Neuroscience 250 (2013) 121–128

PROLINE-INDUCED CHANGES IN ACETYLCHOLINESTERASE ACTIVITY AND GENE EXPRESSION IN ZEBRAFISH BRAIN: REVERSAL BY ANTIPSYCHOTIC DRUGS

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Abstract—Hyperprolinemia is an inherited disorder of proline metabolism and hyperprolinemic patients can present neurological manifestations, such as seizures, cognitive dysfunctions, and schizoaffective disorders. However, the mechanisms related to these symptoms are still unclear. In the present study, we evaluated the *in vivo* and *in vitro* effects of proline on acetylcholinesterase (AChE) activity and gene expression in the zebrafish brain. For the *in vivo* studies, animals were exposed at two proline concentrations (1.5 and 3.0 mM) during 1 h or 7 days (short- or long-term treatments, respectively). For the *in vitro* assays, different proline concentrations (ranging from 3.0 to 1000 μM) were tested. Long-term proline exposures significantly increased AChE activity for both treated groups when compared to the control (34% and 39%). Moreover, the proline-induced increase on AChE activity was completely reverted by acute administration of antipsychotic drugs (haloperidol and sulpiride), as well as the changes induced in *ache* expression. When assessed *in vitro*, proline did not promote significant changes in AChE activity. Altogether, these data indicate that the enzyme responsible for the control of acetylcholine levels might be altered after proline exposure in the adult zebrafish. These findings contribute for better understanding of the pathophysiology of hyperprolinemia and might reinforce the use of the zebrafish as a complementary vertebrate model for studying inborn errors of amino acid metabolism. **© 2013 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: zebrafish, acetylcholinesterase, proline, inherited diseases, hyperprolinemia, haloperidol, supiride.

INTRODUCTION

Hyperprolinemia can be caused by two distinct inherited disorders of proline metabolism. Hyperprolinemia type I (HPI) occurs due to the deficiency of proline oxidase (POX; EC 1.5.1.2). The hyperprolinemia type II (HPII) is caused by deficiency of Δ^1-pyrroline-5-carboxylic acid dehydrogenase (P5CDh; EC 1.5.1.12) activity. These enzymatic defects cause proline accumulation in blood and others tissues, such as the brain (Phang et al., 2001). As a result, some hyperprolinemic patients can present epilepsy and cognitive dysfunctions whereas others are asymptomatic (Flynn et al., 1989; Phang et al., 2001; Di Rosa et al., 2008). Although proline metabolism seems to be specifically related to psychotic disorders, such as schizophrenia (Phang et al., 2001; Jacquet et al., 2005; Oresic et al., 2011), the mechanisms underlying these neurological manifestations still remain poorly understood.

Several reports proposed that high proline levels have a detrimental effect on neuronal integrity, inducing changes in different neurotransmitter systems. Studies showed that proline may activate NMDA and AMPA receptors, suggesting that it potentiates the glutamatergic neurotransmission (Nadler, 1987; Nadler et al., 1992; Cohen and Nadler, 1997). Moreover, high proline levels were able to decrease glutamate uptake in the rat brain, as well as the Na^+, K^+-ATPase and creatine kinase activities, which are crucial enzymes for normal brain function (Pontes et al., 1999, 2001; Kessler et al., 2003; Delwing et al., 2007). Additionally, proline also impaired memory (Bavaresco et al., 2005; Delwing et al., 2006) and altered the

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Abbreviations: AChE, acetylcholinesterase; ANOVA, analysis of variance; BuChE, butyrylcholinesterase; DA, dopamine; DTNB, 5,5′-dithiobis-2-nitrobenzoic acid; EDTA, diethylene-dinitrilo-tetraacetic acid; EGTA, ethylene glycol bis(betaaminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HPI, Hyperprolinemia type I; HPII, hyperprolinemia type II; RT-qPCR, quantitative real-time reverse transcription polymerase chain reaction; SCh, thiocholine; S.E.M., standard error of mean.

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http://dx.doi.org/10.1016/j.neuroscience.2013.07.004

acetylcholinesterase activity in the rat brain (Delwing et al., 2005; Ferreira et al., 2011).

It is currently accepted that the cholinergic neurotransmission plays an important role in the CNS by regulating many biological processes such as learning, memory, sensory perception, and cortical organization of movement (Mesulam et al., 2002; Sarter and Bruno, 2004). At synaptic cleft, acetylcholine triggers muscarinic (metabotropic) and nicotinic (ionotropic) acetylcholine receptors. The inactivation of cholinergic signaling is promoted by the cholinesterases, which cleave acetylcholine into choline and acetate. Two different types of cholinesterases hydrolyze acetylcholine: acetylcholinesterase (AChE) (EC 3.1.1.7) and butyrylcholinesterase (BuChE) (EC 3.1.1.8) (Soreq and Seidman, 2001).

Zebrafish (*Danio rerio*) have gained popularity as an organism for neurobehavioral studies. This species has several features that complement the existing mammalian models such as low maintenance, translucent embryos, rapid development, and high fecundity. Zebrafish has also been used for drug screening and toxicological assays (reviewed in Lele and Krone, 1996; Parng et al., 2002; Kari et al., 2007; Mathur and Guo, 2010). In this sense, it can be easily and continuously exposed to different concentrations of amino acids for different periods (Rosemberg et al., 2010; Savio et al., 2012a). Furthermore, zebrafish genes present a high degree of conservation sharing a 70–80% homology with human genes, which is an additional attractive feature to study genetic and biochemical mechanisms of neurological diseases (Barbazuk et al., 2000; Dooley and Zon, 2000; Best and Alderton, 2008). Parameters of cholinergic signaling have already been characterized in the zebrafish brain (Clemente et al., 2004; Rico et al., 2006). It has been shown that AChE is encoded by a single gene, while BuChE has not been detected in the zebrafish genome (Clemente et al., 2004; Ninkovic et al., 2006). Thus, the effects of high amino acid concentrations on the gene expression and neurochemical changes can be evaluated in this species, as well as several parameters of neurotoxicity during development, including teratogenicity, cell death, and selected neuronal subtypes (Ton et al., 2006; Parng et al., 2007; David and Pancharatna, 2009; Long et al., 2011; Pan et al., 2011). Previous study from our group had already characterized the effects of proline exposure on behavioral parameters in the zebrafish (Savio et al., 2012a). We demonstrated that proline-induced behavioral changes are reverted by acute administration of antipsychotic drugs in this species; however, there is no evidence regarding the neurochemical mechanisms that may contribute to these behavioral responses.

Considering that: (i) the hyperprolinemic patients can present neurological dysfunctions, (ii) the cholinergic system is associated with several neurological disorders, (iii) recent studies suggest an influence of proline on cholinergic neurotransmission, and, finally, (iv) the zebrafish has become a prominent vertebrate to study neurological disorders related to human inherited diseases, here, we sought to investigate the effects of short- and long-term proline exposure on AChE activity and gene expression in the zebrafish brain. Furthermore, we also verified whether typical and atypical antipsychotic drugs, such as haloperidol and sulpiride, are able to revert the proline-induced changes in biochemical and molecular parameters of cholinergic signaling.

EXPERIMENTAL PROCEDURES

Animals

Adult males and females (approximately in the ratio of 1:1) of the "wild type" (short fin – SF) zebrafish (*D. rerio*) strain (6–8-months-old) were obtained from a commercial supplier (Redfish, RS, Brazil). 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 28 ± 2 °C, under a 14–10 h-light/dark photoperiod. The fish were kept at a density of up to five animals per liter (Westerfield, 2007). Animals were acclimated for at least 2 weeks before the experiments and fed three times a day to satiety with TetraMin Tropical Flake Fish®. All protocols were approved by the Ethics Committee of Federal University of Rio Grande do Sul (UFRGS) under License No.: 19636 and followed Brazilian legislation, the guidelines of the Brazilian Collegium of Animal Experimentation (COBEA), and the Canadian Council for Animal Care (CCAC) Guide on the care and use of fish in research, teaching, and testing.

Chemicals

L-Proline, Trizma Base, EDTA, EGTA, sodium citrate, Coomassie Blue, bovine serum albumin, acetylthiocholine, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) were obtained from Sigma–Aldrich (St. Louis, MO, USA). All reagents used were of analytical grade.

In vivo treatments

For the *in vivo* studies, animals were exposed at two proline concentrations (1.5 and 3.0 mM) during 1 h (short-term exposure) or 7 days (long-term exposure). During the treatments, the animals were maintained in 4 L test tanks (30 × 15 × 10 cm, length × height × width) with 3 L of water (control group) or water plus proline (1.5 or 3.0 mM) and were kept in the same environmental conditions of the housing tanks. To ensure a similar amount of amino acid present in the aquarium, the tank water was replaced daily. Immediately after the treatments, the fish were cryoanesthetized and further euthanized by decapitation. The whole brains were dissected and the homogenates were prepared.

In order to verify the effects of antipsychotics on proline-induced effects on AChE activity and gene expression, fish were exposed to proline (1.5 and 3.0 mM) during 7 days (long-term exposure) or water (control group). Afterward, the following acute

treatments were performed in a beaker for 15 min: (i) a control group plus water; (ii) a control group plus DMSO (5%); (iii) a proline group (1.5 mM) plus DMSO (5%); (iv) a proline group (3.0 mM) DMSO (5%); (v) a control group plus haloperidol (9 μM); (vi) a control group plus sulpiride (250 μM); (vii) a proline group (1.5 mM) plus water; (viii) a proline group (3.0 mM) plus water; (ix) a proline group (1.5 mM) plus haloperidol (9 μM); (x) a proline group (3.0 mM) plus haloperidol (9 μM); (xi) a proline group (1.5 mM) plus sulpiride (250 μM); and (xii) a proline group (3.0 mM) plus sulpiride (250 μM). DMSO (5%) per se did not affect the proline-induced changes in AChE parameters (group iii and iv; data not shown). Importantly, the proline and antipsychotic concentrations, as well as the protocols of exposure, have already been described in a previous study from our group, which evaluated the effects of proline on zebrafish behavior (Savio et al., 2012a). The proline concentrations were tested based on plasma proline levels verified in human hyperprolinemic patients (0.5–3.0 mM) in order to mimic the conditions promoted by hyperprolinemia (Phang et al., 2001).

In vitro treatments

For the *in vitro* assays, proline (final concentrations of 3, 30, 500, and 1000 μM) was added directly to the reaction medium (described below), preincubated with the brain samples and maintained throughout the enzyme assay. For the control group, the experiments were performed in the absence of proline (no drug added in the reaction medium). The *in vitro* assays were performed based on the cerebrospinal fluid proline concentration verified in hyperprolinemic patients (Phang et al., 2001; Savio et al., 2012b).

Determination 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 Ellman et al. (1961) 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 DTNB. Briefly, samples containing 5-μg protein and the reaction medium described above were preincubated 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. Importantly, the linearity of absorbance toward 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 (Bradford, 1976).

Gene expression analysis by quantitative real-time RT-PCR (RT-qPCR)

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 *EF1a* and *β-actin* primers were used as constitutive genes for data analysis, as described previously (Tang et al., 2007). After 7 days of proline and/or antipsychotics treatments, the animals were euthanized and the brains were removed for total RNA extraction with Trizol® reagent (Invitrogen, Carlsbad, California, USA) in accordance with the manufacturer's instructions. Three independent experiments were performed and a pool of three whole zebrafish brains was used for each independent experiment. The total RNA was quantified spectrophotometrically and the cDNA was synthesized with ImProm-II Reverse Transcription System (Promega) from 1 μg of total RNA, following suppliers. Quantitative PCR was performed using SYBR® Green I (Invitrogen) to detect double-strand cDNA synthesis. Reactions were carried out in a volume of 25 μL using 12.5 μL of diluted cDNA (1:50 for *EF1α* and 1:20 for *β-actin*), containing a final concentration of 0.2 × SYBR® Green I (Invitrogen), 100 μM dNTP, 1× PCR Buffer, 3 mM $MgCl_2$, 0.25 U Platinum® Taq DNA Polymerase (Invitrogen) and 200 nM of each reverse and forward primers (Table 1). The PCR cycling conditions were: an initial polymerase activation step for 5 min at 95 °C, 40 cycles of 15 s at 95 °C for denaturation, 35 s at 60 °C for annealing and 15 s at 72 °C for elongation. At the end of cycling protocol, a melting-curve analysis was included and fluorescence measured from 60 to 99 °C. The efficiency per sample was calculated using LinRegPCR 11.0 Software (http://LinRegPCR.nl) and the stability of the references genes, *EF1α* and *β-actin* (*M-value)* and the optimal number of reference genes according to the pairwise variation (*V*) were analyzed by GeNorm 3.5 Software (http://medgen.ugent.be/genorm/). Relative RNA expression levels were determined using the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

Results were expressed as means ± standard error of mean (S.E.M.). Statistical analysis was performed by a one-way analysis of variance (ANOVA), followed by a Tukey multiple range test as *post-hoc*. Statistically significant differences between groups were considered for a $p < 0.05$.

Table 1. PCR primers design.

Genes	Primer sequences (5′–3′)	GenBank Accession Number (mRNA)
EF1α[*]	F-CTGGAGGCCAGCTCAAACAT R-ATCAAGAAGAGTAGTACCGCTAGCATTAC	NSDART00000023156
β-Actin[*]	F-CGAGCTGTCTTCCCATCCA R-TCACCAACGTAGCTGTCTTTCTG	ENSDART00000055194
ache[**]	F-GCTAATGAGCAAAAGCATGTGGGCTTG R-TATCTGTGATGTTAAGCAGACGAGGCAGG	NP_571921

[*] According to Tang et al. (2007).
[**] Designed by authors.

RESULTS

In vivo and *in vitro* effects proline on AChE activity in the zebrafish brain

After the short-term exposure (1 h), proline did not cause significant changes in AChE activity ($p > 0.05$) (Fig. 1A). However, after long-term exposure (7 days), both 1.5 and 3.0 mM proline significantly increased AChE activity (34% and 39%) when compared to control group ($p < 0.001$) (Fig. 1B). In order to evaluate whether this amino acid could act directly on AChE activity, we tested the effect of different proline concentrations (ranging from 3.0 to 1000 μM) added in the reaction medium. The results showed that proline did not alter AChE activity ($p > 0.05$) when tested *in vitro* (Fig. 2).

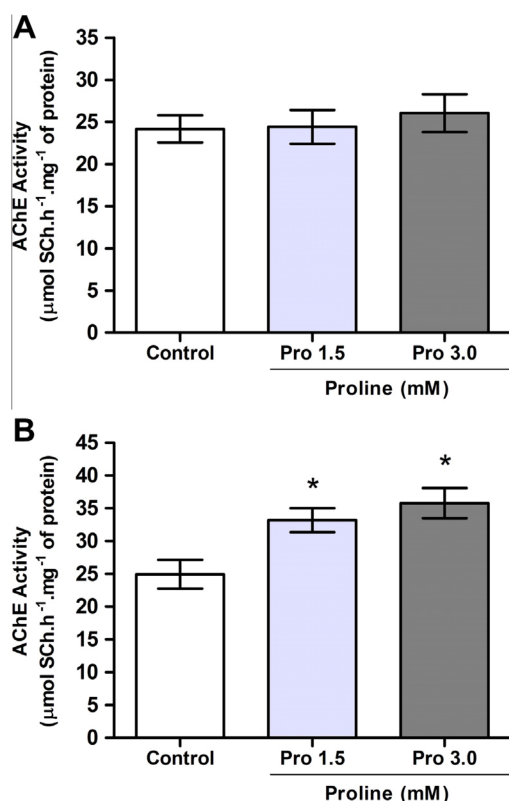

Fig. 1. *In vivo* effect of short-term (1 h) (A) and long-term (7 days) (B) proline exposure (1.5 and 3.0 mM) on AChE activity in zebrafish brain. Data are expressed as means ± S.E.M. of five independent experiments ($n = 8$ at least). Results were analyzed statistically by a one-way ANOVA followed by Tukey test as *post-hoc*. The asterisks (*) represent significant differences when compared to untreated group (white bars) ($p < 0.001$).

Antipsychotic drugs revert the increase of AChE activity induced by long-term proline exposure

Since long-term proline exposure increased AChE activity, we investigated whether typical (haloperidol) and atypical (sulpiride) antipsychotic drugs are able to revert this effect. Our data showed that only sulpiride reverted the effect promoted by 1.5 and 3.0 mM proline on AChE activity as compared to the untreated group ($p > 0.05$). On the other hand, haloperidol was able to revert the increase on AChE activity only at 3.0 mM proline ($p < 0.05$) (Fig. 3).

Effects of proline on ache gene expression in the zebrafish brain: reversal by antipsychotic drugs

We performed a quantitative RT-PCR analysis in order to evaluate the influence of long-term proline exposure on *ache* gene expression in the zebrafish brain. As depicted in Fig. 4, *ache* gene expression was decreased after long-term treatment at both concentrations tested (1.5 and 3.0 mM) ($p < 0.05$). The treatment with antipsychotic drugs showed that both sulpiride and haloperidol revert the proline-induced effects on *ache* transcripts in comparison to the untreated group ($p > 0.05$).

DISCUSSION

The present report showed that long-term, but not short-term proline exposure induces changes in AChE activity and gene expression in the zebrafish brain. We also showed that these effects were reverted by acute administration of antipsychotic drugs. However, proline added directly to the reaction medium did not promote significant changes in AChE activity, suggesting that it may act indirectly in the zebrafish brain.

Although the underlying mechanisms which lead to abnormal brain function in hyperprolinemic patients still remain poorly understood, studies have demonstrated that hyperprolinemia induces neurochemical and behavioral changes mainly affecting the glutamatergic neurotransmission (Vorstman et al., 2009; Wyse and Netto, 2011). Authors have reported that high proline concentrations activate NMDA and AMPA receptors, suggesting that proline may potentiate the glutamatergic neurotransmission, consequently, increasing glutamate

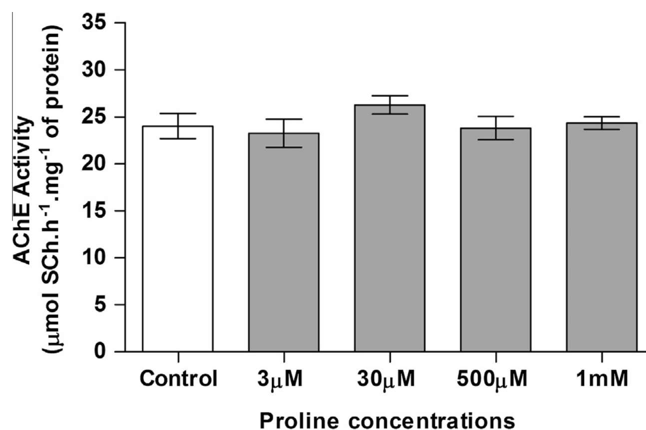

Fig. 2. *In vitro* effect of proline on AChE activity in zebrafish brain. Data are expressed as means ± S.E.M. of four independent experiments (n = 4). Results were analyzed statistically by a one-way ANOVA.

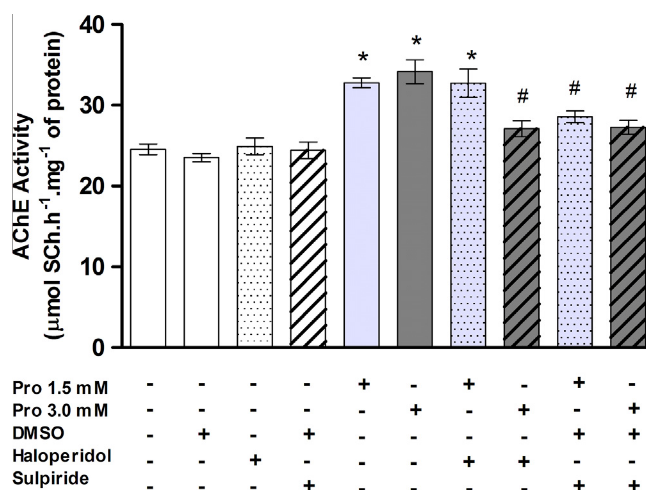

Fig. 3. Effects of haloperidol and sulpiride on proline-induced increase in AChE activity. Fish were exposed to proline (1.5 and 3.0 mM) during 7 days (long-term exposure). Afterward, acute treatments with antipsychotic drugs (haloperidol – 9 μM and sulpiride – 250 μM) were performed in a beaker for 15 min. Data are expressed as mean ± S.E.M. of four independent experiments performed in quadruplicate (n = 6 at least). Results were analyzed by a one-way ANOVA followed by followed by Tukey test as *post-hoc*. The symbols represent statistical differences when compared to control (white bar) (p < 0.001, ∗) and proline-exposed groups (p < 0.05, #).

release (Nadler, 1987; Nadler et al., 1992; Cohen and Nadler, 1997). The increased glutamate levels induced by proline seem to lead to secondary dopamine (DA) release, inducing schizophrenia-like symptoms in animal models. Therefore, high proline concentrations appear to mimic the neurobehavioral effects induced by NMDA receptor antagonists, such as dizocilpine (MK-801) and phencyclidine (PCP) (Paterlini et al., 2005; Vorstman et al., 2009; Savio et al., 2012a). Interestingly, studies have demonstrated that the blockade of NMDA receptors increases the extracellular DA and acetylcholine concentrations in the brain as well as motor activity (Del Arco and Mora, 2005; Del Arco et al., 2008). Considering the effects of proline on ionotropic glutamate receptors, is possible to hypothesize that high concentrations of this amino acid increase the acetylcholine release, inducing behavioral changes, such as hyperlocomotion (Savio et al., 2012a). In addition, we showed a significant increase on AChE activity in the zebrafish brain after long-term proline exposure (at 1.5 and 3.0 mM). Our data are in agreement with a previous study performed in rodents which reported that chronic proline administration significantly increased hippocampal AChE activity (Ferreira et al., 2011). Therefore, these findings could be related to a compensatory response decreasing the acetylcholine levels at synaptic cleft in order to minimize the effects triggered by this neurotransmitter in the brain. On the order hand, the enhancement on AChE activity could be also associated to detrimental effects on cognitive functions, such as learning and memory processes, after chronic proline exposure (Ferreira et al., 2011).

Antipsychotic drugs are widely used for the treatment of neuropsychiatric disorders (Terry et al., 2007; Tadori et al., 2011). In a previous study, we showed that sulpiride, an atypical antipsychotic drug, completely reverted the hyperlocomotion and social deficits induced by proline exposure, whereas a typical antipsychotic (haloperidol) has only attenuated the social interaction

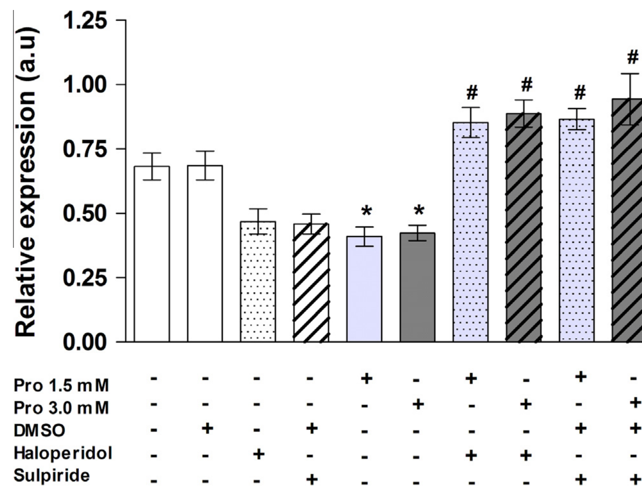

Fig. 4. Effects of haloperidol and sulpiride on proline-induced changes in *ache* gene expression. Fish were exposed to proline (1.5 and 3.0 mM) during 7 days (long-term exposure). Afterward, acute treatments with antipsychotic drugs (haloperidol – 9 μM and sulpiride – 250 μM)/were performed in a beaker for 15 min. Data are expressed as mean ± S.E.M. of three independent experiments performed in triplicate ($n = 3$ at least). Results were analyzed by a one-way ANOVA followed by followed by Tukey test as *post-hoc*. The symbols represent statistical differences when compared to control (white bar) ($p < 0.001$, $*$) and proline-exposed groups ($p < 0.05$, #).

impairment (Savio et al., 2012a). In the current report, we demonstrated that the proline-induced increase on AChE activity was completely reverted by sulpiride. However, haloperidol kept the AChE activity at control levels only at 3.0 mM proline. These data are in agreement with our previous study, which showed that haloperidol failed to revert the hyperlocomotion induced by long-term proline exposure (at 1.5 mM) in the zebrafish (Savio et al., 2012a), suggesting that, similarly to what occur in rodent models, the cholinergic and dopaminergic signaling may play a role in the locomotor effects of proline in this species.

Several neuronal pathways could be involved in the actions of antipsychotic drugs on the enhancement of AChE activity induced by proline administration. Ichikawa et al. (2002) reported that olanzapine, rispiridone, and ziprasidone increased acetylcholine release in the rat medial prefrontal cortex, whereas haloperidol and sulpiride were unable to induce such effect. Moreover, Del Arco et al. (2008) showed that the administration of D_1 and D_2 antagonists reduced the motor effects induced by the blockade of NMDA receptors in the prefrontal cortex. Therefore, considering that haloperidol acts via DA D_2 receptor blockade (Heusler et al., 2008) and sulpiride acts preferentially via D_2 and D_3 DA receptor blockade (Jaworski et al., 2001; Tadori et al., 2011), it is possible that these drugs revert the proline-induced enhancement on AChE activity by a similar mechanism, reducing the acetylcholine availability. However, it is also important to emphasize that atypical antipsychotics have affinity for a wide range of other receptors, such as serotonergic 5-HT_{2A} and 5-HT_6, adrenergic $\alpha 1$, histaminergic H1, and muscarinic M1 (Jones et al., 2008). In this regard, the involvement of different mechanisms associated to the effects of antipsychotics and proline in the zebrafish brain cannot be ruled out and further studies are still required to elucidate the contribution of dopaminergic and serotoninergic systems in this model.

We also investigate whether long-term proline exposure alters *ache* gene expression by performing quantitative RT-PCR assays. Our results demonstrated a significant decrease on *ache* mRNA levels at 1.5 and 3.0 mM proline, which were reverted by typical and atypical antipsychotic treatments. Importantly, the antipsychotics drugs per se did not induce significant changes in ache expression. Although, Seibt et al. (2009) demonstrated that the haloperidol (9 μM) treatment increases the *ache* expression in the zebrafish brain, in their study, the animals were exposed to haloperidol for 2 h. Moreover, the apparent discrepancies between the results of molecular and biochemical experiments could be attributed to the various factors that regulate gene expression, which involve cell machinery and signal transduction pathways. Thus, enzyme activity cannot be directly correlated with the gene expression pattern or with protein levels due to the existence of several post-translational events, such as phosphorylation (Nedeljkovic et al., 2005). It has been previously shown that long-term methionine exposure could influence AChE post-translational modulation, increasing ACh hydrolysis, that in turn down regulates its own expression, via a phenomenon known as negative feedback loop (Salgado et al., 2001; Keseler et al., 2005; Vuaden et al., 2012) in the zebrafish brain. Therefore, post-translational events could contribute, at least in part, to the distinct effects on AChE activity and gene expression profile after proline exposure.

CONCLUSIONS

Our findings demonstrate that long-term proline exposure alters AChE activity and gene expression in the zebrafish brain. Furthermore, the proline-induced increase in AChE activity was completely reverted by acute administration of antipsychotic drugs, as well as the changes in *ache* gene expression pattern. These findings might facilitate

the use of the zebrafish for studying metabolic diseases due to its pharmacological validity and contribute to elucidate the mechanisms underlying cognitive and psychiatry dysfunctions observed in hyperprolinemic patients.

CONFLICT OF INTEREST

The authors declare that no conflict of interest exists.

Acknowledgments—This work was supported by grants from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS), and by DECIT/SCTIE-MS through CNPq and FAPERGS (Proc. 10/0036-5–PRONEX/Conv. 700545/2008).

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(Accepted 6 July 2013)
(Available online 16 July 2013)