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The neuropharmacology of prolactin secretion elicited by 3,4-methylenedioxymethamphetamine (“ecstasy”): A concurrent microdialysis and plasma analysis study

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Abstract

3,4-methylenedioxymethamphetamine (MDMA) is a substituted phenethylamine that is widely abused as the street drug “ecstasy”. Racemic MDMA (S,R(+/-)-MDMA) and its stereoisomers elicit complex spectrums of psychobiological, neurochemical, and hormonal effects. In this regard, recent findings demonstrated that S,R(+/-)-MDMA and its stereoisomer R(-)-MDMA elicit increases in striatal extracellular serotonin levels and plasma levels of the hormone prolactin in rhesus monkeys. In the present mechanistic study, we evaluated the role of the serotonin transporter and the 5-HT_{2A} receptor in S,R(+/-)-MDMA- and R(-)-MDMA-elicited prolactin secretion in rhesus monkeys through concurrent microdialysis and plasma analysis determinations and drug interaction experiments. Concurrent neurochemical and hormone determinations showed a strong positive temporal correlation between serotonin release and prolactin secretion. Consistent with their distinct mechanisms of action and previous studies showing that the serotonin transporter inhibitor fluoxetine attenuates the behavioral and neurochemical effects of S,R(+/-)-MDMA, pretreatment with fluoxetine attenuated serotonin release elicited by either S,R(+/-)-MDMA or R(-)-MDMA. As hypothesized, at a dose that had no significant effects on circulating prolactin levels when administered alone, fluoxetine also attenuated prolactin secretion elicited by S,R(+/-)-MDMA. In contrast, combined pretreatment with both fluoxetine and the selective 5-HT_{2A} receptor antagonist M100907 was required to attenuate prolactin secretion elicited by R(-)-MDMA, suggesting that this stereoisomer of S,R(+/-)-MDMA elicits prolactin secretion through both serotonin release and direct agonism of 5-HT_{2A} receptors. Accordingly, these findings inform our understanding of the neuropharmacology of both S,R(+/-)-MDMA and R(-)-MDMA and the regulation of prolactin secretion.

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None of the authors of this manuscript have any conflicts of interest related to its content.

Keywords

MDMA; serotonin; prolactin; fluoxetine; M100907; rhesus

Introduction

3,4-methylenedioxymethamphetamine (MDMA) is a substituted phenethylamine that is widely abused as the street drug “ecstasy”. Racemic MDMA (S,R(+/-)-MDMA) and its stereoisomers elicit complex spectrums of *in vivo* effects, some of which share similarities with psychomotor stimulant-type compounds whereas others share similarities with hallucinogen-type compounds (Baker *et al*, 1995; Fantegrossi *et al*, 2002; Glennon *et al*, 1988; Murnane *et al*, 2009). Moreover, S,R(+/-)-MDMA elicits distinct effects that have been described as “entactogenic” (Nichols, 1986). In humans, these effects include increased empathy, communication, understanding, and feelings of closeness to others. To date, the underlying pharmacological mechanisms of these complex effects remain unknown.

The complexity of the *in vivo* pharmacological effects of S,R(+/-)-MDMA is paralleled by the complexity of its protein targets and hormonal effects. *In vitro* studies have shown that S,R(+/-)-MDMA binds to or activates the serotonin transporter (SERT), the dopamine and norepinephrine transporters, 5-HT₁ and 5-HT₂ serotonergic receptors, α_1 , α_2 , and β noradrenergic receptors, M1 and M2 muscarinic receptors, and H1 histaminergic receptors (Battaglia *et al*, 1988). These findings have been supported by work demonstrating that a broad set of receptors and transporters contribute to the *in vivo* pharmacology of S,R(+/-)-MDMA (Bubar *et al*, 2004; Fantegrossi *et al*, 2003; Liechti and Vollenweider, 2001). In addition to receptor and transporter effects, *in vivo* administration of S,R(+/-)-MDMA elicits secretion of the hormones oxytocin, cortisol, and prolactin (Harris *et al*, 2002). Although still an unanswered question, it is possible that these hormones may mediate some of the subjective effects of S,R(+/-)-MDMA, such as its prosocial, anxiogenic, and empathogenic effects. Therefore, despite the complexity of the neuropharmacological effects of S,R(+/-)-MDMA, its effects on hormone secretion may have particular relevance for its distinct psychobiology.

The relationship between hormone secretion and the psychobiological effects of S,R(+/-)-MDMA and its stereoisomers remains to be determined. Nevertheless, a significant positive correlation has been demonstrated between S,R(+/-)-MDMA-elicited oxytocin secretion and its prosocial effects (Dumont *et al*, 2009). In addition to eliciting secretion of the hormone oxytocin, S,R(+/-)-MDMA also elicits secretion of the hormone prolactin. Prolactin is a structural analog of growth hormone that is secreted from the anterior pituitary (Emiliano and Fudge, 2004; Muller *et al*, 1983). The most well established functions of prolactin are mammotropic and lactogenic effects. Indeed, prolactin dysregulation has been associated with impairments of fertility and breast cancer. However, prolactin has also been shown to play a role in behavioral and psychobiological effects, such as regulation of food intake, grooming behavior, and anxiety (Emiliano *et al*, 2004). Accordingly, prolactin may have marked relevance for the psychobiological effects of S,R(+/-)-MDMA and its stereoisomers.

The pharmacological mechanisms mediating prolactin secretion have not yet been fully established. Nevertheless, it is well known that drugs that increase extracellular levels of serotonin or are direct agonists of serotonin receptors elicit prolactin secretion; whereas, conversely, drugs that increase extracellular levels of dopamine or are direct agonists of dopamine receptors suppress prolactin secretion (Aloi *et al*, 1984; Baumann *et al*, 2008). In this regard, previous data from our laboratory demonstrated that meta-

chlorophenylpiperazine significantly increased both extracellular serotonin and circulating prolactin levels, whereas (+)-amphetamine significantly increased extracellular dopamine levels but significantly decreased circulating prolactin levels (Murnane *et al.*, 2010). Likewise, S,R(+/-)-MDMA and its stereoisomer R(-)-MDMA-elicited increases in extracellular serotonin levels and plasma levels of prolactin. However, these sorts of correlational determinations do not mechanistically establish a causal relationship between serotonin release and prolactin secretion.

In the present study, we have extended previous findings that correlated drug-elicited increases in serotonin and prolactin by concurrently determining the relationship between the time courses of these effects, through combined microdialysis and plasma analysis. Furthermore, drug interaction studies were carried out with the selective serotonin reuptake inhibitor (SSRI) fluoxetine and the selective receptor antagonist M100907 (Kristiansen *et al.*, 2005) to establish the role of the SERT and the 5-HT_{2A} receptor, respectively, in prolactin secretion elicited by S,R(+/-)-MDMA or R(-)-MDMA. Because previous studies have consistently shown that fluoxetine attenuates both S,R(+/-)-MDMA-elicited serotonin release (Gudelsky *et al.*, 1996; Mehan *et al.*, 2002) and the behavioral and subjective effects of S,R(+/-)-MDMA (Fantegrossi *et al.*, 2009; Liechti *et al.*, 2000; Liechti *et al.*, 2001; McClung *et al.*, 2010; Stein and Rink, 1999), we expected that fluoxetine would attenuate serotonin release elicited by either S,R(+/-)-MDMA or R(-)-MDMA in the primate. That fluoxetine attenuates serotonin release elicited by S,R(+/-)-MDMA or S,R(-)-MDMA is likely a consequence of their distinct mechanisms of action as fluoxetine is a reuptake inhibitor whereas MDMA is a substrate-based releaser, and this effect is likely mediated through direct competition for the SERT. The specific hypothesis tested was whether attenuation of serotonin release would attenuate prolactin secretion. As an extension of this hypothesis, we predicted that pretreatment with fluoxetine would attenuate serotonin release and prolactin secretion elicited by either form of MDMA. Accordingly, this work has marked relevance for the *in vivo* pharmacology of S,R(+/-)-MDMA and R(-)-MDMA. Furthermore, in a wider context, it has relevance for prolactin-related disorders—such as hyperprolactinemia, altered fertility, and breast cancer—which may be associated with long-term exposure to drugs that modulate serotonin systems.

Material and methods

Subjects

Four female rhesus monkeys (*Macaca mulatta*) weighing between 6.5 and 8.0 kgs served as subjects for these experiments. Subjects were housed individually within a primate colony with continuous access to water and were fed daily after their experiments had been completed. Their diet consisted of Purina monkey chow (Ralston Purina, St. Louis, MO) supplemented with fresh fruit and vegetables, and food restriction protocols were not used. Ambient conditions within the colony were maintained at a temperature of 22±2°C, 45–50% humidity, and a 12-h light/dark cycle. Environmental enrichment was regularly provided. All procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee of Emory University.

Surgery

Before the start of this study, each subject was implanted with a chronic indwelling venous catheter attached to a subcutaneous vascular access port using aseptic surgical techniques as previously described (Wilcox *et al.*, 2002). Subjects were also implanted with bilateral CMA/11 guide cannulae (CMA, North Chelmsford, MA, USA) that were stereotactically targeted for the head of the caudate nucleus as previously described (Czoty *et al.*, 2000;

Wilcox *et al*, 2005). During each surgery, subjects were prophylactically administered an antibiotic (Rocephin), an analgesic (Buprenorphine), and a non-steroidal anti-inflammatory agent (Banamine) to minimize any pain or discomfort that may result from the surgery. Catheters were regularly flushed with heparinized (100 U/ml) saline to maintain patency.

Drugs

R(-)- and S,R(+/-)-methylenedioxymethamphetamine (Figure 1A) were supplied by the National Institute on Drug Abuse (Research Technology Branch, Research Triangle Park, NC). Fluoxetine (Figure 1B) was purchased from Sigma-Aldrich (St. Louis, MO). M100907 (Figure 1C) was synthesized at the Chemical Biology Research Branch, National Institute on Drug Abuse and National Institute on Alcohol Abuse and Alcoholism at the National Institutes of Health (Bethesda, MD) and provided as a generous gift from Dr. Kenner C. Rice. Doses were calculated and are expressed as salts. All drugs were dissolved in 0.9% sterile saline and administered intravenously. The doses of each compound were chosen on the basis of typical human dose ranges, positive results obtained in preliminary experiments, or the peer-reviewed literature.

Procedures

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 each form of MDMA prior to the initiation of this study (Murnane *et al.*, 2010). Treatments were counterbalanced within a given experiment, and there was a minimum of six days between each treatment. Doses of S,R(+/-)-MDMA (1.7 and 3.0 mg/kg) and R(-)-MDMA (1.0 and 1.7 mg/kg) were chosen on the basis that they are equivalent, on a mg/kg basis, to the doses of MDMA typically abused by humans (Cole *et al*, 2002; Green *et al*, 2003; Harris *et al*, 2002), are within the range that rhesus monkeys will voluntarily self-administer (Banks *et al*, 2008; Fantegrossi *et al*, 2002), and have been shown to effectively increase prolactin levels (Murnane *et al*, 2010). The dose (3.0 mg/kg) and pretreatment time (15 min) of fluoxetine were chosen based on preliminary experiments and attenuation of the behavioral effects of S,R(+/-)-MDMA (Fantegrossi *et al*, 2009; McClung *et al*, 2010). Finally, the dose (0.3 mg/kg) and pretreatment time of M100907 (15 min) was chosen based on preliminary experiments and previous findings of effective attenuation of S,R(+/-)-MDMA and R(-)-MDMA self-administration (Fantegrossi *et al*, 2002).

In vivo microdialysis—Microdialysis samples were collected and analyzed in a similar fashion to procedures that have been previously described (Banks *et al*, 2009; Kimmel *et al*, 2007). Briefly, 24 mm stainless steel microdialysis probes with a 4 mm membrane (CMA/Microdialysis) were inserted into the subject's surgically implanted guide cannulae, and subjects were then placed in sound-attenuated chambers. Drugs were administered through the indwelling venous catheter via the vascular access port. Following probe insertion, experiments consisted of a 1 hr equilibrium period after which experimental samples were collected. Serotonin levels were determined both before and after all drug administrations. Probe recovery of a known concentration of serotonin was verified both before and after each experimental session. Furthermore, the viability of the sampling site was verified through retrodialysis of a potassium-enriched (100 mM) solution ionically matched to cerebrospinal fluid (aCSF). Neurochemical concentrations within the dialysate were quantified via electrochemical detection (ECD) using high pressure liquid chromatography (HPLC), and analyzed in comparison to known concentration curves with EZChrom Elite software (version 3.1, Scientific Software, Pleasanton, CA).

HPLC

HPLC and ECD were used to quantify serotonin levels as previously described (Murnane *et al*, 2010). Briefly, the HPLC system was composed of a small bore column (3.2 mm X 150 mm X 3 μ m), an ESA 582 model solvent delivery pump set to a flow rate of 0.6 ml/min, and a ESA model 542 autosampler (ESA, Inc., Chelmsford, MA). ECD was carried out via 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. MD-TM2 (ESA, Inc.) was used as the mobile phase and was composed of sodium dihydrogen phosphate (90 mM), octanesulfonic acid (1.7 mM), citric acid (50 mM), EDTA (50 μ M), and acetonitrile (10%); upon mixing, it was brought to a final pH of 3 via addition of phosphoric acid.

Plasma prolactin collection—Subjects were acclimated to having an acute catheter (BD Saf-T-Intima Closed Catheter System, Franklin Lakes, NJ) unilaterally inserted into a saphenous vein prior to experimental collection of blood. For this assay, drug administration and blood collection were carried out 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 were collected 15 min before, immediately before, and 15, 30, 60, and 120 min after the injection of S,R(+/-)-MDMA or R(-)-MDMA. Samples were refrigerated in 3.5 ml serum separating vacutainers (BD, Franklin Lakes, NJ), 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 by the Biomarkers Core Laboratory of Yerkes National Primate Research Center using a fluorescence based enzyme linked immunosorbent assay (UV-ELISA) as previously described (Mook *et al*, 2005).

Data Analysis

The primary dependent variables in this study were extracellular concentrations of serotonin in the caudate nucleus and plasma concentrations of prolactin. Basal levels of either serotonin or prolactin across experiments were analyzed via a one-way repeated measures (RM) analysis of variance (ANOVA). Data were then normalized to the measured baseline levels in a given experimental session. Drug-induced changes in serotonin concentrations were analyzed by two-way RM ANOVA with the main factors of time and pretreatment. Across time, individual comparisons were determined at each time point, and corrected for multiple comparisons using Dunnett's method versus baseline levels. Across pretreatment, individual post-hoc comparisons were analyzed using a paired t-test between each time point during or after pretreatment with fluoxetine or saline. Pearson correlation analysis was used to determine the relationship between S,R(+/-)-MDMA-elicited serotonin release and prolactin secretion. Graphical presentation of all data depicts mean $\pm$ SEM. All graphical data presentations were created using GraphPad Prism 4 (La Jolla, CA), all statistical tests were performed using SigmaStat 3 (San Jose, CA), and significance was arbitrated at a $p < 0.05$.

Results

Basal serotonin and prolactin levels

Over all experimental sessions, mean basal levels of extracellular serotonin, uncorrected for probe recovery, and plasma prolactin were 0.836 ± 0.182 nM and 38.504 ± 18.131 ng/mg, respectively. Analysis by one-way RM ANOVA revealed no significant difference in basal levels of serotonin $F_{3,4} = 1.482$; $p = 0.282$) or prolactin ($F_{3,9} = 0.885$; $p = 0.551$) among experimental sessions. The powers of these tests were 0.126 and 0.050, respectively.

Correlation between S,R(+/-)-MDMA-elicited serotonin release and prolactin secretion—Concurrent microdialysis and plasma analysis was carried out to determine the temporal relationship between S,R(+/-)-MDMA-elicited serotonin release and prolactin secretion (Figure 2A). Pearson correlation analysis revealed that plasma prolactin concentrations were significantly and positively correlated with striatal extracellular serotonin levels ($r^2 = 0.850$; $p < 0.0001$) following administration of 1.7 mg/kg S,R(+/-)-MDMA (Figure 2B). Furthermore, there was also a significant relationship between striatal extracellular levels of the major serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) and plasma prolactin concentrations ($r^2 = 0.485$; $p = 0.01$; data not shown).

Effects of fluoxetine on serotonin release elicited by S,R(+/-)-MDMA or R(-)-MDMA—The effects of fluoxetine pretreatment were initially determined for serotonin release elicited by S,R(+/-)-MDMA (Figure 3A) or R(-)-MDMA (Figure 3B). A two-way RM ANOVA revealed a significant main effect of treatment with S,R(+/-)-MDMA on extracellular serotonin levels ($F_{3,6} = 4.496$; $p = 0.006$), no significant main effect of pretreatment with fluoxetine ($F_{3,1} = 5.282$; $p = 0.105$), but a significant interaction ($F_6 = 5.173$; $p = 0.003$). The powers of these tests were 0.847, 0.302, and 0.912, respectively. Post-hoc analysis revealed a significant effect of S,R(+/-)-MDMA following pretreatment with vehicle ($F_{3,6} = 5.029$; $p = 0.003$), and that serotonin levels following this treatment were significantly different from baseline at 20, 30, and 40 minutes. Following pretreatment with fluoxetine, there was no significant effect of treatment with S,R(+/-)-MDMA on extracellular serotonin levels ($F_{3,6} = 1.161$; $p = 0.369$). The powers of these tests were 0.901 and 0.080, respectively. A two-way RM ANOVA revealed a significant main effect of treatment with R(-)-MDMA on extracellular serotonin levels ($F_{3,6} = 4.292$; $p = 0.007$), no significant main effect of pretreatment with fluoxetine ($F_{3,1} = 8.905$; $p = 0.058$), but a significant interaction ($F_6 = 4.300$; $p = 0.007$). The powers of these tests were 0.820, 0.485, and 0.821, respectively. Post-hoc analysis revealed a significant effect of R(-)-MDMA following pretreatment with vehicle ($F_{3,6} = 4.305$; $p = 0.007$), and that serotonin levels following this treatment were significantly different from baseline at 20 minutes. Following pretreatment with fluoxetine, there was no significant effect of treatment with R(-)-MDMA on extracellular serotonin levels ($F_{3,6} = 2.169$; $p = 0.010$). The powers of these tests were 0.822 and 0.563, respectively.

Effects of fluoxetine at 3mg/kg on prolactin secretion—The dose (3.0 mg/kg) and pretreatment time (15 min) of fluoxetine were chosen based on preliminary experiments and attenuation of the behavioral effects of S,R(+/-)-MDMA (Fantegrossi *et al*, 2009; McClung *et al*, 2010). In those previous studies, fluoxetine did not have any overt behavioral effects in its own right at 3.0 mg/kg. Accordingly, we did not expect that it would have overt neurochemical and hormonal effects at that dose. Nevertheless, we collected prolactin measures before and after treatments with fluoxetine followed by the vehicle of MDMA, saline, over the same time-course used for the subsequent MDMA determinations (Figure 4). A two-way RM ANOVA revealed no significant main effect of fluoxetine treatment on prolactin levels ($F_{3,5} = 2.096$; $p = 0.123$). The power of this test was 0.281.

Effects of fluoxetine on S,R(+/-)-MDMA-elicited prolactin secretion—The role of the SERT in prolactin secretion elicited by S,R(+/-)-MDMA at 1.7 (Figure 5A) and 3.0 mg/kg (Figure 5B) was determined by pretreatments with fluoxetine. At 1.7 mg/kg of S,R(+/-)-MDMA, a two-way RM ANOVA revealed a significant main effect of both treatment with S,R(+/-)-MDMA ($F_{3,5} = 8.267$; $p < 0.001$) and pretreatment with fluoxetine ($F_{3,1} = 12.565$; $p = 0.038$), and a significant interaction ($F_5 = 14.962$; $p < 0.001$). The power of each of these tests was 0.987, 0.630, and 1.000, respectively. Post-hoc analysis revealed a significant effect of S,R(+/-)-MDMA following pretreatment with vehicle ($F_{3,5} = 10.448$; p

< 0.001), and that prolactin levels following this treatment were significantly different from baseline at 30 and 60 minutes. Following pretreatment with fluoxetine, there was also a significant effect of treatment with S,R(+/-)-MDMA ($F_{3,5} = 6.404$; $p = 0.002$); however, no time point was significantly different from baseline. The powers of these tests were 0.998 and 0.942, respectively. Post-hoc analysis via a paired t-test revealed that following pretreatment with either fluoxetine or its vehicle the effects of S,R(+/-)-MDMA on plasma prolactin levels were significantly different at 30 ($t_4 = 4.785$; $p = 0.017$) and 60 minutes ($t_4 = 4.401$; $p = 0.022$). Furthermore, fluoxetine had no significant effect on prolactin levels in its own right as prolactin levels were not significantly different, following administration of fluoxetine but prior to administration of S,R(+/-)-MDMA, at time point 0 ($t_4 = -2.317$; $p = 0.103$). The powers of these tests were 0.866, 0.809, and 0.307, respectively. The effects of S,R(+/-)-MDMA following pretreatment with fluoxetine were then evaluated at 3.0 mg/kg of S,R(+/-)-MDMA to determine whether a higher dose of S,R(+/-)-MDMA would surmount the effects of fluoxetine. One-way RM ANOVA revealed a significant main effect of S,R(+/-)-MDMA ($F_{3,5} = 7.767$; $p < 0.001$) despite pretreatment with fluoxetine. The power of this test was 0.980. Post-hoc comparisons showed that prolactin levels were significantly different from baseline at 15 and 30 minutes ($p < 0.050$) following this treatment.

Effects of fluoxetine on R(-)-MDMA-elicited prolactin secretion—The role of the SERT in prolactin secretion elicited by R(-)-MDMA at 1.0 (Figure 6A) and 1.7 mg/kg (Figure 6B) was then evaluated via the same pretreatment regimen used to determine its role in S,R(+/-)-MDMA-elicited prolactin secretion. Surprisingly, in contrast to its effects on S,R(+/-)-MDMA-elicited prolactin secretion, fluoxetine pretreatment did not significantly attenuated secretion of prolactin elicited by R(-)-MDMA. At 1.0 mg/kg of R(-)-MDMA, a two-way RM ANOVA revealed a significant main effect of treatment with R(-)-MDMA ($F_{3,5} = 6.745$; $p = 0.349$), but not pretreatment with fluoxetine ($F_{3,1} = 0.001$; $p = 0.969$), and no significant interaction ($F_5 = 1.215$; $p = 0.349$). The powers of these tests were 0.955, 0.052, and 0.086, respectively. Post-hoc analysis by means of Dunnett's test revealed a significant effect of R(-)-MDMA following pretreatment with vehicle ($F_{3,5} = 8.665$; $p < 0.001$), and that prolactin levels were significantly different from baseline at 15 and 30 minutes ($p < 0.05$) following this treatment. The same analysis procedure revealed no significant effect of R(-)-MDMA following pretreatment with fluoxetine ($F_{3,5} = 2.905$; $p = 0.050$). The powers of these tests were 0.991 and 0.480, respectively. At 1.7 mg/kg of R(-)-MDMA, a two-way RM ANOVA revealed a significant main effect of treatment with R(-)-MDMA ($F_{3,5} = 11.204$; $p < 0.001$), but not pretreatment with fluoxetine ($F_{3,1} = 0.110$; $p = 0.762$), and no significant interaction ($F_5 = 0.528$; $p = 0.752$). The powers of these tests were 0.999, 0.052, and 0.050, respectively. Post-hoc analysis by means of Dunnett's test showed that there was a significant effect of R(-)-MDMA following pretreatment with vehicle ($F_{3,5} = 4.062$; $p < 0.016$), and that prolactin levels were significantly different from baseline at 15 min ($p < 0.05$) following this treatment. The same analysis procedure revealed a significant effect of R(-)-MDMA following pretreatment with fluoxetine ($F_{3,5} = 29.680$; $p < 0.001$), and that prolactin levels were significantly different from baseline at 15 and 30 minutes ($p < 0.05$) following this treatment. The powers of these tests were 0.715 and 1.000, respectively.

Effects of 5-HT_{2A} antagonism on R(-)-MDMA-elicited prolactin secretion—Since fluoxetine pretreatment attenuated R(-)-MDMA-elicited serotonin release but not prolactin secretion and previous work has suggested that R(-)-MDMA can function as a direct agonist of the 5-HT_{2A} receptor (Fantegrossi *et al*, 2005; Nash *et al*, 1994), we determined the role of the 5-HT_{2A} receptor in prolactin secretion elicited by R(-)-MDMA (Figure 7). A two-way RM ANOVA revealed a significant main effect of treatment with R(-)-MDMA ($F_{3,5} = 5.660$; $p = 0.004$), but no main effect of pretreatment with M100907

($F_{3,1} = 0.010$; $p = 0.925$), and no significant interaction ($F_5 = 1.059$; $p = 0.421$). The powers of these tests were 0.899, 0.052, and 0.059, respectively. Following pretreatment with M100907, post-hoc analysis revealed no significant effect of R(-)-MDMA ($F_{3,5} = 2.573$; $p = 0.071$). The power of this test was 0.400.

Effects of combined 5-HT_{2A} antagonism and fluoxetine administration on R(-)-MDMA-elicited prolactin secretion—Finally, since M100907 pretreatment alone did not significantly attenuate prolactin secretion elicited by R(-)-MDMA, experiments were carried out to determine whether combined pretreatment with M100907 and fluoxetine (at the same doses previously tested) would effectively antagonize R(-)-MDMA-elicited prolactin secretion (Figure 8). A two-way RM ANOVA revealed a significant main effect of treatment with R(-)-MDMA ($F_{3,5} = 9.710$; $p < 0.001$), a significant main effect of combined pretreatment with M100907 and fluoxetine ($F_{3,5} = 3.173$; $p = 0.031$), but no significant interaction ($F_5 = 1.445$; $p = 0.265$). The powers of these tests were 0.996, 0.322, and 0.131, respectively. Post-hoc analysis revealed no significant effect of treatment with R(-)-MDMA ($F_{3,5} = 2.594$; $p = 0.070$) following combined pretreatment with M100907 and fluoxetine. The power of this test was 0.405. Post-hoc analysis via a paired t-test revealed a significant difference due to pretreatment at 15 ($t_4 = 4.261$; $p = 0.012$) and 30 minutes ($t_4 = 4.341$; $p = 0.031$). Furthermore, this combined pretreatment had no significant effect on prolactin levels in its own right as prolactin levels were not significantly different at time point 0 ($t_4 = -1.317$; $p = 0.243$). The powers of these tests were 0.842, 0.871, and 0.452, respectively.

Discussion

The major finding of this study is that S,R(+/-)-MDMA-elicited prolactin secretion is mediated by serotonin release whereas R(-)-MDMA-elicited prolactin secretion is mediated by serotonin release or direct agonism of 5-HT_{2A} receptors. In initial experiments, we used concurrent microdialysis and plasma analysis to establish the temporal relationship between changes in extracellular serotonin and changes in plasma prolactin. Previous work has shown that serotonin neurons in the dorsal raphe innervate neurons in the paraventricular nucleus of the hypothalamus that stimulate prolactin secretion (Emiliano *et al*, 2004), and that serotonin releasers (Aloi *et al*, 1984; Baumann *et al*, 2008; Hatzidimitriou *et al*, 2002; Murnane *et al*, 2010) and SSRIs (Cowen and Sargent, 1997; Muller *et al*, 1983; Spigset and Mjorndal, 1997) can elicit secretion of prolactin. However, the direct temporal correspondence between brain serotonin and plasma prolactin levels established in the present experiments further supports the hypothesized relationship between increased serotonin release and prolactin secretion. Indeed, using this approach, brain serotonin levels were positively correlated with plasma prolactin levels. Nevertheless, an important caveat to these results is the site directed nature of microdialysis measures. As such, it is possible that there is a less pronounced relationship between release of serotonin in other brain regions and prolactin secretion. Future research should examine this possibility.

In subsequent experiments, we determined the role of the SERT in S,R(+/-)-MDMA- and R(-)-MDMA elicited prolactin secretion in rhesus monkeys. It is well established in rodent models that S,R(+/-)-MDMA induces a rapid and robust *in vivo* increase in extracellular serotonin levels (Baumann *et al*, 2008; Gough *et al*, 1991; Gudelsky and Nash, 1996; Mehan *et al*, 2002; Yamamoto *et al*, 1995), and these findings have found recent support in a nonhuman primate model (Murnane *et al*, 2010). Moreover, S,R(+/-)-MDMA-elicited serotonin release can be attenuated by fluoxetine in the rat (Gudelsky *et al*, 1996; Mehan *et al*, 2002) or in synaptosomes (Berger *et al*, 1992). This functionally antagonistic effect of fluoxetine on MDMA-elicited increases in extracellular serotonin levels is likely mediated by the distinct mechanisms of action of these drugs (Green *et al*, 2003; Sulzer *et al*, 2005; Fleckenstein *et al*, 2007). As fluoxetine is a reuptake inhibitor and MDMA is a substrate-

based releaser, and substrate-based releasers generally have more pronounced effects on extracellular neurotransmitter levels than reuptake inhibitors, this could be conceptualized as being similar to the antagonistic effects of a partial agonist on the effects of a full agonist of the same receptor, and is likely mediated through direct competition for the SERT. Despite these findings, similar drug interaction work has not been conducted with S,R(+/-)-MDMA in primate models or with R(-)-MDMA in any *in vivo* model system. In the present study, fluoxetine attenuated serotonin release by either S,R(+/-)-MDMA or R(-)-MDMA in rhesus monkeys. Accordingly, similar to rodents, these findings demonstrate that the SERT is a critical mediator of serotonin release elicited by these forms of MDMA in nonhuman primates.

In subsequent experiments, we determined the role of the SERT in S,R(+/-)-MDMA- and R(-)-MDMA-elicited prolactin secretion. Previous work has shown that pretreatment with SERT inhibitors attenuates many of the behavioral of S,R(+/-)-MDMA in mice, rats, and monkeys and subjective effects of S,R(+/-)-MDMA in humans (Fantegrossi *et al*, 2009; Liechti *et al*, 2000; Liechti *et al*, 2001; McClung *et al*, 2010; Stein and Rink, 1999). Moreover, in both the present work and previous studies (Gudelsky *et al*, 1996; Mechan *et al*, 2002), S,R(+/-)-MDMA-elicited serotonin release was also attenuated by pretreatment with an SSRI. This suggests that the SERT-mediated effects of MDMA may contribute to its behavioral and subjective effects. In this regard, pretreatment with the SSRI fluoxetine attenuated prolactin secretion elicited by administration of S,R(+/-)-MDMA. Furthermore, this effect was surmountable as a higher dose of S,R(+/-)-MDMA elicited prolactin secretion even in the presence of fluoxetine, suggesting a competitive interaction at the SERT. These results indicate that prolactin secretion may contribute to the behavioral and subjective effects of S,R(+/-)-MDMA, and some of the behavioral or subjective effects of S,R(+/-)-MDMA may be prolactin dependent. To dissociate the relative role of prolactin secretion and serotonin release in these effects, the use of selective antagonists for serotonin and prolactin receptors may be required. Regardless of which molecule mediates specific behavioral or subjective effects of S,R(+/-)-MDMA, these data clearly support the role of the SERT in prolactin secretion by S,R(+/-)-MDMA, and likely other compounds.

Despite the clear role of the SERT in S,R(+/-)-MDMA-elicited prolactin secretion, the role of the SERT in R(-)-MDMA-elicited prolactin secretion was more complex. Although it effectively attenuated S,R(+/-)-MDMA-elicited prolactin secretion, fluoxetine was largely ineffective at attenuating R(-)-MDMA-elicited prolactin secretion. This could have resulted from fluoxetine ineffectively attenuating serotonin release by R(-)-MDMA. However, direct microdialysis experiments eliminated this as a possible explanation, suggesting that some effect other than serotonin release mediated R(-)-MDMA-elicited prolactin secretion. Previous work has shown that direct 5-HT_{2A} receptor agonists can elicit prolactin secretion (Aulakh *et al*, 1994; Biezonski *et al*, 2009), and that R(-)-MDMA can function as a direct agonist of the 5-HT_{2A} receptor both *in vitro* (Nash *et al*, 1994) and *in vivo* (Fantegrossi *et al*, 2005). Based on this work, it is reasonable to suggest *in vivo* agonism of 5-HT_{2A} receptors by R(-)-MDMA may contribute to its prolactin secreting effects. Nevertheless, selective antagonism of the 5-HT_{2A} receptor only partially attenuated prolactin secretion elicited by R(-)-MDMA. It is important to note that other serotonin receptors have also been implicated in prolactin secretion (Aulakh *et al*, 1994; Meltzer and Maes, 1995). In other words, prolactin secretion is unlikely to be a 5-HT_{2A} receptor selective effect. As such, R(-)-MDMA may retain its prolactin secreting effects in the presence of blockade of 5-HT_{2A} receptors by increasing extracellular levels of serotonin and thereby indirectly activate other serotonin receptors. In support of this contention, combined pretreatment with fluoxetine and M100907 completely attenuated R(-)-MDMA-elicited prolactin secretion. This pattern of findings supports previous suggestions that R(-)-MDMA is both a serotonin releaser and a direct agonist of the 5-HT_{2A} receptor *in vivo*, and combined treatment with a

SSRI and a 5-HT_{2A} receptor antagonism may be necessary to attenuate some of the behavioral and subjective effects of R(–)-MDMA. Moreover, these data further elucidate the neuropharmacology of prolactin regulation.

In summary, in the present report, administration of S,R(+/-)-MDMA and its stereoisomer R(–)-MDMA resulted in elevated extracellular serotonin levels and increased secretion of prolactin in rhesus monkeys. Following administration of S,R(+/-)-MDMA, serotonin levels significantly and positively predicted prolactin levels, and pretreatment with the SSRI fluoxetine attenuated prolactin secretion elicited by this form of MDMA. In contrast, combined pretreatment with fluoxetine and the selective 5-HT_{2A} receptor antagonist M100907 was required to attenuate R(–)-MDMA-elicited prolactin secretion. These results indicate that S,R(+/-)-MDMA elicits prolactin secretion through serotonin release, whereas R(–)-MDMA elicits prolactin secretion through both serotonin release and direct agonism of the 5-HT_{2A} receptor. Despite the complexity of the underlying pharmacology, this work extends previous findings and significantly strengthens the support for the causative role of serotonin in prolactin secretion. Accordingly, these findings have important implications for our understanding of the psychobiology of S,R(+/-)-MDMA and R(–)-MDMA, and they further elucidate the regulatory role of serotonin in prolactin secretion.

Research highlights

Serotonin release and prolactin secretion are tightly and positively correlated
 Fluoxetine attenuates racemic MDMA-elicited serotonin release and prolactin secretion
 Agonism of 5-HT_{2A} receptors contributes to R(–)-MDMA-elicited prolactin secretion
 Supports a causal role for serotonin activity in prolactin secretion
 Suggests SSRIs and 5-HT_{2A} agonists may facilitate hyperprolactinemia

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Figure 1.
Chemical structures of the test compounds used in the study; including MDMA (A), fluoxetine (B), and M100907 (C).

Figure 2. Time course of serotonin release (closed squares; A) and prolactin secretion (open circles; A) elicited by S,R(+/-)-MDMA (1.7 mg/kg, IV) and the correlation between each effect (B). *Abcissae*: Time expressed in minutes in reference to the administration of S,R(+/-)-MDMA and plotted on a linear scale (A) or absolute change in plasma prolactin

concentration following administration of S,R(+/-)-MDMA (B). *Ordinates*: Absolute change in plasma prolactin concentration following administration of S,R(+/-)-MDMA (A, left) or absolute change in extracellular serotonin concentration following administration of S,R(+/-)-MDMA (A, right and B, left).

Figure 3.

Effects of pretreatment with fluoxetine (3 mg/kg, IV) on serotonin release within the striatum elicited by IV administration of 1.7 mg/kg of S,R(+/-)-MDMA (A) or R(-)-MDMA (B). *Abcissae*: 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 caudate nucleus expressed as a percent of baseline levels. An * indicates a significant difference from baseline assessed via a one-way RM ANOVA with post-hoc analysis carried out by Dunnett's method.

Figure 4. Effects of fluoxetine (3 mg/kg, IV) on circulating prolactin levels. *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.

Figure 5.

Effects of pretreatment with fluoxetine (3 mg/kg, IV) on S,R(+/-)-MDMA-elicited prolactin secretion. S,R(+/-)-MDMA was administered intravenously at 1.7 (A) and 3.0 mg/kg (B) following pretreatment with fluoxetine (closed symbols) or saline (open symbols). Abscissae and ordinates are the same as in Figure 4. An * indicates a significant difference from baseline assessed via a one-way RM ANOVA with post-hoc analysis carried out using Dunnett's test. A # indicates a significant effect of fluoxetine pretreatment compared to saline pretreatment at a given time point as assessed by a paired t-test.

Figure 6. Effects of pretreatment with fluoxetine (3 mg/kg, IV) on R(-)-MDMA-elicited prolactin secretion. R(-)-MDMA was administered intravenously at 1.0 (A) and 1.7 mg/kg (B)

following pretreatment with fluoxetine (open symbols) or saline (closed symbols). Abscissae and ordinates are the same as in Figure 4, and asterisks are the same as in Figure 5.

Figure 7. Determination of the effects of pretreatment with M100907 (0.3 mg/kg, IV) on R(-)-MDMA-elicited prolactin secretion. Abscissae and ordinates are the same as in Figure 4, and asterisks are the same as in Figure 5.

Figure 8.

Effects of combined pretreatment with both fluoxetine (3.0 mg/kg, IV) and M100907 (0.3 mg/kg, IV) on R(-)-MDMA-elicited prolactin secretion. Abscissae, ordinates, and asterisks are the same as in Figure 4. A # indicates a significant effect of fluoxetine pretreatment compared to saline pretreatment at a given time point as assessed by a paired t-test.