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GM1 ganglioside prevents seizures, Na^+,K^+-ATPase activity inhibition and oxidative stress induced by glutaric acid and pentylenetetrazole

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Monosialoganglioside (GM1) is a glycosphingolipid that protects against some neurological conditions, such as seizures and ischemia. Glutaric acidemia type I (GA-I) is an inherited disease characterized by striatal degeneration, seizures, and accumulation of glutaric acid (GA). In this study, we show that GA inhibits Na^+,K^+-ATPase activity and increases oxidative damage markers (total protein carbonylation and thiobarbituric acid-reactive substances—TBARS) production in striatal homogenates from rats in vitro and ex vivo. It is also shown that GM1 (50 mg/kg, i.p., twice) protects against GA-induced (4 μmol/striatum) seizures, protein carbonylation, TBARS increase, and inhibition of Na^+,K^+-ATPase activity ex vivo. Convulsive episodes induced by GA strongly correlated with Na^+,K^+-ATPase activity inhibition in the injected striatum but not with oxidative stress marker measures. Muscimol (46 pmol/striatum), but not MK-801 (3 nmol/striatum) and DNQX (8 nmol/striatum) prevented GA-induced convulsions, increase of TBARS and protein carbonylation and inhibition of Na^+,K^+-ATPase activity. The protection of GM1 and muscimol against GA-induced seizures strongly correlated with Na^+,K^+-ATPase activity maintenance ex vivo. In addition, GM1 (50–200 μM) protected against Na^+,K^+-ATPase inhibition induced by GA (6 mM) but not against oxidative damage in vitro. GM1 also decreased pentylenetetrazole (PTZ)-induced (1.8 μmol/striatum) seizures, Na^+,K^+-ATPase inhibition, and increase of TBARS and protein carbonyl in the striatum. These data suggest that Na^+,K^+-ATPase and $GABA_A$ receptor-mediated mechanisms may play important roles in GA-induced seizures and in their prevention by GM1.

Keywords: Seizure; EEG; Glutaric acid; Oxidative damage; GM1; Protein carbonylation; TBARS; Na^+,K^+-ATPase activity; PTZ

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Introduction

Gangliosides, particularly GM1 ganglioside, are known to modulate adaptive functions, such as neuronal plasticity (Perry et al., 2004), and memory formation (Silva et al., 2000). In addition, they have been proposed as neuroprotective agents against various excitotoxic agents or conditions, such as excitatory amino acid exposure and ischemia (Lombardi et al., 1989; Carolei et al., 1991). Accordingly, GM1 inhibits lipid peroxidation (Defeudis et al., 1979; Fighera et al., 2003) and may directly scavenge free radicals generated during reperfusion and protect receptors and enzymes from oxidative damage (Tyurin et al., 1991; Maulik et al., 1993). In addition, GM1 increases ascorbic acid levels in rat striatum and catalase activity in rat cerebral cortex (Fighera et al., 2003, 2004) and protects against ischemia-induced Na^+,K^+, and Mg^{2+} ATPase activity inhibition (Mahadik et al., 1989).

GM1 has also been proposed as an anticonvulsant, since it abolishes the recurrent epileptiform activity in rats induced by the intracerebral injection of antiserum to brain gangliosides (Karpiak et al., 1976, 1981) and attenuates methylmalonate-induced convulsions (Fighera et al., 2003). On the other hand, GM1 does not alter kindled-amygdaloid seizures (Albertson and Walby, 1987), and it is not known whether GM1 inhibits seizure induced by other chemical convulsants.

Glutaric acidemia type I (GA-I) is an inherited neurometabolic disorder caused by glutaryl-CoA dehydrogenase deficiency, which leads to accumulation in body fluids and in brain of glutaric acid (GA), 3-hydroxyglutaric (3-OHGA), and, to a lesser extent, of glutaconic acids (Goodman and Kohlhoff, 1975). It is characterized by acute neurological compromise, including generalized convulsions, acute loss of motor skills, and dystonia (Freudenberg et al., 2004). If untreated, the disease is complicated by acute encephalopathic crises, resulting in neurodegeneration of vulnerable brain regions, particularly the putamen (Kölker et al., 2003).

Brain imaging shows frontotemporal atrophy, acute striatal degeneration, and shrinkage of the caudate nuclei (Goodman and Frerman, 1995; Tortorelli et al., 2005). Although the mechanisms underlying brain damage in this disorder are not well established, growing evidence suggests that excitotoxicity (Lima et al., 1998; Kölker et al., 1999) and oxidative stress (Oliveira Marques et al., 2003) play a central role in the neuropathogenesis of this disease. In fact, GA causes convulsions in animals through GABAergic function impairment and glutamatergic mechanisms activation (Lima et al., 1998) and neuronal damage by NMDA receptors (NMDAr) activation (Kölker et al., 1999). Accordingly, in this study, we investigated whether GA increases total striatal TBARS and protein carbonylation (indicators of lipid and protein oxidative damage, respectively—see Brown and Kelly, 1996; Shacter et al., 1996) and causes Na^+,K^+-ATPase activity inhibition ex vivo and if these neurochemical markers correlate with seizure activity.

Since pharmacological and neurochemical evidence support that GM1 decreases convulsions induced by glutamatergic activation (Fighera et al., 2003; Liu et al., 2004), increases antioxidant defenses in brain of rats (Fighera et al., 2003; 2004) and Na^+,K^+-ATPase activity in vitro (Maulik et al., 1993), we decided to investigate whether (1) the systemic administration of GM1 protects against behavioral and electroencephalographic convulsions elicited by the intrastriatal injection of GA; (2) GM1 decreases GA-induced oxidative damage to proteins and lipids ex vivo and in vitro; (3) GM1 prevents GA-induced Na^+,K^+-ATPase inhibition ex vivo and in vitro; (4) GA-induced convulsions and Na^+,K^+-ATPase inhibition are sensitive to ionotropic glutamate antagonists and to a $GABA_A$ agonist; (5) GM1 prevents the convulsions, oxidative damage, and Na^+,K^+-ATPase inhibition induced by $GABA_A$ receptor blockade.

Experimental procedures

Animal and reagents

Adult male Wistar rats (270–300 g) maintained under controlled light and environment (12:12-h light–dark cycle, 24 ± 1°C, 55% relative humidity) with free access to food (Guabi, Santa Maria, Brazil) and water were used. Animal utilization protocols followed the Official Government Ethics guidelines and were approved by the University Ethics Committee.

All reagents were purchased from Sigma (St. Louis, MO), except thiobarbituric acid (TBA), which was obtained from Merck (Darmstadt, Germany) and GM1 ganglioside, which was donated by TRB Pharma, the brazilian representative of Fidia Research Laboratories.

Behavioral evaluation and surgical procedure

Animals were anesthetized with Equitesin (1% phenobarbital, 2% magnesium sulfate, 4% chloral hydrate, 42% propylene glycol, 11% ethanol; 3 ml/kg, i.p.) and placed in a rodent stereotaxic apparatus. Under stereotaxic guidance, a cannula was inserted unilaterally into the striatum (coordinates relative to bregma: AP 0 mm, ML 3.0 mm, V 3.0 mm from the dura) (Paxinos and Watson, 1986). Chloramphenicol (200 mg/kg, i.p.) was administrated immediately before the surgical procedure.

The effect of the systemic administration of GM1 ganglioside on seizure activity, on striatal TBARS and protein carbonyl content increase and striatal Na^+,K^+-ATPase inhibition induced by GA was investigated by injecting the animals twice with GM1 ganglioside (50 mg/kg, i.p.) or saline (0.9% NaCl; 1 ml/kg, i.p.), spaced 24 h apart. Thirty minutes after the second 'GM1 or saline injection, buffered GA (4 μmol/2 μl) or NaCl (5.5 μmol/2 μl) was injected into the right striatum. This experimental protocol of GM1 administration has been shown to be effective in decreasing methylmalonate-induced convulsions and TBARS production in rat striatum (Fighera et al., 2003).

The effect of the intrastriatal administration of ionotropic glutamate antagonists and $GABA_A$ agonist on seizure activity and striatal Na^+,K^+-ATPase inhibition induced by GA was investigated by intrastriatally injecting the animals with DNQX (8 nmol/0.5 μl), MK-801 (3 nmol/0.5 μl), muscimol (46 pmol/0.5 μl), or saline (0.9% NaCl/0.5 μl), 30 min before GA administration. GA (3 μmol/1.5 μl, pH set to 7.4 with NaOH) or NaCl (4.1 μmol/1.5 μl) was injected into the right striatum.

The effectiveness of GM1 to prevent the convulsions and Na^+,K^+-ATPase inhibition induced by $GABA_A$ receptor blockade was assessed by giving the animals NaCl (0.9%, i.p, twice) or GM1 ganglioside (50 mg/kg, i.p., twice), as described above, 30 min before the injection of PTZ (1.8 μmol/2 μl) or NaCl (5.5 μmol/2 μl) into the right striatum (Oliveira et al., 2004).

Immediately after the intrastriatal injection, the animals were transferred to a round open field (54.7 cm in diameter). During 15 min, the animals were observed for the appearance of clonic movements of hindlimbs and forelimbs contralateral to the injected striatum. The number of clonic movement episodes and duration of such clonic movements were recorded with a stopwatch (de Mello et al., 1996).

Placement of cannula and electrodes for EEG recordings

Rats were surgically implanted with a cannula and electrodes under stereotaxic guidance. In brief, rats were deeply anesthetized with Equitesin (3 ml/kg, i.p.). Two screw electrodes were placed bilaterally over the parietal cortex, along with a ground lead positioned over the nasal sinus. Bipolar nichrome wire Teflon-insulated depth electrodes (100 μm) were implanted unilaterally into the striatum. For the intrastriatal infusion of drugs, a guide cannula (28 gauge) was glued to a multipin socket and inserted through a previously opened skull orifice. The coordinates from bregma for implantation of the electrodes were (in mm) AP, 0; L, 3; and DV, 3.5, which corresponds to the dorsal striatum (Paxinos and Watson, 1986). The electrodes were connected to a multipin socket and, together with the injection cannula, were fixed to the skull with dental acrylic cement. The experiments were performed 7–9 days after surgery when animals did not show any sign of pain, infection, or discomfort.

EEG recordings and intrastriatal injection of drugs

The procedures for EEG recording and intracerebral injection of drugs were carried out as previously described (Cavalheiro et al., 1992). Briefly, the animals were allowed to habituate to a Plexiglas cage (25 × 25 × 60 cm) for at least 10 min before the EEG recording. The rats were then connected to the lead socket in a swivel inside a Faraday's cage. Routinely, a 10-min baseline recording was obtained to establish an adequate control period. The protocol of drug injection used in this set of experiments was the same used in those experiments that evaluated the protective effect