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Effectiveness of creatine monohydrate on seizures and oxidative damage induced by methylmalonate☆

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Abstract

Methylmalonic acidemias are metabolic disorders caused by a severe deficiency of methylmalonyl CoA mutase activity, which are characterized by neurological dysfunction, including convulsions. It has been reported that methylmalonic acid (MMA) accumulation inhibits succinate dehydrogenase (SDH) and β-hydroxybutyrate dehydrogenase activity and respiratory chain complexes in vitro, leading to decreased CO_2 production, O_2 consumption and increased lactate production. Acute intrastriatal administration of MMA also induces convulsions and reactive species production. Though creatine has been reported to decrease MMA-induced convulsions and lactate production, it is not known whether it also protects against MMA-induced oxidative damage. In the present study we investigated the effects of creatine (1.2–12 mg/kg, i.p.) and MK-801 (3 nmol/striatum) on the convulsions, striatal content of thiobarbituric acid reactive substances (TBARS) and on protein carbonylation induced by MMA. Moreover, we investigated the effect of creatine (12 mg/kg, i.p.) on the MMA-induced striatal creatine and phosphocreatine depletion. Low doses of creatine (1.2 and 3.6 mg/kg) protected against MMA-induced oxidative damage, but did not protect against MMA-induced convulsions. A high dose of creatine (12 mg/kg, i.p.) and MK-801 (3 nmol/striatum) protected against MMA-induced seizures (evidenced by electrographic recording), protein carbonylation and TBARS production ex vivo. Furthermore, acute creatine administration increased the striatal creatine and phosphocreatine content and protected against MMA-induced creatine and phosphocreatine depletion. Our results suggest that an increase of the striatal high-energy phosphates elicited by creatine protects not only against MMA-induced convulsions, but also against MMA-induced oxidative damage. Therefore, since NMDA antagonists are limited value in the clinics, the present results indicate that creatine may be useful as an adjuvant therapy for methylmalonic acidemic patients.

Keywords: Creatine; Convulsion; TBARS; Protein carbonylation; L-methylmalonic acid; *N*-methyl-D-aspartate; Striatum; EEG

1. Introduction

Methylmalonic acidurias comprise a group of inherited metabolic disorders caused by a deficiency of the mitochondrial enzyme methylmalonyl CoA mutase (MCM, EC 5.4.99.2), or by defects in the synthesis of 5′-deoxyadenosylcobalamin, the cofactor of MCM. Deficient MCM, which physiologically catalyses the reaction of methylmalonyl CoA to succinyl CoA, leads to the primary accumulation of methylmalonyl CoA, and to the secondary accumulation of other metabolites, such as propionate, 3-hydroxypropionate and 2-methylcitrate (Fenton et al., 2001). The affected infants present a variable degree of mental retardation and severe neurological dysfunction, such as delayed development, seizures, demyelination and cerebral edema of the white matter (Roodhooft et al., 1990; Brismar and Ozand, 1994; Fenton et al., 2001). Histopathology and neuroimaging studies revealed severe necrosis as well as symmetric degeneration of the basal ganglia in these patients (Brismar and Ozand, 1994). Furthermore, it has been shown that patients with methylmalonic acidemia, during acute metabolic crises, present elevated amounts of lactate in globus pallidus suggesting neuronal damage, via inhibition of mitochondrial energy metabolism (Trinh et al., 2001). In this context, MMA seems to generate other neurotoxins, such as malonic acid, an

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inhibitor of complex II, and 2-methylcitrate, a compound with multiple inhibitory effects on the tricarboxylic acid cycle (Okun et al., 2002), which might contribute for the metabolic collapse and secondary excitotoxic mechanism induced by MMA exposure. Interestingly, the SDH substrate, succinate, has been recently proposed to play a neurotoxic role in methylmalonic acidemia (Roehrs et al., 2004). In fact, there is a considerable body of evidence suggesting that MMA impairs mitochondrial function, since it increases lactate production ex vivo and in vitro (Wajner et al., 1992; Greenamyre et al., 1994; Royes et al., 2003), decreases ATP (McLaughlin et al., 1998) and phosphocreatine levels (Royes et al., 2003), CO_2 production (Wajner et al., 1992) and O_2 utilization (Toyoshima et al., 1995).

Acute creatine administration increases striatal phosphocreatine levels and protects against MMA-induced convulsions and phosphocreatine depletion (Royes et al., 2003). In line with this view, creatine protects against MMA-induced neurotoxicity in primary neuron cultures, probably by increasing energy phosphates (Kölker et al., 2000).

Besides its inhibitory role on the energetic metabolism, an excitatory role for MMA was demonstrated. Accordingly, it has been shown that MMA causes convulsive behavior through glutamatergic mechanisms (de Mello et al., 1996; Malfatti et al., 2003), and striatal degeneration (Narasimhan et al., 1996). The depolarizing effect of MMA on isolated neurons and astrocytes, has also been demonstrated (McLaughlin et al., 1998), as well as its ability to induce LTP in the striatum (Calabresi et al., 2001). Therefore, excessive glutamate receptor stimulation, in particular the NMDA receptor, has been implicated as a major pathway that leads to MMA-induced convulsions (de Mello et al., 1996; Royes et al., 2003). More recently, reactive oxygen species (ROS) have been implicated in the convulsive behavior elicited by MMA, since it has been shown that intrastriatal MMA administration, besides causing convulsive behavior, increases local thiobarbituric acid reacting substances (TBARS) content and inhibits Na^+,K^+-ATPase activity (Malfatti et al., 2003). Moreover, while the systemic administration of antioxidants, such ascorbic acid, α-tocopherol and GM1 (Fighera et al., 1999, 2003) attenuate, ammonia, a pro-oxidant agent, increases MMA-induced convulsions (Marisco et al., 2003). However, until the present moment, it is not known if the ergogenic compound creatine, which has been proposed as a possible adjunct treatment for methylmalonic acidemic patients, also protects against MMA-induced oxidative damage. Therefore, in the present study we decided to investigate whether creatine protects against the behavioral, electrographic and oxidative effects of the intrastriatal injection of MMA.

2. Materials and methods

2.1. Animals 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. All experimental protocols were designed aiming to keep the number of animals used to a minimum, as well as their suffering. All experimental protocols were conducted in accordance with national and international legislation (guidelines of Brazilian College of Animal Experimentation (COBEA) and of U.S. Public Health Service's Policy on Humane Care and Use of Laboratory Animals — PHS Policy), and with the approval of the Ethics Committee for Animal Research of the Federal University of Santa Maria. All reagents were purchased from Sigma (St. Louis, USA), except thiobarbituric acid (TBA), which was obtained from Merck (Darmstadt, Germany).

2.2. Surgical procedure and drug administration protocol

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, DV 4.2 mm for the striatum) (Paxinos and Watson, 1986). Chloramphenicol (200 mg/kg, i.p.) was administrated immediately before the surgical procedure. Three days after the surgery, the animals were injected with creatine (1.2, 3.6 or 12 mg/kg, i.p.) or saline (0.9% NaCl; 10 ml/kg, i.p.) 30 min before the intrastriatal administration of MMA (6 μmol/2 μl) or saline (9 μmol/2 μl). The involvement of NMDA receptor activation on the convulsions as well as alterations of striatal TBARS and carbonyl protein content induced by MMA was investigated by intrastriatally injecting the animals with 0.5 μl of saline (0.9% NaCl) or MK-801 (3 nmol) 30 min before the intrastriatal administration of MMA (4.5 μmol/1.5 μl) or saline (6.7 μmol/1.5 μl).

Immediately after the injections the animals were transferred to a round open field (54.7 cm in diameter) with a floor divided into 10 equal areas. The open field session lasted 15 min, and during this time the animals were observed for the appearance of convulsions. The number and duration of convulsive episodes were recorded (de Mello et al., 1996).

2.3. Placement of cannula and electrodes for EEG recordings

Rats were surgically implanted with a cannula and electrodes under stereotaxic guidance. In brief, rats were anesthetized with Equitesin and 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 ipsilaterally into striatum. For intrastriatal infusion of drugs, a guide cannula (27 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, −4.5; L, 2.5; and DV, 2 for the cortex and AP, 0; L, 3; DV, 4.2 for the 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.