Progress in Neuropsychopharmacology & Biological Psychiatry 85 (2018) 128–135

Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

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

Hyperglycemia elicits anxiety-like behaviors in zebrafish: Protective role of dietary diphenyl diselenide

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ARTICLE INFO

Keywords:
Diabetes mellitus
Zebrafish
Hyperglycemia
Anxiety
Selenium
Diphenyl diselenide

ABSTRACT

Diabetes mellitus (DM) is a chronic metabolic disease that may comorbid with various psychiatric disorders, such as anxiety and depression. The search for effective therapeutics to alleviate hyperglycemia and complications resulting from DM is continuous. Here we investigate the effects of diphenyl diselenide (DD), an organoselenium compound with several pharmacological properties, in a zebrafish model of hyperglycemia. Fish were fed for 74 days with a diet containing 3 mg/Kg DD, a concentration chosen after experiments based in a dose-response curve (DD 1, 2 and 3 mg/Kg) that did not cause overt toxicity (mortality, weight loss and neurobehavioral deficits). In the last 14 days of the experimental period, fish were concomitantly exposed to a glucose solution (111 mM). Afterwards, blood glucose levels, brain selenium (Se) content, and behavioral analysis aiming to assess anxiety-like behaviors and locomotor/exploratory activities were performed. In the novel tank diving test, glucose decreased vertical exploration and fish spent less time in the lit area when tested in the light-dark test, suggesting increased anxiety-like behavior. Moreover, DD decreased blood glucose levels in hyperglycemic fish as well as prevented the development of anxiety-related symptoms. DD diet alone did not change glycemia and behavioral parameters, but increased Se levels in the brain without affecting the cellular viability. Collectively, our findings highlight the growing utility of this zebrafish hyperglycemia model as a valuable strategy for further research in DM field and neuroprotective approaches.

1. Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by a chronic hyperglycemic state, which occurs through insulin deficiency or insulin resistance (Öztürk et al., 2017; Sharma et al., 2014). There are approximately 415 million diabetic adults worldwide and this number is projected to increase to 642 million until 2040 (IDF, 2015). Type 2 diabetes (T2D) is the most prevalent form and the number of cases has increased along with people's lifestyle changes (IDF, 2015).

A close association between hyperglycemia and the development of DM chronic complications, such as nephropathy, neuropathy, retinopathy, cardiovascular and cerebrovascular dysfunctions has been postulated (Bannier et al., 2015; Huang et al., 2017). In the brain, chronic hyperglycemia impairs cognition, synaptic transmission, and neural plasticity (Kodl and Seaquist, 2008; Malone, 2016). Moreover, some psychiatric conditions (e.g., depression and anxiety) are highly prevalent in diabetics (Moulton et al., 2015; Purewal and Fisher, 2018). This association is bidirectional, where diabetic complications may elicit anxiety/depression symptoms as well as these neuropsychiatric disorders may increase the risk of DM onset (Gambeta et al., 2016; Vancampfort et al., 2015). However, the underlying mechanisms involved in the relationship between DM and psychiatric diseases are still poorly understood.

Despite rodent models have been used to assess anxiogenic- and anxiolytic-like behaviors under different experimental conditions (Faturi et al., 2010; Hilber and Chapillon, 2005; Tyree et al., 2016), the zebrafish (*Danio rerio*) emerges as a novel and useful alternative model to evaluate anxiety-like behaviors (Rosemberg et al., 2011; Stewart et al., 2012). Different behavioral paradigms used in rodent models of anxiety have been adapted for zebrafish eliciting similar responses (Maximino et al., 2010; Stewart et al., 2012). Importantly, zebrafish have also been used as a promising organism to study hyperglycemia

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https://doi.org/10.1016/j.pnpbp.2018.04.017
Received 17 January 2018; Received in revised form 27 April 2018; Accepted 29 April 2018
Available online 30 April 2018

and its complications (Capiotti et al., 2014a, 2014b; Dorsemans et al., 2017; Gleeson et al., 2007). As occur in rodents, a hyperglycemic state in zebrafish may be induced via destruction of pancreatic beta cells (Moss et al., 2009; Shin et al., 2012). Another protocol involves immersing zebrafish in a glucose solution, based in the conception that fish can easily absorb molecules from the water and regulate their internal water and total solute concentrations (Capiotti et al., 2014a; Gleeson et al., 2007).

Due to the large number of people with DM worldwide and considering the severity of its chronic complications, the search for therapeutic agents to prevent and/or treat this metabolic disorder is necessary. Here, we targeted the organic selenium compound diphenyl diselenide (DD), which chronically diminished the levels of glucose and improved the antioxidant status of diabetic rats (Barbosa et al., 2006). Although the anti-diabetogenic and anxiolytic effects of DD have already been reported in rodents, little is known about the molecular/cellular mechanisms underlying their actions in the central nervous system. Thus, considering the growing utility of zebrafish to investigate the neural basis involved in various metabolic diseases, the neurobehavioral characterization of novel DM/hyperglycemia models will help elucidate how organochalcogens modulate brain functions, thereby fostering future discovery of potential therapeutic agents in translational research. In this report, we aimed to ascertain the relationship between hyperglycemia and anxiety onset, and whether dietary DD could be helpful under these conditions using an alternative hyperglycemia model in zebrafish.

2. Materials and methods

2.1. Animals and housing

Adult male and female (approximately 50:50 ratio) *short-fin* zebrafish (*Danio rerio*) were purchased from a local commercial supplier (Hobby Aquarios, RS, Brazil). Fish were maintained in 40 L aquariums (at a maximum density of 2 fish per liter) for at least 2 weeks before the experiments. Tanks were filled with non-chlorinated water (height: 25 cm) previously treated with AquaSafe™ (2 drops/L). Water was maintained at constant filtration and aeration at a target temperature of 26 ± 2 °C and pH adjusted to 7.0–8.0. Illumination was performed by a 14:10 light-dark photoperiod cycle (lights on at 7:00 AM). Before the treatments, all animals were fed twice daily with commercially available fish flake food (Alcon Basic™, Alcon, Brasil). Animals were maintained following the recommendations of the National Institute of Health Guide for the Care and Use of Laboratory Animals (2011). A total of 230 animals were used in the study and all experimental protocols were approved by the Ethics Commission on Animal Use of the Federal University of Santa Maria (process number: 2649140717/2017).

2.2. Diet preparation

Diet containing DD were made into pellets (5 mm of diameter) and kept at 4 °C until fed as described previously (Menezes et al., 2014). In order to establish a safe concentration of DD in the diet, we firstly tested a curve of dietary DD. Briefly, 70 fish were divided into 5 groups with 14 animals each. Experiments were performed in duplicate, with 7 animals allocated per 3-L aquarium. The aquariums were filled with non-chlorinated water at a height of 14 cm. Water and illumination conditions were similar to those of housing tanks (item 2.1). Group 1 received commercial food; group 2 an unsupplemented DD diet and groups 3 to 5 a diet supplemented with DD at concentrations of 1.0, 2.0 and 3.0 mg/Kg, respectively. Fish were fed twice daily, with the different diets (3% of body mass) for 74 days. Survival rate was measured by a daily count of the number of fish and the wet weight at 0, 15, 30, 45, 60 and 74 day. Afterwards, fish were submitted to the behavioral tests (novel tank diving and light-dark tests) in two consecutive days. Then, animals were anesthetized with 0.25 g/L tricaine and euthanized by decapitation. Brains were quickly removed, transferred to microtubes and kept at −80 °C for biochemical analysis and Se determination.

2.3. Hyperglycemia induction and DD supplementation

Since dietary DD concentrations tested did not cause apparent signals of toxicity (weight loss, mortality and neurobehavioral deficits), we chose 3.0 mg/Kg DD to use in the hyperglycemia model. Hyperglycemia was induced by maintaining the fish in a glucose solution as previously described by Capiotti et al. (2014a, 2014b). Fish were divided into four groups of 20 animals each, as follows: Group 1 (Control: unsupplemented DD diet), Group 2 (DD 3.0 mg/Kg), Group 3 (Glucose + unsupplemented DD diet), and Group 4 (Glucose + DD 3.0 mg/Kg). Fish were maintained in 3 L aquariums (6–7 fish per aquarium) and fed twice a day for 74 days. Before glucose exposure, the aquarium water (height: 14 cm) was changed once a week. The conditions of water and illumination were maintained as cited in the item 2.1. In the last 14 days, a 111 mM glucose solution was added to the water of groups 3 and 4. Glucose solution was changed every day to avoid a possible contamination.

2.4. Measurement of blood glucose levels

Blood glucose levels were measured after a 12-h fasting period. Before measurement, animals were kept for 15 min in water without glucose to eliminate possible glucose remnants in the fish (Gleeson et al., 2007) and then anesthetized by hypothermia induction to reduce the variability of blood glucose. Afterwards, fish tail was cut and blood glucose measured by placing a glucometer (G-Tech Free 1) directly on the docked tail.

2.5. Brain Selenium quantification by inductively coupled plasma mass spectrometer (ICP-MS)

For Se quantification, 3 brains of each group were pooled ($n = 4$, total of 12 brains) and digested by microwave assisted acid digestion using a Multiwave 3000 microwave sample preparation system (Anton Paar, Graz, Austria) equipped with eight high-pressure quartz vessels (internal volume of 80 mL, maximum temperature and pressure of 280 °C and 80 bars, respectively). For this procedure, samples were weighted inside of the quartz vessels and 6 mL of 7 mol L^{-1} HNO_3 solution was added. The operational conditions and the heating program used were carried out according to recommendations of the manufacturer. After digestion of samples, selenium was determined by inductively coupled plasma mass spectrometer (PerkinElmer Sciex, Model ELAN DRC II, Thornhill, Canada), equipped with a concentric nebulizer (Meinhard Associates, Golden, USA), a cyclonic spray chamber (Glass Expansion, Inc., West Merbourne, Australia) and a quartz torch with a quartz injector tube (2 mm i.d.). Instrumental performance optimization, including nebulizer gas flow rate, ion lens voltage, and torch alignment, was carried out following the instructions of manufacturer (Perkin Elmer-SCIEX 2003).

2.6. Cell viability measurements

2.6.1. MTT assay

Brain cell viability was evaluated by MTT assay as previously reported (Zenki et al., 2014). The assay is based on the enzymatic reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to formazan crystals by dehydrogenases from viable cells. Briefly, whole brain ($n = 7$ per group) was immersed in a 0.5 mg/mL MTT solution and placed in a water bath shaker for 20 min at 37 °C. Then, MTT solution was removed and 300 µL of DMSO was added in each sample that was maintained in the dark for 24 h for total

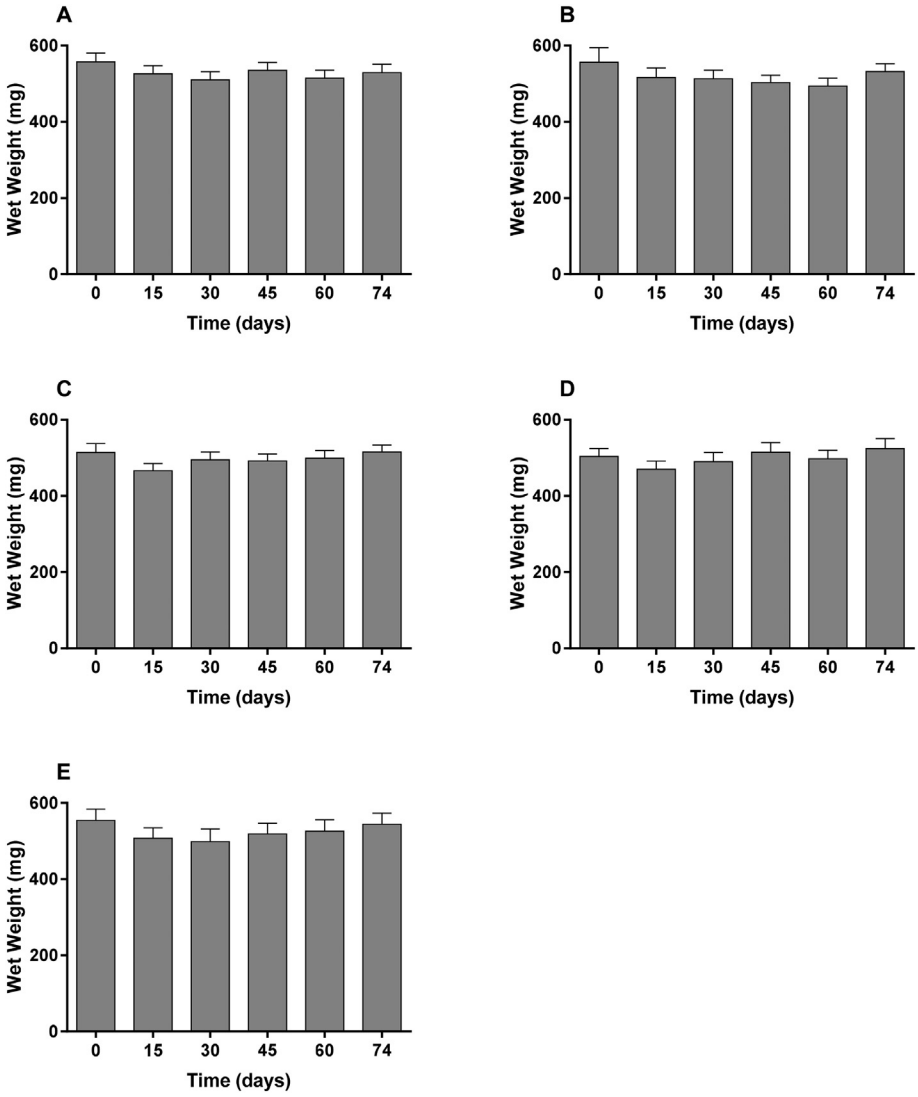

Fig. 1. Body wet weight of fish fed with DD and control diets. Fish were fed with commercial food (A), DD unsupplemented diet (B) and diets containing DD 1, 2 or 3 mg/Kg (C, D, and E, respectively) for 74 days. Data were analyzed by two-way ANOVA followed by Student-Newman-Keuls multiple comparison test when appropriate and expressed as mean ± SEM and ($p < .05$, $n = 14$ per group).

solubilization of formazan crystals. Afterwards, 200 µL aliquots were placed in a 96-well plate and the absorbance was measured in a microplate reader at 560 and 650 nm. Results were expressed as percentage of control.

2.6.2. Lactate dehydrogenase (LDH) activity

LDH activity was performed as described elsewhere (Zenki et al., 2014). Briefly, each whole brain ($n = 7$ per group) was incubated for 10 min at 37 °C in a medium containing HBSS buffer (137 mM NaCl; 0.63 mM Na_2HPO_4; 3.0 mM $NaHCO_3$; 5.36 mM KCl; 0.44 mM KH_2PO_4; 1.26 mM $CaCl_2$; 0.90 mM $MgSO_4$; 5.55 mM glucose). Afterwards, the medium containing the released LDH was removed and 30 µL was added in a reaction medium containing: 7.5 mM NAD^+, 260 mM lactate, and 1.6 mM 1,10-phenantroline. The reaction of 1,10-phenantroline with NADH results in the formation of a colored complex with absorption at 490 nm. Independent and untreated brains (2 per group, in duplicate) were mechanically lysed to obtain the total LDH activity. Results were expressed as percentage of total LDH activity per sample.

2.7. Behavioral parameters

Behavioral tests were performed immediately after the treatments in a same period of the day (11:00 AM to 4:00 PM). All tests were recorded using a webcam (Genius Eye 212®). Fish were gently placed in the apparatus using a dip net. Then, the swimming activities were recorded for 6 min as described elsewhere (Mezzomo et al., 2016; Nunes et al., 2016). All experimental sessions were analyzed using appropriate video-tracking software (ANY-maze®, Stoelting CO., USA) at 30 frames/s. All apparatuses were filled with non-chlorinated water adjusted to the same home tank conditions. Water was substituted after each trial and the aquariums used to assess the different behavioral tasks were similar for all fish.

2.7.1. Novel tank diving test

After treatment, fish were placed in the novel tank diving apparatus that consist in a rectangular tank (25 cm length × 15 cm height × 6 cm width) filled with 1.5 L of water (12 cm height) in the same conditions of home tank water (Rosemberg et al., 2011). To analyze the swimming activity, the aquarium was virtually divided into three equal horizontal sections (bottom, middle, and top). Then, the following endpoints were evaluated: time spent in the top, transitions to the top area, total distance traveled and absolute turn angle. A total of 80 fish were used in the test ($n = 20$ per group).

2.7.2. Light-dark test

The light-dark test consisted in a rectangular aquarium (30 cm length × 15 cm height × 10 cm width) divided into two equal parts colored by black or white self-adhesive film. The apparatus was filled with 2.5 L of water (8 cm height) at the same conditions of home tank water. During the trial, fluorescent lamps were used for illumination (approximately 250 lx above the tank). All procedures were performed based in a protocol previously described (Maximino et al., 2010). After treatments, fish were removed from the home tank and gently placed in the white side of the apparatus. Then, during a session of 6 min, the following endpoints were determined: latency to enter in the dark area, time spent in lit area, shuttling, and number of risk assessment episodes. Risk assessment was counted when the fish enter in the white side and immediately (< 1 s) return to the dark compartment, or just partially enter in the lit area (Maximino et al., 2011). Here, the number of risk assessment episodes was measured manually by three trained researchers (inter-rater reliability > 0.85). A total of 80 animals were used in the test (n = 20 per group).

2.8. Statistical analysis

Data of latency to enter in the dark side were expressed as median ± interquartile range and the statistical analysis was carried out using the Kruskal-Wallis test, followed by Dunn's post hoc test. All other results were expressed as means ± standard error of mean (S.E.M) and analyzed by one or two-way analysis of variance (ANOVA), followed by Student-Newman-Keuls multiple comparison test. Results were analyzed using the Graphpad Prism software (version 7.0) and the significance level was set at $p \leq .05$.

3. Results

3.1. Curve of dietary DD and brain Se quantification

Based on the assessment of some toxicological and behavioral parameters, the first set of experiments was performed to choose an appropriate dietary DD concentration for subsequent tests with hyperglycemic fish. Fish were fed with control diets (commercial food and unsupplemented DD diet) or diets containing DD (1.0, 2.0, and 3.0 mg/Kg) for 74 days. Fig. 1 shows that chronic DD intake did not change the body weight of fish when compared to the groups fed with commercial food and/or unsupplemented DD diet. Diets did not affect the survival rate of fish (data not shown). Moreover, DD did not modify the brain cellular viability analyzed by MTT assay and LDH activity (Fig. 2). Dietary DD did not change the behavior of fish in the novel tank diving (Fig. 3) and in the light-dark (Fig. 4) tasks when compared to control.

Based on these results, we chose the diet containing DD 3 mg/Kg to fed fish in the hyperglycemia protocol. We also quantified Se in brain of fish fed with commercial food, unsupplemented DD and DD 3 mg/Kg diets for 74 days. Fig. 5 shows that the amount of Se found in brain of fish fed with DD 3.0 mg/Kg was approximately 2.5-fold higher than the content found in fish fed with unsupplemented DD diet and/or commercial food.

3.2. Hyperglycemia protocol

3.2.1. Fasting blood glucose

Continuous exposure to glucose caused a 3.5- fold increase in blood glucose levels of fish when compared to the control. Fish exposed to glucose and fed with DD diet had levels of blood glucose lower than hyperglycemic fish fed with unsupplemented DD diet (± 2.5-fold). DD diet alone did not affect the glycemia. Statistical analysis also revealed a significant DD × glucose interaction ($F_{1,76}$ = 16.21, p = .0001) and a main effect of glucose ($F_{1,76}$ = 79.54, p < .0001) and DD supplementation ($F_{1,76}$ = 9.324, p = .0031) (Fig. 6).

3.3. Behavioral analysis in hyperglycemic fish

3.3.1. Novel tank diving test

Glucose and dietary DD did not affect the locomotor activity (distance traveled and turn angle) (Fig. 7A and B). Conversely, glucose decreased the time spent in the top, which was prevented by dietary DD. A significant DD × glucose interaction ($F_{1,76}$ = 5.433, p = 0,0224) and a main effect of glucose ($F_{1,76}$ = 21.67, p < .0001) and DD supplementation ($F_{1,76}$ = 5.177, p = .0257) were observed (Fig. 7C). Similarly, the number of transitions to the top area was reduced in fish exposed to glucose. DD diet intake also counteracted this effect induced by hyperglycemia. Statistical analysis of transitions to the top area revealed a significant DD × glucose interaction ($F_{1,76}$ = 10.19, p = 0,0021) and a main effect of glucose ($F_{1,76}$ = 21.11, p < .0001) and DD supplementation ($F_{1,76}$ = 10.92, p = .0015) (Fig. 7D).

3.3.2. Light-dark test

Except for latency to enter in the dark area (Fig. 8B), all other parameters evaluated in the light-dark test were significantly altered by glucose exposure. Fish exposed to glucose spent less time in the lit area. This effect induced by hyperglycemia was counteracted by DD diet intake. Statistical analysis also revealed a significant effect of glucose exposure ($F_{1,76}$ = 8.23, p = .0053) and DD supplementation ($F_{1,76}$ = 5.821, p = .0182). No significant glucose × DD interaction were observed ($F_{1,76}$ = 2.742, p = .1019) (Fig. 8A). Moreover, the number of crossings decreased in fish exposed to glucose. Differently, hyperglycemic fish fed with DD diet did not exhibit changes in this behavior. Here, a significant DD × glucose interaction ($F_{1,76}$ = 11.75, p = .0010) and a main effect of DD supplementation ($F_{1,76}$ = 4.773, p = .0320) were observed (Fig. 8C).

Glucose exposure caused a marked increase in the number of risk episodes, which was not observed in hyperglycemic fish fed with DD diet. Statistical analysis also revealed a significant DD × glucose interaction ($F_{1,76}$ = 4.311, p = .0413) and a main effect of glucose exposure ($F_{1,76}$ = 4.584, p = .0355) (Fig. 8D).

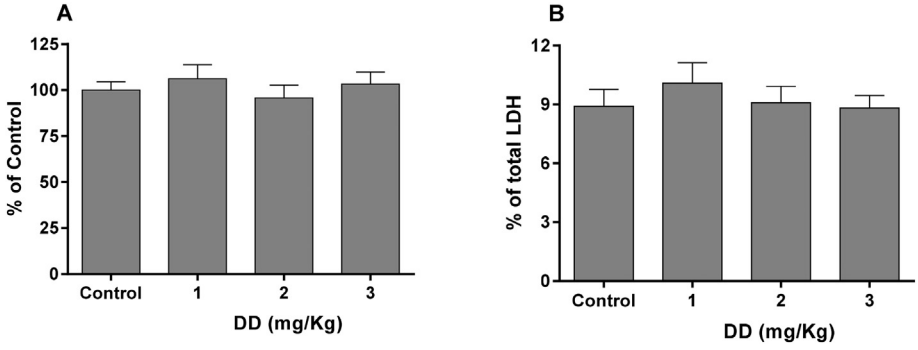

Fig. 2. Effect of dietary DD on brain cellular viability. MTT assay (A) and LDH leakage (B) of brain from fish fed with DD unsupplemented diet or diets containing DD 1, 2 or 3 mg/Kg for 74 days. Data were analyzed by one-way ANOVA, followed by Student-Newman-Keuls multiple comparison test when appropriate and expressed as mean ± SEM and (p < .05, n = 14 per group).

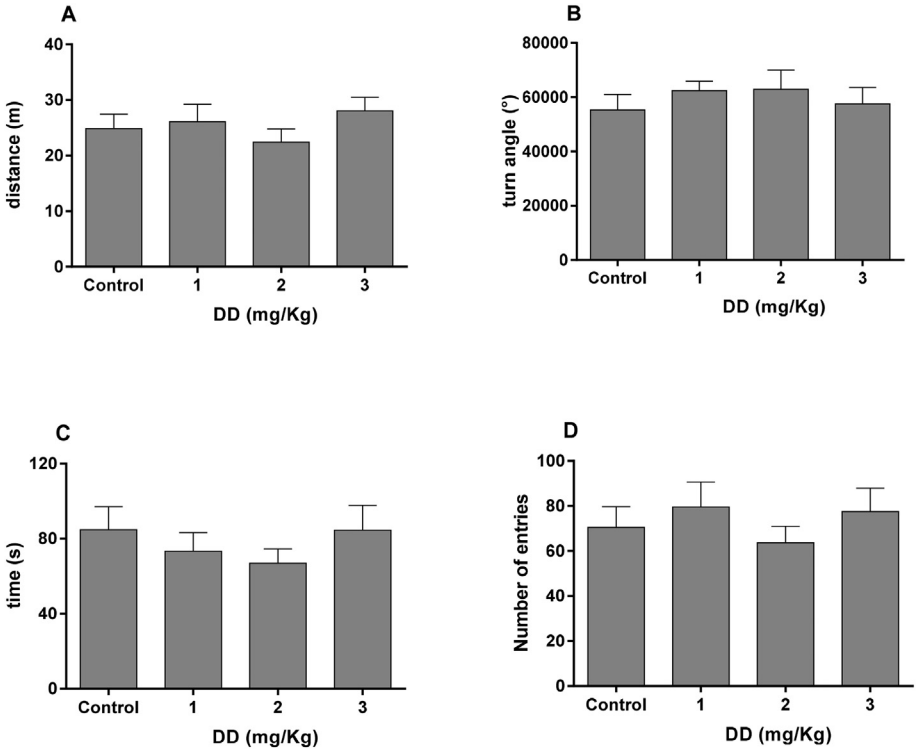

Fig. 3. Effects of dietary DD on locomotor and exploratory parameters in the novel tank diving test. Parameters of distance traveled (A), absolute turn angle (B), time spent in top (C), and transitions to top area (D) from fish groups fed with DD unsupplemented diet or diets containing DD 1, 2 or 3 mg/Kg for 74 days were quantified. Data were analyzed by one-way ANOVA, followed by Student-Newman-Keuls multiple comparison test when appropriate and expressed as mean ± SEM and ($p < .05$, $n = 14$ per group).

4. Discussion

Various protocols that induce a hyperglycemic state in animal models are considered suitable to assess DM complications and putative therapeutic approaches (Ecker et al., 2017; Ou et al., 2016; Tzschentke et al., 2015). In this scenario, some zebrafish features make it a promising model organism for translational studies in DM field. Zebrafish present endocrine islet tissue, which contains hormone-producing β cells; and the teleost insulin is homologous to human insulin (Gleeson et al., 2007; Moon, 2001). Zebrafish also has glucose transporters homologous to the human GLUTs and expresses other important proteins involved in insulin-mediated signaling (Jensen et al., 2006; Maures et al., 2002; Pozios et al., 2001).

The relationship between DM and neuropsychiatric conditions, such as depression and anxiety, has been increasingly studied in recent years (Alba-Delgado et al., 2016; Mast et al., 2017; Roy and Lloyd, 2012). There are different anxiety models, mainly with rodents (Almeida-Souza et al., 2015; Hughes and Otto, 2013; Kangussu et al., 2017;

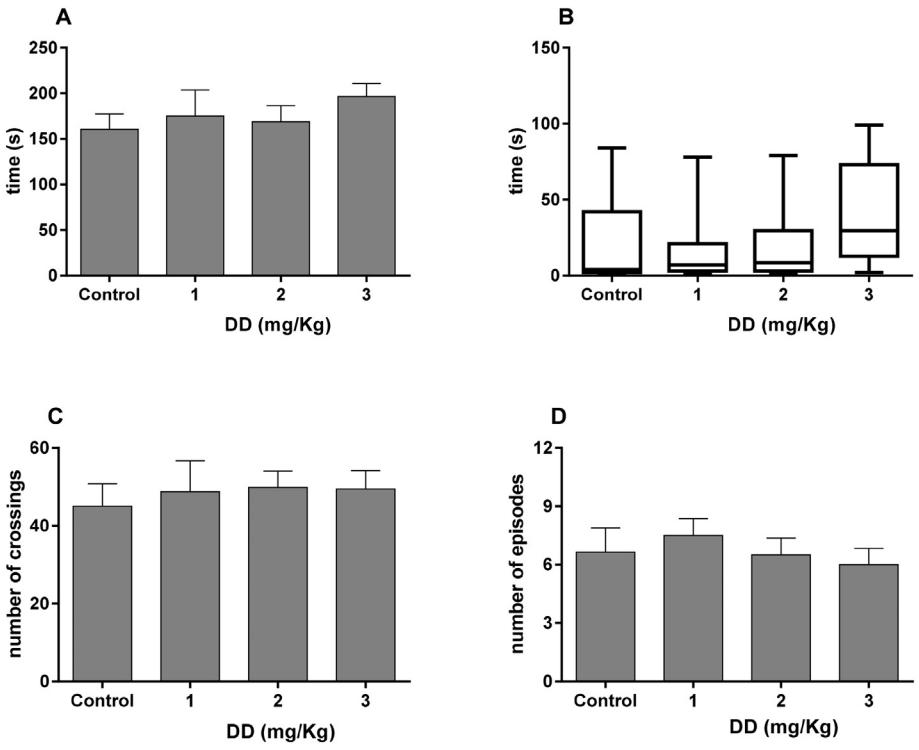

Fig. 4. Effects of dietary DD on anxiety-like behavior in the light-dark test. Parameters of time spent in lit area (A), latency to enter the dark area (B), shuttling (C), and risk assessment (D) from fish groups fed with DD unsupplemented diet or diets containing DD 1, 2 or 3 mg/Kg for 74 days were measured. Latency to enter in dark area was analyzed by Kruskal-Wallis test, followed by Dunn's post hoc test and expressed as median ± interquartille range. The other parameters were analyzed by one-way ANOVA, followed by Student-Newman-Keuls multiple comparison test when appropriate and expressed as mean ± SEM and ($p < .05$, $n = 14$ per group).

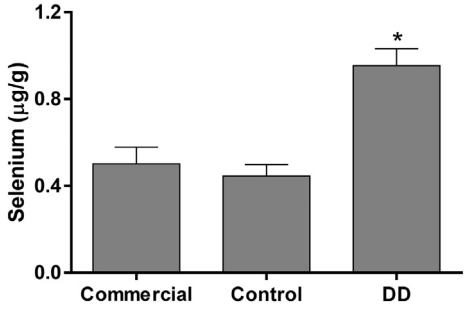

Fig. 5. Brain Se content. Se levels were determined in brain of fish fed with commercial diet, DD unsupplemented diet or DD 3 mg/Kg for 74 days. The quantification was performed by ICP-MS. Data were analyzed by one-way ANOVA, followed by Student-Newman-Keuls multiple comparison test when appropriate and expressed as mean ± SEM. The asterisk indicates statistical differences as compared to the other groups ($p < .05$, $n = 4$ per group).

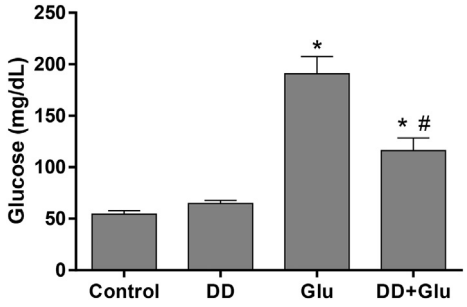

Fig. 6. Effects of glucose exposure and DD diet intake on fasting blood glucose levels. Control (unsupplemented DD diet); DD (DD 3.0 mg/Kg); Glu (glucose) and DD + Glu (DD 3.0 mg/Kg plus glucose). Data were analyzed by two-way ANOVA, followed by Student-Newman-Keuls multiple comparison test when appropriate and expressed as mean ± SEM. The asterisk indicates statistical differences as compared to the control. The hash indicates statistical differences as compared to glucose group ($p < .05$, $n = 20$ per group).

Oliveira et al., 2014). In zebrafish, the novel tank diving test and light-dark test are two behavioral paradigms to measure anxiety-like behaviors (Cachat et al., 2010; Egan et al., 2009; Stewart et al., 2012). The novel tank diving test evaluates the exploratory profile of zebrafish, which have a natural tendency to spend more time on the bottom and gradually enter in the top area of the tank when introduced into a novel environment. Moreover, changes in some behavioral endpoints (e.g. time spent in top and number of entries in the upper area) may reflect a reduced anxiety state. The light-dark test assesses the natural preference of zebrafish for dark environments, which is called scototaxis. Fish with increased anxiety-like behavior usually spend more time in the dark compartment (Stewart et al., 2012). Here, the immersion of zebrafish in a glucose solution did not alter locomotion. However, hyperglycemic fish spent less time in the top and showed reduced transitions to top. In the light-dark test, hyperglycemic fish decreased the number of crossings, spent less time in the lit area, and exhibited a higher number of risk assessment episodes. Together, these phenotypes reflect increased anxiety-like behaviors, showing a relationship between increased blood glucose levels and anxiety in a zebrafish hyperglycemia model. A similar protocol, evidenced that fish immersed in glucose solution had increased glycation of proteins from eyes, which could potentially interfere with the behavioral responses measured here (Capiotti et al., 2014a). However, although we did not examine parameters related with retina damage, the increased number of risk assessment episodes by hyperglycemic fish provides a strong indication of their preserved visual acuity and environment perception.

Among the various chemical elements with biological activity, Se exhibits a wide spectrum of physiological effects, fact that makes it target of many studies toward to human diseases (Álvarez-Pérez et al., 2018; Cardoso et al., 2015; Nogueira and Rocha, 2011). Although Se is essential for all vertebrates, there is a narrow window between its essentiality and toxicity (Nogueira and Rocha, 2011; Roman et al., 2014). Se is required for the synthesis of various selenoproteins including antioxidant enzymes, showing a key role in thyroid, brain, reproductive and immune system functions. However, high levels of Se can be toxic to organisms via thiols oxidation and free radicals generation (Barbosa et al., 2017; Cardoso et al., 2015). In this study, we targeted the diphenyl diselenide as therapeutic molecule toward hyperglycemia and related anxiety mainly due previous findings showing the beneficial effects of dietary DD in diabetic rats (Barbosa et al., 2006). In analogy, here the chronic consumption of diet containing DD reduced partially

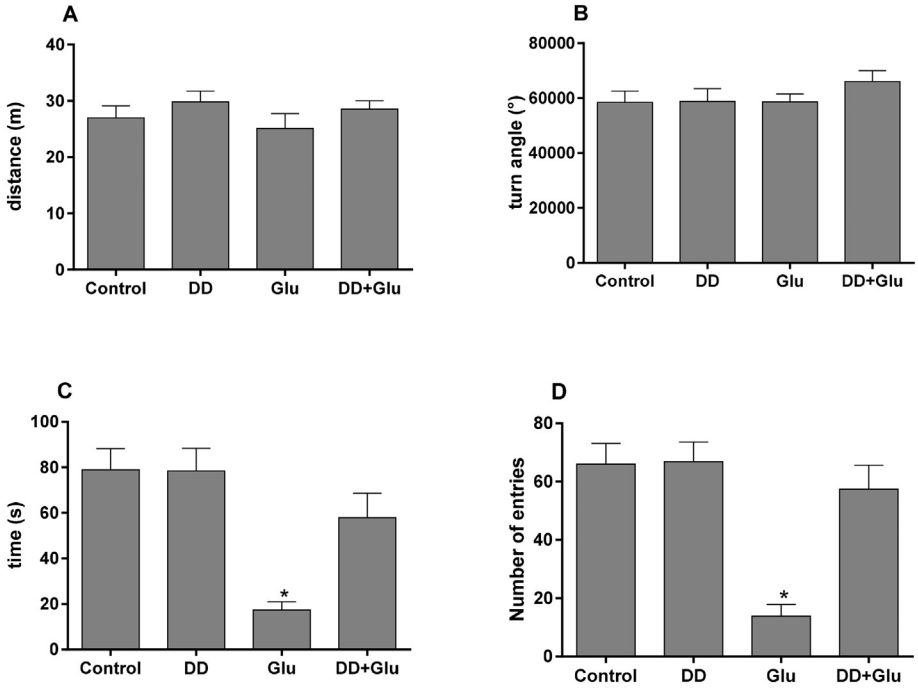

Fig. 7. Effects of glucose exposure and DD diet intake on locomotor and exploratory parameters of fish evaluated by novel tank diving test. Distance traveled (A), absolute turn angle (B), time spent in top (C), and transitions to top area (D). Control (unsupplemented DD diet); DD (DD 3.0 mg/Kg); Glu (glucose) and DD + Glu (DD 3.0 mg/Kg plus glucose). Data were analyzed by two-way ANOVA, followed by Student-Newman-Keuls multiple comparison test when appropriate and expressed as mean ± SEM and. The asterisk indicates statistical differences as compared to the other groups ($p < .05$, $n = 20$ per group).

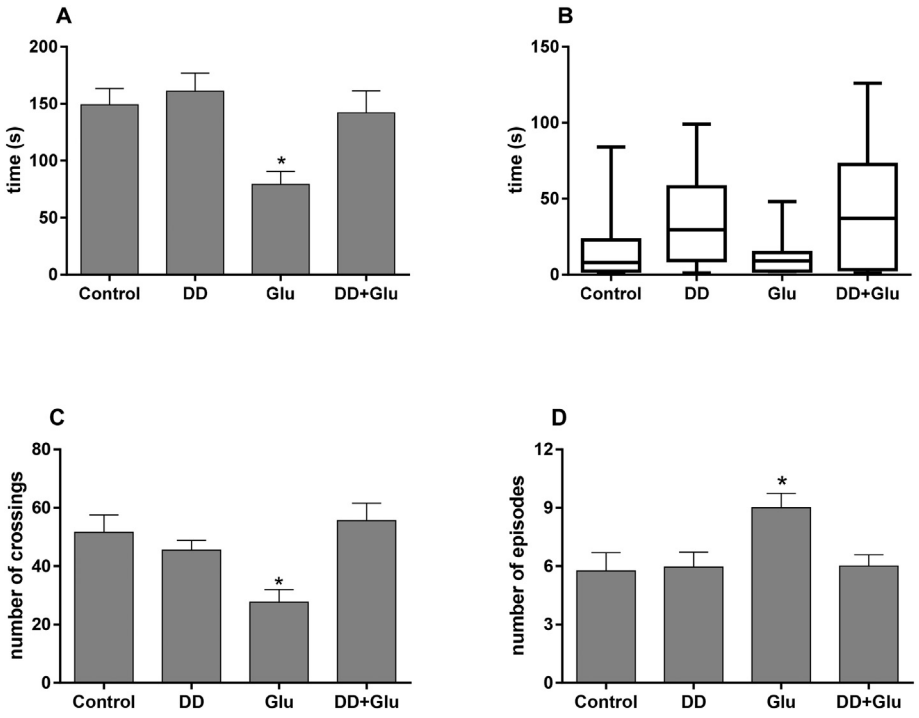

Fig. 8. Effects of glucose exposure and DD diet intake on anxiety-like behavior of fish evaluated by light-dark test. Time spent in lit area (A), latency to enter the dark area (B), shuttling (C), and risk assessment (D). Control (unsupplemented DD diet); DD (DD 3.0 mg/Kg); Glu (glucose) and DD + Glu (DD 3.0 mg/Kg plus glucose). Latency to enter the dark area was analyzed by Kruskal-Wallis test, followed by Dunn's post hoc test and expressed as median ± interquartille range. The other parameters were analyzed by two-way ANOVA, followed by Student-Newman-Keuls multiple comparison test when appropriate and expressed as mean ± SEM and. The asterisk indicates statistical differences as compared to the other groups ($p < .05$, $n = 20$ per group).

the glycemic levels elevated by glucose exposure without causing toxicity in zebrafish. Although the mechanisms underlying the protective effects of Se have not been properly studied so far, some studies with rodent models have suggested that in addition their antioxidant action in pancreatic β-cells, selenium compounds could alleviate the hyperglycemia by mimicking insulin (Stapleton, 2000).

Mounting evidence has highlighted the importance of Se for normal brain functions, showing that Se deficiency is associated with several neurological disorders (Cardoso et al., 2015; Roman et al., 2014). As a corollary, organic and inorganic forms of Se have been extensively studied and expected as therapeutics (Álvarez-Pérez et al., 2018; Nogueira and Rocha, 2011; Roman et al., 2014; Zheng et al., 2017). In this scenario, diphenyl diselenide has been widely explored as an antioxidant and neuroprotective molecule (Dias et al., 2014; Nogueira and Rocha, 2011). Specifically with zebrafish, the anxiolytic role of DD has been demonstrated after exposing the fish to DD diluted in the aquarium water for a short time period (Ibrahim et al., 2014). Here, the chronic intake of dietary DD increased Se levels in brain and reduced the anxiety-like behavior associated to hyperglycemia, showing positive effects in the zebrafish model.

Se preferentially reaches the brain under conditions of dietary selenium deficiency (Cardoso et al., 2015; Roman et al., 2014). This high demand for Se is critical for synthesis of essential selenoproteins, which are highly expressed and distributed throughout the central nervous system (Roman et al., 2014; Steinbrenner and Sies, 2013; Wrobel et al., 2016). Therefore, we presume that DD diet had provided to zebrafish brain an optimal Se status, important to maintain neurological functions. As dietary DD also attenuated hyperglycemia, the neuroprotective effects of DD might involve both hypoglycemia and anxiolysis.

5. Conclusion

In summary, our results show the relationship of hyperglycemia with anxiety onset in zebrafish. In a pharmacological perspective, we also demonstrated that increased Se in the brain could contribute to the hypoglycemic and anxiolytic effects provided by DD supplementation. Although future studies are necessary to elucidate the molecular mechanisms underlying DD actions in the brain, these findings support the relevance of zebrafish hyperglycemia model as a suitable strategy for further behavioral and pharmacological studies in DM field.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors thank the financial support and fellowships from FAPERGS/CNPq/PRONEX n° 16/2551-0000 499-4, Pronem, CAPES and CNPq - Brazil. NVB, DBR and VLL are recipients of CNPq fellowships.

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