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Up-regulation of insulin-like growth factor 2 by ketamine requires glycogen synthase kinase-3 inhibition

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ABSTRACT

An antidepressant dose of the rapidly-acting ketamine inhibits glycogen synthase kinase-3 (GSK3) in mouse hippocampus, and this inhibition is required for the antidepressant effect of ketamine in learned helplessness depression-like behavior. Here we report that treatment with an antidepressant dose of ketamine (10 mg/kg) increased expression of insulin-like growth factor 2 (IGF2) in mouse hippocampus, an effect that required ketamine-induced inhibition of GSK3. Ketamine also inhibited hippocampal GSK3 and increased expression of hippocampal IGF2 in mice when administered after the induction of learned helplessness. Treatment with the specific GSK3 inhibitor L803-mts was sufficient to up-regulate hippocampal IGF2 expression. Administration of IGF2 siRNA reduced ketamine's antidepressant effect in the learned helplessness paradigm. Mice subjected to the learned helplessness paradigm were separated into two groups, those that were resilient (non-depressed) and those that were susceptible (depressed). Non-depressed resilient mice displayed higher expression of IGF2 than susceptible mice. These results indicate that IGF2 contributes to ketamine's antidepressant effect and that IGF2 may confer resilience to depression-like behavior.

1. Introduction

Ketamine treatment induces a rapid antidepressant effect in depressed patients with mood disorders (Newport et al. 2015; Niciu et al. 2014; Scheuing et al., 2015). The mechanism that underlies this action is currently unknown. In mice, ketamine's antidepressant effect in the learned helplessness model is dependent on its inhibition of glycogen synthase kinase-3 (GSK3) (Beurel et al. 2011). GSK3 refers to two isoforms that are primarily regulated by inhibitory phosphorylation on ser-21-GSK3α and ser-9-GSK3β, and this inhibitory phosphorylation of both GSK3 isoforms in mouse brain is increased following ketamine administration (Beurel et al. 2015). Ketamine also increased the serine-phosphorylation of GSK3 in lymphocytes of patients with depression (Yang et al. 2013). In GSK3 knockin mice the serines are mutated to alanines to prohibit this major mechanism of GSK3 inhibition, leaving GSK3 constitutively active (McManus et al. 2005). Ketamine treatment fails to induce an antidepressant effect in GSK3 knockin mice, demonstrating the necessity for ketamine-induced inhibitory serine-phosphorylation of GSK3 for this effect (Beurel et al. 2011). Further studies showed that administration of GSK3 inhibitors enhanced the antidepressant effects of ketamine (Chiu et al. 2014; Ghasemi et al. 2010; Liu et al. 2013). Thus, ketamine-induced inhibition of GSK3 is linked to its antidepressant action, but it remains unknown how this contributes to the antidepressant effects of ketamine (Zunszain et al. 2013).

Several reports have implicated insulin-like growth factor 2 (IGF2) in regulating depression and actions of antidepressants. IGF2 is one of the most abundant growth factors present in cerebrospinal fluid (Åberg et al. 2015; Tham et al. 1993), and IGF2 is expressed and binds receptors throughout the human hippocampus (Caracausi et al. 2016; Wilczak et al. 2000). Variability in the regulation of IGF2 expression has been associated with depression status in adult monozygotic twins (Córdova-Palomera et al. 2015). In rodents, antidepressant treatments up-regulate IGF2 in the hippocampus (Cline et al. 2012; Lisowski et al. 2013) and other brain regions (Lauterio et al. 1993), and IGF2 is down-regulated in the hippocampus in rodent models exhibiting depression-like behaviors (Andrus et al. 2012; Luo et al. 2015). Hippocampal overexpression of IGF2 ameliorated sucrose consumption and immobility time in the forced swim test displays of depression-like behaviors induced by chronic restraint stress in rats (Luo et al. 2015). Administration of IGF2 also increased neurogenesis in the hippocampus, a process that may contribute to the action of antidepressants (Bracko et al. 2012; Chen et al. 2007; Ferrón et al. 2015; Kita et al. 2014). In vivo

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administration of IGF2 in the hippocampus increased the expression of several neurotrophins and growth factors, such as brain-derived neurotrophic factor (BDNF) (Mellott et al. 2014). Thus, diminished IGF2 may contribute to susceptibility to depression and increased IGF2 is linked to antidepressant responses.

Here we report that administration of an antidepressant dose of ketamine up-regulated IGF2 expression in mouse hippocampus, and that this requires inhibition of GSK3. Furthermore, administration of a specific GSK3 inhibitor was sufficient to up-regulate hippocampal IGF2 expression. In mice with GSK3 activation induced by the learned helplessness paradigm, ketamine restored GSK3 inhibition and increased expression of IGF2. Administration of IGF2 siRNA reduced ketamine's antidepressant effect in the learned helplessness paradigm, and mice that were resilient to learned helplessness displayed higher hippocampal expression of IGF2 than control mice. Thus, increased hippocampal IGF2 contributes to ketamine's antidepressant effect and may contribute to resilience or susceptibility of mice to depression-like behavior.

2. Methods

2.1. Mice

Adult (8–12 weeks of age) male C57BL/6 wild-type or homozygous GSK3α/β$^{21A/21A/9A/9A}$ knockin mice (McManus et al. 2005) were used. Adult female C57BL/6 mice (8–12 weeks of age) were used where indicated. GSK3 knockin mice reproduce and develop normally with no overt phenotypes (Eom and Jope 2009; McManus et al. 2005; Polter et al. 2010). Mice were housed in standard cages in a temperature and light controlled room. Mice were treated in accordance with the regulations of the National Institutes of Health and the University of Miami Institutional Animal Care and Use Committee. Mice were injected intraperitoneally (i.p.) with saline or ketamine (10 mg/kg; Vedco Inc.). Mice were treated intranasally with L803-mts, a substrate-competitive peptide GSK3 inhibitor (Plotkin et al. 2003), (60 μg/mouse; 24 h pretreatment; produced in the Eldar-Finkelman laboratory) diluted in DDX1 vehicle (128 mM NaCl, 8 mM citric acid, 17 mM Na_2HPO_4, 0.0005% benzalkonium chloride) as described previously (Beurel et al., 2013a; Kaidanovich-Beilin et al. 2004). Mice were treated with IGF2 siRNA 2 h after ketamine treatment (10 mg/kg; i.p.) and again 2 h before exposure to escapable foot shocks (10 μg/mouse/treatment in DDX1; intranasal; 5 μL/nostril; #J-043,709-09, GE Healthcare Dharmacon, Inc). Scrambled siRNA (AM4642; Ambion) was used as control.

2.2. Western blotting

The hippocampus was rapidly dissected in ice-cold phosphate-buffered saline and stored at −80 °C after being snap-frozen. Brain regions were homogenized in Triton lysis buffer containing 20 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton-100, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 5 μg/ml pepstatin, 1 mM phenylmethanesulfonyl fluoride, 1 mM sodium vanadate, 50 mM sodium fluoride, and 100 nM okadaic acid. Tissue lysates were centrifuged at 14,000 rpm for 10 min at 4 °C to remove the insoluble fraction. The Bradford protein assay was used to determine the concentration of protein in the supernatants. Hippocampal proteins (5–20 μg) were resolved with SDS-PAGE, transferred to nitrocellulose membranes and then immunoblotted. Antibodies used were to phospho-Ser9-GSK3β (#9336, Cell Signaling Technology), total GSK3β (#610,202, BD Transduction Laboratories), phospho-Ser21-GSK3α (#9316, Cell Signaling Technology) and total GSK3α/β (clone 4G-1E, #05–412, Millipore). The membranes were reblotted with β-actin (#A5441, Sigma Aldrich) to ensure equal protein loading.

2.3. Enzyme-linked Immunosorbent assay (ELISA)

Proteins from hippocampal extracts were prepared as described above, and IGF2 ELISAs were performed according to the manufacturer's instructions (RayBiotech) using 100 μg protein.

2.4. Quantitative real-time polymerase chain reaction

Total RNA from mouse hippocampus or prefrontal cortex was isolated using TRIzol extraction according to the manufacturer's instructions (Invitrogen). RNA was converted to cDNA using the ImProvII reverse transcriptase (Promega) according to the manufacturer's instructions. qRT-PCR was performed using SYBR Green master mix, according to the manufacturer's instructions (Applied Biosystems), and the primers described below. Quantification of cDNAs was made using the $2^{-\Delta\Delta Ct}$ method. The primers for IGF2 were: (Forward-TGTGCTGCATCG CTGCTTAC; Reverse-CGGTCCGAACAGACAAACTGA). The primers used for GAPDH, which was used as housekeeping gene, were: (Forward-AGGTCGGTGTGAACGGATTTG; Reverse-TGTAGACCATGTAGTTGA GGTCA). Experiments were performed on a 7900 HT Fast instrument (Applied Biosystems).

2.5. Learned helplessness

Learned helplessness depression-like behavior was measured as described previously (Beurel et al., 2013b; Polter et al. 2010). Mice were placed inside the Modular Shuttle Box (Med Associates Inc., St. Albans, VT, USA), with the gate between the chambers in the closed position. A total of 180 inescapable foot shocks were given at an amplitude of 0.3 mA at random durations of 6–10 s. Random inter-shock intervals were 5–45 s. Learned helplessness was tested 24 or 48 h later by exposing mice to 30 trials of escapable 0.3 mA foot shocks that lasted for a maximum of 24 s if the mouse did not escape, and inter-shock intervals of 30 s. The latency to escape from these shocks was acquired using MED-PC® Data Acquisition Software. Escape failures were tallied when mice did not escape the shock within 24 s, and >15 escape failures out of the 30 trials was defined as learned helpless behavior.

2.6. Statistical analysis

Statistical significance was analyzed with the Student's *t*-test, one- or two-way ANOVA with a Bonferroni post-hoc test as indicated or Kruskal-Wallis test with a Dunn's post hoc test, or a Chi-square test, and $p < 0.05$ was considered significant.

3. Results

3.1. Ketamine treatment increases expression of IGF2 in hippocampus

IGF2 mRNA expression was measured in the hippocampus of male mice 0.5, 12, 24, and 48 h after administration of a sub-anesthetic, antidepressant dose of ketamine (10 mg/kg; i.p.). There was an up-regulation of IGF2 mRNA 24 h after ketamine treatment (2.75 ± 0.2-fold of control levels), which then returned to control levels by 48 h, indicating that ketamine induces a transient increase in IGF2 (one-way ANOVA; $F_{(4,32)} = 3.070$; Bonferroni post-hoc test, $^*p < 0.05$, compared to saline-treated mice; Fig. 1A). Female mice had higher basal levels of hippocampal IGF2 mRNA than male mice (Student's *t*-test; $t_{(22)} = 2.772$, $^*p < 0.05$; Fig. 1B), but also exhibited an increase in IGF2 mRNA levels 24 h after ketamine treatment (Student's *t*-test; $t_{(12)} = 2.658$, $^*p < 0.05$; Fig. 1C). IGF2 protein levels were also significantly increased in the hippocampus of male mice 24 h after ketamine administration (Student's *t*-test; $t_{(8)} = 2.622$, $^*p < 0.05$; Fig. 1D). Basal hippocampal IGF2 mRNA levels were equivalent in male wild-type and GSK3 knockin mice. However, the increased level of IGF2 mRNA in response to ketamine was absent 24 h after ketamine administration in GSK3 knockin