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Behavioural and Toxic Interaction Profile of Ketamine in Combination with Caffeine

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Abstract: Ketamine street tablets often contain several other compounds in addition to ketamine, among them is caffeine. The purpose of this study was to examine whether caffeine interacts with ketamine-induced behavioural and toxic effects. Male ICR mice were treated with ketamine alone or ketamine combined with various doses of caffeine, then the locomotor activity, rotarod test, prepulse inhibition of acoustic startle, loss of righting reflex, and mortality rate were examined. Caffeine enhanced the locomotor hyperactivity, caused disruption of the rotarod performance, and mortality rates due to ketamine, whereas prepulse inhibition deficits and anaesthesia remained unaffected. These findings demonstrate that use of ketamine in combination with caffeine enhances its stimulant responses and lethal risk, suggesting that a potentially toxic interaction exists between ketamine and caffeine.

Ketamine is used in human beings and animal medicine as a general anaesthetic. The illegal use of ketamine as a recreational drug is rapidly growing worldwide. It is classified as a schedule III controlled substance. Recreational use of ketamine is related to its hallucinogenic and euphoric properties. In addition, as this drug causes powerful dissociative effects, loss of the ability to judge, and amnesia, it has also been inappropriately used as a date-rape drug.

It should be noted that most of the ketamine street tablets seized in Taiwan are impure. Caffeine is one of the main components detected in the ketamine tablets [1]. In addition to reduction of the cost, the reason for this intentional combination by tablet makers is unclear. It has been reported that motor activity produced by low doses of ketamine was significantly increased by caffeine [2,3] in animal studies. It is of interest to know if caffeine can enhance the other behavioural effects of ketamine. Actually, subanaesthetic dose of ketamine induces not only hyperlocomotion, but also stereotypes and ataxia, and impairs sensorimotor gating in rodents [4–6]. The purpose of this study was to examine the effect of concomitant administration of caffeine on ketamine-induced behavioural alterations to reveal the possible reason for and outcome of the combination use.

While caffeine is a substance that is generally regarded as safe and is freely available in beverages such as tea, coffee, and soft drinks, caffeine has been shown to increase mortality in combination with amphetamine, cocaine [7], and MDMA [8,9]. As it is possible that caffeine may also have the potential to exacerbate the fatal intoxication of ketamine for recreational drug users and victims of date rape, the toxic interaction of ketamine and caffeine was also investigated.

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Materials and Methods

Animals and drugs. Male ICR mice (8–9 weeks, 33–40 g) were supplied from the BioLASCO Charles River Technology (Taiwan) and housed four to five per cage in a 12 hr light/dark cycle with *ad libitum* access to water and food. The experimental protocol was approved by Review Committee of the Tzu Chi University for the Use of Animals.

Ketamine and caffeine (Sigma Chemical Co., St. Louis, MO, USA) were dissolved in saline, respectively. Ketamine and caffeine were concomitantly administered by mixing various ratios of ketamine and caffeine solutions in the same syringe. There were no visible insoluble particles in the mixed solution. The injection volume was 10 ml/kg.

Locomotor activity. 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, OH, USA) for habituation for 2 hr. The observation period started 90 min prior to administration of ketamine (20 mg/kg), caffeine (20 mg/kg), ketamine (20 mg/kg) plus caffeine (5–20 mg/kg) or saline. Distance (cm) travelled was recorded totally for 150 min. A 75% alcohol solution was used to clean the inner surface of the apparatus between trials to remove any potentially interfering odours left by the previous mouse.

Rotarod test. Motor coordination was assessed by means of an automated rotarod apparatus (TSE systems, Bad Homburg, Germany). A computer recorded the latency to fall in seconds. First, the mice were trained in the rotarod at a constant speed of 20 rpm until all the mice were able to spend at least 3 min on the rod. Then, the mice were tested 5, 10, 15, and 20 min after treatment with ketamine (20 mg/kg), ketamine (20 mg/kg) plus caffeine (5–20 mg/kg) or saline.

Prepulse inhibition. SR-LAB (San Diego Instruments, San Diego, CA, USA) acoustic startle chambers were used. SR-LAB software

controlled the delivery of all stimuli to the animals and recorded the response. The animals were moved from the home cage, weighed, and placed into the restrainers in the startle chambers for a 30 min habituation. After administration of ketamine (30 mg/kg), caffeine (20 mg/kg), ketamine (20 mg/kg) plus caffeine (20 mg/kg), or saline, the experiment started with a 5-min adaptation period during which the animals were exposed to 67 dB background white noise; 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 msec duration). After the first five initial stimuli, the mice received five different trial types: pulse alone trials (120 dB bursts of white noise, 40 msec duration); three prepulse and pulse trials (76, 81, or 86 dB) white noise bursts (9, 14, and 19 dB above background) of 20 msec duration, preceded by 120 dB pulse by 100 msec 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 randomised order. The intertrial interval was 7–23 sec and the test lasted 15 min in total. Prepulse inhibition (PPI) was calculated as the percent inhibition of the startle amplitude evoked by the pulse alone: % PPI = (magnitude on pulse alone trial – magnitude on prepulse *and* pulse trial / magnitude on pulse alone trial) × 100.

Loss of righting reflex test. After an intraperitoneal injection of ketamine (100 mg/kg) and ketamine (100 mg/kg) plus caffeine (5–100 mg/kg), the mice were placed in a clean cage until the righting reflex was lost. They were then placed in the supine position until recovery and the duration of the loss of righting reflex was recorded. Recovery of the righting reflex was defined as the ability to perform three successive rightings.

Mortality rates. After the administration of ketamine (200 mg/kg) in combination with caffeine (20–100 mg/kg) the mice were placed in individual cages for observation.

Statistical analyses. Data are expressed as the mean ± SEM. The data from locomotor activity and rotarod test were analyzed by repeated two-way ANOVA and time as the within subject factor. The % of PPI was analysed by repeated two-way ANOVA (prepulse intensity as the within subject factor). The data for onset and duration of loss of righting reflex was analyzed by one-way ANOVA. Multiple comparisons were performed using the Student–Newman–Keuls test. $P < 0.05$ was considered statistically significant. Mortality rates were analyzed using chi-square test.

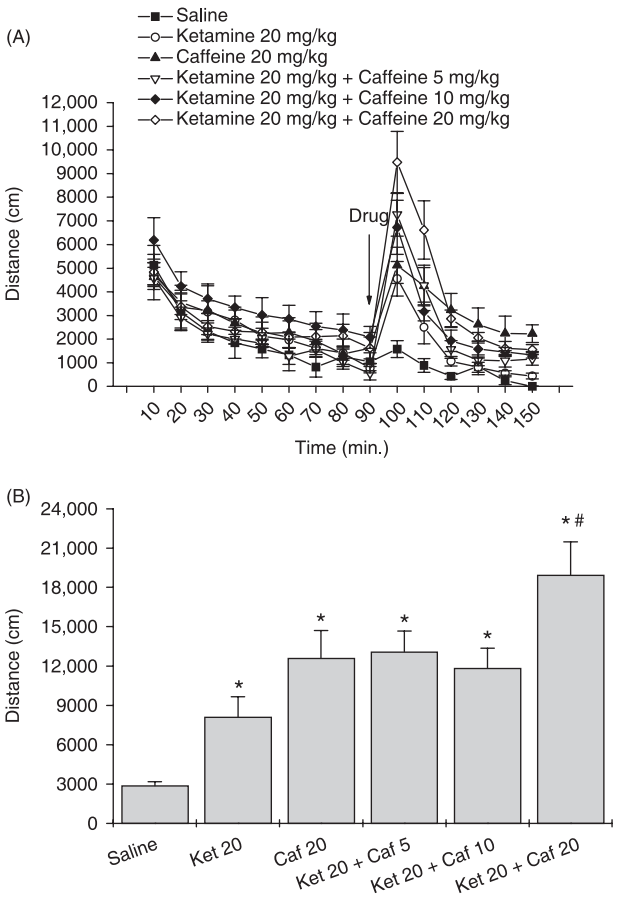

Fig. 1. Combined effects of ketamine and caffeine on locomotor hyperactivity. Mice were habituated in testing cages for 90 min and challenged with saline, ketamine (20 mg/kg, intraperitoneally), caffeine (20 mg/kg, intraperitoneally), and combined various doses of caffeine (5–20 mg/kg, intraperitoneally) with ketamine (20 mg/kg, intraperitoneally). The traveled distances were recorded for 60 min (A). The total distances during 30 min after drug treatment were calculated and compared (B). All values are expressed as the mean ± SEM (n = 6). Data were analyzed by one-way ANOVA followed by Student–Newman–Keuls test. *$P < 0.05$, compared with the saline group, #$P < 0.05$, compared with the ketamine group.

Results

As shown in fig. 1A, the locomotor activity was significantly enhanced after treatment with ketamine, caffeine, or the two in combination. The effects were maintained for 30 min. Ketamine (20 mg/kg) and caffeine (20 mg/kg) alone significantly increased the total distances during 30 min after treatment. A high dose of caffeine (20 mg/kg) plus ketamine (20 mg/kg) significantly increased the total locomotor activity compared to ketamine ($P < 0.01$) and caffeine ($P < 0.05$) alone. However, lower doses of caffeine (5 and 10 mg/kg) did not affect the ketamine-induced locomotor hyperactivity (fig. 1B).

In the experiment for assessing the effect of ketamine and caffeine on rotarod performance, the ANOVA revealed a main effect of different treatment ($F_{5,68} = 32.72$, $P < 0.001$) and time ($F_{3,68} = 69.519$, $P < 0.001$), and a significant interaction effect of treatment and time ($F_{12,68} = 7.048$, $P < 0.001$). Ketamine alone induced motor in coordination. Caffeine alone did not affect the duration on the rotarod. Caffeine at 20 mg/kg, but not 5 and 10 mg/kg sigificantly delayed the recovery of ketamine-induced motor incoordination (fig. 2).

Two-way ANOVA revealed that ketamine and caffeine did not affect startle amplitude to pulse alone ($F_{3,43} = 0.29$, $P = 0.83$) (fig. 3A). As for PPI, two-way ANOVA revealed a main effect of treatment ($F_{3,129} = 11.95$, $P < 0.001$) and noise ($F_{2,129} = 5.09$, $P < 0.01$). Ketamine alone and ketamine plus caffeine significantly reduced the PPI. However, there was no significant difference between these two groups, indicating that caffeine did not affect the PPI deficits induced by ketamine (fig. 3B).

As shown in fig. 4, caffeine did not affect ketamine-induced onset ($F_{3,23} = 1.07$, $P = 0.38$) and duration ($F_{3,23} = 0.33$, $P = 0.79$) of loss of righting reflex (fig. 4). The interactions of caffeine and ketamine on mortality rates were shown in

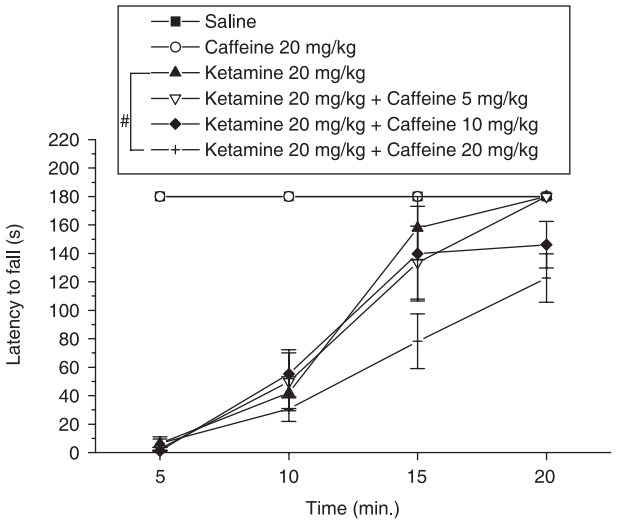

Fig. 2. Effects of caffeine on ketamine-induced motor incoordination in the rotarod test. Mice were pretreated with saline, ketamine (20 mg/kg, intraperitoneally) alone, caffeine alone (20 mg/kg, intraperitoneally), or ketamine (20 mg/kg, intraperitoneally) plus caffeine (5–20 mg/kg, intraperitoneally) and the latency to fall was recorded 10, 15, 20, and 25 min after treatment. All values are expressed as the mean ± SEM (n = 5–6). Data were analyzed by two-way repeated measures ANOVA. $^{\#}P < 0.05$, compared with the ketamine group.

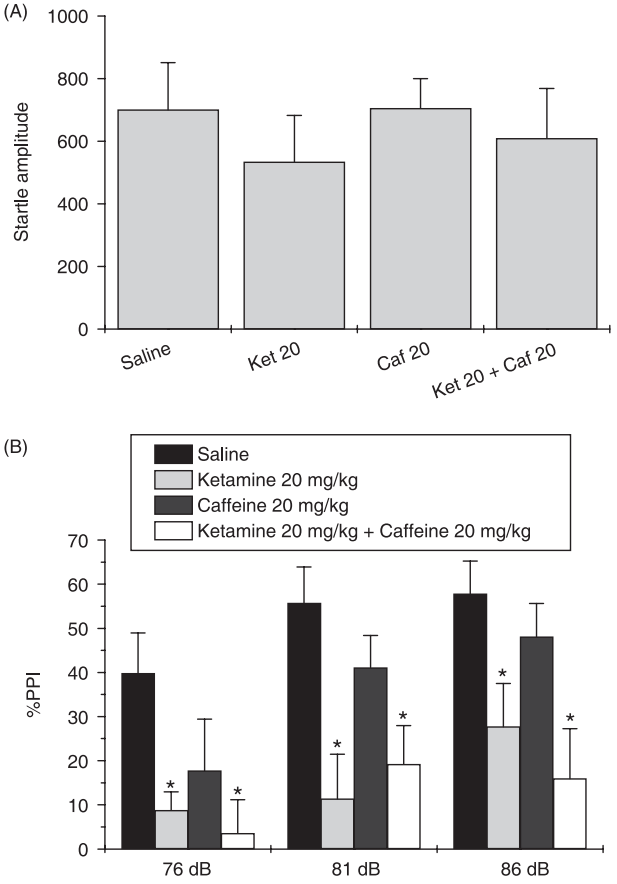

Fig. 3. Combined effects of ketamine and caffeine on startle amplitude (A) and prepulse inhibition (PPI) (B). Mice were pretreated with saline, ketamine (20 mg/kg, intraperitoneally), caffeine (20 mg/kg, intraperitoneally), and combined ketamine (20 mg/kg, intraperitoneally) and caffeine (5–20 mg/kg, intraperitoneally) 15 min prior to startle amplitude and PPI test. All values are expressed as the mean ± SEM (n = 9–14). Data were analysed by one-way ANOVA (startle amplitude) and two-way repeated measures ANOVA (PPI). *P < 0.05, compared with the saline group (post-hoc Student–Newman–Keuls test).

table 1. Caffeine (20–100 mg/kg) dose-dependently increased the incidence of lethality of ketamine. Most of the animals treated with ketamine in combination with caffeine exhibited convulsions before death.

Discussion

In this study, co-administration of caffeine with ketamine produced additive effects on locomotor hyperactivity, increased motor incoordination, and incidence of death, but did not significantly affect sensory motor gating deficits and anaesthesia induced by ketamine. The promotion by caffeine of the acute toxicity associated with ketamine is a serious drug interaction. Although ketamine is a safe and rapidly acting, lipid-soluble anaesthetic with a short elimination half-life that is used for medical and veterinary purposes, fatalities attributed to ketamine intoxication have been documented [10,11]. With the increased use of ketamine street tablets as a recreational drug, the lethal risk is likely to be enhanced by consumption with caffeine, as caffeine is often found in ketamine tablets.

Pharmacologically, ketamine is classified as a non-competive NMDA receptor antagonist and at high, fully anaesthetic doses, ketamine has also been found to bind to opioid μ-receptors, σ-receptors [12], and nicotinic acetylcholine receptors [13,14]. At low doses, stimulant effects predominate; with higher doses, psychedelic effects predominate [15,16]. Consistent with a previous report [3], our data show that ketamine-induced locomotor hyperactivity was enhanced by caffeine. Caffeine, a non-selective A_1 and A_2 adenosine receptor antagonist, have been reported to produce motor-activating

Table 1.

Incidence of death after administration of ketamine and caffeine alone or in combination.

Treatment (mg/kg)	Incidence of lethality
Ketamine 200	1/13
Caffeine 100	0/6
Ketamine 200 + caffeine 20	2/9
Ketamine 200 + caffeine 50	5/13*
Ketamine 200 + caffeine 100	12/13**

Ketamine and caffeine were administered to animals alone or in combination. Data represent the incidence of lethality (number of fatalities/number of animals in the group). *P < 0.05, **P < 0.01 compared with the ketamine group.

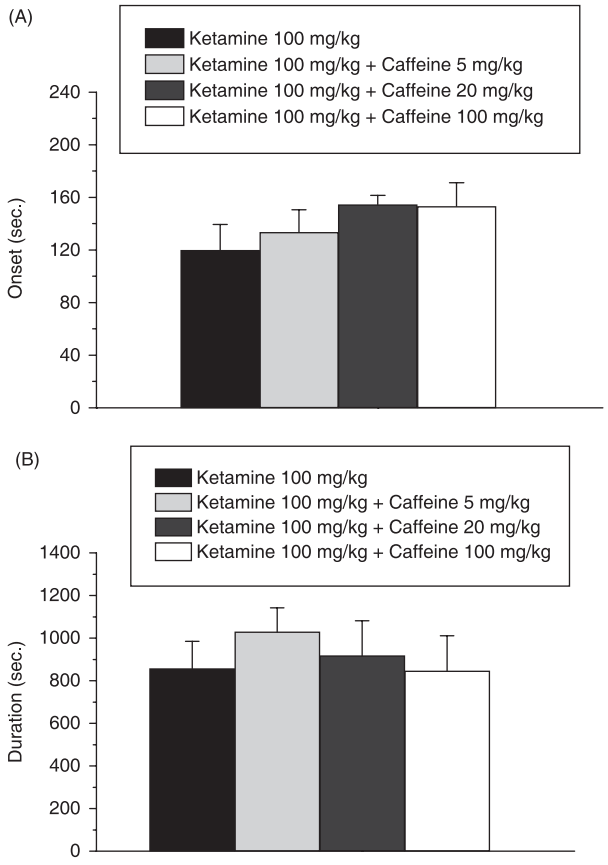

Fig. 4. Effects of caffeine on ketamine anaesthesia. Mice were treated with ketamine (100 mg/kg, intraperitoneally) alone or ketamine (100 mg/kg) plus caffeine (5–100 mg/kg, intraperitoneally) and the latency (A), and the duration (B) of loss of righting reflex were recorded. All values are expressed as the mean ± SEM (n = 6–7).

effects mediated by central blockade of both A_1 and A_2 adenosine receptors [17,18]. A_1 receptors may play a predominant role [19]. However, the A_{2A} receptor agonist, but not the A_1 adenosine receptor agonist, can significantly attenuate ketamine-induced locomotor hyperactivity [3], indicating that A_{2A} adenosine receptors play a more important role in ketamine hyperactivity. It is likely that the enhancing effect of caffeine on ketamine-induced locomotor hyperactivity is primarily due to A_1 receptor blockade.

Motor incoordination or ataxia is a general effect of ketamine and its analogues, such as PCP and MK-801 [20,21]. Caffeine alone does not produce adverse effects on motor coordination. Our results indicate that caffeine enhanced the ketamine-induced motor incoordination at a higher dose (20 mg/kg). It should be studied whether adenosine receptor blockade also contributes to the potentiated effect of caffeine on ketamine-induced motor incoordination.

Caffeine alone did not cause PPI deficits [22] and anaesthesia. Our data show that caffeine did not affect ketamine-induced PPI deficits and anaesthesia. Consistently, chronic caffeine affects locomotor hyperactivity, but not ataxia and cognitive dysfunction by MK-801, a ketamine analogue and also a NMDA receptor channel blocker [23]. Recently, it has been found that a low dose (3 mg/kg), but not higher doses of caffeine, significantly lowers brain stimulation reward thresholds when given together with MK-801 [24]. Caffeine might specifically modulate locomotor hyperactivity and rewarding effects associated with NMDA receptor antagonists including ketamine. However, the sensorimotor gating and anaesthetic effects of ketamine that have been reported to be closely correlated with its antagonistic effects on NMDA receptors [25,26] were not affected by caffeine. Ketamine might induce behavioural manifestations associated with distinct neuronal circuits and are apparently distinctly modulated by caffeine.

A serious intoxication of ketamine can induce aspiration, epileptic seizures [27], respiratory depression [28], and cardiac arrest [29], leading to death. Death by caffeine overdose may result from convulsions, arrhythmia, and cardiac arrest [30]. Our results demonstrate that caffeine dose-dependently increased the incidence of convulsions and lethality of ketamine. It is well known that antagonism of the A_1 and A_2 receptors can cause seizures and convulsions. It has been demonstrated that treatment of ketamine in combination with aminophylline, a caffeine analogue, and A_1 and A_2 receptor antagonist, resulted in decreases in seizure threshold [31]. Alternatively, both ketamine and caffeine can affect the cardiovascular system. It is highly possible that combined treatment with ketamine and caffeine produces synergistic effects on convulsions and the cardiovascular system to enhance the mortality rates.

In summary, combined treatment with ketamine and caffeine enhanced the locomotor hyperactivity at low doses and mortality rates at high doses, reflecting that the impure ketamine tablets containing caffeine exhibit more stimulant and lethal effect. The increase in lethality is important and clinically relevant. If the interaction of ketamine and caffeine which we observed in this rodent model generalizes to human beings, such a pattern of drug use could have serious acute health consequences for ketamine abusers and date rape victims taking ketamine. This interaction has not been previously reported or documented in clinical case reports, but may well offer some insight into the contributing factors to reported idiosyncratic reactions to ketamine.

Acknowledgements

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