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Research report

Repeated ketamine treatment induces sex-specific behavioral and neurochemical effects in mice

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HIGHLIGHTS

- Repeated ketamine treatment induced opposite behavioral effects in the two sexes.
- Repeated ketamine treatment induced antidepressant-like effects in male mice.
- Repeated ketamine treatment induced anxiogenic and depressogenic effects in females.
- Ketamine enhanced hippocampal synapsin levels and serotonin turnover in males.

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Abbreviations:
5-HIAA, 5-hydroxy-indoleacetic acid
5-HT, serotonin (5-hydroxytryptamine)
ANOVA, analysis of variance
ADRs, adverse drug reactions
DRN, dorsal raphe nucleus
EAA, excitatory amino acid neurotransmitters
FST, forced swim test
HIPP, hippocampus
HNK, hydroxynorketamine
MDD, major depressive disorder
NMDA, *N*-methyl-D-aspartate
OFT, open field test
PFC, prefrontal cortex
SYX, syntaxin-I
SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor
VEH, vehicle

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ABSTRACT

One of the most striking discoveries in the treatment of major depression was the finding that infusion of a single sub-anesthetic dose of ketamine induces rapid and sustained antidepressant effects in treatment-resistant depressed patients. However, ketamine's antidepressant-like actions are transient and can only be sustained by repeated drug treatment. Despite the fact that women experience major depression at roughly twice the rate of men, research regarding the neurobiological antidepressant-relevant effects of ketamine has focused almost exclusively on the male sex. Importantly, knowledge regarding the sex-differentiated effects, the frequency and the dose on which repeated ketamine administration stops being beneficial, is limited. In the current study, we investigated the behavioral, neurochemical and synaptic molecular effects of repeated ketamine treatment (10 mg/kg; 21 days) in male and female C57BL/6J mice. We report that ketamine induced beneficial antidepressant-like effects in male mice, but induced both anxiety-like (i.e., decreased time spent in the center of the open field arena) and depressive-like effects (i.e., enhanced immobility duration in the forced swim test; FST) in their female counterparts. Moreover, repeated ketamine treatment induced sustained sex-differentiated neurochemical and molecular effects, as it enhanced hippocampal synapsin protein levels and serotonin turnover in males, but attenuated glutamate and aspartate levels in female mice. Taken together, our findings indicate that repeated ketamine treatment induces opposite behavioral effects in male and female mice, and thus, present data have far-reaching implications for the sex-oriented use of ketamine in both experimental and clinical research settings.

1. Introduction

During the past decade, one of the most striking discoveries in the treatment of major depressive disorder (MDD) was the

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finding that infusion of a single sub-anesthetic dose of the *N*-methyl-D-aspartate (NMDA) receptor antagonist ketamine induces rapid and sustained antidepressant effects in treatment-resistant MDD patients and in rodents subjected to various antidepressant-predictive animal models, such as the forced swim test (FST) and the chronic mild stress (CMS) model of depression [1].

The intricate behavioral and neurobiological mechanisms underlying ketamine's antidepressant actions appear to be brain region- and dose-dependent and have not yet been fully elucidated [1]. Compelling preclinical evidence indicates that ketamine's antidepressant potential lies on its rapid synaptogenic effects in the medial prefrontal cortex (mPFC) and the hippocampus (HIPP), two brain regions that have been strongly implicated in the pathophysiology of MDD [2,3]. Acute ketamine-induced NMDA antagonism in the brain accounts for the first step in the neurobiological cascade of events leading to regional alterations in the activity of brain's neurotransmitter systems and synaptic protein synthesis, eventually resulting in synaptogenesis [1]. Specifically, it is currently believed that ketamine-induced alterations of glutamatergic tone in the HIPP and the PFC trigger neuroplasticity-related molecular cascades that in turn regulate the synthesis of synaptic proteins involved in synaptogenesis (e.g., *synapsin-I*) and in the pre-synaptic release machinery (e.g., *syntaxin-I*;SYX) [1,3,4]. Indeed, acute low-dose ketamine administration has been shown to rapidly increase *synapsin-I* protein levels and to decrease the accumulation of SYX-consisting SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) complexes in synaptoneurosomal preparations from the HIPP and/or the PFC [3,4]. Moreover, it has been reported that enhancement of central serotonergic activity also underlies the antidepressant-like effects of ketamine [3]. Specifically, activation of the dorsal raphe nucleus (DRN) and subsequent serotonin (5-hydroxytryptamine; 5-HT) release in the PFC and the HIPP [5], is possibly implicated in the sustained antidepressant-like effects of ketamine in the FST [6].

A battery of evidence indicates that behaviorally females are more sensitive to NMDA receptor antagonists such as dizocilpine (MK-801), phencyclidine and ketamine than males [7–9]. For instance, female rats have been reported to be more responsive to the motor-enhancing properties of MK-801 and to respond to lower doses of the drug that are not effective in their male counterparts [8]. Notably, in an early study it was also shown that female rats tend to sleep longer than males following administration of anesthetic doses of ketamine [10]. Interestingly, ketamine's (40–80 mg/kg; s.c.) neurotoxic effects in the retrosplenial cortex have been found to be more severe in female rats [11]. Moreover, women have also been reported to experience more psychotropic effects (i.e., emergence hallucinations) than men upon ketamine anesthesia [12,13]. Despite the fact that women experience MDD at roughly twice the rate of men [14–16], research regarding the neurobiological antidepressant-relevant effects of ketamine has focused almost exclusively on the male sex. Recent preclinical data from our group and others show that female rodents are more sensitive and/or reactive to the rapid and the sustained antidepressant-like effects of acute ketamine treatment, as assessed in antidepressant-predictive behavioral tasks, such as the FST and the CMS model of depression [17,18]. Most importantly, we recently reported that acute ketamine administration affects the levels of excitatory amino acid neurotransmitters (EAAs; glutamate and aspartate) and serotonergic activity in the HIPP and the PFC of stress-naïve mice in a sex- and brain region-dependent manner [18]. Indeed, both these brain regions have been strongly implicated in sex-related neurobehavioral responses to stress and antidepressant treatments [19–25].

Strikingly, the clinical antidepressant-like effects of ketamine are transient and can only be sustained by repeated drug treatment [26]. However, our knowledge regarding the frequency and the dose on which repeated ketamine administration stops being beneficial and becomes harmful is limited [1]. Following our recent demonstration that female mice are more sensitive to the antidepressant-like effects of a single dose of ketamine in the FST [18], we hypothesized that the female sex may also be at greater risk for developing adverse drug reactions (ADRs) upon repeated ketamine treatment.

2. Material and methods

2.1. Animals

C57BL/6J mice used in the present study were bred at the University of Dayton from a mouse colony originally obtained from the Jackson Laboratory (ME, USA). Adult (8–12 week-old) mice of both sexes were maintained on a 12 light:12 dark schedule (lights on at 8:00). Experimental procedures and animal husbandry were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23; revised 1996) and approved by the University of Dayton Animal Care and Use Committee (IACUC; No: 015-01).

2.2. Experimental design and drug treatments

Mice (N = 6–8 per group) were administered a single intraperitoneal injection of 3, 5 or 10 mg/kg of ketamine hydrochloride (Henry-Schein; NY; USA) or vehicle (VEH; 0.9% NaCl), once daily for 21 days. The sub-anesthetic doses of ketamine implemented and route of administration selected are commonly used to screen for the antidepressant-like effects of ketamine in preclinical rodent models [18,26]. Moreover, earlier studies have implemented similar drug regimens to investigate the antidepressant-like effects of repeated ketamine treatment in rodents [27–32] (Table 1). In an initial dose-dependent study we tested whether male and female mice would display differential responsiveness to increasing doses of repeated ketamine administration in the open field test (OFT) and the FST (Fig. 1a). Based on this behavioral experiment, the 10 mg/kg ketamine dose was further selected for neuromolecular assessments, as it induced behavioral effects in both sexes. Neuromolecular analyses were conducted in a different mouse cohort of behaviorally-naïve mice treated repeatedly with ketamine (10 mg/kg) or VEH for 21 days and sacrificed on day 22 (i.e., at 24 h after last injection). Following sacrifice the HIPP and the PFC were rapidly isolated on ice, split into right and left parts, snap frozen and stored at −80 °C until processing for neurochemical analysis or western blotting, respectively.

Given our aim to determine whether there is a basic sex difference in the way males and females respond to repeated ketamine treatment, the female reproductive state was not taken into account [33]. Since it is generally agreed that the first stage of any sex-difference study should be a comparison of gonadally intact adult females and males [34], a two-group design (males and females) was implemented for testing for sex-differentiated traits, as in previous studies from our laboratory [18,24,25].

2.3. Spontaneous locomotor activity in the open field test (OFT)

In order to assess the effects of ketamine administration on spontaneous locomotor activity and anxiety, mice were placed in the center of an OFT arena (45 × 45 × 45 cm), as previously described [18]. Horizontal locomotor activity was recorded for 30 min and the time spent in the center was evaluated for the first 15 min of the test, using a video tracking system (SMART v3.0; PanLab; Barcelona, Spain).