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Research paper

The role of taurine on anxiety-like behaviors in zebrafish: A comparative study using the novel tank and the light–dark tasks

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HIGHLIGHTS

- The effects of taurine were evaluated in zebrafish using two behavioral tasks.
- Taurine did alter neither locomotor activity nor vertical exploration.
- Taurine had anxiolytic-like effects in the light–dark test.
- Behavioral endpoints are differently altered by acute taurine treatment.

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ABSTRACT

Taurine (TAU) is an amino sulfonic acid with several functions in central nervous system. Mounting evidence suggests that it acts in osmoregulation, neuromodulation and also as an inhibitory neurotransmitter. However, the effects of TAU on behavioral functions, especially on anxiety-related parameters, are limited. The adult zebrafish is a suitable model organism to examine anxiety-like behaviors since it presents neurotransmitter systems and behavioral functions evolutionary conserved. Anxiety in zebrafish can be measured by different tasks, analyzing the habituation to novelty, as well as the response to brightly lit environments. The aim of this study was to investigate whether acute TAU treatment alters anxiety-like behavior in zebrafish using the novel tank and the light-dark tests. Fish were individually treated with TAU (42, 150, and 400 mg/L) for 1 h and the behaviors were further analyzed for 6 min in the novel tank or in the light–dark test. Control fish were handled in a similar manner, but kept only in home tank water. Although TAU did not alter locomotor and vertical activities, all concentrations significantly increased shuttling and time spent in lit compartment. Moreover, TAU 150 group showed a significant decrease in the number of risk assessment episodes. Overall, these data suggest that TAU exerts an anxiolytic-like effect in zebrafish and the comparative analysis of behavior using different tasks is an interesting strategy for neuropsychiatric studies related to anxiety in this species.

1. Introduction

Zebrafish (*Danio rerio*) is a small vertebrate model that has been considered a promising tool for the advancement of neuroscience and behavioral researches [1,2]. Several features like the presence of evolutionary conserved genes, sensitivity to pharmacological compounds, easy maintenance and low cost, have stimulated the use of zebrafish to model human diseases in a medium/large scale manner [3]. Additionally, different pharmacological agents that modulate synaptic transmission and neural membrane stability in humans show analogous activities in zebrafish, suggesting the existence of similar neural networks [4]. In this context, the use of zebrafish as an animal model to study defensive behaviors and anxiety-like parameters has increased considerably over the past few years [5–7].

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The behavior of rodents in response to anxiety has been extensively documented, but research with zebrafish is still limited [8]. Anxiety-like behavior is a complex phenotype evoked by dangerous or potentially dangerous environment/stimuli. It includes reduced exploration and typically manifests as geotaxis (diving), thigmotaxis, scototaxis, increased freezing, opercular movements, and erratic movements. Anxiolytic drugs generally reduce anxiety-like behaviors, while anxiogenic agents potentiate these responses [1,9]. In the literature, several neural mechanisms have been associated with anxiety-like behaviors (*e.g.*, modulation of $GABA_A$ receptors, strychnine-sensitive glycine receptors, and NMDA receptors) [10–12].

In the central nervous system (CNS), taurine (TAU) (2-aminoethanesulfonic acid) is a simple amino sulfonic acid which is not incorporated into proteins [13,14]. Its biosynthesis is dependent on sequential oxidative steps of cysteine, catalyzed by cysteine dioxygenase and cysteine sulfinate decarboxylase [15,16]. After glutamate, TAU is the second most abundant molecule in CNS [17] and plays multiple roles, including osmoregulation, neuroprotection, neuromodulation and inhibitory neurotransmission [18–20]. Previous reports showed that TAU acts as a ligand that activates $GABA_A$ and strychnine-sensitive glycine receptors [12]. Recent evidence also pointed a role of TAU as an activator of putative TAU receptors as well as an inhibitory molecule of the NMDA receptor complex through multiple mechanisms [10]. Since TAU interacts with GABAergic, glycinergic and glutamatergic receptors, it is a promising therapeutic tool for the treatment of anxiety-related disorders [21].

Recent studies have proposed the use of different protocols to measure anxiety-like behavior in zebrafish. The novel tank diving test is based on the natural instinct of zebrafish to initially seek protection in an unfamiliar environment avoiding the surface ('diving response') [22]. This test was introduced by Levin et al [23], who reported that adult zebrafish spent about 50% of a 5-min session in the bottom of a novel tank and showed that nicotine decreases this preference. Anxiolytic (anxiety-reducing) drugs, such as buspirone, diazepam and (chronic) fluoxetine, also decrease it, as well as the panicolytic agents (panic-reducing) [9,24]. A second task that evaluates anxiety-like behaviors is the light–dark test, which was proposed by Serra et al. [25]. and further validated by Maximino et al [26]. Zebrafish have a natural preference to dark (scototaxis) and such behavior is also modulated by pharmacological manipulations. Considering that different factors affect animal behavior, it is important to perform an integrated analysis of various anxiety-like parameters [8]. The comparison of the results obtained in multiple behavior tests can improve the knowledge of the variables modulated in the presence of different molecules. Thus, we aimed to investigate whether acute TAU treatment alters anxiety-like behavior of zebrafish using the novel tank and the light–dark tasks.

2. Materials and methods

2.1. Animals

Zebrafish (*Danio rerio*) were obtained from a local distributor (Hobby Aquarios, RS, Brazil). The animals used were approximately 50:50 male:female ratio of heterogeneous wild-type stock (short fin phenotype). Fish were acclimatized for 2 weeks in 40-L tanks filled with non-chlorinated water (at a maximum density of 2-3 animals per liter) under constant filtration and aeration before onset of experiments. The water temperature was set at $26 \pm 2\,^{\circ}C$, with pH adjusted to 7.0–7.5. The room illumination was provided by ceiling-mounted fluorescent light tubes and adjusted to a 14:10 light–dark photoperiod cycle (lights on at 7:00 am). The animals were fed thrice daily with a commercial fish flake food (Alcon Basic®, Alcon, Brazil) and maintained in accordance to the National Institute of Health Guide for Care and Use of Laboratory Animals. All protocols of this study were approved by the Ethics Commission on Animal Use of the Federal University of Santa Maria under process number 026/2014.

2.2. Treatments

To evaluate the role of taurine (Sigma, St. Louis, MO, USA) on anxiety-like behavior, we exposed the zebrafish individually for 1 h in 500-mL beakers at distinct TAU concentrations (42, 150, and 400 mg/L), named as TAU 42, TAU 150, and TAU 400, respectively. These concentrations has been extensively used in previous studies from our group which assessed neurochemical effects of TAU in zebrafish [16,27]. Additionally, this range (varying from 0.33 to 3.2 mM) is commonly used for *in vitro* and *in vivo* assays [18,28]. Control group was kept for 1 h under the same conditions, except that no TAU was added in the home tank water. A total of 80 fish were used for the experiments.

2.3. Behavioral tasks

The behavioral tests were performed during the same time frame each day (between 11:00 am and 4:00 pm) immediately after the treatments. All experiments were recorded using a webcam (Vtrex X6000®) in order to register the location and swimming activity of the zebrafish and analyzed by three trained observers (inter-rater reliability >0.85). The behavioral activity of zebrafish was recorded in a single session of 6 min and the behaviors were assessed using appropriate video-tracking software (ANY-maze®, Stoelting CO., USA) at a rate of 30 frames/s. Importantly, each animal was tested in a single apparatus (novel tank or light–dark task) to avoid possible stress promoted by subsequent manipulation and experiments. Thus, different cohorts were used for both experiments. All apparatuses were filled with water adjusted to home tank conditions and the experiments were performed on a stable surface with all environmental distractions kept to a minimum. The tank water was changed after each trial.

2.3.1. Novel tank diving test

The apparatus was consisted in a rectangular tank (25 cm length × 15 cm height × 6 cm width) filled with 1.5 L of home tank water. The aquarium was divided into three equal horizontal areas (bottom, middle, and top) and all experimental conditions were similar to those previously described [9,16,29]. The following endpoints were measured: total distance travelled, absolute turn angle, number of immobile episodes, latency to enter the top, time spent in top, and transitions to top area. For the novel tank diving test, a total of 48 fish were used ($n = 12$ per group).

2.3.2. Light–dark test

The light–dark test was performed based in the protocol described previously [26]. A rectangular glass tank (30 cm length × 10 cm width × 15 cm height) was divided into two equally sized partitions using black or white self-adhesive film externally covering the walls, floor, and the corresponding sides of the tank. Illumination was provided by fluorescent lamps at the ceiling (approximately 250 lux above the tank) and the apparatus was filled with 2.5 L of home tank water. After the treatments, fish were removed from the beakers and gently placed in the white partition of the tank. The trial was immediately started and behaviors were recorded in a single 6-min session. The following endpoints were automatically determined: time spent in lit area, shuttling, latency to enter the dark area, and number of risk assessment episodes. According to the literature, risk assessment is defined as a fast (>1 s) entry in the white compartment followed by re-entry