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Omega-3 fatty acids prevent the ketamine-induced increase in acetylcholinesterase activity in an animal model of schizophrenia

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ABSTRACT

Aims: Schizophrenia is a debilitating neurodevelopmental disorder that is associated with dysfunction in the cholinergic system. Early prevention is a target of treatment to improve long-term outcomes. Therefore, we evaluated the preventive effects of omega-3 fatty acids on AChE activity in the prefrontal cortex, hippocampus and striatum in an animal model of schizophrenia.
Main methods: Young Wistar rats (30 days old) were initially treated with omega-3 fatty acids or vehicle alone. Animals received ketamine to induce an animal model of schizophrenia or saline plus omega-3 fatty acids or vehicle alone for 7 consecutive days beginning on day 15. A total of 22 days elapsed between the treatment and intervention. Animals were sacrificed, and brain structures were dissected to evaluate AChE activity and gene expression.
Key findings: Our results demonstrate that ketamine increased AChE activity in these three structures, and omega-3 fatty acids plus ketamine showed lower values for the studied parameters, which indicate a partial preventive mechanism of omega-3 fatty acid supplementation. We observed no effect on AChE expression. Together, these results indicate that omega-3 fatty acid supplementation effectively reduced AChE activity in an animal model of schizophrenia in all studied structures. In conclusion, the present study provides evidence that ketamine and omega-3 fatty acids affect the cholinergic system, and this effect may be associated with the physiopathology of schizophrenia. Further studies are required to investigate the mechanisms that are associated with this effect.

Introduction

Schizophrenia is a highly debilitating neurodevelopmental disorder that affects the lives of young people during a critical period. Early diagnosis and prevention improve long-term outcomes in schizophrenia [25,28]. However, the need for improved treatments for schizophrenia is illustrated by evidence that approximately two-thirds of schizophrenics do not respond optimally to antipsychotic drugs [5]. Moreover, newer antipsychotic drugs have side effects that are poorly tolerated, which results in low levels of medication compliance [15]. Many neurotransmitter systems have been studied to improve the quality of life of these patients, and some evidence suggests the involvement of muscarinic receptors in the regulation of dopamine [44]. This evidence presents an alternate target for the treatment of psychosis and suggests that the modulation of cholinergic receptors may ameliorate some symptoms in schizophrenia.

Acetylcholine (ACh) plays an important role in a wide variety of cognitive tasks, such as perception, selective attention, associative learning, and memory [43]. However, ACh neurons represent only 5–10% of the cortical and hippocampal neurons [9]. Cholinergic disturbances may contribute to neuropsychiatric manifestations of schizophrenia, particularly hallucinations and delusions [8]. Therefore, one major marker of cholinergic metabolism is the activity of the hydrolytic enzyme acetylcholinesterase (AChE), which enables precise temporal control of synaptic activation by rapidly hydrolyzing the neurotransmitter acetylcholine

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(ACh) into acetate and choline. AChE is implicated in many cognitive functions, and it may play important roles in neurodegenerative disorders, such as Alzheimer's disease [12,37].

Many researchers evaluated the use of omega-3 fatty acids in individuals who are under high risk (UHR) for psychosis. A recent double-blinded clinical trial evaluated the use of omega-3 fatty acids over a 12-week intervention in UHR individuals. This study found that omega-3 fatty acids reduced the risk of progression to a psychotic disorder, and this intervention may offer a safe and efficacious strategy for prevention [2]. Omega-3 fatty acids, which are major structural components of cell membranes, are decreased in patients with schizophrenia [26,33,36,42]. Recent evidence suggests that decreased levels of nervonic acid, a monounsaturated omega-9 fatty acid that is important in myelin biosynthesis, correlate with prodromal symptoms and predict conversion to psychosis in young people who are at high clinical risk for psychosis [3]. This study implicates arachidonic acid (omega-6) [36] and nervonic acid (omega-9) [4] in the lipid metabolism dysfunction that is involved in the etiology of psychotic disorders [2,21,22,39].

Studies of postmortem central nervous system (CNS) tissues report decreased choline acetyltransferase (CHAT) activity in the inferior parietal cortex of schizophrenic subjects with poor cognitive functioning as assessed by case history reviews. These data suggest that ACh production may be deficient in schizophrenic subjects [35]. Our research group recently showed that omega-3 administration in adolescent rats prevents positive, negative and cognitive symptoms in a ketamine animal model of schizophrenia [16]. Previous clinical findings [2] and the potential of omega-3 fatty acids as a promising treatment for individuals who are at risk for psychosis prompted our evaluation of the efficacy of omega-3 fatty acid supplementation on AChE activity and Ach gene expression in the prefrontal cortex, hippocampus and striatum in an animal model of schizophrenia.

Methods

Animals

Young male Wistar rats (30 days old) weighing between 80 g and 150 g were obtained from the Central Animal House of UNESC. Animals were maintained in a climate-controlled (22 °C) environment with a light–dark cycle of 12 h. Water and food were provided ad libitum throughout the experiment. All experimental procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care. Approval was obtained from the Ethics Committee of UNESC.

Drugs and treatment

Animals were randomly divided into four groups: 1) Vehicle + Saline, 2) Omega-3 + Saline, 3) Vehicle + Ketamine 25 mg/kg, and 4) Omega-3 + Ketamine 25 mg/kg. Omega-3 fatty acids were administered once daily at a dose of 0.8 g/kg body weight via orogastric gavage. Treatment with omega-3 fatty acids or vehicle alone was initiated in young rats on the 30th day of life for a total duration of 15 days. Animals subsequently received ketamine or saline plus omega-3 fatty acids or vehicle alone for 7 days, for a total of 22 days of intervention. The vehicle was chosen as a placebo because it is an inert oil (Tween 80, SIGMA ALDRICH Co., St. Louis, USA) that does not impact omega-3 fatty acid metabolism. Fish oil capsules (NATURE'S BOUNTY, INC.) contained 1200 mg of oil that was rich in omega-3 fatty acids. The capsules were composed of eicosapentaenoic acid (EPA) (18%) and docosahexaenoic acid (DHA) (12%) diluted in Tween 80.

Ketamine (CU Chemie Uetikon, Lahr, Germany) was administered to induce an animal model of schizophrenia at a dose of 25 mg/kg intraperitoneally (i.p.). The injection solution was prepared in saline at a volume of 1 mL/100 g [40]. The 25 mg/kg dose was used to mimic psychotic symptoms, such as blunted affect and hyperlocomotion [23]. Rats were sacrificed by decapitation after the last day of treatment, and brain structures (i.e., striatum, hippocampus and prefrontal cortex) were carefully removed for subsequent biochemical analyses (the schematic experimental design is shown in Fig. 1). All procedures for the disposal of animals followed the standards of RDC No. 306/2004 ANVISA (National Agency for Sanitary Vigilance).

AChE activity

AChE activity was measured according to a previous study [14]. Briefly, hydrolysis rate was measured using an ACh (S) concentration of 0.8 mM in 1-mL assay solution of 100 mM of phosphate buffer (pH 7.5) and 1.0 mM of DTNB. Fifty microliters of brain structure homogenate was added to the reaction mixture and pre-incubated for 3 min. Hydrolysis was monitored by the formation of the thiolated anion of DTNB at 412 nm for 2–3 min (intervals of 30 s) at 25 °C. All samples were run in duplicate. Proteins were measured using Lowry's method with bovine serum albumin as a standard [29].

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

Gene expression analysis was performed only when kinetic alterations occurred. Total RNA was isolated using Trizol® reagent (Invitrogen, Carlsbad, California, USA) according to the manufacturer's instructions and quantified using spectrophotometry. cDNA was synthesized using an ImProm-II™ Reverse Transcription System (Promega) from 1 μg of total RNA following the manufacturer's instructions. Quantitative PCR was performed using SYBR® Green I (Invitrogen) to detect double-stranded cDNA synthesis. Reactions were performed in a volume of 25 μL using 12.5 μL of diluted cDNA (1:50 for Hprt1, Rlp13A and AChE), which contained a final concentration of 5 M betaine (Sigma-Aldrich), 0.2× SYBR® Green I (Invitrogen), 100 μM dNTP, 1× PCR Buffer, 3 mM $MgCl_2$, 0. 25U Platinum® Taq DNA Polymerase (Invitrogen) and 200 nM each of reverse and forward primers (Table 1). The following PCR cycling conditions were used: an initial polymerase activation step for 5 min at 95 °C, 40 cycles of 15 s each at 95 °C for denaturation, 35 s at 60 °C for annealing and 15 s at 72 °C for elongation. A melting curve analysis was performed at the end of the cycling protocol, and fluorescence was measured from 60 to 99 °C. Relative expression levels were determined using 7500 Fast Real-Time System Sequence Detection Software v.2.0.5 (Applied Biosystems). The efficiency per sample was calculated using LinRegPCR 11.0 Software (http://LinRegPCR.nl). The stability of the reference genes, Hprt1 and Rlp13A (M-value), and the optimal number of reference genes, were calculated according to pair-wise variation (V) and analyzed using GeNorm 3.5 Software (http://medgen.ugent.be/genorm/). Relative RNA expression levels were determined using the 2-ΔΔCT method [6].

Statistical analyses

Molecular data and AChE activity were analyzed using a two-way analysis of variance (ANOVA). Post-hoc comparisons were made using Tukey's test. Significance was set at $p \leq 0.05$.

Results

Fig. 2 shows the levels of AChE activity in different brain structures. A two-way ANOVA indicated that the observed differences between groups on Tukey's post-test arose from the effect of ketamine [$F(1,16) = 293.72$, $p < 0.01$ in the cortex; $F(1, 16) = 421.91$, $p < 0.01$ in the hippocampus; $F(1,16) = 336.80$ $p < 0.01$ in the striatum], omega-3 [$F(1,16) = 56.13$, $p < 0.01$ in the cortex; $F(1,16) = 144.65$, $p < 0.01$ in the hippocampus; $F(1,16) = 64.62$, $p < 0.01$ in the striatum], and the interaction between variables [$F(1,16) = 49.83$, $p < 0.01$ in the