Available online at www.sciencedirect.com
ScienceDirect
Neuroscience Letters 408 (2006) 151–154
Neuroscience Letters
www.elsevier.com/locate/neulet

Propionic acid induces convulsions and protein carbonylation in rats

Flávia Karine Rigo [a], Liana Pasquetti [a], Carlos Ricardo Maneck Malfatti [a,b], Michele Rechia Fighera [a], Rafael Correa Coelho [a], Cristiane Zanardo Petri [a], Carlos Fernando Mello [a,*]

[a] *Laboratório de Neurotoxicologia e Psicofarmacologia, Departamento de Fisiologia e Farmacologia, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil*
[b] *Departamento de Educação Física e Saúde, Universidade de Santa Cruz do Sul, 96815-900 Santa Cruz do Sul, RS, Brazil*

Received 19 May 2006; received in revised form 29 August 2006; accepted 30 August 2006

Abstract

Propionic acid (PA) accumulates in patients with propionic acidemia, an inherited metabolic disorder caused by the deficiency of propionyl-CoA carboxylase activity that is clinically characterized by neurological dysfunction, including seizures. However, it is not known whether PA causes seizures in experimental animals. In the current study, we investigated whether intrastriatal injection of PA (0.6–6 μmol) causes seizures and alters protein carbonyl content in the striatum of adult rats. The injection of PA caused the appearance of seizures and increased protein carbonyl content in injected and noninjected striata. PA-induced seizures and increased protein carbonylation in the striatum were prevented by the injection of MK-801 (3 nmol/0.5 μL). Our results suggest that PA causes seizures and oxidative damage by NMDA receptor-mediated mechanisms. The involvement of NMDA receptors in the pathogenesis of propionic acidemia is suggested.

Keywords: Propionic acidemia; Seizure; NMDA receptor; Neurotoxicity; Oxidative stress; Protein carbonyl

Propionic acid (PA) is found in high amounts in patients with propionic acidemia, an inherited disorder of the metabolism caused by severe deficiency of propionyl-CoA carboxylase activity [8,11]. While the affected patients present psychomotor delay/mental retardation and generalized seizures, the pathophysiology of the disease is poorly known. Recently, it has been proposed that primary or secondary excitotoxicity may play a significant role in a number of organic acidemias [9,10,22,23]. In this context, it has been suggested that PA metabolism generates methylcitrate [1], a citric acid cycle inhibitor [5]. It is well known that energy impairment can lead to secondary excitotoxicity due to failure of ATP-dependent ion pumps and consequent ionic gradient collapse, which results in neuronal depolarization, overactivation of NMDA glutamate receptors [25], and generation of reactive oxygen species [2]. In fact, PA can increase thiobarbituric acid reactive substances (TBARS) and chemiluminescence in brain homogenates, as well as the production of superoxide by human neutrophils [10,15]. Additionally, decreased erythrocyte tocopherol levels have been described in a propionic acidemic patient, suggesting the role of oxidative stress in this disorder [14].

It has also been proposed that reactive species are involved in the development of convulsions [17,19–21]. Since reactive species may play a role in the pathogenesis of propionic acidemia and seizures are a major finding in propionic acidemic patients, the present study investigated whether: (1) PA causes seizures; (2) glutamatergic mechanisms are involved in the currently reported convulsant action of PA; (3) PA increases protein carbonylation, a marker of protein oxidative damage [4].

All reagents were purchased from Sigma (St. Louis, MO, USA). Adult male Wistar rats (270–300 g) were used. All animal utilization protocols followed the Official Government Ethics guidelines and were approved by the University Ethics Committee.

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 dorsal striatum (coordinates relative to bregma: AP 0 mm, ML 3.0 mm, V 3.0 mm from the

* Corresponding author. Tel.: +55 552208031; fax: +55 55 3220 8241.
E-mail address: cf.mello@smail.ufsm.br (C.F. Mello).

0304-3940/$ – see front matter © 2006 Published by Elsevier Ireland Ltd.
doi:10.1016/j.neulet.2006.08.075

dura). Chloramphenicol (200 mg/kg, i.p.) was administrated immediately before the surgical procedure.

Seven to nine days after cannula placing, the animals were injected with 2 μl of propionic acid (0.6; 2; 6 μmol, pH adjusted to 7.4 with NaOH) or NaCl (9 μmol), which served as control. Immediately after the injections the animals were transferred to a round open field (54.7 cm in diameter), and during 15 min the latency for convulsions, number of convulsive episodes and total time spent convulsing were recorded.

The involvement of NMDA receptors in PA-induced convulsions was determined by intrastriatally injecting the animals with MK-801 (3 nmol/0.5 μl) or NaCl (0.9%) 30 min before the administration of PA (4.5 μmol/1.5 μl) or NaCl (8.25 μmol/1.5 μl). Seizure activity was confirmed electroencephalographically in a subset of animals by using the same protocol of injection used in the behavioral studies.

The procedures for EEG recording and intracerebral injection of drugs were carried out as previously described [9]. In brief, rats were deeply anesthetized and two screw electrodes placed bilaterally over the parietal cortex, with a ground lead positioned over the nasal sinus. Bipolar nichrome wire, Teflon-insulated depth electrodes (100 μm) were implanted unilaterally into the dorsal striatum [18]. The electrodes were connected to a multipin socket and, together with an injection cannula, were fixed to the skull with dental acrylic cement.

The animals were allowed to habituate a Plexiglas cage (25 cm × 25 cm × 60 cm) and then were connected to the lead socket in a swivel inside a Faraday's cage. 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 effect of MK-801 on PA-induced behavioral convulsions. EEG signals were amplified, filtered (0.1–50.0 Hz, bandpass), and recorded using an analogical encephalographer (Berger TP 119). Epochs (30 s) were selected from preinfusion and postinfusion periods to determine significant EEG changes.

Immediately after behavioral evaluation, the animals were sacrificed and the striata rapidly removed. Tissues were homogenized in 10 volumes (w/v) of 10 mM Tris–HCl buffer (pH 7.4), and the protein carbonyl content determined through methods described by Yan et al. [24], with some modifications [16]. Each sample was read at 370 nm and total carbonylation was calculated using a molar extinction coefficient of 22,000 M^{-1} cm^{-1}. Protein content was measured by the Bradford [3] method using bovine serum albumin as a standard.

The intrastriatal administration of PA (6 μmol/2 μl) initially caused the appearance of clonic convulsions involving fore and/or hindlimbs contralateral to the injected striatum, that eventually spread to the ipsilateral limbs after a time of continuous seizure activity [$H(3) = 18.16$; $p < 0.05$ (Fig. 1A) for number, and $H(3) = 18.03$; $p < 0.05$ (Fig. 1B) for duration of convulsive episodes].

Electroencephalographic recording confirmed that the intrastriatal administration of PA generated a depolarizing focus in the injected area, which spread to the ipsi- and, eventually, to the contralateral cerebral cortex (Fig. 2B and C). Seizures were defined by episodic incidence, consisting of the simultaneous occurrence of at least two of the following alterations in all three recording leads: high-frequency, polyspike complexes, and/or high-voltage synchronized spike activity. Seizures initiated 4–5 min after PA injection (Fig. 2B) and persisted (Fig. 2C) up to the end of the observation period (15 min). PA infusion induced the appearance of polyspikes and high-frequency complexes (Fig. 2B), as well as synchronous spiking involving the ipsilateral and contralateral cerebral cortex (Fig. 2C).

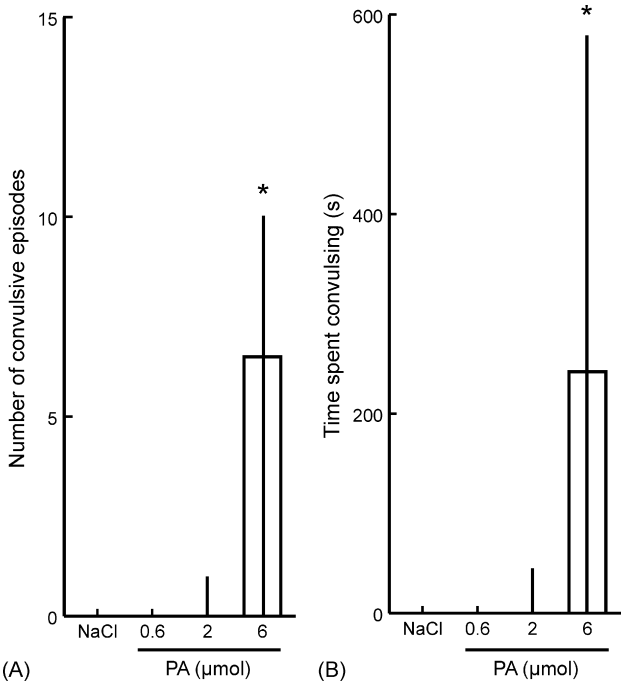

Fig. 1. Propionic acid induces clonic convulsions measured by number (A) and duration of convulsive episodes (B). Animals injected with NaCl or 0.6 μmol PA did not present convulsions. Data are median and interquartile ranges, $n = 9$–12 per group. $^{*}p < 0.05$ compared with control (NaCl) by the Kruskal–Wallis test, followed by the Dunn nonparametric multiple comparisons test.

PA-induced seizures were prevented by MK-801 (Fig. 2D and E). Statistical analysis of behavioral data (Scheirer–Ray–Hare extension of the Kruskal–Wallis test) revealed that MK-801 (3 nmol/0.5 μl) decreased the number [PA-injected: median (interquartiles): 2.5 (2–4.5) episodes; PA plus MK-801: 1 (0–1) episodes: $H(1) = 3.85$; $p < 0.05$], and duration [PA-injected: median (interquartiles): 89 (56–135) s; PA plus MK-801: 9 (0–15) s: $H(1) = 3.89$; $p < 0.05$] of convulsive episodes induced by PA, suggesting that NMDA receptors may play a role in PA-induced seizures. MK-801 also increased the latency to seizures [PA-injected: median (interquartiles): 251 (217–287) s; PA plus MK-801: 743 (478–900) s: $H(1) = 3.98$; $p < 0.05$].

Fig. 3 shows the effect of PA and MK-801 on the total protein carbonyl content of the striatum. Statistical analysis (three-way ANOVA followed by Student–Newman–Keuls test) revealed that the intrastriatal injection of MK-801 prevented the increase of total protein carbonyls induced by PA [$F(3,36) = 2.33$; $p < 0.05$], supporting previous evidence that PA induces oxidative damage [10,14].

In this study we showed that intrastriatal injection of PA causes seizures and protein carbonyl increase in the injected