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Combinational effects of ketamine and amphetamine on behaviors and neurotransmitter systems of mice

Chuan-Ching Lai [a], Li-Jen Lee [a,b,c], Hsiang-Shu Yin [a,b,c,*]

[a] Graduate Institute of Anatomy and Cell Biology, National Taiwan University, Taipei, Taiwan
[b] Graduate Institute of Brain and Mind Sciences, National Taiwan University, Taipei, Taiwan
[c] Neurobiology and Cognitive Science Center, National Taiwan University, Taipei, Taiwan

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ABSTRACT

The combined ingestion of ketamine (Ket) and amphetamine (Amph) by drug-users has been rampant and produced more severe behavioral abnormality. However, the interactive consequences of the two drugs are still unclear. In this study, we treated adult male mice with a single i.p. injection of saline, Amph (5 mg/kg), low Ket (LK, 10 mg/kg), high Ket (HK, 50 mg/kg), or Amph and LK or HK (ALK or AHK) and examined their behavioral and neurochemical changes at 0.5 and 2 h post-injection. Compared with saline, Amph, LK or HK treatment alone increased the levels of motor activities such as locomotion, stereotypy or ataxia of mice. Notably, at combined treatments, LK and HK differentially exacerbated Amph-induced locomotion and stereotypy, whereas Amph worsened LK or HK-produced ataxia. The higher striatal dopamine levels of A, ALK and AHK groups correlated with their greater motor activities. The prolonged increase of dopamine in the motor cortex of ALK and AHK mice may associate with the longer duration of behavioral hyperactivity and greater peak score of locomotion; the greater dopamine level in the somatosensory cortex probably contributes to the more severe ataxia. Furthermore, in the striatum of all drug-treated groups, the expression of GAD_{67} mRNA and GAD_{67}-positive punctates was higher than respective saline controls, indicating the involvement of GABAergic system in the drug-induced behavioral changes. Our results demonstrate the acute interplay between Amph and Ket in both behavioral and neurochemical aspects for the first time. Dopaminergic and GABAergic systems were affected differentially by the drugs in the striatum.

1. Introduction

Ketamine (Ket), a noncompetitive antagonist of NMDA (N-methyl-D-aspartic acid) receptor, is often used as an anesthetic for veterinary medicine. In human, however, Ket may produce a sense of detachment or dissociation, thus it is rarely used in general surgical procedure but as a recreational drug and known as a dissociative anesthetic (for review, see White et al., 1982). In rodents, administration of a sub-anesthetic dose of Ket results in the release of dopamine (DA), norepinephrine, glutamate, and serotonin in the medial prefrontal cortex and striatum, and induces behavioral abnormality characterized by increased locomotion, stereotypy, and ataxia (Martin et al., 1982; Martin and Smith, 1982; Kubota et al., 1999; Imre et al., 2006). By contrast, amphetamine (Amph) may act as a false substrate that binds to monoamine transporters such as DA transporter and thereby elicits hyper-locomotion and stereotypy in rodents, but not ataxia (for review, see White and Kalivas, 1998).

Ataxia is a neuropathic sign of lack of coordination and may be resulted from the dysfunction of distinct brain regions, such as cerebellum, motor and sensory areas (Bastian, 1997; Middleton and Strick, 2000). The functional connectivity among these areas might be affected by Ket (Nicolás et al., 2011; Liao et al., 2012). Although Amph alone does not produce ataxia, it is unknown whether Amph affects the severity of Ket-evoked ataxia.

The combined abuse of Ket and Amph by humans has been rampant and produced more severe behavioral hyperactivity, lack of coordination, thought disorder, and euphoria, compared to single use of either drug (Dillon et al., 2003; Lankenau et al., 2005; Liu et al., 2005). This behavioral potentiation in human is probably associated with the Ket-mediated enhancement of dopamine release induced by Amph (Kegeles et al., 2000). Nevertheless, the detailed mechanisms remain to be clarified underlying the combinational effects of Ket and Amph.

As a NMDA receptor antagonist, Ket administration may reduce excitatory signals. Therefore the Ket-induced activation of dopamine system is apparently not directly mediated by inhibition of

* Corresponding author at: Graduate Institute of Anatomy and Cell Biology, College of Medicine, National Taiwan University, No. 1, Ren-Ai Rd, Section 1, Taipei 100, Taiwan. Tel.: +886 2 23123456x88173; fax: +886 2 23915292.
E-mail address: hsyin@ntu.edu.tw (H.-S. Yin).

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glutamatergic neurotransmission. To reconcile this paradox, a GABA-mediated mechanism has been suggested in the rat medial prefrontal cortex (Kegeles et al., 2000). We have previously shown the immunohistochemical changes of the GABAergic markers, such as GAD_{67} and calcium binding proteins in various brain regions of rat and mouse following Amph treatment (Yin et al., 2004, 2009, 2010, 2012; Yin and Tan, 2007). However, the activities of GABA system in response to Ket or combined use of Ket and Amph have not been carefully characterized.

In this study, we hypothesize that both dopaminergic and GABAergic systems are influenced by mixed administration of Ket and Amph and these changes could contribute to the potentiated behavioral alterations. To test this hypothesis, we established a mouse model that mimics the mixed abuse of Ket and Amph. Behavioral changes and neurochemical features in the dopaminergic and GABAergic systems were addressed.

2. Materials and methods

2.1. Animals

Adult male FVB mice of 8 weeks old were used in this study. Animals were housed in the Laboratory Animal Center of National Taiwan University College of Medicine under 12-h light/dark cycle with free access to food and water. Animal handling was in accordance with a protocol approved by Institutional Animal Care and Use Committee of College of Medicine and College of Public Health, National Taiwan University. All efforts were made to minimize animal suffering and reduce the number of animals used.

2.2. Drug treatments

Each mouse first received an intraperitoneal injection of saline or Amph (5 mg/kg), a dosage used in our previous studies (Yin et al., 2009, 2012). Immediately after the first treatment, a second injection of saline, or Ket was given. It is known that 50 mg/kg of Ket is a subanesthesia dosage, yet a lower dose, 10 mg/kg, has been shown to induce significant behavioral changes (White et al., 1982; Hunt et al., 2006). In the present study, the effects of high dose Ket, 50 mg/kg (HK) and low dose Ket, 10 mg/kg (LK) were tested. The dosages of Amph and Ket were selected by similar behavioral response and neurochemical changes of the animals to that of humans after the drug administration (White and Kalivas, 1998; Martin et al., 1982; Martin and Smith, 1982; Kubota et al., 1999; Imre et al., 2006). Six groups of animals (saline + saline [S]; 5 mg/kg Amph + saline [A]; saline + 10 mg/kg Ket [LK]; Amph + 10 mg/kg Ket [ALK]; saline + 50 mg/kg Ket [HK]; and Amph + 50 mg/kg Ket [AHK]) were then subjected to behavioral observation and sacrificed at 30 or 120 min post-injection for neurochemical examinations.

2.3. Behavioral observation

Seven to nine mice in each group were used for behavioral observation. Thirty min prior to the first injection, each mouse was placed in an open field apparatus (30 cm × 30 cm × 40 cm) for habituation. The behavior of the animals was rated every 5 min, beginning from 5 min before drug injection until 120 min post-injection. According to a ranking criterion of locomotion, stereotypy, or ataxia (Jerram et al., 1996), 0–5 was given for each type of the behavior. Briefly, the scores of mouse locomotion were determined by the following criteria: 0. stationary or normal ambulation/exploration behaviors; 1. intermittent moving activity at a low rate within a localized area; 2. moving intermittently at a low-moderate rate over a small area; 3. moving continuously at a moderate-rapid rate over a small area; 4. moving intermittently at a low to moderate rate over a large area; 5. moving continuously at a moderate to rapid rate over a large area. The ranks of stereotypy were: 0. inactive or in-place non-repetitive activity; 1. slow rate of locomotion, sniffing, and grooming; 2. gagging, weaving, occasional reciprocal forepaw treading (RFT), and moderate rate of rearing or sniffing; 3. intermittent turning at a moderate rate, backpeddling, praying, RFT, sniffing, weaving, and gagging; 4. continuous turning at a rapid rate, backpeddling, praying, sniffing weaving, and gagging; 5. dyskinetic extension and flexion of limbs, head, and neck. For ataxia: 0. inactive or in place activity with coordinated movement; 1. jerky movement, loss of balance, and occasional falling on side; 2. awkward-jerky movements with a moderate rate of falling on side; 3. frequent falling on back and/or side; 4. unable to move beyond a restricted area; 5. unable to move except for twitching/convulsive movements. The most frequent ranking was recorded within every 1 min interval and verified by two experienced observers who were blind to the treatments. Values were recorded for peak scores and latencies of the peak scores for each type of the behavior. Duration of hyperactivity was defined by after the drug injections, the time of the presence of scores higher than that of saline-injected mice.

2.4. Measurement of dopamine (DA) level

Thirty minutes or 2 h after drug injections, mice (n = 5–7 mice per group in each time point) were killed and the brains were rapidly removed and dissected on an ice-cold plate. Brain regions such as the striatum, motor cortex and somatosensory cortex were dissected and rapidly placed in 1.5 ml microcentrifuge tubes that contained 0.3 ml 0.16 N perchloric acid for HPLC (high performance liquid chromatography) analysis. After sonication (Sonic Dismembrator, Fisher Scientific, Hampton, NH, USA), the homogenates were centrifuged at 14,000 rpm for 15 min at 4 °C, and the supernatant was filtered through a 0.2 μm PVDF membrane (PALL, Port Washington, NY, USA). Protein concentrations were quantified with the bicinchoninic acid (BCA) assay. The supernatants were subjected to the reverse-phase HPLC (626 LC system, Waters, Milford, MA, USA). The mobile phase for detecting DA consisted of 1.5 M of acetonitrile, 0.27 mM EDTA, 0.03 M triethylamine, 10.3 mM heptane-sulfonic acid and 35 mM phosphoric acid, pH 2.44 (Champney et al., 1992; Yadid et al., 1994). The level of DA was determined based on respective standards and normalized by the total protein concentration of each sample.

2.5. Immunocytochemistry

After treatments, a number of mice (n = 4–6 mice per group in each time point) were used for immunochemical examinations. In brief, mice were killed and perfused with 4% paraformaldehyde in 0.1 M PB, pH 7.4. Brains were taken and embedded in paraffin (Yin et al., 2004). Serial coronal paraffin sections (7 μm thick) were prepared and deparaffinized with xylene. After rehydration, sections were incubated with 1% H_2O_2 in PBS for 30 min. Samples were then blocked with 2.5% nonfat milk in PBS for 1 h at room temperature (RT) and then incubated with the anti-GAD_{67} (1:1000, Merck Millipore, Billerica, MA, USA) at 4 °C for 20 h. Thereafter, the sections were incubated with a biotinylated secondary antibody (Vector lab, Burlingame, CA, USA) for 1 h at RT and avidin–biotin peroxidase complex (ABC kit, Vector lab) for 1 h. Finally, the sections were reacted with 0.05% 3,3′-diaminobenzidine tetrachloride (with 0.01% H_2O_2 in PBS), dehydrated and coverslipped with Permount. Some deparaffinized sections were stained with the cresyl violet (Nissl stain) to show the cellular organization of the brain region. The numbers of GAD_{67}-immunopositive punctates and processes were conducted using a computerized image analysis system (Image-Pro plus, Media Cybernetics, Bethesda,