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The role of the glutathione system in seizures induced by diphenyl diselenide in rat pups

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ABSTRACT

The present study investigated the role of the glutathione system in seizures induced by diphenyl diselenide $(PhSe)_2$ (50 mg/kg) in rat pups (post natal day, 12–14). Reduced glutathione (GSH) (300 nmol/site; i.c.v.), administered 20 min before $(PhSe)_2$, abolished the appearance of seizures, protected against the inhibition of catalase and δ-aminolevulinic dehydratase (δ-ALA-D) activities and increased glutathione peroxidase (GPx) activity induced by $(PhSe)_2$. Administration of L-buthionine sulfoximine (BSO, a GSH-depleting compound) (3.2 μmol/site; i.c.v.) 24 h before $(PhSe)_2$ increased the percentage (42–100%) of rat pups which had seizure episodes, reduced the onset for the first convulsive episode. In addition, BSO increased thiobarbituric acid reactive species (TBARS) levels and decreased GSH content, catalase, δ-ALA-D and Na^+, K^+-ATPase activities. Treatment with sub effective doses of GSH (10 nmol/site) and D-2-amino-7-phosphonoheptanoic acid (AP-7, an antagonist of the glutamate site at the NMDA receptor; 5 mg/kg, i.p.) abolished the appearance of seizures induced by $(PhSe)_2$ in rat pups. Sub effective doses of GSH and kynurenic acid (an antagonist of strychnine-insensitive glycine site at the NMDA receptor; 40 mg/kg, i.p.) were also able in abolishing the appearance of seizures induced by $(PhSe)_2$. In conclusion, administration of GSH protected against seizure episodes induced by $(PhSe)_2$ in rat pups by reducing oxidative stress and, at least in part, by acting as an antagonist of glutamate and glycine modulatory sites in the NMDA receptor.

1. Introduction

Epilepsy affects approximately 1 in 130 people, and has a lifetime cumulative incidence approaching 1 in 25, thus representing one of the most common serious neurological disorders [1]. Clinical and experimental data show that there is a period of increased susceptibility to seizures during early postnatal development. The cause of this increased susceptibility is probably a combination of enhanced excitation and diminished inhibition throughout the brain, as well as developmental differences in subcortical circuits, such as the substantia nigra-mediated seizure-suppression system [2].

Some studies have demonstrated that diphenyl diselenide $[(PhSe)_2]$, an organoselenium compound, crosses the blood–brain barrier [3] and induces seizure episodes in rat pups, depending on the dose administered [4,5]. It is important to point out that seizures induced by $(PhSe)_2$ are related to oxidative stress, characterized by an increase in lipid peroxidation levels and catalase activity, as well as, a reduction in the activity of δ-ALA-D and Na+, K+ ATPase, sulfhydryl containing enzymes [4]. Additionally, $(PhSe)_2$ induces over stimulation of the glutamate system, by acting on N-methyl-D-aspartate (NMDA) receptors and inhibits [^{3}H]glutamate uptake [5]. In the GABAergic system, $(PhSe)_2$ reduces the GABAergic transmission by stimulating [^{3}H]GABA uptake in cortex and hippocampal slices from rat pups that presented seizure episodes [6].

Reid and Jahoor [7] have demonstrated that alterations in the glutathione metabolism in association with increased oxidative stress have been implicated in the pathogenesis of many diseases, including seizures. Brain is susceptible to free radical damage, considering the large lipid content of myelin sheaths and the high rate of brain oxidative metabolism [8]. In this context, the thiol redox state is an essential parameter associated with major biologic processes, such as oxidative stress, intracellular redox homeostasis and gene expression [9]. γ-Glutamylcysteinylglycine (GSH and GSSG, reduced and oxidized glutathione) is the most abundant thiol in the central nervous system (CNS), the major part in the reduced form [10]. In the CNS, glutathione has been shown to exert a number of specialized effects. Reduced glutathione (GSH) can react non-enzymatically with oxidative compound or support glutathione peroxidase (GPx) activity in the clearance of hydrogen and organic peroxides. Moreover, GSH protects neurons against glutamate-induced excitotoxicity [11], affects the functions of second messengers [12] and protects against Ca^{2+}-mediated cell death [13].

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Based on the considerations above, the specific aim of the present study was to investigate the possible involvement of the glutathione system in seizures induced by $(PhSe)_2$ in rat pups.

2. Material and methods

2.1. Chemicals

Reduced glutathione (GSH), L-buthionine sulfoximine (BSO), kynurenic acid, D-2-amino-7-phosphonoheptanoic acid (AP-7), δ-aminolevulinic acid (δ-ALA), *p* dimethylaminobenzaldehyde, epinephrine, adenosine triphosphate (ATP) and ouabain were purchased from Sigma (St. Louis, MO, USA). All other chemicals were obtained of analytical grade or from standard commercial suppliers. GSH, BSO, AP-7 and kynurenic acid were dissolved in 0.9% physiological saline.

$(PhSe)_2$ was prepared in our laboratory according to the literature method Paulmier [14]. Analysis of the ^1H NMR and ^{13}C NMR spectra showed that $(PhSe)_2$ obtained presented analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of compound (99.9%) was determined by GC/MS. This drug was dissolved in canola oil.

2.2. Animals

Wistar rats post natal day (PND) 12–14 of both sexes were obtained from a local breeding colony. PND 12–14 was chosen based on Mikulecká [15]. The animals were kept in a separate animal room, on a 12 h light/12 dark cycle, with light on at 7:00 a.m., in an air conditioned room (22 ± 2 °C). Commercial diet (GUABI, RS, Brazil) and tap water were supplied *ad libitum*. The dams were allowed to deliver and wean their pups until PND 12–14. All animal experiments were carried out according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil. All efforts were made to minimize animal suffering, to reduce the number of animals used.

2.3. In vivo Experiments

2.3.1. Effect of the glutathione system on $(PhSe)_2$-induced seizures

$(PhSe)_2$ was administered at the dose of 50 mg/kg by oral route (p.o., 10 ml/kg of body weight) to pup rats. The dose of $(PhSe)_2$ used in this study was chosen based on Prigol [4]. To address the role played by GSH in seizures induced by $(PhSe)_2$, distinct groups of animals were treated with GSH (300 nmol/site, intracerebroventricular, i.c.v), 24 h before $(PhSe)_2$ administration. BSO, a GSH-depleting compound, was administered at 3.2 μmol/site (i.c.v.) 20 min before $(PhSe)_2$ administration. The concentrations used for GSH or BSO were administrated at the volume of 2 μl/site, based on Abe et al. [42]. Intracerebroventricular injections were given as described by Haley and McCormick [16] with the bregma fissure as a reference. Appropriate vehicle-treated groups were simultaneously assessed.

Experimenters were blind to the drugs given to rat pups. Rats were divided into groups as follows: *Control group* – (saline i.c.v.) + (canola oil, p.o); *$(PhSe)_2$ 50 group* – (saline i.c.v.) + $(PhSe)_2$ (50 mg/kg, p.o.); *$(PhSe)_2$ 50 + BSO 3.2 group* – (BSO (3.2 μmol/site, i.c.v.)) + $(PhSe)_2$ (50 mg/kg, p.o.); *$(PhSe)_2$ 50 + GSH 300 group* – GSH (300 nmol/site, i.c.v.) + $(PhSe)_2$ (50 mg/kg, p.o.).

The animals were observed for one hour in Plexiglas chambers for the appearance of tonic-clonic seizures lasting more than 5 s. The latency for the onset of the first tonic-clonic seizure episode was also recorded. Seizures were classified as generalized (tonic-clonic) or stage 5 according to Racine [17].

Subsequently to the seizure episode, rat pups were anesthetized and decapitated. Animals which did not display seizures were considered protected and decapitated one hour after compound administration. The whole brains of all animals were removed and used for *ex vivo* assays. Based on the results obtained in *in vivo* experiments related to the appearance of seizure episodes (Table 1), the *ex vivo* analyses were performed using five groups, since animals of *$(PhSe)_2$ 50 group* were subdivided in two groups: *$(PhSe)_2$ 50 NS* (animals that did not present seizure episodes) and *$(PhSe)_2$ 50 S* (animals that presented seizure episodes).

2.3.2. Involvement of glutamate and glycine modulatory sites in the NMDA receptor in $(PhSe)_2$ induced seizures

To evaluate the role played by GSH (in glutamate and glycine modulatory sites in the NMDA receptor) in seizures induced by $(PhSe)_2$, distinct groups of animals were treated with distinct drugs. For this purpose, rat pups were pretreated with sub effective doses of AP-7 (an antagonist of glutamate site at the NMDA receptor) (5 mg/kg, i.p., 10 ml/kg of body weight) and GSH (10 nmol/site).

Another group was treated with sub effective doses of kynurenic acid (an antagonist of strychnine-insensitive glycine site at the NMDA receptor) (40 mg/kg, i.p., 10 ml/kg of body weight) and GSH (10 nmol/site).

Twenty minutes after AP-7 and GSH or kynurenic acid and GSH administration, $(PhSe)_2$ (50 mg/kg of body weight, p.o.) or canola oil was administered to rat pups. The appearance of seizures was quantified as described on 2.3.1 section. Subsequently to the seizure episode, rat pups were anesthetized and decapitated. Animals which did not display seizures were considered protected and decapitated 1 h after drug administration.

2.4. Ex vivo Experiments

The samples of brains were homogenized in 50 mM Tris–HCl, pH 7.5 (1/5, w/v), centrifuged at 2400*g* for 10 min. The low-speed supernatants (S1) were separated and used for biochemical assays, excepting for GSH assay.

2.4.1. Lipid peroxidation

The reaction product was determined using an aliquot (200 μl) of S1, 500 μl thiobarbituric acid (0.8%), 200 μl sodium dodecil sulfate (SDS, 8.1%) and 500 μl acetic acid, the mixture was incubated at 95 °C for 2 h. TBARS (thiobarbituric acid reactive species) were determined as described by Ohkawa [18]. TBARS levels were expressed as nmol MDA/mg protein.

Table 1
Influence of treatment with BSO and GSH on $(PhSe)_2$-induced seizures in rat pups.

Groups	Appearance of Seizures[a]	Latency[b] (min)
Control	0/11	ns
$(PhSe)_2$ 50	5/12[*] (42%)	53.00 ± 4.54[**]
$(PhSe)_2$ 50 + BSO 3.2	9/9[*] (100%)	46.68 ± 5.09[**] [##]
$(PhSe)_2$ 50 + GSH 300	0/10[#]	ns

[a] Number of animals which presented seizures/N of animals per group and percentage of animals which presented seizures.
[b] time (min) to the appearance for the first seizure episode. "ns" animals which did not present seizure in 60 min of observation. $(PhSe)_2$ (mg/kg), BSO (μmol per site) and GSH (nmol per site). Data are reported as means ± S.D.
[*] Denotes $p < 0.05$ as compared to the control group.
[#] Denotes $p < 0.05$ as compared to the $(PhSe)_2$ 50 + BSO 3.2 group (X2 method and Fischer's exact probability test).
[**] Denotes $p < 0.05$ as compared to the control and GSH groups.
[##] Denotes $p < 0.05$ as compared to the $(PhSe)_2$ 50 group (one-way ANOVA/Duncan).