

Research paper

Pharmacodynamic interactions between MDMA and concomitants in MDMA tablets
on extracellular dopamine and serotonin in the rat brain

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Abstract

3,4-Methylenedioxymethamphetamine (MDMA) is a psychoactive stimulant abused by young people as the recreational drug ecstasy. Other compounds, either deliberately added or present as byproducts, are often found in MDMA tablets and can unexpectedly interact with each other. The aim of this study was to evaluate the pharmacodynamic effects of interactions caused by concomitants in MDMA tablets on extracellular dopamine and serotonin (5-HT) by microdialysis in the striatum of ethylcarbamate-anesthetized rats.

Baseline levels of dopamine and 5-HT in the striatum were 16.5 ± 7.7 and 3.5 ± 1.7 nM (mean \pm standard deviation), respectively. After a single administration of MDMA (10 mg/kg, *i.p.*), a dramatic increase in extracellular dopamine (C_{max} : 36.1-fold vs. baseline) and 5-HT levels (C_{max} : 9.3-fold vs. baseline) was observed. When rats were co-administered with methamphetamine (1, 5 or 10 mg/kg) with MDMA, the dopamine levels induced by MDMA increased in a methamphetamine-dose-dependent manner (C_{max} : 2.5-, 3.5-, and 3.8-fold vs. MDMA). A similar trend was observed in 5-HT levels (C_{max} : 1.1-, 1.3-, and 1.8-fold vs. MDMA). In contrast, ketamine and caffeine showed synergistic effects on the monoamine levels induced by MDMA, whereas the individual administration of either of these compounds did not affect monoamine levels. Ketamine (1, 5 mg/kg) decreased the dopamine levels induced by MDMA (C_{max} : 0.9- and 0.7-fold vs. MDMA) and increased the 5-HT levels induced by MDMA (C_{max} : 1.4- and 1.6-fold vs. MDMA), and co-administration of caffeine (20

mg/kg) with MDMA increased dopamine levels (C_{max} : 1.7-fold vs. MDMA). These results suggest that exposure to multiple drugs in addition to MDMA can have neurotoxic effects.

249 words

Keywords: 3,4-methylenedioxymethamphetamine, methamphetamine, ketamine, caffeine, dopamine, serotonin, interaction

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA, “ecstasy”) is a designer drug with pharmacological and toxicological characteristics similar to amphetamines and mescaline as a central nervous system (CNS) stimulant and hallucinogen, respectively (Duterte et al., 2009; Kalant, 2001). MDMA was popular with “rave” party participants in the early 1990s (Wu et al., 2006). Its present illegal use is spreading rapidly among youth in the U.S. (Banken, 2004) and East Asian countries (Ahmad, 2002) and causes serious social problems.

MDMA is a potent releaser and/or reuptake inhibitor of presynaptic serotonin (5-HT), dopamine, noradrenaline and acetylcholine (Cole and Sumnall, 2003). It is also a substrate for monoamine transporter, and acts as an inhibitor of vesicular monoamine transporter and/or monoamine oxidase (Cole and Sumnall, 2003). Intake of MDMA results in increased extracellular monoamine levels, and induces intense feelings of euphoria, friendliness, comfort, intimacy, pleasure, empathy and hyperactivity (Parrot, 2001). Its acute physiological effects cause hyperthermia, rhabdomyolysis, acute renal failure, cardiac arrest, disseminated intravascular coagulation, liver failure, convulsions or cerebral hemorrhage, potentially resulting in death (Capela et al., 2009; Parrot, 2001). Moreover, long-term MDMA intake results in persistent neurotoxicity due to neurodegenerative changes (Escobedo et al., 2005). These physiological and psychological effects of MDMA abuse arise from changes in 5-HT and dopamine levels, so evaluation of monoamine levels in the brain after MDMA

intake might be an important pharmacodynamic parameter for elucidating the mechanisms by which MDMA causes neurotoxicity.

MDMA is commonly prepared as brightly-colored tablets imprinted with a friendly logo (Duterte et al., 2009; Makino et al., 2003) and is taken orally. This, and the widespread though erroneous belief that “ecstasy is safe”, have promoted the vogue for MDMA among youth (Eede et al., 2009; Kalant et al., 2001). Wide variations in the concentration of MDMA (1-207 mg/tablet) and the presence of several concomitants in seized tablets have been reported (Cole et al., 2002; Duterte et al., 2009; Makino et al., 2003; Teng et al., 2006; Vogels et al., 2009). For example, abused drugs (methamphetamine, amphetamine, ketamine and 3,4-methylenedioxyamphetamine (MDA)) and/or CNS-effective substances (caffeine, ephedrine, and dextromethorphan) have been detected in MDMA tablets. Furthermore, the percentage of MDMA tablets containing other substances is increasing (Tanner-Smith, 2006). When legal substances which affect the CNS are used as concomitants, a stronger stimulant effect may be obtained. Such MDMA tablets would therefore be extremely harmful and could result in the user unintentionally abusing multiple drugs, leading to enhanced adverse effects or poisoning.

This investigation aimed to evaluate the acute interaction effects caused by ingestion of MDMA tablets by studying dopamine and 5-HT levels in the rat brain after a single co-administration of MDMA with methamphetamine, ketamine or caffeine.

2. Materials and methods

2.1 Animals

Male Wistar rats (7 weeks old, 230-250 g, Kyudo Co. Ltd., Saga, Japan) were purchased and housed in plastic cages containing wood chip bedding material under standard environmental conditions (ambient temperature $22\pm 1^{\circ}\text{C}$, humidity $55\pm 5\%$, and 12 h light-dark cycle) with free access to laboratory diet (MD, Oriental Yeast, Tokyo, Japan) and tap water. Animals were used for experiments at 8 weeks of age and with weights of 280-320 g, considered to be the rat equivalent of human adolescence (Tsuruta et al., 2000). Each group consisted of three rats. All animal studies were approved by the Nagasaki University Animal Care and Use Committee.

2.2 Drugs and reagents

MDMA, a narcotic used for research, was kindly donated by the National Institute of Health Sciences (Tokyo, Japan). Methamphetamine hydrochloride (Philopon[®]) was purchased from Dainippon Pharma (Osaka, Japan). Anhydrous caffeine and dopamine were purchased from Wako Pure Chemicals (Osaka, Japan). Ketamine (Ketalar[®] for intramuscular injection) was purchased from Daiichi Sankyo (Tokyo, Japan). Sodium 1-decanesulfonate (SDS) was purchased from Sigma Aldrich Japan (Tokyo), and ethylenediamine-*N,N,N',N'*-tetraacetic acid disodium salt dihydrate (EDTA·2Na) was purchased from Dojindo Laboratories (Kumamoto, Japan). 5-HT was purchased from Kanto Chemical (Tokyo, Japan).

Dopamine and 5-HT were dissolved in 0.1 M HCl containing 100 mg/l EDTA·2Na to prepare 10 mM stock solutions, which were stored in a freezer to avoid decomposition. Artificial cerebrospinal fluid (aCSF) prepared with analytical grade reagents consisted of KCl 2.5 mM, NaCl 125 mM, MgCl·6H₂O 1.0 mM, NaH₂PO₄·2H₂O 0.5 mM, Na₂HPO₄·12 H₂O 2.5 mM and CaCl₂ 1.2 mM.

2.3 Experimental design

MDMA (5, 10, and 20 mg/kg), methamphetamine (1, 5, and 10 mg/kg), ketamine (5, 10, 20, and 40 mg/kg) or caffeine (20 mg/kg) in 0.9% saline was intraperitoneally administered to rats either individually or as combinations of two drugs at 0.1 ml per 100 g body weight. Saline alone was used for control experiments. For co-administration of two drugs, the second drug was administered immediately following administration of the first drug. According to interspecies dose scales (McCann and Ricaurte, 2001; Mehan et al., 2002), the dose of MDMA used in this study (10 mg/kg) is equivalent to a dose of 136 mg in a 70-kg human. Since the MDMA content of seized tablets ranges from 1 to 207 mg/tablet (Cole et al., 2002; Duterte et al., 2009; Makino et al., 2003; Teng et al., 2006; Vogels et al., 2009), a dose of 10 mg/kg is comparable to the ingestion of one or two tablets.

2.4 *In vivo* brain microdialysis

Rats were anesthetized with ethylcarbamate (1.5 g/kg, *i.p.*) and fixed on a stereotaxic

system (SR-5R, Narishige Scientific Instrument, Tokyo, Japan). A CMA microdialysis system (Carnegie Medicine, Stockholm, Sweden) was used. A MAB6 microdialysis probe with a 4-mm, 15-kDa cutoff polyethersulfone membrane (ALS/Microbiotech, Stockholm, Sweden) was implanted in the left striatum (coordinates: A, +0.6 mm; L, +3.0 mm from bregma; H, -0.7 mm from the skull surface; Paxinos and Watson, 2005) and was perfused with aCSF at a flow rate of 2.0 μ l/min.

To estimate baseline levels, samples were taken twice at 10-min intervals before drug administration, and then baseline samples were collected 60 min after implantation of the probe to avoid interference caused by the operation. After drug administration, samples were collected every 10 min for 3 h into a sampling tube containing 5 μ l of 0.1 M phosphate buffer, and 0.1 M EDTA·2Na, pH 3.5.

The *in vivo* recoveries of the dialysis probe ($R_{in\ vitro}$) were calculated from four replicate measurements of *in vitro* recoveries ($R_{in\ vitro}$), *in vitro* losses ($L_{in\ vitro}$), and *in vivo* losses ($L_{in\ vivo}$), according to previous reports (Sun et al., 2002; Wada et al., 2008). For these calculations, aCSF spiked with dopamine (25 nM) and 5-HT (25 nM) was used. The relative $R_{in\ vitro}$, $L_{in\ vitro}$ and $L_{in\ vivo}$ values were calculated to be 8.5%, 96.6% and 96.9% for dopamine, and 14.4%, 95.0% and 99.1% for 5-HT, respectively. The percent *in vivo* recoveries for dopamine and 5-HT were estimated to be 8.5% and 15.0%, respectively.

2.5 HPLC-electrochemical detection

The dopamine and 5-HT levels in the brain were determined by HPLC using an HTEC-500 system (Eicom, Kyoto, Japan) equipped with electrochemical detection. Twenty microliters of sample was injected and analytes were separated on an Eicompak PP-ODS column (30 × 4.6 mm, i.d., 2 μm, Eicom) using a mixture of 0.1 M phosphate buffer (pH 6.0)/methanol (=99:1, v/v) containing 500 mg/l SDS and 50 mg/l EDTA·2Na. The mobile phase flow rate was 0.5 ml/min and the column temperature was set at 25°C. The working electrode was WE-3G (graphite, Eicom) and the reference electrode was RE-100 (Ag/AgCl, Eicom). The applied voltage of the conditioning cell was set at +400 mV.

2.6 Calculations and statistics

The dopamine and 5-HT levels in dialysates were calculated from calibration curves using the formulas: $Y = 5.99 \cdot X - 2.43$ (for dopamine; Y, peak area (mV·s); X, concentration of standard (nM)) and $Y = 13.06 \cdot X + 4.39$ (for 5-HT). The measured dopamine and 5-HT concentrations in dialysates were corrected for *in vivo* recovery, and the pharmacokinetic parameters of the amines were obtained with the corrected values.

All data are presented as mean ± standard error of mean (S.E.M., n = 3). The dopamine and 5-HT parameters were calculated by moment analysis (Yamaoka et al., 1978). The peak concentrations (C_{max}) and concentration peak times (T_{max}) were

obtained from the original data. The area under the curve (AUC) for concentration vs. time was calculated using the linear trapezoidal rule until 180 min after drug administration. The mean residence time (MRT) was calculated from the equation for area under the moment curve/AUC. The data for time-concentration profile of MDMA with its 10 mg/kg single administration in each figures were same to that in Fig. 1.

The profiles of dopamine and 5-HT levels vs. time were analyzed by a two-way mixed model analysis of variance (ANOVA) with a split plot design followed by Scheffe's post-test. The moment parameters obtained were analyzed by one-way ANOVA followed by Scheffe's post-test, and Student's *t*-test was used to compare two groups. A *P*-value less than 0.05 was considered to be statistically significant. Statistical calculations were done using IBM SPSS[®] Statistics 17.0 (SPSS Japan Inc., Tokyo, Japan).

3. Results

3.1 Pharmacodynamic effects of MDMA on dopamine and 5-HT levels in the brain

The baseline levels of dopamine and 5-HT were 16.5 ± 7.7 nM ($n = 48$) and 3.5 ± 1.7 nM ($n = 48$), respectively. Pre-treatment dopamine levels were relatively high for the MDMA 10 mg/kg group, and low for the ketamine 20 mg/kg administered group ($P = 0.016$, MDMA 10 mg/kg group vs. ketamine 20 mg/kg group, Scheffe's post-hoc test). No other significant differences in the pre-administration levels of dopamine and 5-HT among the administered groups were observed.

The profiles of extracellular dopamine and 5-HT concentrations vs. time after a single *i.p.* administration of saline or MDMA, and the moment parameters, are shown in Fig. 1 and Table 1, respectively.

Extracellular dopamine levels were significantly increased by administration of MDMA at a dose above 10 mg/kg compared to the saline group (5 mg/kg, $P = 0.603$; 10 mg/kg, $P = 0.046$; 20 mg/kg, $P = 0.007$; vs. saline group, Scheffe's post-hoc test). The C_{max} of dopamine was 23-, 36- and 65-fold compared to the baseline level (MDMA 5, 10 and 20 mg/kg, respectively). The AUC also increased dose-dependently compared to the saline group (5 mg/kg, $P = 0.598$; 10 mg/kg, $P = 0.047$; 20 mg/kg $P = 0.007$; vs. saline group, Scheffe's post-hoc test).

After MDMA administration, 5-HT levels in the rat striatum significantly increased. The AUC increased significantly from low dosage (5 mg/kg, $P = 0.003$; 10 mg/kg, $P = 0.002$; 20 mg/kg, $P < 0.001$; vs. saline group, Scheffe's post-hoc test), and it increased

non-linearly. In MDMA 20 mg/kg administration group, 5-HT levels were extremely increased and the C_{max} were 8-, 9- and 23-fold increased from baseline level (MDMA 5, 10 and 20 mg/kg, respectively).

Fig. 1, Table 1

3.2 Effect of methamphetamine on the pharmacodynamics of MDMA

The effects of methamphetamine co-administered with MDMA on extracellular dopamine and 5-HT concentrations are shown in Fig. 2 and Table 2.

Similar to an acute effect of MDMA, the extracellular levels of dopamine and 5-HT increased significantly compared to baseline levels after a single administration of methamphetamine (10 mg/kg). The C_{max} of the dopamine and 5-HT levels were increased 84.8- and 14.2-fold over the baseline level.

Methamphetamine co-administered with MDMA dose-dependently increased the extracellular dopamine level compared to the administration of MDMA alone (1 mg/kg, $P = 0.831$; 10 mg/kg, $P = 0.202$, 20 mg/kg, $P = 0.091$; vs. MDMA 10 mg/kg group, Scheffe's post-hoc test). The C_{max} and AUC of MDMA and methamphetamine co-administration ($2,265.2 \pm 157.8$ nM and $(201.5 \pm 14.5) \times 10^3$ nM·min in Table 2 for MDMA 10 mg/kg + methamphetamine 10 mg/kg) was 4 and 3 times higher than MDMA single administration in table 1. However, the amount of dopamine released (AUC 201.5×10^3 nM·min) by the co-administration of MDMA (10 mg/kg) and methamphetamine (10 mg/kg) was comparable to the theoretical sum of the amount of

dopamine released ($AUC\ 161.8 \times 10^3\ \text{nM} \cdot \text{min}$) calculated by the basal level and each drug caused an increase from each sole administration of MDMA or methamphetamine alone.

When rats were co-administered with methamphetamine with MDMA, the C_{max} of extracellular 5-HT increased in a methamphetamine dose-dependent manner. Although the C_{max} (MDMA 10 mg/kg + methamphetamine 10 mg/kg: $57.0 \pm 13.0\ \text{nM}$, Table 2) increased by a factor of two compared to the group administered MDMA alone ($32.5 \pm 4.0\ \text{nM}$, Table 1), the AUC was comparable to that of the group administered with methamphetamine alone.

[Fig. 2, Table 2](#)

3.3 Effect of ketamine on the pharmacodynamics of MDMA

The profiles of extracellular dopamine and 5-HT levels vs. time after each single administration of saline, ketamine, MDMA and/or MDMA + ketamine are shown in Fig. 3, and the corresponding parameters are given in Table 3. The administration of ketamine alone at any dose (5, 10, 20, and 40 mg/kg) did not affect the extracellular dopamine and 5-HT levels.

Co-administration of ketamine tended to dose-dependently decrease extracellular DA levels compared to the administration of MDMA alone (5 mg/kg, $P = 0.308$; 10 mg/kg, $P = 0.107$; ANOVA). However, ketamine increased the C_{max} and AUC of 5-HT induced by MDMA. C_{max} and AUC increased 1.6-fold and 1.8-fold compared to the

administration of 10 mg/kg MDMA alone ($P = 0.269$, $P = 0.078$; vs. MDMA 10 mg/kg group, Scheffe's post-hoc test).

When ketamine (20, 40 mg/kg) was co-administered with MDMA, the rats died 10-20 min after administration, but did not die when ketamine alone was administered.

Fig. 3, Table 3

3.4 Effect of caffeine on the pharmacodynamics of MDMA

The profiles of extracellular dopamine and 5-HT levels vs. time for each single administration of saline, caffeine, MDMA and/or MDMA + caffeine are shown in Fig. 4, and the corresponding parameters calculated by moment analysis are shown in Table 4.

Caffeine alone did not have any significant effect on dopamine and 5-HT levels compared to the group administered with saline alone. However, caffeine co-administered with MDMA significantly shortened the T_{max} of dopamine ($P = 0.038$, vs. MDMA 10 mg/kg group, Student's t -test) and increased dopamine levels in the striatum, and the C_{max} of dopamine was increased 2-fold compared to the MDMA group ($P = 0.526$, vs. MDMA 10 mg/kg group, Student's t -test). The T_{max} and MRT of dopamine were shortened to 25 min ($P = 0.184$, vs. MDMA 10 mg/kg group, Student's t -test) and 66 min ($P = 0.035$, vs. MDMA 10 mg/kg group, Student's t -test) by co-administration of caffeine with MDMA.

Fig. 4, Table 4

4. Discussion

We evaluated the effects of concomitants found in MDMA tablets on brain monoamine (dopamine and 5-HT) levels. To our knowledge, this is the first study of the pharmacodynamic interactions of MDMA with methamphetamine, ketamine and/or caffeine based on the monitoring of dopamine and 5-HT levels in rat striatum.

Although MDMA produces many adverse effects, hyperthermia is the most lethal. Hyperthermia is believed to be caused by multiple factors and to be involved in a complex fashion with serotonergic, dopaminergic and adrenergic neuronal functions (Capela et al., 2009). MDMA increases dopamine levels in the hypothalamic and other thermoregulatory areas (Benamar et al., 2008), and a direct relationship between core temperature and long-term loss of 5-HT induced by MDMA has been suggested (Goni-Allo et al., 2008). Dopamine and 5-HT are believed to be involved in the MDMA-induced acute physiological effect. The striatum is known to be a main target of both dopaminergic and serotonergic projections from the midbrain, so monitoring dopamine and 5-HT levels in the striatum using microdialysis sampling should be useful for assessing the risks caused by MDMA.

Analysis of seized MDMA tablets revealed the presence of several concomitants including methamphetamine, ketamine and caffeine. Methamphetamine is the most popular drug of abuse in Japan. Ketamine has an anesthetic pharmacological effect, whereas caffeine stimulates the CNS. All three compounds can be present in MDMA tablets in very large amounts (methamphetamine, 1-850%; ketamine, 7-74%; and

caffeine, 4-113% vs. MDMA content) (Makino et al., 2003; Teng et al., 2006). In this study, MDMA acutely increased extracellular dopamine and 5-HT levels compared to the saline control group. MDMA directly acts on monoamine transporter (MAT) at nerve terminal and could be a substrate for all the presynaptic MATs, including serotonin transporter (SERT), dopamine transporter and norepinephrine transporter. Of these, SERT has a lower affinity than the norepinephrine transporter, but a higher affinity than the dopamine transporter for MDMA, as shown for the rat (Rothman et al., 2001) and for the human protein (Piffl et al., 2005; Verrico et al., 2007). In addition, MDMA might indirectly affect dopamine levels via 5-HT release (Koch et al., 1997). Once 5-HT is released from the striatum, it binds to 5-HT_{2A/2C} receptor, thereby attenuating γ -aminobutylic acid (GABA) mediated feedback control at the substantia nigra, leading to dopamine release from the striatum (Yamamoto et al., 1995). This indirect effect might cause the observed differences in T_{max} between dopamine and 5-HT.

5-HT levels were significantly increased by 20 mg/kg administration of MDMA, and dopamine levels were increased in a MDMA dose-dependent manner. Several studies suggest a non-linear pharmacokinetic response to MDMA in human, monkey and rat (Kolbrich et al., 2008; de la Torre et al., 2000; Chu et al., 1996; Mueller et al., 2008). Consistent with this, MDMA overdosing (20 mg/kg) caused extreme enhancement of dopamine and 5-HT release in the current study, suggesting that the deleterious effects of MDMA overdosing are particularly focused on the brain neurological effects of

extracellular dopamine and 5-HT.

Once MDMA enters nerve terminal through SERT or by diffusion, it acts as 1) an enhancer of 5-HT release from storage vesicles, 2) an inhibitor of tryptophan hydroxylase, a 5-HT synthesis enzyme, and 3) an inhibitor of monoamine oxidase B (MAO-B), a 5-HT degradation enzyme; moreover, it acts as 4) an agonist to the 5-HT_{2A} receptor at the synapse (Capela et al., 2009).

There have been a few reports regarding the physical or pharmacological effects resulting from interactions caused by MDMA tablet intake or multi-drug abuse. Kuwayama et al. (2007) reported the effects of MDMA, ketamine and caffeine interactions on intestinal absorption by Caco-2 cells and suggested that a common transport system for MDMA, methamphetamine, ketamine and caffeine affects MDMA absorption. The effects of a combination of MDMA and methamphetamine on anxiety behavior and neurochemistry (monoamines measured following brain dissection) following a single administration (Clemens et al., 2004) and long-term administration (four times, Clemens et al., 2005; 10 weeks, Clemens et al., 2007) were reported and showed that drugs used in combination enhanced the adverse effects of a drug used alone. Ke et al. (2008) reported that the administration of ketamine 12 h before the administration of MDMA aggravated MDMA-induced dopaminergic toxicity, determined by measuring monoamine levels in brain dissection samples. Caffeine co-administrated with MDMA induced tachycardia (McNakamura et al., 2007); furthermore, caffeine promotes MDMA-induced toxicity such as

hyperthermia and increased 5-HT levels in brain tissue (McNakamura et al., 2006) and 5-HT₂ receptor down regulation (Camarasa et al., 2006). In a previous study, we investigated the pharmacokinetic interactions of MDMA with caffeine in rat brain and blood and found that caffeine decreased MDMA levels and prolonged MRT of MDMA in the brain (Tomita et al., 2007).

In this study, co-administration of methamphetamine enhanced the release of dopamine induced by MDMA, but did not affect the release of 5-HT significantly. Ketamine suppressed extracellular dopamine release induced by MDMA and accelerated 5-HT release, while ketamine alone had no effect on dopamine and 5-HT release. On the other hand, caffeine co-administration increased the dopamine levels and shortened the T_{max} of the dopamine levels, whereas caffeine alone had no effect on dopamine and 5-HT levels. These results suggest that methamphetamine, ketamine and caffeine might enhance the toxicity of MDMA.

Methamphetamine stimulates the CNS, and methamphetamine-induced neurotoxicity damages the striatal dopaminergic and serotonergic nerve terminal. Methamphetamine is a substrate for MAT and can more potently affect DAT than SERT (Rothman et al., 2001; Baumann et al., 2007). Co-administration of methamphetamine with MDMA caused additive dopamine release at the striatum in rats, indicating that each drug might independently diffuse into the brain and affect dopamine levels in neurons. On the other hand, extracellular 5-HT levels are believed to attain threshold levels by the administration of MDMA alone. Therefore, methamphetamine might not affect

MDMA-induced 5-HT levels additively. However, Clemens et al. (2004) reported that a combination of MDMA and methamphetamine enhanced dopamine and 5-HT depletion; therefore, the long-term effects and acute effects of methamphetamine may be different.

Ketamine is a noncompetitive antagonist of *N*-methyl-D-aspartate receptor, and has been used for anesthesia in animals. Verma and Moghaddam (1996) reported that ketamine increased dopamine release at the prefrontal cortex, but not at the striatum. These results are compatible with our results, in which ketamine alone did not affect dopamine and 5-HT levels in the rat striatum under ethyl carbamate anesthesia. On the other hand, our results showed that co-administration of ketamine produced the opposite effect on dopamine and 5-HT: ketamine decreased dopamine release induced by MDMA, but enhanced 5-HT release. This might be due to acute toxicity. However the effect of ketamine on monoamine decomposition involving monoamine oxidase or catechol *O*-methyltransferase remains unclear.

Caffeine, a psychoactive substance, is a nonselective adenosine receptor antagonist. Caffeine antagonizes adenosine A_{2A} receptor, which is thought to regulate dopamine signaling (Singh et al., 2009). In this study, caffeine had a synergistic effect on dopamine release induced by MDMA. Pharmacokinetic interactions should be also taken into consideration when drugs are co-administered. Amphetamine, an active metabolite of methamphetamine, is a substrate for CYP2D6 (Sweeney and Bromilow, 2006), as is MDMA. MDMA is metabolized through *N*-demethylation and

O-demethylation processes. MDMA is mainly *N*-demethylated by CYP1A2 in rodents, and caffeine is also metabolized by the same enzyme (Singh et al., 2009). Thus, competitive interactions between MDMA and caffeine for CYP should be considered. Tomita et al. (2007) reported that the T_{max} and MRT of MDMA (5 mg/kg, *i.p.*) in the brain were considerably prolonged by the co-administration of caffeine (20 mg/kg, *i.p.*) and that C_{max} was drastically decreased, while the C_{max} of MDMA in blood was not affected. Therefore, caffeine might affect the pharmacokinetics to the brain towards MDMA.

In addition to interactions involved with neurotoxicity, the physiological effects of MDMA have also been examined. Caffeine produced unexpected hyperthermia and tachycardia (McNakamura et al., 2006, 2007) when co-administered with MDMA. In our study, co-administration of ketamine (20 and 40 mg/kg) with MDMA was lethal, while the administration of ketamine or MDMA alone was not. These results show that the co-administration of ketamine not only has neurological effects, but also enhances the systemic physical toxicity of MDMA.

Increases in MDMA-induced dopamine and 5-HT release, and the neurotoxicity of 5-HT by MDMA, suggest the involvement of MDMA metabolites (Escobedo et al., 2005; Erives et al., 2008; Colad et al., 2004) and the formation of reactive oxygen species (Erives et al., 2008; Gudelsky and Yamamoto, 2008; Quinton and Yamamoto, 2006). Clarification of the mechanism of drug interactions with MDMA, especially with respect to metabolism and the formation of reactive oxygen species, will require

further study.

This study was performed in ethyl carbamate anesthetized rat. Some researches showed that ethyl carbamate would inhibit CYP3A and increase CYP 1E and 1A activity in rat (Loch et al., 1995; Meneguz et al., 1999). However, influence of extracellular dopamine in rat striatum by volatile anesthetic, halothane has been reported (Adachi et al., 2001), therefore, ethyl carbamate was selected. Further study in freely-moving microdialysis is needed to elucidate physiological effect by the interactions.

In conclusion, co-administration of methamphetamine, ketamine and caffeine with MDMA seriously affected MDMA-induced dopamine and 5-HT release in the rat striatum, indicating the potential risks associated with MDMA abuse.

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Figure captions

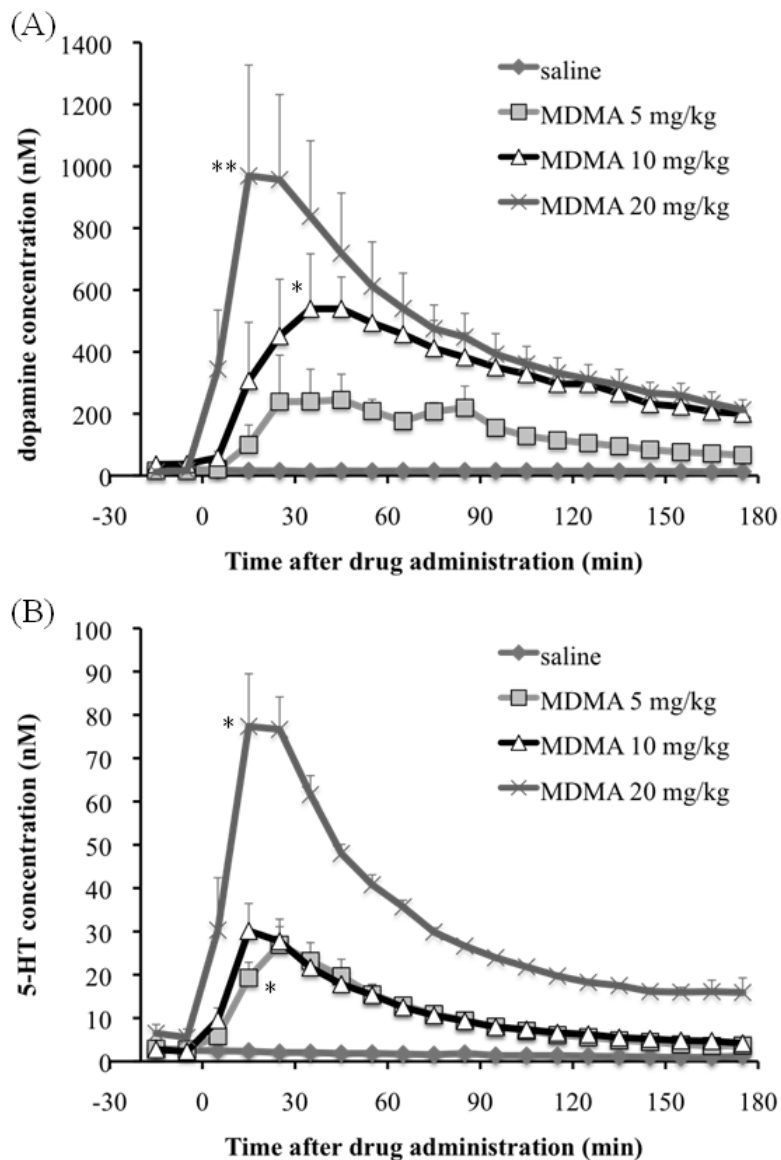


Fig. 1 Time-concentration profiles of extracellular dopamine (A) or 5-HT (B) after a single administration of saline or MDMA (5, 10, and 20 mg/kg, *i.p.*) as determined by microdialysis in ethylcarbamate-anesthetized rats.

Data represent the mean + S.E.M. (n = 3). *P*-values were calculated by two-way mixed mode ANOVA with Scheffe's post-hoc test for the saline group and the three MDMA-administered groups. ** *P* < 0.01, * *P* < 0.05 vs. saline group.

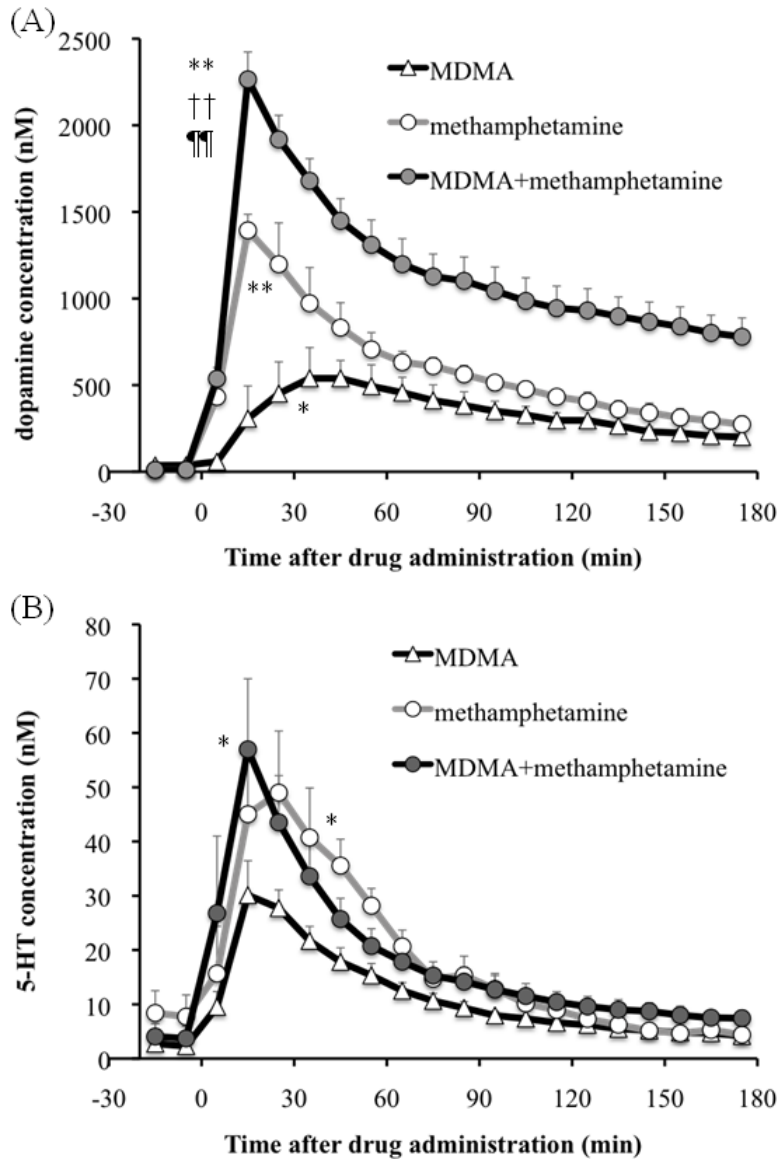


Fig. 2 Time-concentration profiles of extracellular dopamine (A) or 5-HT (B) after a single administration of MDMA (10 mg/kg, *i.p.*, Fig. 1), methamphetamine (10 mg/kg, *i.p.*) or MDMA (10 mg/kg, *i.p.*) + methamphetamine (10 mg/kg, *i.p.*) as determined by microdialysis in ethylcarbamate-anesthetized rats.

Data represent the mean + S.E.M. (n = 3). *P*-values were calculated by two-way mix mode ANOVA with Scheffe's post-hoc test. ** *P* < 0.01, * *P* < 0.05 vs. saline group; †† *P* < 0.01 vs. MDMA 10 mg/kg group; and ††† *P* < 0.01 vs. methamphetamine group.

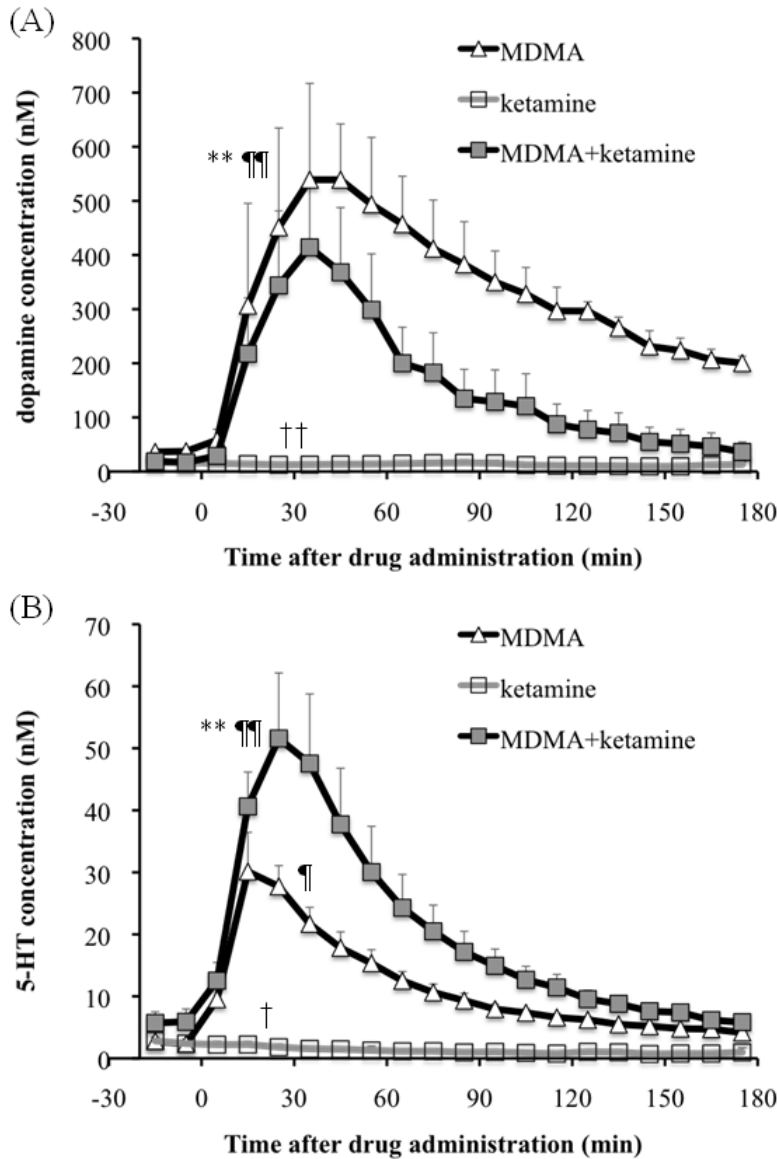


Fig. 3 Time-concentration profiles of extracellular dopamine (A) or 5-HT (B) after a single administration of saline, ketamine (10 mg/kg, *i.p.*), MDMA (10 mg/kg, *i.p.*) or MDMA (10 mg/kg, *i.p.*) + ketamine (10 mg/kg, *i.p.*) as determined by microdialysis in ethylcarbamate-anesthetized rats.

Data represent the mean + S.E.M. ($n = 3$). P -values were calculated by two-way mix mode ANOVA with Scheffe's post-hoc test. ** $P < 0.01$ vs. saline group; †† $P < 0.01$, † $P < 0.05$ vs. MDMA 10 mg/kg group; and ††† $P < 0.01$, † $P < 0.05$ vs. ketamine group.

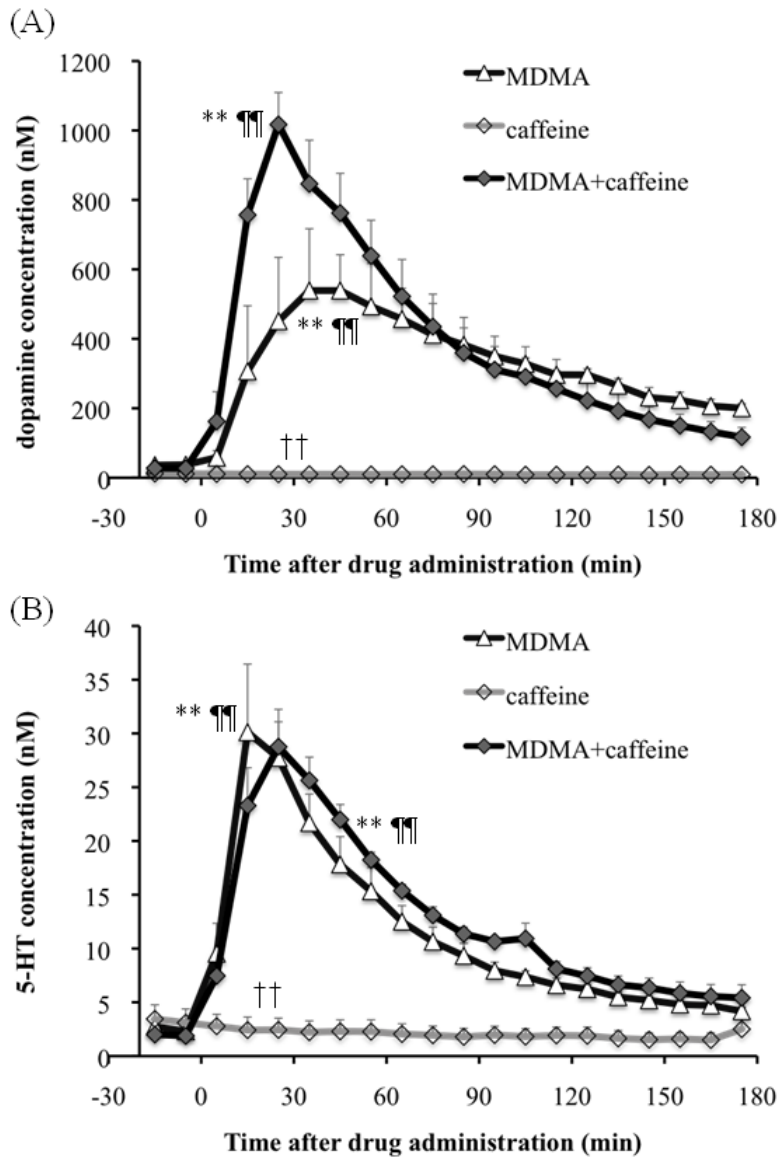


Fig. 4 Time-concentration profiles of the dopamine (A) or 5-HT (B) response after a single administration of saline, caffeine (20 mg/kg, *i.p.*), MDMA (10 mg/kg, *i.p.*) or MDMA (10 mg/kg, *i.p.*) + caffeine (20 mg/kg, *i.p.*) as determined by microdialysis in ethylcarbamate-anesthetized rats.

Data represent the mean + S.E.M. ($n = 3$). P -values were calculated by two-way mix mode ANOVA with Scheffe's post-hoc test. ** $P < 0.01$ vs. saline group; †† $P < 0.01$ MDMA 10 mg/kg group; and ¶¶ $P < 0.01$ vs. caffeine group.

Table 1 Moment parameters of dopamine and 5-HT levels after single administration of MDMA (5, 10, 20 mg/kg, *i.p.*).

	Saline ^a	MDMA			<i>P</i>
		5 mg/kg ^a	10 mg/kg ^b	20 mg/kg ^a	
dopamine					
<i>C_{max}</i> (nM)	–	372.3 ± 90.3	595.9 ± 151.6	1,065.2 ± 330.2	0.148 ^c
<i>T_{max}</i> (min)	–	55 ± 17	42 ± 3	22 ± 3	0.152 ^c
AUC (×10 ³ nM·min)	2.6 ± 0.3	25.1 ± 3.0	59.3 ± 12.2 ^d	83.8 ± 18.9 ^e	0.004 ^f
MRT (min)	(84 ± 3)	79 ± 9	82 ± 5	70 ± 5	0.495 ^c
5-HT					
<i>C_{max}</i> (nM)	–	26.9 ± 5.9	32.5 ± 4.0	82.0 ± 10.4	0.003 ^c
<i>T_{max}</i> (min)	–	25 ± 0	18 ± 3	22 ± 3	0.296 ^c
AUC (×10 ³ nM·min)	0.08 ± 0.0	1.9 ± 0.2 ^e	2.0 ± 0.1 ^e	5.8 ± 0.3 ^e	<0.001 ^f
MRT (min)	(72 ± 2)	63 ± 3	61 ± 3	64 ± 7	0.833 ^c

Data were represented as mean ± S.E.M.

^a Administration of saline or MDMA (5 and 20 mg/kg) to rats (n = 3).

^b Simultaneous administration of MDMA (10 mg/kg) and saline to rats (n = 3).

^c *P*-values were calculated by one-way ANOVA of three MDMA groups (5, 10, and 20 mg/kg).

^d *P* < 0.05, vs. saline group (Scheffe's post-hoc test).

^e *P* < 0.01, vs. saline group (Scheffe's post-hoc test).

^f *P*-values were calculated by one-way ANOVA of saline and MDMA groups (5, 10, and 20 mg/kg).

Table 2 Moment parameters of dopamine and 5-HT levels after single administration of methamphetamine and co-administration of MDMA with methamphetamine.

MDMA	–	10 mg/kg	10 mg/kg	10 mg/kg	<i>P</i>
methamphetamine	10 mg/kg ^a	1 mg/kg ^b	5 mg/kg ^b	10 mg/kg ^b	
dopamine					
<i>C_{max}</i> (nM)	1,399.7 ± 98.4	1,491.6 ± 262.7	2,061.6 ± 822.8	2,265.2 ± 157.8	0.096 ^c
<i>T_{max}</i> (min)	18 ± 3 ^f	25 ± 0 ^f	28 ± 3	15 ± 0 ^g	<0.001 ^c
AUC (×10 ³ nM·min)	105.1 ± 12.3	118.3 ± 20.8	178.8 ^a ± 63.3	201.5 ^e ± 14.5	0.004 ^d
MRT (min)	68 ± 1	67 ± 2	76 ± 5	76 ± 4	0.041 ^d
5-HT					
<i>C_{max}</i> (nM)	49.7 ± 11.2	36.9 ± 5.8	41.3 ± 8.9	57.0 ± 13.0	0.388 ^c
<i>T_{max}</i> (min)	28 ± 9	15 ± 0	22 ± 7	15 ± 0	0.382 ^c
AUC (×10 ³ nM·min)	3.4 ^e ± 0.8	2.3 ± 0.4	2.8 ± 0.3	3.3 ^e ± 0.7	0.005 ^d
MRT (min)	56 ± 1	58 ± 2	65 ± 4	60 ± 3	0.024 ^d

Data were represented as mean ± S.E.M.

^a Administration of methamphetamine (10 mg/kg) to rats (n = 3).

^b Simultaneous administrations of MDMA (10 mg/kg) and methamphetamine (1, 5 or 10 mg/kg) to rats (n = 3, respectively).

^c *P*-values were calculated by one-way ANOVA of methamphetamine (10 mg/kg), MDMA (10 mg/kg, Table 1), MDMA (10 mg/kg) + methamphetamine (1, 5, 10 mg/kg) groups.

^d *P*-values were calculated by one-way ANOVA of saline, methamphetamine (10 mg/kg), MDMA (10 mg/kg, Table 1), MDMA (10 mg/kg) + methamphetamine (1, 5, 10 mg/kg) groups.

^e *P* < 0.05, vs saline group (Scheffe's post-hoc test).

^f *P* < 0.05, ^g *P* < 0.01, vs MDMA (10 mg/kg, Table 1) sole-administration group (Scheffe's post-hoc test).

Table 3 Moment parameters of dopamine and 5-HT levels after single administration of ketamine and co-administration of MDMA and ketamine.

MDMA (mg/kg)	–	–	–	–	10	10	<i>P</i>
Ketamine (mg/kg)	5 ^a	10 ^a	20 ^a	40 ^a	5 ^b	10 ^b	
dopamine							
<i>C_{max}</i> (nM)	–	–	–	–	551.4 ± 19.7	443.7 ± 140.7	0.672 ^c
<i>T_{max}</i> (min)	–	–	–	–	25 ± 6	35 ± 6	0.145 ^c
AUC (×10 ³ nM·min)	2.7 ^g ± 0.2	2.3 ^g ± 0.4	0.8 ^g ± 0.1	1.4 ^g ± 0.7	34.8 ^e ± 5.1	28.4 ^e ± 10.0	<0.001 ^d
MRT (min)	(79 ± 2)	(82 ± 3)	(76 ± 3)	(81 ± 4)	60 ± 7	59 ± 6	0.062 ^c
5-HT							
<i>C_{max}</i> (nM)	–	–	–	–	45.0 ± 10.8	51.6 ± 18.3	0.260 ^c
<i>T_{max}</i> (min)	–	–	–	–	18 ± 3	25 ± 0	0.216 ^c
AUC (×10 ³ nM·min)	0.1 ^f ± 0.0	0.2 ^f ± 0.0	0.2 ^f ± 0.0	0.2 ^f ± 0.0	2.5 ^e ± 0.3	3.6 ^e ± 0.7	<0.001 ^d
MRT (min)	(73 ± 10)	(70 ± 2)	(70 ± 2)	(67 ± 6)	58 ± 4	61 ± 2	0.749 ^c

Data were represented as mean ± S.E.M.

^a Simultaneous administration of saline and ketamine (5, 10, 20, 40 mg/kg) to rats (n = 3, respectively).

^b Simultaneous administration of MDMA (10 mg/kg) and ketamine (5 or 10 mg/kg) to rats (n = 3, respectively).

^c *P*-values were calculated by one-way ANOVA of MDMA (10 mg/kg, Table 1) and MDMA (10 mg/kg) + ketamine (5, 10 mg/kg) groups.

^d *P*-values were calculated by one-way ANOVA of saline, ketamine (5, 10, 20, 40 mg/kg), MDMA (10 mg/kg, Table 1), MDMA (10 mg/kg) + ketamine (5, 10 mg/kg) groups.

^e *P* < 0.01 vs saline group (Scheffe's post-hoc test).

^f *P* < 0.05, ^g *P* < 0.01, vs MDMA (10 mg/kg, Table 1) sole-administration group (Scheffe's post-hoc test).

Table 4 Moment parameters of dopamine and 5-HT levels after single administration of MDMA or caffeine and co-administration of MDMA and caffeine.

MDMA	–	10 mg/kg	<i>P</i>
Caffeine	20 mg/kg ^a	20 mg/kg ^b	
dopamine			
<i>C_{max}</i> (nM)	–	1,017.3 ± 92.1	0.076 ^c
<i>T_{max}</i> (min)	–	25 ^f ± 0	0.038 ^c
AUC (×10 ³ nM·min)	1.7 ^g ± 0.6	72.5 ^e ± 10.8	<0.001 ^d
MRT (min)	(85 ± 4)	63 ^f ± 3	0.035 ^c
5-HT			
<i>C_{max}</i> (nM)	–	28.8 ± 3.5	0.526 ^c
<i>T_{max}</i> (min)	–	25 ± 0	0.184 ^c
AUC (×10 ³ nM·min)	0.4 ^g ± 0.2	2.3 ^e ± 0.1	<0.001 ^d
MRT (min)	(79 ± 5)	66 ± 4	0.406 ^c

Data were represented as mean ± S.E.M.

^a Simultaneous administration of saline and caffeine (20 mg/kg) to rats (n = 3).

^b Simultaneous administration of MDMA (10 mg/kg) and caffeine (20 mg/kg) to rats (n = 3).

^c *P*-values were calculated by Student's *t*-test comparing to MDMA (10 mg/kg, Table 1) group and MDMA (10 mg/kg) + caffeine (20 mg/kg).

^d *P*-values were calculated by one-way ANOVA of saline, caffeine (20 mg/kg), MDMA (10 mg/kg, Table 1) and MDMA (10 mg/kg) + caffeine (20 mg/kg) groups.

^e *P* < 0.01, vs saline group (Scheffe's post-hoc test).

^f *P* < 0.05, ^g *P* < 0.01, vs MDMA (10 mg/kg, Table 1) sole-administration group (Scheffe's post-hoc test)