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N,*N*-dimethylglycine differentially modulates psychotomimetic and antidepressant-like effects of ketamine in mice

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ABSTRACT

Ketamine, a dissociative anesthetic, produces rapid and sustained antidepressant effects at subanesthtic doses. However, it still inevitably induces psychotomimetic side effects. *N*,*N*-dimethylglycine (DMG) is a derivative of the amino acid glycine and is used as a dietary supplement. Recently, DMG has been found acting at glycine binding site of the *N*-methyl-D-aspartate receptor (NMDAR). As blockade of NMDARs is one of the main mechanisms responsible for the action of ketamine on central nervous system, DMG might modulate the behavioral responses to ketamine. The present study determined the effects of DMG on the ketamine-induced psychotomimetic, anesthetic and antidepressant-like effects in mice. DMG pretreatment reversed the ketamine-induced locomotor hyperactivity and impairment in the rotarod performance, novel location and novel object recognition tests, and prepulse inhibition. In addition, DMG alone exhibited antidepressant-like effects in the forced swim test and produced additive effects when combined with ketamine. However, DMG did not affect ketamine-induced anesthesia. These results reveal that DMG could antagonize ketamine's psychotomimetic effects, yet produce additive antidepressant-like effects with ketamine, suggesting that DMG might have antipsychotic potential and be suitable as an add-on therapy to ketamine for patients with treatment-resistant depression.

1. Introduction

Ketamine, a dissociative anesthetic, produces multiple effects on the central nervous system. Acute or chronic administration of ketamine at subanesthestic doses produces schizophrenia-like psychotic symptoms and cognitive disturbances in healthy humans (D'Souza et al., 2012; Krystal et al., 2000; Newcomer et al., 1999) and animals (Becker et al., 2003; Chan et al., 2013, Chan et al., 2008). Recently, accumulating evidence reveals that ketamine exerts rapid and lasting antidepressant effects (Koike et al., 2011; Maeng and Zarate, 2007), particularly in treatment-resistant patients in clinical studies (Diamond et al., 2014; Kallmunzer et al., 2016; Messer et al., 2010; Singh et al., 2016). These observations implicate that ketamine may exert its effects through different action sites and neural circuits.

In fact, ketamine affects a wide range of cellular processes. It is well known that ketamine binds to the PCP-binding site of the *N*-methyl-D-aspartate receptor (NMDAR) complex, which is located within the ion channel, thereby blocking the transmembrane ion flux. Ketamine also modulates the activity of nicotinic, muscarinic, and opioid receptors, and voltage-gated ion channels, but at significantly higher concentrations than needed to block NMDAR (Mion and Villevieille, 2013). Moreover, ketamine affects several important players of neuronal signaling pathways, including mammalian target of rapamycin (mTOR), eukaryotic elongation factor 2 (eEF2), glycogen synthase kinase-3 (GSK-3), brain-derived neurotrophic factor (BDNF), extracellular signal-regulated kinase (ERK) and protein kinase B (PKB/Akt) (Zunszain et al., 2013).

The psychotomimetic effects of ketamine have been associated with noncompetitive inhibition of NMDARs. Numerous studies have shown that enhancing NMDAR function, via activation of glycine binding site or modulation of metabotropic glutamate receptors, represents a promising approach to reverse psychotomimetic effects of ketamine (Chan et al., 2008; Krystal et al., 2005; Roberts et al., 2010; Yang et al., 2010) or

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other NMDAR antagonists (Kanahara et al., 2008; Kawaura et al., 2015; Le Pen et al., 2003; Lipina et al., 2005; Santini et al., 2014; Shimazaki et al., 2010). On the other hand, facilitation of NMDAR-mediated transmission via direct activation of NMDAR glycine site or inhibition of the glycine transporter 1, such as GLYX-13, D-cycloserine, and sarcosine, has shown potential benefits in treatment of major depression (Burgdorf et al., 2013; Huang et al., 2013; Karcz-Kubicha et al., 1999; Papp and Moryl, 1996). Modulation of NMDAR glycine binding site has been proposed as the next wave of drug development for schizophrenia (Chang et al., 2014), depression (Dutta et al., 2015), and autism spectrum disorders (Santini et al., 2014). It is of interest to examine the antipsychotic and antidepressant potential of the compounds which act as modulators of the glycine site of NMDAR.

DMG is an important unit in one-carbon metabolism and a commonly used nutrient supplement. DMG lowers the mortality rate in rodents with penicillin and strychnine-induced seizures (Freed, 1985; Ward et al., 1985). In addition, the beneficial effect of DMG has been revealed in young children with autism spectrum disorders (Chia, 2009; Xia, 2011). DMG might have the potential in treatment of certain neurological disorders. In fact, sarcosine, a metabolite of DMG, has been shown to have efficacy as monotherapy and an add-on treatment for schizophrenia (Lane et al., 2005; Lane et al., 2010, 2008; Lin et al., 2015) and major depression (Huang et al., 2013). Sarcosine, an NMDAR co-agonist (Zhang et al., 2009) and a competitive inhibitor of the type I glycine transporter (GlyT1) (Eulenburg et al., 2005; Lopez-Corcuera et al., 1998; Smith et al., 1992), can directly and indirectly activate glycine binding site of NMDARs. As DMG is a precursor of sarcosine and acts like a partial agonist at NMDAR glycine binding site in the cortical neurons and in the medial prefrontal cortex (unpublished data), DMG may also have antipsychotic and/or antidepressant effects like sarcosine through direct activation of glycine binding sites of NMDA receptors or indirectly via its metabolite sarcosine. The present study assessed the acute effects of DMG on ketamine-induced psychotomimetic, antidepressant-like and anesthetic effects.

2. Materials and methods

2.1. Animals and drugs

Male ICR mice (8–10 weeks, 30–45 g) were supplied from the BioLASCO Charles River Technology (Taiwan) and housed 4–6 per cage in a 12 h light/dark cycle with ad libitum access to water and food. All experiments were carried out between 10:00 and 17:00 h and in accordance with the Republic of China animal protection law (Chapter III: Scientific Application of Animals) and approved by the Review Committee of the institutional animal care and use committees of Tzu Chi University and National Health Research Institutes, Taiwan.

Ketamine and *N*,*N*-dimethylglycine (Sigma Chemical Co., St. Louis, MO, USA) were dissolved in saline and intraperitoneally injected in volumes of 10 ml/kg. Low dose of ketamine (10 mg/kg, i.p.) was chosen for forced swimming test based on the previous studies (Garcia et al., 2008). Ketamine (30 mg/kg, i.p.) was applied in open field test, rotarod performance, novel object recognition memory and prepulse inhibition to produce the psychotomimetic effect (Chan et al., 2008; Moghaddam et al., 1997). The anesthetic dose of ketamine (100 mg/kg i.p.) was used to induce loss of righting reflex. The mice were randomly divided into different treatment groups in each experiment and were used in only one experiment.

2.2. Rotarod test

Motor coordination was examined using an automated rotarod device (Singa; Technology Co., Ltd., Taiwan) for a maximum of 6 mice. A computer recorded the latency to fall in seconds. During two-three days training period, the mice were first trained on the rotarod at a constant speed of 20 rotations per minute (rpm) until all of the mice were able to spend at least 3 min on the rod. DMG (0, 30 and 100 mg/kg) was administered 30 min prior to the ketamine (30 mg/kg) or saline injection. Then, the mice were tested 10, 15, 20, 25, and 30 min after ketamine injection.

2.3. Prepulse inhibition test (PPI)

The PPI was operated as described in our previous work (Chan et al., 2012a). Briefly, the animals were initially moved from the home cage, weighed, and then placed into the restrainers in the SR-LAB (San Diego Instruments, San Diego, CA, USA) acoustic startle chambers for 30-min habituation. DMG (0, 30 and 100 mg/kg) was administered 30 min prior to ketamine (30 mg/kg) or saline injection. After administration of ketamine or saline, the experiment started with a 5-min adaptation period during which the animals were exposed to 67-dB background white noise, and this background noise was continued throughout the session. Then, the following adaptation period startle session began with five initial startle stimuli (120 dB bursts of white noise, 40 ms duration). After the first five initial stimuli, mice received five different trial types: pulse alone trials (120 dB bursts of white noise, 40 ms duration), three prepulse and pulse trials in which 76, 81, or 86 dB white noise bursts (9, 14, and 19 dB above background) of 20 ms duration preceded 120 dB pulse by 100 ms prepulse onset to pulse onset, and no-stimuli trials during which only background noise was applied. Each of these trial types was presented five times in randomized order. The inter trial interval was 7–23 s, and the test lasted 15 min in total. Prepulse inhibition was calculated as the percent inhibition of the startle amplitude evoked by the pulse alone: % PPI = (magnitude on pulse alone trial − magnitude on prepulse + pulse trial / magnitude on pulse alone trial) × 100.

2.4. Open field test

To evaluate the effect of DMG on ketamine-induced locomotor hyperactivity, the animals were moved from the home cage, weighed and placed into an activity cage (Columbus Auto-Track System, Version 3.0 A, Columbus Institute, Columbus, OH, USA) for 2 h. Thereafter, DMG (0, 30, and 100 mg/kg) was given 30 min prior to ketamine (30 mg/kg) or saline. The distance (cm) travelled was recorded for totally 180 min. A 70% alcohol solution was used to clean the inner surface of all the testing apparatus between trials to remove any potentially interfering odors left by the previous mouse.

2.5. Novel location and novel object recognition tests

The novel location recognition test (NLRT) and novel object recognition test (NORT) were examined in a Plexiglas open field box (35 × 35 × 30 cm) located in a sound-attenuated room and illuminated with a 20-W light bulb. The novel location and novel object recognition procedure consisted of habituation, training, and retention sessions. Habituation was conducted in two consecutive daily sessions, during which each mouse was allowed to individually explore the box in the absence of objects for 20 min. The animal is then removed from the arena and placed in its home cage. During the sample phase, each animal was placed in the box, and after 5 min, two identical sample objects (A + A) were simultaneously introduced in two corners. Each animal was allowed to explore the objects for 5 min. An animal was considered to explore the object when its head was facing the object at a distance of approximately 1 cm or less between the head and object or when it was touching or sniffing the object. The time spent exploring each object was recorded using stopwatches by an experimenter blind to the treatment condition. After the sample phase, the mice were immediately returned to their home cages. The novel location recognition test was conducted 30 min after the training session. The animals were returned to the same box as during the sample phase, and one of the two objects was replaced with a novel local corner (A + A′) to test the location-