Decreased BDNF levels in amygdala and hippocampus after intracerebroventricular administration of ouabain

Diminuição dos níveis de BDNF em amígdala e hipocampo após a administração intracerebroventricular de ouabaína

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Abstract

Objective: The present study aims to investigate the effects of ouabain intracerebroventricular injection on BDNF levels in the amygdala and hippocampus of Wistar rats. **Methods:** Animals received a single intracerebroventricular injection of ouabain (10^{-3} and 10^{-2} M) or artificial cerebrospinal fluid and immediately, 1h, 24h, or seven days after injection, BDNF levels were measured in the rat's amygdala and hippocampus by sandwich-ELISA (n = 8 animals per group). **Results:** When evaluated immediately, 3h, or 24h after injection, ouabain in doses of 10^{-2} and 10^{-3} M does not alter BDNF levels in the amygdala and hippocampus. However, when evaluated seven days after injection, ouabain in 10^{-2} and 10^{-3} M, showed a significant reduction in BDNF levels in both brain regions evaluated. **Discussion:** In conclusion, we propose that the ouabain decreased BDNF levels in the hippocampus and amygdala when assessed seven days after administration, supporting the Na/K ATPase hypothesis for bipolar illness.

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Keywords: BDNF, mania, Na/K ATPase, ouabain.

Resumo

Objetivo: O presente estudo tem como objetivo investigar os efeitos da injeção intracerebroventricular de ouabaína sobre os níveis de BDNF na amígdala e no hipocampo de ratos Wistar. **Métodos:** Os animais receberam uma única injeção intracerebroventricular de ouabaína (10^{-3} and 10^{-2} M) ou fluido cerebroespinhal artificial e, imediatamente, 3h, 24h ou sete dias após a injeção, os níveis de BDNF foram mensurados na amígdala e hipocampo dos ratos por ELISA sandwich (n = 8 animais por grupo). **Resultados:** Quando avaliados imediatamente após a injeção, 3h ou 24h, ouabaína nas doses 10^{-2} e 10^{-3} M não alterou os níveis de BDNF em ambas as estruturas avaliadas. Entretanto, quando avaliados sete dias após a injeção, ouabaína nas doses 10^{-2} e 10^{-3} M mostrou uma significante redução nos níveis de BDNF em amígdala e hipocampo. **Conclusão:** Em conclusão, propõe-se que a administração de ouabaína diminuiu os níveis de BDNF em amígdala e hipocampo quando avaliados sete dias após a injeção, suportando a hipótese da participação da Na/K ATPase no transtorno bipolar.

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Palavras-chave: BDNF, mania, Na/K ATPase, ouabaína.

Introduction

Bipolar disorder (BD) is a prevalent, highly disabling, and chronic mood disorder, characterized by the presence of manic and depressive symptoms, but, the key clinical factor of the bipolar condition is a manic episode, characterized by an extremely elevated mood, energy, psychomotor activation, and sometimes psychosis. This is a disease with unclear pathophysiology and pathogenesis.

Sodium and potassium-activated adenosine triphosphatase (Na/K ATPase) plays an important role in regulating neural activity and neurotransmitter release[1-7]. Several studies have found direct and indirect evidence for a mood-state related decrease in the activity of the Na/K ATPase or Na pump in bipolar illness[8]. This change can directly alter neuronal activity[9] and may be associated with activation of a second message in the absence of a first message (neurotransmitter)[10]. The small reduction in sodium pump activity may alter the excitability of neurons and produces symptoms like-mania behavior, such as hyperactivity, aggressiveness, and risk-taking behavior[9,11,12].

In more recent studies, BD has been associated with impairments in neuroplasticity and cell survival. Brain-derived neurotrophic factor (BDNF) is a member of the nerve growth factor family and has involvement in promoting synaptic efficacy, neuronal connectivity and neuroplasticity[13], being highly expressed in brain areas that are known to regulate cognitive and emotional behavior, such as the hippocampus and amygdala[14]. BDNF regulates neuronal development and survival, and controls the activity of many neurotransmitters, including the serotoninergic, dopaminergic, and glutamatergic systems[15]. Studies support the notion that changes in BDNF levels may be involved in the pathophysiology of BD[16,17]. Several studies show that serum BDNF is decreased during manic and depressive episodes[16-21].

It is known that the intracerebroventricular (ICV) injection of ouabain (OUA), a potent Na/K ATPase pump inhibitor, induces hyperlocomotion[22,23], which may persist for several days after a single injection[24]. Thus, the present study aims to investigate the effects of ICV administration of OUA in rats on BDNF expression in the hippocampus and amygdala immediately, 1h, or 24h, to mimic an acute episode of mania, and seven days, to mimic the persistence of a manic episode, after ouabain injection.

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Methods

Animals

We conducted the study using 96 (n = 8 animals per group) adult male Wistar rats (250-300 g – approximately 2 months of age) obtained from our breeding colony. The animals were housed 5 to a cage, on a 12-hour light/dark cycle (lights on at 7:00 am), with free access to food and water. All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behaviour (SBNeC). This study was approved by the local ethics committee (*Comitê de Ética em Uso de Animais da Universidade do Extremo Sul Catarinense*, Protocol n° 536/2007), and all efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to *in vivo* techniques.

Surgical procedure and treatment

Animals were intraperitoneally anesthetized with ketamine (80 mg/kg) and xylasine (10 mg/kg). In a stereotaxic apparatus, the skin of the rat skull was removed and a 27 gauge 9 mm guide cannula was placed at 0.9 mm posterior to bregma, 1.5 mm right from de midline and 1.0 mm above the lateral brain ventricle. Through a 2 mm hole made at the cranial bone, a cannula was implanted 2.6 mm ventral to the superior surface of the skull, and fixed with jeweler acrylic cement. Animals were tested on the third day following surgery. A 30 gauge cannula was fitted into the guide cannula and connected by a polyethylene tube to a microsyringe. The tip of the infusion cannula protruded 1.0 mm beyond the guide cannula aiming the right lateral brain ventricle. Each animal was administered 5 µl of either artificial cerebrospinal fluid (aCSF) or OUA (10^{-3} and 10^{-2} M; Sigma Chemical, Saint Louis, USA; dissolved in aCSF), over 30 sec[23,25].

Locomotor activity

Locomotor activity was measured immediately after (10 minutes approximately), 3h, 24h or seven days after ouabain or aCSF injection. Locomotor activity was assessed using the open-field task as previously described[26]. This task was performed in a 40 × 60 cm open field surrounded by 50 cm high walls, made of brown plywood, with the floor divided into 12 equal rectangles by black lines. The animals were gently placed on the left rear rectangle, and left free to explore the arena for 5 min. Crossings of the black lines (locomotor activity/horizontal activity) was counted.

BDNF levels measurement

BDNF levels in hippocampus and amygdala were measured immediately after (10 minutes approximately), 3h, 24h or seven days after ouabain or aCSF injection by anti-BDNF sandwich-ELISA, according to the manufacturer instructions (Chemicon, USA). Briefly, brain slices were homogenized in phosphate buffer solution (PBS) with 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM ethylene glycol tetraacetic acid (EGTA). Microtiter plates (96-well flat-bottom) were coated for 24 hr with the samples diluted 1:2 in sample diluent and standard curve ranged from 7.8 to 500 pg/ml of BNDF. The plates were then washed four times with sample diluent and a monoclonal anti-BNDF rabbit antibody diluted 1:1000 in sample diluent was added to each well and incubated for 3 hr at room temperature. After washing, a peroxidase conjugated anti-rabbit antibody (diluted 1:1000) was added to each well and incubated at room temp room temperature for 1 h. After addition of streptavidin-enzyme, substrate and stop solution, the amount of BDNF was determined by absorbance in 450 nm. The standard curve demonstrates a direct relationship between Optical Density (OD) and BDNF concentration. Total protein was measured by Lowry's method using bovine serum albumin as a standard.

Statistical analysis

Data are presented as mean and standard error of the mean. Differences among the experimental groups were determined by one-way analysis of variance (ANOVA) followed by the Tukey *post-hoc* test. In all comparisons, statistical significance was set at $P < 0.05$.

Results

Results for locomotor activity are shown in figure 1. When evaluated immediately, 3h, 24h or seven days after ICV injection, the ouabain (10^{-2} and 10^{-3} M) administration increased rat spontaneous locomotion, compared to the control group.

As illustrated in figure 2, when evaluated immediately, 1h, 3h, or 24h after ICV injection, ouabain in doses of 10^{-2} and 10^{-3} M does not alter BDNF levels in both the amygdala and hippocampus. However, when evaluated seven days after ICV injection (Figure 2D), ouabain in doses of 10^{-2} and 10^{-3} M, showed a significant reduction in the BDNF levels in the amygdala and hippocampus.

Discussion

The Na/K ATPase (Na pump) maintains the concentration gradients of Na and K ions across the surface membrane of animal cells. It has been proposed that the ICV administration of ouabain in rats induces the neuronal ATPase hypoactivity, which is proposed to occur in mania and depression in humans. Previous studies showed that the ICV injection of ouabain induces hyperlocomotion (manic-like behavior), which persist for seven days after a single injection[25,27], suggesting that inhibition of brain Na/K ATPase activity causes hyperactivity. We also demonstrated that when evaluated immediately, 3h, 24h or seven days after ICV injection, the ouabain administration increased rat spontaneous locomotion.

In the present study, we demonstrate that ouabain does not alter BDNF levels in the hippocampus and amygdala when evaluated immediately after a single ICV injection. Inhibition of Na/K ATPase induces alterations in intracellular ion concentrations, which can induce secondary changes in the activity of intracellular signal pathways[28]. However, these changes can occur in a late time course, as observed in the evaluation seven days following the injection. Moreover, BDNF protein levels may not be altered immediately after an ICV ouabain injection because synthesis or degradation of BDNF takes time. In this evaluation, we showed that ouabain causes a substantial reduction in BDNF levels of both structures in 10^{-2} and 10^{-3} M doses, when assessed seven days after administration. Previous studies from our research laboratory showed that after seven days the ouabain administration causes damage to lipids and proteins in the rat brain, but not immediately after ouabain administration[25,29]. Whereas acute reactions to ouabain in animals have considerable homology to a manic episode (as reduction in the Na/K ATPase and consequent hyperactivity), the persistent effect of ouabain resembles aspects of illness progression. From these observations we suggest that ICV administration of ouabain is a good model to study the chronicity of BD.

Palomino *et al.*, in a study with bipolar patients that experienced a first psychotic episode, observed a dramatic decrease in levels of plasma BDNF of the patients[30]. Interestingly, BDNF levels in all instances progressively increased towards control values during 1-year follow-up subsequent to the first episode[31], which should be related to the neuroprotective effect of the treatment used in this disease.

A growing body of evidence has showed that the pathophysiology of BD could be the result of deregulation of synaptic plasticity with downstream alterations of neurotrophins[31]. Neuroimaging studies suggest that decreased BDNF levels may account for structural brain changes in bipolar patients[32,33]. Kapczinski *et al.*[17] recently showed that serum levels of BDNF are decreased during both manic and depressive mood episodes, being normalized in euthymia. Moreover, acute treatments with psichostimulant drugs, such as amphetamine, decrease BDNF levels in rat cerebral tissues accompanied with hyperlocomotion[26].

This study presents the following limitations: a) this animal model mimics only aspects of manic episodes and b) only evaluated BDNF levels in the brain of rats, however it is known that other neurotrophins (NT-3, NT-4 and NGF)[34-36] and other biochemical changes (oxidative protein, lipid and DNA damage)[37,38] are involved in aspects of BD progression.

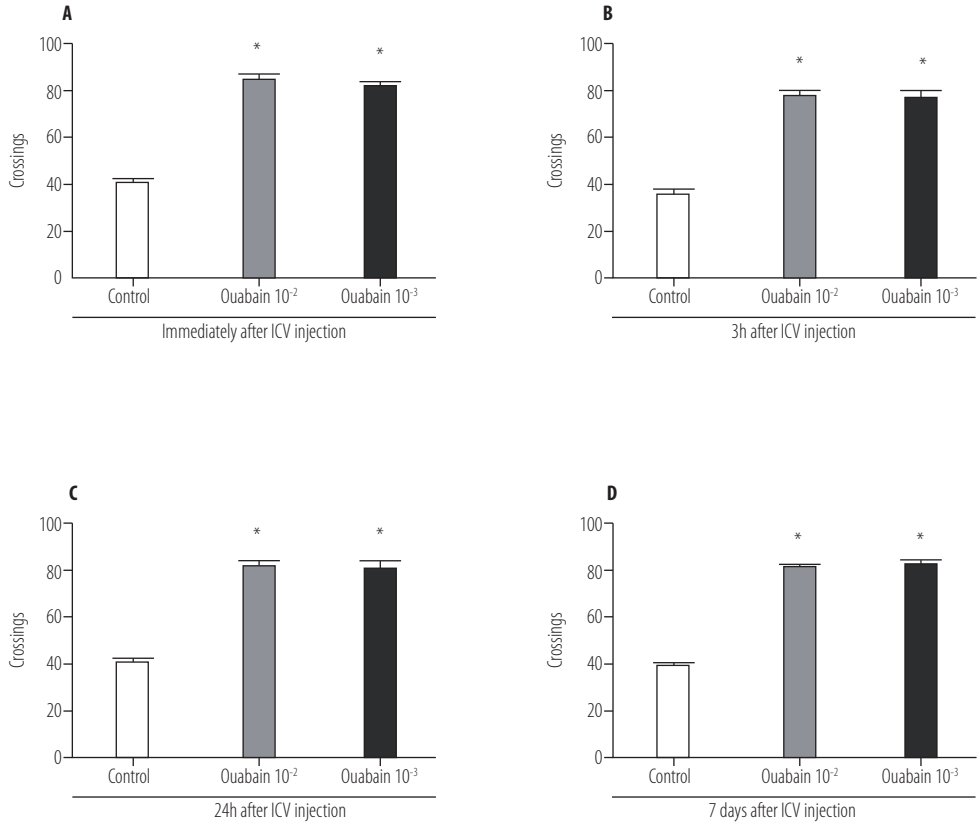

Figure 1. Locomotor activity immediately **(A)**, 3h **(B)**, 24h **(C)** or seven days **(D)** after ouabain or aCSF ICV injection. Bars represent means ± standard error of means of 7 animals. * $P < 0.05$ *vs.* aCSF group, according to ANOVA followed by the Tukey test.

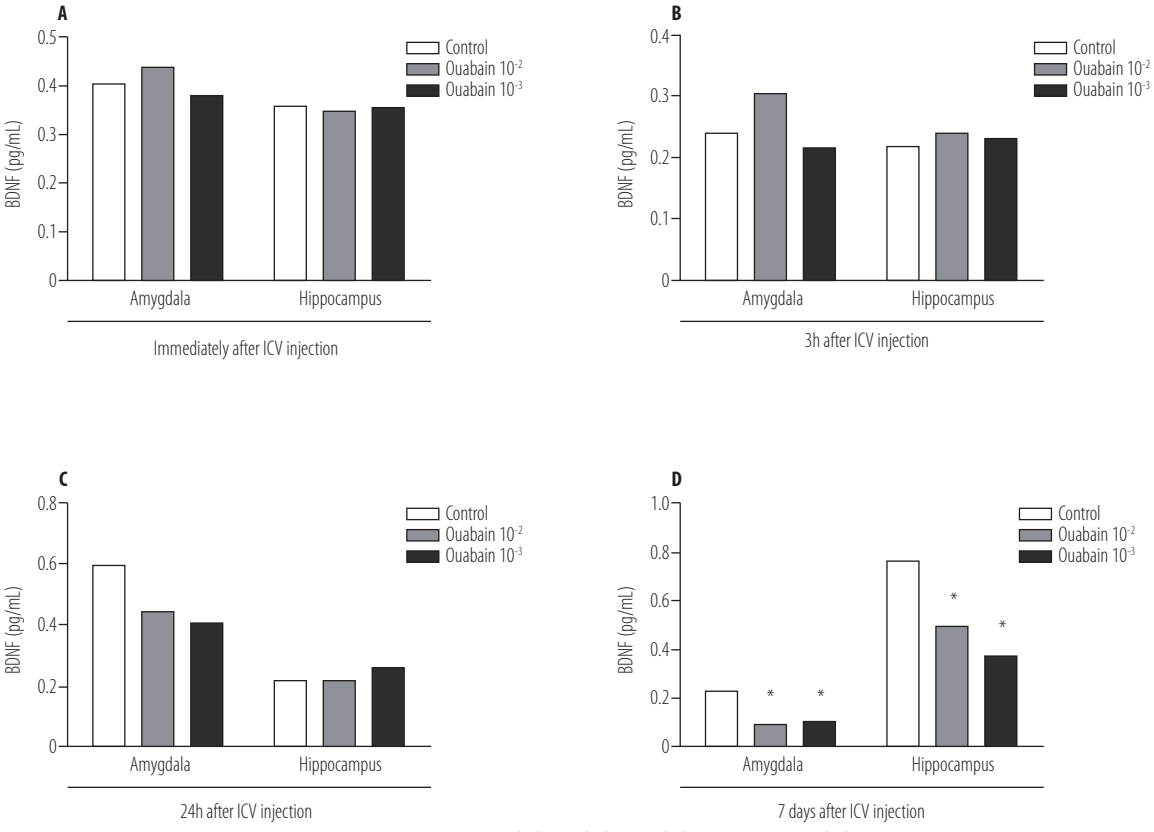

Figure 2. BDNF levels in rat hippocampus and amygdala immediately **(A)**, 3h **(B)**, 24h **(C)** or seven days **(D)** after ouabain or aCSF ICV injection. Bars represent means ± standard error of means of 7 animals. * $P < 0.05$ *vs.* aCSF group, according to ANOVA followed by the Tukey test.

Conclusion

Impairment of brain Na/K ATPase has an important role in the pathogenesis of BD and these findings suggest the possible link between BDNF and Na/K ATPase induced by ouabain in rats. Our findings support the Na/K ATPase hypothesis for bipolar illness, but, further studies must be conducted to define the model, explore its utility in understanding bipolar illness and in potential drug screening.

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Conflicts of interest

Prof. Quevedo has received grant/research support from CNPq, Capes, Fapesc and Unesc and has been a member of the speakers' boards for Eli Lilly. Prof. Kapczinski has received speaker fees, educational grants and travel assistance from Eli Lilly.

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