Disruption of prepulse inhibition by 3,4-methylenedioxymethamphetamine (MDMA): comparison between male and female wild-type and 5-HT\textsubscript{1A} receptor knockout mice

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Abstract

The aim of this study was to investigate the involvement of serotonin-1A (5-HT\textsubscript{1A}) receptors in the effects of 3,4-methylenedioxymethamphetamine (MDMA) on prepulse inhibition of acoustic startle (PPI) by comparing male and female wild-type (WT) mice and 5-HT\textsubscript{1A} receptor knockout (1AKO) mice. MDMA dose-dependently decreased PPI in male and female mice although female mice were more sensitive at the 100-ms inter-stimulus interval (ISI). In male mice, 10 mg/kg MDMA disrupted PPI in 1AKO but not in WT controls. There was no genotype difference at higher or lower doses of MDMA. In female mice, there was no difference between genotypes at any dose of MDMA. Average startle was reduced by 10 mg/kg and 20 mg/kg MDMA similarly in male and female mice and all genotypes. These results show an involvement of 5-HT\textsubscript{1A} receptors in the effect of MDMA on PPI in male, but not female mice.

Key words: Ecstasy, 5-HT\textsubscript{1A} receptor, MDMA, prepulse inhibition, schizophrenia, serotonin.

Introduction

The increased recreational use of the illicit drug, ‘Ecstasy’ (3,4-methylenedioxymethamphetamine; MDMA), and its potential detrimental long-term effects (Kalant, 2001) have sparked many investigations into its neurophysiological and neuropharmacological mechanism of action. In human studies, acute treatment with MDMA increases feelings of elation and intimacy and reduced anxiety without major perceptual changes or hallucinations (Dumont & Verkes, 2006; Parrott, 2001). These effects are predominantly mediated by central serotonin (5-HT) release (Rudnick & Wall, 1992) and could be inhibited by pretreatment with 5-HT transporter inhibitors, such as citalopram (Liechti & Vollenweider, 2000b). The 5-HT\textsubscript{2A} receptor (5-HT\textsubscript{2A}R) antagonist, ketanserin, or the dopamine D\textsubscript{2} receptor antagonist, haloperidol, modulated some aspects of the neuropsychological and physiological effects of MDMA (Liechti & Vollenweider, 2000a, b; Liechti et al. 2000).

Several MDMA studies have used prepulse inhibition of acoustic startle (PPI) to more precisely delineate its actions. PPI is a model of sensory gating, a mechanism allowing filtering of sensory stimuli and focused attention (Braff et al. 2001; van den Buuse, 2010). There were no changes in PPI in chronic MDMA users (Quednow et al. 2004). However, acute administration of MDMA increased PPI in human volunteers but decreased it in rats (Vollenweider et al. 1999). The effect of MDMA on PPI in humans was attenuated by citalopram, but not ketanserin or haloperidol (Liechti et al. 2001b). A role of 5-HT\textsubscript{1B}R\textsubscript{S} was suggested by the observation that MDMA increased PPI in 5-HT\textsubscript{1B}R knockout mice, but not wild-type (WT) controls (Dulawa et al. 2000b). Similar experiments have not been done to assess the role of 5-HT\textsubscript{1A}R\textsubscript{S} in the effects...
of MDMA. Therefore, the aim of the present study was to compare the effect of MDMA on PPI in WT C57Bl/6 mice and 5-HT1A knockout mice. We also compared male and female mice as previous studies have suggested sex differences in the action of MDMA, generally showing that females are more sensitive to its effects than males (Allott & Redman, 2007; Liechti et al. 2001a; Palenicek et al. 2005; Walker et al. 2007).

Methods

Animals

Male and female mice were obtained from a breeding colony at the Mental Health Research Institute which was established with breeders kindly provided by Professor Mark Geyer and Dr Victoria Risbrough (Department of Psychiatry, University of California at San Diego, USA). The colony had been backcrossed for over 10 generations to a C57Bl/6 background. We used 5-HT1A+/− (heterozygous) mice as breeders and offspring were therefore either wild-type (5-HT1A+/+, WT), heterozygous (5-HT1A+/−, 1AHet) or knockout (5-HT1A−/−, 1AKO). The genotypes were confirmed at weaning by polymerase chain reaction (PCR) methodology. DNA was extracted from tail samples by the ‘Hotshot’ method and primers were as follows:

Primer A (upper primer):

5′-AAC TAT CTC ATC GCC TCC TTG-3′,

Primer B (upper neo primer):

5′-GTT AAG AAG GGT GAG AAC AGA-3′,

Primer C (lower primer):

5′-CTT CTT TTC CAC CTG CTT GAC-3′,

with primers A and C forming the WT product and primers B and C forming the KO product (Dulawa et al. 2000a).

The animals were housed in same-sex groups of 1–5 in standard plastic mouse cages with free access to pellet food and tap water. The environment was temperature-controlled (21 ± 2 °C) with a 12-h light/dark cycle (lights on 07:00 hours). At the time of PPI testing, the mice were aged 12–20 wk. PPI experiments were performed between 09:00 and 17:00 hours. All experiments were approved and conducted in accordance to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes set out by the National Health and Medical Research Council of Australia.

PPI

PPI was measured as previously described (van den Buuse & Gogos, 2007; van den Buuse et al. 2009) using eight automated startle chambers (SR-Lab; San Diego Instruments, USA). Over a continuous 70-dB intensity background noise, a total of 104 stimuli were delivered. There were eight pulse-alone trials, consisting of a 40-ms burst of 115-dB white noise, at the beginning and end of the session. The middle 88 trials consisted of the pseudo-random presentation of 16 additional pulse-alone trials, and four groups of eight prepulse-pulse trials at 2, 4, 8 and 16 dB over baseline. The inter-stimulus interval (ISI) between the prepulse and pulse was set at 30 ms or 100 ms (van den Buuse & Gogos, 2007) and these ISIs were pseudo-randomly mixed throughout the session. In addition, there were eight NOSTIM trials during which no stimulus was delivered. Data obtained with the 32 P115 pulse-alone trials and the four different prepulse intensities were averaged and used for statistical analysis (Fig. 1).

Experimental protocol

We obtained (+)3,4-methylenedioxymethamphetamine (MDMA) from the National Measurement Institute, Linfield, NSW, Australia. Drug doses were dissolved and diluted in sterile 0.9% saline and administered intraperitoneally (i.p) with an injection volume of 10 ml/kg 5–10 min before the start of the PPI session.

There were two series of within-animal, repeated-measures experiments. In the first series of four PPI experiments, animals were tested after injection of saline, 10 mg/kg MDMA, 20 mg/kg MDMA, and saline, respectively, with 3–4 d between treatments. The last saline session was used to ascertain that MDMA did not cause long-lasting changes in PPI. Because of the results in the first series of experiments, a second series of four PPI tests was conducted in a separate cohort of mice with saline, 5 mg/kg MDMA, 10 mg/kg MDMA, and saline as the respective treatments. In both experiments, a pseudo-randomized within-animal repeated-measures protocol was used (van den Buuse & Gogos, 2007; van den Buuse et al. 2009). Because of the similarity between the results in the two main cohorts, data obtained in the two cohorts of animals were combined (see Table 1).

Data analysis

All data were expressed as the mean ± standard error of the mean (S.E.M.) and analysed with analysis of variance (ANOVA) with repeated measures (Systat 9.0, SPSS Inc., USA). The between-group statistical factor was genotype and repeated-measures factors were prepulse (four different prepulse intensities) and drug dose (vs. their respective ‘saline’ result). To simplify data analysis and presentation, data from
male and female mice and at the 100-ms and 30-ms ISIs were analysed separately. Furthermore, because the prepulse level always resulted in a significant main effect, irrespective of the group or treatment, details of this factor will not be included here. A p value of <0.05 was considered to be statistically significant.

Results

Male mice

In male mice, there was no effect of 5 mg/kg MDMA on PPI at the 100-ms ISI. In contrast, 10 mg/kg MDMA significantly disrupted PPI (main effect of dose: $F_{1,53} = 36.1, p < 0.001$) and this effect differed between genotypes (dose $\times$ genotype interaction: $F_{1,53} = 3.7, p = 0.031$). Further analysis by genotype revealed that this dose of MDMA had no effect in WT mice, but significantly disrupted PPI in 1AHet mice and 1AKO mice ($F_{1,17} = 15.4$ and 21.9, $p = 0.001$ and $p < 0.001$, respectively). However, comparison of the effect of 10 mg/kg MDMA on PPI between 1AHet mice and WT controls failed to show a significant difference between the genotypes. Comparison of the effect of this dose between 1AKO mice and WT controls confirmed a genotype-dependent PPI disruption in 1AKO mice only (dose $\times$ genotype interaction: $F_{1,36} = 6.8, p = 0.013$). Treatment with 20 mg/kg MDMA significantly disrupted PPI ($F_{1,13} = 118.2, p < 0.001$) but this effect was similar in the three genotypes (Fig. 1). There was no difference between baseline PPI after saline injection in the first or last session of the series of tests, suggesting there were no lasting effects of MDMA on baseline PPI (data not shown).

In male mice, all doses of MDMA caused disruption of PPI at the 30-ms ISI ($F_{1,53} = 27.5, F_{1,53} = 82.4, F_{1,23} = 38.3$; all $p < 0.001$) but there were no differences between the genotypes at this ISI (Fig. 1). PPI was slightly higher in the last control PPI session compared to the first ($F_{1,17} = 8.8, p = 0.004$) although this was similar between the genotypes (data not shown).

Average startle was not affected by 5 mg/kg MDMA in male mice (Table 1). In contrast, both the
was done comparing the effect of 10 mg/kg MDMA in male, but not in female mice, an additional ANOVA because statistical analysis suggested genotype effects.

Sex differences

Between baseline PPI after saline injection in the first or last session of the series of tests (Fig. 1). There was no difference between the first and last PPI control session in terms of average startle (data not shown).

<table>
<thead>
<tr>
<th>Treatment/genotype</th>
<th>WT mice (n)</th>
<th>1AHet mice (n)</th>
<th>1AKO mice (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male mice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>106 ± 8 (20)</td>
<td>109 ± 10 (18)</td>
<td>131 ± 9 (18)</td>
</tr>
<tr>
<td>MDMA 5 mg/kg</td>
<td>112 ± 20 (11)</td>
<td>120 ± 21 (12)</td>
<td>149 ± 27 (7)</td>
</tr>
<tr>
<td>MDMA 10 mg/kg</td>
<td>80 ± 8* (20)</td>
<td>80 ± 10* (18)</td>
<td>90 ± 12* (18)</td>
</tr>
<tr>
<td>MDMA 20 mg/kg</td>
<td>73 ± 8* (10)</td>
<td>73 ± 14* (6)</td>
<td>72 ± 10* (11)</td>
</tr>
<tr>
<td>Female mice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>102 ± 8 (20)</td>
<td>101 ± 11 (21)</td>
<td>95 ± 10 (20)</td>
</tr>
<tr>
<td>MDMA 5 mg/kg</td>
<td>75 ± 8* (13)</td>
<td>77 ± 10* (13)</td>
<td>79 ± 8* (13)</td>
</tr>
<tr>
<td>MDMA 10 mg/kg</td>
<td>49 ± 3* (20)</td>
<td>56 ± 5* (21)</td>
<td>58 ± 6* (20)</td>
</tr>
<tr>
<td>MDMA 20 mg/kg</td>
<td>55 ± 44* (7)</td>
<td>62 ± 5* (8)</td>
<td>63 ± 7* (7)</td>
</tr>
</tbody>
</table>

WT, Wild type; 1AHet, heterozygous 5-HT₁A receptor knockout; 1AKO, homozygous 5-HT₁A receptor knockout.

* p < 0.05 for difference with saline treatment (ANOVA main effect of dose).

10 mg/kg and 20 mg/kg dose significantly reduced startle (F₁,₅₈ = 48.9 and F₁,₂₉ = 34.6, both p < 0.001), but this was similar in all genotypes (Table 1). There was no difference between the first and last PPI control session in terms of average startle (data not shown).

**Female mice**

In female mice, there was no effect of 5 mg/kg MDMA on PPI at the 100-ms ISI (Fig. 1). In contrast, both the 10 mg/kg and 20 mg/kg dose significantly disrupted PPI at this ISI (F₁,₅₈ = 59.2 and F₁,₂₉ = 100.7, respectively, both p < 0.001) although there were no differences between genotypes (Fig. 1). There was no difference between baseline PPI after saline injection in the first or last session of the series of tests (Fig. 1).

All doses of MDMA significantly disrupted PPI at the 30-ms ISI (F₁,₅₈ = 31.9, F₁,₅₈ = 103.1, F₁,₁₉ = 29.1, respectively; all p < 0.001) and this effect was not different between the genotypes (Fig. 1). Again, baseline PPI after saline injection tended to be higher in the last control session compared to the first (F₁,₅₈ = 5.5, p = 0.022), but this was similar in all three genotypes.

Average startle was significantly reduced by all doses of MDMA in female mice (F₁,₅₈ = 33.1, F₁,₅₈ = 164.8 and F₁,₁₉ = 46.2 for 5, 10 and 20 mg/kg, respectively; all p < 0.001). However, as in male mice, again this effect was similar between the genotypes (Table 1). Startle was slightly, but significantly lower in the last saline-control session compared to the first (F₁,₅₈ = 11.9, p = 0.001) (data not shown).

**Sex differences**

Because statistical analysis suggested genotype effects in male, but not in female mice, an additional ANOVA was done comparing the effect of 10 mg/kg MDMA between the sexes. In WT mice, a significant dose × sex interaction (F₁,₃₈ = 5.4, p = 0.026) confirmed the observation that female WT mice show a PPI disruption after this dose of MDMA whereas male mice do not (Fig. 1). There were also main effects of sex (F₁,₃₈ = 9.0, p = 0.005), confirming that female mice were more sensitive to MDMA at this dose than male mice, and a main effect of dose (F₁,₃₈ = 17.4, p < 0.001), confirming that MDMA disrupts PPI. No differential effect of MDMA was found at the 30-ms ISI (main effect of dose only: F₁,₃₈ = 38.4, p < 0.001) or when male and female 1AKO mice were compared either at the 100-ms ISI (main effect of sex and of dose only: F₁,₃₈ = 7.1 and 47.8, p = 0.011 and p < 0.001, respectively) or at the 30-ms ISI (main effect of dose only: F₁,₃₈ = 81.3, p < 0.001). Finally, although male 1AKO mice were more sensitive to the action of MDMA on PPI at the 100-ms ISI than male WT mice (see above), there was no difference in the effect of 10 mg/kg MDMA between male 1AKO and female WT mice (Fig. 1).

**Discussion**

The main results of this study were: (1) MDMA dose-dependently disrupted PPI and reduced startle amplitude in male and female mice; (2) male 1AKO mice were more sensitive than male WT mice to the effect of MDMA at the 100-ms ISI; (3) female mice were more sensitive to the effect of MDMA than male mice but showed no difference between the genotypes; (4) no genotype differences were observed for PPI at the 30-ms ISI; (5) no genotype differences were observed for startle amplitudes. Therefore, these results suggest an involvement of the 5-HT₁A R in the effect of MDMA on PPI at the longer ISI in male, but not female mice.
An involvement of 5-HT\textsubscript{1A}Rs in the action of MDMA has not been clearly established. In human volunteers, there were only limited effects of pre-treatment with the 5-HT\textsubscript{1A}R antagonist, pindolol, on the MDMA-induced changes in sustained attention and visual-spatial memory (Hasler et al. 2009), while in rats, MDMA-induced locomotor hyperactivity was modulated only to a minor extent by pre-treatment with the 5-HT\textsubscript{1A}R antagonist, WAY 100,635 (McCreary et al. 1999). On the other hand, acute facilitation of social interaction by MDMA in rats was prevented by pre-treatment with WAY 100,635, but not by a 5-HT\textsubscript{1B}R or 5-HT\textsubscript{1A}R antagonist (Morley et al. 2005).

The effect of MDMA on PPI has not been studied in 1AKO mice before. In mice, 5-HT\textsubscript{1A}R and 5-HT\textsubscript{1B}R activation has opposing effects on PPI, with increasing and decreasing effects, respectively (Dulawa et al. 2000a). Thus the most parsimonious explanation for the present results – increased sensitivity to PPI disruptive effects of MDMA in male 1AKO mice – is that PPI increases via 5-HT\textsubscript{1A} activation are not occurring in these mice, resulting in a greater net decrease in PPI, probably via 5-HT\textsubscript{1B} activation. Alternatively, compensatory changes in remaining 5-HT receptor signalling in 1AKO mice (e.g. increased 5-HT\textsubscript{1B}R signalling) may also have contributed to the increased sensitivity to MDMA effects (Ramboz et al. 1998). Overall, however, these data indicate that 5-HT\textsubscript{1A} contributions to MDMA effects are relatively subtle, and also suggest a number of reasons why the role of 5-HT\textsubscript{1A} activation has been unclear so far. First, the difference between male 1AKO mice and WT controls in our study was limited to one dose only (10 mg/kg) and was seen only at the 100-ms ISI. However, it should be noted that at the 30-ms ISI, the effect of even the lowest dose of MDMA was already near maximum and a ‘floor-effect’ can therefore not be excluded at this ISI. Further studies will have to be performed in order to show if a similar genotype effect at the 30-ms ISI would be found if lower doses of MDMA were tested. Second, a difference between 1AKO and WT controls was not seen in female mice. This means that studies using female mice (or rats) or mixed male/female cohorts could miss an involvement of 5-HT\textsubscript{1A}Rs. Indeed, female mice have been shown to be less sensitive to 5-HT\textsubscript{1A} null mutation effects on anxiety-like and exploratory behaviours as well (Ramboz et al. 1998), suggesting that there are sex differences in 5-HT\textsubscript{1A} effects on a number of behaviours.

Our results also suggest that female mice were more sensitive to the disruption of PPI than male mice, at least at the 100-ms ISI. This observation is consistent with previous studies which have suggested sex differences in the effects of MDMA (reviewed in Allott & Redman, 2007). For example, the subjective neuropsychological effects of MDMA were stronger in women than in men (Liechti et al. 2001a) although physiological effects and long-term side-effects were more pronounced in men than in women (Allott & Redman, 2007; Liechti et al. 2001a). Moreover, in rats, the behavioural effects of MDMA have been shown to be more marked in females than in males (Palenicek et al. 2005; Walker et al. 2007). It has been suggested that, at least in rats, sex differences in the metabolism of MDMA to 3,4-methylenedioxymethamphetamine (MDA) might account for some of the reported male/female differences in the behavioural effects of MDMA (Fonsart et al. 2009). Further studies are needed comparing effects of MDMA metabolites on PPI to investigate this possibility.

In conclusion, using 1AKO mice, we were able to show a subtle, but significant involvement of this receptor in the effect of MDMA on PPI. This involvement was seen in male mice, but not female mice. These results extend our insight into the acute pharmacology of MDMA.

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Statement of Interest

None.

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