Epilepsy & Behavior 27 (2013) 212–219

Contents lists available at SciVerse ScienceDirect

Epilepsy & Behavior

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

Oral gabapentin suppresses pentylenetetrazole-induced seizure-like behavior and cephalic field potential in adult zebrafish

Rakesh Kumar Banote, Srinivas Koutarapu, Keerthana Sarma Chennubhotla, Kiranam Chatti, Pushkar Kulkarni *

Dr. Reddy's Institute of Life Sciences, University of Hyderabad Campus, Gachibowli, Hyderabad 500046, India
Zephase Therapeutics Pvt. Ltd. (an incubated company at Dr. Reddy's Institute of Life Sciences), University of Hyderabad Campus, Gachibowli, Hyderabad 500046, India

ARTICLE INFO

Article history:
Received 9 November 2012
Revised 28 December 2012
Accepted 29 January 2013
Available online 5 March 2013

Keywords:
Zebrafish
Epilepsy
Gabapentin
Pentylenetetrazole
Neurobehavior
Cephalic field potential

ABSTRACT

We report the effect of orally administered gabapentin (GBP) on pentylenetetrazole (PTZ)-induced seizure-like activity in adult zebrafish. Zebrafish were pretreated with vehicle or GBP using a novel method of precise oral administration, followed by an intraperitoneal administration of PTZ. Behavioral assessment was carried out using locomotion-based video-tracking analysis and seizure score assignment using visual observation. Cephalic field potential recordings of the zebrafish brain were conducted using an electrical data acquisition system. Orally administered GBP significantly suppressed the seizure-like locomotor activity and strong slow-wave (~3 Hz) activity in the cephalic field potential caused by PTZ. This work is the first report of the activity of an orally delivered anticonvulsant in adult zebrafish. Our study provides behavioral and physiological evidence in support of an adult zebrafish model for studying seizures including excitotoxic brain injury and a novel *in vivo* framework for the evaluation of pharmacological modulators of epilepsy.

1. Introduction

The physiological balance between inhibitory and excitatory neurotransmission is a distinctive feature of the brain. γ-Aminobutyric acid (GABA) and glutamate, the main neurotransmitters involved in inhibitory and excitatory neurotransmissions, play a vital role in both physiological and pathological processes such as epilepsy [1]. Epilepsy is the term used for a group of disorders characterized by recurrent spontaneous seizures [2] and an imbalance in excitatory/inhibitory neurotransmission [3].

Gabapentin (1-(aminomethyl) cyclohexaneacetic acid) is a marketed antiepileptic drug and is shown to be active in a variety of animal seizure models [4–6]. In clinical practice, the use of gabapentin results in significant alleviation of various kinds of seizures [7]. γ-Aminobutyric acid is the most abundant inhibitory neurotransmitter in the CNS, and gabapentin is a structural analogue of GABA [8]. Administration of centrally active gabapentin has been used as an effective therapeutic approach for the treatment of epilepsy [9]. While the precise mechanism of action of gabapentin is not entirely clear, it is known to bind to the $\alpha_2\delta$ subunit of voltage-gated calcium channels [10,11] and to inhibit calcium current [12].

Pentylenetetrazole (PTZ) acts at the picrotoxin site of the $GABA_A$ receptor [13] and reduces chloride conductance [14–16] which further leads to glutamate excitation. Pentylenetetrazole is a popular chemoconvulsant used for the assessment of antiepileptic drugs (AEDs) [17] and for eliciting seizure activity in animal experiments [18–20].

Zebrafish (*Danio rerio*), a simple vertebrate species, have emerged as a popular model to study the basis of epilepsy and provide specific advantages compared to rodents such as a lower requirement of drug, higher fecundity, and ease of manipulation. Moreover, seizure-like behavior can be evoked in zebrafish similar to those in rodent models [21,22]. Pentylenetetrazole can produce a continuum of epileptiform activity in adult zebrafish [22–26] and zebrafish larvae [27,28]. Zebrafish have been reported for the assessment of the antiepileptic potential of drug candidates in a PTZ-induced seizure model [29,30].

Measurement of the brain's electrical activity is achieved using electroencephalography (EEG) [31]. An EEG equivalent referred to as cerebral field potential measurement has been recently reported in a detailed study on zebrafish [32], in which the authors successfully demonstrated the effect of PTZ-induced seizure activity on the cerebral field potential of adult zebrafish. An elevated epileptiform activity has also been recorded from zebrafish larvae [33]. Even though the potential of zebrafish for the assessment of the effects of antiepileptic drugs and the amenability of adult zebrafish for electric field recordings have been reported [29,30,32,33], there are no reports that demonstrate the effects of antiepileptic drugs on both behavior as well as electric field potential in adult zebrafish.

* Corresponding author at: Dr. Reddy's Institute of Life Sciences, University of Hyderabad Campus, Gachibowli, Hyderabad 500046, India. Fax: +91 40 66571581.
E-mail addresses: pushkark@drils.org, kk.pushkar@gmail.com (P. Kulkarni).

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http://dx.doi.org/10.1016/j.yebeh.2013.01.018

With the intent of establishing a framework for evaluating convulsant and anticonvulsant effects in zebrafish, we sought to investigate the effect of the orally administered anticonvulsant gabapentin (GBP) on pentylenetetrazole (PTZ)-induced seizures in adult zebrafish, and we monitored the corresponding behavioral and electrophysiological changes that occurred in the fish. Our results are reported below.

2. Material and methods

2.1. Animal care and maintenance

All procedures for zebrafish experimentation were as per guidelines published by the National Institutes of Health for care and use of zebrafish. Indigenous wild-type male adult zebrafish strains (5–6 months old) were used for this study (obtained from Vikrant Aquaculture, Mumbai, India). Fish were maintained in a custom-built recirculation system with polysulfone housing tanks containing purified water (Millipore ELIX system grade) with 200-mg/L sea salt at 28 °C under a 14:10-h light and dark cycle [34]. Fish were fed three times daily with live hatched brine shrimp and dry food. All fish were maintained at a constant temperature (~28 °C) during all experimental procedures, and all solutions administered were prepared fresh at room temperature (~28 °C).

2.2. Oral drug administration

Conventional studies in adult zebrafish are conducted by the addition of chemicals to the aquarium water at different concentrations which requires large amounts of drugs in order to meet therapeutic concentrations, and the actual oral dosage of the drug in terms of milligrams per kilogram of body weight cannot be ascertained as the fish would be exposed to the drug through multiple routes through the water. Our laboratory is the first to develop a method for conducting oral drug administration in zebrafish (provisional patent: CBR no. 10389, application no. 3644/CHE/2011: Oral drug administration in the zebrafish (*D. rerio*)). Briefly, this method involves the use of a standard micropipette with a small tip that is gently inserted into the mouth and pharynx of the fish. The drug solution/suspension is then gently released into the fish ensuring that the administered solution is not regurgitated. Permitted food-grade colored solutions were used during the training sessions of personnel to ensure that no spillage occurs either through the oral cavity or through the gill filaments. Furthermore, the fish did not show any symptoms of bleeding, prolapse, damage to the oral cavity, distress, discomfort, or any other clinical sign of trauma proving that this oral administration method is nontraumatic, painless, and ethical.

2.3. Behavioral monitoring

Prior to each experiment, fish were placed in an experimentation room for 2 h to allow acclimatization. Acclimatized fish were pretreated with 200-mg/kg, 400-mg/kg, and 600-mg/kg gabapentin (obtained as a gift sample from Dr. Reddy's Laboratories Ltd., Hyderabad, India) administered orally (p.o.). Ten minutes later, intraperitoneal (i.p.) injection of 220-mg/kg PTZ (Sigma-Aldrich, USA) was done (dose volume: p.o. GBP—4 μL; i.p. PTZ—15 μL). Control fish were treated with p.o. water and i.p. saline. Behavioral testing was performed using an observation tank (10.5-cm height × 15.5-cm width × 30-cm length) maximally filled with aquarium water. Videos were recorded using a digital camera attached to a dial-gauge stand looking down at the tank for 8 min, beginning 2 min after PTZ injection. All behavioral tests took place between 11 AM and 4 PM to ensure consistency and reduce potential variation in behavior. The quantification of seizure-like locomotor activity was performed using the video-tracking software EthoVision® XT 8.0 (Noldus Information Technology, Wageningen, Netherlands), and movement-tracking plots were obtained using ZebraLab™ (ViewPoint, Lyon, France). Qualitative seizure scoring was conducted by trained observers in a blinded fashion. All fish were observed for 8 min to evaluate seizure-like behavior, and a score was assigned according to the following scale—stage 1: intermittent immobility and hyperventilation; stage 2: rotational swimming; stage 3, side-to-side movements; stage 4: visible muscular spasms and contractions; stage 5: quick convulsions of the entire body; stage 6: spasms and high frequency convulsions lasting several minutes including sinking of fish to tank bottom and; stage 7: complete immobility and death.

Thirty male fish were distributed in five treatment groups with six fish per group. The different groups (with treatment) were the following: control (p.o. water + i.p. saline), PTZ (p.o. water + i.p. PTZ 220 mg/kg), GBP 200 + PTZ (p.o. GBP 200 mg/kg + i.p. PTZ 220 mg/kg), GBP 400 + PTZ (p.o. GBP 400 mg/kg + i.p. PTZ 220 mg/kg), and GBP 600 + PTZ (p.o. GBP 600 mg/kg + i.p. PTZ 220 mg/kg).

2.4. Cephalic field potential measurement

Zebrafish were acclimatized for a period of 2 h in a polysulfone tank, taking care to avoid sources of electromagnetic radiation. During the period of acclimatization, fish were provided with adequate oxygen supply by aeration. Treatment groups were as described above. Fish were anesthetized by immersion in 0.003% eugenol [35] with or without GBP pretreatment and maintained with continuous perfusion of buffer containing 0.0015% eugenol in a polyethylene tray containing 3.3% agarose cast in a mould. Eugenol was prepared in 20-mM HEPES dissolved in E3 medium (5-mM NaCl, 0.17-mM KCl, 0.33-mM $CaCl_2$, and 0.33-mM $MgSO_4$). Anesthetized fish were gently placed with their dorsal side facing up in a slightly hollowed-out cut made in the agarose and the mould, and each fish was restricted with a horseshoe-shaped (∩) insulated hard wire to prevent motion artifacts (Fig. S2). Prevention of motion artifacts was attempted to the best possible extent using eugenol and insulated wires as suggested previously [32]. The tray and mould contained a small opening precisely beneath the fish, thus providing access for injection. A small passage was made in the agarose for draining excess buffer. Cephalic field potential (electric field potential from the head region) was monitored continuously for variations in peak amplitude. Intraperitoneal administration of PTZ was performed while the recording was being done. The time taken for seizure induction, variation in peak amplitude, and recovery time were the parameters measured.

Electrical recordings were done using a PowerLab ML856 26 T data acquisition system (ADInstruments). Twenty-nine-gauge 5-mm electrodes (MLA1203) were placed superficially over the brain region of the zebrafish. The positive electrode was placed over the mesencephalon region, while the negative electrode was placed over the telencephalon region down the anterio-dorsal surface on the central axis of the body (Fig. S2). The electrodes were held immobile using a coarse manipulator (Narishige Instruments). Potential difference between the electrodes was amplified by a factor of 1000 using an ECG amplifier and sampled at a rate of 2000 samples/s and 12-bit resolution. The acquisition was controlled using the software LabChart Pro. A steady baseline was obtained for a period of at least 2–3 min allowing for signal stabilization before each recording. Power spectrum was calculated on 25-s segments at different time points using GNU Octave fast Fourier transforms (FFT) for three male fish per treatment group.

2.5. Statistical analysis

Statistical analysis was performed using the GraphPad Prism® software. Data were represented using mean and standard error of the mean (± SEM). The behavioral effects of the drug and vehicle treatments were evaluated statistically using analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Seizure score and the power spectrum of cephalic field potential were evaluated statistically