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Chronic high-dose creatine has opposing effects on depression-related gene expression and behavior in intact and sex hormone-treated gonadectomized male and female rats

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ABSTRACT

Creatine is an antioxidant, neuromodulator and key regulator of energy metabolism shown to improve depressive symptoms in humans and animals, especially in females. To better understand the pharmacological effects of creatine, we examined its influence on depression-related hippocampal gene expression and behaviors in the presence and absence of sex steroids. Sham-operated and gonadectomized male and female rats were fed chow alone or chow blended with either 2% or 4% w/w creatine monohydrate for five weeks before forced swim, open field, and wire suspension tests, or seven weeks total. Before supplementation, males were chronically implanted with an empty or a testosterone-filled (T) capsule (10-mm surface release), and females were administered progesterone (P, 250 μg), estradiol benzoate (EB, 2.5 μg), EB + P, or sesame oil vehicle weekly. Relative to non-supplemented shams, all hippocampal plasticity-related mRNAs measured, including brain-derived neurotrophic factor (BDNF), tyrosine kinase B, doublecortin, calretinin, and calbindin, were downregulated in sham males given 4% creatine, and BDNF, doublecortin, and calbindin mRNAs were downregulated in sham females given 4% creatine. In contrast, combined 4% creatine + T in castrates prevented downregulation of BDNF, doublecortin, and calretinin mRNAs. Similarly, combined 4% creatine + EB + P in ovariectomized females attenuated downregulation of BDNF and calbindin mRNA levels. Moderate antidepressant and anxiolytic-like behaviors were observed in EB + P-treated ovariectomized females fed creatine, with similar trends in T-treated castrates fed creatine. Altogether, these data show that chronic, high-dose creatine has opposing effects on neuroplasticity-related genes and depressive behavior in intact and gonadectomized male and female rats. The dose and schedule of creatine used negatively impacted hippocampal neuronal integrity in otherwise healthy brains, possibly through negative compensatory changes in energy metabolism, whereas combined creatine and sex steroids acted in a neuroprotective manner in gonadectomized rats, potentially by reducing metabolic complications associated with castration or ovariectomy.

1. Introduction

Creatine, *N-(aminoiminomethyl)-N-methylglycine*, is a naturally occurring nutrient found in high protein foods such as beef, fish and milk, and it can also be synthesized by the body from the amino acids L-arginine, glycine, and L-methionine (Wyss and Kaddurah-Daouk, 2000). Creatine has traditionally been understood as a vital regulator of adenosine triphosphate (ATP) levels in muscle and brain tissue (Wallimann et al., 1992), and the peripheral effects of creatine supplements have been studied most extensively in athletes and military personnel. More recent studies have demonstrated creatine's neuromodulatory, antioxidant, anti-apoptotic, and anti-inflammatory properties in the central nervous system, motivating researchers to assess the therapeutic utility of creatine monohydrate for treating brain-related disorders (Allen, 2012; Andres et al., 2008; Gaulano et al., 2010).

Of clinical relevance, previous evidence has shown that impaired creatine metabolism has deleterious effects on brain integrity (in 't Zandt et al., 2004; Streijger et al., 2005) and cognitive development (Braissant and Henry, 2008; Jost et al., 2002; Schulze et al., 2003; Streijger et al., 2004, 2005). Conversely, supplementation with creatine has been shown to protect the brain from the negative effects of mild stress (McMorris et al., 2006, 2007a; Watanabe et al., 2002), age-related memory decline (Bender et al., 2008; McMorris et al., 2007b), brain and spinal cord injury (Rabchevsky et al., 2003; Sakellaris et al., 2006; Scheff and Dhillon, 2004; Sullivan et al., 2000), and muscle and neurodegenerative disorders such as Huntington's disease (Rosas

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et al., 2014). The necessity of endogenous creatine for healthy brain function combined with evidence that dietary creatine improves brain-related conditions underscores its potential for treating mental illness (Andres et al., 2008; Gualano et al., 2010).

An increasing number of human and animal studies indicate that creatine is particularly promising for the treatment of depressive disorders (Allen, 2012). Rodent studies have found that creatine supplementation reduces immobility in the forced swim and tail suspension tests, which are animal models that robustly predict antidepressant treatment response in humans (Allen et al., 2010, 2012; Cryan et al., 2005; Cunha et al., 2012, 2013a,b). Of particular interest, our laboratory has found sex differences in the effects of creatine on behavior in rats. Specifically, daily creatine supplementation (+4% w/w, five weeks, per os) decreased immobility behavior in female rats, an effect that is indicative of antidepressant-like properties, whereas the same dose and duration produced either no effect or increased immobility behaviors in male rats (Allen et al., 2010, 2012). Two clinical trials reported that adjunctive creatine accelerated treatment response in depressed female adolescents (Kondo et al., 2011) and women (Lyoo et al., 2012) resistant to conventional treatment, underscoring its translational potential and clinical utility.

The present research aimed to build upon and extend our previous rodent findings by examining two plausible neurobiological mechanisms that could explain the sex-specific antidepressant-like effects of dietary creatine in rats and possibly humans, namely the activational influence of sex steroids and the involvement of neurotrophin-related neuronal activity. Briefly, sex hormones exert important influences on physiology and behavior that extend well beyond reproduction, including the regulation of cognition, mood, mitochondrial function, neurogenesis and synaptic plasticity (for review, see Baudry et al., 2012; Walf and Frye, 2006). The effects of sex hormones on creatine metabolism are sex-specific. For instance, creatine kinase activity, which regulates the use and consumption of energy, increases and decreases in sync with the rat estrous cycle (Sömjen et al., 1991). Moreover, brain-type creatine kinase has been shown to be upregulated sex-specifically by estrogens in female rats and by testosterone in male rats (Malnick et al., 1983; Sömjen et al., 1989, 1997, 2011). Our previous forced swim data indicated that creatine supplementation produced antidepressant-like effects in females particularly during the proestrus and estrus phases of the estrous cycle, when levels of ovarian hormones are high (Allen et al., 2012). We hypothesized that cycling levels of estrogens augment the antidepressant-like effect of creatine in female rats by stimulating energy production and consumption. In males, it is possible that the stable, as opposed to cyclical, nature of testosterone precludes significant increases in creatine metabolism during testing.

Another consideration is that creatine may improve depressive symptoms by enhancing the growth or survival of neurons (Zainuddin and Thuret, 2012). Increasing evidence supports a relationship between creatine and brain-derived neurotrophic factor (BDNF), an essential mediator of synaptic plasticity, neural survival and energy metabolism associated with depression (Duman and Monteggia, 2006). For instance, in mice, oral supplementation with 1% creatine upregulated BDNF expression levels in whole brain hemisphere samples by 1.27 times compared to controls (Bender et al., 2008). In humans, three studies found that a single nucleotide polymorphism of the BDNF gene (val66met) that interferes with BDNF function is associated with lower levels of total creatine in hippocampal and cortical tissue in healthy and mood disordered patients (Frey et al, 2007; Gallinat et al, 2010; Stern et al, 2008). Given this evidence, we examined the effects of creatine on mRNA levels of hippocampal BDNF, and its receptor tyrosine kinase B (TrkB), in the presence and absence of sex hormones to determine whether creatine alters neuroplasticity-related factors akin to conventional antidepressants.

Additionally, to develop a more complete profile of creatine's effects on factors related to neural growth and survival, we also examined hippocampal mRNA levels of doublecortin (DCX), calretinin (CALR), and calbindin (CALB). DCX is classified as a microtubule-associated protein (MAP) and is involved in hippocampal cell division, differentiation and migration (des Portes et al., 1998). CALB and CALR are calcium-binding proteins that frequently colocalize with GABAergic interneurons and maintain intracellular calcium balance that is integral for neurotransmission (Todkar et al., 2012). These three markers have distinct developmental expression patterns within the subgranular zone of the dentate gyrus in adult hippocampus (see Brandt et al., 2003). DCX is expressed in newly born progenitor cells, whereas CALR and CALB are expressed in immature and mature granule cells, respectively.

We conducted two experiments in male (Experiment 1) and female (Experiment 2) rats using three doses of creatine monohydrate (+0%, +2%, or +4%, per os) to determine whether the previously observed effects of creatine on depression-related behavior are attributable to the actions of gonadal steroids during behavioral testing. This work paired classic hormone manipulation paradigms, namely bilateral castration or ovariectomy ± sex hormone replacement, with standard behavioral assays to evaluate potential hormone–creatine interactions on affective behavior. A secondary aim was to conduct a focused analysis on depression-related hippocampal gene expression to determine whether creatine alters mRNA levels of neurotrophic- and neurogenesis-related markers in adult hippocampal tissue. Due to the large number of experimental groups, we planned a priori to narrow the scope of this initial molecular investigation to compare the effects no-dose (+0%) and high-dose (+4%) creatine in pre-selected hormone groups, as 4% creatine is the dose previously shown to robustly alter affective behavior (Allen et al., 2010, 2012). We hypothesized that creatine would increase expression of plasticity-related markers in the same manner shown by chronic studies of conventional antidepressant medication, and that any positive mRNA alterations would correspond with antidepressant-like effects. Together, this work aimed to increase understanding of the behavioral, pharmacological, and neurobiological mechanisms of creatine and its potential to improve depressive symptoms.

2. Material and methods

2.1. Experiment 1: males

2.1.1. Animals and housing

Seventy-two male Sprague–Dawley rats, approximately six weeks old, were obtained from Charles River Laboratories (Raleigh, NC). Rats were housed individually in hanging stainless-steel cages in a climate-controlled vivarium (22 ± 1 °C) on a 12:12 h reverse light–dark cycle (lights on at 1900 h). Rats were given seven days to acclimate to handling and housing conditions prior to the beginning of each experiment.

All procedures were approved by the Tufts University Institutional Animal Care and Use Committee (IACUC).

2.1.2. General experimental procedure

The duration of the experiment was a total of eleven weeks: one week habituation period, two weeks for surgery and recovery, five weeks of daily creatine supplementation, and two weeks for behavioral testing before euthanasia (Fig. 1). Following habituation, rats were randomly assigned to undergo bilateral castration (GDX) or sham-surgery and to receive testosterone (T) maintenance or no T (described below). Upon recovery, equal numbers of rats from each hormone group (i.e., sham, GDX, GDX + T) were randomly assigned to receive 0%, 2%, or 4% creatine supplementation (Allen et al., 2010, 2012). Following five weeks of daily creatine treatment, rats performed in the forced swim, open field, and wire suspension tests, within a two-week period, prior to euthanasia. Rats were tested in two separate but consecutive squads under identical conditions and counterbalanced by diet and hormone conditions. This was necessary