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Intracerebroventricular D-galactose administration impairs memory and alters activity and expression of acetylcholinesterase in the rat

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ABSTRACT

Tissue accumulation of galactose is a hallmark in classical galactosemia. Cognitive deficit is a symptom of this disease which is poorly understood. The aim of this study was to investigate the effects of intracerebroventricular administration of galactose on memory (inhibitory avoidance and novel object recognition tasks) of adult rats. We also investigated the effects of galactose on acetylcholinesterase (AChE) activity, immunocontent and gene expression in hippocampus and cerebral cortex. Wistar rats received a single injection of galactose (4 mM) or saline (control). For behavioral parameters, galactose was injected 1 h or 24 h previously to the testing. For biochemical assessment, animals were decapitated 1 h, 3 h or 24 h after galactose or saline injection; hippocampus and cerebral cortex were dissected. Results showed that galactose impairs the memory formation process in aversive memory (inhibitory avoidance task) and recognition memory (novel object recognition task) in rats. The activity of AChE was increased, whereas the gene expression of this enzyme was decreased in hippocampus, but not in cerebral cortex. These findings suggest that these changes in AChE may, at least in part, to lead to memory impairment caused by galactose. Taken together, our results can help understand the etiopathology of classical galactosemia.

1. Introduction

Classical galactosemia (OMIM #230400) is a genetic disorder caused by recessive mutations in the gene encoding the enzyme galactose-1-phosphate uridylyltransferase (GALT, EC 2.7.7.12) and about 200 mutations were found in humans (Calderon et al., 2007; Fridovich-Keil and Walter, 2008). These mutations decrease or null the GALT activity leading to the accumulation of galactose (GAL), galactose-1-P and galactitol (Berry, 2011; Mahmood et al., 2012; McCorvie and Timson, 2011), metabolites believed to cause cellular toxicity in brain and other tissues (Fridovich-Fridovich-Keil and Walter, 2008; Fridovich-Keil, 2006; Ridel et al., 2005). GAL is the first metabolite in the pathway (Fridovich-Keil and Walter, 2008) and is found in high concentration in liver and brain of untreated patients (Berry, 2011).

The first signs of the disease appear in the first weeks of life and include diarrhea, vomiting, hepatomegaly, jaundice, septicemia and kidney malfunction; untreated newborns might die within the firsts days of life (Fridovich-Keil and Walter, 2008; Holton, 1996; Waggoner et al., 1990). The main treatment is dietary restriction of GAL, nevertheless symptoms like impaired cognitive and motor function are commonly found (Fridovich-Keil and Walter, 2008; Fridovich-Keil et al., 2011; Jumbo-Lucioni et al., 2013; Kaufman et al., 1995; Potter et al., 2013). However, Berry et al. (1995) have shown that even patients following a GAL restrict diet might present increased GAL circulating levels due to endogenous production, a finding possibly associated to brain damage.

As the exact mechanism behind the pathology of classical galactosemia remains obscure (Jumbo-Lucioni et al., 2013), an experimental murine model of this condition has been described where GAL levels are similar to those found in the plasma of patients with classical galactosemia. However this has been considered a poor model in reproducing the common complications found in the

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suckling period, like the behavioral dysfunction (Leslie et al., 1996; Ridel et al., 2005). Moreover, GAL was found to promote oxidative stress and a decrease of life span in the drosophila GALT *knockout* model (Jumbo-Lucioni et al., 2013).

The cholinergic system plays a key role in the modulation of learning and memory in mammals (Kukolja et al., 2009) and has also been associated with inflammation processes (Pavlov et al., 2009; Scherer et al., 2014). Acetylcholinesterase (AChE), the enzyme responsible for the termination of cholinergic transmission, is attached to the outside of the plasmatic membrane (Pohanka, 2011) and its hydrolytic activity may be altered by oxidative stress (Melo et al., 2003).

In the present study, we investigated the effects of an intracerebroventricular administration of GAL on the memory of inhibitory avoidance and novel object recognition tasks, as well as on the activity, the immunocontent and gene expression of AChE in the hippocampus and the cerebral cortex of Wistar rats. These cerebral structures were chosen because of their roles in memory modulation (Izquierdo and Medina, 1997) and their involvement in chemical models of inborn errors of metabolism using wild-type rats (Ferreira et al., 2011; Schweinberger et al., 2014; Stefanello et al., 2011; Streck et al., 2002; Vuaden et al., 2016; Wyse et al., 1995).

2. Material and methods

2.1. Animals and ethics approval

Sixty-days-old male Wistar rats were used; all animals were obtained from the Central Animal House of Biochemistry Department of Universidade Federal do Rio Grande do Sul (UFRGS). Rats were kept on a 12:12 h light-dark cycle in an air-conditioned room under constant temperature (22 ± 1 °C), with free access to water and 20% (w/w) protein commercial chow. All animal procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication number 80-23 revised 1996) and the Brazilian Society for Neuroscience and Behavior recommendations for animal care. The research project was previously approved by the Ethics Committee of the University under the protocol number 27786.

2.2. Stereotaxic surgery and cannula placement

Rats were anesthetized with ketamine and xylazine (75 and 10 mg/kg i.p., respectively) and placed in a rodent stereotaxic apparatus. Under stereotaxic guidance, a 27- gauge stainless cannula (0.9 mm o.d.) with an inner needle guide was inserted unilaterally into the right ventricle (coordinates relative from bregma: AP, −0.9 mm; L, −1.5 mm; DV, −2.6 mm) (Paxinos et al., 1985). The cannula was fixed to the skull with dental cement. Two days after surgery, a 30-gauge needle was inserted into the guide cannula in order to inject 5 μL of D-galactose (4 mM) diluted in saline or vehicle (saline) into the right ventricle, over a 5 min interval. The tip of the infusion needle protruded 1.0 mm beyond the guide cannula towards the right cerebral ventricle. Animals were randomly divided into two groups: group 1 (vehicle treated), rats that received intracerebroventricular saline and group 2 (GAL treated), rats that received intracerebroventricular GAL solution (Bavaresco et al., 2008). Since untreated patients accumulate millimolar concentration of GAL in the brain (Berry, 2011), we have chosen to adjust the GAL concentration based on previous reports of Tsakiris et al. (2002, 2005). For biochemical assays, animals were decapitated 1 h, 3 h or 24 h after drug administration, the brain was removed and cerebral structures (hippocampus and cerebral cortex) were dissected out.

2.3. Behavioral procedures

All behavioral procedures were performed between 10 a.m. to 3 p.m. in a controlled light and sound room, by a researcher blind to the animal's experimental condition. For the assessment of cognitive parameters (step-down inhibitory avoidance and novel object recognition) GAL was injected 1 h before the training session, in order to evaluate the process of memory acquisition. The test session was performed 24 h after the training to assess long-term memory (Bavaresco et al., 2008).

2.3.1. Step-down inhibitory avoidance

Animals were subjected to training and test sessions in a step-down inhibitory avoidance task with an interval of 24 h between the sessions. The task was performed in an automatically operated, brightly illuminated box. The left extreme of the grid was covered by a 7.0 cm-wide, 2.5 cm-high formic platform. In the training session, animals were placed on the platform and their latencies to step down, placing their four paws on the grid (42.0 × 25.0 cm grid of parallel 0.1 cm caliber stainless steel bars spaced 1.0 cm apart), was measured. In the test session, no foot shock was delivered and step-down latency (with a ceiling of 180s) was used as a measure of memory retention as described by Wyse et al. (2004).

2.3.2. Novel object recognition

One day before the training session, all animals were habituated to walk freely in the empty arena for 10 min. The arena used was a black wooden box (50 × 50 × 50 cm). In the training session, two identical objects were placed equidistant from the sidewalls. In this chamber, each animal performed a trial of 10 min. After each trial, the apparatus was cleaned to alleviate olfactory cues. In the second trial, the test session, one of the objects was substituted by a different. An experimenter registered the time of object exploration, i.e., touching it with paws or exploring it by olfaction with direct contact of the snout (Plamondon et al., 2006). The object discrimination index was calculated in the test session, as follows: the difference in exploration time divided by the total time spent exploring the two objects {[(B − A)/(A + B)] where B is the new object and A is the familiar object}. This task was performed according to Pereira et al. (2008); Rojas et al. (2013).

2.4. Biochemical analyses

2.4.1. AChE activity assay

Hippocampus and cerebral cortex were homogenized in ten volumes (1:10; w/v) of 0.1 mM potassium phosphate buffer, pH 7.5 and centrifuged for 10 min at 1.000 *g*. Hydrolysis rates were measured at acetylcholine concentration of 0.8 mM in 300 μL assay solution with 30 mM phosphate buffer, pH 7.5, and 1.0 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) at 25 °C. Supernatant from homogenized tissue was added to the reaction mixture and pre incubated for 3 min. The hydrolysis was monitored by formation of the thiolate dianion of DTNB at 412 nm for 2–3 min (intervals of 30 s) (Ellman et al., 1961), with modifications (Delwing et al., 2003; Scherer et al., 2010). All samples were run in triplicate.

2.4.2. AChE western blot

Hippocampus and cerebral cortex were homogenized in lysis solution (2 mM EDTA, 50 mM Tris-HCl, pH 6.8, plus 4% SDS) and a part of samples was taken for protein determination. The remainder was mixed (v/v) in laemmli buffer (40% glycerol, 5% mercaptoethanol, 50 mM Tris–HCl, pH 6.8) and then boiled for 3 min. In this analysis, samples were loaded (30 μg of protein/lane) in 10% polyacrylamide SDS-PAGE. The proteins were then transferred (Trans-blot SD semidry transfer cell; Bio-Rad) to nitrocellulose membranes, for 1 h at 15 V in transfer buffer (48 mM Trizma, 39 mM