Epilepsy Research 135 (2017) 14–18

Contents lists available at ScienceDirect

Epilepsy Research

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

The influence of temperature on adult zebrafish sensitivity to pentylenetetrazole

Fabiano Peres Menezes, Rosane Souza Da Silva*

Laboratório de Neuroquímica e Psicofarmacologia, Departamento de Biologia Celular e Molecular, Faculdade de Biociências, PUCRS, Porto Alegre, RS, Brazil

ARTICLE INFO

Keywords:
Gender
NMDA receptor
Seizure
Temperature
Weight and Zebrafish

ABSTRACT

Pentylenetetrazole (PTZ) is one of the most valuable drugs used to induce seizure-like state in zebrafish especially considering the pharmacological screening for anticonvulsants and the study of basic mechanisms of epilepsy. Here, the effect of gender, weight and changes in temperature on latency to adult zebrafish reach classical seizure states induced by PTZ (10 mM) was evaluated. Gender and weight (200–250 mg *versus* 400–500 mg) did not affect the profile of response to PTZ. When water temperature was changed from 22 to 30 °C the lower temperature increased the latency time to reach seizure states and the higher temperature significantly decreased it, in comparison to the control group maintained at 26 °C. The blockage of kainate receptors by DNQX (10 μM) were unable to prevent the increased susceptibility of adult zebrafish exposed to hyperthermia and PTZ-induced seizures. The NMDA block by MK-801 (2.5 μM) prevented the additive effect of hyperthermia on PTZ effects in adult zebrafish. This report emphasize that PTZ model in adult zebrafish exhibits no confounder factors from gender and weight, but water temperature is able to directly affect the response to PTZ, especially through a mechanism related to NMDA receptors.

1. Introduction

The search for models that mimic diseases and/or manifestation of symptoms observed in specific diseases has been the subject of many studies, since as more accurate the model becomes more effective is that tool to the search for treatments. In diseases such as epilepsy, which affects approximately 50 million people in worldwide (Prilipko et al., 2005; World Health Organization, 2016), there is a number of models that seek to emulate in a reliable way the characteristics found in this disease (Albala et al., 1984; Bonan et al., 2000; Haas et al., 2001; Porter et al., 2006).

The use of zebrafish to model seizure has proved to be very efficient as regards the understanding of the various mechanisms involved in the etiology of this pathology, by the possibility of use in large-scale of the genetic and pharmacologic screening (Cunliffe, 2016; Hortopan et al., 2010). Pentylenetetrazole (PTZ) is the widest validated pharmacological proconvulsant used in zebrafish to perform anticovulsivant screenings and elucidate the pathogenetic mechanism of epilepsy (Baraban et al., 2005; Baxendale et al., 2012; Cunliffe, 2016; Da Silva et al., 2016) PTZ-based models offer behavioral changes similar to clonic-like convulsion (Mussulini et al., 2013; Orellana-paucar et al., 2013), epileptiform discharges (Baraban et al., 2005), reduced neurogenesis (Kim et al., 2010) and c-*fos* expression (Baraban et al., 2005; Baxendale et al., 2012). The basis of PTZ mechanism is the block of GABAA receptors, which is soon expressed in zebrafish embryos promoting responsiveness to PTZ as early as 50 hours post-fertilization (Baxendale et al., 2012).

To promote accuracy in the translation of research findings using zebrafish, several particularities of the zebrafish biology, and also must be considered. Here, we evaluated the effect of gender, weight and changes in water temperature on PTZ-sensitivity of adult zebrafish. Additionally, we evaluated the influence of antagonists of glutamatergic receptors on the PTZ effects under hyperthermia conditions.

2. Methods

2.1. Animals

All animals were from the local breeding of Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil. They were kept on a shelf of aquariums with circulating water system and automated controllers of water quality ZEBTEC (Tecniplast Group Buguggiate (VA), Italy). The light/dark cycle was 14/10 h, the water temperature was 27 °C ± 1 and a maximum density of 5 animals/L was maintained. A total of 169 animals were used in the range of 5-7 months post-fertilization. No deaths were registered during the experi-

* Corresponding author at: Faculdade de Biociências, PUCRS, Avenida Ipiranga, 6681,90619 – 900, Porto Alegre, RS, Brazil.
E-mail address: rosane.silva@pucrs.br (R.S. Da Silva).

http://dx.doi.org/10.1016/j.eplepsyres.2017.05.009
Received 16 March 2017; Received in revised form 26 April 2017; Accepted 19 May 2017
Available online 22 May 2017

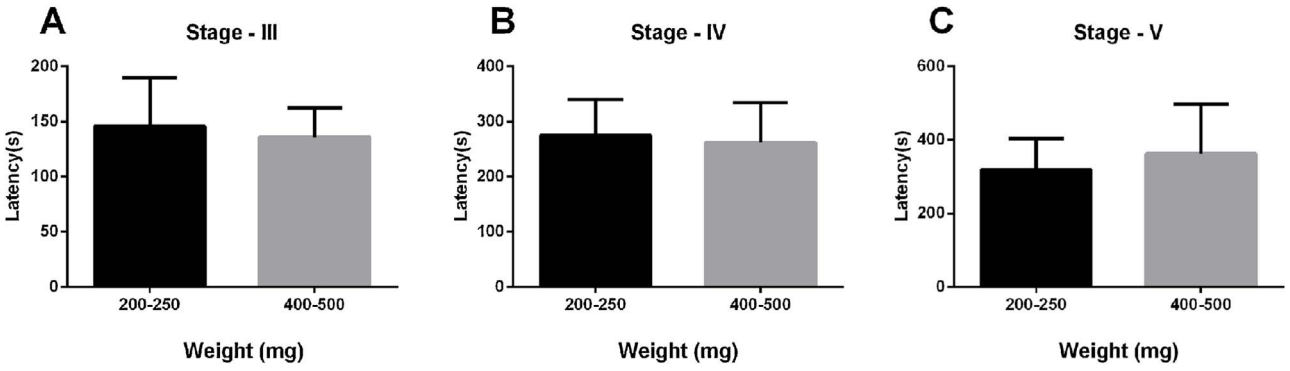

Fig. 1. Latency time of male zebrafish weighted 200–250 mg (N = 13) and 400–500 mg (N = 10) to reach the seizure stages caused by exposure to PTZ (10 mM) at 26 °C. Latency times to reach the stage III (A), IV (B) and V (C) of seizure are expressed as mean ± C.I. N represents the number of animals per group.

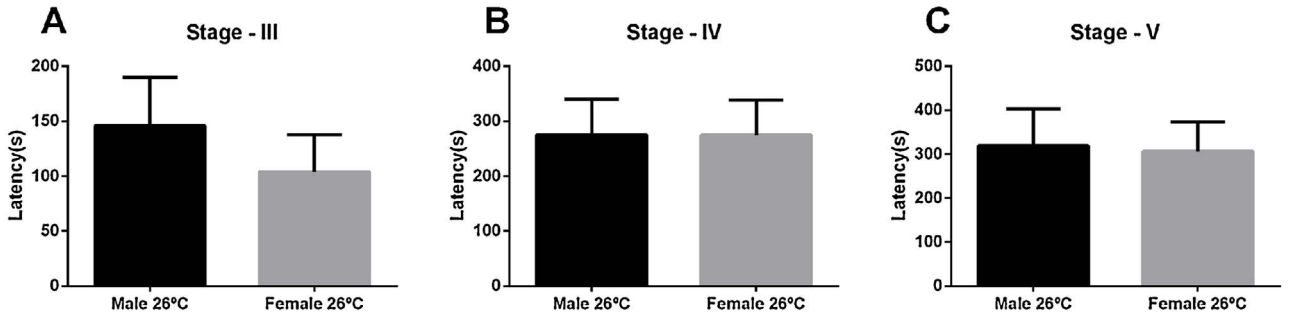

Fig. 2. Latency time of male (N = 13) and female (N = 10) zebrafish to reach the seizure stages caused by exposure to PTZ (10 mM) at 26 °C. Latency times of animals to reach the stage III (A), IV (B) and V (C) of seizure are expressed as mean ± C.I. N represents the number of animals per group.

ments. All animal experiments were conform with the ARRIVE guidelines and carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and the Brazilian legislation. The experimental protocols were approved by the Institutional Animal Care Committee of PUCRS registered under the protocol number 14/00416 − CEUA/PUCRS.

2.2. PTZ exposure

The exposure to PTZ (10 mM) (Sigma-St. Louis, Missouri, EUA) was performed by 10 minutes in an aquarium in dimensions 13 × 11.5 × 8 cm (length × height × width) and volume of 500 mL. Control animals were kept in the water system free of drugs. PTZ exposure was performed between females *versus* males with similar weight (200–250 mg), males weighted 200–250 mg *versus* males weighted 400–500 mg and males (200–250 mg) in three water temperatures (22, 26 and 30 °C). The temperature was monitored with a digital thermometer and the solution was changed after each test. To check if the highest temperature was able to promote convulsive behavior *per se*, an additional control group was kept at 30 °C without the presence of PTZ.

2.3. Seizure scores

The differences in susceptibility to PTZ seizure were measured by the latency (seconds) for the animals reach the three most obvious seizure stages observed in zebrafish, standardized by Mussulini et al. (2013) as stage III: a circular motion; stage IV: behavior convulsive clonic type; and stage V: fall to the bottom of the aquarium and convulsive behavior of tonic type. The animals were exposed to PTZ until to reach the score V, or up to 10 minutes of exposure.

2.4. Pretreatment with antagonist of glutamatergic receptors

To assess the role of NMDA and Kainic receptors in PTZ-induced seizure in combination with hyperthermia, two groups were considered, the animals pretreated with NMDA receptor antagonist, MK-801 (2.5 μM), and animals pretreated with Kainic receptor antagonist, DNQX (10 μM) (Sigma-St. Louis, Missouri, EUA). The exposure was carried out for 10 minutes in an aquarium in the dimensions of 13 × 11.5 × 8 cm containing 400 ml of solution of MK-801, DNQX or water-free drug at 26 °C. Locomotor activity during the pretreatment period was recorded by Logitech cam (Romanel-sur-Morges, Switzerland), located frontally to the apparatus. The analysis of locomotor activity was performed considering the digitally division of aquarium into upper and lower part, using any-maze software. The analyzed period was 5 minutes between the third and the eighth minute of exposure. The parameters measured were; Average speed (m/s), distance traveled (m) and time at the bottom (s) of the aquarium.

After 10 minutes of pretreatment, the animals were transferred immediately to the aquarium containing the PTZ solution (10 mM) at temperatures of 26 °C or 30C. The analysis of seizure score was performed as described above. For these tests only males between 200–250 mg were used.

2.5. Statistical analysis

Statistical tests were performed using Graphpad-Prism software version 6.0 (La Jolla, CA 92037 USA). Normality test was performed in all groups through the test D'Agostino & Pearson normality test. To check for significant differences between groups One-way-ANOVA was used followed by Dunnett's test post-hoc for multiple comparisons. To test difference among the genders or weights unpaired t-test was used with Welch's correction. Statistical significant levels considered $p < 0.05$. Values were expressed as means with confidence intervals.