPx, GR, CAT and SOD) at the gene level might have taken place as a compensatory mechanism in response to increased formation of reactive species. Furthermore, considering that SOD scavenges superoxide anions (Fridovich, 1989), whereas GPx and CAT scavenge preferentially fatty acid hydroperoxides and H_2O_2 (Shull et al., 1991; Matés et al., 1999), we presume that these reactive species were elicited by Gly administration. This is in line with our findings showing that Gly did not increase nitrate and nitrite formation, signaling that Gly-induced lipid and protein oxidative damage probably occurred via reactive oxygen species (ROS).

On the other hand, it is possible that a higher GR activity may explain the normal GSH concentrations found in the present study despite the increase of GPx activity. Similar findings showing normal GSH levels with a higher activity of GPx were observed in brainstem of hypoglycemic rats (Singh et al., 2004).

We emphasize that, although Gly induced lipid oxidative damage probably as a consequence of overstimulation of NMDA receptors, we cannot exclude the possibility that this amino acid also caused the generation of free radicals via other mechanisms, as previously demonstrated in vitro (Leipnitz et al., 2009).

At this point it should be emphasized that the brain has low cerebral antioxidant defenses compared with other tissues (Halliwell, 1992), a fact that makes this tissue more vulnerable to increased formation of reactive species (Halliwell, 1992). The lower capacity of the brain to react against free radicals is reinforced by the fact that this tissue has a high rate of oxidative metabolism coupled to ROS production, high amount of iron and higher peroxidation potential because of its high content of polyunsaturated fatty acids (Halliwell and Gutteridge, 2007).

It is difficult to determine the pathophysiological relevance of our present data. However, considering that brain Gly concentrations in NKH-affected patients are at the millimolar range and that Gly-pro-oxidant effects occurred at these concentrations, it is feasible that our results might contribute, at least in part, to explain the brain damage found in patients affected by NKH.

Conclusion

We report for the first time that Gly induces oxidative stress in vivo in the striatum. It is presumed that overproduction of reactive radicals due to high brain Gly concentrations is potentially deleterious to the CNS and may represent a relevant pathomechanism of brain injury in NKH and other pathologies with high Gly concentrations.

Conflict of interest

The authors declare that there are no conflict of interest.

Acknowledgments

This work was supported by grants from CNPq, PRONEX II, FAPERGS, PROPESQ/UFRGS and FINEP research grant Rede Instituto Brasileiro de Neurociência (IBN-Net) # 01.06.0842-00, and Instituto Nacional de Ciência e Tecnologia – Excitotoxicidade e Neuroproteção (INCT-EN).

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