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Mutual enhancement of central neurotoxicity induced by ketamine followed by methamphetamine

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Abstract

We hereby report that repeated administration of ketamine (350 mg/kg in total) and methamphetamine (30 mg/kg in total) causes specific glutamatergic and dopaminergic neuron deficits, respectively, in adult mouse brain. Acute ketamine did not affect basal body temperature or the later methamphetamine-induced hyperthermia. However, pretreatment with repeated doses of ketamine aggravated methamphetamine-induced dopaminergic terminal loss as evidenced by a drastic decrease in the levels of dopamine, 3,4-dihydroxyphenylacetic acid, and dopamine transporter density as well as poor gait balance performance. In contrast, methamphetamine-induced serotonergic depletion was not altered by ketamine pretreatment. Likewise, the subsequent treatment with methamphetamine exacerbated the ketamine-induced glutamatergic damage as indicated by reduced levels of the vesicular glutamate transporter in hippocampus and striatum and poor memory performance in the Morris water maze. Finally, since activation of the D1 and AMPA/kainate receptors has been known to be involved in the release of glutamate and dopamine, we examined the effects of co-administration of SCH23390, a D1 antagonist, and CNQX, an AMPA/kainate antagonist. Intraventricular CNQX infusion abolished ketamine's potentiation of methamphetamine-induced dopamine neurotoxicity, while systemic SCH23390 mitigated methamphetamine's potentiation of ketamine-induced glutamatergic toxicity. We conclude that repeated doses of ketamine potentiate methamphetamine-induced dopamine neurotoxicity via AMPA/kainate activation and that conjunctive use of methamphetamine aggravates ketamine-induced glutamatergic neurotoxicity possibly via D1 receptor activation.

Keywords: Norodin; NMDA antagonist; Serotonin; Dopamine; Glutamate; AMPA/Kainate; D1 receptor

Introduction

Many lines of evidence have shown that repeated doses of ketamine (KE), a noncompetitive antagonist for glutamate NMDA receptor, trigger acute neuronal apoptotic death by a direct NMDA blockade and produce late-onset excitotoxic neurodegeneration via a compensatory up-regulation of NMDA receptors during the developmental period of synaptogenesis (Olney, 2002; Slikker et al., 2005). In fact, repeated doses of KE have been found to induce hydroxyl radical generation, oxidative stress and neuronal degeneration in both neonates and adults (Hayashi et al., 2002; Scallet et al., 2004; Nishizawa et al., 2000; Sharp et al., 1991). Since a previous report suggested that NMDA receptors were localized not only in the postsynaptic density but also in presynaptic terminals of the glutamatergic neurons (Berretta and Jones, 1996), we decided to examine the neurotoxic effects of repeated doses of KE on glutamatergic neurons of the adult mouse.

Repeated doses of methamphetamine (MA) have been known to produce dopaminergic terminal degenerations, as indicated by long-lasting depletions in dopamine (DA) and its primary metabolite levels, decreases in DA transporter number, tyrosine

Abbreviations: MA, methamphetamine; KE, ketamine; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; NMDA, *N*-methyl-D-aspartic acid; vGLUT-1, vesicular glutamate transporter type I.

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hydroxylase activity, and vesicular monoamine transporter binding sites in striatum (Wagner et al., 1979, 1980; Ricaurte et al., 1982; Hess et al., 1990; Stephans and Yamamoto, 1996; Frey et al., 1997). Endogenous DA release, blockade of DA reuptake, and subsequent oxidative stress/free radical formation have been identified as the most critical events associated with MA-produced neurotoxicity (Kita et al., 2003; Cadet et al., 1994; LaVoie and Hastings, 1999). Dizocilpine (MK-801) and ketamine (KE), both NMDA antagonists, were found to provide substantial protection against MA-induced dopaminergic neurotoxicity (Pu and Vorhees, 1995; Sonsalla et al., 1989). The neuroprotective effects provided by these NMDA antagonists did not appear to involve inhibition of DA release (Finnegan and Taraska, 1996). However, it is of importance to note that, in these studies, KE and MA were administered essentially at the same time and the dosage for KE was relatively low. Since the KE and MA were given so close in time, complicated pharmacokinetics and a possible effect on MA-elicited hyperthermia may compromise the conclusion that KE exerts a protective effect against MA-induced neurotoxicity. Therefore, we examined the modulating effects of KE pretreatment on MA-induced neurotoxicity when these two drugs were delivered at least 12 h apart.

The abuse of KE and the designer amphetamines, such as MA and 3,4-methylenedioxymethamphetamine, is increasing worldwide (Wu et al., 2006). Surveys revealed that young adults who frequent certain night clubs as well as street-involved adolescents were likely to self-administer combinations of KE and MA (Kelly et al., 2006; Martin et al., 2006). Although use of KE or MA alone seldom results in fatal intoxication (Gill and Stajic, 2000; Sribanditmongkoi et al., 2000), long-lasting and irreversible central neuronal toxicity associated with their combined binge use is of concern. Polydrug mixing can be categorized in terms of the period of time for using two or more drugs, as simultaneous, alternating or sequential mixing within hours, days, weeks or longer (Schensul et al., 2005). Thus, a second purpose of this study was to examine the reciprocal modulation of glutamatergic and dopaminergic neurotoxicity by delivering both KE and MA with the KE dosing protocol 12 h ahead of the MA treatment. In an attempt to reveal KE and MA-produced neurotoxicity pertaining to dopaminergic and glutamatergic neurons, assays for these neuron-specific markers, including dopamine and primary metabolite contents, dopamine transporter, vesicular glutamate transporter were used. In addition, frequently used behavioral tests for determining deficits in dopaminergic and glutamatergic functions were employed.

Materials and methods

Animals. Male C57BL/6J mice, aged 8–9 weeks, were housed in a facility located at National Cheng Kung University Laboratory Animal Center (NCKULAC, Tainan, Taiwan, ROC) with free access to food (Purina Mouse Chow, Richmond, IN, USA) and tap water. The colony room was temperature and humidity controlled and maintained on a 12-h light/dark cycle (lights on at 0700). All experiments were conducted in a laboratory with temperature maintained at 24±1 °C. This study was performed in accordance with the *Guiding Principles in the Use of Animals in Toxicology* adopted by the Society of Toxicology. All procedures were approved by the local Animal Care Committee at NCKU College of Medicine.

KE and MA dosing regimens. Mice received 7 consecutive doses of KE (50 mg/kg each, intraperitoneally) or an equivalent volume of saline injections at 1.5-h intervals. This dosing regimen caused minimal lethality in our preliminary study. Twelve hours after the last dose of KE, mice received 3 subcutaneous doses of MA (10 mg/kg/dose) or a comparable volume of saline injections at a 2-h interval. In an attempt to examine long-lasting drug-induced neuronal deficits, mice were killed 2 weeks after the MA dosing regimen.

Previous reports indicated that pretreatment with the D1 receptor antagonist, SCH23390, reduced spontaneous glutamate release, amphetamine-stimulated glutamate release, dopamine release and development of amphetamine-induced behavioral sensitization (Wolf and Xue, 1999; Kalivas and Duffy, 1995). Although the role of NMDA receptor up-regulation has been demonstrated in KE-produced neurotoxicity (Olney, 2002; Slikker et al., 2005), D1 activation-mediated glutamate release was suspected to further aggravate the KE-induced neurotoxicity. Therefore, in an effort to study the role of D1 activation in the predicted potentiation of KE-induced toxicity by MA, 3 doses (0.5 mg/kg/dose) of SCH23390 were intraperitoneally injected 30 min before the subsequent MA injection.

Cannula implantation and intraventricular infusion of CNQX. An AMPA/kainate receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) was purchased from Sigma-RBI. Sodium pentobarbital was from Tokyo Chem. Co. (Tokyo, Japan). Stereotaxic guide cannula (26 G) implantation was made bilaterally under sodium pentobarbital anesthesia (50 mg/kg, i.p.) 1 week before the KE dosing regimen. Intraventricular infusion of CNQX was performed with injection needles being inserted bilaterally through guide cannulas and toward the ventricle (coordinates: anteroposterior, −0.58 mm; lateral, ±1.2 mm; dorsoventral, −2.2 mm) 10 min before the first dose of KE. CNQX (a total amount of 100 μM in 2 μl) was infused at a flow rate of 1 μl/min by a microdialysis pump (CMA/102, CMA/Microdialysis, Stockholm, Sweden).

Tissue DA, DOPAC, 5-HT, and 5-HIAA concentrations. Following a 2-week recovery period after the MA dosing regimen, mice were killed by rapid decapitation and the brain was removed within 20–30 s and placed on the dorsal surface on a glass dish sitting on crushed ice. Prefrontal cortex sample was obtained

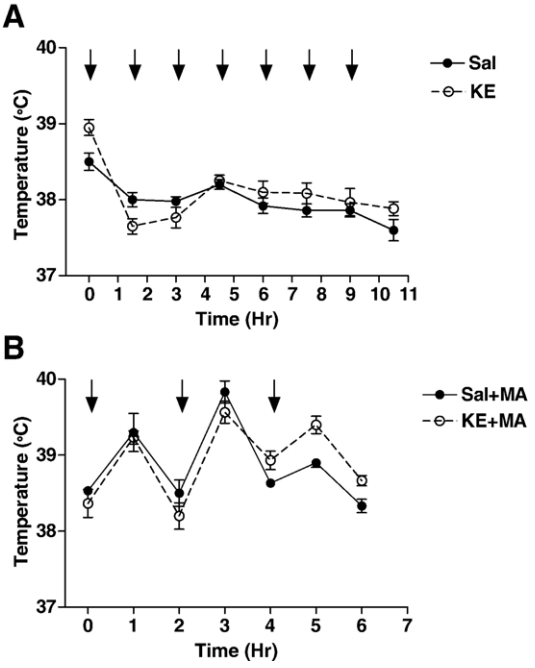

Fig. 1. Rectal temperature in mice treated with ketamine (KE) and methamphetamine (MA). (A) Repeated intraperitoneal KE injections (50 mg/kg/dose for 7 doses) at 1.5-h intervals do not affect rectal temperature. $n=6$ for each group. (B) Single subcutaneous MA injection (10 mg/kg) enhances rectal temperature an hour after injection. Such elevated temperature returns to the baseline 2 h after the injection. Pretreatment with KE does not affect later MA-induced hyperthermia. $n=3$ for each group. Data are expressed as mean±SEM. Arrows stand for the time points for drug injection.