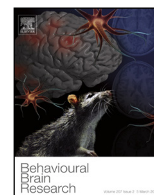
Behavioural Brain Research 316 (2017) 205–214

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr



Research report

Hippocampal changes in STZ-model of Alzheimer's disease are dependent on sex

Regina Biasibetti, João Paulo Almeida dos Santos, Letícia Rodrigues, Krista Minéia Wartchow, Lucas Zingano Suardi, Patrícia Nardin, Nicholas Guerini Selistre, Dandara Vázquez, Carlos-Alberto Gonçalves*

Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Ramiro Barcelos, 2600-anexo, 90035-003, Porto Alegre, RS, Brazil

HIGHLIGHTS

- Alzheimer's disease (AD) has prevalence dependent on gender.
- We investigated alterations dependent on sex in a streptozotocin model of AD in rats.
- Hippocampal parameters were investigated 2, 4 and 8 weeks after streptozotocin.
- Cholinergic neurons and glucose uptake decrease were dependent on sex.
- S100B alteration in the hippocampus were also dependent on sex, but not GFAP.

ARTICLE INFO

Article history:
Received 25 May 2016
Received in revised form 24 August 2016
Accepted 28 August 2016
Available online 29 August 2016

Keywords:
Alzheimer
Astrocyte
ChAT
Glucose uptake
Hippocampus
Sex

ABSTRACT

The majority of Alzheimer's disease (AD) cases are sporadic and aging is the major risk factor for developing the disease, affecting more women than men. In spite of different gender prevalence, most experimental studies in animal models have been performed in male. This study investigates the streptozotocin (STZ)-induced AD model at three different times (2, 4 and 8 weeks afterwards) and in male and female rats, evaluating cognitive deficit, cholinergic neurotransmission, glucose uptake, glutathione content and specific glial markers (GFAP and S100B protein) in the hippocampus of the rat. Our data reinforce the relevance of alterations in STZ model of dementia, reported in the genesis and/or progression of AD such as cholinergic deficit and glucose uptake decrease. All alterations in these parameters (except GFAP) were dependent on sex. It is unclear, at this moment, which alterations are due to sex steroid modulation. In spite of limitations of this experimental model, these data may contribute to understand AD susceptibility and progression dependent on sex.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Alzheimer's disease (AD) is the most common type of dementia, comprising 60–80% of the cases and is characterized by the memory loss and multiple cognitive dysfunctions [26]. The majority of AD cases are sporadic (about 95%) and aging seems to be the major non-genetic risk factor for the developing the disease, with the prevalence doubling every five years after the age of 65. It is possible to highlight other factors, such as life style, medical history, level of education and gender [34]. There is a higher proportion of women suffering from AD than men [63].

These sex differences are likely to be mediated both via actions of sex steroid hormones, as well as by differences in neurochemical substrates between men and women. According to [35], several normal age-related changes significantly increase AD risk including (i) estrogen depletion associated with menopause, (ii) age-related decreases in testosterone in men, and (iii) increasing adiposity in men and women. Since both estrogen and testosterone regulate adiposity, there are possible interactions between sex steroid hormones, adiposity, and AD risk that may be expected to exhibit sex differences. This sex bias has also been studied in some mouse models of AD and female mice exhibited more aggressive senile plaque

* Corresponding author: Departamento de Bioquímica, ICBS, Universidade Federal do Rio Grande do Sul, Ramiro Barcelos, 2600-Anexo, 90035-003, Porto Alegre, RS, Brazil.

E-mail address: casg@ufrgs.br (C.-A. Gonçalves).

http://dx.doi.org/10.1016/j.bbr.2016.08.057
0166-4328/© 2016 Elsevier B.V. All rights reserved.

pathology compared to male mice. However, the molecular basis underlying this gender bias in mouse has not been fully solved.

There are many types of animal models to help us study and comprehend Alzheimer's disease. One of the non-transgenic models used for this aim is the intracerebroventricular (ICV) injection of streptozotocin (STZ). When STZ is injected in both lateral ventricles, it provides a relevant model of chronic brain dysfunction that is characterized by long-term and progressive deficits in learning, memory, and cognitive behavior.

Histopathologically, the AD is characterized by extensive cortical neuronal loss (where cholinergic fibers in the hippocampus are early affected), presence of senile plaques and intracellular neurofibrillary tangles. In transgenic murine models of AD the cholinergic neurotransmission alterations are also more pronounced in the hippocampus [12]. It is also known that brain tissue from AD patients presents a solid disturbed metabolism of glucose – defective insulin signaling and oxidative stress [24,54]. Such alterations are found in STZ model of AD and are very useful to understand the mechanisms of disease.

We have used the STZ model of AD to test the neuroprotection of several compounds such as aminoguanidine [47], statins [58], antioxidants [4] as well as non-pharmacological strategies [48], such as physical exercise, focusing on astroglial parameters. In fact, our data reinforce the importance of astrocyte in the basis of AD, as well as therapeutic targets. Moreover considering the brain role of astrocytes, in health and disease, has been proposed that these cells are key elements to understanding the genesis of many neuropsychiatric disorders whose prevalence exhibit a gender-bias [13,51].

In spite of different gender prevalence pointed out in epidemiological studies, most experimental studies in animal models have been performed in male, and the effects of gender in a STZ-model of AD have not been examined. Therefore, the present study has been designed to investigate the STZ-induced AD model at three different times (2, 4 and 8 weeks after STZ administration) and in both genders, evaluating cognitive deficit, cholinergic neurotransmission, glucose uptake, glutathione content and specific glial markers in the hippocampus of the rat brain.

2. Materials and methods

2.1. Chemicals

Streptozotocin, 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), o-phenylenediamine (OPD), o-phthaldialdehyde (OPA), *meta*-phosphoric acid, sodium nitrate, nitrate reductase, o-phenylenediamine, and monoclonal anti-S100B antibody were purchased from Sigma. Anti-S100 antibody conjugated with peroxidase and anti-GFAP antibody were from Dako. Peroxidase secondary antibodies were from Amersham. All other chemicals were purchased from local commercial suppliers.

2.2. Animals

A total of a hundred and six, three to four-month-old, males and females Wistar rats weighing 300–400 g were obtained from our breeding colony (at the Department of Biochemistry, Universidade Federal do Rio Grande do Sul), and were maintained under controlled light and environmental conditions (12 h light/12 h dark cycle at a constant temperature of 22 ± 1 °C) with free access to food and water. All animal experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23) revised 1996, and following the regulations of the local animal house authorities.

Rats were divided into 2 groups: sham and STZ. After behavioral tasks, rats were anaesthetized, hippocampi were cut out from the brain and hippocampal slices prepared to glucose uptake assay or to be immediately frozen and stored at −80 °C, aiming to evaluate S100B and GFAP contents, choline acetyltransferase (ChAT) and glutathione contents (GSH).

2.3. Surgical procedure

Streptozotocin was intracerebroventricular infused, based on previous studies [4,47]. Briefly, on the day of the surgery animals were anesthetized with ketamine/xylazine (75 and 10 mg/kg, respectively, i.p.) and placed in a stereotaxic apparatus. A midline sagittal incision was made in the scalp. Burr holes were drilled in the skull on both sides over the lateral ventricles. The lateral ventricles were accessed using the following coordinates: 0.9 mm posterior to bregma; 1.5 mm lateral to sagittal suture; 3.6 mm beneath the surface of the brain. Rats received a single bilateral infusion of 5 μL STZ (3 mg/kg) or vehicle (Hank's balanced salt solution – HBSS – containing in mM: 137 NaCl; 0.63 Na_2HPO_4; 4.17 $NaHCO_3$; 5.36 KCl; 0.44 KH_2PO_4; 1.26 $CaCl_2$; 0.41 $MgSO_4$; 0.49 $MgCl_2$ and 10 glucose, in pH 7.4) using a Hamilton syringe. After the surgical procedure, rats were placed on a heating pad to maintain body temperature at 37.5 ± 0.5 °C and were kept there until recovery from anesthesia. The animals were submitted to behavioral tasks and biochemical analysis at 2, 4 and 8 weeks after STZ injection. A schematic representation of the experimental procedure is shown in Fig. 1, indicating times of surgery (STZ or vehicle infusion), cognitive behavior and biochemical analysis.

2.4. Cognitive evaluation

The object recognition test (ORT) is a simple behavioral assay of memory that primarily relies on a rodent's innate exploratory behavior in the absence of externally applied rules or reinforcement [6]. This task comprised three phases: habituation, training and test; each session had five-minute duration and was performed in an open field apparatus (50 cm side). To habituate the animals, rats were placed in the center of the apparatus and allowed to freely explore the open-field arena in the absence of objects. Twenty-four hours after the habituation phase, the animals underwent the training phase: the rat was returned to the apparatus that contained two identical sample objects (A + A). 2 h and 24 h after the training phase, the rats returned to the apparatus to test short-term (STM) or long-term (LTM) memory, respectively. In the test session, the rat was returned to the open-field arena that contained two objects; one object was identical to the training session and the other object was novel (A + B). For LTM, the object 'B' was replaced by a third one, and the 'A' object was maintained the same. The recognition index in each session was calculated as follows: time exploring the novel object/time exploring both objects. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. The apparatus and the objects were thoroughly cleaned with 90% ethanol between the trials to ensure the absence of olfactory cues.

2.5. Quantification of S100B and GFAP

S100B content in the hippocampus was measured by ELISA [33]. Briefly, 50 μL of sample plus 50 μL of Tris buffer were incubated for 2 h on a microtiter plate previously coated with monoclonal anti-S100B (SH-B1). Polyclonal anti-S100B was incubated for 30 min and then peroxidase-conjugated anti-rabbit antibody was added for a further 30 min. A colorimetric reaction with o-phenylenediamine was measured at 492 nm. The standard S100B curve ranged from 0.020 to 10 ng/mL. ELISA for GFAP [59] was carried out by coating the microtiter plate with 100 μL samples containing 30 μg of pro-

Download English Version:

https://daneshyari.com/en/article/4312015

Download Persian Version:

https://daneshyari.com/article/4312015

Daneshyari.com