

Effect of GBR 12909 and fluoxetine on the acute and long term changes induced by MDMA ('ecstasy') on the 5-HT and dopamine concentrations in mouse brain

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Abstract

We examined the long term effect of 3,4-methylenedioxymethamphetamine (MDMA, 10, 20 and 30 mg/kg, i.p.) on the cerebral 5-hydroxytryptamine (5-HT) and dopamine content in Swiss Webster mice. Three injections of MDMA (20 or 30 mg/kg, i.p.) given 3 h apart produced a marked depletion in the striatal content of dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) 7 days later. None of the doses administered altered the concentration of 5-HT or its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in several brain areas. Pre-treatment with the dopamine uptake inhibitor GBR 12909 (10 mg/kg, i.p.), 30 min before each of the three MDMA (30 mg/kg, i.p.) injections, completely prevented the long term loss in the striatal catechol concentrations. However, GBR 12909 (10 mg/kg, i.p.) not only failed to prevent the acute effects induced by MDMA (30 mg/kg $\times$ 3, i.p.) on dopamine metabolism 30 min later, but in fact potentiated them. The 5-HT uptake inhibitor, fluoxetine (10 mg/kg, i.p.) failed to prevent both the acute and long term dopaminergic deficits. MDMA (30 mg/kg $\times$ 3) altered the body temperature of the mice biphasically, producing a rapid hyperthermia followed by prolonged hypothermia. In contrast, MDMA (20 mg/kg $\times$ 3) produced an initial hypothermia followed by hyperthermia. The present experiments therefore appear to rule out any direct relationship between the neurotoxic effects of MDMA and its acute effects on body temperature in mice. Fluoxetine administered 30 min before each MDMA (30 mg/kg) injection prevented these temperature changes, while GBR 12909 was without effect. This suggests that the neuroprotective effect of GBR 12909 against MDMA-induced neurotoxicity is not directly related to its ability to inhibit the MDMA-induced acute effects on dopamine metabolism or alter the MDMA-induced temperature change. The data illustrate major differences in the neurotoxic profile of MDMA in mice and rats.

Keywords: 3,4-Methylenedioxymethamphetamine; MDMA; Ecstasy; GBR 12909; Fluoxetine; Dopamine; 5-Hydroxytryptamine; Neuroprotection; Hyperthermia

1. Introduction

Administration of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') to rats produces 2 major acute events. The first is a marked release of 5-hydroxytryptamine (5-HT) in several regions of the brain. This has been demonstrated both in vitro using a synaptosomal preparation (Schmidt et al., 1987) and also by using in

vivo microdialysis (Schmidt et al., 1987; Gough et al., 1991; Gudelsky and Nash, 1996). The second is a major release of dopamine. Again this is demonstrable using both in vitro techniques (Koch and Galloway, 1997) and microdialysis (Koch and Galloway, 1997; Sabol and Seiden, 1998; Colado et al., 1999). Interestingly the dopamine release appears to result from both an 'amphetamine-like' release and as an indirect result of the 5-HT release (Gudelsky and Nash, 1996; Koch and Galloway, 1997). Administration of either 5-HT neurotoxins or antagonists in vivo prevents a major component of the dopamine release (Nash, 1990; Schmidt et al., 1990; Gudelsky and Nash, 1996), while 5-HT agonists

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enhance release (Gudelsky et al., 1994), indicating the importance of increased 5-HT release in the mechanism of the enhanced dopamine release (Gudelsky and Nash, 1996; Koch and Galloway, 1997).

MDMA administration also produces long term changes in the brain of rats and also the brain of guinea pigs and several species of non-human primates. In rats, for example, there is clear and unequivocal evidence for a substantial and sustained long term neurotoxic loss of 5-HT nerve terminals in several regions of the brain (Stone et al., 1986; Schmidt, 1987; Schmidt and Kehne, 1990; Steele et al., 1994; Green et al., 1995; White et al., 1996; Colado et al., 1997, 1999; Huether et al., 1997). The degeneration has been demonstrated histologically (O'Hearn et al., 1988; Molliver et al., 1990) and biochemically and is reflected in a substantial decrease in the concentration of 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), and a decrease in the density of 5-HT uptake sites labelled with [3 H]-paroxetine (Sharkey et al., 1991; Hewitt and Green, 1994; Colado et al., 1997). Two early studies suggested that there was also a long term loss in striatal dopamine content (Logan et al., 1988; Schmidt et al., 1987). However Logan et al. (1988) only saw this change following a very large dose (150 mg/kg in 24 h) and not at half this dose and Schmidt's later papers appeared not to confirm his initial report (see for example, Schmidt and Kehne, 1990). Indeed there are many other reports indicating that MDMA, given at a dose that produces a major neurotoxic loss of 5-HT leaves long term dopamine and noradrenaline concentrations unaltered (Stone et al., 1986; Battaglia et al., 1987; Schmidt and Kehne, 1990; Lew et al., 1996; Sabol et al., 1996; Colado et al., 1997, 1999).

There have been relatively few studies on the effects of MDMA in mice when compared to the number of studies in rats. However the consensus is that MDMA behaves as a relatively selective dopaminergic neurotoxin in mice having little effect on 5-HT containing neurons, but inducing a sustained loss in the concentration of dopamine and its metabolites in the striatum (Stone et al., 1987; Logan et al., 1988; O'Callaghan and Miller, 1994). The MDMA-induced loss in the striatal catechol content induced by MDMA in mice probably reflects a neurotoxic degeneration of dopamine nerve terminals similar to that which occurs following methamphetamine administration (Green et al., 1992; Baldwin et al., 1993; Bowyer et al., 1994; Itzhak and Ali, 1996).

In spite of the existence of a substantial amount of information on the neurotoxic effects induced by MDMA in rats, studies focusing on evaluating the mechanism by which MDMA produces selectively toxic damage to dopamine containing neurones in mice are scarce. We have now therefore conducted a systematic study to further characterise the acute and long term effects induced by MDMA on 5-HT and dopamine concen-

trations in the brain of Swiss Webster mice and to investigate the possible relationship between MDMA-induced changes in body temperature and the long term neurotoxicity. The role of dopamine and 5-HT transporters on both these effects has also been evaluated since there is some evidence that blockade of either the 5-HT (Schmidt, 1987) or dopamine (Stone et al., 1988) transporter attenuates MDMA-induced neurodegeneration in the rat (and see Discussion).

2. Methods

2.1. Animals, drug administration and experimental protocol

Adult male Swiss-Webster mice (CFW1, Interfauna, Barcelona, Spain) weighing 30–35 g were used. They were housed in groups of 10, in conditions of constant temperature ($21^{\circ}\text{C} \pm 2^{\circ}\text{C}$) and a 12 h light/dark cycle (lights on: 07 h.00 min) and given free access to food and water.

The following drugs were used: ($\pm$) 3,4-methylenedioxymethamphetamine HCl (MDMA, Ministry of Health, Spain), fluoxetine (Lilly S.A., Spain) and GBR 12909 (R.B.I., Spain). MDMA, fluoxetine and GBR 12909 were dissolved in 0.9% w/v NaCl (saline) and injected i.p. in a volume of 10 ml/kg. Control animals were injected with saline. Doses are always quoted in terms of the base.

The effects of repeated administration of MDMA (10, 20, 30 mg/kg, 3 times, every 3 h) on brain catechol and indole concentrations were examined either 3 h or 7 days later. We used this regimen, because, when applied to methamphetamine, it produces a loss of dopamine nerve terminals (Itzhak and Ali, 1996). In the neuroprotection studies, GBR 12909 (10 mg/kg), fluoxetine (10 mg/kg) or saline were always given 30 min before each of three MDMA (30 mg/kg) or saline injections 3 h apart, animals being sacrificed 30 min or 7 days after the last MDMA administration.

2.2. Measurement of rectal temperature

Temperature was measured by use of a digital readout thermocouple (Type K thermometer, Portec, UK) with a resolution of $\pm 0.1^{\circ}\text{C}$ and accuracy of $\pm 0.2^{\circ}\text{C}$ attached to a CAC-005 Rodent Sensor which was inserted 2 cm into the rectum of the mouse, the animal being lightly restrained by holding in the hand. A steady readout was obtained within 10 s of probe insertion. Mice receiving different doses of MDMA as well as those injected with MDMA (30 mg/kg) plus GBR 12909 or fluoxetine were evaluated in parallel.

2.3. Measurement of monoamines and their metabolites in cerebral tissue

Mice were killed by cervical dislocation and decapitation, the brains rapidly removed and cortex, hippocampus and striatum dissected out on ice. Tissue was homogenised and 5-HT, 5-HIAA, dopamine, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) measured by high performance liquid chromatography (HPLC). Catechol concentration was only determined in the striatum since in this area the majority (95%) of monoaminergic terminals are dopaminergic. Briefly, the mobile phase consisted of KH_2PO_4 (0.05 M), octanesulphonic acid (0.16 mM), EDTA (0.1 mM) and methanol (16%), and was adjusted to pH 3 with phosphoric acid, filtered and degassed. The flow rate was 1 ml/min and the working electrode potential was set at +0.8 V.

The HPLC system consisted of a pump (Waters 510, Spain) linked to an automatic sample injector (Loop 200 μl , Waters 712 WISP), a stainless steel reversed-phase column (Spherisorb ODS2, 5 μm , 150 $\times$ 4.6 mm) with a precolumn and an amperometric detector (Waters M460). The current produced was monitored by using an integrator (Waters M745).

2.4. Statistics

Data from the monoamine studies were analysed using one-way ANOVA followed by the Tukey multiple comparison test when a significant *F* value was obtained. Statistical analyses of the temperature measurements were performed using the statistical computer package BMDP/386 Dynamic (BMDP Statistical Solutions, Cork, Eire). Data were analysed by analysis of variance (ANOVA) with repeated measures (program 2V) or, where missing values occurred, an unbalanced repeated measure model (program 5V) was used. Both used treatment as the between subjects factor and time as the repeated measure. ANOVA was performed on both pre-treatment and post-treatment data.

3. Results

3.1. Acute effects of MDMA on dopamine and 5-HT concentrations in mouse brain

Repeated administration of MDMA (10, 20 and 30 mg/kg, i.p.), 3 times, every 3 h, resulted in a dose-dependent depletion in the DOPAC content and a modest decrease in dopamine concentration 3 h after injection of the higher dose tested. The HVA concentration remained unaltered (Fig. 1).

At this time, MDMA (10, 20 and 30 mg/kg, i.p.) also produced a modest decrease in the 5-HT and 5-HIAA

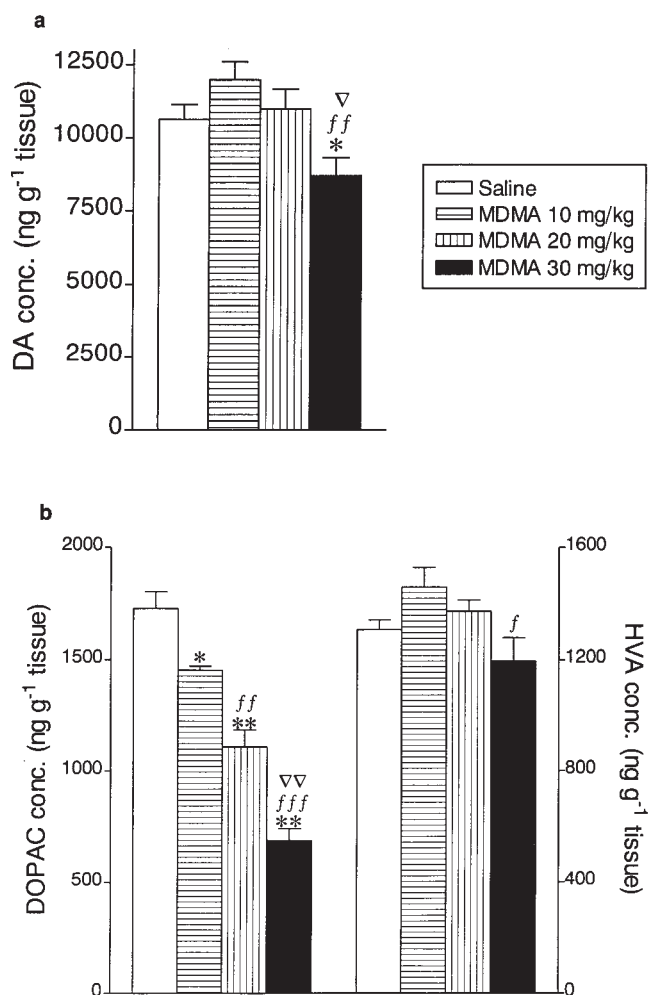


Fig. 1. Effect of MDMA (10, 20 and 30 mg/kg) given 3 times 3 h apart on the striatal concentrations of (a) dopamine (DA) and (b) 3,4-dihydroxyphenylacetic (DOPAC) and homovanillic (HVA) acids 3 h after last MDMA injection. The results are shown as mean $\pm$ SEM ($n=5-6$). Different from saline: * $p<0.01$, ** $p<0.001$. Different from 10 mg/kg MDMA: $^{\dagger}p<0.05$, $^{\#}p<0.01$, $^{\text{fff}}p<0.001$. Different from 20 mg/kg MDMA: $^{\nabla}p<0.05$, $^{\nabla\nabla}p<0.01$.

concentration in cortex and hippocampus and a decrease in the 5-HIAA concentration in the striatum (Fig. 2). These changes were similar in the examined interval of doses.

3.2. Effects of GBR 12909 and fluoxetine on acute changes induced by MDMA in dopamine concentration

In these experiments, fluoxetine or GBR 12909 was administered 30 min before each of the 3 MDMA (30 mg/kg, i.p.) injections, the animals being sacrificed 30 min after the last injection. Repeated administration of the dopamine uptake inhibitor, GBR 12909 (10 mg/kg, i.p.) to saline-treated mice produced a significant decrease in the concentration of dopamine and DOPAC in the striatum (Fig. 3). When GBR 12909 was given 30 min before MDMA it enhanced the MDMA-induced

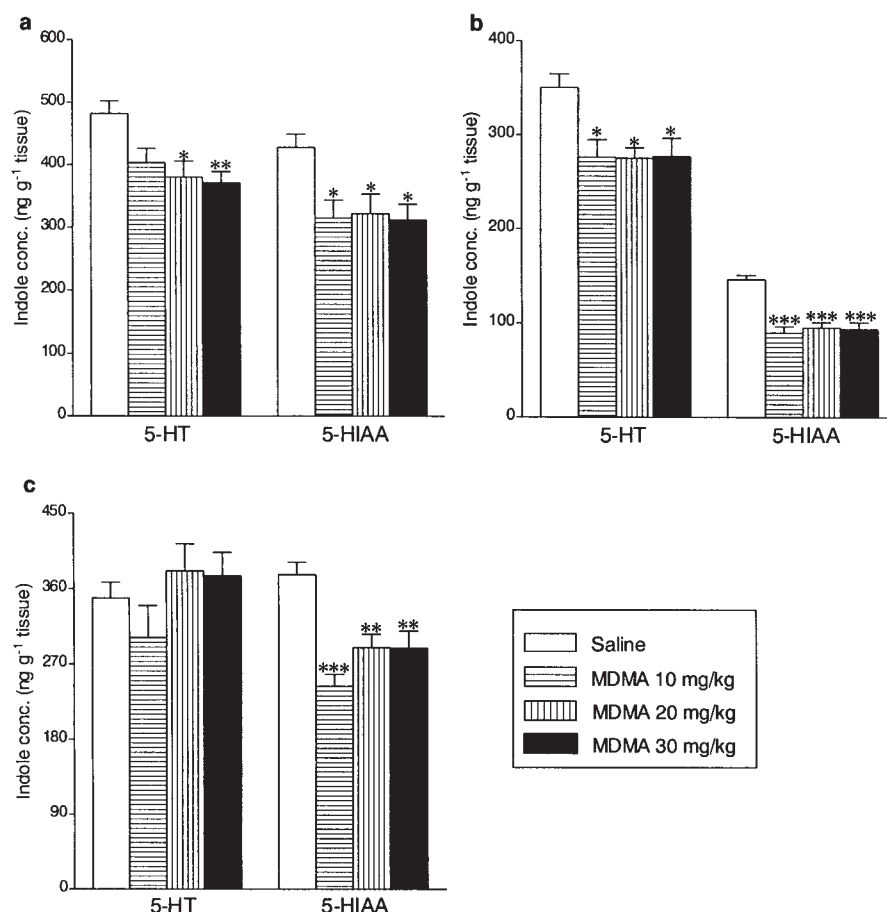


Fig. 2. Effect of MDMA (10, 20 and 30 mg/kg, i.p.) given 3 times 3 h apart on the indole concentrations of (a) hippocampus, (b) cortex and (c) striatum 3 h after the last MDMA injection. The results are shown as mean $\pm$ SEM ($n=5-6$). Different from saline: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

depletion of dopamine, produced no change in DOPAC content and increased the HVA concentration in the striatum (Fig. 3).

In contrast, fluoxetine (10 mg/kg, i.p.) did not produce any effect on dopamine metabolism in saline-treated animals nor did it modify the acute changes induced by MDMA on dopamine metabolism (data not shown).

3.3. Effect of MDMA on brain monoamine concentrations 7 days later

Repeated administration of MDMA (20 or 30 mg/kg, i.p. $\times$ 3) produced a dose-dependent loss in the striatal concentration of dopamine and its metabolites, DOPAC and HVA (Fig. 4), the effect following the dose of 10 mg/kg not being significant (Fig. 4). No effect was seen on the concentration of 5-HT, or its metabolite 5-HIAA, in the hippocampus, cortex and striatum, even at the highest dose (Table 1).

3.4. Effects of fluoxetine and GBR 12909 on MDMA-induced dopamine loss

Administration of GBR 12909 (10 mg/kg, i.p.) 30 min before each of the three MDMA (30 mg/kg, i.p.)

doses completely prevented the MDMA-induced loss in the striatal content of dopamine and its metabolites (Fig. 5). In contrast, fluoxetine (10 mg/kg, i.p.) failed to provide protection (Fig. 5). Neither GBR 12909 nor fluoxetine administration altered the striatal concentration of dopamine and its metabolites in saline-treated mice (Fig. 5).

3.5. Effect of repeated administration of MDMA on rectal temperature

The first injection of MDMA (10 mg/kg, i.p.) induced a marked hypothermic response lasting 2.5 h, the second and third injections not producing any effect on body temperature. The dose of 20 mg/kg of MDMA also induced a decrease in body temperature after the first injection and a modest hyperthermia after the second injection which was maintained throughout the rest of the experiment. Following MDMA (30 mg/kg) mice became hyperthermic immediately after the first injection. The second injection progressively decreased rectal temperature, mice showing marked hypothermia during 1 h after the third MDMA dose (Fig. 6).

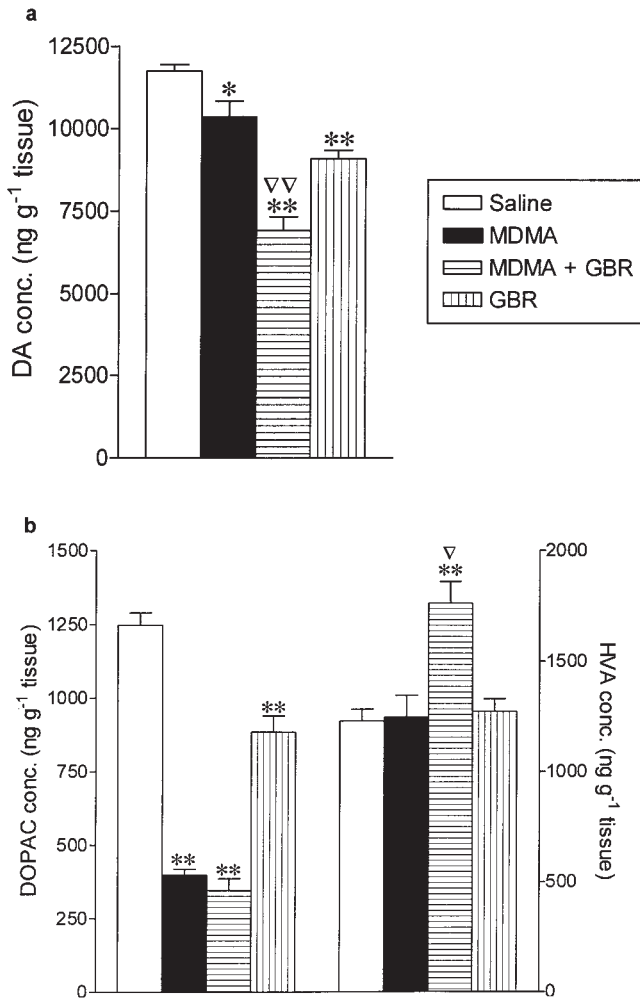


Fig. 3. The effect of GBR 12909 on the changes induced by MDMA (30 mg/kg, i.p.) on the concentrations of (a) dopamine (DA) and (b) 3,4-dihydroxyphenylacetic (DOPAC) and homovanillic (HVA) acids in the striatum of mice receiving saline or GBR 12909 (GBR, 10 mg/kg) 30 min before each of 3 MDMA injections 3 h apart. Mice were sacrificed 30 min after last MDMA administration. The results are shown as mean $\pm$ SEM ($n=5-10$). Different from saline: * $p<0.05$, ** $p<0.001$. Different from MDMA: $\nabla p<0.05$, $\nabla\nabla p<0.001$.

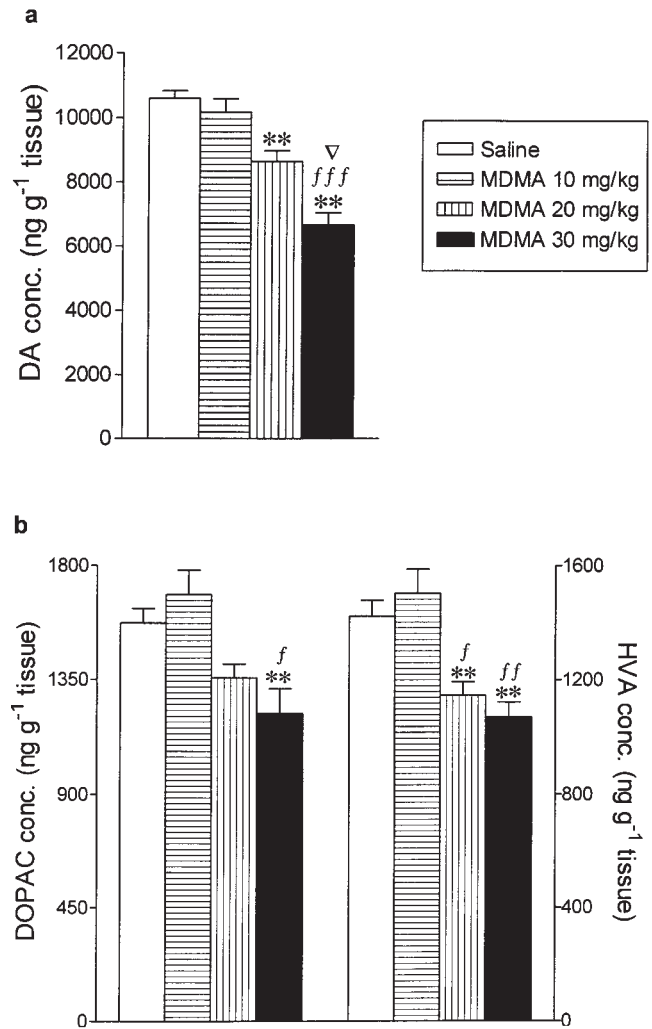


Fig. 4. Effect of repeated administration of MDMA (10, 20 and 30 mg/kg, i.p.) on the striatal concentrations of (a) dopamine (DA) and (b) 3,4-dihydroxyphenylacetic (DOPAC) and homovanillic (HVA) acids 7 days after treatment. The results are shown as mean $\pm$ SEM ($n=5-11$). Different from saline: ** $p<0.001$. Different from 10 mg/kg MDMA: $\nabla p<0.05$, $\nabla\nabla p<0.01$, $\nabla\nabla\nabla p<0.001$. Different from 20 mg/kg MDMA: $\nabla p<0.01$.

3.6. Effects of GBR 12909 and fluoxetine on rectal temperature of MDMA-treated mice

Pre-treatment with GBR 12909 (10 mg/kg, i.p.) did not modify the changes on rectal temperature induced by MDMA (30 mg/kg) (Fig. 7) whilst pretreatment with fluoxetine (10 mg/kg, i.p.) abolished the effect of MDMA on body temperature in such a way that mice receiving fluoxetine and MDMA showed a temperature similar to that observed in saline-treated animals (Fig. 7). The administration of either GBR 12909 or fluoxetine to saline-treated mice did not produce any change in the rectal temperature (data not shown).

4. Discussion

The current studies suggest that both the acute and the long term neurotoxic effects of MDMA in the mouse brain are markedly different from its effects in the rat brain. This appears to be true in terms of its effects on both dopamine and 5-HT biochemistry. The effects will now therefore be discussed in turn.

4.1. Effect of MDMA on 5-HT biochemistry in the brain

Three hours after the last dose of MDMA to mice there was a modest decrease in the 5-HT concentration in the cortex and hippocampus, but not striatum. There was also a small decrease in the 5-HT metabolite, 5-

Table 1

The indole concentrations in hippocampus, cortex and striatum 7 days after MDMA (10, 20 and 30 mg/kg, i.p.) given 3 times 3 h apart^a

	Saline	MDMA		
		10 mg/kg	20 mg/kg	30 mg/kg
<i>Hippocampus</i>				
5-HT	343±19	335±20	325±23	354±17
5-HIAA	387±8	374±18	367±29	376±19
<i>Cortex</i>				
5-HT	444±43	408±31	380±32	415±41
5-HIAA	194±11	181±14	180±7	180±8
<i>Striatum</i>				
5-HT	502±18	455±22	455±29	443±25
5-HIAA	480±19	456±25	490±18	436±50

^a Results shown as mean ± SEM, *n*=5–9. Indole concentrations expressed in ng/g tissue.

HIAA, in all 3 regions. Stone et al. (1987) previously reported that MDMA produced a slight and reversible depletion of 5-HT and 5-HIAA concentration in mouse striatum. The effect was not dose-related and may reflect enhanced 5-HT release from nerve terminals (Steele et al., 1987), since mouse tryptophan hydroxylase does not appear to be inhibited by MDMA (Stone et al., 1987) in contrast to rat brain (Schmidt and Taylor, 1987, 1988). These data contrast strongly with the effect of MDMA in rat brain where single or repeated doses of MDMA produce a profound decrease in the cerebral 5-HT content in the first few hours following MDMA administration (see Colado and Green, 1994).

In addition, in contrast to the data reported by Logan et al. (1988), but in agreement with others (Battaglia et al., 1988; O'Callaghan and Miller, 1994), our study found MDMA administration to mice did not produce any long lasting effect on the indole content. This is again in marked contrast to the effect of MDMA in rats where it produces sustained neurotoxic damage to 5-HT nerve terminals, which is reflected in a long term loss in the 5-HT concentration of several brain regions (Stone et al., 1986; Schmidt, 1987; Schmidt and Kehne, 1990; Steele et al., 1994; Green et al., 1995; White et al., 1996; Colado et al., 1997, 1999; Huether et al., 1997).

4.2. Effect of MDMA on dopamine biochemistry in the brain

Three hours after the last dose of MDMA there was a dose dependent decrease in dopamine and its metabolite DOPAC in mouse brain. Our study therefore confirmed the report of Logan et al. (1988) showing that as early as 3 h after administration, MDMA produced a modest reduction in dopamine concentration. Measures of dopamine metabolites do not allow accurate interpretations in terms of dopamine release and microdialysis studies are essential to clarify whether MDMA actually releases

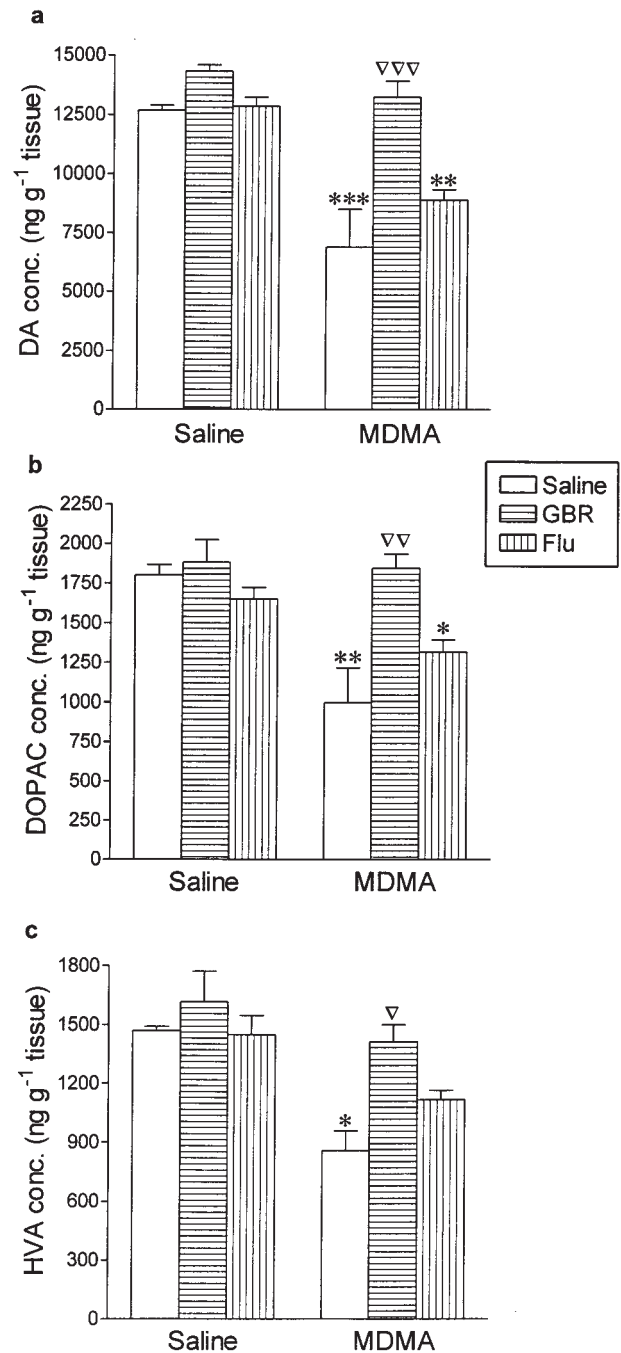


Fig. 5. The striatal concentrations of (a) dopamine (DA), (b) 3,4-dihydroxyphenylacetic (DOPAC) and (c) homovanillic (HVA) acids 7 days after saline, GBR 12909 (GBR, 10 mg/kg) or fluoxetine (Flu, 10 mg/kg) 30 min before saline or each of 3 MDMA (30 mg/kg) injections 3 h apart. The results are shown as mean ± SEM (*n*=5–7). Different from saline: **p*<0.05, ***p*<0.01, ****p*<0.001. Different from MDMA: ▽*p*<0.05, ▽▽*p*<0.01, ▽▽▽*p*<0.001.

dopamine in mouse brain. However the decrease in dopamine content, even after 3 doses of MDMA was small, suggesting a modest effect. In rats, there is substantial evidence that MDMA produces a large acute increase in dopamine release (Koch and Galloway, 1997; Sabol and Seiden, 1998; Colado et al., 1999), an effect

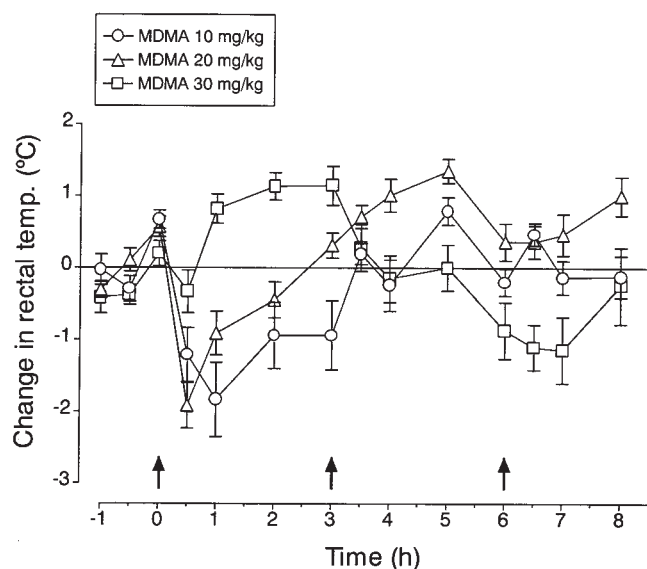


Fig. 6. Effect of MDMA (10, 20 and 30 mg/kg, i.p.) given 3 times 3 h apart (indicated by the arrow) on rectal temperature in mice. Results show change in temperature after subtracting all data by a baseline value corresponding to saline-treated mice. The symbols are shown as mean and the vertical lines indicate SEM ($n=5-12$). There was no difference in the basal temperature of the groups. There was a significant difference between the response of the mice to MDMA 20 mg/kg compared to 30 mg/kg after the first ($F(1,22)=26.53$, $p<0.001$) and second injection ($F(1,22)=12.32$, $p<0.01$). Changes induced by MDMA 20 mg/kg were not significantly different from those of MDMA 10 mg/kg ($F(1,15)=3.73$, $p=0.0725$).

that is, in part, due to the enhanced 5-HT release occurring at the same time. It has been shown, for example, that fluoxetine prevents the increased dopamine release (Gudelsky and Nash, 1996; Koch and Galloway, 1997). For this reason we examined the effect of both fluoxetine and GBR 12909 on the acute effects of MDMA on dopamine metabolism.

In contrast to the effect in rats (Gudelsky and Nash, 1996; Koch and Galloway, 1997), fluoxetine had no effect on the MDMA-induced change in dopamine metabolism. Administration of GBR 12909 not only failed to prevent the acute effects of MDMA on the dopamine system, but in fact potentiated them. This is hard to reconcile with the evidence in rats that GBR 12909 prevents MDMA-induced dopamine release in rat brain slices (Koch and Galloway, 1997), but is consistent with the observation that mazindol failed to block methamphetamine-induced dopamine depletion in rat brain in vivo (Marek et al., 1990a). Thus one may propose that the mechanisms involved in dopamine release in rat and mouse brain differ and the question arises as to whether the acute change in dopamine concentration following MDMA involves the dopamine carrier.

Differences between the action of MDMA on dopamine in mouse and rat brain are also emphasised in our studies on the long term consequences of drug administration. While there have been some reports that MDMA

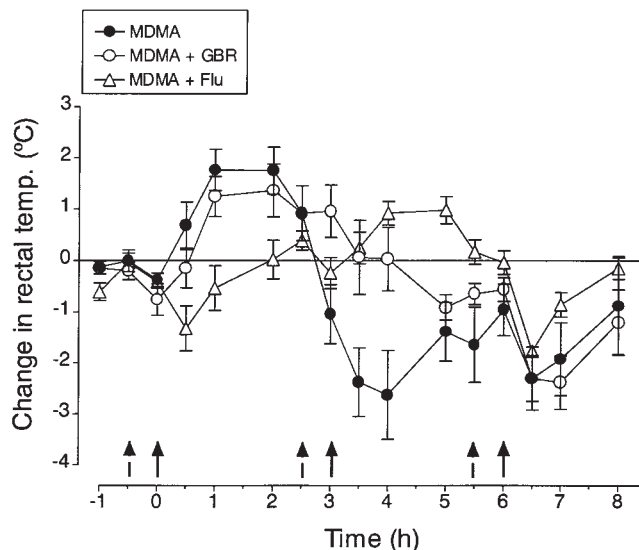


Fig. 7. Effect of pretreatment of mice with GBR 12909 (GBR, 10 mg/kg, i.p.) or fluoxetine (Flu, 10 mg/kg, i.p.) 30 min before MDMA (30 mg/kg, i.p.) on rectal temperature. MDMA was given 3 times 3 h apart. Results show change in temperature after subtracting all data by a baseline value corresponding to saline-treated mice. The symbols are shown as mean and the vertical lines indicate SEM ($n=5-8$). There was no difference in the basal temperature of the groups. Whilst GBR did not alter the changes induced by MDMA on temperature either after the first ($F(1,8)=0.004$, n.s.) or second injections ($F(1,8)=0.952$, n.s.), fluoxetine prevented the first ($F(1,11)=8.053$, $p<0.01$) and second response ($F(1,11)=10.839$, $p<0.001$).

produces long term neurotoxic loss of dopamine nerve endings in rat brain, the majority of studies indicate that there is no long term damage to dopaminergic systems (see Introduction). In contrast, MDMA is clearly damaging to dopaminergic terminals in mouse brain, producing a major long term loss in cerebral dopamine content (this paper; Logan et al., 1988; Laverty and Logan, 1990; Miller and O'Callaghan, 1994) which probably reflects a neurotoxic degeneration of dopamine nerve terminals in the striatum (Mann et al., 1997). In rats, the dopamine uptake inhibitor GBR 12909 protects against neurodegeneration of 5-HT neurones (Stone et al., 1988), but in mice GBR 12909 protects against damage to dopamine nerve terminals (this paper). It is noteworthy that the dopamine uptake inhibitor mazindol prevents methamphetamine-induced damage to dopamine nerve endings (Marek et al., 1990b). In contrast, the 5-HT uptake inhibitor protects against MDMA-induced damage to 5-HT nerve endings in rats (Schmidt, 1987; Malberg et al., 1996), but is ineffective against MDMA-induced damage to dopaminergic neurones in mice (this paper).

It has been suggested that MDMA acts as a substrate of the dopamine uptake system in rat brain (Crespi et al., 1997). If that were so in mouse brain then GBR 12909 would be expected to inhibit the entry of MDMA into the nerve terminal. Our data does not support this hypothesis because co-administration of GBR 12909 with MDMA produced a greater effect on dopamine

release than that obtained with each compound separately. On the other hand, MDMA is relatively lipid soluble and could therefore enter the nerve terminal by passive diffusion as occurs in synaptosomes (Zaczek et al., 1990). Taken together, these observations indicate that the neuroprotective effect of GBR 12909 against MDMA-induced toxicity in mice is probably not due to its ability to prevent either the entry of MDMA into the neuron or inhibit dopamine release into the synaptic cleft. In this context, it is worth noting that MDMA-induced dopamine release involves both a Ca^{2+} -independent process mediated by the transporter (Schmidt et al., 1987; Nash and Brodtkin, 1991; Crespi et al., 1997) and a Ca^{2+} -dependent mechanism which involves an increase in the Ca^{2+} intracellular concentration leading to an exocytotic-like release (Crespi et al., 1997).

The proposal that the mechanism of neurotoxic damage induced by MDMA is not due to the parent compound but to a metabolite is supported by the fact that, in the rat, i.c.v. injection, or the continuous perfusion of MDMA into the hippocampus does not produce any long term effect on 5-HT nerve terminals or cell bodies (Paris and Cunningham, 1991; Esteban et al., 1999). MDMA must therefore have to be transformed to both quinone and catechol metabolites which, on further metabolism, would result in the formation of free radicals in the rat hippocampus (Colado et al., 1997) and in changes in the activities of various antioxidant enzymes (CuZn superoxide dismutase, catalase and glutathione peroxidase) in several areas of the mouse brain (Cadet et al., 1995; Jayanthi et al., 1999). The current results appear to allow two possible interpretations: (1) GBR 12909 is preventing the entry into the terminal of a neurotoxic metabolite of MDMA via the dopamine transporter or, (2) GBR 12909 may have additional actions on dopaminergic neurones. The first possibility seems to be the most likely if we take into account that GBR 12909 is a rather selective dopamine uptake inhibitor (Van Der Zee et al., 1980; Andersen, 1989), not producing appreciable effects on either norepinephrine (Heikkilä and Manzino, 1984) or 5-HT (this work) uptake mechanisms. Our findings are thus consistent with those of Marek et al. (1990a) showing that other dopamine uptake inhibitors such as amfonelic acid or mazindol, did not block the acute depletion of dopamine neostriatal levels induced by administration of a single high dose of methamphetamine, but did prevent the long term loss of cerebral dopamine (Marek et al., 1990b).

4.3. Effects on rectal temperature

Traditionally the release of 5-HT and dopamine induced by amphetamine derivatives has been associated with the drug-induced hyperthermia (Bowyer et al., 1993; White et al., 1996). However, the acute 5-HT depletion induced by MDMA in mice was similar for all

the doses tested while the temperature change followed different patterns. In addition, the current experiments also appear to rule out any direct relationship between the neurotoxic effects of MDMA and its acute effects on body temperature. MDMA (30 mg/kg) altered the body temperature biphasically, producing rapid hyperthermia followed by prolonged hypothermia. Fluoxetine administered before each MDMA injection prevented these changes but did not provide protection. Furthermore, GBR 12909 did not modify the MDMA-induced change in body temperature, indicating not only that its neuroprotective effect is unrelated to effects on body temperature but also that changes in dopaminergic activity are not the mechanism by which MDMA alters rectal temperature.

Finally, the MDMA-induced acute change in body temperature, specifically acute hyperthermia, plays a major role in the occurrence of the subsequent neurodegeneration in rats (Malberg et al., 1996; Colado et al., 1998, 1999), but temperature changes appear to have little if any role in the neurotoxic effect of MDMA in mice.

4.4. Summary

The current results favour the view that MDMA has very different effects on both 5-HT and dopamine neurochemistry in mouse brain when compared with its effects in rat brain. Further in vitro and in vivo studies are required to clarify the reasons for these differences.

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References

- Andersen, P.H., 1989. The dopamine uptake inhibitor GBR 12909: selectivity and molecular mechanism of action. *European Journal of Pharmacology* 166, 493–504.
- Baldwin, H.A., Colado, M.I., Murray, T.K., De Souza, R.J., Green, A.R., 1993. Striatal dopamine release in vivo following neurotoxic doses of methamphetamine and effect of the neuroprotective drugs, chlormethiazole and dizocilpine. *British Journal of Pharmacology* 108, 590–596.
- Battaglia, G., Yeh, S.Y., O'Hearn, E., Molliver, M.E., Kuhar, M.J., De Souza, E.B., 1987. 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: quantification of neurodegeneration by measurement of [^3H]paroxetine-labeled serotonin uptake sites. *Journal of Pharmacology and Experimental Therapeutics* 242, 911–916.

- Battaglia, G., Yeh, S.Y., De Souza, E.B., 1988. MDMA-induced neurotoxicity: parameters of degeneration and recovery of brain serotonin neurons. *Pharmacology, Biochemistry and Behavior* 29, 269–274.
- Bowyer, J.F., Gough, B., Slikker, W. Jr., Lipe, G.W., Newport, G.D., Holson, R.R., 1993. Effects of a cold environment or age on methamphetamine-induced dopamine release in the caudate putamen of female rats. *Pharmacology, Biochemistry and Behavior* 44, 87–98.
- Bowyer, J.F., Davies, D.L., Schmued, L., Broening, H.W., Newport, G.D., Slikker, W. Jr., Holson, R.R., 1994. Further studies of the role of hyperthermia in methamphetamine neurotoxicity. *Journal of Pharmacology and Experimental Therapeutics* 268, 1571–1580.
- Cadet, J.L., Ladenheim, B., Hirata, H., Rothman, R.B., Ali, S., Carlson, E., Epstein, C.J., Moran, T.H., 1995. Superoxide radicals mediate the biochemical effects of methylenedioxymethamphetamine (MDMA): evidence from using CuZn-superoxide dismutase transgenic mice. *Synapse* 21, 169–176.
- Colado, M.I., Green, A.R., 1994. A study of the mechanism of MDMA ('ecstasy')-induced neurotoxicity of 5-HT neurones using chlormethiazole, dizocilpine and other protective compounds. *British Journal of Pharmacology* 111, 131–136.
- Colado, M.I., Granados, R., O'Shea, E., Esteban, B., Green, A.R., 1998. Role of hyperthermia in the protective action of clomethiazole against MDMA ('ecstasy')-induced neurodegeneration, comparison with the novel NMDA channel blocker AR-R15896. *British Journal of Pharmacology* 124, 479–484.
- Colado, M.I., O'Shea, E., Granados, R., Murray, T.K., Green, A.R., 1997. In vivo evidence for free radical involvement in the degeneration of rat brain 5-HT following administration of MDMA ('ecstasy') and *p*-chloroamphetamine but not the degeneration following fenfluramine. *British Journal of Pharmacology* 121, 889–900.
- Colado, M.I., O'Shea, E., Granados, R., Esteban, B., Martin, A.B., Green, A.R., 1999. Studies on the role of dopamine in the degeneration of 5-HT nerve endings in the brain of Dark Agouti rats following 3,4-methylenedioxymethamphetamine (MDMA or 'ecstasy') administration. *British Journal of Pharmacology* 126, 911–924.
- Crespi, D., Mennini, T., Gobbi, M., 1997. Carrier-dependent and Ca²⁺-dependent 5-HT and dopamine release induced by (+)-amphetamine, 3,4-methylenedioxymethamphetamine, *p*-chloroamphetamine and (+)-fenfluramine. *British Journal of Pharmacology* 121, 1735–1743.
- Esteban, B., O'Shea, E., Martínez, I., Green, A.R., Colado, M.I., 1999. Similar brain concentrations of MDMA have different neurotoxic effects following central or peripheral drug administration. *British Journal of Pharmacology* 127, 70P.
- Gough, B., Ali, S.F., Slikker, W. Jr., Holson, R.R., 1991. Acute effects of 3,4-methylenedioxymethamphetamine (MDMA) on monoamines in rat caudate. *Pharmacology, Biochemistry and Behavior* 39, 619–623.
- Green, A.R., De Souza, R.J., Williams, J.L., Murray, T.K., Cross, A.J., 1992. The neurotoxic effects of methamphetamine on 5-hydroxytryptamine and dopamine in brain: evidence for the protective effect of chlormethiazole. *Neuropharmacology* 31, 315–321.
- Green, A.R., Cross, A.J., Goodwin, G.M., 1995. Review of the pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA or 'ecstasy'). *Psychopharmacology* 119, 247–260.
- Gudelsky, G.A., Yamamoto, B.K., Nash, J.F., 1994. Potentiation of 3,4-methylenedioxymethamphetamine-induced dopamine release and serotonin neurotoxicity by 5-HT₂ receptor agonists. *European Journal of Pharmacology* 264, 325–330.
- Gudelsky, G.A., Nash, J.F., 1996. Carrier-mediated release of serotonin by 3,4-methylenedioxymethamphetamine: implications for serotonin–dopamine interactions. *Journal of Neurochemistry* 66, 243–249.
- Heikkilä, R.E., Manzino, L., 1984. Behavioral properties of GBR 12909, GBR 13069 and GBR 13098: specific inhibitors of dopamine uptake. *European Journal of Pharmacology* 103, 241–248.
- Hewitt, K.E., Green, A.R., 1994. Chlormethiazole, dizocilpine and haloperidol prevent the degeneration of serotonergic nerve terminals induced by administration of MDMA ('Ecstasy') to rats. *Neuropharmacology* 33, 1589–1595.
- Huether, G., Zhou, D., Rüther, E., 1997. Causes and consequences of the loss of serotonergic presynapses elicited by the consumption of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') and its congeners. *Journal of Neural Transmission* 104, 771–794.
- Itzhak, Y., Ali, S.F., 1996. The neuronal nitric oxide synthase inhibitor, 7-nitroindazole, protects against methamphetamine-induced neurotoxicity in vivo. *Journal of Neurochemistry* 67, 1770–1773.
- Jayanthi, S., Ladenheim, B., Andrews, A.M., Cadet, J.L., 1999. Overexpression of human copper/zinc superoxide dismutase in transgenic mice attenuates oxidative stress caused by methylenedioxymethamphetamine (ecstasy). *Neuroscience* 91, 1379–1387.
- Koch, S., Galloway, M.P., 1997. MDMA induced dopamine release in vivo: role of endogenous serotonin. *Journal of Neural Transmission* 104, 135–146.
- Lavery, R., Logan, B.J., 1990. Protection by MK 801 and other drugs of methylenedioxymethamphetamine (MDMA) neurotoxicity in rats and mice. *European Journal of Pharmacology* 183, 451–452.
- Lew, R., Sabol, K.E., Chou, C., Vosmer, G.L., Richards, J.B., Seiden, L.S., 1996. Methylenedioxymethamphetamine-induced serotonin deficits are followed by partial recovery over a 52-week period. Part II: Radioligand binding and autoradiography studies. *Journal of Pharmacology and Experimental Therapeutics* 276, 855–865.
- Logan, B.J., Lavery, R., Sanderson, W.D., Yee, Y.B., 1988. Differences between rats and mice in MDMA (methylenedioxymethylamphetamine) neurotoxicity. *European Journal of Pharmacology* 152, 227–234.
- Malberg, J.E., Sabol, K.E., Seiden, L.S., 1996. Co-administration of MDMA with drugs that protect against MDMA neurotoxicity produces different effects on body temperature in the rat. *Journal of Pharmacology and Experimental Therapeutics* 278, 258–267.
- Mann, H., Ladenheim, B., Hirata, H., Moran, T.H., Cadet, J.L., 1997. Differential toxic effects of methamphetamine (METH) and methylenedioxymethamphetamine (MDMA) in multidrug-resistant (*mdr1a*) knockout mice. *Brain Research* 769, 340–346.
- Marek, G.J., Vosmer, G., Seiden, L.S., 1990a. The effects of monoamine uptake inhibitors and methamphetamine on neostriatal 6-hydroxydopamine (6-OHDA) formation, short-term monoamine depletions and locomotor activity in the rat. *Brain Research* 516, 1–7.
- Marek, G.J., Vosmer, G., Seiden, L.S., 1990b. Dopamine uptake inhibitors block long term neurotoxic effects of methamphetamine. *Brain Research* 513, 274–279.
- Miller, D.B., O'Callaghan, J.P., 1994. Environment-, drug- and stress-induced alterations in body temperature affect the neurotoxicity of substituted amphetamines in the C57BL/6J mouse. *Journal of Pharmacology and Experimental Therapeutics* 270, 752–760.
- Molliver, M.E., Berger, U.V., Mamounas, L.A., Molliver, D.C., O'Hearn, E., Wilson, M.A., 1990. Neurotoxicity of MDMA and related compounds: anatomic studies. *Annals of the New York Academy of Science* 600, 640–661.
- Nash, J.F., 1990. Ketanserin pretreatment attenuates MDMA-induced dopamine release in the striatum as measured by in vivo microdialysis. *Life Science* 47, 2401–2408.
- Nash, J.F., Brodtkin, J., 1991. Microdialysis studies on 3,4-methylenedioxymethamphetamine-induced dopamine release: effect of dopamine uptake inhibitors. *Journal of Pharmacology and Experimental Therapeutics* 259, 820–825.
- O'Callaghan, J.P., Miller, D.B., 1994. Neurotoxicity profiles of substi-

- tuted amphetamines in the C57BL/6J mouse. *Journal of Pharmacology and Experimental Therapeutics* 270, 741–751.
- O'Hearn, E., Battaglia, G., De Souza, E.B., Kuhar, M.J., Molliver, M.E., 1988. Methylenedioxymethamphetamine (MDA) and methylenedioxymethamphetamine cause ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence. *Journal of Neuroscience* 8, 2788–2803.
- Paris, J.M., Cunningham, K.A., 1991. Lack of serotonin neurotoxicity after intraraphe microinjection of (+)-3,4-methylenedioxymethamphetamine (MDMA). *Brain Research Bulletin* 28, 115–119.
- Sabol, K.E., Lew, R., Richards, J.B., Vosmer, G.L., Seiden, L.S., 1996. Methylenedioxymethamphetamine-induced serotonin deficits are followed by partial recovery over a 52-week period. Part I: Synaptosomal uptake and tissue concentrations. *Journal of Pharmacology and Experimental Therapeutics* 276, 846–854.
- Sabol, K.E., Seiden, L.S., 1998. Reserpine attenuates D-amphetamine and MDMA-induced transmitter release in vivo: a consideration of dose, core temperature and dopamine synthesis. *Brain Research* 806, 69–78.
- Schmidt, C.J., 1987. Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine. *Journal of Pharmacology and Experimental Therapeutics* 240, 1–7.
- Schmidt, C.J., Levin, J.A., Lovenberg, W., 1987. In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain. *Biochemical Pharmacology* 36, 747–755.
- Schmidt, C.J., Taylor, V.L., 1987. Depression of rat brain tryptophan hydroxylase activity following the acute administration of methylenedioxymethamphetamine. *Biochemical Pharmacology* 36, 4095–4102.
- Schmidt, C.J., Taylor, V.L., 1988. Direct central effects of acute methylenedioxymethamphetamine on serotonergic neurons. *European Journal of Pharmacology* 156, 121–131.
- Schmidt, C.F., Abbate, G.M., Black, C.K., Taylor, V.L., 1990. Selective 5-hydroxytryptamine₂ receptor antagonists protect against the neurotoxicity of methylenedioxymethamphetamine in rats. *Journal of Pharmacology and Experimental Therapeutics* 255, 478–483.
- Schmidt, C.J., Kehne, J.H., 1990. Neurotoxicity of MDMA: neurochemical effects. *Annals of the New York Academy of Science* 600, 665–681.
- Sharkey, J., McBean, D.E., Kelly, P.A., 1991. Alterations in hippocampal function following repeated exposure to the amphetamine derivative methylenedioxymethamphetamine ('Ecstasy'). *Psychopharmacology* 105, 113–118.
- Steele, T.D., Nichols, D.E., Yim, G.K., 1987. Stereochemical effects of 3,4-methylenedioxymethamphetamine (MDMA) and related amphetamine derivatives on inhibition of uptake of [³H]monoamines into synaptosomes for different regions of rat brain. *Biochemical Pharmacology* 36, 2297–2303.
- Steele, T.D., McCann, U.D., Ricaurte, G.A., 1994. 3,4-Methylenedioxymethamphetamine (MDMA, 'Ecstasy'): pharmacology and toxicology in animals and humans. *Addiction* 89, 539–551.
- Stone, D.M., Stahl, D.C., Hanson, G.R., Gibb, J.W., 1986. The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxymethamphetamine (MDA) on monoaminergic systems in the rat brain. *European Journal of Pharmacology* 128, 41–48.
- Stone, D.M., Hanson, G.R., Gibb, J.W., 1987. Differences in the central serotonergic effects of methylenedioxymethamphetamine (MDMA) in mice and rats. *Neuropharmacology* 26, 1657–1661.
- Stone, D.M., Johnson, M., Hanson, G.R., Gibb, J.W., 1988. Role of endogenous dopamine in the central serotonergic deficits induced by 3,4-methylenedioxymethamphetamine. *Journal of Pharmacology and Experimental Therapeutics* 247, 79–87.
- Van Der Zee, P., Koger, H.S., Gootjes, J., Hespe, W., 1980. Aryl 1,4-dialk(en)ylpiperazines as selective and very potent inhibitors of dopamine uptake. *European Journal of Medicinal Chemistry* 15, 363–370.
- White, S.R., Obradovic, T., Imel, K.M., Wheaton, M.J., 1996. The effects of methylenedioxymethamphetamine (MDMA, 'Ecstasy') on monoaminergic neurotransmission in the central nervous system. *Progress in Neurobiology* 49, 455–479.
- Zaczek, R., Culp, S., De Souza, E.B., 1990. Intrasyntosomal sequestration of [³H]amphetamine and [³H]methylenedioxymethamphetamine: characterization suggest the presence of a factor responsible for maintaining sequestration. *Journal of Neurochemistry* 54, 195–204.