Endocrine and Neurochemical Effects of 3,4-Methylenedioxymethamphetamine and Its Stereoisomers in Rhesus Monkeys

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ABSTRACT

3,4-Methylenedioxymethamphetamine (MDMA) is an amphetamine derivative that elicits complex biological effects in humans. One plausible mechanism for this phenomenon is that racemic MDMA is composed of two stereoisomers that exhibit qualitatively different pharmacological effects. In support of this, studies have shown that *R*(–)-MDMA tends to have hallucinogen-like effects, whereas *S*(+)-MDMA tends to have psychomotor stimulant-like effects. However, relatively little is known about whether these stereoisomers engender different endocrine and neurochemical effects. In the present study, the endocrine and neurochemical effects of each stereoisomer and the racemate were assessed in four rhesus monkeys after intravenous delivery at doses (1–3 mg/kg) that approximated voluntary self-administration by rhesus monkeys and human recreational users. Specifically, fluorescence-based enzyme-linked immunosorbent assay was used to assess plasma pro-

lactin concentrations, and in vivo microdialysis was used to assess extracellular dopamine and serotonin concentrations in the dorsal striatum. *R*(–)-MDMA, but not *S*(+)-MDMA, significantly increased plasma prolactin levels and the effects of *S*,*R*(±)-MDMA were intermediate to each of its component stereoisomers. Although *S*(+)-MDMA did not alter prolactin levels, it did significantly increase extracellular serotonin concentrations. In addition, *S*(+)-MDMA, but not *R*(–)-MDMA, significantly increased dopamine concentrations. Furthermore, as in the prolactin experiment, the effects of the racemate were intermediate to each of the stereoisomers. These studies demonstrate the stereoisomers of MDMA engender qualitatively different endocrine and neurochemical effects, strengthening the inference that differences in these stereoisomers might be the mechanism producing the complex biological effects of the racemic mixture of MDMA in humans.

Racemic 3,4-methylenedioxymethamphetamine (MDMA; ecstasy) is a substituted phenethylamine with significant abuse liability. Although MDMA was placed into Schedule 1 of the Controlled Substances Act, its behavioral effects do not clearly fit into traditional delineations of drugs of abuse. Specifically, the racemic mixture of MDMA has both stimulant and hallucinogen-like effects (Shulgin, 1986; Harris et al., 2002). Moreover, Nichols (1986) postulated that MDMA

represented a new class of compounds categorized as “entactogens.” In support for this new categorization, Johanson et al. (2006) reported that in a three-choice discrimination procedure approximately half of the human subjects reported that MDMA was similar to the substrate-based dopamine releaser *S*(+)-amphetamine, whereas the other half reported that it was similar to the serotonin releaser *meta*-chlorophenylpiperazine (mCPP).

Previous studies have suggested that these complex effects are mediated by qualitative differences (i.e., apparent efficacy differences) between MDMA's stereoisomers. For example, one of the earliest studies contrasted the subjective effects of these stereoisomers in humans (Anderson et al., 1978). This work has been supported by a series of studies using drug discrimination, the preclinical analog of subjective effects in humans (Schuster and Johanson, 1988; Brauer et al., 1997), showing marked differences in the interoceptive

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ABBREVIATIONS: MDMA, 3,4-methylenedioxymethamphetamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; mCPP, *meta*-chlorophenylpiperazine; HPLC, high-performance liquid chromatography; RM, repeated measures; ANOVA, analysis of variance; 5-HT, 5-hydroxytryptamine.

effects of each stereoisomer. Specifically, these studies support the notion that *S*(+)-MDMA more readily functions as a psychomotor stimulant, whereas *R*(-)-MDMA more readily functions as a hallucinogen (Glennon et al., 1988; Baker et al., 1995; Murnane et al., 2009). This work has been further supported by studies showing that *S*(+)-MDMA and *S,R*(±)-MDMA, but not *R*(-)-MDMA, functioned as locomotor stimulants (Fantegrossi et al., 2003) or positive reinforcers, under a progressive ratio schedule (Wang and Woolverton, 2007). Taken together, this literature suggests that the stereoisomers of MDMA have distinct behavioral and interoceptive effects.

Despite these findings, other results call this hypothesis into question. For example, Taffe et al. (2006) found that all three forms of MDMA elicited hyperthermia and did not function as locomotor stimulants. Furthermore, Fantegrossi et al. (2004) found that all three forms of MDMA functioned as positive reinforcers, under a fixed-ratio schedule. Therefore, further study of the *in vivo* effects of these stereoisomers is warranted.

The complex biological effects of MDMA may be mediated by a number of endocrine and neurochemical effects elicited by this compound. Some of the most well established endocrine and neurochemical effects elicited by MDMA include secretion of prolactin and release of dopamine and serotonin in the central nervous system. For example, when administered 1.5 mg/kg of the racemate, human MDMA abusers not only reported complex subjective effects but also exhibited an increased level of circulating prolactin (Harris et al., 2002). As such, if the stereoisomers of MDMA have qualitatively different behavioral or interoceptive effects they should concomitantly exhibit qualitatively different endocrine and neurochemical effects. In support of this contention, it has been shown that *S*(+)-MDMA increases extracellular dopamine turnover in the striatum (Hatzidimitriou et al., 2002; Acquas et al., 2007) and significantly occupies the dopamine transporter (Fantegrossi, 2008), whereas *R*(-)-MDMA does not. However, to date, the effects of the stereoisomers of MDMA on release of dopamine and serotonin and secretion of prolactin have not been comprehensively studied.

In summary, MDMA produces complex behavioral and interoceptive effects that may be mediated via qualitative endocrine and neurochemical differences in its stereoisomers. However, relatively little data regarding the endocrine and neurochemical effects of these stereoisomers have been published. Therefore, in the present study the effects of each stereoisomer and the racemic mixture on circulating prolactin levels were assessed by enzyme-linked immunosorbent assay in rhesus monkeys. Given the novelty of these prolactin procedures in rhesus monkeys, initial experiments used amphetamine and mCPP as positive controls because of the selectivity of their monoamine-releasing effects (Owens et al., 1997; Davids et al., 2002), their established effects on prolactin secretion (Muller et al., 1983; Aloï et al., 1984; Baumann et al., 2008), and the similarity of their subjective

effects to MDMA (Tancer and Johanson, 2003; Johanson et al., 2006). Furthermore, the effects of each form of MDMA on striatal extracellular dopamine and serotonin levels were assessed by *in vivo* microdialysis and high-pressure liquid chromatography (HPLC). The specific hypothesis tested was that *S*(+)-MDMA would function as a mixed dopamine/serotonin releaser, whereas *R*(-)-MDMA would selectively release serotonin. As an extension of this hypothesis, we predicted that these neurochemical effects would result in *R*(-)-MDMA engendering more pronounced effects on prolactin secretion than *S*(+)-MDMA.

Materials and Methods

Subjects

Four female rhesus monkeys (*Macaca mulatta*) weighing between 6.5 and 8.0 kg served as subjects for these experiments. Subjects were housed individually within a primate colony with continuous access to water and were fed daily in the early evening after their experiments had been completed. Their diet consisted of monkey chow (Purina, St. Louis, MO) supplemented with fresh fruit and vegetables. Food restriction protocols were not used. Ambient conditions within the colony were maintained at a temperature of $22 \pm 2^\circ\text{C}$ and 45 to 50% humidity; room lighting was set to a 12-h light/dark cycle. Environmental enrichment was provided on a regular basis. All procedures and studies strictly followed the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (Publication 85-23, revised 1985) and were approved by the Institutional Animal Care and Use Committee of Emory University.

Surgery

Before initiation of this study, subjects were implanted with chronic indwelling venous catheters by using aseptic surgical techniques as described previously (Wilcox et al., 2002). Subjects were also implanted with bilateral CMA/11 guide cannulae (CMA Microdialysis, North Chelmsford, MA) that were stereotactically targeted for the head of the caudate nucleus as described previously (Czoty et al., 2000; Wilcox et al., 2005). During each surgery, subjects were prophylactically administered an antibiotic (Rocephin), an analgesic (Buprenorphine), and a nonsteroidal anti-inflammatory agent (Bannamine) to minimize any pain or discomfort that might result from the surgery. Catheters were regularly flushed with heparinized (100 U/ml) saline to maintain patency.

Drugs

S(+)-MDMA, *R*(-)-MDMA, and *S,R*(±)-MDMA were supplied by the National Institute on Drug Abuse (Research Technology Branch, Research Triangle Park, NC). *S*(+)-amphetamine and mCPP (Fig. 1) were purchased from Sigma-Aldrich (St. Louis, MO). Doses were calculated and are expressed as salts. All drugs were dissolved in 0.9% sterile saline.

Procedure

Dosing Schedule and Drug History. All procedures were carried out in fully conscious subjects while they sat in commercially available primate chairs (Primate Products, Woodside, CA). All subjects had a history of contingent and noncontingent administration of both cocaine and MDMA before the initiation of this study. However,

Fig. 1. Chemical structures of all test compounds used in this study: mCPP (A), amphetamine (B), and MDMA (C).

no subject received either drug within 3 months of the start of the study. The order of drug administration was randomized without regard to which assay was carried out on a particular day, and there was a minimum of 5 days between sessions. The doses studied were chosen on the basis that they were equivalent, on a milligram/kilogram basis, to the doses of MDMA typically abused by humans (Cole et al., 2002; Harris et al., 2002; Green et al., 2003) and were within the range that rhesus monkeys will voluntarily self-administer (Fantegrossi et al., 2002; Banks et al., 2008). However, the first time 3.0 mg/kg *S*(+)-MDMA was administered, the subject presented acute symptoms of untoward effects, so it was decided that this dose of *S*(+)-MDMA would not be repeated because of ethical and safety concerns. The randomized dose order was thus continued while omitting any administrations of this dose of *S*(+)-MDMA. Plasma prolactin collection and microdialysis procedures were alternated such that a given assay was carried out every other week. Because of the need to develop new HPLC procedures (see *HPLC*), the effects of each form of MDMA on serotonin release were determined subsequent to all other experiments.

Plasma Prolactin Collection. Subjects were seated in restraint chairs and acclimated to having an acute catheter (BD Saf-T-Intima Closed Catheter System; BD, Franklin Lakes, NJ) unilaterally inserted into a saphenous vein before experimental data collection. For this assay, drug administration and plasma collection were carried out by using an acute catheter, rather than the chronic indwelling catheter, because a sufficient number of blood samples could not be reliably withdrawn from the chronic catheter in all subjects. During experiments, 2.0 ml of blood was collected 15 min before an intravenous drug injection and 15, 30, 60, and 120 min after the injection. Samples were refrigerated in 3.5-ml serum-separating vacutainers (BD), centrifuged (at 3000 rpm for 15 min) to isolate the plasma, and frozen in a cryogenic freezer at -20°C (range -15 to -25°C) until they were assayed. Samples were assayed at the Yerkes National Primate Research Center's Biomarkers Core Laboratory using a fluorescence-based enzyme-linked immunosorbent assay as described previously (Mook et al., 2005).

In Vivo Microdialysis. Microdialysis measurements were collected and samples were analyzed in a similar fashion to procedures that have been described previously (Kimmel et al., 2007; Banks et al., 2009). In brief, subjects were placed in sound-attenuated testing chambers after 24-mm stainless-steel microdialysis probes with a 4-mm membrane (CMA Microdialysis) were inserted into the subject's surgically implanted guide cannulae. Drugs were administered through the previously implanted indwelling venous catheter via the subcutaneous vascular access port. Experiments consisted of a 1-h equilibrium period after which samples were collected every 10 min for 3 h. Drugs were administered 1 h after the sampling phase was initiated to provide a before and after drug sampling period. Probe function was verified for each experimental session, both before and after the session, via determination of the change in neurotransmitter concentration as a function of traversing the probe with a known concentration of dopamine (i.e., probe recovery). Furthermore, the viability of the sampling site was verified through retrodialysis of a potassium-enriched (100 mM) solution ionically matched to artificial cerebrospinal fluid. Neurochemical concentrations within the dialysate were quantified by electrochemical detection using HPLC and analyzed in comparison with known concentration curves with EZChrom Elite software (version 3.1; Scientific Software, Pleasanton, CA).

HPLC. High-pressure liquid chromatography and electrochemical detection were used to quantify dopamine levels as described previously (Kimmel et al., 2007; Banks et al., 2009). In brief, the HPLC system was composed of a small bore column ($3.2\text{ mm} \times 150\text{ mm} \times 3\text{ }\mu\text{m}$), an ESA 582 model solvent delivery pump set to a flow rate of 0.6 ml/min, and an ESA model 542 autosampler (ESA, Inc., Chelmsford, MA). Electrochemical detection was carried out with a guard cell (ESA model 5020; potential 350 mV), a dual-channel analytical cell (ESA model 5040), and an ESA model Coulochem II detector. The

analytical cell's oxidative channel was set to a voltage of -150 mV , and its reductive channel was set to 275 mV . Different commercially available mobile phases were used for dopamine (MD-TM; ESA, Inc.) or serotonin (MD-TM2; ESA, Inc.) quantification. MD-TM is composed of sodium dihydrogen phosphate (75 mM), octanesulfonic acid (1.7 mM), triethylamine (100 $\mu\text{l/l}$), EDTA (25 μM), and acetonitrile (10%); upon mixing, it is brought to a final pH of 3 by the addition of phosphoric acid. MD-TM2 is composed of sodium dihydrogen phosphate (90 mM), octanesulfonic acid (1.7 mM), citric acid (50 mM), EDTA (50 μM), and acetonitrile (10%); upon mixing, it is brought to a final pH of 3 by the addition of phosphoric acid. All other microdialysis and HPLC procedures were identical to those used for dopamine quantification.

Data Analysis

Graphical presentation of all data depicts mean $\pm$ S.E.M., and any points without error bars indicate instances in which the S.E.M. is encompassed by the data point or data derived from a single subject. All graphical data presentations were created by using Prism 4 (GraphPad Software Inc., San Diego, CA), all statistical tests were performed by using SigmaStat 3 (Systat Software, San Jose, CA), and significance was arbitrated at $p < 0.05$.

Plasma Prolactin Analysis. The primary dependent variable tested in this experiment was plasma concentration of prolactin. Differences in basal levels across days were assessed by one-way repeated measures (RM) analysis of variance (ANOVA). Data were then normalized to the measured baseline levels in a given experimental session. Main effects of condition and time were analyzed by two-way RM ANOVA. Individual comparisons were then drawn at each time point with correction for multiple comparisons by using Dunnett's method versus baseline levels. Assessment of the effects of MDMA administration on basal prolactin levels (basal levels during the first or final prolactin determination; Table 1) were analyzed by using a paired *t* test.

Neurochemical Analysis. The primary dependent variable tested in this experiment was striatal extracellular concentration of the neurotransmitters serotonin or dopamine. For dopamine, differences in basal levels across days were assessed by one-way RM ANOVA. Assessment of the effects of MDMA administration on basal levels without correction for probe recovery of dopamine and its major metabolites DOPAC and HVA (basal levels during the first or

TABLE 1

Basal levels of prolactin, dopamine, DOPAC, and HVA in rhesus monkeys

First and last determination data were derived from the first and last days of endocrine or dopamine data collection within this study regardless of which treatment was subsequently administered on that particular day.

Hormone/ Neurochemical	Monkey	First Determination	Last Determination
Prolactin (ng/ ml)	RHp	8.57	9.30
	RNb	36.70	57.90
	RJt	24.99	35.82
	RLt	12.77	51.21
	Mean (S.E.M.)	20.76 (6.35)	38.55 (10.79)
Dopamine (nM)	RHp	11.19	6.64
	RNb	8.92	6.23
	RJt	3.40	4.92
	RLt	1.40	2.76
	Mean (S.E.M.)	6.23 (2.29)	5.13 (0.87)
DOPAC (nM)	RHp	217.57	181.37
	RNb	166.87	127.81
	RJt	212.55	55.12
	RLt	119.39	134.61
	Mean (S.E.M.)	179.10 (22.93)	124.73 (26.08)
HVA (nM)	RHp	3503.35	3819.53
	RNb	2825.19	3562.68
	RJt	3286.21	2129.02
	RLt	1927.12	2735.76
	Mean (S.E.M.)	2885.47 (349.34)	3061.75 (387.45)

final microdialysis determination; Table 1) were analyzed by using a paired *t* test. Basal levels of extracellular serotonin in the three experimental sessions carried out to determine the effects of each form of MDMA on this neurochemical were analyzed by one-way RM ANOVA. Dopamine and serotonin data were then normalized to the measured baseline levels in a given experimental session. The main effect of treatment was analyzed by one-way RM ANOVA. Individual comparisons were then drawn at each time point with correction for multiple comparisons by using Tukey's test versus baseline levels.

Results

Basal Hormone and Dopamine Levels

Basal Levels of Prolactin, Dopamine, DOPAC, and HVA. Table 1 shows the basal hormone and neurochemical levels determined during the first or last endocrine (prolactin) or dopamine microdialysis (dopamine, DOPAC, and HVA) experiment in each subject. Analysis by paired *t* test revealed that there were no significant differences in basal prolactin ($t_3 = -2.21$; $p = 0.114$), dopamine ($t_3 = 0.722$; $p = 0.114$), DOPAC ($t_3 = 1.487$; $p = 0.234$), or HVA ($t_3 = -0.385$; $p = 0.726$) levels across these time points. However, the power of each test was only 0.28, 0.52, 0.12, or 0.52, respectively.

Effects on Circulating Prolactin Levels

Amphetamine Versus mCPP. Under the procedures used, circulating levels of plasma prolactin could be reliably obtained from rhesus monkeys (Fig. 2). Two-way RM ANOVA revealed a significant main effect of both drug ($F_{3,2} = 16.656$; $p = 0.004$) and time ($F_{3,4} = 12.614$; $p < 0.001$) and a significant interaction ($F_8 = 12.988$; $p < 0.001$). The power of this test for the main effect of drug was 0.974, main effect of time

was 0.998, and the interaction was 1. Basal levels of prolactin did not differ depending on the day of the treatment as determined by one-way RM ANOVA ($F_{3,2} = 1.457$; $p = 0.305$). However, the power of this test was only 0.095. Post hoc analysis by Dunnett's test showed that saline administration did not significantly alter circulating prolactin compared with baseline at any time point ($F_{3,4} = 0.720$; $p = 0.595$). However, intravenous administration of 2.5 mg/kg mCPP, used as a positive control for the effects of a serotonin releaser, significantly elevated prolactin ($F_{3,4} = 12.934$; $p < 0.001$) at 15, 30, and 60 min ($p < 0.05$) but not at 120 min. In contrast, intravenous administration of 1.0 mg/kg (+)-amphetamine, used as a positive control for the effects of a dopamine releaser, significantly decreased prolactin levels ($F_{3,4} = 9.150$; $p = 0.001$) at 30, 60, and 120 min ($p < 0.05$) but not at 15 min. Consistent with our expectations regarding their underlying neurochemical effects, analysis of microdialysis experiments by one-way RM ANOVA revealed a significant main effect of treatment with 1.0 mg/kg amphetamine on extracellular levels of dopamine ($F_{3,8} = 6.678$; $p < 0.001$) but not serotonin ($F_{3,8} = 1.726$; $p = 0.143$), whereas, in contrast, the same analysis revealed a significant main effect of treatment with 2.5 mg/kg mCPP on levels of extracellular serotonin ($F_{3,8} = 1.555$; $p = 0.191$) but not dopamine ($F_{3,8} = 2.699$; $p = 0.028$). The powers of these tests were 0.996, 0.255, 0.198, and 0.591, respectively. Post hoc analysis by Tukey's test showed that extracellular levels of dopamine were significantly different from baseline after amphetamine administration at 20 and 30 min, whereas extracellular levels of serotonin were significantly different from baseline after mCPP administration at 20 min.

Fig. 2. Effects of mCPP (2.5 mg/kg i.v.) and amphetamine (1.0 mg/kg i.v.) on plasma prolactin levels and extracellular levels of dopamine and serotonin. A, effects of administration of mCPP (■) or saline (□) on plasma prolactin levels. B, effects of administration of amphetamine (●) or saline (□) on plasma prolactin levels. C, effects of administration of mCPP on extracellular levels of dopamine (▲) or serotonin (△) levels. All points represent the mean $\pm$ S.E.M. Abscissae, time expressed in minutes in reference to the administration of the test compound and plotted on a linear scale. Ordinates, plasma prolactin concentration expressed as an absolute change from baseline (A and B) or extracellular neurochemical concentration expressed as a percentage of baseline levels (C and D). * indicates a significant difference from baseline assessed by one-way repeated measures analysis of variance with post hoc analysis carried out by using Dunnett's test (A and B) or Tukey's test (C and D).

MDMA Stereoisomers. One-way RM ANOVA revealed that basal levels of prolactin did not differ across day ($F_{3,9} = 1.771$; $p = 0.121$); however, the power of this test was only 0.292 (Fig. 3). Two-way RM ANOVA showed a significant main effect of treatment (drug and dose; $F_{3,7} = 5.585$; $p < 0.001$), time ($F_{3,4} = 11.432$; $p < 0.001$), and a significant interaction ($F_{28} = 5.315$; $p < 0.001$). The power of this test for

Fig. 3. Dose-effect determination of the effects of *S*(+)-MDMA (0.3–3.0 mg/kg i.v.; A), *S,R*(±)-MDMA (0.3–1.7 mg/kg i.v.; B), and *R*(-)-MDMA (0.3–1.7 mg/kg i.v.; C) in comparison with saline (□; A–C redrawn from Fig. 2) on circulating prolactin levels. The lowest dose administered of each form of MDMA is represented by ▲, the intermediate dose is shown by ●, and the highest dose is indicated by ■. The data at the highest dose of *S*(+)-MDMA (3.0 mg/kg) came from a single subject; in all other cases, the sample size was four. Abscissae, ordinates, and asterisks are the same as in Fig. 2.

the main effect of treatment was 0.966, effect of time was 0.996, and the interaction was 1. The 3.0 mg/kg *S*(+)-MDMA data were not included in this analysis because they were derived from a single subject (see *Materials and Methods*). Post hoc analysis by Dunnett's test showed that at 15 min there was a significant main effect of treatment ($F_{3,8} = 6.641$; $p < 0.001$) that was exclusively attributable to the effects of 1.7 mg/kg *R*(-)-MDMA ($p < 0.05$). At 30 min a significant main effect remained ($F_{3,8} = 6.289$; $p < 0.001$) but treatment isolation by Dunnett's method showed that this was attributable to both 1.7 mg/kg *R*(-)-MDMA and 1.7 mg/kg *S,R*(±)-MDMA ($p < 0.05$). A significant main effect was also found at 60 min ($F_{3,8} = 2.693$; $p < 0.031$) that was due to the effects of 1.7 mg/kg *R*(-)-MDMA ($p < 0.05$). No significant main effect of treatment was found at 120 min ($F_{3,8} = 1.122$; $p = 0.387$). In contrast to these effects of *R*(-)-MDMA, *S*(+)-MDMA had no significant effect compared with baseline on prolactin levels at any of the measured time points.

Effects on Neurochemical Levels

Serotonergic Effects at 1.7 mg/kg. Under the procedures used, measurements of extracellular levels of serotonin in the striatum could be reliably obtained from rhesus monkeys (Fig. 4). Basal extracellular serotonin levels were 0.47 ± 0.27 , 0.15 ± 0.02 , and 0.29 ± 0.14 during the sessions carried out to determine the effects of *S*(+)-MDMA, *R*(-)-MDMA, and *S,R*(±)-MDMA, respectively. One-way RM ANOVA revealed that basal extracellular serotonin levels did not differ by session ($F_{3,2} = 0.741$; $p = 0.516$); however, the power of this test was only 0.052. One-way RM ANOVA also showed that *S*(+)-MDMA ($F_{3,8} = 6.438$; $p < 0.001$), *S,R*(±)-MDMA ($F_{3,8} = 4.868$; $p < 0.001$), and *R*(-)-MDMA ($F_{3,8} = 2.864$; $p = 0.022$) significantly elevated extracellular serotonin levels. Post hoc analysis by Tukey's test showed that these effects were significant for all three compounds at the 20-min time point.

Dopaminergic Effects at 1.7 mg/kg. Under the procedures used, measurements of extracellular levels of dopa-

Fig. 4. Effects of *S*(+)-MDMA (■), *S,R*(±)-MDMA (△), and *R*(-)-MDMA (●) on extracellular serotonin levels within the striatum at 1.7 mg/kg. This dose of each compound was found to be the most effective in the prolactin component of this study and is within the range, on a mg/kg basis, that has been reported to be typically taken by human MDMA abusers. Inset, the peak effect of each treatment is shown. Abscissae, time expressed in minutes in reference to the administration of the test compound and plotted on a linear scale. Ordinates, extracellular concentration of serotonin within the striatum expressed as a percentage change from baseline. * indicates a significant difference from baseline assessed by one-way repeated measures analysis of variance with post hoc analysis carried out by Tukey's test.

Fig. 5. Effects of *S*(+)-MDMA (■), *S,R*(±)-MDMA (△), and *R*(-)-MDMA (●) at 1.7 mg/kg on extracellular dopamine levels within the striatum. Inset, the peak effect of each treatment is shown. Ordinates, extracellular concentration of dopamine within the striatum expressed as a percentage change from baseline. Abscissae and asterisks are the same as in Fig. 4.

mine and its major metabolites DOPAC and HVA could be reliably obtained from rhesus monkeys in the striatum (Fig. 5). One-way RM ANOVA showed that both *S*(+)-MDMA ($F_{3,8} = 3.232$; $p = 0.012$) and *S,R*(±)-MDMA ($F_{3,8} = 5.027$; $p < 0.001$) significantly elevated dopamine levels. Post hoc analysis by the Tukey's test showed that these effects were significant for both compounds at the 20-min time point. In contrast, *R*(-)-MDMA had no significant effects compared with baseline on extracellular dopamine levels ($F_{3,8} = 0.884$; $p = 0.544$) analyzed across the first 60 min after drug administration. Additional analysis (data not shown) revealed that both *S*(+)-MDMA ($F_{3,8} = 18.603$; $p < 0.001$) and *S,R*(±)-MDMA ($F_{3,8} = 4.377$; $p < 0.001$) significantly decreased extracellular levels of DOPAC, whereas *R*(-)-MDMA did not ($F_{3,8} = 1.424$; $p < 0.164$). No form of MDMA significantly altered extracellular levels of HVA (data not shown).

Dose-Effect Determination of *R*(-)-MDMA. To ascertain whether the differential effects of *S*(+)-MDMA and *R*(-)-MDMA, shown in Fig. 5, were attributable to a potency difference, 3 mg/kg *R*(-)-MDMA was administered to determine whether this stereoisomer would produce a change in extracellular dopamine levels at a higher dose (Fig. 6). One-way repeated measures analysis of variance revealed no significant main effect of treatment ($F_{3,8} = 1.963$; $p = 0.098$) with this dose of *R*(-)-MDMA. Higher doses were not tested because of safety concerns regarding the effects of *R*(-)-

Fig. 6. Lack of effect of *R*(-)-MDMA at 1.7 mg/kg (●; redrawn from Fig. 5) or 3.0 mg/kg (▲) on extracellular dopamine levels within the striatum. Inset, the peak effect of each treatment is shown. Abscissae, ordinates, and asterisks are the same as in Fig. 5.

Fig. 7. Comparison of the effect of *S*(+)-MDMA (0.85 mg/kg i.v., □) and *S,R*(±)-MDMA (1.7 mg/kg i.v., △; redrawn from Fig. 5) on extracellular dopamine levels within the striatum. By halving the dose of *S*(+)-MDMA administered compared with *S,R*(±)-MDMA, the total amount of *S*(+)-MDMA administered was equalized between the two conditions because *S,R*(±)-MDMA is composed of equal parts *S*(+)-MDMA and *R*(-)-MDMA. Inset, the peak effect of each treatment is shown. Abscissae, ordinates, and asterisks are the same as in Figs. 5 and 6.

MDMA on heart rate (Fantegrossi, 2008) and the presentation of acute untoward effects of *S*(+)-MDMA at 3 mg/kg in this study.

In Vivo Interaction: 1.7 mg/kg (±)-MDMA Versus 0.85 mg/kg (+)-MDMA. To determine whether coadministration of *R*(-)-MDMA with *S*(+)-MDMA, as in the racemic mixture, potentiates or diminishes the dopaminergic effects of *S*(+)-MDMA, the effects of 1.7 mg/kg *S,R*(±)-MDMA were compared with those of 0.85 mg/kg *S*(+)-MDMA (Fig. 7). Because the racemate is composed of equal parts of the two stereoisomers, this equalized the amount of *S*(+)-MDMA administered. Two-way repeated measures analysis of variance revealed a significant main effect of treatment with these compounds at these doses ($F_{3,8} = 5.460$; $p < 0.001$) but no significant difference between them ($F_{3,1} = 0.610$; $p = 0.492$).

Discussion

The major finding of this study is that the stereoisomers of MDMA have distinct endocrine and neurochemical effects that are consistent with *S*(+)-MDMA functioning as a mixed substrate-based dopamine/serotonin releaser, whereas *R*(-)-MDMA selectively releases serotonin in rhesus monkeys. In the first experiment, mCPP selectively increased extracellular levels of serotonin and concomitantly increased circulating prolactin levels, whereas (+)-amphetamine selectively increased extracellular levels of dopamine and concomitantly decreased circulating prolactin levels. The effects of mCPP are consistent with previous reports that serotonin releasers (Aloi et al., 1984; Baumann et al., 2008) and direct serotonin receptor agonists (Aulakh et al., 1994; Biezonski et al., 2009) elicit an increase in circulating prolactin and that this prolactin increase can be blocked by multiple serotonin receptor antagonists (Aulakh et al., 1994; Meltzer and Maes, 1995; Biezonski et al., 2009). It may be important to note that in addition to serotonin-releasing effects mCPP has been shown to function as a direct agonist of the 5-hydroxytryptamine 2A (5-HT_{2A}), 5-HT_{2B}, and 5-HT_{2C} receptors (Bentley et al., 2004). As such, mCPP elicited prolactin secretion might be mediated by direct or indirect serotonin receptor agonism or a synergistic combination of both. Despite the complexity of its binding profile, mCPP was chosen as a positive control

compound because of the selectivity of its monoamine-releasing effects (Owens et al., 1997), its established effects on prolactin secretion (Muller et al., 1983; Aloï et al., 1984; Baumann et al., 2008), and the similarity of its subjective effects to MDMA (Tancer and Johanson, 2003; Johanson et al., 2006). Furthermore, the effects of amphetamine are consistent with previous findings that dopamine releasers and direct dopamine receptor agonists have been shown to lower circulating prolactin levels and antagonists of dopamine receptors increase prolactin levels (Muller et al., 1983). These positive control experiments extend these findings by showing that these prolactin regulatory processes are also present in rhesus monkeys.

At the doses tested, administration of the stereoisomers of MDMA elicited differential effects on prolactin levels. Previous studies have used variants of allometric scaling to equilibrate the doses of MDMA administered to experimental animals to human consumption (Mueller et al., 2008). However, these allometric models are sensitive to the pharmacokinetic and corrective factors used and can thus yield highly variable estimates (Yates and Kugler, 1986; Baumann et al., 2007; Fantegrossi, 2007). Furthermore, previous studies have shown that the doses of MDMA voluntarily self-administered by rhesus monkeys (Fantegrossi et al., 2002; Banks et al., 2008) are within the range, on a mg/kg basis, that humans abuse (1–2 mg/kg) (Cole et al., 2002; Harris et al., 2002; Green et al., 2003). Therefore, we chose to forgo potentially problematic allometric scaling procedures in favor of simply administering doses voluntarily self-administered by human and nonhuman primates. However, it may be important to note that human MDMA abusers typically consume MDMA orally, which probably yields different pharmacokinetics than the intravenous route used in this study. Nevertheless, unlike *R*(–)-MDMA and *S,R*(±)-MDMA, *S*(+)-MDMA did not significantly alter circulating prolactin levels up to a dose of 3.0 mg/kg. In addition, the effects of *S,R*(±)-MDMA were intermediate to the effects of its two component stereoisomers. Indeed, at the most effective dose tested for each form of MDMA, *S,R*(±)-MDMA was approximately half as effective as *R*(–)-MDMA. In combination with the lack of *S*(+)-MDMA-elicited prolactin secretion, this suggests that MDMA-elicited prolactin secretion is entirely attributable to the *R*(–) stereoisomer. However, at 1.0 mg/kg *S,R*(±)-MDMA significantly increased plasma prolactin levels, whereas at 0.3 mg/kg *R*(–)-MDMA did not. Because *S,R*(±)-MDMA is composed, on average, of an equal mixture of each stereoisomer, this suggests that coadministration of *S*(+)-MDMA may potentiate *R*(–)-MDMA-elicited prolactin secretion at low doses of *R*(–)-MDMA. Nevertheless, there is a clear dissociation, across the dose range tested, of the effects of the stereoisomers of MDMA on prolactin secretion when they are separately administered. It is noteworthy that the effects of *R*(–)-MDMA and *S,R*(±)-MDMA on circulating levels of prolactin are consistent with those of other serotonin-releasing drugs.

Release of norepinephrine may also contribute to MDMA-elicited prolactin release because selective norepinephrine reuptake inhibitors, such as reboxetine, elicit prolactin secretion (Schule et al., 2004). However, the relationship between norepinephrine release and prolactin secretion has not been fully elucidated as, in apparent contrast to the effects of drugs that increase norepinephrine levels, central adminis-

tration of norepinephrine attenuates prolactin secretion (Thomas et al., 1989). Furthermore, the effects of norepinephrine on prolactin secretion seem to depend on whether it is the α or β receptor subtypes that are stimulated (Dodge and Badura, 2004). In addition, the stereoisomers of MDMA exhibit comparable potencies to release norepinephrine in vitro (Setola et al., 2003), perhaps minimizing the possibility that differential in vivo release of norepinephrine accounts for their differential capacities to elicit prolactin secretion. Drug interaction studies may be necessary to disentangle the role of serotonin release from norepinephrine release in MDMA-elicited prolactin secretion.

The results of the microdialysis experiments complement those obtained in the prolactin experiments and further support the hypothesis that *S*(+)-MDMA functions as a mixed substrate-based dopamine/serotonin releaser, whereas *R*(–)-MDMA selectively releases serotonin. The failure of *S*(+)-MDMA to alter plasma prolactin could be due to two possibilities. First, *S*(+)-MDMA may simply not function as a serotonin releaser in rhesus monkeys and therefore would not be expected to elicit a prolactin response. Alternatively, *S*(+)-MDMA may function as a serotonin releaser but have a more complex pharmacology than a drug that selectively releases serotonin, such as mCPP. In this regard, a complex mechanism involving released dopamine might be predicted to functionally antagonize the effects of released serotonin on prolactin secretion (Emiliano and Fudge, 2004).

To determine which alternative best fits the data, HPLC and microdialysis procedures were used to determine the effects of *S*(+)-MDMA on extracellular serotonin and dopamine levels. At 1.7 mg/kg, all three forms of MDMA, including *S*(+)-MDMA, significantly increased extracellular serotonin levels. In contrast, at the same dose, only *S*(+)-MDMA and *S,R*(±)-MDMA significantly increased extracellular dopamine levels. This effect is consistent with the in vitro pharmacological profile of these compounds as *S*(+)-MDMA has an EC_{50} for the dopamine transporter that is approximately 30 times higher than *R*(–)-MDMA (Setola et al., 2003). It is also consistent with previous in vivo neurochemical measures, as *S*(+)-MDMA, but not *R*(–)-MDMA, increases in vivo extracellular dopamine turnover in the striatum of rats (Hiramatsu and Cho, 1990; Acquas et al., 2007). These data complement the prolactin results and lend credence to the hypothesis that some additional effect of *S*(+)-MDMA, presumably dopamine release, functionally antagonizes its effects on prolactin secretion.

One alternative explanation for the different effects of the stereoisomers on dopamine levels in the microdialysis studies could be a difference in potency rather than qualitative differences between the two compounds. Potency differences on this measure are a reasonable expectation because whereas *S*(+)-MDMA is 30 times more potent than *R*(–)-MDMA at releasing dopamine in vitro, *R*(–)-MDMA does release dopamine under these conditions when the dose is escalated (Setola et al., 2003). Therefore, the effects of *R*(–)-MDMA were tested at 3.0 mg/kg. At this dose, *R*(–)-MDMA still did not significantly increase extracellular dopamine levels in the striatum of these subjects. However, it is still possible that, at higher doses, the *R*(–) stereoisomer would significantly increase extracellular dopamine levels. This may in fact be likely because at 3.0 mg/kg *R*(–)-MDMA exhibited a greater peak change in extracellular dopamine

levels (155%) than it did at 1.7 mg/kg (107%). However, it was decided not to continue increasing the dose of *R*(-)-MDMA given the heart rate-increasing effects of this stereoisomer (Fantegrossi, 2008) and the untoward effects produced by 3.0 mg/kg *S*(+)-MDMA; therefore, this possibility remains untested. Nevertheless, it is clear that at the doses that humans abuse and rhesus monkeys self-administer *S*(+)-MDMA is the only component stereoisomer of MDMA that significantly increases extracellular dopamine levels.

Previous studies have also suggested that there is an in vivo interaction between the stereoisomers of MDMA that leads to effects of the racemic mixture that cannot be accounted for by the individual effects of its component stereoisomers. For example, racemic MDMA elicits a greater locomotor-stimulant effect in mice than either stereoisomer does when administered alone (Fantegrossi et al., 2003). This may be caused by facilitation of the dopaminergic effects of *S*(+)-MDMA, via direct agonism of the 5-HT_{2A} receptor by *R*(-)-MDMA, because 5-HT_{2A} receptor agonists potentiate dopamine release by racemic MDMA (Gudelsky et al., 1994) and *R*(-)-MDMA functions as a partial agonist of the 5-HT_{2A} receptor (Nash et al., 1994). To test whether *R*(-)-MDMA facilitates the dopaminergic effects of *S*(+)-MDMA in the striatum of rhesus monkeys, a constant amount of *S*(+)-MDMA was administered in the presence [1.7 mg/kg *S,R*(±)-MDMA] or absence [0.85 mg/kg *S*(+)-MDMA] of an equal dose of *R*(-)-MDMA. The results of this experiment show that there is no significant difference between these treatments. This is not supportive of an in vivo interaction of these stereoisomers on this measure and further suggests that the dopaminergic effects of racemic MDMA are exclusively mediated by its *S*(+) stereoisomer.

Finally, previous work has shown that exposure to MDMA can lead to persistent decreases in the prolactin response to acute administration of serotonin releasers such as mCPP and MDMA. Indeed, this "blunting" of the prolactin response has been suggested to be a marker of the integrity of serotonin systems (Hatzidimitriou et al., 2002; Baumann et al., 2008). However, previous studies have also shown that basal levels of prolactin are unaltered by exposure to MDMA (Hatzidimitriou et al., 2002). The results of the present study support this finding because we did not observe any significant differences in basal prolactin over time. Furthermore, we found no significant change in extracellular levels of dopamine or its major metabolites, DOPAC and HVA, over the course of the study. However, this study was specifically designed to minimize such effects by scheduling drug administration to occur only once per week and in randomized order. Furthermore, subjects had previously received significant amounts of both MDMA and cocaine before the initiation of these studies. Nevertheless, these results further suggest that exposure to MDMA does not alter basal prolactin levels or extracellular levels of dopamine or its metabolites. Whether the response of these substances to drug challenge in rhesus monkeys diminishes with exposure to MDMA remains to be determined.

In summary, the component stereoisomers of MDMA exhibited distinct endocrine and neurochemical effects in rhesus monkeys. Across the dose range tested, these effects are consistent with the hypothesis that *S*(+)-MDMA functions as a mixed dopamine/serotonin releaser, whereas *R*(-)-MDMA selectively releases serotonin. A thorough understanding of

the complex and distinct effects of the stereoisomers of MDMA will probably enlighten our understanding of the complex pharmacology of racemic MDMA and support therapeutic strategies for the treatment of MDMA abuse. To this end, this work supports previous findings by showing that stereoisomers of MDMA engender qualitatively different effects on the release of dopamine and serotonin and the secretion of prolactin and thereby strengthens the inference that distinct in vivo effects of the stereoisomers mediate the complex biological effects of the racemate. Furthermore, this work indicates that additional research into the effects of the stereoisomers of MDMA is warranted, particularly as it may relate to the complex effects of MDMA in humans.

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