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# The pharmacology of the acute hyperthermic response that follows administration of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') to rats

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**1** The pharmacology of the acute hyperthermia that follows 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') administration to rats has been investigated.

**2** MDMA (12.5 mg $kg^{-1}$ i.p.) produced acute hyperthermia (measured rectally). The tail skin temperature did not increase, suggesting that MDMA may impair heat dissipation.

**3** Pretreatment with the 5-$HT_{1/2}$ antagonist methysergide (10 mg $kg^{-1}$), the 5-$HT_{2A}$ antagonist MDL 100,907 (0.1 mg $kg^{-1}$) or the 5-$HT_{2C}$ antagonist SB 242084 (3 mg $kg^{-1}$) failed to alter the hyperthermia. The 5-$HT_2$ antagonist ritanserin (1 mg $kg^{-1}$) was without effect, but MDL 11,939 (5 mg $kg^{-1}$) blocked the hyperthermia, possibly because of activity at non-serotonergic receptors.

**4** The 5-HT uptake inhibitor zimeldine (10 mg $kg^{-1}$) had no effect on MDMA-induced hyperthermia. The uptake inhibitor fluoxetine (10 mg $kg^{-1}$) markedly attenuated the MDMA-induced increase in hippocampal extracellular 5-HT, also without altering hyperthermia.

**5** The dopamine $D_2$ antagonist remoxipride (10 mg $kg^{-1}$) did not alter MDMA-induced hyperthermia, but the $D_1$ antagonist SCH 23390 (0.3–2.0 mg $kg^{-1}$) dose-dependently antagonized it.

**6** The dopamine uptake inhibitor GBR 12909 (10 mg $kg^{-1}$) did not alter the hyperthermic response and microdialysis demonstrated that it did not inhibit MDMA-induced striatal dopamine release.

**7** These results demonstrate that *in vivo* MDMA-induced 5-HT release is inhibited by 5-HT uptake inhibitors, but MDMA-induced dopamine release may not be altered by a dopamine uptake inhibitor.

**8** It is suggested that MDMA-induced hyperthermia results not from MDMA-induced 5-HT release, but rather from the increased release of dopamine that acts at $D_1$ receptors. This has implications for the clinical treatment of MDMA-induced hyperthermia.

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**Keywords:** 3,4-methylenedioxymethamphetamine; ecstasy; hyperthermia; dopamine; 5-hydroxytryptamine; 5-HT antagonists; dopamine antagonists; GBR 12909; fluoxetine

**Abbreviations:** aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; DA, Dark Agouti; EDTA, ethylenediamine tetra-acetic acid; GBR 12909, 1-[2[*bis*(4-fluorophenyl)methoxy ethyl]-4-[3-phenylpropyl]piperazine diHCl; h.p.l.c., high performance liquid chromatography; HVA, homovanillic acid; MDL 100,907, R(+)-α-(2,3-dimethoxyphenyl)-1-[2(4-fluorophenylethyl)]-4-piperidine-methanol; MDL 11939, α-phenyl-1-(2-phenylethyl)-4-piperidinemethanol; MDMA, (±)3,4-methylenedioxymethamphetamine; SB 242084, 6-chloro-5-methyl-methyl-1-[2-(-methylpyridyl-3-oxy)-pyrid-5-yl carbamoyl]indoline; SCH 23390, R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzapine; 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindoleacetic acid

## Introduction

3,4-Methylenedioxymethamphetamine (MDMA or 'ecstasy') is a commonly used recreational drug, often ingested at dance clubs. A major feature of cases presenting with acute MDMA toxicity is hyperthermia, with body temperatures as high as 43°C having been reported (Henry, 1992). It is probable that many of the other, often fatal, toxicological problems that are seen, particularly rhabdomyolysis, disseminated intravenous coagulation and acute renal failure (Brown & Osterloh, 1987; Henry *et al*., 1992; Screaton *et al*., 1992) result from the hyperthermia.

Clinically, the major treatment for the hyperthermia is to decrease body temperature which has been achieved by surrounding the body with ice. Pharmacologically, administration of dantrolene has been used, and this has sometimes led to a successful outcome (Henry, 1992; Tehan, 1993; Mallick & Bodenham, 1997). However, its use has been questioned by Barrett (1992) on the basis of the fact that it is a peripherally acting muscle relaxant with no central effects and is therefore not acting on the primary mechanisms involved in the production of the hyperthermia.

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Surprisingly, there has been limited experimental investigation into the pharmacology of the MDMA-induced acute hyperthermic response, despite the fact that administration of MDMA to rats, as in humans, produces an acute dose-dependent hyperthermic response (Nash *et al*., 1988; Gordon *et al*., 1991; Colado *et al*., 1993; Dafters, 1994; O'Shea *et al*., 1998). It has long been known that acutely increasing 5-HT synthesis and release in rat brain by administration of L-tyrptophan and a monoamine oxidase inhibitor results in hyperthermia (Grahame-Smith, 1971a; Green & Grahame-Smith, 1976). Since MDMA produces an acute and massive release of 5-HT from serotonergic nerve endings (Stone *et al*., 1986; 1987; Schmidt *et al*., 1987; Colado & Green, 1994; Mechan *et al*., 2000) it has sometimes been assumed (e.g. Shankaran & Gudelsky, 1999) that MDMA-induced hyperthermia is a consequence of 5-HT release and subsequent stimulation of the 5-HT receptors involved in thermoregulation. Such a view has been reinforced by the observations that p-chloroamphetamine, another 5-HT releasing drug, also produces hyperthermia (Colado *et al*., 1993) and that 5-$HT_2$ receptor antagonists such as ketanserin and MDL 11,939 antagonize MDMA-induced hyperthermia (Nash *et al*., 1988; Schmidt *et al*., 1990).

One problem encountered in previous studies on MDMA-induced neurotoxicity has been the fact that some drugs produce marked hypothermia in rats when given alone. This effect therefore confounds any evidence that a drug has a selective action on the MDMA-induced hyperthermic response and makes interpretation difficult (see for example Colado *et al*., 1999). We have now investigated the effect of compounds known to alter either 5-HT or dopamine function on their ability to antagonize MDMA-induced hyperthermia by examining their effect on both saline and MDMA treated rats.

## Methods

### *Animals*

Male Dark Agouti (DA) rats, weighing 200–260 g, were used in all experiments (Harlan U.K. Ltd., Bicester, Oxon, U.K. and Interfauna, Barcelona, Spain). The animals were housed in groups of three, at an ambient temperature of $20\pm2^{\circ}C$ and a 12 h light/dark cycle (lights on: 0730 h). Both food (B&K Universal Ltd., Aldbrough, Hull, U.K.) and water were freely provided.

All procedures were carried out following approval by the University Experimental Ethics Committee.

### *Drugs and drug administration*

Drugs were administered, intraperitoneally (i.p.) unless otherwise specified. In the antagonist/uptake inhibitor pretreatment studies all compounds were injected 20 min prior to administration of MDMA or saline. In the microdialysis studies fluoxetine was administered 5 min before and 55 min after the MDMA or saline, while GBR 12,909 was injected 30 min prior to MDMA/saline administration. All drugs were dissolved in normal saline (NaCl 0.9% w $v^{-1}$) at a volume of 1 ml $kg^{-1}$, unless otherwise stated, and doses are quoted as the base weight. Methysergide was dissolved in 20% dimethylsulphoxide in normal saline. Ritanserin and MDL 100,907 were dissolved in 2% glacial acetic acid in deionized water. SB 242084 was dissolved in normal saline and citric acid (25 mM, Sigma) containing a saturated solution of $\beta$-cyclodextrin (Sigma). GBR 12909 was dissolved in peanut oil and administered s.c. in a volume of 2 ml $kg^{-1}$.

Drugs were obtained from the following sources: MDMA (Sigma-Aldrich Co. Ltd., Poole, Dorset, U.K., Dr P. Guiry, University College, Dublin and Ministry of Health, Spain); methysergide hydrogen maleate (Novartis International AG, Basel, Switzerland); $\alpha$-phenyl-1-(2-phenylethyl)-4-piperidine-methanol (MDL 11,939) and R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzapine (SCH 23390) (Tocris Cookson Ltd., Avonmouth, Bristol, U.K.); ritanserin (Janssen Research Foundation, Beerse, Belgium); R-(+)-a-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidinemethanol (MDL 100,907) and 6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbomyl]indoline (SB 242084) (Dr G. Kennett, Vernalis Ltd., Wokingham, U.K.); (cis)-zimeldine and S-(−)-remoxipride hydrochloride (Astra-Zeneca, R&D Södertälje, Sweden); 1-[2-*bis*(4-fluorophenyl) methyoxy]ethyl]-4-3-phenylpropyl]piperazine dihydrochloride (GBR 12909) (Sigma Chemical Co., Poole, Dorset, U.K.).

### *Temperature measurement*

Temperature measurement was performed using an MC 8700 thermometer, with digital readout, and a lubricated H-RB3 rectal temperature probe (EXACON A/S, Roskilde, Denmark). Each rat was lightly restrained by hand for approximately 20 s while the probe was inserted approximately 2.5 cm into its rectum and a steady reading was obtained. In one experiment tail temperature was measured on the upper tail region by binding the thermocouple probe to the tail with plastic tape.

### *Implantation of a microdialysis probe in the hippocampus or striatum*

Rats were anaesthetized with sodium pentobarbitone ('Euta-Lender', 40 mg $kg^{-1}$ i.p.) and secured in a Kopf stereotaxic frame with the tooth bar at −3.3 mm below the interaural zero. For implantation in the hippocampus, the dialysis probe (3.5 mm × 200 $\mu$m, Cuprophan) was implanted +2.2 mm from the interaural line, −4.3 mm lateral and −8 mm below the surface of the brain (König & Klippel, 1963).

For implantation in the striatum, rats were anaesthetized and prepared as above. The dialysis probe was implanted in the right striatum using the following co-ordinates: +7.9 mm from the interaural line, −2.5 mm lateral and − mm below the surface of the brain (König & Klippel, 1963). In both cases probes were secured to the skull as described by Baldwin *et al*. (1994).

### *Measurement of monoamines and their metabolites in the hippocampal and striatal dialysate*

Twenty-four hours after implantation, probes were perfused with aCSF (mM: KCl 2.5, NaCl 125, $MgCl_2.6H_2O$ 1.18, $CaCl_2.2H_2O$ 1.26) at a rate of 1 $\mu$l $min^{-1}$ and samples were collected from the freely moving animals at 30 min intervals

in tubes containing 5 $\mu$l of a solution composed of $HClO_4$ (0.01 M), cysteine (0.2%) and sodium metabisulphite (0.2%). The first 60 min sample was discarded and the next three 30 min baseline samples collected before starting MDMA perfusion.

5-HT, dopamine and their metabolites were measured in the dialysate by h.p.l.c. with electrochemical detection. The mobile phase consisted of $KH_2PO_4$ (0.05 M), octanesulphonic acid (0.4 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 $min^{-1}$.

The h.p.l.c. system consisted of a pump (Waters 510) linked to a manual sample injector (Loop 20 $\mu$l, Rheodyne), a stainless steel reversed-phase column (spherisorb ODS2, 5 $\mu$m, 150 × 4.6 mm) with a precolumn and a coulometric detector (Coulochem 5100 A) with a 5011 analytical cell. The working electrode potential was set at 400 mV with 500 nA gain. The current produced was monitored *via* a computer data handling system (AXXIOM 747).

### *Statistics*

Statistical analyses of the temperature data and microdialysis experiments were performed using the statistical computer package BMDP/386 Dynamic (BMDP Statistical Solutions). Data were analysed by two-way analysis of variance (ANOVA) with repeated measures (program 2V) or, where missing values occurred, an unbalanced repeated measures 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 (starting immediately after the first injection) data.

## Results

### *Effect of MDMA on rectal and tail temperature*

Administration of MDMA (12.5 mg $kg^{-1}$ i.p.) produced an approximate 1.5°C rise in rectal temperature within 20 min, and this increase was sustained for at least 3 h (Figure 1, main graph). In contrast, the tail temperature did not rise but was seen to decrease during the period 40–80 min after injection, compared to the pretreatment value, and was overall similar to the control animal values (Figure 1, inset graph).

### *Effect of methysergide, MDL 11,939 and ritanserin on MDMA-induced hyperthermia*

Administration of the non-selective 5-$HT_{1/2}$ antagonist methysergide, at a dose of 5 mg $kg^{-1}$ (data not shown) or 10 mg $kg^{-1}$ (Figure 2a) had no significant effect on MDMA-induced hyperthermia and no effect on the rectal temperature of control animals. In contrast, the 5-$HT_2$ antagonist MDL 11,939 (5 mg $kg^{-1}$) abolished the MDMA-induced hyperthermic response without altering the rectal temperature of saline-treated rats (Figure 2b). Ritanserin had no effect on the hyperthermic response at a dose of 1 mg $kg^{-1}$ (Figure 2c). At a dose of 5 mg $kg^{-1}$ it did not alter the peak temperature response but modestly attenuated the duration of the response (data not shown).

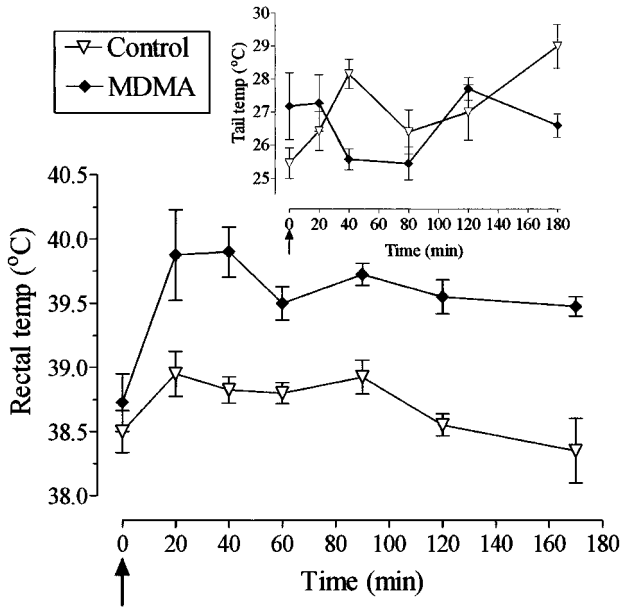


**Figure 1** Effect of MDMA on temperature. Main graph: rectal temperature of rats injected with MDMA (12.5 mg $kg^{-1}$, i.p.) or saline at t = 0. MDMA produced a significant rise in body temperature ($F(1,6) = 61.17$, $P < 0.001$) during $t_{20-180}$. Inset graph: tail skin temperature of rats injected with MDMA (12.5 mg $kg^{-1}$, i.p.) or saline at t = 0. MDMA had no significant effect on tail skin temperature compared to control animals.

### *Effect of selective 5-$HT_{2A}$ and 5-$HT_{2C}$ antagonists on MDMA-induced hyperthermia*

The potent and selective 5-$HT_{2A}$ antagonist MDL 100,907 was without effect on MDMA-induced hyperthermia or basal temperature at a dose of 0.1 mg $kg^{-1}$ (Figure 3a). At a dose of 0.3 mg $kg^{-1}$ it produced a modest attenuation of MDMA-induced hyperthermia (Figure 3b). The 5-$HT_{2C}$ antagonist SB 242084 (3 mg $kg^{-1}$) did not alter MDMA-induced hyperthermia and had no effect on the rectal temperature of control rats (Figure 3c).

### *Effect of 5-HT uptake inhibitors on MDMA-induced hyperthermia*

Pre-treatment with the 5-HT uptake inhibitor zimeldine (10 mg $kg^{-1}$) did not alter MDMA-induced hyperthermia and was without effect on the rectal temperature of saline-injected (control) rats (Figure 4).

A study was also conducted with the 5-HT uptake inhibitor fluoxetine, using a different protocol. Rats that had a microdialysis probe implanted in the hippocampus 24 h earlier were administered fluoxetine (10 mg $kg^{-1}$) 5 min prior to and 55 min after injection of MDMA (15 mg $kg^{-1}$). Both rectal temperature and the concentration of 5-HT in the hippocampal dialysate were measured over the following 4 h.

Fluoxetine administration did not alter the MDMA-induced hyperthermic response (Figure 5a). However, the major increase in 5-HT concentration in the dialysate produced by MDMA injection was almost totally abolished in rats injected with fluoxetine (Figure 5b).

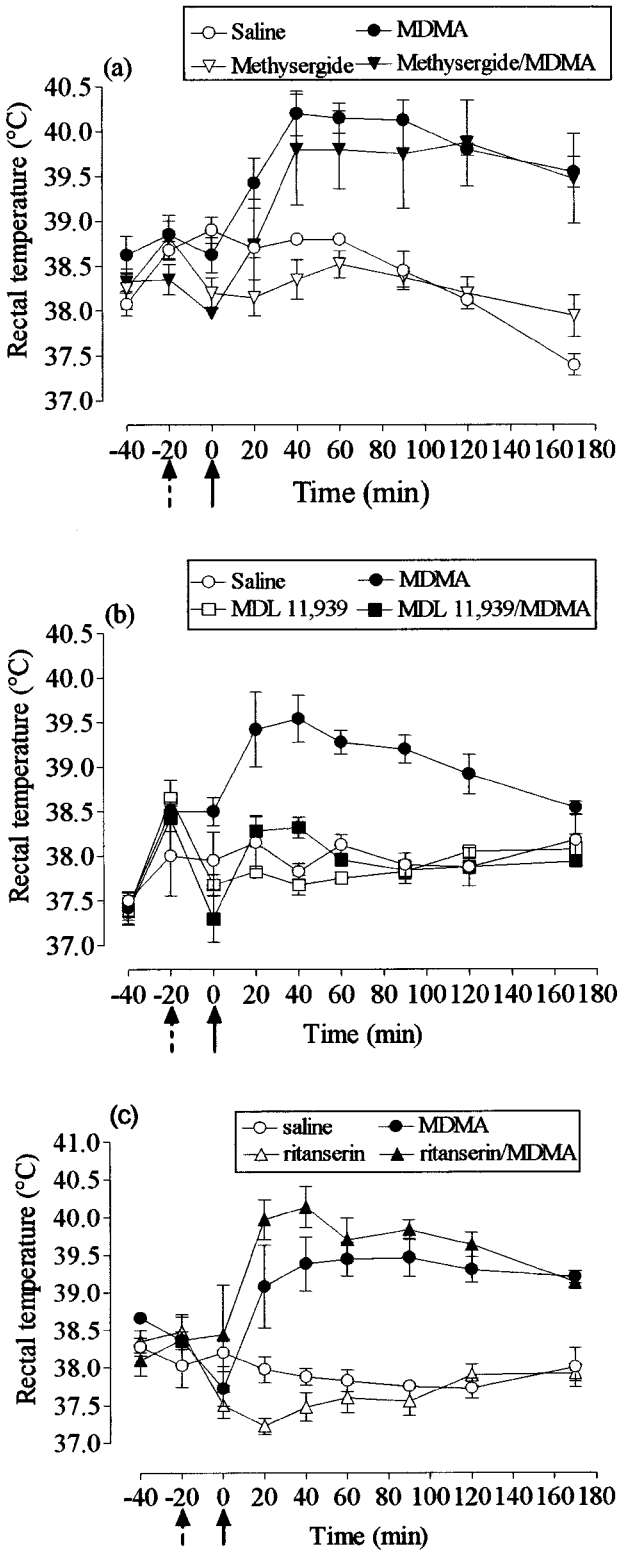


**Figure 2** Effect of methysergide, MDL 11,939 and ritanserin on MDMA-induced hyperthermia. (a) Rectal temperature of rats injected with methysergide (10 mg $kg^{-1}$, i.p.) or saline 20 min before administration of MDMA (12.5 mg $kg^{-1}$, i.p.) or saline. There was no difference in the basal temperature of the groups. MDMA produced a significant rise in body temperature ($F(1,6)=76.36$, $P<0.001$) compared with the saline injected group. Methysergide did not modify the hyperthermic response induced by MDMA ($F(1,6)=0.64$, n.s.) and did not alter the body temperature of saline treated rats ($F(1,6)=2.14$, n.s.). Results shown as mean ± s.e.mean of four rats. (b) Rectal temperature of rats injected with MDL 11,939 (5 mg $kg^{-1}$, i.p.) or saline 20 min before admininstration of MDMA (12.5 mg $kg^{-1}$, i.p.) or saline. There was no difference in the basal temperature of the groups. MDMA produced a significant rise in body temperature ($F(1,7)=36.25$, $P<0.001$) compared with the saline injected group. MDL 11,939 abolished the MDMA-induced hyperthermia ($F(1,8)=73,54$, $P<0.001$) and failed to alter temperature in saline-treated rats ($F(1,6)=1.39$, n.s.). Results shown as mean ± s.e.mean of 4–5 rats. (c) Rectal temperature of rats injected with ritanserin (1 mg $kg^{-1}$, i.p.) or saline 20 min before administration of MDMA (12.5 mg $kg^{-1}$, i.p.) or saline. There was no difference in the basal temperature of the groups. MDMA produced a significant rise in body temperature ($F(1,7)=21.04$, $P<0.01$) compared with the saline injected group. Ritanserin did not modify the hyperthermic response induced by MDMA ($F(1,6)=1.97$, n.s.) and did not alter the body temperature of saline treated rats ($F(1,6)=3.68$, n.s.). Results shown as mean ± s.e.mean of 4–5 rats.

### *Effect of remoxipride and SCH 23390 on MDMA-induced hyperthermia*

Administration of the dopamine $D_2$ receptor selective antagonist remoxipride (10 mg $kg^{-1}$) failed to alter the hyperthermic response to MDMA (Figure 6a). In contrast, the dopamine $D_1$ selective antagonist SCH 23390 attenuated the MDMA-induced hyperthermic response at a dose of 0.3 mg $kg^{-1}$ (data not shown) and 1.0 mg $kg^{-1}$ (Figure 6b). At the highest dose of SCH 23390 (2.0 mg $kg^{-1}$), the temperature of the MDMA + SCH 23390 group was significantly lower than that of the control group for 120 min post MDMA injection (Figure 6c). However, even at the highest dose, SCH 23390 pretreatment was without effect on the rectal temperature of control rats (Figure 6c).

### *Effect of GBR 12909 on MDMA-induced hyperthermia*

Administration of the dopamine uptake inhibitor GBR 12909 (10 mg $kg^{-1}$) was without effect on MDMA-induced hyperthermia and also had no effect on the temperature of control animals (Figure 7).

### *Effect of GBR 12909 on MDMA-induced dopamine release from the striatum*

A previous *in vitro* study (Koch & Galloway, 1997) suggested that GBR 12909 markedly attenuated MDMA-induced dopamine release. This observation was difficult to reconcile with the lack of effect of GBR 12909 on MDMA-induced hyperthermia, given the results of the SCH 23390 study which strongly suggested the involvement of dopamine in the hyperthermic response.

We, therefore, decided to examine the effect of GBR 12909 on MDMA-induced dopamine release *in vivo*, using a microdialysis probe inserted into the striatum.

Following the administration of MDMA there was a marked and sustained increase in concentration of extracellular dopamine (Figure 8) and an increase in rectal temperature (data not shown). Pretreatment with GBR 12909 again failed to inhibit the hyperthermic response (data not shown) and also failed to inhibit the MDMA-induced rise in extracellular dopamine. In control animals GBR 12909 produced a modest enhancement in the extracellular concentration of dopamine and its major metabolite homovanillic acid (HVA) (Figure 8).

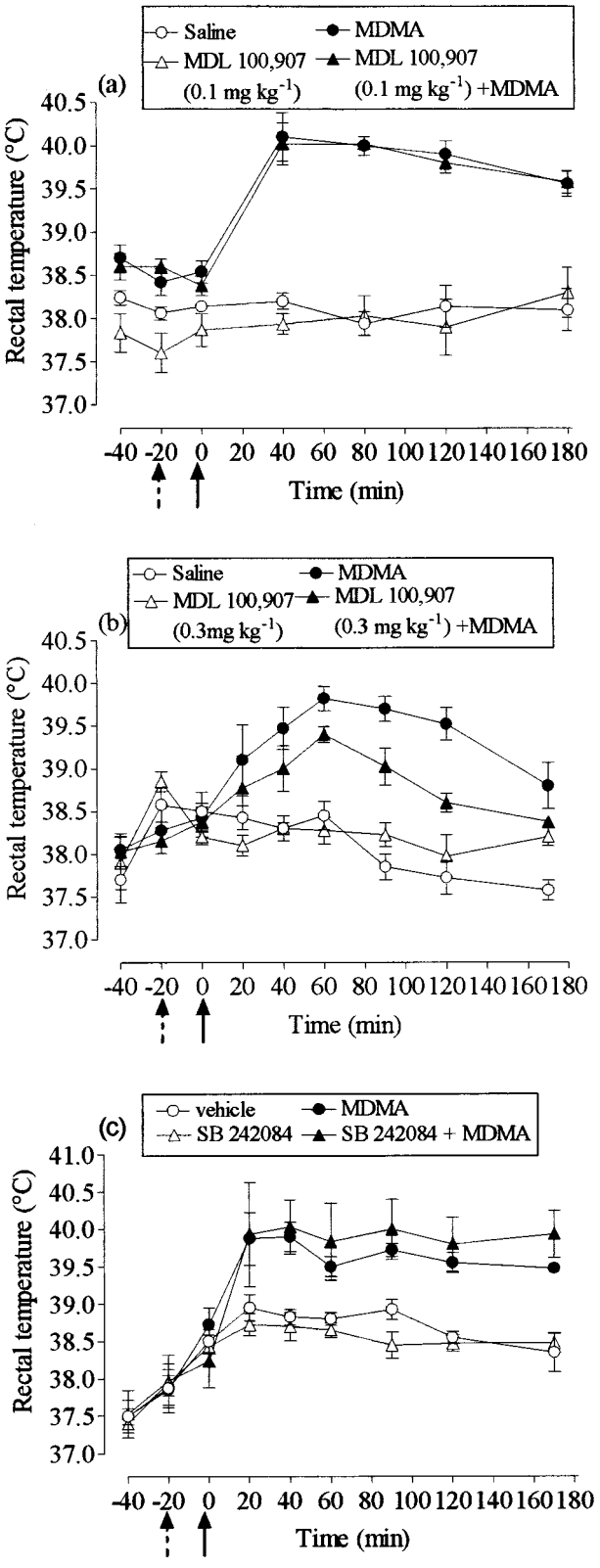


**Figure 3** Effect of selective 5-$HT_{2A}$ and 5-$HT_{2C}$ antagonists on MDMA-induced hyperthermia. (a) Rectal temperature of rats injected with MDL 100,907 (0.1 mg $kg^{-1}$, i.p.) or saline 20 min before administration of MDMA (12.5 mg $kg^{-1}$, i.p.) or saline. MDMA produced a significant rise in body temperature ($F(1,8)=85.96$, $P<0.001$) compared with the saline injected group. MDL 100,907 did not modify the hyperthermic response induced by MDMA ($F(1,8)=0.25$, n.s.) and did not alter the body temperature of saline treated rats ($F(1,6)=0.16$, n.s.). Results shown as mean ± s.e.mean of 3–5 rats. (b) Rectal temperature of rats injected with MDL 100,907 (0.3 mg $kg^{-1}$, i.p.) or saline 20 min before administration of MDMA (12.5 mg $kg^{-1}$, i.p.) or saline. MDMA produced a significant rise in body temperature ($F(1,6)=306.97$, $P<0.001$) compared with the saline injected group. MDL 100,907 attenuated the MDMA-induced hyperthermia ($F(1,6)=22.38$, $P<0.01$), but did not alter temperature in saline-treated rats ($F(1,6)=0.29$, n.s.). Results shown as mean ± s.e.mean of four rats. (c) Rectal temperature of rats injected with SB 242084 (3 mg $kg^{-1}$, i.p.) or saline 20 min before administration of MDMA (12.5 mg $kg^{-1}$, i.p.) or saline. There was no difference in the basal temperature of the groups. MDMA produced a significant rise in body temperature ($F(1,6)=70.44$, $P<0.001$) compared with the saline-injected group. SB 242084 did not modify the hyperthermic response induced by MDMA ($F(1,5)=0.18$, n.s.) and did not alter the body temperature of saline-treated rats ($F(1,6)=1.24$, n.s.). Results shown as mean ± s.e.mean of 3–4 rats.

## Discussion

Any attempt to characterize the hyperthermic response that follows MDMA administration is fraught with difficulties in terms of interpretation of data. Following drug injection any effect seen may result from either an action on the key neurotransmitter or, secondarily, on another neurotransmitter which modulates the action of the initiating transmitter. Also, of course, since drugs are administered peripherally, the effect could also occur *via* a peripheral mechanism such as a change in blood flow. There is also the additional problem of elucidating the brain region, or regions, involved in the hyperthermic response. An observed neurotransmitter change in one region may not reflect a change in another more functionally relevant region. Nevertheless, even with all these provisos, such studies remain relevant since they give indications as to the best approaches that might be employed clinically to alleviate the life-threatening hyperthermia that can occur in persons overdosing on this commonly used recreational drug.

At the outset it should be emphasized that the dose of MDMA chosen in this investigation is clinically relevant. It has previously been shown that a dose of MDMA of 10 mg $kg^{-1}$ (a 20% lower dose than in the current study) produced a plasma drug concentration of 6.3 nmol $ml^{-1}$ 45 min later (Colado *et al.*, 1995). This value is in exactly the same range as the plasma concentrations reported in patients suffering acute adverse responses to ingestion of the drug (Henry *et al.*, 1992; Dowling *et al.*, 1987). Furthermore, as McCann & Ricaurte (2001) have recently pointed out, the principle of interspecies drug dose scaling (see Mordenti & Chappell, 1989) should always be used when extrapolating from doses in rat studies to those used by humans.

Using interspecies dose scaling, the dose used in this current study is the equivalent to a human (70 kg) taking a dose of MDMA of 560 mg. Given the most recent evidence that many tablets now being ingested contain between 200–300 mg of MDMA (see: www.dancesafe.org), this means that the current study is equivalent to a human ingesting 2–3 tablets, a not unusual recreational dose.

Acutely, MDMA produces a major release of both 5-HT (Schmidt *et al.*, 1986; Stone *et al.*, 1986; 1987; Colado & Green, 1994; Mechan *et al.*, 2000) and dopamine (Koch & Galloway, 1997; Sabol & Seiden, 1998; Colado *et al.*, 1999). Methamphetamine also induces the release of both these neurotransmitters (Schmidt & Gibb, 1985a, b; Baldwin *et al.*,

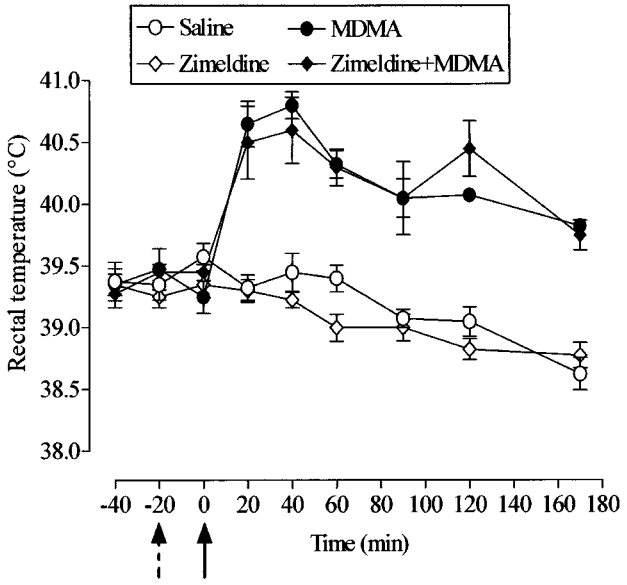


**Figure 4** Effect of zimeldine on MDMA-induced hyperthermia. Rats received zimeldine (10 mg $kg^{-1}$, i.p.) or saline 20 min before MDMA (12.5 mg $kg^{-1}$, i.p.) or saline. There was no difference in the basal temperature of the groups. MDMA produced a significant rise in body temperature ($F(1,6)=65.62$, $P<0.001$) compared with the saline injected group. Zimeldine did not modify the hyperthermic response induced by MDMA ($F(1,6)=0.01$, n.s.) and did not alter the body temperature of saline treated rats ($F(1,6)=2.36$, n.s.). Results shown as mean ± s.e.mean of four rats.

1993) and hyperthermia follows administration to rats of both MDMA (Nash *et al*., 1988; Gordon *et al*., 1991; Colado *et al*., 1993; O'Shea *et al*., 1998; Dafters, 1994) and methamphetamine (Clark & Lipton, 1986; Bronstein & Hong, 1995). Hyperthermia also results from administration of the 5-HT releasing drug *p*-chloroamphetamine (Colado *et al*., 1993; 1997), certain 5-HT agonists such as MK 212 (Yamawaki *et al*., 1983), 5-methoxy N,N-dimethyltryptamine (Grahame-Smith, 1971b) and quipazine (Yamawaki *et al*., 1983) and after injection of a monoamine oxidase inhibitor and L-tryptophan (Grahame-Smith, 1971a). Therefore it has often been assumed that MDMA-induced hyperthermia is also 5-HT mediated (see for example: Shankaran & Gudelsky, 1999). However, since studies with methamphetamine have implicated dopamine in the hyperthermia seen after administration of that compound (Bronstein & Hong, 1995), there is little reason not to assume that dopamine release could also be involved in the hyperthermic action of MDMA.

MDMA induces dopamine release, at least in the striatum, through several mechanisms. It has been reported to have a direct dopamine-releasing effect *via* both a calcium-dependent and -independent mechanism at the level of the nerve ending (Crespi *et al*., 1997; Koch & Galloway, 1997; Yamamoto & Spanos, 1988; Nash & Brodkin, 1991; Johnson *et al*., 1986; Schmidt *et al*., 1987). In addition, the increased 5-HT function resulting from the MDMA-induced 5-HT release has been suggested to stimulate $5\text{-HT}_2$ receptors thereby further enhancing dopamine release (Nash, 1990; Gudelsky *et al*., 1994; Schmidt *et al*., 1990). Finally, recent evidence also supports a role for MDMA in inhibiting the dopamine uptake carrier (Metzger *et al*., 1998).

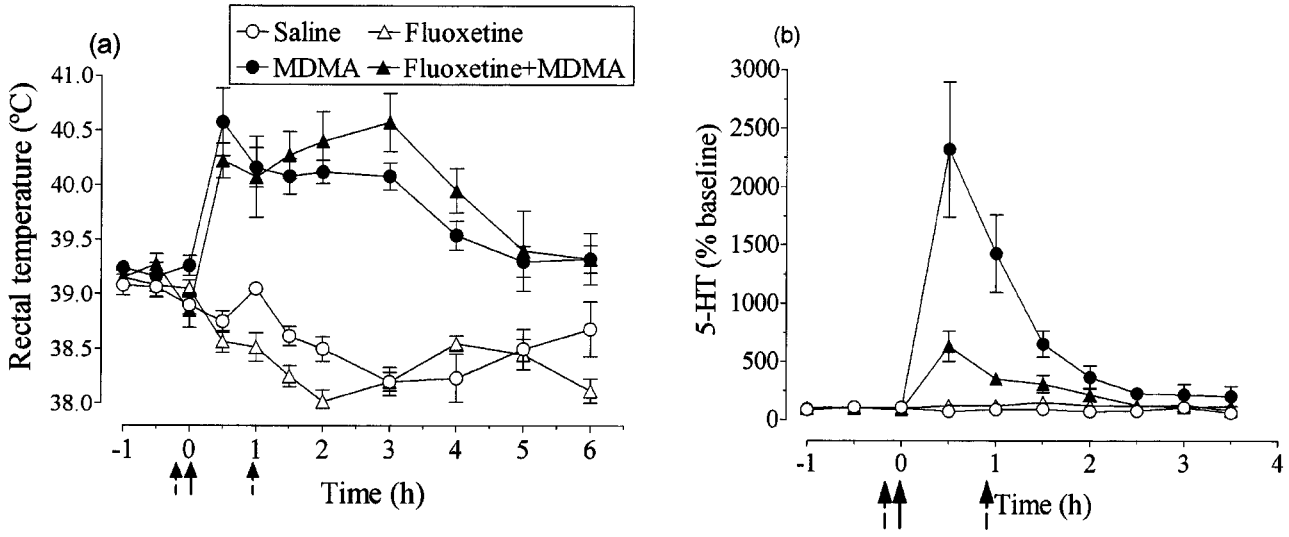


**Figure 5** Effect of fluoxetine on hyperthermia and 5-HT hippocampal release induced by MDMA. (a) Rectal temperature of rats injected with fluoxetine (10 mg $kg^{-1}$, i.p.) or saline 5 min before and 55 min after MDMA (15 mg $kg^{-1}$, i.p.) or saline. There was no difference in the basal temperature of the groups. MDMA produced a significant rise in body temperature ($F(1,9)=74.27$, $P<0.001$) compared with the saline injected group. Fluoxetine did not modify the hyperthermic response induced by MDMA ($F(1,7)=0.82$, n.s.) and did not alter the body temperature of saline treated rats ($F(1,10)=3.34$, n.s.). Results shown as mean ± s.e.mean of 4–6 rats. (b) Changes in the concentration of 5-HT in the hippocampal dialysate of rats treated with fluoxetine (10 mg $kg^{-1}$, i.p.) or saline 5 min before and 55 min after MDMA (15 mg $kg^{-1}$, i.p.) or saline. MDMA produced a significant increase in the content of 5-HT ($F(1,11)=6.57$, $P<0.01$). Administration of fluoxetine significantly attenuated the effect of MDMA on extracellular 5-HT concentrations ($F(1,13)=4.81$, $P<0.01$). Fluoxetine, when given to saline-treated rats, increased 5-HT levels in the dialysate ($F(1,7)=6.70$, $P<0.01$). Values are expressed as percentage of the mean of three measurements before MDMA administration. Each value is the mean ± s.e.mean of 4–8 experiments. The basal concentration of 5-HT in saline-treated rats was: 0.213 ± 0.06 pg $\mu l^{-1}$.

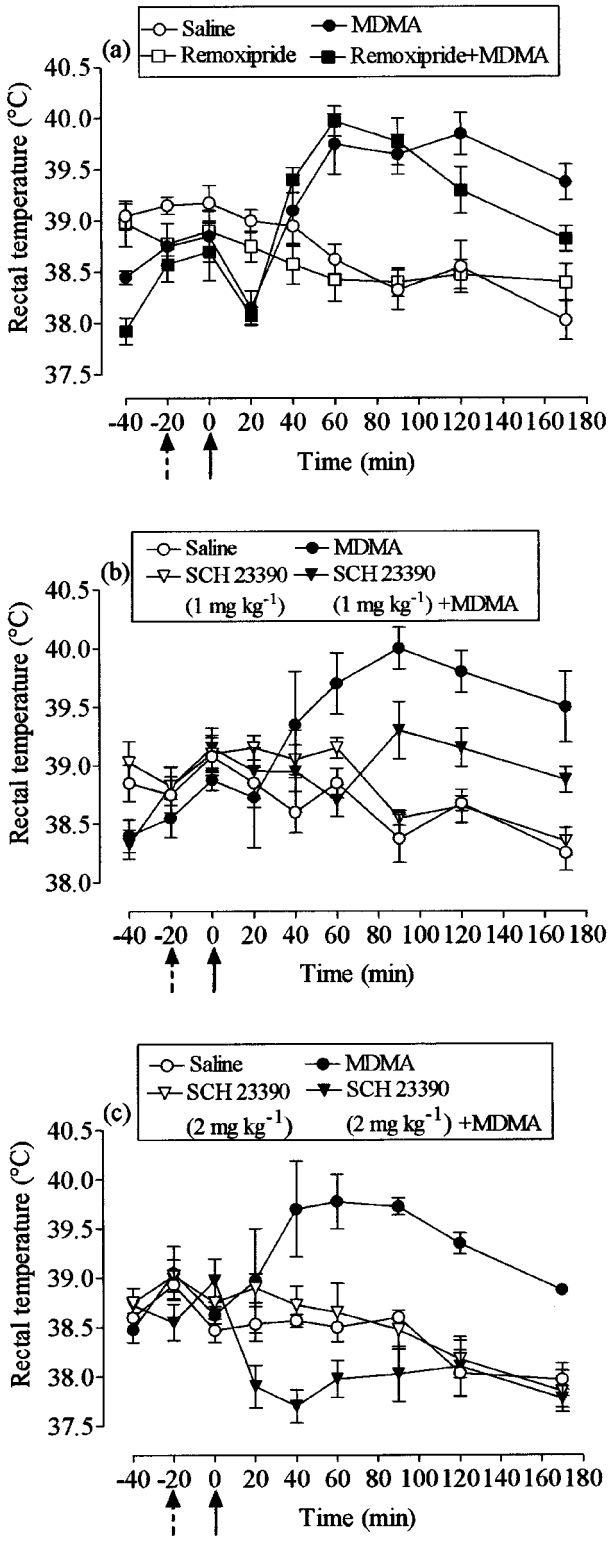


**Figure 6** Effect of remoxipride and SCH 23390 on MDMA-induced hyperthermia. Rats received (a) remoxipride (10 mg $kg^{-1}$, i.p.), (b) SCH 23390 (1 mg $kg^{-1}$, i.p.) or (c) SCH 23390 (2 mg $kg^{-1}$, i.p.), or saline, 20 min before MDMA (12.5 mg $kg^{-1}$, i.p.) or saline. (a) MDMA produced a significant rise in body temperature 40 min after injection ($F(1,6)=15.25$, $P<0.01$) compared with the saline injected group, which was sustained for over 2 h. Remoxipride did not modify the hyperthermic response induced by MDMA ($F(1,6)=0.16$, n.s.) and did not alter the body temperature of saline treated rats ($F(1,6)=0.29$, n.s.). Results shown as mean ± s.e.mean of four rats. (b) MDMA produced a significant rise in body temperature ($F(1,6)=8.00$, $P<0.05$) compared with the saline injected group. SCH 23390 attenuated the MDMA-induced hyperthermia ($F(1,6)=8.80$, $P<0.05$) in the interval 60–170 min and failed to alter temperature in saline-treated rats ($F(1,6)=4.65$, n.s.). Results shown as mean ± s.e.mean of four rats. (c) MDMA produced a significant rise in body temperature ($F(1,5)=16.37$, $P<0.01$) compared with the saline injected group. SCH 23390 abolished the MDMA-induced hyperthermia ($F(1,6)=25.62$, $P<0.01$) and failed to alter temperature in saline-treated rats ($F(1,5)=0.53$, n.s.). Results shown as mean ± s.e.mean of 3–4 rats.

MDMA (12.5 mg $kg^{-1}$) produced a rise in rectal temperature of approximately 1.5°C in the current study. This is a similar rise to that seen in our other recent studies (O'Shea *et al.*, 1998; Mechan *et al.*, 2001). We also briefly examined the tail skin temperature following this dose and saw no evidence for an increase occurring at the same time as the rise in rectal temperature. A similar lack of increase in tail temperature occurs following a hyperthermia-inducing dose of methamphetamine (Mohaghegh *et al.*, 1997). These authors interpreted this to indicate that methamphetamine impaired heat dissipation rather than heat conservation, since tail temperature is a major heat gain/heat loss effector mechanism in rats (Grant, 1963). Thus MDMA may similarly impair heat dissipation mechanisms.

Regardless of whether this mechanistic interpretation is correct or not, our current investigation does not suggest that the acute MDMA-induced release of 5-HT plays a major role in the hyperthermic response. The non-selective 5-$HT_{1/2}$ antagonist methysergide was without effect on the hyperthermic response, while the selective 5-$HT_2$ antagonist MDL 11,939 blocked the MDMA-induced hyperthermia, in agreement with Schmidt *et al.* (1990). However, one has to question whether this latter result was due to a lack of receptor selectivitiy of this compound, or its metabolites, given our observation that another potent and selective 5-$HT_2$ antagonist, ritanserin, was without an effect at a dose of 1 mg $kg^{-1}$, a dose that is still approximately 20 times the $ED_{50}$ for inhibiting 5-$HT_2$ mediated behaviour (Goodwin & Green, 1985). A lack of selectivity might also explain the fact that ketanserin blocks the MDMA-induced hyperthermic effect (Schmidt *et al.*, 1990) since this compound is also an effective $\alpha_1$-adrenoceptor antagonist (McCall & Schuette, 1984). The effect of MDL 11,939 does stand out as being unique. All the other 5-$HT_2$ antagonists in our study failed to antagonize the MDMA-induced hyperthermia, including MDL 100,907 (5-$HT_{2A}$ antagonist) and SB 242084 (5-$HT_{2C}$ antagonist).

Therefore, with the exception of MDL 11,939, the 5-HT antagonist studies reported here suggest that 5-$HT_2$ receptors are not primarily involved in the MDMA-induced hyperthermic response. Supporting this interpretation are the data obtained with 5-HT uptake inhibitors. The 5-HT uptake inhibitor fluoxetine failed to alter the MDMA-induced hyperthermic response. Since both fluoxetine and MDMA are metabolized by the same hepatic enzyme (Crewe *et al.*, 1992), it was conceivable that altered kinetics of one of these two drugs might be suggested as an explanation for this lack of effect. However, a recent study has demonstrated that fluoxetine administration does not alter the concentration of MDMA in the brain following its peripheral administration (Sanchez *et al.*, 2001). Furthermore, administration of the 5-HT uptake inhibitor zimeldine, which is metabolized by an

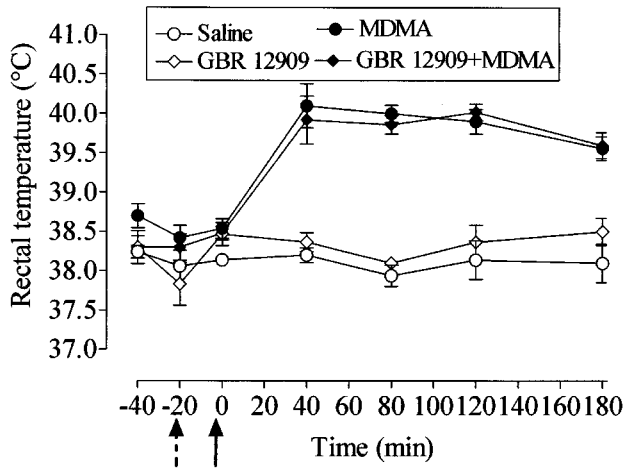


**Figure 7** Effect of GBR 12909 on MDMA-induced hyperthermia. Rats received GBR 12909 (10 mg $kg^{-1}$, s.c.) or saline 20 min before MDMA (12.5 mg $kg^{-1}$, i.p.) or saline. There was no difference in the basal temperature of the groups. MDMA produced a significant rise in body temperature ($F(1,8)=85.96$, $P<0.001$) compared with the saline injected group. GBR 12909 did not modify the hyperthermic response induced by MDMA ($F(1,8)=0.09$, n.s.) and did not alter the body temperature of saline treated rats ($F(1,6)=1.62$, n.s.). Results shown as mean ± s.e.mean of 3–5 rats.

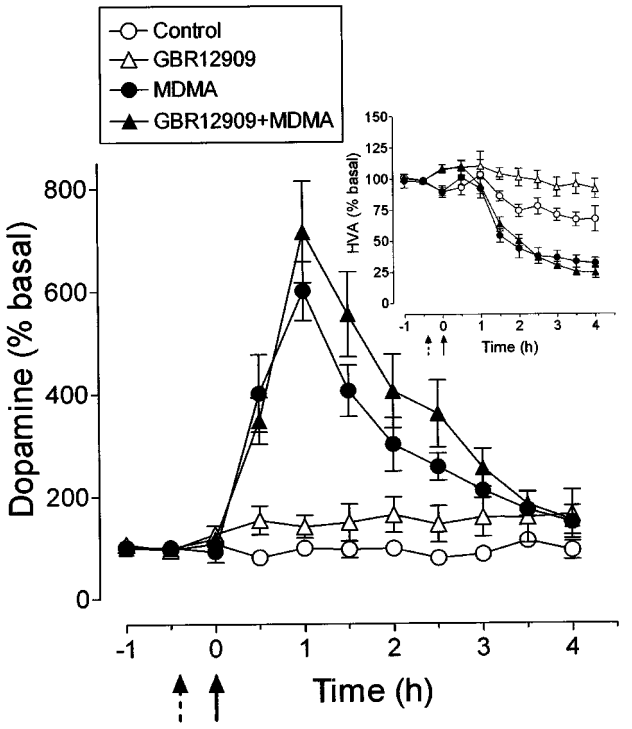


**Figure 8** Changes on the concentration of dopamine and homovanillic acid (HVA, shown in the insert) in the striatal dialysate of rats injected with GBR 12909 and MDMA. GBR 12909 (10 mg $kg^{-1}$, i.p.) or saline was administered 30 min before MDMA (15 mg $kg^{-1}$, i.p.) or saline. MDMA produced an increase in the content of dopamine ($F(1,8)=62.18$, $P<0.001$) and a decrease in the levels of HVA ($F(1,7)=16.18$, $P<0.001$). Administration of GBR 12909 did not modify the effect of MDMA on dopamine ($F(1,9)=2.83$, n.s.) and HVA content ($F(1,9)=0.17$, n.s.). GBR 12909 when given to saline-treated rats increased the extracellular levels of HVA ($F(1,12)=4.18$, $P<0.05$). Values are expressed as percentage of the mean of two measurements before MDMA administration. Each value is the mean ± s.e.mean of 4–10 experiments. The basal concentration of dopamine and HVA in saline treated rats were: 0.472 ± 0.13 pg $\mu l^{-1}$ and 360 ± 25 pg $\mu l^{-1}$, respectively.

entirely different pathway to fluoxetine (Cashman *et al.*, 1988), also had the same lack of effect on MDMA-induced hyperthermia.

Our studies also demonstrated that while fluoxetine treatment did not alter the hyperthermic response, it had nevertheless esssentially abolished the MDMA-induced 5-HT release in the hippocampus. This region was selected as one in which it was relatively easy to implant a microdialysis probe (in contrast to the hypothalamus, a more rational region for examining the relationship between neurotransmitter function and temperature related changes). While inhibition by fluoxetine of MDMA-induced 5-HT release has been reported by others (Berger *et al.*, 1992; Schmidt *et al.*, 1990; Malberg *et al.*, 1996), we think this is the first study to also examine temperature in the same animals. As mentioned above, it is unlikely that the hippocampus is the locus of any 5-HT mediated thermoregulatory response, the fact remains that MDMA has similar actions in all major forebrain regions, including the hypothalamus (Sabol *et al.*, 1996). It is therefore likely that the changes produced by MDMA and fluoxetine in the hippocampus reflect changes also occurring in other brain regions. It is also noteworthy that the rise in the extracellular concentration of 5-HT in the hippocampus following MDMA is of a considerably shorter duration than the rise in rectal temperature (Figure 5). This, in addition to the fact that inhibition of the MDMA-induced acute 5-HT release does not alter the hyperthermia, suggests that these events may be unrelated.

The data with zimeldine and fluoxetine reported here may also have clinical relevance. Liechti & Vollenweider (2000) recently reported that administration of a low dose of MDMA (1.5 mg $kg^{-1}$) to healthy volunteers resulted in a modest increase in body temperature which was not attenuated by the 5-HT uptake inhibitor citalopram, even though other MDMA-induced physiological changes were inhibited by this compound.

Although our data fail to provide a clear role for 5-HT in the hyperthermic response, the current study does indicate a role for dopamine. Administration of SCH 23390, a $D_1$ receptor selective antagonist, produced a dose-dependent inhibition of the hyperthermic response. This indicates that MDMA-induced hyperthermia may primarily result from stimulation of $D_1$ receptors and is consistent with the observation that SCH 23390 also antagonizes methamphetamine-induced hyperthermia (Bronstein & Hong, 1995).

While the $D_2$ selective compound remoxipride was without effect on the hyperthermia, $D_2$ receptors may also be involved in some of the temperature changes seen following MDMA administration under specific conditions. For example, administration of MDMA produces hypothermia in rats kept in low ambient temperature conditions (Gordon *et al.*, 1991; Dafters, 1994) while apomorphine, the prototypic $D_2$ agonist produces hypothermia in rats at normal and low ambient temperature, but hyperthermia at high ambient temperature (Faunt & Crocker, 1987). It is, therefore, possible that $D_2$ stimulation predominates in animals adminstered MDMA at ambient temperatures below approxi-

mately 18°C, which is why hypothermia is then seen in these animals (Gordon *et al*.; 199l Dafters, 1994). Similarly, it seems reasonable to propose that the hypothermia observed in the MDMA + SCH 23390 treated rats given the highest dose of SCH 23390 (Figure 7) is due to the total blockade of $D_1$ sites allowing expression of $D_2$ receptor stimulation by the dopamine released by MDMA. Thus one sees an 'apomorphine-like' effect and hypothermic response which is in agreement with Faunt & Crocker (1987) who reported that apomorphine-induced hypothermia could be potentiated by SCH 23390.

Since Koch & Galloway (1997) showed that GBR 12909 prevented MDMA-induced dopamine release *in vitro* in brain slices and our results suggested that dopamine is the neurotransmitter primarily involved in MDMA-induced hyperthermia, it seemed surprising that pretreatment with the dopamine uptake inhibitor GBR 12909 had no effect on MDMA-induced hyperthermia. However, the current data on the role of the dopamine uptake site in MDMA-induced dopamine release is conflicting. The data of Koch & Galloway (1997) using an *in vitro* technique are hard to reconcile with the fact that, *in vivo*, in uptake inhibitor mazindol failed to block the acute dopamine depeletion which follows administration of the MDMA-related compound methamphetamine (Marek *et al*., 1990a).

We therefore decided to examine further the effect of GBR 12909 on MDMA-induced dopamine release *in vivo*. Nash & Brodkin (1991) had previously studied the effect of GBR 12909 on MDMA-induced dopamine release *in vivo* but they had infused MDMA directly into the brain which we postulated might have resulted in a different effect to that seen when MDMA was given peripherally. We obtained clear evidence that GBR 12909 has no effect on MDMA-induced dopamine release in the rat when both drugs are given peripherally. This is consistent with a recent study in mice which also demonstrated that GBR 12909 had no effect on MDMA-induced dopamine release *in vivo* (O'Shea *et al*., 2001). In addition, our experiment explained why (if we assume from our other results that the hyperthermia is dopamine mediated) MDMA-induced hyperthermia was unaffected by GBR 12909.

One explanation for the failure of GBR 12909 to block MDMA-induced dopamine release in the current study could be that we gave an insufficient dose. While Rothman *et al*. (1991) suggested that the $ED_{50}$ dose for inhibiting the uptake site was 10 mg $kg^{-1}$ (the dose used in the current study) and Stephens & Yamamoto (1994) previously found this dose to be sufficient to block the dopamine releasing effect of methamphetamine, other studies have indicated that a much higher dose of GBR 12909 (45 mg $kg^{-1}$) is required to completely block the dopamine uptake carrier (Nakachi *et al*., 1995). Further studies on the role of the dopamine uptake site in MDMA-induced dopamine release are, therefore, clearly required to finally determine whether MDMA is or is not transported by the dopamine uptake carrier. However, the fact that a high dose of mazindol (40 mg $kg^{-1}$) failed to block the dopamine-releasing action of methamphetamine (Marek *et al*., 1990b) does cast some doubt on the role of the dopamine uptake site in the dopamine releasing action of amphetamine compounds.

There are several studies that demonstrate that MDMA, by increasing 5-HT release indirectly increases dopamine release, *via* an action at 5-$HT_2$ receptors. For example, it has been shown that co-administration of fluoxetine and MDMA enhances dopamine release (Koch & Galloway, 1997), 5-$HT_2$ receptor antagonists block the MDMA-induced increase in dopamine (Nash, 1990; Schmidt *et al*., 1992) and 5-HT agonists potentiate dopamine release (Gudelsky *et al*., 1994). The question therefore arises as to why MDMA-induced hyperthermia was not altered by administration of either fluoxetine or 5-$HT_2$ antagonists if indeed the hyperthermia results from increased dopamine function as we are proposing. At present the most plausible explanation is that this association between 5-HT and dopamine function has only been demonstrated to occur in the striatum. It is probable that the dopamine release involved with hyperthermia is an event associated with a direct effect of MDMA on release in a different brain area, probably the hypothalamus.

In conclusion, we have demonstrated a clear dissociation between MDMA-induced hyperthermia and a change in 5-HT function, particularly that mediated by 5-$HT_2$ receptors. However, a probable association between an MDMA-induced increase in dopamine release, $D_1$ receptor activity and the hyperthermia has been demonstrated. This result suggests that administration of a $D_1$ receptor antagonist could be a logical way to treat patients presenting with an acute and potentially life-threatening MDMA-induced hyperthermia.

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