Pharmacology, Biochemistry and Behavior 159 (2017) 90–99

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Chronic choline supplementation improves cognitive and motor performance via modulating oxidative and neurochemical status in rats

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ARTICLE INFO

Keywords:
Learning
Memory
Locomotion
Choline bitartrate
Acetylcholine
Monoamines
Antioxidants

ABSTRACT

Choline, an essential nutrient, accounts for multiple functions in the body and brain. While its beneficial effects on healthy adults are not clear, choline supplementation is important during pregnancy for brain development, in elderly patients for support of cognitive performance and in patients with neurological disorders to reduce memory deficits. Thus, the aim of this study is to investigate whether choline administration in healthy adult rats beneficially impacts cognitive and locomotor performance, and associated oxidative and neurochemical outcomes. Two groups, control and choline, received tap water and choline bitartrate, respectively at the dose equivalent to adequate intake for five weeks. Food intake and body weight were monitored daily. Behavioral analysis comprising assessment of cognitive performance (by novel object recognition, passive avoidance and Morris Water Maze test) and locomotor performance (by Open field, Kondziela's inverted screen and beam walking test) were performed. Following testing, rats were decapitated and brain samples were collected for estimation of acetylcholine, redox profile and monoamine measurements. The results showed that chronic choline administration significantly improves cognitive and locomotor performance accompanied by a reduction in oxidative stress, enhanced cholinergic neurotransmission and monoamine levels in the brain of healthy adult rats. Hence, chronic choline intake was found to improve behavioral, oxidative and neurochemical outcomes in the normal population, so it can be suggested that choline tablets can be used as a safe and effective supplement for improving the neurological health of normal individuals and that they might also be beneficial in preventing cognitive and motor disorders later in life.

1. Introduction

For a healthy and long life, humans require consumption of a complex set of nutrients (Naber et al., 2015). Choline is one such vital nutrient, which accounts for multiple functions in the body and brain (Zeisel and Da Costa, 2009; Glenn et al., 2012) including cell membrane integrity, methyl group metabolism, cell signaling pathways, lipid transport, myelination, growth factor signaling (Borges et al., 2015), and synthesis of phospholipids, very low density lipoprotein and acetylcholine (ACh) (Zeisel and Da Costa, 2009; Glenn et al., 2012; Wallace et al., 2012; Ueland, 2011). According to the US Institute of Medicine's Food and Nutrition Board, choline is an essential water-soluble dietary nutrient (Glenn et al., 2012; Wallace et al., 2012) with an adequate intake (AI) of 550 mg/day for men and 425 mg/day for women (Wallace et al., 2012; Institute of Medicine, US Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1998). Reports show that choline deficiency is implicated in different metabolic consequences that include fatty liver disease, DNA damage, cell apoptosis, altered gene expression and cognitive impairments (Wallace et al., 2012; Ueland, 2011). Previously, it has been reported that average dietary choline intakes among adult men and women were lower than the AI (Wallace et al., 2012; Price et al., 2010). In 1998, choline was recognized as 'an essential nutrient' (Zeisel and Da Costa, 2009), and it was recommended that humans consume choline in their diet for maintenance of normal bodily functions (Glenn et al., 2012; Wallace et al., 2012). Choline supplements are commercially available for treating liver diseases, asthma, to prevent neural tube defects during pregnancy (WebMD, 2009), for clearing fatty liver, maintaining cell membranes, protecting breast tissue and for improving cardiovascular function (Power City). Choline supplements are ineffective for improving memory function and motor activity (WebMD, 2009). However, studies have shown that following oral administration, exogenous choline enters the circulation, crosses the blood–brain barrier (Borges et al., 2015) and can form ACh (Borges et al., 2015; Leermakers et al., 2015). This ACh may be involved in cognitive function (Gandhi et al., 2000), brain development (Ueland, 2011; Leermakers et al., 2015) and may be required for neuronal survival (Borges et al., 2015). Researchers have determined that choline

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http://dx.doi.org/10.1016/j.pbb.2017.05.011
Received 17 October 2016; Received in revised form 30 March 2017; Accepted 30 May 2017
Available online 19 June 2017

supplementation supports brain development (Borges et al., 2015), reduction of neuropathological memory deficits (Wong-Goodrich et al., 2008; Yang et al., 2000), cognition (Borges et al., 2015) and hippocampal responsiveness to cholinergic stimulation (Montoya et al., 2000). Evidence that choline content is lower in Alzheimer's patients suggests that supplementation of choline may improve the cognitive status of dementia patients (Leermakers et al., 2015). Extensive evidence shows memory improvement following prenatal choline supplementation (Glenn et al., 2012; Borges et al., 2015; Meck and Williams, 2003). This effect is likely via an increased ACh concentration due to increased choline acetyltransferase activity and decreased acetylcholinesterase activity in the hippocampal cholinergic system (Jadavji et al., 2015). The majority of choline intake studies involve choline supplementation either during pregnancy or in elderly patients, and very few were conducted in adults. Other studies are based on monitoring effects of choline administration in different pathological states including stroke, traumatic brain injury (Borges et al., 2015; Guseva et al., 2008), dementia (Conant and Schauss, 2004; Lee et al., 2015), and epilepsy (Wong-Goodrich et al., 2008). A recent review (Leermakers et al., 2015) of choline supplementation studies also shows that varying choline intervention durations and use of multiple choline compounds have made clear conclusions regarding the beneficial effects of choline difficult to assess. Effects of choline supplementation specifically in healthy adults is still a matter of debate, as a recent study observed improved vasomotor performance and decreased pupil size after choline ingestion (Naber et al., 2015), while others reported no beneficial effects of acute choline supplementation on memory function (Lippelt et al., 2016; Nagrecha et al., 2013). The effects of chronic choline supplementation on cognitive performance and motor function in healthy adult rats have not yet been systematically studied. Therefore, the aim of the current study is to investigate the effects of choline bitartrate tablets on cognitive and locomotor behavior and associated alterations in neurochemical and oxidative systems in healthy adult rats at the dose equivalent to AI recommended in humans.

2. Materials and methods

2.1. Animals

Twelve locally bred Albino-Wistar rats purchased from Dow University of Health Sciences, OJHA campus, Karachi, Pakistan, were used in this study. Animals were caged individually (to avoid effects of social interaction) with ad libitum access to cubes of standard rodent diet (Bocarsly et al., 2012) and tap water under a 12:12 h light/dark cycle (lights on at 7:00 am) at controlled room temperature (22 ± 2 °C). For seven days prior to the experiments, animals were subjected to an acclimation period and to various handling procedures to nullify novelty and handling stress. All animal experiments were approved by the institutional ethics and animal care committee and performed in strict accordance with the National Institute of Health Guide for Care and Use of laboratory Animals (Publication No. 85-23, revised 1985). All treatments and behavioral monitoring were conducted in a balanced design to avoid order and time effects.

2.2. Drugs and chemicals

Choline bitartrate tablets purchased from Nature's way, New York, USA were used in the experiment. All chemicals were of analytical grade. All reagents were freshly prepared before the start of the experiment. Drug solutions were freshly made in tap water each day for administration at the dose equivalent to AI recommended in humans (500 mg/day) (Wallace et al., 2012). Controls received an equal volume of tap water. Hydrogen peroxide (H_2O_2) stock (35%) solution, thiobarbituric acid (TBA), trichloroacetic acid (TCA), nitro blue tetrazolium (NBT), and dithiobisnitrobenzoic acid (DTNB) were purchased from the British Drug House (BDH, Dorset, UK). Hydroxylamine hydrochloride ($H_3NO{\cdot}HCl$), acetylthiocholine (ATC), and all other analytical grade reagents were purchased from Sigma Chemical Co. (St. Louis, USA).

2.3. Experimental protocol

Animals (n = 12) (weight, 150–200 g) were randomly divided into two experimental groups (n = 6); Control and Choline. Control rats received tap water daily via the oral route in a volume of 0.2 ml/150 g body weight. Test rats received an aqueous solution of choline bitartrate tablet powder via the oral route, at a dose of 52 mg/kg/day body weight daily in a volume of 0.2 ml/150 g body weight, for the duration of five weeks. The dose selection is equivalent to the recommended dose for humans (500 mg/day/60 kg body weight) mentioned by the manufacturer, and previous reports (Zeisel and Da Costa, 2009), and from a pilot study conducted in our laboratory (Tabassum and Haider, 2016). At the end of four weeks' treatment schedule, behavior studies were performed as outlined in Fig. 1. Behavioral tests included the open field test (OFT), the inverted screen test (KIST), the beam walking test (BWT) to assess locomotor activity, muscular strength and motor coordination, respectively, the novel object recognition test (NORT), Morris Water Maze test (MWM) and the passive avoidance test (PAT) to determine recognition ability, spatial and associative learning and memory performance. After monitoring behavioral activities, rats were decapitated, and their brains were removed within 30 s and dissected to collect hippocampal tissue, as described previously (Haider et al., 2016).

2.4. Behavioral protocols

2.4.1. Food intake and body weight

Food intake was monitored daily during the five weeks of treatment by giving rats a weighed amount of food and weighing the remaining food at next feeding. Body weights of the rats were also monitored daily during the five weeks of the treatment. Animals were weighed at the beginning of the experiment, and were followed up with daily until the end of this study.

2.4.2. Assessment of locomotor activity

Following chronic choline supplementation, OFT, KIST, and BWT were performed to assess locomotor activity, muscular strength and motor coordination, respectively.

2.4.2.1. Kondziela's inverted screen test. Kondziela's inverted screen test has been used previously for measure of muscular strength using all four limbs (Kondziela, 1964). Nearly all healthy animals easily score maximum on this task. The inverted screen is a 43 cm square of wire mesh consisting of 12 mm squares of 1 mm diameter wire. It is bordered by a 4 cm deep wooden beading (which prevents animals from climbing on to the other side). The test was done by placing the rat in the center of wire mesh screen and the screen was rotated to an inverted position over 120 s with the rat's head declining first. The time when the rat falls off from the screen was noted. Animals were scored for inverted screen as follows: falling between 1 and 10 s = 1, falling between 11 and 25 s = 2, falling between 26 and 60 s = 3, falling between 61 and 90 s = 4, falling after 90s = 5.

2.4.2.2. Beam walking test. Beam walking is a test of motor coordination (Goldstein and Davis, 1990). The rats have to cross a beam which is suspended between a start platform and their home cage at a height of 50 cm and is supported by two pillars. A cushion was placed under the beam to protect the animals from the bang into the floor. The difficulty of this task can be assorted by using beams with different shapes and widths (Jover et al., 2006). Motor coordination and balance was assessed by the ability of a rat to crossways a graded series of beams. Three circular beams of different diameter were used in this study such as 3 cm, 2 cm, 1 cm and length of 100 cm. In the training phase animals were trained to traverse the beam (from widest