



Clinical Manifestations of Combined Methamphetamine with Morphine and their Effects on Brain Dopamine and 5-hydroxytryptamine Release in Mice

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Background: Methamphetamine (MA) is often mixed with morphine by polydrug addicts, and polydrug abuse has become a serious health problem worldwide. The purpose of this study was to investigate the major signs and symptoms of combined MA and morphine abuse in the Emergency Department (ED). In addition, we used a mouse model to study their effects on the release of dopamine (DA) and 5-hydroxytryptamine (5-HT) in the central nervous system. **Materials and Methods:** Seventy-two patients with combined MA and morphine abuse were collected during a 3-year period, and their medical records were reviewed. Mice were intraperitoneally administered MA (0.75 and 2.5 mg/kg/day) and morphine (5 mg/kg/day) either alone or in combination for 5 consecutive days. The mechanisms underlying the interaction between MA and morphine were explored by measuring the extracellular levels of DA and 5-HT in the shell of the nucleus accumbens using an *in vivo* microdialysis technique. **Results:** The most common manifestations of combined MA and morphine abuse included tachypnea, tachycardia, confusion, anxiety, delirium, insomnia, and diaphoresis in the ED. Of those, 25% of acute intoxication required hospitalization for intensive care. The group of mice treated with a combination of MA and morphine had higher concentrations of DA and 5-HT in the accumbens than with either drug alone. **Conclusion:** These findings suggest that MA pharmacologically interacts with morphine to induce characteristic signs and symptoms. Our preclinical results also implicate the involvement of increased DAergic and 5-HTergic neurotransmission among polydrug abusers with a combination of MA and morphine.

Key words: Methamphetamine, morphine, combined abuse, dopamine, 5-hydroxytryptamine

INTRODUCTION

According to the epidemiologic survey of the National Bureau of Controlled Drugs in Taiwan from 2000 to 2003, amphetamines and opiates are frequently abused together by addicts [Table 1]. Moreover, patients with their combination abuse are more often than morphine abuse alone, and polydrug abuse has become a serious health problem in the Emergency Department (ED). Methamphetamine (MA) is a powerful psychostimulant that has more potent and more efficacious effects than amphetamine on the central nervous system (CNS). Repeated exposure to MA leads to drug dependence, which is characterized by drug-seeking behavior after withdrawal. Opiates such as morphine and heroin produce

euphoric feelings and are highly addictive. Morphine (mu opioid receptors agonist) potentiates MA-induced behavioral responses, increases rewarding effects, and augments behavior sensitization in the mouse model.¹ Moreover, a behavioral study in humans showed that mixed regimens of d-amphetamine and morphine produced highly positive subjective responses in nondependent individuals.²

Multiple brain regions and neurotransmitter systems are associated with drug addiction to MA and morphine. The reinforcing effects of MA on behavior are mainly mediated through dopamine (DA) and 5-hydroxytryptamine (5-HT) neurotransmission in the brain and primarily depend on its ability to increase DA and 5-HT release in the terminal regions of the mesoaccumbens and neostriatum.^{3,4} Pharmacological evidence also indicates that systemic administration of morphine increases the extracellular levels of DA and 5-HT in the nucleus accumbens (NAc) and striatum of mice.⁵ Furthermore, a series of studies to explore neural correlates of rewarding effects and drug-seeking behavior induced by MA and morphine focused on the interaction between the dopaminergic and opioidergic systems.^{6,7} For example, systemic administration of MA enhances endorphin

Received: June 21, 2015; Revised: July 03, 2015;
Accepted: July 13 2015

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neurotransmission and expression of opioid peptide mRNA in the NAc.⁸ In addition to DA pathways, a number of investigators provided significant evidence for the interaction between the central 5-HTergic and opioidergic systems.^{9,10} Indeed, 5-HT release in the NAc was modified when the NAc was receiving projections from the dorsal raphe nucleus after systemic morphine administration in rats.¹¹

In the present study, we investigated and characterized the signs and symptoms of combined MA and morphine abuse in the ED. Decoding the interactions between MA and morphine relating to brain neurotransmission may unveil the mechanisms leading to the high prevalence of polydrug abuse among humans. We hypothesize that the mesolimbic pathway plays a critical role in the reinforcement of drug abuse, and the NAc is a crucial brain region associated with drug reward. Therefore, we applied brain microdialysis to investigate alterations of individual and combined administration of MA and morphine in mice. Furthermore, we characterized their combined effects on extracellular levels of DA and 5-HT in the mouse NAc shell.

MATERIALS AND METHODS

Selection of patients

The Committee on Human Research for Tri-Service General Hospital approved this observational study (TSGHIRB: 1-103-05-103). We retrospectively reviewed the medical records of patients from January 2011 to December 2013 who were consecutively registered to Military Poison Control Center and treated for combined MA and morphine abuse in the ED of a university-affiliated teaching hospital.

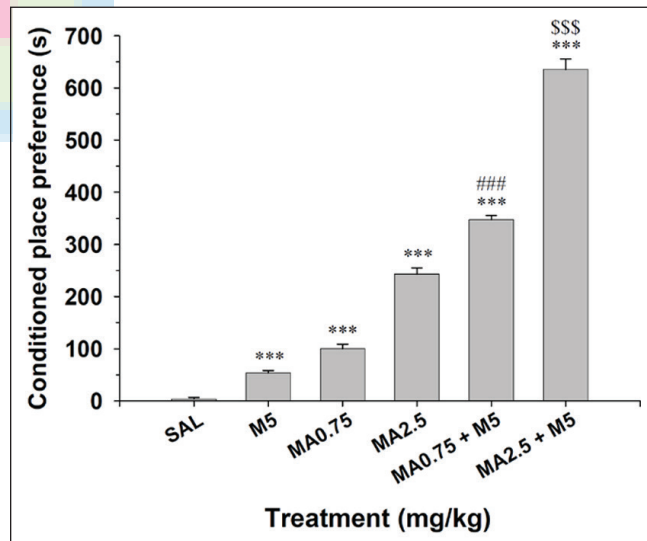
The diagnosis of combined MA and morphine abuse was based on patients' significant history, clinical features, and toxicological confirmation of the drugs in their urine. Urine samples were collected on ED arrival and then submitted for preliminary drug screening using an enzyme-multiplied immunoassay technique. The same samples with positive findings of amphetamines and opiates were further confirmed with gas chromatography/mass spectrometry (HP 5890, 5971A MSD, Hewlett-Packard Co., Palo Alto, CA, USA). Data were

collected, including gender, age, route of exposure, signs and symptoms, physical findings, laboratory abnormalities, and associated complications.

Animals and drug treatments

All procedures for animal care were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals and were approved by the Animal Research Committee of National Taiwan University College of Medicine. The obtained male Institute of Cancer Research mice (an albino mouse strain stock originally established in the ICR), weighing 30–35 g from in the Animal Center of National Taiwan University. Mice were maintained in an animal room with a 12-h light/dark cycle and at a constant temperature ($22 \pm 2^\circ\text{C}$) with food and water available *ad libitum*.

MA hydrochloride and morphine hydrochloride were purchased from the National Bureau of Controlled Drugs in Taipei, Taiwan, ROC. In this study, dosing of 0.75 mg/kg and 2.5 mg/kg of MA (MA0.75 and MA2.5, respectively) and the dose of 5 mg/kg of morphine (M5) were chosen on the basis of our behavioral experiments (appendices) in which their combination proved to induce conditioned place preference [Supplementary Figure S1]. Mice ($n = 6$ for each group) were



Supplementary Figure S1: Enhancing effects of morphine on methamphetamine-induced conditioned place preference (CPP) in mice. Methamphetamine (0.75 and 2.5 mg/kg) and morphine (5 mg/kg, M5) either alone or in combination (MA0.75 + M5 and MA2.5 + M5) was intraperitoneally administered to mice once daily for 5 consecutive days. Conditioned place preference was tested on the 6th day. Each experimental group included eight mice. Data are presented as mean $\pm$ standard error of mean calculated as the total mean time of conditioned place preference (postconditioning minus preconditioning) in a period of 15 min (900 s) as described in methods. The significance of differences between treatments was determined by one-way ANOVA followed by Dunnett's test, *** $P < 0.001$ versus saline (SAL); ### $P < 0.001$ versus MA0.75; SSS $P < 0.001$ versus MA2.5

Table 1: Prevalence of combined MA and M abuse in Taiwan

Drug	Year			
	2000	2001	2002	2003
MA, <i>n</i> (%)	40,882 (56.2)	19,141 (40.5)	9252 (22.1)	7448 (23.3)
M, <i>n</i> (%)	6396 (9.7)	7806 (17.3)	10,276 (24.6)	9685 (30.1)
MA+M, <i>n</i> (%)	9531 (14.5)	8368 (18.5)	6489 (15.5)	6415 (19.9)

Epidemiological data of positive drug screens of urine samples reported by the National Bureau of Controlled Drugs in Taiwan from 2000 to 2003. Website: <http://www.nbcd.gov.tw>. MA = Methamphetamine; M = Morphine

Effects of combined methamphetamine with morphine

given MA or morphine alone or in combination once a day for 5 consecutive days. MA and morphine were dissolved in physiological-saline (0.9% NaCl) and administered intraperitoneally (i.p.) to mice with separate syringes at the same time on test days. Control mice received 10 ml/kg i.p. injections of physiological saline.

Surgical procedures and microdialysis study

A guide cannula was implanted into each test animal's left NAc shell 5 days prior to drug administration [Figure 1]. The coordinates for the implantation relative to the bregma were as follows: Anteriorly 1.4 mm, and laterally 0.7 mm and 3.6 mm below the dorsal skull surface [Supplementary Figure S2].¹² After repeated administration of drugs or saline, microdialysis procedures, and high-performance liquid chromatography analyses were conducted according to the method reported by our laboratory previously.¹ A freshly calibrated microdialysis probe (CMA/7, membrane length = 1 mm) was placed into the implanted guide cannula. The collection of consecutive 20 min dialysate samples for the determination of basal values of DA and 5-HT began after equilibration for a period of 2-3 h. Following a collection of three basal dialysate samples (with no more than 10% inter-sample concentration variation), a single i.p. injection of drugs or saline was administered to the mice, and consecutive 20 min dialysate samples were again collected for the next 120 min.

Statistical analysis

All values were expressed as means $\pm$ standard error of means and statistical analyses were performed using SPSS for Windows (Version 20, SPSS Inc., Chicago, IL, USA). The changes in dialysate concentrations of NAc DA and 5-HT subsequent to the administration of MA and morphine alone or together were expressed as a proportion (percentage) of the corresponding basal value for each mouse. We examined the differences in the microdialysate levels of DA and 5-HT in the NAc shell for six treatment groups during a 120 min perfusion period using repeated measures analysis of variance (ANOVA). These statistical analyses were followed by *post-*

hoc multiple comparisons using Dunnett's test. A value of $P < 0.05$ was considered to indicate a statistically significant difference between data sets.

RESULTS

Patients data analysis

Of 2580 total urine drug screens in the ED, 387 (15%) were positive for MA and of those, 89 (23%) were positive for morphine. ED physicians treated 88 patients for acute intoxication of combined MA and morphine. We excluded 16 patients because alcohol was also detected in their blood. Subsequently, 72 typical patients with combined MA and morphine abuse were collected during a 3-year period. Forty-four patients (61%) had a cigarette smoking habit. Several routes of administration of MA and morphine were reported by the patient, including oral ingestion (51/72, 71%), nasal snorting (11/72, 15%), intravenous injection (6/72, 8%), or a combination of these (4/72, 6%). There were 46 men and 26 women, with a mean age of 26.8 years (range: 20-52 years).

The patients most often manifested neuropsychiatric signs and symptoms due to drug abuse with a combination of MA and morphine. These signs and symptoms included confusion (66/72, 92%), anxiety (66/72, 92%), delirium (65/72, 90%), and insomnia (62/72, 86%), which were observed in over 80% of the patients [Table 2]. In addition, the majority of the patients (43/72, 60%) with insomnia persisted to abuse with a combination of MA and morphine more than 3 months. These patients experienced tachypnea (49/72, 68%), tachycardia (48/72, 67%), and diaphoresis (47/72, 65%), which occurred in over 60% of them in our study. Eighteen of 72 patients (25%) with acute intoxication had serious complications related to

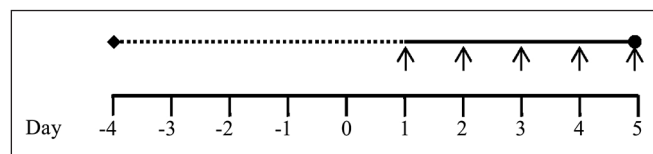
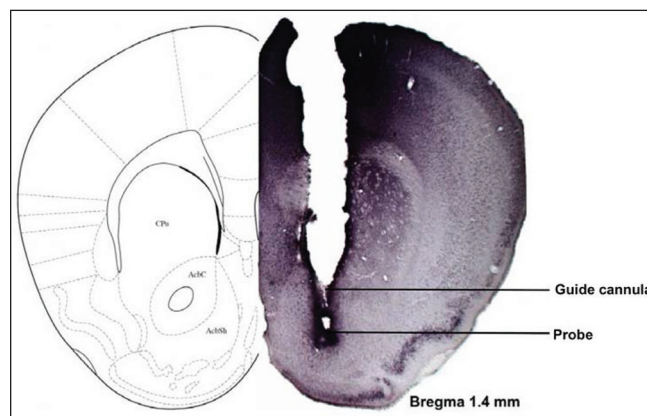


Figure 1: Time schedule for surgery, drug administration and high-performance liquid chromatography analyses. Mice were injected with methamphetamine at either 0.75 or 2.5 mg/kg and morphine at 5 mg/kg alone or in combination once a day for 5 consecutive days. Surgical implantation of the guide cannula was performed five days prior to drug administration; surgery (♦), dosage (↑) and high-performance liquid chromatography analysis (•)



Supplementary Figure S2: Schematic showing the location of the probe in the left nucleus accumbens of a mouse. The right planes are taken from the atlas of Paxinos and Franklin (2001) and coded according to the anterior distance (mm) from the bregma. AcbC: Accumbens core; AcbSh: Accumbens shell; CPu: Caudate putamen

combined MA and morphine abuse such as acute pulmonary edema, myocardial ischemia, seizure, coma, rhabdomyolysis, and acute renal failure; these patients were admitted to Intensive Care Unit of the hospital. The laboratory findings of these critically ill patients always showed leukocytosis (white blood cell count > 11000/ μ l), high serum concentrations of aspartate transaminase, creatine kinase, and troponin-I, and low values of arterial pH and bicarbonate. Five patients (7%) died of respiratory compromise and circulatory instability, and the predominant cause of death was multiple organs failure.

Effects of methamphetamine and morphine on the dialysate levels of dopamine in the nucleus accumbens of mice

There were no significant differences among the experimental groups of mice in the basal concentrations (mean of three individual samples) of DA in the NAc prior to the final injection of drugs or saline [Table 3]. The repeated measures ANOVA model revealed a main effect of treatment on DA levels (treatment: $F(5,30) = 170.48$, $P < 0.001$) and an interaction between treatment and every 20 min period (treatment $\times$ time: $F(25,150) = 34.22$, $P < 0.001$). A *post-hoc* analysis revealed that repeated administration of combined MA and morphine significantly increased DA levels in the NAc compared with repeated administration of MA or morphine alone [Figure 2]. Treatment with MA produced a dose and time-dependent increase in DA levels compared with saline controls. The time points of maximal impact of treatment with MA in combination with M5 on DA levels were quite similar to MA alone during the 40 min and 60 min perfusion periods. Treatment with M5 did not produce significant differences in DA levels compared to the saline controls.

Effects of methamphetamine and morphine on the dialysate levels of 5-hydroxytryptamine in the nucleus accumbens of mice

The basal concentrations of 5-HT in the NAc did not

significantly differ among the experimental groups [Table 3]. The repeated measures ANOVA model revealed a main effect of treatment on 5-HT levels (treatment: $F(5,30) = 54.64$, $P < 0.001$) and an interaction between treatment and every 20 min period (treatment $\times$ time: $F(25,150) = 37.78$, $P < 0.001$). A *post-hoc* analysis revealed that repeated administration of combined MA and morphine significantly increased 5-HT levels in the NAc compared with repeated administration of MA or morphine alone [Figure 3]. Treatment with MA produced a dose and time-dependent increase in

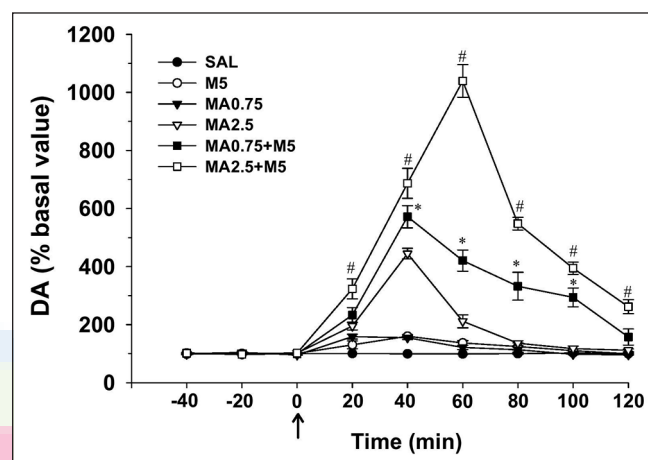


Figure 2: Time course of changes in the release of dopamine in the dialysate of the nucleus accumbens after repeated administration of methamphetamine, morphine or their combination in mice. The regimens of pretreatment with methamphetamine (0.75 mg/kg/day, MA0.75 and 2.5 mg/kg/day, MA2.5), morphine (5 mg/kg/day, M5) or their combination (MA0.75 + M5 and MA2.5 + M5) are described in the methods. The drugs were administered on day 5 as indicated by the arrow. Dialysate levels of dopamine in the nucleus accumbens were measured once every 20 min prior to and after treatment with drugs or saline. The combination of methamphetamine and morphine significantly increased dopamine levels compared with methamphetamine or morphine alone. Each experimental group included six mice. The data are expressed as a percentage (mean $\pm$ standard error of mean) of the respective basal values. Repeated measures analysis of variance followed by Dunnett's test was used to analyze the significance of differences between treatments during the 120 min perfusion period in levels of dopamine (MA0.75 + M5 vs. MA0.75, * $P < 0.001$; MA2.5 + M5 vs. MA2.5, * $P < 0.001$).

Table 2: Clinical features and complications of combined MA and M abuse

Respiratory	Cardiovascular	Neuropsychiatric	Gastrointestinal	Skin and others
Tachypnea ^a	Tachydysrhythmia ^a	Confusion*	Abdominal pain	Diaphoresis ^a
Bradypnea	Bradycardia	Anxiety*	Nausea	Hyperthermia
Wheezing	Chest pain	Delirium*	Vomiting	Cyanosis
Crackles	Hypertension	Insomnia*		Rhabdomyolysis
Pulmonary edema	Myocardial ischemia	Seizure		Renal failure
Respiratory arrest	Cardiac arrest	Coma		

* $>80\%$ and $>60\%$ of study patients, respectively. Seventy-two patients with combined MA and M abuse were collected during a 3-year period, and their medical records were reviewed. Patients with acute intoxication had positive urine samples for the combination of MA and M in the Emergency Department. An abuser may visit the Emergency Department several times and have different signs and symptoms at different times in their clinical course. MA = Methamphetamine; M = Morphine

Effects of combined methamphetamine with morphine

Table 3: Basal values (pg/20 μ L) of the concentrations of DA and 5-HT prior to the administration of MA, M, and combination of MA and M measured in the nucleus accumbens of mice

Group	DA	5-HT
SAL	0.42 $\pm$ 0.01	0.07 $\pm$ 0.01
M5	0.43 $\pm$ 0.01	0.05 $\pm$ 0.01
MA0.75	0.43 $\pm$ 0.01	0.05 $\pm$ 0.01
MA2.5	0.42 $\pm$ 0.01	0.06 $\pm$ 0.01
MA0.75+M5	0.42 $\pm$ 0.01	0.06 $\pm$ 0.01
MA2.5+M5	0.41 $\pm$ 0.01	0.06 $\pm$ 0.01

SAL = Physiological saline; M5 = Morphine (5 mg/kg/day); MA0.75 = Methamphetamine (0.75 mg/kg/day); MA2.5 = Methamphetamine (2.5 mg/kg/day); MA0.75 + M5 = Methamphetamine (0.75 mg/kg/day) in combination with morphine (5 mg/kg/day); MA2.5 + M5 = Methamphetamine (2.5 mg/kg/day) in combination with morphine (5 mg/kg/day). The regimens of drug administration are described in methods. Representative data for basal concentrations (mean of three individual samples) of extracellular dialysate measured prior to the final injection of drugs or saline. Data are presented as means $\pm$ SEMs. Each experimental group included six mice. The data reveal that the differences between drug treatment and the control saline are statistically nonsignificant (one-way ANOVA). DA = Dopamine; 5-HT = 5-hydroxytryptamine; SEM = Standard error of mean

5-HT levels compared with saline controls. The time points of maximal impact of treatment with MA in combination with M5 on 5-HT levels were quite similar to MA alone during the 40 min and 60 min perfusion periods. Treatment with M5 did not produce significant differences in 5-HT levels compared with saline controls.

DISCUSSION

The clinical presentation of MA and morphine is reported in several phenomenological surveys of abused drugs, but it is a serious problem to recognize the characteristic signs and symptoms of co-administration of MA with morphine. The drug effects of MA in combination with morphine apparently differ from those of either drug alone. For example, most patients with a combination of MA and morphine had normal pupil size and did not exhibit mydriasis typical of MA abuse or pinpoint pupils typical of morphine abuse. In addition, the physical findings did not show psychomotor hyperactivity as with MA abuse or analgesic sedation as with morphine abuse in this study. Instead, our patients with combination of MA and morphine manifested with increased neuropsychiatric toxicity in the CNS and catecholamine hyperstimulation in the respiratory, cardiovascular, and peripheral nervous systems including seizure, tachypnea, tachycardia, hypertension, and diaphoresis. In the ED, observations of clinical features are more relevant to the severity of drug intoxication than

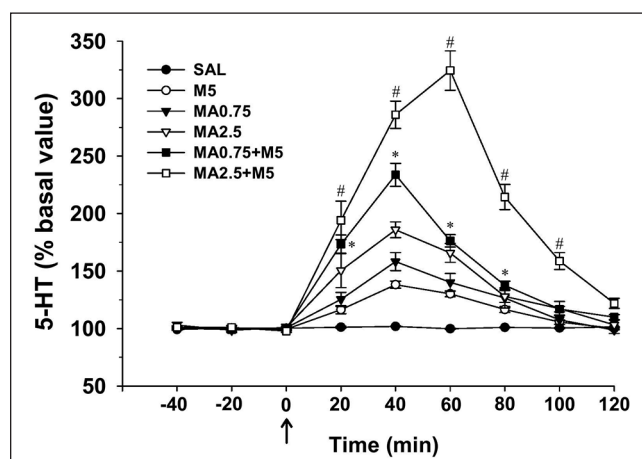


Figure 3: Time course of changes in the release of 5-hydroxytryptamine in the nucleus accumbens dialysate after repeated administration of methamphetamine, morphine or their combination in mice. The regimens of pretreatment with methamphetamine (0.75 mg/kg/day, MA0.75 and 2.5 mg/kg/day, MA2.5), morphine (5 mg/kg/day, M5) or their combination (MA0.75 + M5 and MA2.5 + M5) are as described in Figure 1. The administration of the respective drugs was given on day 5 as indicated by an arrow. Dialysate levels of 5-hydroxytryptamine in the nucleus accumbens were measured once every 20 min prior to and after treatment with drugs or saline. The combination of Methamphetamine and morphine significantly increased 5-hydroxytryptamine levels compared with methamphetamine or morphine alone. Each experimental group included six mice. Data are expressed as a percentage (mean $\pm$ standard error of mean) of the respective basal values. Repeated measures analysis of variance followed by Dunnett's test was used to analyze the significance of differences between treatments during the 120 min perfusion period in levels of 5-hydroxytryptamine (MA0.75 + M5 vs. MA0.75, * P < 0.01; MA2.5 + M5 vs. MA2.5, * P < 0.001)

estimates of abused doses because repeated administration of the drugs results in tachyphylaxis.¹³

Approximately, 25% of the study patients experienced serious complications related to acute intoxication with a combination of MA and morphine including acute pulmonary edema, myocardial ischemia, coma, rhabdomyolysis, and acute renal failure. In fact, these complications were also observed in ED patients with high doses of acute MA intoxication resulting in fatal outcomes.¹⁴ According to our preliminary study in ICR mice, systemic co-administration of MA and morphine (10 mg/kg) was more toxic than administration of MA alone (LD₅₀: 22.2 mg/kg vs. 49.2 mg/kg). This observation is consistent with previous a report showing that systemic administration of MA produced greater toxicity for morphine-dependent mice than for saline-treated mice.¹⁵

In the mouse model, the combined effects of MA and morphine are mediated by potentiating the release of DA and 5-HT in the mesolimbic system of the brain, especially as it relates to polydrug abuse. It is very interesting to research why patients favor to abuse combination of MA and morphine than either drug alone. Of particular importance is the high prevalence

APPENDICES

Conditioned place preference test

Place conditioning studies were conducted using an apparatus, consisting two identically sized compartments (30 cm × 25 cm × 25 cm) at both ends, which was separated by a narrower compartment (10 cm × 25 cm × 25 cm). Guillotine doors connected the compartments (10 cm × 10 cm) to the central unit. Mosaic tape covered one of the large compartments (2.5 cm × 2.5 cm black and white squares) on three of the walls, and the other large compartment was covered with purely white tape to provide visual cues. Additional visual cues were provided by a blue light bulb, and a red light bulb hung separately above the two large compartments. During the experiments, the conditioned place preference (CPP) apparatus was kept in an isolated dark room which was free from noise. The mice were placed into the central area of the apparatus and allowed free access to each compartment for 15 min (900 s). After each place conditioning, the whole apparatus was thoroughly cleaned to prevent interference from the scent of feces and urine. The preconditioning time spent by the animal in each compartment was considered to measure the extent of baseline place preference (day 0). Animals showing a strong unconditioned aversion (<33% of the session time, i.e., 300 s) or preference (more than 67%, i.e., 600 s) for any compartment were excluded. Six out of the total 54 mice were discarded on the basis of preexisting aversion (three) or preference (another three) before drug conditioning. The remaining 48 mice were randomly divided into two sets of 24 mice.

The first set of 24 mice, divided into six groups (SAL, M5, MA0.75, MA2.5, MA0.75 + M5 and MA2.5 + M5), were drug-conditioned in the morning and saline-conditioned in the afternoon once daily for 5 consecutive days (days 1, 2, 3, 4, and 5). The mice were kept for 40 min in the drug-association compartment with the guillotine doors closed promptly after drug administration to ensure recognition of visual cues (i.e., illumination differences). After an interval of 4 h, saline injections were given, and the mice were placed in the saline-association compartment. After the mice had been conditioned, their post-CPP was measured and recorded for 15 min on day 6. Another 24 mice were reversely drug-conditioned in the different compartments at the same time. The time difference in the drug-paired compartments between postconditioning and preconditioning represented the degree of conditioning induced by the drugs. If this difference was positive then the drug was considered to have induced a preference for the drug-paired compartment, while the opposite indicated the induction of an aversion. Mice in the control group were injected with saline.

Surgical procedures and microdialysis study

A guide cannula was implanted into the test animal's left nucleus accumbens shell (NAc) 5 days prior to drug administration. The mice were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally) and then placed in a stereotaxic apparatus (Stoelting Co., Wood Dale, IN, USA). The coordinates for the implantation relative to the bregma were as follows: Anteriorly 1.4 mm, laterally 0.7 mm and 3.6 mm below the dorsal skull surface [Supplementary Figure S2]. Dental cement (Durelon; 3M ESPE AG, Seefeld, Germany) was applied to the surface of the skull. On completion of the experimental sequence, the brain was removed, and the probe track was verified histologically on serial coronal sections (50 µm). Only data obtained from mice with correctly implanted probes into the shell of the NAc were included in the results.

After repeated administration of drugs or saline, microdialysis procedures and high-performance liquid chromatography (HPLC) analyses were conducted according to the method reported by our laboratory previously. A freshly calibrated microdialysis probe (CMA/7, membrane length = 1 mm) was placed into the implanted guide cannula. The microdialysis probe was then perfused with artificial cerebrospinal fluid (pH 6.0, purchased from CMA Microdialysis AB, Solna, Sweden) containing: NaCl-147 mmol/L, KCl-2.7 mmol/L, CaCl₂-1.2 mmol/L, and MgCl₂-0.85 mmol/L at a flow rate of 1.2 µl/min. The collection of consecutive 20-min dialysate samples for the determination of basal values of DA and 5-hydroxytryptamine (5-HT) began after equilibration for a period of 2-3 h. Following collection of three basal dialysate samples (with no more than 10% of the inter-sample variation of concentration), a single intraperitoneally injection of drugs or saline was administered to the mice, and consecutive 20-min dialysate samples were collected for the next 120 min.

DA and 5-HT levels were measured in 20 µl of dialysate by HPLC (HTEC500, Eicom, Kyoto, Japan) coupled with an Eicompack CAX cation exchange column (length = 200 mm; i.d. = 2 mm) and a graphite working electrode set at +450 mV versus an Ag/AgCl reference electrode. The mobile phase for HPLC consisted of 300 ml methanol, 0.1 M of acetate ammonium, 0.1 M of acetic acid, 50 mg of EDTA-2Na, and 7.1 g sodium sulfate in 1 L double-distilled water (pH 6.0). Peak detection limits were approximately 50-100 fg/sample with signal-to-noise ratios of at least 2. The peak levels of DA and 5-HT were verified by retention time, peak shape, and comparison of samples with standards consisting of each chemical.

of drug abuse of combined MA and morphine among polydrug addicts; whether DAergic and 5-HTergic neurotransmission in the NAc play critical roles in addiction to co-administered MA with morphine still awaits elucidation. A mixture of equal amounts of amphetamine and morphine with a dose of 12.5 or 20 mg in 70 kg male adults enhanced the abuse liability, which was greater than that of either drug alone.² Similarly, several behavioral studies in mice have demonstrated that the effects of combined MA and morphine on conditioned rewarding effects

and locomotor activity exhibited a synergistic interaction and are seen at low-dose combinations.^{1,16} The neurochemical effect of combined MA and morphine leading to increased levels of DA and 5-HT in the NAc was expected by either additive or synergistic interactions. The mesolimbic DA system in the CNS is thought to play a prominent role in the development of drug reward and is strongly modulated by other neurotransmitter systems, including the opioidergic, glutamatergic, and γ -aminobutyric acid (GABA-ergic) systems. The most possible

Effects of combined methamphetamine with morphine

neural mechanism by which morphine enhances MA-induced DA release in the NAc is by dis-inhibition of DA neurons in the ventral tegmental area. That is, μ -OR stimulation inhibited local GABA-ergic interneurons,¹⁷ resulting in subsequent increase in DA outflow in the NAc. There are many factors that affect the extracellular concentrations of DA and 5-HT induced by morphine including genetic factors, doses of drugs, interval between injections, and the duration of drug administration.^{18,19} Although behavioral interaction between MA and morphine produced the greatest effect at 5 mg/kg of morphine in combination with MA,²⁰ morphine itself elicited not apparently increased dialysate concentrations of accumbal DA and 5-HT in this study. The drug effects of a broad dosage range on the interaction between MA and morphine remains to be evaluated.

CONCLUSION

The present study provides an important basis for understanding the major toxidromes of combined MA and morphine abuse in the ED. Our results demonstrated that the co-administration of MA with morphine not only resulted in significantly increased levels of DA in the NAc, but also produced profoundly increased 5-HT release in the NAc compared to the corresponding doses of MA in a dose-dependent manner. Our findings support the hypothesis that opioidergic systems participate in the potentiation of the effects of MA on brain DA and 5-HT release.

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