ARTICLE IN PRESS

Pharmacology, Biochemistry and Behavior xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

FGIN-1-27, an agonist at translocator protein 18 kDa (TSPO), produces anti-anxiety and anti-panic effects in non-mammalian models

Monica Gomes Lima-Maximino[a], Jonathan Cueto-Escobedo[b], Juan Francisco Rodríguez-Landa[b], Caio Maximino[c,*]

[a] Laboratório de Neurofarmacologia e Biofísica, Departamento de Morfologia e Ciências Fisiológicas, Universidade do Estado do Pará – Campus VIII, Marabá, Brazil
[b] Laboratorio de Neurofarmacología, Instituto de Neuroetología, Universidad Veracruzana, Xalapa, Mexico
[c] Instituto de Estudos em Saúde e Biológicas, Universidade Federal do Sul e Sudeste do Pará, Marabá, Brazil

ARTICLE INFO

Keywords:
Translocator protein 18 kDa
Fear
Anxiety
Zebrafish
Wall lizard
Benzodiazepines

ABSTRACT

FGIN-1-27 is an agonist at the translocator protein 18 kDa (TSPO), a cholesterol transporter that is associated with neurosteroidogenesis. This protein has been identified as a peripheral binding site for benzodiazepines; in anamniotes, however, a second TSPO isoform that is absent in amniotes has been implicated in erythropoiesis. Functional conservation of the central benzodiazepine-binding site located in the $GABA_A$ receptors has been demonstrated in anamniotes and amniotes alike; however, it was not previously demonstrated for TSPO. The present investigation explored the behavioral effects of FGIN-1-27 on an anxiety test in zebrafish (*Danio rerio*, Family: Cyprinide) and on a mixed anxiety/panic test on wall lizards (*Tropidurus oreadicus*, Family: Tropiduridae). Results showed that FGIN-1-27 reduced anxiety-like behavior in the zebrafish light/dark preference test similar to diazepam, but with fewer sedative effects. Similarly, FGIN-1-27 also reduced anxiety- and fear-like behaviors in the defense test battery in wall lizards, again producing fewer sedative-like effects than diazepam; the benzodiazepine was also unable to reduce fear-like behaviors in this species. These results A) underline the functional conservation of TSPO in defensive behavior in anamniotes; B) strengthen the proposal of using anamniote behavior as models in behavioral pharmacology; and C) suggest TSPO/neurosteroidogenesis as a target in treating anxiety disorders.

1. Introduction

The translocator protein 18 kDa (TSPO, mitochondrial benzodiazepine receptor [MBR], peripheral benzodiazepine receptor) was first identified as a peripheral binding site for diazepam, but later identified as part of the mitochondrial cholesterol transport pathway that is associated with the regulation of cellular proliferation, immunomodulation, porphyrin transport and heme biosynthesis, anion transport, regulation of steroidogenesis, and apoptosis (Casellas et al., 2002). This transporter is highly expressed in steroidogenic tissues. In the central nervous system, its expression is mainly restricted to ependymal cells and glia, in which it is responsible for the local synthesis of neuroactive steroids such as allopregnanolone (Papadopoulos et al., 2006). This latter neurosteroid, in its turn, positively modulates $GABA_A$ receptors, especially those involved in tonic inhibition (Smith et al., 2009; Maguire et al., 2012), producing anxiolytic- (Fernández-Guasti and Picazo, 1995; Schüle et al., 2014) and antidepressant-like effects (Khisti et al., 2000; Rodríguez-Landa et al., 2007, 2009), and modulates panic attacks (Bali and Jaggi, 2014; Lovick, 2014). As such, TSPO has been proposed as a pharmacological target for the treatment of neurological and psychiatric disorders associated with decreased GABAergic tone, such as anxiety disorders (Romeo et al., 1993; de Mateos-Verchere et al., 1998; Kita et al., 2004; Costa et al., 2011; Matsuda et al., 2011; Nin et al., 2011; Pinna and Rasmusson, 2012; Perna et al., 2014) and epilepsy (Ugale et al., 2004), as well as for the fine control of stress responses (Gunn et al., 2011; Maguire et al., 2012; Maguire, 2014). TSPO agonists produce anti-anxiety and anti-conflict effects in rodents with both systemic (Kita et al., 2004; Costa et al., 2011) and intra-hippocampal (Bitran et al., 2000) injections; these effects are blocked by $GABA_A$ receptor antagonists (i.e. picrotoxin) and/or 5α-reductase blockers (i.e. 4-MA) and TSPO antagonists (PK 11195), implicating neurosteroidogenesis and chloride ion channel at the $GABA_A$ receptors in these responses (Bitran et al., 2000). Additionally, octadecaneuropeptide, a diazepam-binding inhibitor peptide that acts through both the central benzodiazepine receptor (CBR) and TSPO produces anxiety-like behavior in mice and rats (de Mateos-Verchere et al., 1998), as well

---

[*] Corresponding author at: Instituto de Estudos em Saúde e Biológicas, Universidade Federal do Sul e Sudeste do Pará, Campus III, Av. dos Ipês, s/n, Marabá, PA, Brazil.
E-mail address: cmaximino@unifesspa.edu.br (C. Maximino).

https://doi.org/10.1016/j.pbb.2018.04.007
Received 24 January 2018; Received in revised form 20 April 2018; Accepted 23 April 2018

Please cite this article as: Lima, M.G., Pharmacology, Biochemistry and Behavior (2018), https://doi.org/10.1016/j.pbb.2018.04.007

as in goldfish (Matsuda et al., 2011).

These effects are spared in adrenalectomized and castrated animals, suggesting that they are not mediated by peripheral steroidogenesis, but rather by the production of neurosteroids in the brain (Romeo et al., 1993).

TSPO is highly conserved, being present in Bacteria, Archaea and Eukarya domains (Fan and Papadopoulos, 2013). Anamniotes and invertebrates possess a single isoform, while amniotes possess two TSPO isoforms (Fan et al., 2009). Interestingly, while no functional divergence is predicted to appear between *tspo* (found in invertebrates and basal vertebrates) and *tspo1* (found in amniotes), a functional divergence was detected in TSPO2 (Fan and Papadopoulos, 2013). Some of the neurobehavioral functions of this protein, on the other hand, seem to be conserved. In zebrafish, for example, benzodiazepines have been shown to affect a plethora of anxiety-like behaviors, from bottom-dwelling (Bencan et al., 2009; Egan et al., 2009) and dark preference (Maximino et al., 2010; Maximino et al., 2011) to shoal cohesion (Gebauer et al., 2011) and cocaine withdrawal-induced anxiety-like behavior (López-Patiño et al., 2008). Likewise, benzodiazepines decrease tonic immobility duration and the following freezing and explosive behavior in a defensive behavior battery in the wall lizard (*Tropidurus oreadicus*), and also increase exploratory behavior in the same test (Maximino et al., 2014). In the separation stress paradigm, benzodiazepines attenuate separation stress-induced distress vocalizations in chicks in the anxiety phase, but not in the depression phase (Warnick et al., 2009). Thus, agonists at the CBR decrease fear- and anxiety-like behavior in both amniotes and anamniotes. Moreover, some evidence regarding the neurosteroidogenesis pathway in behavioral control has been suggested by the observation that allopregnanolone has an anticonvulsant effect in zebrafish (Baxendale et al., 2012), and that chronic fluoxetine treatment upregulates the expression of genes from the neurosteroidogenesis pathway in this species (Wong et al., 2013). These results suggest that some downstream effectors of neurosteroidogenesis are conserved, although little is known about the role of TSPO in behavioral control per se. A comparative approach could untangle this question, especially if species at the base of the amniote and anamniote clades are used. In this paper, we describe the behavioral effects of FGIN-1-27, a TSPO agonist, in zebrafish (*Danio rerio* Hamilton 1822, Family: Cyprinidae) and wall lizards (*Tropidurus oreadicus* Rodrigues, Family: Tropiduridae) and compare these responses with the effects of diazepam, an agonist at the CBR with tested anxiolytic and anticonvulsant actions at preclinical and clinical research.

2. Experimental procedures

2.1. Experiment 1: Effects of FGIN-1-27 and diazepam on dark preference in zebrafish

2.1.1. Animals and husbandry

120 adult zebrafish from the *longfin* phenotype were acquired in a local aquarium shop and kept in collective tanks at the laboratory for at least 2 weeks before experiments started. Conditions in the maintenance tank were kept stable, as described by Lawrence (2007). Recommendations in the Brazilian legislation (Conselho Nacional de Controle de Experimentação Animal - CONCEA, 2017) were followed to ensure ethical principles in animal care and throughout experiments. This manuscript is a complete report of all the studies performed to test the effect of diazepam or FGIN-1-27 on anxiety-like behavior in zebrafish. We report how the sample size was determined, all data exclusions (if any), all manipulations, and all measures in the study.

2.1.2. Drug administration

Diazepam was dissolved in 40% propylene glycol, 10% ethyl alcohol, 5% sodium benzoate, and 1.5% benzyl alcohol (Maximino et al., 2010). FGIN-1-27 was dissolved in 1% DMSO to which one or two drops of Tween 80 was added before sonication into a fine suspension (Auta et al., 1993). Drugs were diluted to their final concentrations and injected *i.p.* in a volume of 1 μL/0.1 g b.w. (Kinkel et al., 2010). For both diazepam and FGIN-1-27, the doses used were 0.14, 0.28, 0.57, 1.1, and 2.3 mg/kg. The three highest doses were chosen based on reported effects of diazepam on zebrafish (Maximino et al., 2010) or wall lizard (Maximino et al., 2014) defensive behavior, and were identical for both drugs to facilitate comparison; the lower doses were chosen in order to define a minimal effect. All drugs were acquired from Tocris Bioscience (UK).

2.1.3. Experimental groups and dosage

The adult zebrafish were assigned to 12 independent groups ($n = 10$ fish per group): a vehicle group (propylene glycol:ethyl alcohol:sodium benzoate:benzyl alcohol for the diazepam controls, DSMO for the FGIN-1-27 controls) that received 1 μL/0.1 g b.w.; and 10 additional groups that received the doses described above for diazepam (5 groups) or FGIN-1-27 (5 groups). Sample sizes were based on Maximino et al. (2010). All drugs were *i.p.* injected in a volume of 1 μL/0.1 g b.w., 30 min before of the behavioral test. Animals were randomly drawn from the holding tank immediately before injection, and the order with which doses were tested was randomized via generation of random numbers using the randomization tool in http://www.randomization.com/. Experimenters were blinded to treatment by using coded vials for drugs. The data analyst was blinded to phenotype by using coding to reflect treatments in the resulting datasets; after analysis, data was unblinded.

2.1.4. Scototaxis assay

The light/dark preference (scototaxis) assay was performed as described by Maximino et al. (2013a). Briefly, 30 min after injection animals were transferred individually to the central compartment of a black/white tank (15 cm height × 10 cm width × 45 cm length) for a 3-min acclimation period, after which, the doors which delimit this compartment were removed and the animal was allowed to freely explore the apparatus for 15 min. While the whole experimental tank was illuminated from above by an homogeneous light source, due to the reflectivity of the apparatus walls and floor average illumination (measured just above the water line) above the black compartment was 225 ± 64.2 (mean ± S.D.) lux, while in the white compartment it was 307 ± 96.7 lx. The following variables were recorded:

time spent on the white compartment: the time spent in the white half of the tank (percentage of the trial);

squares crossed: the number of 10 cm^2 squares crossed by the animal in the white compartment;

latency to white compartment: the time in seconds (s) to first entry in the white compartment;

erratic swimming: defined as the number of zig-zag, fast, and unpredictable swimming behavior of short duration;

freezing: the proportional duration of freezing events (in % of time in the white compartment), defined as complete cessation of movements with the exception of eye and operculum movements;

thigmotaxis: the proportional duration of thigmotaxis events (in % of time in the white compartment), defined as swimming in a distance of 2 cm or less from the white compartment's walls;

Number of risk assessment: defined as a fast (< 1 s) entry in the white compartment followed by re-entry in the black compartment, or as a partial entry in the white compartment (i.e., the pectoral fin does not cross the midline);

A digital video camera (Samsung ES68, Carl Zeiss lens) was installed above the apparatus to record the behavioral activity of the zebrafish. Two independent observers, blinded to treatment, manually measured the behavioral variables using X-Plo-Rat 2005 (https://github.com/lanec-unifesspa/x-plo-rat). Inter-observer reliability was at least > 0.85.