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Additive antidepressant-like effects of fasting with imipramine via modulation of 5-HT_2 receptors in the mice

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ABSTRACT

Recently, studies show that intermittent fasting and caloric restriction may improve symptoms of depression. However, there is little scientific evidence regarding the literature on the antidepressant-like effects of acute fasting. The present study aims to investigate the antidepressant-like effects and its influence on brain levels of the transcription factor cAMP response element-binding protein (CREB) and its phosphorylated form (p-CREB) in different time periods of fasting mice. Furthermore, the additive antidepressant-like effects of fasting with imipramine and the possible involvement of the 5-HT_2 receptors were examined. In the present study 9 h, but not 3 h and 18 h of fasting significantly reduced immobility time in the forced swimming test (FST) without alteration in locomotor activity in the open field test. 9 h fasting also enhanced the ratio of p-CREB/CREB in the frontal cortex and hippocampus. Co-administration of 9 h of fasting and imipramine (30 mg/kg, i.p) produced the additive antidepressant-like effects in the FST and increased the ratio of p-CREB/CREB. Meanwhile, the additive effects were partially reversed by treatment with a 5-$HT_{2A/2C}$ receptor agonist, (±)-1-(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI) (5 mg/kg, s.c). Furthermore, the antidepressant-like effects of 9 h fasting was also blocked by DOI compared to the non-fasting control group. Serum corticosterone level, but not 5-HT and noradrenaline, was significantly increased in a time-dependent manner following different time periods of fasting. Taken together, these results suggest that acute fasting produces antidepressant-like effects via enhancement of the p-CREB/CREB ratio, and additive antidepressant-like effects of fasting with imipramine may be related to modulating 5-HT_2 receptors.

1. Introduction

Recently the physiological consequences of controlled feeding behavior have received much attention in the literature with benefits ranging from overall health and quality of life to anti-aging (Fond et al., 2013; Trepanowski et al., 2011). Caloric restricted mice are more socially active compared to their control counterparts (Govic et al., 2009). Manzanero et al. (2011) reported that dietary energy restriction may benefit neurons, as suggested by experimental evidence showing that caloric restriction and intermittent fasting protected neurons against degeneration in rodents. Furthermore, feeding behavior alters the process of neurogenesis, including brain-derived neurotrophic factor (BDNF) and brain neuronal plasticity (Del Arco et al., 2011; Duan et al., 2001). Lutter et al. (2008) reported that 10 days of caloric restriction causes a marked antidepressant-like response in rodent models of depression. However, there is little evidence available on the antidepressant-like effects of acute fasting.

In the past, the monoamine hypothesis of depression was the most widely accepted by the scientific community (Delgado, 2000; Li et al., 2006; Liu et al., 2012), A large number of studies have focused on monoamine neurotransmitters and their receptors in depression (Cardoso et al., 2009; Freitas et al., 2010; Jesse et al., 2010; Li et al., 2006). Evidence has shown that 5-HT_2 receptors are upregulated in depressive conditions (Arango et al., 1990; Pandey et al., 1990; Yates et al., 1990) and some of the effective therapies elicit their antidepressant effects by down-regulating 5-HT_{2A} receptor activity. In addition to monoamine pathways, CREB-BDNF (CREB, cAMP response element binding)

Abbreviations: ANOVA, analysis of variance; 5-HT, serotonin; FST, forced swimming test; i.p, intraperitoneal; NMDA, N-methyl-D-aspartate; DOI, (±)-1-(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride; OFT, open field test; FST, forced swimming test; CREB, cAMP response element-binding protein; p- CREB, phosphorylated cAMP response element-binding protein; s.c, subcutaneous; BDNF, brain derived neurotrophic factor.

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is another important signaling pathways that contributes to antidepressant activity (Liu et al., 2012). CREB is a transcription activator that plays a critical role in multiple signal transduction cascades activated by cellular stimuli implicated in neuronal plasticity and is also implicated in antidepressant-induced neuronal transcription regulation (Conti et al., 2002; Réus et al., 2011). The CREB activation pathway involves phosphorylation of CREB on Ser-133 to facilitate the transcription of genes with the CRE motif. It is a bona fide neuron survival factor, as Ser-133 phosphorylated CREB (p-CREB) can activate the relative gene transcription to further promote protein expression such as c-Fos (Chan et al., 1999) and neurotrophins (Alboni et al., 2011). Several antidepressants also enhance the level of CREB and p-CREB (Nibuya et al., 1996; Réus et al., 2011). Furthermore, another group reported that p-CREB was enhanced in chronic caloric restricted mice (Fusco et al., 2012). Therefore, the effects of acute fasting on monoamine and CREB were examined in the present study.

Several studies have demonstrated the effect of dietary restriction as a mild stressor on the elevation of corticosterone concentrations (Chacón et al., 2005; Levay et al., 2010). In response to stress, glucocorticoides are slightly elevated as a physiological mechanism so that the organism may respond to perceived or actual stressful events and return to normal when the stress subsides, while chronic excessive elevation often results in detrimental long term effects (Levay et al., 2010). In the present study we investigated the effects of acute fasting in the forced swimming test (FST), a widely used behavioral test that predicts the efficacy of antidepressant treatments (Bourin et al., 2005; Li et al., 2006; Liu et al., 2012). We also examined whether the 5-HT_2 receptor system and CREB phosphorylation were involved in mechanisms of the antidepressant-like effects of acute fasting. Finally, the effects on serum corticosterone, norepinephrine (NE) and serotonin (5-HT) concentrations and the possible additive effects of fasting with imipramine were investigated. Imipramine is mainly used in the treatment of depression. It affects many neurotransmitters, including 5-HT, NE, dopamine, acetylcholine, opioid and histamine. Therefore, in the present study imipramine was a positive control drug as our previous report (Li et al., 2006; Liu et al., 2012).

2. Materials and methods

2.1. Animals

Imprinting Control Region (ICR) strain male mice were purchased from Jilin University. Each mouse was housed in an individual cage (25.5 × 15 × 14 cm) and left to acclimatize to the laboratory conditions for 5 days, during which time they had *ad-libitum* access to standard food and water. The mice were maintained in standard laboratory conditions: 23 ± 1 °C and 12 h light/dark cycle (lights on/off at 6:00 a.m/6:00 p.m). All handling and experiments were done during the light phase. All experiments were conducted in accordance with the Chinese Council on Animal Care Guidelines.

2.2. Drugs

The following drugs were used: imipramine hydrochloride and (±)-1-(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI) (Sigma-Aldrich, ST. Louris, MO). The drugs were dissolved in distilled water, which also served as the vehicle. All drugs were administered in a constant volume of 10 ml/kg body weight. To compare the effect of fasting and other drugs, mice were treated with either the vehicle or the respective drug. The doses of drug were based on our previous report (Li et al., 2006; Liu et al., 2012).

2.3. Measurement of immobility time in the forced swimming test

The test employed was essentially similar to that described by our reports previously (Li et al., 2006; Liu et al., 2012). Mice were divided into 5 groups: no fasting (negative control), 3 h fasting, 9 h fasting, 18 h fasting and imipramine (positive control). The mice were forced to swim individually into glass cylinders (height: 25 cm, diameter: 11 cm) containing 12 cm water (25 ± 1 °C) for 6 min, and recorded by camera. At the end of each session, mice were removed from the glass cylinder and dried with a paper towel before returning them to their home cages. The time of immobility was recorded during the last 4 min of the 6-min testing period, thus after 2 min of habituation. A mouse was judged to be immobile when it floated in an upright position and made only small movements to keep its head above water. Results are expressed as the immobility time during the 240 sec test period (mean ± S.E.M.).

2.4. Involvement of 5-HT_2 system and additive antidepressant-like effects of fasting with imipramine

To investigate the possible additive effects of fasting with imipramine, as well as the involvement of the 5-HT_2 receptor system, six groups of mice were used as follows: no fasting, imipramine, 9 h fasting, 9 h fasting + imipramine, 9 h fasting + imipramine + DOI and 9 h fasting + DOI. Imipramine (30 mg/kg, i.p) and DOI (5 mg/kg, s.c) were administered 30 min and 5 min before open field test (OFT) and FST, respectively.

2.5. Measurement of locomotor activity in the open field test

Round acrylic apparatus (48.8 cm in diameter × 16 cm height wall) with a gray floor divided into 19 equal squares were used according to our reports (Li et al., 2006; Liu et al., 2012). Each mouse was individually placed in the center of the open field apparatus for 6min, and recorded on an overhead video camera. The behaviors scored were: horizontal locomotor activity (grid lines crossed with all four paws) and vertical locomotor activity (rearing). The apparatus were cleaned with 70% alcohol before each successive test. Results are expressed as mean ± S.E.M.

2.6. p-CREB/CREB protein analysis

2.6.1. Sample collection

Mice were sacrificed by decapitation. The brain was quickly dissected out and placed on ice. The frontal cortex and hippocampus from both hemispheres were removed and quickly frozen to -80°C until later use.

2.6.2. Protein extraction

The extracted frontal cortex and hippocampus tissues were weighed and homogenized in lysis buffer (137 mM NaCl, 20 mM TRIS, 1% NP40, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml aprotinin, 1 μg/ml leupeptin, 0.5 mM sodium vanadate, 0.5 mM sodium flouride) on ice. The homogenates were then centrifuged at 10,000 rpm, 4 °C for 20 min. The supernatant was collected into new tubes and stored at −80 °C until use.

2.6.3. SDS-PAGE and western blot analysis

Western blot analysis was performed according to our previous report (Liu et al., 2012). The samples were resolved on 10% SDS polyacrylamide resolving gel using running buffer (25 mM Tris-glycine buffer, pH 8.3, containing 1% SDS) at 110 V, room temperature. A pre-stained molecular weight marker was loaded alongside the sample. The portion containing CREB (molecular weight 43 kd) was cut out and electrophoretically transferred to a PVDF membrane using transfer buffer (25 mM Tris-glycine buffer, pH 8.3, containing 20% methanol) at a constant voltage of 80 V, at about 4 °C for 2 h. The membranes were subjected to the following treatments: (i) washed in TBST (TBS-0.2% Tween-20) for 5 min, (ii) incubated with 5% non-fat milk in TBST for 2 h at room temperature with gentle shaking; (iii) incubated with primary rabbit polyclonal antibodies against either Ser133-phosphorylated CREB (p-CREB) or total CREB (Cell Signaling Technology, Beverly, MA)