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Sex differences in the antidepressant-like effects of ketamine

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ABSTRACT

Current medications for major depression suffer from numerous limitations. Once the right drug for treatment has been determined, it still takes several weeks for it to take effect and improve mood. This time lag is a serious concern for the healthcare community when dealing with patients with suicidal thoughts. However, recent clinical studies have shown that a single low-dose injection of ketamine, an N-methyl D-aspartate receptor (NMDAR) antagonist, has rapid antidepressant effects that are observed within hours and are long lasting. Although major depression affects twice as many women as men, all studies examining the rapid antidepressant effects of ketamine have focused on male subjects. Thus, we have investigated the behavioral and molecular effects of ketamine in both male and female rats and demonstrated greater sensitivity in female rats at a low dose of ketamine, a dose does not have antidepressant-like effects in male rats. The antidepressant-like effects of this low dose of ketamine were completely abolished when female rats were ovariectomized (OVX), and restored when physiological levels of estrogen and progesterone were supplemented, suggesting a critical role for gonadal hormones in enhancing the antidepressant-like effects of ketamine in female rats. In preclinical studies, the mammalian target of rapamycin (mTOR) in the medial prefrontal cortex and the eukaryotic elongation factor (eEF2) in the hippocampus have been proposed as critical mediators of ketamine's rapid antidepressant actions. In our hands, the increased sensitivity of female rats to a low dose of ketamine was not mediated through phosphorylation of mTOR or eEF2.

1. Introduction

In any given year, more women than men are diagnosed with depression (Holden, 2005; Kessler et al., 2005; Steiner et al., 2005). This has been attributed to the pronounced sex differences that exist in both the anatomy and function of the brain, as well as to the sexually dimorphic hormonal environment (Cosgrove et al., 2008; Kessler, 2003). In particular, women are more likely to suffer from depression and anxiety during periods of when levels of estrogen and progesterone are at their lowest during the premenstrual, postpartum and perimenopausal periods (Douma et al., 2005). In both sexes, the current available antidepressants have serious limitations in that they require weeks to months to ameliorate symptoms, and only one third of patients respond to the first prescribed antidepressant (Trivedi, 2006; Trivedi et al., 2006). Recent clinical studies have shown that acute treatment with ketamine produces rapid antidepressant effects that last for up to 7 days (Berman et al., 2000; Zarate et al., 2006a), and ketamine infusion has been applied by practitioners in off-label use for treatment resistant major depressive disorder (Covvey et al., 2012).

Ketamine is a non-competitive antagonist acting at the NMDA glutamate receptor. Since its discovery more than 50 years ago, ketamine has been used very efficiently in anesthesia and in pain management and is showing great promises for its antidepressant effects. Indeed, unlike classical antidepressants whose therapeutic effects take weeks to be observed, an acute intravenous injection of ketamine is sufficient to induce quick and long-lasting antidepressant effects (Berman et al., 2000; Zarate et al., 2006a, 2006b). The rapid antidepressant effect of ketamine is of utmost interest when dealing with depressed patients who have suicidal thoughts. In this population, a single injection of small dose of ketamine induced a rapid resolution of suicidal ideation (DiazGranados et al., 2010; Price et al., 2009).

In preclinical studies, two independent research groups have identified mTOR in the mPFC and eEF2 in the HPC for mediating the antidepressant effects of ketamine (Autry et al., 2011; Li et al., 2010). Since these studies were carried out only in male subjects, we aimed to determine if ketamine also has antidepressant-like effects in female rats. Our findings clearly show that female rats are much more sensitive to ketamine when compared to male rats, as they respond to a low dose of ketamine (2.5 mg/kg), a dose that is clearly not antidepressant in male rats, and that the gonadal hormones estrogen and progesterone mediate this high sensitivity to

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ketamine in female rats. Understanding the behavioral and molecular basis of sex differences in NMDAR function and responses to estrogen and progesterone are vitally important for designing novel therapeutic agents that have optimal effectiveness in men and women. Due to the multimodal mechanism of action of NMDAR antagonists and estrogen, the rapidity and the breadth of action spectrum, and their combination could lead to more rapid effects – in both men and women – as compared to current antidepressant therapy.

In male rats, administration of low doses of ketamine reportedly leads to rapid phosphorylation of synaptoneurosomal mTOR and other associated proteins (PSD-95, GluR1, and synapsin) in the prefrontal cortex which are responsible for increased formation of dendritic spines (Li et al., 2010). Alternatively, increased BDNF translation mediated by reduced activity of eEF2 kinase within the hippocampus has also been implicated in mediating the antidepressant-like effects of ketamine (Autry et al., 2011). To understand the molecular mechanisms underlying sex differences in the antidepressant-like response to ketamine, we investigated the role of mTOR activation within total protein extracts and synaptoneurosomal fractions of the medial prefrontal cortex as well as hippocampal eEF2 kinase phosphorylation in male and female rats following acute exposure to various doses of ketamine.

2. Methods

2.1. Experimental design

2.1.1. Experiment 1. Sex differences in the antidepressant and anxiolytic-like effects of ketamine

Male and female rats ($n = 6$ per group) were exposed to a single intraperitoneal (i.p.) injection of 0, 2.5, 5.0, or 10.0 mg/kg of ketamine hydrochloride (Butler Schein Animal Health, Inc.), or 20 mg/kg imipramine hydrochloride (Sigma–Aldrich, Co.), and their behaviors tested 30 min later in the forced swim test, novelty suppressed feeding test, or light–dark box. Twenty-four and 48 h later, behaviors were tested in the elevated plus maze and sucrose preference, respectively (Supplementary Fig. S1). The drugs were injected at a volume of 1 ml/kg.

2.1.2. Experiment 2. The role of estrogen and progesterone in mediating sex differences in forced swim behavior at a low dose of ketamine

As illustrated in Fig. 3A, 10 days following ovariectomy, female rats ($n = 6$–10 per group) received subcutaneous injections of 0, 2 or 10 μg estrogen benzoate (Sigma, St. Louis, MO) in sesame oil 24 h prior to testing, and 0 or 500 μg progesterone (Sigma, St. Louis, MO) in sesame oil 4 h prior to testing. These doses produce near physiological levels of estrogen (Asarian and Geary, 2002) and progesterone (al-Dahan and Thalmann, 1996) and the cyclic regimen models the four day estrous cycle of intact rats (Yu et al., 2011). OVX and intact female rats were injected (i.p) with 0 or 2.5 mg/kg ketamine and tested 30 min later in the forced swim test.

2.1.3. Experiment 3. Activation of mTOR in medial prefrontal cortex synaptoneurosomes and sex differences in phosphorylated eEF2 in the hippocampus in response to ketamine

Male and female rats ($n = 6$ per group) were exposed to a single injection (i.p.) of 0, 2.5, or 5.0 mg/kg ketamine and sacrificed 30 min later. The phosphorylation status of mTOR within the entire medial prefrontal cortex and the synaptoneurosomal fraction were determined. The phosphorylation status of eEF2 within the hippocampus was also determined.

2.2. Animals

Adult male (weighing 250–270 g) and female (weighing 200–225 g) Sprague–Dawley rats, were purchased from Charles River (Wilmington, MA, USA). All rats were pair-housed in 43 × 21.5 × 25.5 cm Plexiglas cages and kept on a 12 h:12 h light:dark cycle (lights on at 0700 h) and each cage received identical treatment. Food and water were available *ad libitum* except during testing. All behavioral experiments, except the sucrose preference test, were conducted during the first 4 h of the light phase of the light/dark cycle and all animal protocols were carried out in accordance with the NIH Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Florida State University. For ovariectomy, a 2–3 cm ventral midline incision was made in the lower abdominal region of adult female rats to expose the uterus. Visible blood vessels were ligated, the ovaries removed, and the muscle layers and skin were sutured.

2.3. Behavioral tests

2.3.1. Forced swim test

The FST is a two day procedure performed as described previously (Carrier and Kabbaj, 2012a). On day 1, rats were placed for 15 min in large inescapable Plexiglas cylinders (30 × 45 cm) filled with 25 °C water to a depth of 30 cm in a dimly lit room. On day 2, the rats were again forced to swim for 5 min under the same conditions. Rats were exposed to ketamine before the second swim exposure. The cylinders were emptied and cleaned between rats. Rats' behavior for both swim sessions was videotaped, and the latency to the first immobility, and the total time spent immobile were analyzed by a scorer that was blind to the experimental treatment. Immobility was defined as minimal movements required only to remain afloat (Lucki, 1997).

2.3.2. Novelty suppressed feeding

In the novelty suppressed feeding test, rats were food-deprived for 24 h and then placed in a novel open field (1 m × 1 m) with a small amount of food available in the middle of the field. The amount of time to take the first bite was recorded as the latency to feed. The animal was removed immediately after feeding, or after 15 min had elapsed, whichever came first.

2.3.3. Sucrose preference test

The sucrose preference test consisted of a two-bottle choice paradigm (Carrier and Kabbaj, 2012a; Chen et al., 2012; Dagyte et al., 2011; Kentner et al., 2010). Rats were allowed to drink from two water bottles for five days prior to testing. The rats were given access to two preweighed bottles, one containing water and the other containing 0.25% sucrose for 48 h. The bottles were weighed at 8 am and 5 pm and the preference for sucrose over water was used as a measure of anhedonia. The position of the bottles was counterbalanced daily to prevent a place preference.

2.3.4. Elevated plus maze

Rats were placed into the elevated plus maze (MED Associates Inc., St. Albans, Vermont) facing a closed arm and were allowed to freely explore the maze for 10 min under dim light as described previously (Carrier and Kabbaj, 2012b). Rats' behavior was recorded by a digital camcorder placed directly above the elevated plus maze. Time spent in the open arms and number of entries into the open arms were analyzed in EthoVision XT version 6 (Noldus Information Technology, Leesburg, VA). The elevated plus maze was cleaned with 70% ethanol between trials.

2.3.5. Light–dark box

Rats were placed into the dark compartment (200 × 310 mm) of the dual chamber apparatus (Model LE-812, EB Instruments, Pinellas Park, FL) and allowed to freely explore both compartments for 10 min as described previously (Carrier and Kabbaj, 2012b). Time spent and frequency of entries in the light compartment (310 × 310 mm) were analyzed using PPCWIN software. The apparatus was cleaned with 70% ethanol between trials.

2.4. Synaptoneurosome preparation and western blot

The medial prefrontal cortex (cingulate, infralimbic, and prelimbic regions) and the dorsal hippocampus were tissue punched using a cryostat and frozen at −80 °C until further processing. A crude synaptoneurosomal fraction was prepared from the medial prefrontal cortex as previously described (Li et al., 2010). Briefly, tissue from the medial prefrontal cortex was homogenized in a solution containing 0.32 M sucrose, 20 mM HEPES (pH 7.4), 1 mM EDTA, 1× protease cocktail inhibitor cocktail, 5 mM NaF and 1 mM sodium vanadate and centrifuged at 4 °C for 10 min at 2800 rpm. The pellet contains nuclei and large debris (nuclear fraction). The supernatant was centrifuged at 4 °C for 10 min at 12,000 rpm. Following this centrifugation, the supernatant (cytosolic fraction) was removed and the pellet (crude synaptoneurosome fraction) was sonicated in 50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 2 mM EDTA, 1 mM $NaVO_3$, 5 mM NaF, and 1× protease inhibitor cocktail. Total proteins were extracted from both hippocampi, or from the medial prefrontal cortex. Protein samples were processed as described previously (Carrier and Kabbaj, 2012a). Equal concentrations of proteins (10–20 μg) were loaded into 10–12% SDS PAGE gel for electrophoresis. Immunoblots were incubated overnight (4 °C) with phospho-mTOR (1:500), mTOR (1:1000), phosphor-eEF2 (1:1000), eEF2 (1:1000), or GAPDH (1:5000) antibodies (Cell Signaling Technology), washed and incubated 1 h with goat anti-rabbit IR Dye 680LT (Li-COR Biosciences; 1:20,000) or donkey anti-goat IR Dye 800CW (Li-COR; 1:10,000) fluorescent secondary antibodies, and visualized using an Odyssey infrared imaging system (Li-COR Biosciences). Quantification was done using NIH ImageJ (http://rsbweb.nih.gov/ij). Normalized data are expressed as percent of control, with saline injected control animals set to 1.

2.5. Statistical analysis

Results were analyzed using one-way or two-way analysis of variance (ANOVA) followed by post-hoc Fisher tests where appropriate. For behavioral testing, unless specified, interactions were not significant and planned comparison tests were