Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Behavioural Brain Research 187 (2008) 411–416
BEHAVIOURAL BRAIN RESEARCH
www.elsevier.com/locate/bbr

Research report

# Chronic early postnatal glutaric acid administration causes cognitive deficits in the water maze

Gustavo da Costa Ferreira [a], Patrícia Fernanda Schuck [a], Carolina Maso Viegas [a], Anelise Tonin [a], César Augusto João Ribeiro [a], Leticia Ferreira Pettenuzzo [a], Lenir Orlandi Pereira [a], Carlos Alexandre Netto [a], Moacir Wajner [a,b,c,*]

[a] *Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*
[b] *Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil*
[c] *Universidade Luterana do Brasil, Canoas, RS, Brazil*

Received 30 July 2007; received in revised form 1 October 2007; accepted 7 October 2007
Available online 11 October 2007

**Abstract**

Glutaric acidemia type I (GA I) is an autosomal recessive metabolic disorder caused by glutaryl-CoA dehydrogenase deficiency leading to predominant accumulation of glutaric acid (GA), and to a lesser extent of 3-hydroxyglutaric acid (3HG) in body fluids and tissues. The clinical manifestations of GA I are predominantly neurological. Although the pathophysiological mechanisms responsible for the brain damage of this disease are virtually unknown, they are thought to be due to the neurotoxic actions of GA and 3HG. Therefore, in the present work we investigated whether chronic exposure of GA (5 μmol g of body weight$^{-1}$, twice per day), the major metabolite accumulating in GA I, during early development (from the 5th to the 28th day of life) could alter the cognitive performance of adult rats in the Morris water maze, open field and elevated plus maze tasks. Control rats were treated with saline in the same volumes. GA administration provoked an impairment of spatial performance in the water maze since adult rats pretreated with GA were not able to remember the previous location of the platform spending significantly less time in the training quadrant. In contrast, GA chronic administration did not affect rat performance in the open field and elevated plus maze tasks, indicating that motor activity and anxiety was not changed by GA. The results provide evidence that early chronic GA treatment induces long-lasting spatial behavioral deficit.
© 2007 Elsevier B.V. All rights reserved.

*Keywords:* Glutaric acidemia type I; Glutaric acid; Memory; Water maze; Rat

## 1. Introduction

Glutaric acidemia type I (GA I, McKusick 23167; OMIM #231670) is an autosomal recessive disease caused by deficiency of the activity of the mitochondrial enzyme glutaryl-CoA dehydrogenase (EC 1.3.99.7), which is involved in the catabolic pathway of lysine, hydroxylysine and tryptophan [1]. Increased concentrations of glutaric acid (GA), as well as 3-hydroxyglutaric (3HG), but in lesser amounts, are found in the body fluids and brain tissue of GA I patients [2,3]. Clinical manifestations of GA I are predominantly neurological, occurring especially after encephalopathic crises, which are accompanied by bilateral destruction of caudate and putamen with severe loss of medium-sized GABAergic neurons [3,4]. Fronto-operculo-temporal hypoplasia frequently detected at birth is a distinctive radiological appearance that may be pathognomonic for GA I. Over subsequent years, progressive involvement of the cortical white matter with vacuolization of cerebral structures (cerebral atrophy) occurs leading to cognitive impairment [5]. Although the neuropathological findings are pronounced in this disease, the mechanisms underlying the acute and chronic progressive brain damage of GA I are not completely defined to date. However, various *in vivo* and *in vitro* studies have shown neurotoxic effects of GA and 3HG, including excitotoxicity [6–10],

* Corresponding author at: Departamento de Bioquímica, ICBS, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2600 Anexo, CEP 90035-003, Porto Alegre, RS, Brazil. Tel.: +55 51 3308 5571; fax: +55 51 3308 5539.
*E-mail address:* mwajner@ufrgs.br (M. Wajner).

0166-4328/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2007.10.003

oxidative damage [11–15] and disruption of energy metabolism [16–21].

We have recently induced in our laboratory high sustained brain GA concentrations similar to those found in GA I patients by injecting GA subcutaneously to developing rats [20]. In the present study, we tested this chemically-induced animal model during early development (5th to the 28th day of life) on the cognitive performance of adult rats in various behavioral tasks in order to test whether chronic GA administration to infantile rats could provoke long-standing or permanent brain damage involving cerebral structures involved in learning/memory.

## 2. Material and methods

### 2.1. Chemicals

Unless otherwise stated, reagents were purchased from Sigma (St. Louis, MO, USA).

### 2.2. Subjects

A total of 16 male Wistar rats obtained from the Central Animal House of the Departamento de Bioquímica, ICBS, UFRGS, were used. Pregnant rats were housed in individual cages and left undisturbed during gestation. Forty-eight hours after delivery, litters were culled to eight male pups; rats were weaned at 21 days of life. The animals were divided so that in each cage there was the same number of rats for each treatment. The animals had free access to water and to a standard commercial chow and were maintained on a 12:12 h light/dark cycle in an air-conditioned constant temperature ($22 \pm 1\,^{\circ}$C) colony room. The "Principles of Laboratory Animal Care" (NIH publication no. 80-23, revised 1996) were followed in all experiments and the experimental protocol was approved by the Ethics Committee for Animal Research of the Federal University of Rio Grande do Sul, Porto Alegre, Brazil. All efforts were made to minimize the number of animals used and their suffering.

### 2.3. In vivo treatment

Saline-buffered GA, pH 7.4 (5 $\mu$mol g of body weight$^{-1}$) was administered subcutaneously, twice a day, from the 5th to the 28th day of life to produce brain concentrations of GA of around 0.6 $\mu$mol g$^{-1}$, ~0.72 mM [20]. Control animals received saline subcutaneously in the same volumes and frequency. All solutions were prepared so that each animal received 10 $\mu$L solution. g of body weight$^{-1}$.

### 2.4. Physical development

Sixteen male rats were used for the neurobehavioral development studies. Maturation of physical characteristics was determined daily at the appropriate ages by one experimenter that was not aware of the subject condition. Litters were inspected between 12:00 and 15:00 h, and progress of physical development was followed throughout the experiment. The date of appearance of hair, eruption of upper incisors, and eye opening was recorded using previously reported criteria [22,23] described in detail by Smart and Dobbing [24].

### 2.5. Free-fall righting task

On postnatal day 14, animal ability to turn in midair to land on all fours after being dropped back downwards from 35 cm onto a cotton wool pad was measured. Each animal was tested in three consecutive trials spaced 15 s apart, and scored one point if it landed ventrally, with all legs distant from the body in each trial [22]. Therefore, a maximum of three points could be assigned to each animal.

### 2.6. Cognitive tasks

All behavioral tests were performed between 14:00 and 17:00 h. Each experimental group was tested in the different cognitive tasks with a two-day interval in between. Animals were then tested in the elevated plus maze on the 60th and 61st postnatal day, and on the open field task on postnatal days 64 and 65. Finally, behavior of rats in the Morris water maze was assessed from the 68th to the 77th postnatal day.

### 2.7. Morris water maze task

The experimental procedure was carried out as previously described [25]. Briefly, the animals were left to recover for approximately one month after GA treatment. Spatial learning/memory was tested in the Morris water maze [26], which consisted of a black circular pool (200 cm in diameter, 100 cm high), theoretically divided into four equal quadrants for the purpose of analysis. The pool was filled to a depth of 50 cm with water ($23 \pm 1\,^{\circ}$C) made opaque by the addition of milk. The escape platform was transparent, had a diameter of 10 cm and was placed 2 cm below the water surface. The experimenter remained at the same location on each trial, corresponding to the adjacent target quadrant, approximately 50 cm from the outside edge of the tank, on each trial. A video camera was mounted above the center of the tank and all trials were recorded. The room was dimly illuminated in order to provide extra-maze clues so allowing rats to develop a spatial map strategy. Two black and white large cartoons were hanged on the walls.

#### 2.7.1. Reference memory test

Rats had daily sessions of 4 trials per day for 5 days to find the submerged platform that was located in the center of a quadrant of the tank and remained there throughout training. We observed that all animals of each group were able to swim in a normal way during all trials. On each trial the rat was placed in the water, facing the edge of the tank, in one of the four standard start locations (N, S, W and E). The order of the start locations was varied in a quasi-random sequence so that, for each block of four trials, any given sequence was not repeated on consecutive days. The rat was then allowed 60 s to search for the platform. Latency to find the platform (escape latency) and swimming speed were measured in each trial. Once the rat located the platform, it was permitted to remain on it for 10 s. If it did not find the platform within this time, it was guided to it and allowed to remain on it for 10 s. After each trial, the rats were removed, dried in a towel and put back in their home cages. The interval between trials was 15–20 min [27].

#### 2.7.2. Probe trial

One day after the last training session, each rat was subjected to a probe trial (60 s) in which there was no platform present. The time spent in the quadrant of the former platform position, the time spent in the opposite quadrant, the correct annulus crossing, i.e., the number of times animals passed through the circular area that formerly contained the submerged platform during acquisition, and the latency to cross over the platform place for the first time were taken as a measure for spatial memory.

#### 2.7.3. Working memory (repeated acquisition) test

Working memory test was tested a week after the probe test, consisting of four trials per day during four consecutive days. The working memory test was procedurally similar to reference memory test except that the platform location was changed daily. The first trial of the day was an informative sample trial in which the rat was allowed to swim to the platform in its new location. Spatial working memory was regarded as the mean escape latency of the second until the fourth trial.

### 2.8. Open field task

The apparatus consisted of a wooden box measuring 60 cm × 40 cm × 50 cm with a glass front wall, whose floor was divided by black lines into 12 equal squares. The animals were gently placed facing the rear left corner of the arena and the number of squares crossed with the four paws recorded for 5 min to eval-

Download English Version:

https://daneshyari.com/en/article/4315536

Download Persian Version:

https://daneshyari.com/article/4315536

Daneshyari.com