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The hyperthermic and neurotoxic effects of 'Ecstasy' (MDMA) and 3,4 methylenedioxyamphetamine (MDA) in the Dark Agouti (DA) rat, a model of the CYP2D6 poor metabolizer phenotype

[1]M.I. Colado, J.L. Williams & [2]A.R. Green

Astra Neuroscience Research Unit, 1 Wakefield Street, London WC1N 1PJ

1 The effect of administration of 3,4-methylenedioxymethamphetamine (MDMA or 'Ecstasy') and its N-demethylated product, 3,4-methylenedioxyamphetamine (MDA) on both rectal temperature and long term neurotoxic loss of cerebral 5-hydroxytryptamine (5-HT) has been studied in male and female Dark Agouti (DA) rats. The female metabolizes debrisoquine more slowly than the male and its use has been suggested as a model of the human debrisoquine 4-hydroxylase poor metabolizer phenotype.

2 A novel h.p.l.c. method was developed and used to measure plasma MDMA and MDA concentrations in the DA rats.

3 The hyperthermic response following MDMA was enhanced in female rats. Plasma MDMA concentrations were also 57% higher than in males 45 min post-injection, while plasma concentrations of MDA were 48% lower.

4 Plasma concentrations of MDMA and MDA in male rats were unaffected by pretreatment with proadifen (15 mg kg^{-1}) or quinidine (60 mg kg^{-1}), but the hyperthermic response to MDMA (10 mg kg^{-1}, i.p.) was enhanced by quinidine pretreatment.

5 The hyperthermic response following MDA was greater in male DA rats, despite plasma drug concentrations being 40% higher in females 60 min after injection.

6 Seven days after a single dose of MDMA (10 mg kg^{-1}, i.p.) there was a substantial loss in the concentration of 5-HT and 5-hydroxyindoleacetic acid (5-HIA) in cortex and hippocampus. [^{3}H]-paroxetine binding was also decreased by 27% in the cortex, indicating that the amine loss reflected a neurodegenerative change. MDMA (5 mg kg^{-1}, i.p.) was without effect on brain 5-HT content.

7 A single dose of MDA (5 mg kg^{-1}, i.p.) produced a major (approximately 40%) loss of 5-HT content of cortex and hippocampus 7 days later. The loss was similar in males and females.

8 These data demonstrate that female DA rats are more susceptible to the acute hyperthermic effects of MDMA, probably because of impaired N-demethylation and indicate that in human subjects acute MDMA-induced toxicity may be exacerbated in poor metabolizer phenotypes. Low debrisoquine hydroxylase activity did not appear to impair the formation of a MDMA or MDA neurotoxic metabolite. Both severe acute hyperthermia and delayed neurotoxicity occurred following plasma levels of MDMA comparable to those reported in persons misusing the drug.

Keywords: 3,4-Methylenedioxymethamphetamine; 3,4-methyldioxyamphetamine; 'Ecstasy'; hyperthermia; neurotoxicity; debrisoquine hydroxylase; Dark Agouti rats; neurodegeneration; quinidine; 5-hydroxytryptamine

Introduction

'Ecstasy' (3,4-methylenedioxymethamphetamine or MDMA) is a commonly misused recreational drug (Peroutka, 1987). Administration of this compound produces behavioural abnormalities resembling the 'serotonin syndrome' and marked hyperthermia in both rats (Nash *et al.*, 1988; Schmidt *et al.*, 1990; Gordon *et al.*, 1991; Colado *et al.*, 1993; Dafters 1994; Spanos & Yamamoto 1989; Slikker *et al.*, 1989; Callaway *et al.*, 1992) and man (Brown & Osterloh, 1987; Chadwick *et al.*, 1991; Henry *et al.*, 1992). The compound also produces a long term neurotoxic loss of 5-hydroxytryptamine (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in several brain regions of rats (Stone *et al.*, 1986; Battaglia *et al.*, 1987; Schmidt 1987; Colado *et al.*, 1993) and several species of non-human primates (Ricaurte *et al.*, 1988a,b; Insel *et al.*, 1989; Wilson *et al.*, 1989). There is good histological evidence that this amine loss reflects neurodegenerative changes that have occurred (O'Hearn *et al.*, 1988; Molliver *et al.*, 1990) and this is supported by biochemical data. For example, following MDMA administration, there is a loss of [^{3}H]-paroxetine binding to the presynaptic 5-HT transporter in the cortex (Battaglia *et al.*, 1987; Sharkey *et al.*, 1991; Nash *et al.*, 1991; Hewitt & Green 1994) and also reduced high affinity uptake of [^{3}H]-5-HT in cortical homogenates (Commins *et al.*, 1987; Hewitt & Green, 1994). It is not known whether similar changes occur in the brains of humans who misuse the drug, but a recent review of the indirect clinical evidence gave no grounds for reassurance (Green *et al.*, 1995).

One of the clinical problems concerning the acute toxic effect of MDMA is that the hyperthermia does not appear to be closely related to the amount of drug ingested (Henry *et al.*, 1992), although the veracity of subjects and ingestion of other drugs complicates interpretation. A recent possible explanation for the apparent lack of a dose-response relationship was suggested by Tucker *et al.* (1994) based on their work and that of Kumagai *et al.* (1994). These authors demonstrated that *in vitro*, MDMA could be shown to be demethylenated by the P450 enzyme, debrisoquine 4-hydroxylase or CYP2D6 (see Figure 1). This enzyme is expressed polymorphically in man and 5–9% of caucasians are deficient in the enzyme (Gonzalez

[1] Permanent address: Departmento de Farmacologia, Facultad de Medicina, Universidad Complutense, Madrid 28040, Spain.
[2] Author for correspondence.

& Meyer, 1991). It seems reasonable to propose that the hyperthermia results from the rapid release of cerebral 5-HT which follows administration of the drug (Schmidt, 1987; Gibb *et al.*, 1990; Colado & Green, 1994) and that this effect is induced by the parent drug (Johnson *et al.*, 1986; Steele *et al.*, 1987). Tucker *et al.* (1994) therefore suggested that persons deficient in CYP2D6 and who metabolize the drug more slowly (poor metabolizers or PM phenotypes) might have an exaggerated hyperthermic response. Tucker *et al.* (1994) also proposed the corollary, namely that, given the evidence that the long term neurotoxicity is the result of the action of metabolites of MDMA (Molliver *et al.*, 1986; Lim & Foltz, 1991; Paris & Cunningham, 1992; Johnson *et al.*, 1992), rather than a consequence of released 5-HT (Hekmatpanah *et al.*, 1989; Colado & Green, 1994), poor metabolizers might be at less risk of neurotoxic damage.

We have now undertaken studies to evaluate these two hypotheses by examining both the hyperthermic and neurotoxic effect of (±)-MDMA in male and female Dark Agouti (DA) rats. Al-Dabbagh *et al.* (1981) first proposed that the DA rat might be an acceptable model for the PM phenotype, with other strains being analagous to the human extensive metabolizer (EM) phenotype. Vincent-Viry *et al.* (1988) subsequently demonstrated that the liver of the female DA rat cleared debrisoquine at a significantly slower rate than that of the male. This suggests that females have lower debrisoquine hydroxylase activity and is consistent with evidence that female DA liver microsomes have lower debrisoquine oxidising activity than males (Larney *et al.*, 1984; Boobis *et al.*, 1986).

An investigation has also been made of the hyperthermic and neurotoxic effects of a major MDMA metabolite (±)-3,4-methylenedioxyamphetamine (MDA; Figure 1) in male and female DA rats since this compound also induces hyperthermia (Miller & O'Callaghan, 1994) and long term neurotoxicity (Ricaurte *et al.*, 1985; Battaglia *et al.*, 1987).

Methods

Animals and drugs

Adult DA rats, male and female (Harlan Olac, Bicester, Oxon) weighing 140–160 g were used. They were housed in groups, in conditions of constant temperature (21°C) and a 12 hour light/dark cycle (lights on 07 h 00 min) and given free access to food and water. (±)-3,4-Methylenedioxymethamphetamine HCl, (±)-3,4-methylenedioxyamphetamine HCl, proadifen and quinidine HCl were all obtained from Sigma Chemical Co., Poole, Dorset. With the exception of quinidine and proadifen all the drugs were dissolved in NaCl (0.9% w/v). Quinidine was dissolved in ethanol before addition of saline (3:1 ethanol) and proadifen was dissolved in saline containing 20% dimethylsulphoxide (DMSO). All drugs were injected intraperitoneally and doses are quoted in terms of the base. Control animals were injected with the appropriate vehicle.

Measurement of monoamines and their metabolites

Rats were killed by cervical dislocation and decapitation, the brains removed and cortex and hippocampus dissected out. Tissue was homogenized and 5-HT and 5-HIAA measured by high performance liquid chromatrography (h.p.l.c.) with electrochemical detection by the method previously described in detail elsewhere (Colado *et al.*, 1993).

Measurement of [^{3}H]-paroxetine binding

The binding of [^{3}H]-paroxetine to cerebral tissue was essentially by the method of Nash *et al.* (1991) as described by Hewitt & Green (1994).

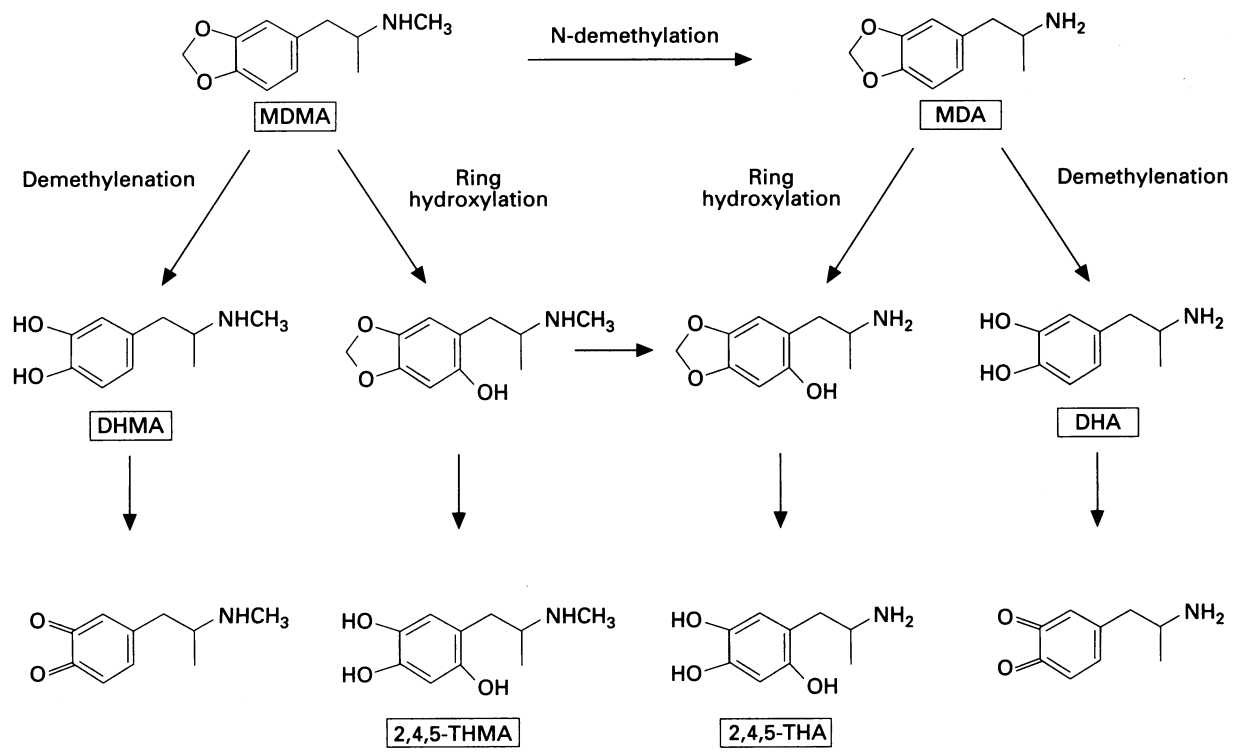

Figure 1 The major postulated pathways of MDMA metabolism. DHA: dihydroxyamphetamine, DHMA: dihydroxymethamphetamine, MDA: 3,4-methylenedioxyamphetamine, MDMA: 3,4-methylenedioxymethamphetamine, 2,4,5-THA: 2,4,5-trihydroxyamphetamine, 2,4,5-THMA: 2,4,5-trihydroxymethamphetamine.

Measurement of plasma concentrations of MDMA and MDA

Following cervical dislocation and decapitation blood was rapidly collected from the trunk into heparinized tubes. MDMA and MDA were extracted from plasma using a 1 mg C8 end-capped SPE column (International Sorbent Technology, Hengoed, Glamorgan). The column was washed with methanol (2 ml) followed by distilled H_2O (2 ml) before plasma (100 µl diluted to 750 µl with distilled H_2O) was applied. The column was washed with H_2O (2 ml) before selective elution of MDMA and MDA with the h.p.l.c. mobile phase. The mobile phase consisted of CH_3CN (20%), KH_2PO_4 (0.025M, pH 3.5; 80%) and dimethyloctylamine added at a concentration of 100 µl l^{-1} to improve peak shape.

An aliquot (50 µl) of the resulting eluent was injected into a Kontron h.p.l.c. system with a 422 pump, 360 autosampler and 332 ultraviolet detector. Peaks were separated on a 250 × 4.6 mm, 5 µm Spherisorb ODS2 column (Anachem). Flow rate was normally set to 1 ml min^{-1} and u.v. absorption measured at 235 nm. Peaks were integrated and concentrations calculated with Kontron PC integration pack software. Internal standards were not used as intra-assay variation was less than 5% and recovery greater than 90%. Plasma concentrations of MDMA 60 min following a dose of 10 mg kg^{-1} (see Table 1) were essentially identical to those reported by Cho *et al.* (1990) using a gas chromatography-mass spectrometric (GC-MS) assay.

Measurement of rectal temperature

Temperature was measured by insertion of a thermocouple probe, connected to a digital readout, 2.5 cm into the rectum, the rat being lightly restrained by holding in the hand. A steady temperature readout was obtained within 10 s of probe insertion.

Statistics

Statistical analysis was performed using the statistical computer package, BMDP/386 Dynamic (BMDP Statistical Software, Cork, Eire). Two-way analysis of variance (ANOVA, programme 7D) with sex and treatment as the between subjects factor and amine level as the within subjects factor were performed. Temperature data were analysed by repeated measures ANOVA (programme 2V) with sex as the between subject factor and time as the repeated measure. Where a significant effect of drug occurred, *post-hoc* pairwise camparisons (Tukey) were carried out.

Results shown in Tables 1, 2 and 4 were analysed by use of Students *t* test (unpaired).

Results

Effects of MDMA injection on rectal temperature in male and female DA rats

Male and female rats were injected intraperitoneally with MDMA (10 mg kg^{-1}, i.p.) and rectal temperature measured for the next 240 min. The compound increased rectal temperature in both sexes, the peak occurring approximately 40 min after injection (Figure 2a). The temperature response of the females was greater over the whole observation period (Figure 2a). There was a sigmoid dose-response curve, whether response was measured as the maximum temperature increase (Figure 2b) or the area under the response curve (data not shown) with female rats displaying markedly greater temperature rise to every dose of MDMA examined compared to males (Figure 2b).

Plasma concentrations of MDMA and MDA in male and female DA rats following MDMA injection

Rats were injected with MDMA (10 mg kg^{-1}) and blood collected 45 min later (the time of the maximum temperature response) for measurement of MDMA and its major N-demethylated metabolite, MDA (see Figure 1).

Forty-five min after MDMA the plasma level of parent drug was almost 60% higher in females and MDA concentrations were lower by a similar amount (Table 1). The plasma concentration of MDMA plus MDA was similar in both sexes with the proportion of MDMA therefore being approximately 50% higher in the female animals (Table 1).

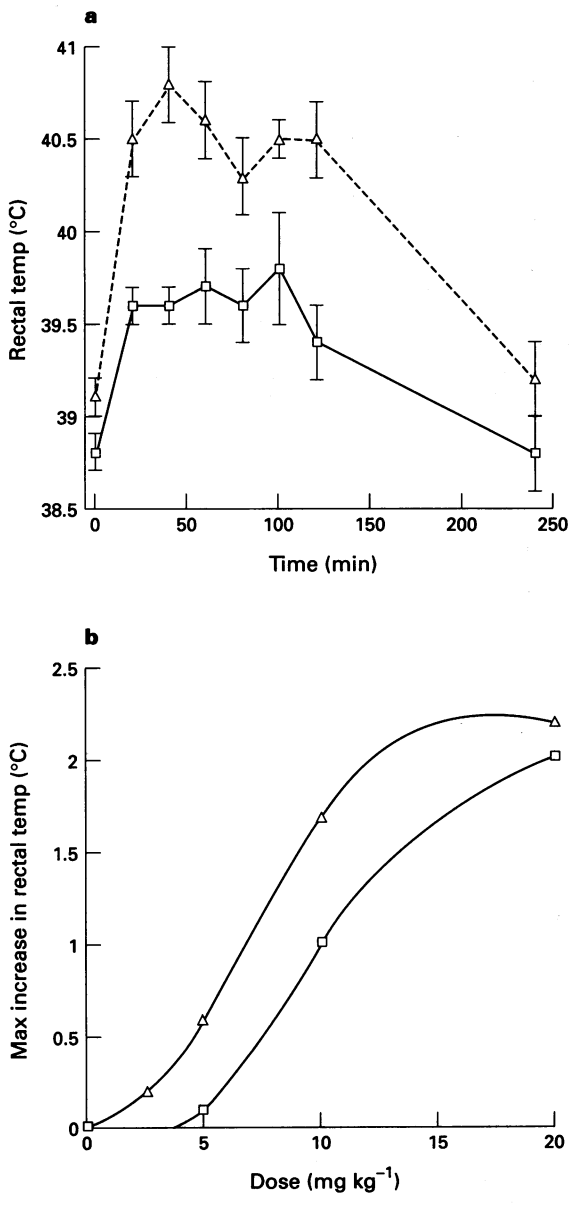

Figure 2 Effect of MDMA on rectal temperature in male and female DA rats. (a) Rectal temperature in male (□, $n=5$) and female (△, $n=4$) rats in the 4 h following MDMA (10 mg kg^{-1}, i.p.). Mean shown by symbol with s.e.mean . There was a significant effect of sex on the size of the response ($F=14.83$, d.f. 1,7, $P<0.006$) as well as an effect of time ($F=35$, d.f. 7,49, $P<0.0001$). (b) Dose of MDMA versus maximum temperature increase-response curve in male (□) and female (△) rats.

Table 1 The plasma concentration of MDMA and MDA in male and female DA rats 45 min after injection of MDMA (10 mg kg^{-1}, i.p.)

	Male	*Female*	P
MDMA	6.27±0.38 (8)	9.85±0.73 (8)	<0.001
MDA	5.29±0.27 (8)	2.49±0.23 (8)	<0.001
MDMA + MDA	11.55±0.60 (8)	12.26±0.89 (8)	N.S.
Ratio of MDMA/ MDMA + MDA	0.54±0.01	0.80±0.01	<0.001

Results show means±s.e.mean in nmol drug ml^{-1} plasma with number of observations in parentheses. For abbreviations, see text.

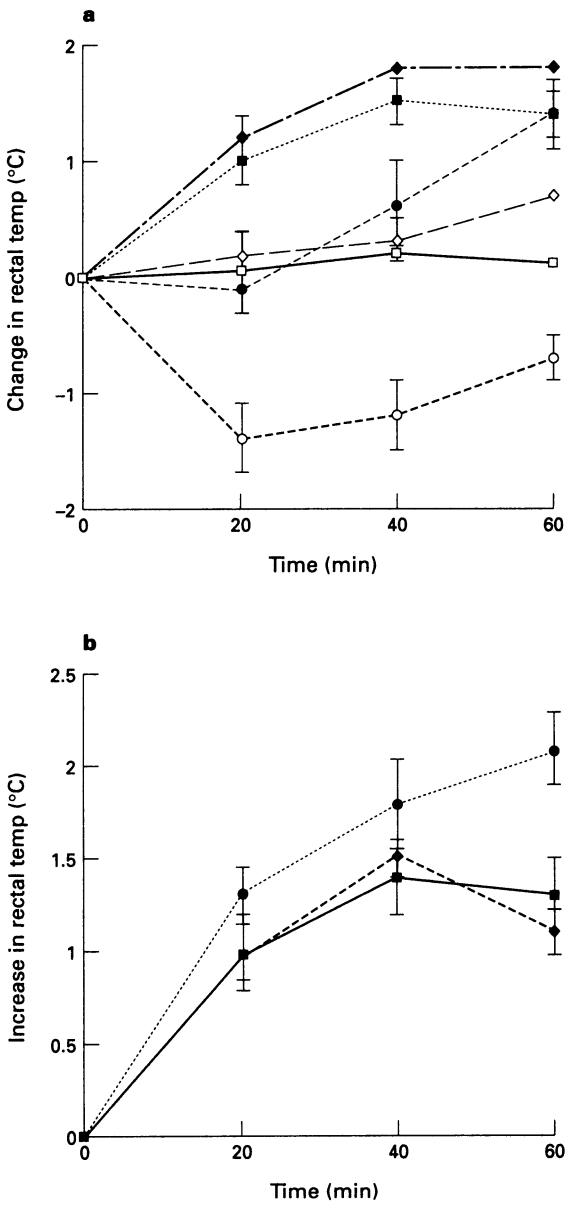

Figure 3 The effect of pretreatment of male DA rats with proadifen (15 mg kg^{-1}, i.p.) or quinidine (60 mg kg^{-1}, i.p.) 30 min before MDMA (10 mg kg^{-1}, i.p.) on rectal temperature. (a) Change in temperature in the 60 min following injection of either saline (open symbols) or MDMA (10 mg kg^{-1}, i.p.) (closed symbols) in rats pretreated 30 min earlier with either saline (□,■), quinidine (○,●) or proadifen (◇,◆). Rectal temperature of the groups at the time of saline or MDMA injection (time zero on the graph) was: saline pretreated 39.1±0.1 (□), 38.7±0.1 (■); quinidine pretreated 37.7±0.1 (○), 36.7±0.2 (●); proadifen pretreated 38.5±0.2 (◇), 38.4±0.1 (◆). (b) The same data plotted as a change in rectal temperature following MDMA (10 mg kg^{-1}, i.p.) with the change in temperature following proadifen or quinidine subtracted from the response. Results show mean±s.e. mean; no s.e. is shown if it is smaller than symbol. There was a significant difference between the response of the rats given MDMA when pretreated with quinidine compared with the control animals given MDMA alone ($F=6.02$, d.f. 1,11, $P<0.03$).

The effect of proadifen or quinidine on plasma levels of MDMA and hyperthermia response to MDMA

The previous experiments suggested that the larger temperature response seen in the females following MDMA injection might have been due to a higher plasma MDMA concentration, but that this resulted from an impairment of N-demethylation rather than demethylenation. To investigate this further, rats were pretreated with either the non-selective cytochrome P450 enzyme inhibitor, proadifen (SKF 525A) or quinidine, a relatively selective inhibitor of debrisoquine hydroxylase.

Male rats were injected with either proadifen (15 mg kg^{-1}, i.p.) or quinidine (60 mg kg^{-1}, i.p.) 30 min before MDMA (10 mg kg^{-1}). Rectal temperature was measured during the next 60 min at which time the rats were killed and blood collected for measurement of plasma MDA and MDMA concentrations.

Quinidine alone produced a pronounced hypothermia (Figure 3a). The increase in rectal temperature following MDMA was therefore attenuated by quinidine pretreatment (Figure 3a). The temperature response nevertheless appeared enhanced when the change induced by quinidine alone had been subtracted (Figure 3b). Proadifen had little effect on temperature (Figure 3a) and did not alter MDMA-induced hyperthermia (Figure 3b).

The plasma concentration of MDMA or MDA was unaltered by pretreatment with proadifen or quinidine (Table 2).

Effect of MDA on rectal temperature in male and female DA rats

Male and female rats were injected with MDA (10 mg kg^{-1}, i.p.) and rectal temperature measured for the next 120 min. Male rats showed a marked hyperthermic response to MDA, while females had a small and variable response (Figure 4). A lower dose of MDA (5 mg kg^{-1}) produced a smaller hyperthermic response in male rats (Figure 4) and a small and variable response in females (data not shown, but see Figure 5a).

Plasma MDA concentrations in male and female DA rats

Male and female rats were injected with MDA (5 mg kg^{-1}, i.p.) and rectal temperature measured for the next 60 min at which time they were killed and plasma MDA concentrations measured. The observation that MDA-induced a greater temperature response in males was confirmed with this lower dose (Figure 5a). However, plasma levels of MDA were almost 40% higher in the females (Figure 5b).

Table 2 Effect of pretreatment with proadifen (15 mg kg^{-1}, i.p.) or quinidine (60 mg kg^{-1}, i.p.) on plasma levels on MDMA and MDA, 60 min after injection of MDMA (10 mg kg^{-1}, i.p.)

Injected	n	*MDMA*	*MDA*	*MDMA + MDA*	*Ratio MDMA/ MDMA + MDA*
Saline	6	5.40±0.08	5.30±1.21	10.34±0.82	0.49±0.03
Proadifen	6	5.06±0.38	4.70±0.22	9.76±0.59	0.49±0.01
Vehicle	4	4.38±0.17	6.55±0.96	10.93±0.87	0.41±0.02
Quinidine	6	4.17±0.16	5.64±0.52	9.80±0.55	0.42±0.03

Results show mean±s.e.mean in nmol drug ml^{-1} plasma with number of observations (n) shown in column.

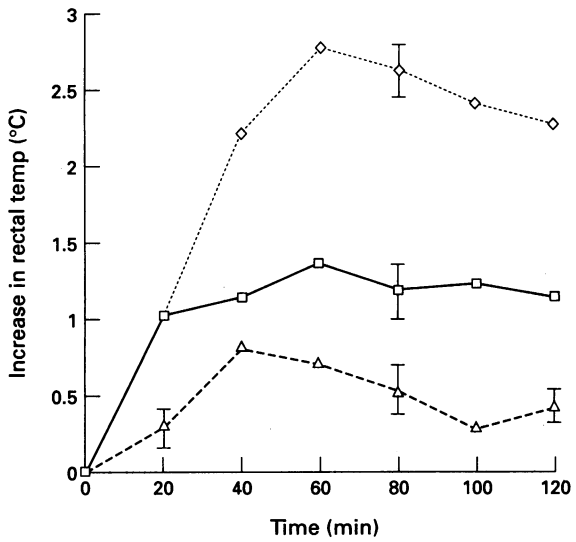

Figure 4 Effect of MDA (5 or 10 mg kg^{-1}, i.p.) on rectal temperature in male and female DA rats. Results show change in temperature from pre-injection value in males (□ = 5 mg kg^{-1}; ◇ = 10 mg kg^{-1}) and females (△ = 10 mg kg^{-1}). Symbols show mean±s.e.mean; if no s.e. is shown it is smaller than symbol (n=4–5).

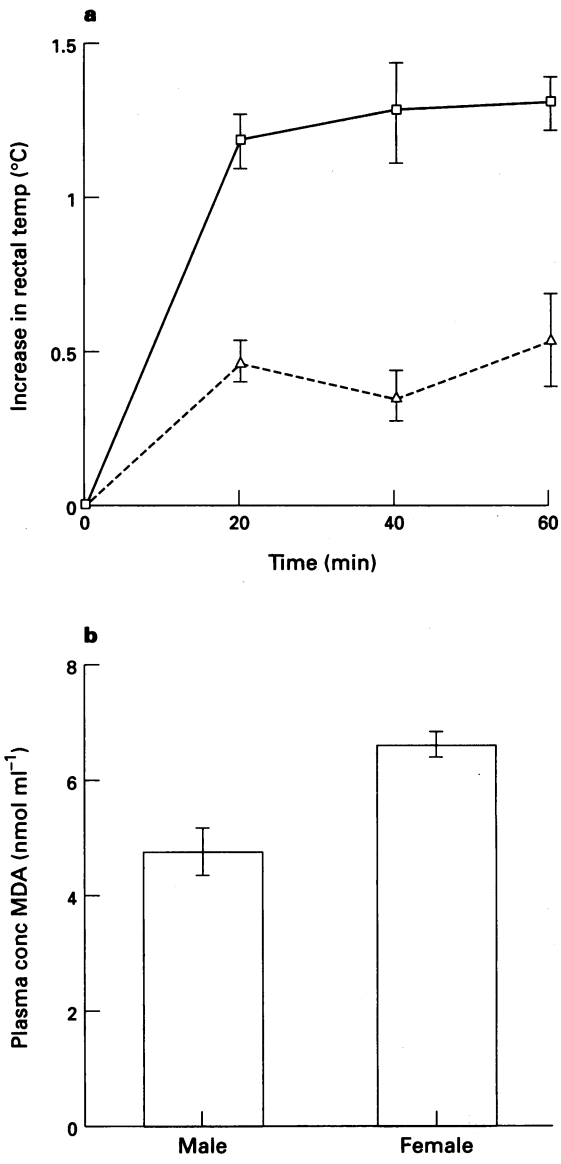

Figure 5 Effect of MDA (5 mg kg^{-1}) on (a) rectal temperature in male (□, n=7) and female (△, n=8) rats during the next 60 min and (b) plasma MDA concentration of the same animals at 60 min. Results shown as mean±s.e.mean. The response of the female rats was significantly different from males (F=10.88, d.f. 1,13, P<0.01). The concentration of MDA was also different between the sexes (P<0.001).

Effect of MDMA administration on brain 5-HT and 5-HIAA concentration seven days later

Male and female rats were injected with MDMA (5 or 10 mg kg^{-1}, i.p.) and killed seven days later for analysis of brain 5-HT and 5-HIAA content in cortex and hippocampus.

MDMA (5 mg kg^{-1}) failed to produce a significant loss of 5-HT or 5-HIAA in either brain region (Table 3). A dose of 10 mg kg^{-1}, in contrast, produced a substantial indole loss in both cortex and hippocampus, the percentage loss being greater in the hippocampus. There was no sex difference in either brain region (Table 3).

Effect of MDMA administration on [^{3}H]-paroxetine binding in the cortex of male rats

To confirm that the MDMA-induced neurotoxic loss of 5-HT in DA rats resulted from a neurodegenerative change, male rats were injected with MDMA (10 mg kg^{-1}) and [^{3}H]-paroxetine binding measured in cortical tissue 7 days later.

The binding of [^{3}H]-paroxetine to the presynaptic 5-HT transporter site was decreased by 27% in MDMA treated rats (Table 4). The loss of 5-HT and 5-HIAA measured in the same tissue was 40% (Table 4).

The effect of MDA administration on cerebral 5-HT content in male and female rats seven days later

Male and female DA rats were injected with MDA (5 mg kg^{-1}) and the 5-HT and 5-HIAA concentration measured in cortex and hippocampus seven days later.

MDA administration resulted in an approximate 40% loss of 5-HT on both brain regions seven days later (Table 5). The loss of 5-HIAA was similar (data not shown). There was no evidence for a sex difference in this effect.

Table 3 The concentration of 5-HT and 5-HIAA in the cortex and hippocampus of male and female rats 7 days after injection of MDMA

Measured	*Saline*	*MDMA* (5 mg kg^{-1})	*MDMA* (10 mg kg^{-1})
Cortex 5-HT			
Male	415±30	351±14	309±21*
Female	417±23	341±43	216±18**
Cortex 5-HIAA			
Male	153±9	155±9	112±10*
Female	187±11	171±10	97±4**
Hippocampus 5-HT			
Male	317±33	309±19	193±27*
Female	311±32	306±32	152±26*
Hippocampus 5-HIAA			
Male	290±13	329±20	178±16**
Female	347±8	367±23	199±11**

Results show mean±s.e.mean of indole concentration in ng g^{-1} tissue ($n=4-5$). There was no significant effect of sex on the response but a significant effect of drug as follows: cortex 5-HT: $F=4.25$; d.f. 1,16., $P<0.001$; cortex 5-HIAA: $F=57.2$, d.f. 1,16., $P<0.001$; hippocampus 5-HT: $F=21.4$, d.f. 1,15., $P<0.001$; hippocampus 5-HIAA: $F=73.1$, d.f. 1,15., $P<0.001$. *Post-hoc* comparisons of drug-treated versus saline-injected controls are as follows: *$P<0.05$, **$P<0.01$. For abbreviations, see text.

Table 4 The effect of MDMA (10 mg kg^{-1}, i.p.) on 5-HT neurochemical markers in the cortex 7 days after administration

Measurement	*Saline*	*MDMA*
[^{3}H]-paroxetine	7.44±0.16	5.41±0.34 [73]*
5-HT	377±13	228±22 [60]*
5-HIAA	141±5	84±4 [60]*

Table shows values of [^{3}H]-paroxetine binding (in fmol mg^{-1} tissue) and 5-HT and 5-HIAA content (in ng g^{-1} tissue) 7 days after MDMA administration. Values expressed as mean±s.e.mean. Value in square brackets is the % values in MDMA-treated rats compared to saline-injected control values (100%). *Results different from control value: $P<0.01$.

Table 5 The effect of MDA (5 mg kg^{-1}, i.p.) on 5-HT concentration in cortex and hippocampus of male and female DA rats 7 days later

	Tissue 5-HT concentration (ng g^{-1})	
	Saline	*MDA*
Cortex		
Male	366±12 (4)	278±24 (6)*
Female	429±26 (4)	250±23 (5)**
Hippocampus		
Male	438±19 (4)	234±6 (5)**
Female	458±19 (4)	261±14 (4)**

Results shown as mean±s.e.mean with number of observations in parentheses. There was no effect of sex on the response but a significant effect of drug as follows: cortex 5-HT: $F=34.1$, d.f. 1,15, $P<0.001$; hippocampus 5-HT: $F=93.8$, d.f. 1,13, $P<0.001$. *Post hoc* comparisons of drug-treated versus saline-injected controls are as follows: *$P<0.05$, **$P<0.01$.

Discussion

The hypotheses advanced by Tucker *et al.* (1994) can be stated simply. The first hypothesis was that low activity of the debrisoquine hydroxylase enzyme in poor metabolizers would result in a lower rate of MDMA demethylenation, higher plasma levels of MDMA and therefore a greater risk of acute toxicity. The major acute toxic effect of the drug in man is hyperthermia (see introduction and Green *et al.*, 1995), which in turn results in other, often fatal problems such as rhabdomyolysis, disseminated intravenous coagulation and acute renal failure (Chadwick *et al.*, 1991; Henry *et al.*, 1992; Green *et al.*, 1995). The second hypothesis was that poor metabolizers might produce lower concentrations of neurotoxic metabolites of MDMA since these may be formed via the demethyleneation pathway. These subjects might therefore be less susceptible to any long term neurotoxic loss of 5-HT in the brain.

To examine these hypotheses we used the Dark Agouti strain of rat since the female has been shown to metabolize debrisoquine more slowly than the male and thus might be considered to be a model of the human PM phenotype (see Introduction).

The initial experiments performed appeared to support the first hypothesis, since female DA rats had an enhanced hyperthermic response to MDMA at all doses examined and also 60% higher plasma concentrations of MDMA 45 min after drug administration. However females also had much lower concentrations of MDA, while the total plasma MDA+MDMA concentration was the same in both sexes. This suggests that N-demethylation might be impaired in the female rather than demethylenation, the debrisoquine hydroxylase step (Figure 1).

We next attempted to alter MDMA metabolism in male rats by using both the non-selective P450 enzyme inhibitor, proadifen, and the reasonably selective debrisoquine hydroxylase inhibitor, quinidine. Proadifen was without effect on MDMA metabolism which is consistent with previous observations, both *in vitro* (Lim *et al.*, 1992) and *in vivo* (Cho *et al.*, 1990). Although quinidine is a better inhibitor than quinine of debrisoquine hydroxylase in man the reverse is true in rats (Kobayashi *et al.*, 1989). Nevertheless at the dose used in our study quinidine is an effective inhibitor of this enzyme (Muralidharan *et al.*, 1991) and pretreatment with this compound resulted in an enhanced MDMA-induced hyperthermia. However the marked hypothermia produced by quinidine injection makes this a tentative observation. What was notable however was that quinidine injection did not alter the plasma levels of MDMA or MDA, so the increased temperature response is not the result of an increased plasma MDMA concentration. The data also suggest that metabolism of MDMA by debrisoquine hydroxylase is a minor pathway in the DA rat *in vivo* since inhibiting the enzyme with quinidine had no effect on the plasma concentration of the drug.

We next examined the effect of MDA in male and female DA rats since this compound is also demethylenated, albeit possibly not by the same enzymes as MDMA (Kumagai *et al.*, 1992; Fukuto *et al.*, 1991). MDA has also been shown to enhance 5-HT release *in vitro* (McKenna *et al.*, 1991) and produce hyperthermia (Miller & O'Callaghan, 1994). In contrast to the effect of MDMA, it was found that MDA produced a

larger hyperthermic response in males. Indeed MDA injection induced only a small and variable hyperthermia in females despite the fact that they had significantly higher plasma levels of MDA. This suggests that MDA may be metabolized more slowly in females.

However, a major problem in interpretation is that both MDMA and MDA exist in enantiomeric forms. The current study used racemic compounds since these would be the form in which people both obtain and misuse them. While Schmidt *et al.* (1986) reported that (+)-MDMA and (–)-MDMA were equipotent in releasing 5-HT *in vitro*, evidence from behavioural studies indicates that *in vivo*, the (+)-enantiomer is as much as fourfold more potent (Hiramatsu *et al.*, 1989; Glennon *et al.*, 1987). Similarly, (+)-MDA is more potent than (–)-MDA in evoking 5-HT-mediated behaviour (Hiramatsu *et al.*, 1989).

Gollamudi *et al.* (1989) has reported that microsomes prepared from female rat livers metabolized (+)-MDMA at least twice as rapidly as (–)-MDMA, an effect not seen in microsomes prepared from males. Indeed males metabolized both isomers at the 'lower' rate. Therefore the more active isomer of MDMA was cleared more rapidly in females. If this were also to be true of MDA enantiomers, then the plasma MDA concentrations seen in females might consist predominantly of the less active enantiomer, thereby explaining the attenuated temperature response.

An alternative explanation is that it is not the parent MDA which releases 5-HT and is responsible for the hyperthermia, but rather an active metabolite, the formation of which is impaired in females. This however seems unlikely given the speed with which hyperthermia appears post-injection and the *in vivo* evidence for the potency of MDA in releasing 5-HT from cerebral tissue (Harvey *et al.*, 1993).

These problems of interpretation will only be finally answered by examining the effect of the enantiomers on both temperature and 5-HT release *in vivo* using microdialysis techniques. Extrapolation from *in vitro* studies remains problematical in the light of evidence that the rates of metabolism of enantiomeric forms of MDMA were the opposite in *in vitro* studies compared to *in vivo* observations (Cho *et al.*, 1990).

With regard to the long term neurotoxicity, we were unable to detect any clear sex difference in the effect of either MDMA or MDA. This means either that if there is a slower rate of formation of toxic metabolites in animals with debrisoquine hydroxylase deficiency it has little importance, or that demethylenation is not a primary route for the production of these putative toxins.

There are however major problems in extrapolating these data to the situation in human subjects and in using the results to indicate that the hypothesis of Tucker *et al.* (1994) might be flawed. The major problem is that debrisoquine is metabolized by different enzymes in different species and even within strains of rats. In man the major debrisoquine hydroxylase enzyme is CYP2D6 and it is this enzyme which Tucker *et al.* (1994) have shown to be responsible for MDMA demethylenation. In several rat strains the equivalent enzyme is CYP2D1 (Matsunaga *et al.*, 1989). However even this enzyme appears to be absent in DA rats (Matsunaga *et al.*, 1989). Nevertheless the DA rat can metabolize debrisoquine and this metabolism is slower in the female (Vincent-Viry *et al.*, 1988). This suggests that another P450 enzyme is both responsible for debrisoquine metabolism and that its activity is sex-linked. Whether this enzyme is also responsible for MDMA metabolism, particularly demethylenation is unknown. A further point is that while the rate of MDMA metabolism in the liver may well be crucial to the acute toxic hyperthermic response (since the amphetamine will have good brain penetration) it is likely to be the metabolism of MDMA in the brain that is responsible for long term neurotoxicity. This is because the postulated neurotoxic metabolites are catechols and quinones (Hiramatsu *et al.*, 1990; Figure 1) which would be expected to have poor brain penetration.

It is also difficult to compare the data now obtained in a CYP2D1-deficient rat with earlier data obtained in our previous studies using rats, which presumably have this enzyme, without extensive knowledge of the pharmacology of MDMA in the two strains. It might be proposed that a CYP2D1 deficiency would result in an enhanced hyperthermic response to MDMA, but reduced neurotoxicity. However, the hyperthermic response of DA rats appears similar to Lister Hooded rats (see Colado *et al.*, 1993) while the DA rat is clearly very susceptible to MDMA-induced neurotoxic loss of 5-HT compared to the Lister rats (see Colado *et al.*, 1993; Hewitt & Green, 1994). Furthermore we do have some indirect evidence that the neurotoxic catechols and quinone compounds may be being formed in the DA rat brain even if it is lacking the CYP2D1 enzyme. Catechols and quinones have been shown by others to produce free radicals on further metabolism (e.g. Chieuh *et al.*, 1992) and we have demonstrated that administration of the spin trap reagent PBN prevented MDMA-induced neurodegeneration (Colado & Green, 1995). PBN is an effective agent for preventing free radical formation in the brain (Carney & Floyd, 1991) and has been shown to prevent neurodegeneration resulting from an acute ischaemic insult (Cao & Phyllis, 1994).

Since the DA rat is a limited model of human CYP2D6 deficiency, having as it does other metabolic differences compared to man (Barnham *et al.*, 1994), conclusions on the application of the current results to the clinical problems of MDMA toxicity are of necessity, tentative. It is however reasonable to propose that poor metabolizer phenotypes will be more susceptible to the acute toxic effects of MDMA.

A recent review of the pharmacology and clinical pharmacology of MDMA highlighted the substantial similarities of the effects of this drug in laboratory animals and man (Green *et al.*, 1995). What is striking about the current study is that the plasma concentrations of MDMA in the rats 60 min after a dose of 10 mg kg^{-1} are in *exactly the same range* as those reported in patients suffering acute fatal responses to ingestion of the drug (e.g. Henry *et al.*, 1992; Dowling *et al.*, 1987). These animals also displayed an acute hyperthermic response similar to that seen clinically. Since subsequently there was also unequivocal evidence for neurodegeneration of 5-HT pathways in the brain of these rodents, concern must be expressed that similar changes could be occurring in people who regularly use the drug for recreational purposes.

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