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ORIGINAL PAPER

Long-Term Methionine Exposure Induces Memory Impairment on Inhibitory Avoidance Task and Alters Acetylcholinesterase Activity and Expression in Zebrafish (*Danio rerio*)

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Abstract Hypermethioninemic patients exhibit a variable degree of neurological dysfunction. However, the mechanisms involved in these alterations have not been completely clarified. Cholinergic system has been implicated in many physiological processes, including cognitive performances, as learning, and memory. Parameters of cholinergic signaling have already been characterized in zebrafish brain. Since zebrafish is a small freshwater teleost which is a vertebrate model for modeling behavioral and functional parameters related to human pathogenesis and for clinical treatment screenings, in the present study we investigated the effects of short- and long-term methionine exposure on cognitive impairment, AChE activity and gene expression in zebrafish. For the studies, animals were exposed at two methionine concentrations (1.5 and 3.0 mM) during 1 h or 7 days (short- or long-term treatments, respectively). We observed a significant increase in AChE activity of zebrafish brain membranes after long-term methionine exposure at 3.0 mM. However, AChE gene expression decreased significantly in both concentrations tested after 7 days of treatment, suggesting that post-translational events are involved in the enhancement of AChE activity. Methionine treatment induces memory deficit in zebrafish after long-term exposure to this amino acid, which could be related, at least in part, with cognitive impairment observed in hypermethioninemia. Therefore, the results here presented raise a new perspective to use the zebrafish as a complementary vertebrate model for studying inborn errors of metabolism, which may help to better understand the pathophysiology of this disease.

Keywords Acetylcholinesterase · Hypermethioninemia · Memory · Zebrafish

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Introduction

Methionine (Met) is an essential amino acid that plays an important role in the development of the central nervous system (CNS), contributing to brain function, such as myelin formation. However, abnormally elevated Met levels and/or its metabolites are potentially toxic [1–3]. Hypermethioninemia can occur under different conditions and, at present, six genetic conditions which lead to elevated methionine rates were identified, such as methionine adenosyltransferase (MAT) I/III deficiency, classical homocystinuria (due to cystathionine beta-synthase deficiency), deficiencies of citrin, glycine N-methyltransferase, S-adenosylhomocysteine hydrolase, and fumarylacetoacetate hydrolase (tyrosinemia type I) [4].

Hypermethioninemic patients present neurological dysfunction, including cognitive deficit, demyelination, memory impairment, and cerebral edema formation [2, 4–8]. Although the mechanisms involved in these alterations

have not been completely clarified, studies performed in animals submitted to an experimental model of hypermethioninemia, with plasma Met concentration similar to those found in patients with MAT deficiency [9], present biochemical and physiological alterations on the CNS. In this context, it has been shown that elevated Met concentrations increase lipid peroxidation and reduce Na^{+}, K^{+}-ATPase activity in synaptic plasma membranes from hippocampus of rats [10]. More recently, Stefanello et al. [11] showed that this inhibition on Na^{+}, K^{+}-ATPase activity caused by Met was partially prevented by antioxidants, suggesting that oxidative stress can be involved in the Met effect. Moreover, hypermetioninemia increases Ach hydrolysis in rat cerebral cortex [12].

Several studies have demonstrated the participation of cholinergic system in neurodegenerative disorders that can cause dementia. Moreover, it has been demonstrated that decreased levels of acetylcholine (ACh) in the brain are associated with deficits in cognitive performances, as learning, behavior, and memory processes [13–17]. ACh is widely distributed in the nervous system and elicits its effects via muscarinic (metabotropic) and nicotinic (ionotropic) ACh receptors in the extracellular space. The inactivation of cholinergic signaling in the synaptic cleft is promoted by acetylcholinesterase (AChE) (E.C.3.1.1.7) and butyrylcholinesterase (BuChE) (E.C.3.1.1.8) [18], which cleave acetylcholine into choline and acetate, allowing its reuptake through the choline transporter.

Zebrafish (*Danio rerio*) is a small freshwater teleost that becomes a promising model organism for developmental neurobiology, neurodegenerative diseases, and toxicological studies [19–25]. Zebrafish is an ideal animal model for laboratory research because they are inexpensive, low-maintenance, and produce a large number of offspring [26–28]. Zebrafish genes are highly conserved, sharing a 70–80 % homology to those of humans [29]. For this reason, it is a tempting vertebrate model for modeling behavioral and functional parameters related to human pathogenesis and for clinical treatments screening, which includes therapeutic strategies. Parameters of cholinergic signaling have already been characterized in zebrafish brain [30, 31]. AChE gene is already cloned and sequenced and its enzyme activity was detected in zebrafish brain [32], but BuChE was not detected in zebrafish genome [30]. Furthermore, cholinergic receptors are also expressed in neuronal tissues of this teleost [33].

Considering that (1) hypermethioninemic patients exhibit a variable degree of neurological dysfunction, (2) the mechanism involved in this pathology remain poorly understood, (3) cholinergic system is associated with neurodegenerative disorders, and (4) zebrafish has become a promising model to many human diseases, such as neurodegenerative diseases, we sought to investigate the effects of short- and long-term Met exposure on cognitive impairment, AChE activity and gene expression in zebrafish.

Materials and Methods

Animals

Wild-type adult zebrafish were obtained from specialized commercial supplier (Redfish, RS, Brazil) and acclimated for at least 2 weeks before the experiments. Animals were kept in 50 L housing tanks with tap water previously treated with Tetra's AquaSafe® (to neutralize chlorine, chloramines, and heavy metals present in the water that could be harmful to fish) and continuously aerated (7.20 mgO_2/L) at 26 ± 2 °C, under a 14–10 h light/dark photoperiod and up a density of five animals per liter [34]. They were fed three times a day with TetraMin Tropical Flake fish®.

Chemicals

L-Methionine, Trizma Base, EDTA, EGTA, sodium citrate, Coomasie Blue G, bovine serum albumin, acetylthiocholine, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) were purchased from Sigma (St. Louis, USA). Trizol® Reagent, dNTPs, oligonucleotides, Taq polymerase, Syber Green, Low DNA Mass Ladder, and SuperScript™ III First-Strand Synthesis SuperMix were purchased from Invitrogen (Carlsbad, CA, USA). Primers were obtained from Integrated DNA Technologies (Coralville, IA, USA). All other reagents used were of analytical grade.

Experimental Protocols

For short- and long-term exposure to Met, animals were introduced in 4-L aquariums containing two different final concentrations of this amino acid (1.5 mM and 3.0 mM) dissolved in tank water (three animals per liter of water). These doses were chosen based on methionine concentrations found in plasma of hypermethioninemic patients [5] and previous reports performed in rodents [9]. For the short-term Met exposure, animals were maintained in the test aquarium during 1 h whereas the long-term Met exposure was performed during 7 days, replacing the water in the fish treatment tanks daily. The aquariums were continuously aerated (7.20 mgO_2/L). Immediately after the treatments, the fish were cryoanaesthetized and further euthanized by decapitation. The brains were dissected before performing biochemical and molecular analysis. For behavior studies, all sessions were performed in the morning.

For AChE activity determination we used a pool of three whole zebrafish brains to prepare the homogenate fraction.

For each group tested (control, Met 1.5 mM, and Met 3.0 mM), we performed five different experiments in both short- and long-term Met exposure, totalizing 90 animals. For gene expression analysis we performed four independent assays for each group tested (control, Met 1.5 mM, and Met 3.0 mM) in long-term Met exposure and a pool of five whole zebrafish brains was used for each independent experiment, totalizing 60 animals. For investigating the effects of short- and long-term Met exposure on exploratory capacity, animals were divided in control, Met 1.5 mM, and Met 3.0 mM-exposed groups ($n = 6$ per group). To establish the effect of long-term Met exposure on zebrafish inhibitory avoidance task long-term memory, animals were divided in control, Met 1.5 mM, and Met 3.0 mM-exposed groups ($n = 12$ per group). In order to perform a control experiment for the inhibitory avoidance task effects, animals from the same groups ($n = 10$) were exposed to an equivalent electric shock and later tested on the inhibitory avoidance. This study was entirely between-subjects and used a total of 234 animals.

Assay of AChE Activity

A pool of three whole zebrafish brains was used to prepare each homogenate fraction. The brains were gently homogenized on ice in 60 volumes (v/w) of Tris–citrate buffer (50 mM Tris, 2 mM EDTA, 2 mM EGTA, pH 7.4, with citric acid) using a Potter–Elvehjen-type glass homogenizer. AChE activity was measured according to the method previously described by Ellman et al. [35]. AChE activity in the homogenate was measured by determining the rate of hydrolysis of acetylthiocholine iodide (0.88 mM) in 300 μL, with 33 μL of 100 mM phosphate buffer, pH 7.5 mixed to 33 μL of 2.0 mM 5,5′-dithionitrobis 2-nitrobenzoic acid (DTNB). Before the addition of substrate, samples containing 5 μg of protein and the reaction medium mentioned above were pre-incubated for 10 min at 25 °C. The hydrolysis of acetylthiocholine iodide was monitored by the formation of thiolate dianion of DTNB at 412 nm for 2–3 min (intervals of 30 s). Controls without the homogenate preparation were performed in order to determine the non-enzymatic hydrolysis of the substrate. The linearity of absorbance towards time and protein concentration was previously determined. All reactions were performed in quadruplicate. AChE activity was expressed as micromole of thiocholine (SCh) released per hour per milligram of protein (μmol thiocholine h^{-1} mg $protein^{-1}$).

Protein Determination

Protein was measured by the Coomassie Blue method using bovine serum albumin as standard [36].

Gene Expression Analysis by Quantitative RT-PCR

Analysis of the *ache* gene expression was performed by a quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR) assay. RT-qPCR was performed on a 7500 Real-Time PCR System (Applied Biosystems) with SYBR green fluorescent label. The *ache* primers were designed using the Oligos 9.6 program. The *EF1α*, *Rpl13α and β-actin* primers were used as constitutive genes for data analysis, as described previously [37] (Table 1). After 7 days of Met treatment, the animals were euthanized and the brains were removed for total RNA extraction with Trizol® Reagent in accordance with the manufacturer's instructions. Four independent assays for each tested group were performed and a pool of five whole zebrafish brains was used for each independent experiment. The cDNA species were synthesized using SuperScript™ III First-Strand Synthesis SuperMix from 3 μg of total RNA following the supplier's instructions. For qPCR assays, 12.5 μL of diluted cDNA (1:50) was used as template. qPCR reaction for all genes of interest were carried out in a volume of 25 μL using a concentration of 0.02 μM of each primer, 0.1 μM dNTP (deoxyribonucleotide triphosphate), 3.0 mM $MgCl_2$, 0.02 μM Syber Green, and 5 U/μL Taq DNA polymerase (Invitrogen). Samples were run in quadruplicate in optically clear 96-well plates (Applied Biosystems). For each qPCR set, a negative control was included. Cycling parameters were as follows: 94 °C × 5 min, then 40 cycles of the following 94 °C × 15 s, 60 °C × 10 s, 72 °C × 15 s, 60 °C × 35 s. For each sample a dissociation step was performed at 94°C × 10 s, 50 °C × 2 min, and 95 °C × 15 s Relative expression levels were determined with 7500 Real-Time Software v.2.0.5 (Applied Biosystems). LinReg was used to calculate the primer efficiency per sample and GeNorm used to estimate the optimal number of reference genes (pairwise variation) and their stability (*M* value). The relative RNA expression levels were determined using the $2^{-\Delta\Delta CT}$ method.

Table 1 qPCR primers sequences

Gene		Sequence (5′-3′)
Ache	Sense	GCTAATGAGCAAAAGCATGTGGGCTTG
Ache	Antisense	TATCTGTGATGTTAAGCAGACGAGGCAGG
EF1α	Sense	CTGGAGGCCAGCTCAAACAT
EF1α	Antisense	ATCAAGAAGAGTAGTACCGCTAGCATTAC
Rpl13α	Sense	TCTGGAGGACTGTAAGAGGTATGC
Rpl13α	Antisense	AGACGCACAATCTTGAGAGCAG
β-actin	Sense	CGAGCTGTCTTCCCATCCA
β-actin	Antisense	TCACCAACGTAGCTGTCTTTCTG

Exploratory Assessment

The animals were individually transferred to a 2.7 L tank (24 cm L × 8 cm W × 20 cm H) with white covered laterals and bottom, except for the front to avoid any visual disturbances, and they were first habituated to the tank for 30 s, as previously described [38]. There was no drug exposure during behavioral experiments. The locomotor activity of the animals was video recorded using Logitech Quickcam PRO 9000 for 5 min after the habituation period and further analyzed using the ANY-Maze recording software (Stoelting Co., Wood Dale, IL, USA). The tank was divided into two equal sections with one horizontal line, and the following behavioral patterns were measured: distance traveled, mean speed, number of line crossings (horizontal line), absolute turn angle, and time spent in upper and lower half.

Inhibitory Avoidance Apparatus

In order to investigate long-term memory of zebrafish in an inhibitory avoidance apparatus, we used adult male animals (>8 months old) according to Blank et al. [39]. For establishing the effect of Met exposure in the one-trial inhibitory avoidance task, we have treated the animals with Met for 7 days, at two different concentrations (1.5 and 3 mM) previously to training session. A glass tank (18 cm L × 9 cm W × 7 cm H) divided by a sliding guillotine-type partition (9 cm × 7 cm) in two equally sized compartments, designated hereon as dark and white, was used. Compartments were defined by opaque plastic self-adhesive films in black or white colors externally covering walls, floor and the corresponding sides of the partition. Briefly, in each session, the animals were individually gently placed in the white tank compartment while the sliding partition between both compartments was closed. After 1 min of habituation and orientation, the partition was raised, allowing the fish to cross to the dark side of the tank through a 1 cm high opening. In the training session, the sliding partition was immediately closed after the fish crossed to the dark compartment and a pulsed electric shock of 3 ± 0.2 V AC was administered for 5 s. After that, the fish was removed from the apparatus. Twenty-four hours after training, animals were submitted to a test session that repeated the training protocol except that no shock was administered. The latency to enter the dark compartment was measured in all sessions.

To ensure the inhibitory avoidance test results were specific to the association of shock and the dark chamber, animals from control and Met treated groups were exposed to an equivalent shock in a glass tank of similar dimensions but with no white/dark compartmentalization and 24 h later evaluated in the inhibitory avoidance apparatus. After 7 days of treatment, animals were gently placed individually on a glass tank (5 cm L × 5 cm W × 4 cm H). After 1 min of habituation and orientation a pulsed electric shock of 3 ± 0.2 V AC was administered for 5 s. Twenty-four hours later, animals were submitted to an identical session to test inhibitory avoidance. Animals were individually gently placed in the white tank compartment of the inhibitory avoidance apparatus with the sliding partition between both compartments closed. After 1 min of habituation and orientation, the partition was raised, allowing fish to cross to the dark side of the tank through a 1 cm high opening. No shock was administered in this session. We measured the latency is seconds to enter the dark compartment in the test session.

Statistical Analysis

For AChE activity and gene expression, results are expressed as means ± standard error (SEM). Statistical analysis was performed by one-way analysis of variance (ANOVA), followed by a Tukey multiple range test. Statistically significant differences between groups were considered for a $P < 0.05$. For behavioral studies, data were analyzed parametrically using SPSS software and shown as means ± SEM. The time spent on each compartment and the number of crossings during each half period of the 5 min tank recognition session was compared by paired *t* test. Inhibitory avoidance training and test latencies for each group were compared using paired *t* test. Comparisons between groups were performed using independent *t* test. In all comparisons, $P < 0.05$ was considered to indicate statistical significance. Latencies in the control experiment protocol for inhibitory avoidance test were compared by paired *t* test.

Results

Effect of Short- and Long-Term Methionine Exposure on Acetylcholinesterase Activity in Zebrafish Brain Membranes

The effect of Met exposure was evaluated on AChE activity in zebrafish brain membranes. The experiments were performed after 1 h (short-term) and 7 days (long-term) in vivo Met exposure at two different concentrations (1.5 and 3 mM). For short-term Met exposure there was no significant difference between the groups analyzed and the control group (Fig. 1a). In Fig. 1b, we observed that long-term Met exposure at 3.0 mM significantly increased the enzyme activity when compared to the control group (approximately 36 %). On the other hand, long-term Met

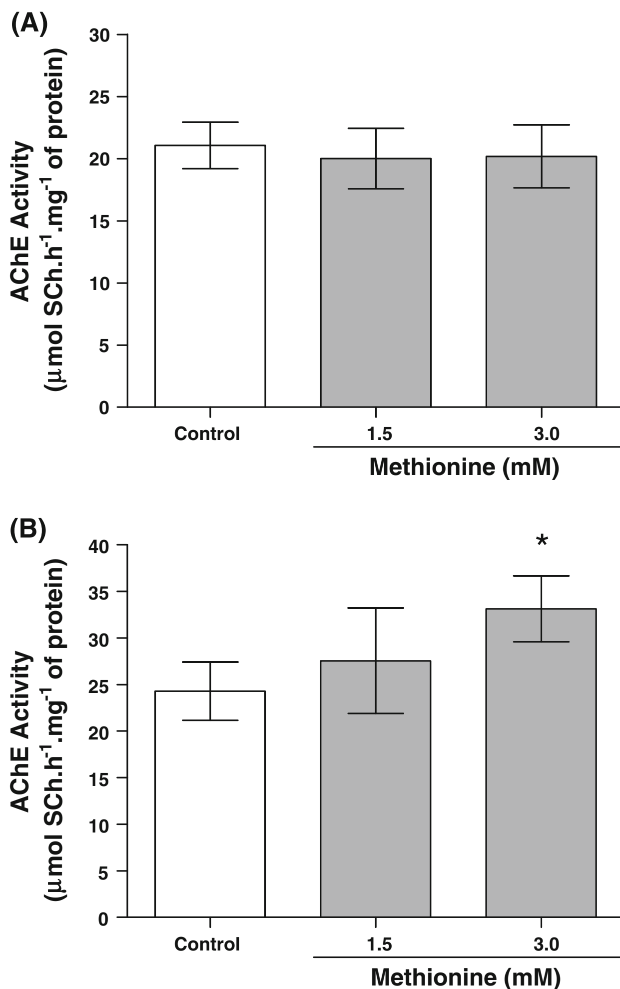

Fig. 1 Effect of short- (1 h) (**a**) and long-term (7 days) (**b**) Met exposure (1.5 and 3.0 mM) on acetylcholinesterase (AChE) activity in zebrafish brain membranes. Data are expressed as mean ± SEM of five independent experiments performed in quadruplicate. Data were analyzed statistically by one-way ANOVA followed by Tukey test as post hoc test. *Asterisk* indicates statistically significant difference between treated and control group ($P < 0.05$)

exposure, at 1.5 mM, did not alter AChE activity when compared to the control group (Fig. 1b).

Effect of Long-Term Methionine Exposure on Acetylcholinesterase Gene Expression in Zebrafish Brain

We performed a quantitative RT-PCR analysis in order to evaluate the influence of Met exposure on *ache* gene expression in zebrafish brain. As it can be observed in Fig. 2, *ache* gene expression was decreased after 7 days of Met treatment in tank water in both concentrations tested (1.5 and 3.0 mM Met) (approximately 16 and 17 %,

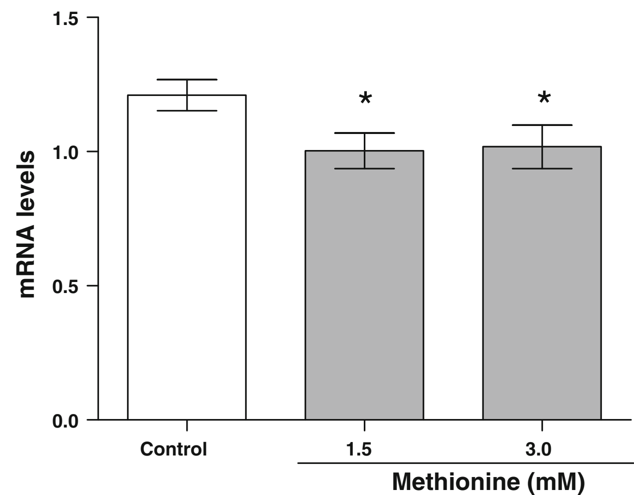

Fig. 2 Effect of long-term Met exposure (1.5 and 3.0 mM) on acetylcholinesterase (ache) gene expression in zebrafish brain. Data are expressed as mean ± SEM of four independent experiments performed in quadruplicate. Data were analyzed statistically by one-way ANOVA followed by Tukey test as post hoc test. *Asterisk* Indicates statistically significant difference between treated and control group ($P < 0.05$)

respectively). For normalization, *β-actin*, *Rpl13α*, and *EF1α* were used as constitutive genes.

Short- and Long-Term Methionine Exposure Effects on Exploratory Assessment

Distinct parameters of zebrafish swimming activity were examined in the tank diving behavioral test. No differences were found in the locomotor activity of animals receiving 1.5 mM and 3.0 mM Met in both short- and long-term exposure, as indicated by the line crossings, distance traveled, and mean speed parameters when compared to the control group (Fig. 3a–c, respectively). No differences in the time spent in the upper half or the lower half were found in the treated animals in comparison with the control group (Fig. 3d) showing no anxiogenic properties of Met exposure on zebrafish.

Long-Term Methionine Exposure Induces Memory Deficits in an Inhibitory Avoidance Paradigm

The water-treated control group and 1.5 mM Met-exposed animals showed a robust long-term memory retention on the inhibitory avoidance test session performed 24 h after training when training and test latencies were compared ($P < 0.05$ for both groups). However, exposure to 3.0 mM Met significantly impaired memory formation (with no significant statistical difference between training and test session latencies in 3.0 mM Met-treated animals) (Fig. 4).

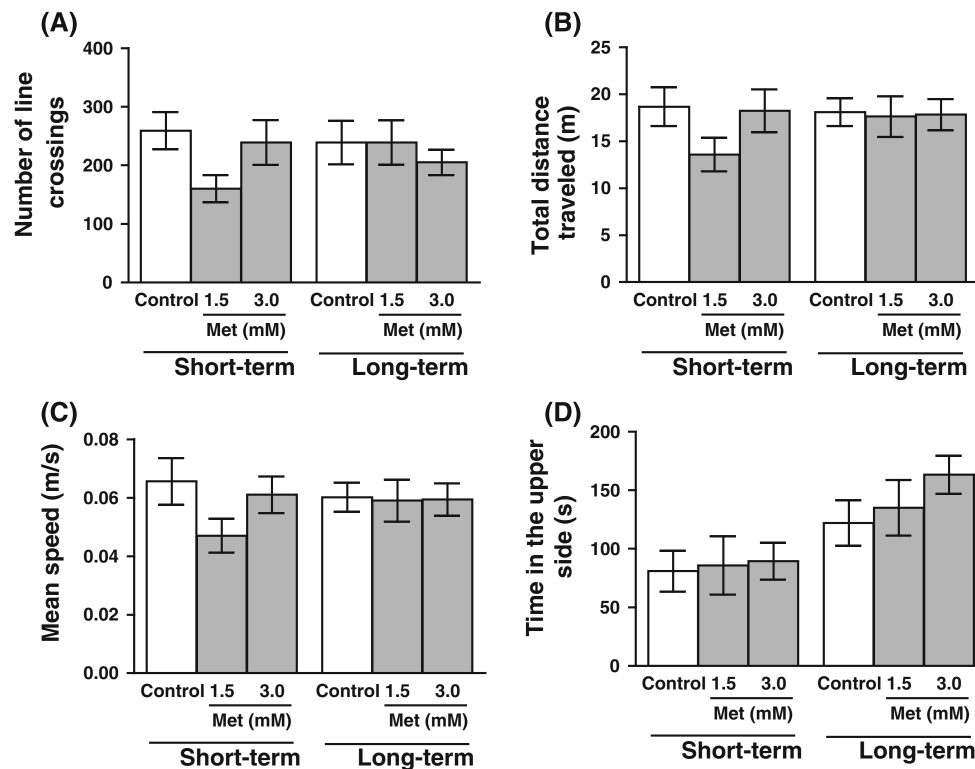

Fig. 3 Short- and long-term Met exposure no affects zebrafish locomotor activity. Effect of exposure to water only, Met 1.5 mM, and Met 3.0 mM, on the number of line crossings (**a**), distance traveled (**b**), mean speed (**c**), and time spent in the upper zone (**d**) determined during 5 min of video recording in the tank diving behavioral test. Data were expressed as mean ± SEM of six animals in each group ($P > 0.05$)

Importantly, no differences were found between training latencies for all groups, demonstrating that their dark-environment preference Blank et al. [39] remained intact and corroborating the lack of treatment effect on exploratory and locomotor parameters evaluated (Fig. 3). In order to demonstrate that the memory impairing effects of 3.0 mM Met was due to an specific cognitive impairment, a control experiment in which animals were submitted to the same treatment but receive an equivalent shock in another environment (not paired with the black chamber) was performed. Animals from control and Met-treated groups that were not trained in the inhibitory avoidance apparatus—but instead received an electric shock in a different environment—showed very low latencies (equivalent to inhibitory avoidance training latencies) to enter the dark compartment 24 h later when exposed to the inhibitory avoidance apparatus (Fig. 5). These results demonstrate the specificity of 3.0 mM Met-exposure induced deficits.

Discussion

Hypermethioninemic patients exhibit a variable degree of neurological dysfunction. Nevertheless, the mechanisms of this pathology remain poorly understood [5–7]. Therefore, in the present study, we investigated the effects of short- and long-term Met exposure on memory and AChE activity, and gene expression pattern. We observed a significant increase in AChE activity of zebrafish brain membranes after long-term Met exposure at 3.0 mM. In order to determine whether long-term Met exposure could alter AChE gene expression, we performed quantitative RT-PCR assays. Interestingly, the results demonstrated that AChE gene expression decreased significantly in both concentrations tested (1.5 and 3.0 mM). Gene expression is regulated by various factors involving cell machinery and signal transduction pathways, so enzyme activity cannot be directly correlated with the gene expression pattern or with protein levels due to the existence of several post-translational events [40]. Therefore, the control exerted by phosphorylation is one possible reason for the changes observed in AChE activity, since zebrafish AChE sequence presents a high predicted score of possible PKC phosphorylation sites (Thr271 and Thr761). Long-term Met exposure could exert an influence on AChE post-translational modulation, increasing ACh hydrolysis, that in turn down regulates its own expression, via a phenomenon named negative feedback loop [41, 42] in zebrafish brain.

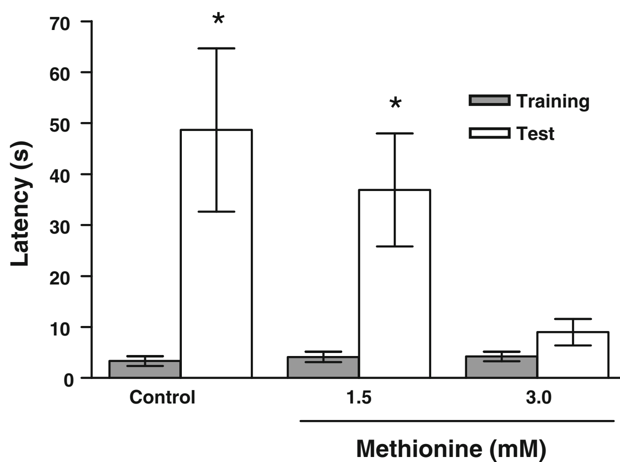

Fig. 4 Methionine induces long-term memory deficits in inhibitory avoidance. Effect of long-term Met 3.0 mM exposure on latency to cross to *dark* compartment in training and long-term memory test sessions in the inhibitory avoidance task. *Columns* indicate mean latencies ± SEM to cross from *white to dark* compartment (in seconds) in training and test sessions for both control and treated groups (n = 12 per group). Control animals were manipulated equally as treated animals, except that they were exposed to water only. Data were analyzed parametrically using SPSS software and compared by paired *t* test. *Asterisk* indicates statistically significant difference between latencies on training and test sessions ($P < 0.05$)

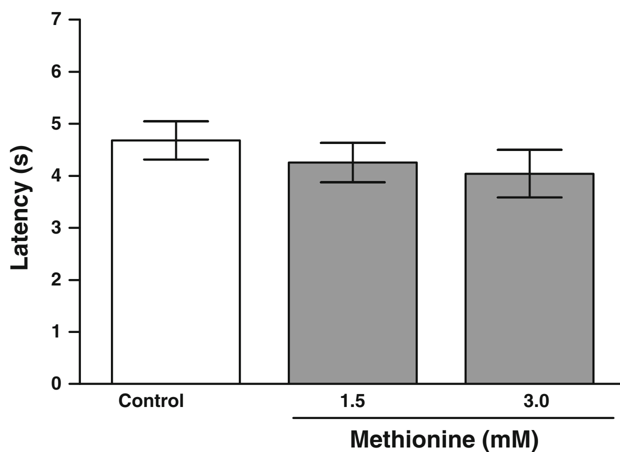

Fig. 5 Isolated shock no affects latency to cross to *dark* compartment after long-term Met exposure. Animals were exposed to water only, Met 1.5 mM, and Met 3.0 mM. *Columns* indicate mean latencies ± SEM to cross from *white to dark* compartment (in seconds) for both control and treated groups (n = 10 per group). Control and treated animals were manipulated equally. Data were analyzed parametrically using SPSS software and compared by paired *t* test ($P > 0.05$)

The cholinergic system has been implicated to play a critical role in many physiological processes, including cerebral cortical development and activity, cognitive performances, such as learning, and memory [14, 43, 44]. Cholinergic hypofunction has been related to the progressing memory deficits with aging [17] and cholinesterase inhibitors have been used as cognitive enhancers for the treatment of neurodegenerative diseases, such as Alzheimer's disease [45]. Regarding this, we also investigated the effect of Met exposure on zebrafish memory. In order to verify whether the alterations in behavior are related to peripheral locomotor effects, instead of memory disruption, we performed a general analysis on zebrafish locomotor behavior. We did not observe changes in any parameters analyzed in both short- and long-term Met exposure. For zebrafish behavioral studies, we used a one-trial passive avoidance task protocol designed by Blank et al. [39], which is comparable to the one used in rodents and may represent a valuable tool to characterize different processes affecting memory in the zebrafish. We observed a significant memory deficit in the group exposed to Met at 3.0 mM after 7 days of treatment. This effect was observed for the same concentration able to increase ACh hydrolysis, reinforcing the correlation between cholinergic system and memory formation. In light of this evidence, we believe that the Met memory deficits observed were due to the effect of high levels of this amino acid on the cholinergic system. Stefanello et al. [12] demonstrated that chronic hypermethioninemia increases AChE activity in rat brain as well as it induces impairment in working memory performance in the water maze task. The authors suggest that the constant stimulation of this enzyme by Met might decrease ACh levels, which could be associated to memory deficits observed in hypermethioninemic rats. Elevated Met concentrations also reduce Na^+, K^+-ATPase activity, which was prevented by antioxidants [10, 11]. Besides, it has also been shown that chronic administration of Met significantly reduced the quantity of the major gangliosides (GM1, GD1a, GD1b and GT1b) in rat cerebral cortex [46]. Since gangliosides are closely associated with neuronal membranes and participate in several neuronal functions [47, 48], these results suggest that elevated Met levels promote alterations in brain plasma membranes, reinforcing the hypothesis that Met, through oxidative damage, could affect the function of membrane-bound enzymes, such as Na^+,K^+-ATPase and AChE, which may have serious consequences on neuronal function.

Zebrafish is used in many areas of biological research, which ranges from developmental biology, biomedicine, drug discovery [49], model for human disease [19, 50], and behavioral analysis [27, 39, 51, 52]. Additionally to the advantages of its use, such as the small size and maintenance cost, the fast development, and the transparency of embryos and larvae throughout its development [53, 54], zebrafish has been suitable for developing chemical models of amino acid inborn errors of metabolism. Moreover, a major advantage for use of this teleost in noninvasive behavioral screenings is its ability to rapidly and efficiently

absorb small molecules in water, when compared to other model organisms [39].

In summary, our findings demonstrated that long-term Met exposure significantly increase ACh hydrolysis and decrease AChE expression, suggesting that post-translational events might be involved in AChE activity enhancement. Besides, Met-induced memory deficit in zebrafish after long-term exposure to this amino acid could be related, at least in part, with cognitive impairment observed in hypermethioninemia. The findings presented here raise a new perspective to use the zebrafish as a complementary vertebrate model for studying inborn errors of metabolism, which may help to better understand the pathophysiology of this disease.

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Conflict of interest The authors report no conflicts of interest.

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