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Vascular actions of MDMA involve α_1 and α_2-adrenoceptors in the anaesthetized rat

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1 We have investigated the effects of methylenedioxymethamphetamine (MDMA, 'ecstasy'), i.v., on diastolic blood pressure (DBP) in pithed and pentobarbitone anaesthetized rats.

2 In pithed rats, the non-selective 5-HT receptor antagonist methiothepin (0.1 mg kg^{-1}) and the α_2-adrenoceptor antagonists methoxyidazoxan and yohimbine (1 mg kg^{-1}) showed significant α_1-adrenoceptor antagonist potency, but methiothepin did not show α_2-adrenoceptor antagonist potency. MDMA (1 and 5 mg kg^{-1}) produced pressor responses which were significantly reduced by the α_1-adrenoceptor antagonist prazosin (0.1 mg kg^{-1}), yohimbine (1 mg kg^{-1}) or methiothepin (0.1 mg kg^{-1}), but not by the 5-HT$_2$ receptor antagonist ritanserin (1 mg kg^{-1}).

3 In anaesthetized rats, antagonists revealed two phases with three components to the effects of MDMA (5 mg kg^{-1}) on DBP: an initial pressor response, a later pressor component at 1 min, the sustained depressor response. Methoxyidazoxan, methiothepin or the combination ritanserin/prazosin significantly reduced the initial pressor response, although neither of the latter compounds alone had any effect.

4 The pressor response to MDMA (5 mg kg^{-1}) at 1 min was converted to a depressor response by prazosin and to a lesser extent methiothepin and methoxyidazoxan.

5 The depressor response to MDMA (5 mg kg^{-1}) was significantly reduced by methoxyidazoxan (0.1 mg kg^{-1}), and by the noradrenaline re-uptake blocker cocaine 10 mg kg^{-1} but not 1 mg kg^{-1}. However, the most marked reduction in the depressor response was produced by the combination of methoxyidazoxan and cocaine.

6 It is concluded that the initial pressor response to MDMA (5 mg kg^{-1}) in anaesthetized rats involves α_2- and possibly α_1-adrenoceptors and 5-HT$_2$ receptors, the pressor component at 1 min is largely α_1-adrenoceptor mediated, and the sustained depressor response involves α_2-adrenoceptors.
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Abbreviations: DBP, diastolic blood pressure; MDMA, methylenedioxymethamphetamine

Introduction

Ecstasy (3,4-methylenedioxymethamphetamine; MDMA) is widely used as a drug of abuse, and as a result adverse effects of this drug have come to the fore. Tachycardia and hypertension (Hayner & McKinney, 1986), and cardiovascular mortality (Dowling *et al*., 1987) have been reported in man. It is reported to have cardiac stimulant actions in rats resulting in tachycardia (Gordon *et al*., 1991) and is also reported to facilitate vasconstriction in the rat (Fitzgerald & Reid, 1994). In addition, MDMA has been linked to intracerebral haemorrhage (Harries & De Silva, 1992), and cerebral hyperperfusion can be demonstrated in rats (Kelly *et al*., 1994). Certainly, chronic use of methamphetamine may also result in serious cardiovascular changes in man including tachycardia and palpitations (Chan *et al*., 1994), and another amphetamine derivative, fenfluramine, has been linked to valvular heart disease (Connolly *et al*., 1997).

Central actions of MDMA has been studied in much greater detail and include acute effects to displace 5-hydroxytryptamine which can result in hyperthermia in experimental animals and man (Green *et al*., 1995). A second action is to cause neuronal damage in rodent brain, particularly to serotonergic nerves (Battaglia *et al*., 1987; 1988), and this action may involve free radical generation (Colado *et al*., 1997). Given the major involvement of serotonergic nerves in the central actions of MDMA, possible effects of MDMA involving the noradrenergic system have been largely overlooked. Depletion of noradrenaline can be shown in mouse heart in response to MDMA (Steele *et al*., 1989). Fitzgerald & Reid (1993), in radioactive overflow studies, found that MDMA increased basal release of noradrenaline (and, indeed, dopamine and 5-hydroxytryptamine) and increased stimulation-evoked release.

The object of this study was to examine the effects of MDMA on blood pressure in the anaesthetized rat, given that we have recently shown that MDMA has major actions as an agonist at α_2-adrenoceptors (Lavelle *et al*., 1999). An α_2-adrenoceptor agonist would be predicted to have central depressor and peripheral pressor actions by analogy to agonists such as clonidine (Haeusler, 1974; Anden *et al*., 1976; Hamilton *et al*., 1980).

Some of these results have been published in abstract form (McDaid & Docherty, 1999).

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Methods

Male wistar rats (250–350 g), obtained from Trinity College Dublin, were employed in this study.

Pithed rat preparation

Rats were intubated under ether anaesthesia, and then pithed using a steel rod (see Docherty, 1988). Pithed animals were immediately respired with 100% O_2 at 60 min^{-1} and a volume of 1 ml 100 g^{-1}. Temperature was maintained at approximately 37°C, employing a heated table. The carotid artery was exposed, cannulated and connected to a blood pressure transducer for pressure monitoring and recording. The jugular vein was then exposed and cannulated for drug injection. Heart rate was extracted from the blood pressure signal using a Columbus Instruments BP-2 blood pressure/heart rate monitor. After 15 min equilibration, two types of protocol were employed: pressor responses to injected agonists; pressor responses to nerve stimulation.

Pressor responses to injected agonists In the first kind of experiment, vehicle or antagonist drug was administered, and 10 min later, a dose response curve was carried out to the α_1-adrenoceptor agonist phenylephrine or the α_2-adrenoceptor agonist xylazine (in both cases, 1 μg kg^{-1}–10 mg kg^{-1}). In the second kind of experiment, a control response was obtained to MDMA (1 mg kg^{-1}), vehicle or antagonist drug or antagonist drug combination was then administered and 5 min later, MDMA (1 mg kg^{-1}) was repeated. The pressor response to MDMA (5 mg kg^{-1}) was assessed in the presence of vehicle or antagonists or antagonist combinations (MDMA 5 mg kg^{-1} was not administered before and after antagonist or vehicle due to the long time course of action in vehicle experiments).

Pressor responses to nerve stimulation The pithing rod was used as an electrode and positioned to stimulate the thoraco-lumbar spinal cord, with a second electrode placed under the skin. In the first kind of experiment, electrical stimulation with 10 pulses at 1 Hz (supramaximal voltage, 0.5 ms pulses) was carried out at 2 min intervals producing consistent rises in DBP. Once consistent control responses had been obtained, MDMA 1 mg kg^{-1} or vehicle was administered 1 min before the next stimulation and 6 min later MDMA 5 mg kg^{-1} was administered. In the second kind of experiment, continuous electrical stimulation at 0.5 Hz (supramaximal voltage, 0.5 ms pulses) was carried out: for these experiments, d-tubocurarine (0.75 mg kg^{-1}) was administered to reduce skeletal muscle twitching. Once a consistent rise in DBP had been obtained, MDMA 1 mg kg^{-1} or vehicle was administered during the stimulation and 5 min later MDMA 5 mg kg^{-1} was administered also during stimulation.

Anaesthetized rat

Animals were anaesthetized with pentobarbitone (Sagatal) (approximately 40 mg kg^{-1}, i.p.), supplemented with pentobarbitone (approximately 10 mg kg^{-1}, i.v. per hour). Animals were respired with room air at 60 min^{-1} and a volume of 1 ml 100 g^{-1}. Temperature was maintained at approximately 37°C, employing a heated table. The jugular vein was exposed and cannulated for drug injection. The carotid artery was then exposed, cannulated and connected to a blood pressure transducer for pressure monitoring and recording. Heart rate was extracted from the blood pressure signal using a Colombus Instruments BP-2 blood pressure/heart rate monitor.

Following a 15 min equilibration period, drug administration commenced. The α_2-adrenoceptor antagonist methoxyidazoxan (0.1 and 1 mg kg^{-1}), the α_1-adrenoceptor antagonist prazosin (0.1 mg kg^{-1}), the non-selective 5-HT receptor antagonist methiothepin (0.1 mg kg^{-1}), the 5-HT_{1A} receptor antagonist WAY 100635 (0.1 mg kg^{-1}), the 5-HT_{1B} receptor antagonist GR 55562 (1 mg kg^{-1}), the 5-HT_{1D} receptor antagonist BRL 15572 (0.1 mg kg^{-1}), the 5-HT_2 receptor antagonist ritanserin (1 mg kg^{-1}) or the noradrenaline re-uptake blocker cocaine (1 and 10 mg kg^{-1}), alone or in combination (multiple drugs were administered at approximately 3 min intervals), or equivalent volumes of vehicle (1 ml kg^{-1}) were infused over 30–60 s through the jugular vein and washed in with saline (1 ml kg^{-1}). MDMA (5 mg kg^{-1}) was administered intravenously 5 min after the last antagonist or vehicle. In some experiments, MDMA (1 or 20 mg kg^{-1}) was employed. Blood pressure effects of MDMA were measured as the initial peak pressor response (at approximately 20–30 s post injection) and at 1, 2, 3, 4, 5 and 10 min post injection. The maximum depressor response was measured at one of these time points. In some preliminary experiments, several doses of antagonist drugs were investigated in order to determine appropriate doses. These experiments are not reported in the results. In all experiments, the effects of a single drug or combination of drugs (or vehicle) were investigated against the response to a single dose of MDMA. The vehicle experiments (appropriate single, double or triple vehicle) were combined as the results obtained were similar. At the end of the experiment, the animal was killed by an overdose of pentobarbitone.

Drugs

BRL 15572 (3-[4-(4-chlorophenyl)piperazin-1-yl]-1,1-diphenyl-2-propanol hydrochloride), GR 55562 (3-[3-(dimethylamino)-propyl]-4-hydroxy-N-[4-(4-pyridinyl)phenyl]benzamide dihydrochloride) (Tocris, Bristol, U.K.); cocaine hydrochloride, phenylephrine hydrochloride, prazosin hydrochloride, yohimbine hydrochloride, WAY 100635 (N-[2-(4-[2-methoxyphenyl]-1-piperazinyl)ethyl]N-2-pyridinylcyclohexanecarboxamide maleate) (Sigma, Dublin, Ireland); D-tubocurarine hydrochloride ('Tubarine', Wellcome, Dublin, Ireland), methiothepin maleate (gift: Roche, Dublin, Ireland); methoxyidazoxan, 3,4-methylenedioxymethamphetamine (MDMA) (Research Biochemicals, Natick, U.S.A.); ritanserin (gift: Janssen, Dublin, Ireland); xylazine hydrochloride (gift: Bayer, Dublin, Ireland).

Drugs were dissolved in distilled water using an ultrasonic bath and diluted in normal saline (NaCl 0.9% w v^{-1}), except for methiothepin and ritanserin, which were dissolved initially in a small volume of HCl (100 μl) prior to addition of distilled water, and BRL 15572 which was dissolved in dimethylsulphoxide (DMSO) and diluted in distilled water.

Statistics

Values are expressed as mean and standard error (s.e.) of the mean. In pithed rat experiments, agonist potency was expressed as an ED_{50} (dose producing a rise in DBP of 50 mmHg, approximately 50% of maximum), and upper and lower standard error limits. In each set of experiments n indicates the number of animals studied. Differences between groups were compared by Analysis of Variance and Dunnett's test (for comparisons with vehicle) or Tukey test (comparison of all groups). Means were considered significantly different when P values were <0.05.

Results

Pithed rat preparation

Pressor responses to injected agonists In pithed rats, resting diastolic blood pressure (DBP) was 35.8 ± 1.7 mmHg ($n=30$), and resting heart rate was 265 ± 6 min^{-1}. In vehicle experiments, the α_1-adrenoceptor agonist phenylephrine produced dose dependent pressor responses with a potency (ED_{50}) of 3.89 μg kg^{-1} (3.24–4.68 μg kg^{-1}) (see Figure 1a). In interaction experiments, following exposure to antagonist, the potency of phenylephrine was significantly shifted by yohimbine (1 mg kg^{-1}), methoxyidazoxan (1 mg kg^{-1}), methiothepin (0.1 mg kg^{-1}) and prazosin (0.1 mg kg^{-1}), with 5.1, 15.4, 18.6 and 339 fold shifts, respectively.

In vehicle experiments, the α_2-adrenoceptor agonist xylazine produced dose dependent pressor responses in pithed rats with an ED_{50} of 190 μg kg^{-1} (151–240 μg kg^{-1}) (see Figure 1b). In interaction experiments, following exposure to antagonist, the potency of xylazine was significantly shifted by yohimbine (1 mg kg^{-1}) and methoxyidazoxan (1 mg kg^{-1}), with 5.2 and 53.7 fold shifts, respectively. Methiothepin (0.1 mg kg^{-1}) and prazosin (1 mg kg^{-1}) did not significantly affect potency of xylazine.

MDMA (1 mg kg^{-1}) produced a pressor response in pithed rats of 49.0 ± 2.5 mmHg ($n=28$). Following vehicle, the response to MDMA (1 mg kg^{-1}) was 53.8 ± 8.8 mmHg ($n=5$). The pressor response was significantly reduced following prazosin (0.1 mg kg^{-1}), yohimbine (1 mg kg^{-1}) or methiothepin (0.1 mg kg^{-1}) (Figure 2a). Ritanserin (1 mg kg^{-1}) did not significantly affect the pressor response.

Following vehicle, the pressor response to MDMA (5 mg kg^{-1}) in pithed rats was 105.5 ± 5.3 mmHg ($n=4$). This pressor response was significantly reduced by prazosin (0.1 mg kg^{-1}), by yohimbine (1 mg kg^{-1}) and prazosin (0.1 mg kg^{-1}) in combination, by methiothepin (0.1 mg kg^{-1}), by yohimbine (1 mg kg^{-1})/prazosin (0.1 mg kg^{-1})/methiothepin (0.1 mg kg^{-1}) in combination, and by ritanserin (1 mg kg^{-1})/prazosin (0.1 mg kg^{-1})/methoxyidazoxan (1 mg kg^{-1}) in combination (Figure 2b). The combinations of yohimbine/prazosin/methiothepin or ritanserin/prazosin/methoxyidazoxan produced significantly greater reductions in the pressor response to MDMA (5 mg kg^{-1}) than produced by prazosin alone (Analysis of Variance and Tukey test, $P<0.05$).

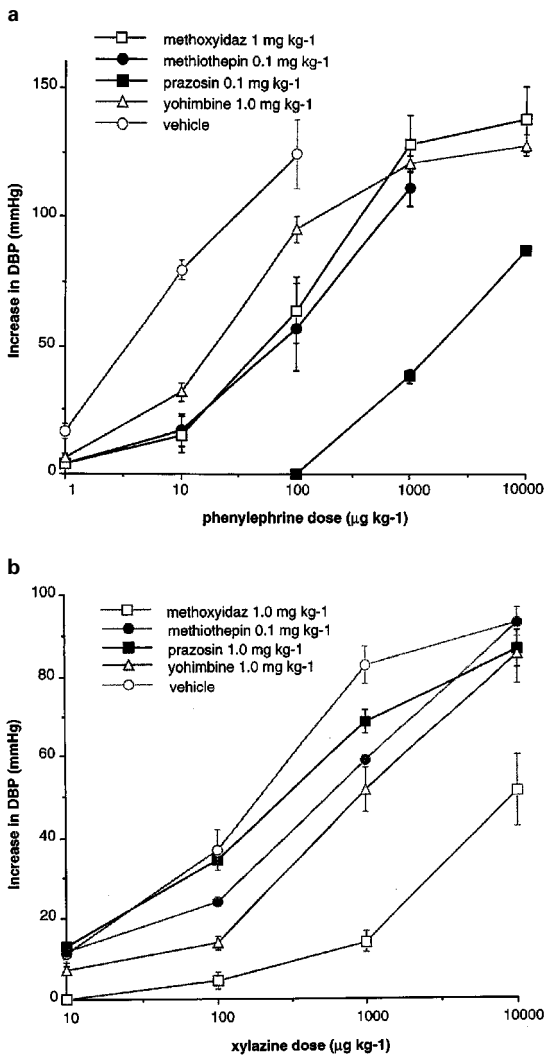

Figure 1 Increase in diastolic blood pressure (DBP) produced by (a) phenylephrine or (b) xylazine in the pithed rat preparation following vehicle, yohimbine (1 mg kg^{-1}), methoxyidazoxan (1 mg kg^{-1}), methiothepin (0.1 mg kg^{-1}) or prazosin (0.1 mg kg^{-1}). Vertical bars represent s.e.mean from four experiments.

Pressor responses to nerve stimulation

Stimulation with 10 pulses at 1 Hz every 2 min produced a pressor response in pithed rats of 52.8 ± 2.9 mmHg ($n=6$). Pressor responses to 1 Hz stimulation were relatively constant over 30 min, and saline vehicle had no significant effect. MDMA (1 mg kg^{-1}) produced a peak rise in DBP of 42.3 ± 5.2 mmHg ($n=3$): by 5 min pressor responses to nerve stimulation had returned to control levels (control: 49.0 ± 2.0 mmHg, $n=3$; post MDMA: 56.0 ± 3.2 mmHg) despite a continued elevation of DBP (15.7 ± 5.8 mmHg). MDMA (5 mg kg^{-1}) produced a pressor response of 87.7 ± 10.3 mmHg ($n=3$), which was still elevated 7.30 ± 13.0 mmHg at 5 min. Hence it was difficult to assess any inhibitory effect of MDMA against pressor nerve responses.

Continuous stimulation at 0.5 Hz produced a sustained pressor response of 57.0 ± 10.0 mmHg ($n=4$), raising DBP

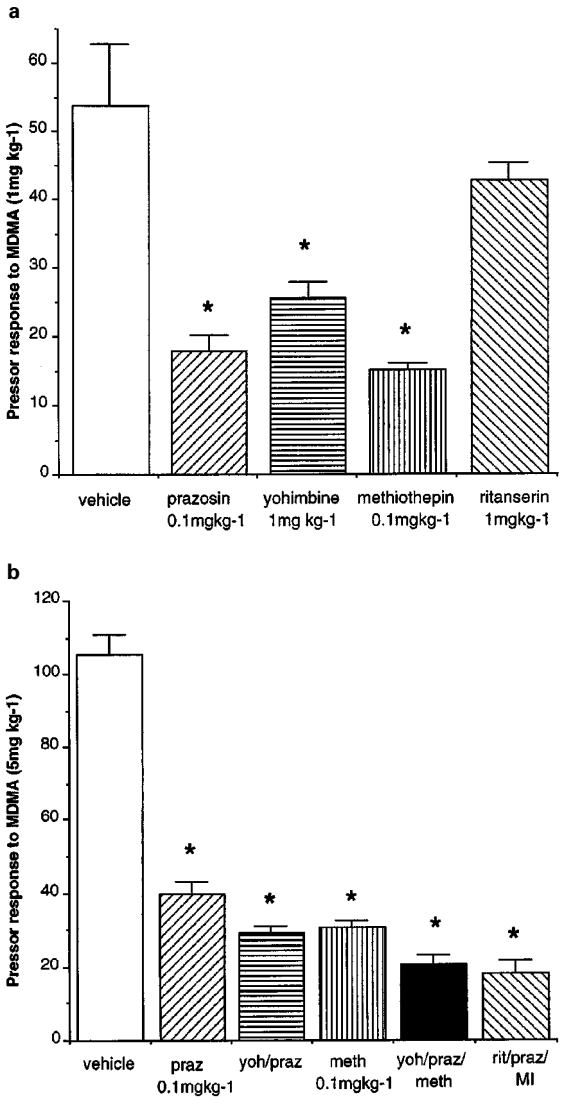

Figure 2 Pressor responses, measured as increase in diastolic blood pressure (DBP), to (a) MDMA (1 mg kg^{-1}) and (b) MDMA (5 mg kg^{-1}) in the pithed rat following vehicle or the antagonists prazosin (praz; 0.1 mg kg^{-1}), yohimbine (yoh; 1 mg kg^{-1}), methiothepin (meth; 0.1 mg kg^{-1}) or ritanserin (rit; 1 mg kg^{-1}), methoxyidazoxan (MI, 1 mg kg^{-1}), alone or in combination. Vertical bars represent s.e.mean from 4–6 experiments. Asterisks denote the significance of difference of effects of MDMA following test drugs from effects of MDMA in vehicle experiments (Analysis of Variance and Dunnett's test: $P<0.05$).

to 96.3 ± 12.9 mmHg ($n=4$). When injected during continuous stimulation, saline had only transient effects. MDMA (1 mg kg^{-1}) produced a peak rise in DBP of 41.0 ± 8.0 mmHg ($n=4$) above the level of the sustained nerve stimulation-evoked pressor response; by 5 min DBP was still elevated by 3.3 ± 2.1 mmHg. MDMA (5 mg kg^{-1}) produced a peak rise in DBP of 46.3 ± 9.2 mmHg ($n=4$), which was still elevated 15.0 ± 9.6 mmHg above the level of the sustained nerve stimulation-evoked pressor response at 5 min. Hence, MDMA 1 and 5 mg kg^{-1} did not reduce DBP below the 0.5 Hz nerve stimulation-evoked level.

Tachycardia to MDMA

MDMA (0.1, 1 and 5 mg kg^{-1}) produced tachycardias in pithed rats of 36 ± 9 ($n=3$), 120 ± 9 ($n=16$) and 145 ± 12 min^{-1} ($n=6$), respectively.

Anaesthetized rat

Systemic blood pressure in anaesthetized rats In anaesthetized rats, baseline systolic blood pressure (SBP) was 150.7 ± 0.9 mmHg and baseline diastolic blood pressure (DBP) was 108.5 ± 0.6 mmHg ($n=209$). The effects of antagonists on DBP prior to injection of MDMA (5 mg kg^{-1}), plus the number of experiments in each group, are shown in Table 1. Prazosin (0.1 mg kg^{-1}), alone or in combination, methiothepin (0.1 mg kg^{-1}) and cocaine (10 mg kg^{-1}) significantly reduced DBP. The combination of methoxyidazoxan (1 mg kg^{-1}) and cocaine (10 mg kg^{-1}) also significantly reduced baseline DBP. Methoxyidazoxan (0.1 mg kg^{-1}) in combination with cocaine (1 mg kg^{-1}), significantly increased baseline DBP. Vehicle and other drugs and combinations did not significantly affect baseline DBP (Table 1).

Effects of MDMA (1, 5 and 20 mg kg^{-1}) in anaesthetized rats In vehicle experiments, MDMA (1, 5 and 20 mg kg^{-1}) produced a biphasic effect on blood pressure (see Figure 3). For MDMA (1 mg kg^{-1}), there was a simple pressor response (Figure 3). However, in the presence of prazosin (0.1 mg kg^{-1}), the response to MDMA (1 mg kg^{-1}) became biphasic: an initial pressor response followed by a depressor response (data not shown). For MDMA (5 mg kg^{-1}) there was an initial pressor response (DBP) of 34.9 ± 2.3 mmHg followed by a fall in DBP below baseline of 47.7 ± 3.4 mmHg ($n=15$) (see Figures 3 and 4). For MDMA 20 mg kg^{-1}, the pressor response was similar to that for 1 and 5 mg kg^{-1}, and the depressor response was similar to that obtained with MDMA 5 mg kg^{-1}, but the time taken to reach the maximum depressor response was greatly prolonged.

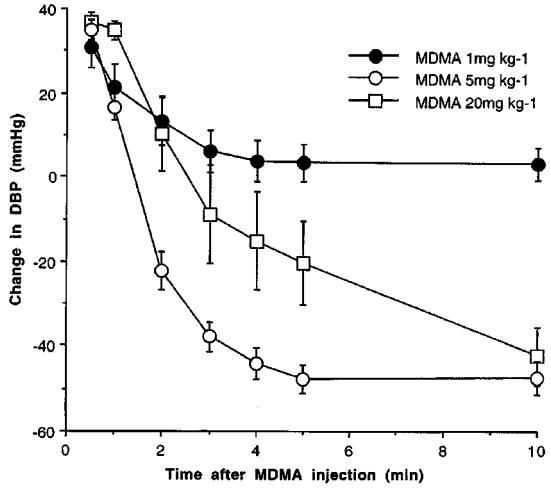

Figure 3 Time course of the change in diastolic blood pressure (DBP) produced by MDMA (1, 5 and 20 mg kg^{-1}) in the anaesthetized rat. Vertical bars represent s.e.mean from 5–15 experiments.

Table 1 Effects of vehicle or antagonist on diastolic blood pressure in anaesthetized rats immediately prior to injection of MDMA (5 mg kg^{-1}). Values are mean ± s.e.mean. Negative values indicate a fall in DBP.

Vehicle	−0.3 ± 1.3 mmHg (n = 15)
Prazosin (0.1 mg kg^{-1})	−22.5 ± 3.0 mmHg (n = 9)*
Methoxyidazoxan (0.1 mg kg^{-1})	7.5 ± 0.7 mmHg (n = 10)
Methoxyidazoxan (1 mg kg^{-1})	−10.5 ± 1.9 mmHg (n = 9)
Methoxyidazoxan (0.1 mg kg^{-1})/ prazosin (0.1 mg kg^{-1})	−24.0 ± 1.6 mmHg (n = 5)*
Methiothepin (0.1 mg kg^{-1})	−14.7 ± 1.3 mmHg (n = 7)*
Methiothepin (0.1 mg kg^{-1})/ prazosin (0.1 mg kg^{-1})	−20.2 ± 3.0 mmHg (n = 9)*
Cocaine (1 mg kg^{-1})	−2.7 ± 1.4 mmHg (n = 6)
Cocaine (10 mg kg^{-1})	−17.2 ± 3.6 mmHg (n = 11)*
Cocaine (1 mg kg^{-1})/ methoxyidazoxan (0.1 mg kg^{-1})	8.9 ± 2.1 mmHg (n = 20)*
Cocaine (10 mg kg^{-1})/ methoxyidazoxan (0.1 mg kg^{-1})	9.6 ± 3.8 mmHg (n = 8)
Cocaine (10 mg kg^{-1})/ methoxyidazoxan (1 mg kg^{-1})	−21.0 ± 7.8 mmHg (n = 6)*
Ritanserin (1 mg kg^{-1})	−4.7 ± 1.5 mmHg (n = 6)
Ritanserin (1 mg kg^{-1})/ prazosin (0.1 mg kg^{-1})	−19.6 ± 2.9 mmHg (n = 5)*
WAY 100635 (0.1 mg kg^{-1})/ GR55562 (1 mg kg^{-1})	−0.8 ± 2.5 mmHg (n = 4)
WAY (0.1 mg kg^{-1})/ GR (1 mg kg^{-1})/ BRL (0.1 mg kg^{-1})	2.0 ± 0.9 mmHg (n = 4)

Asterisks denote effects of test drug(s) significantly different from effects of vehicle (*P < 0.05). Abbreviations: BRL, BRL 15572; GR, GR 55562; WAY, WAY 100635.

Biphasic response to MDMA (5 mg kg^{-1}) with three components in anaesthetized rats The time course of the effects of MDMA (5 mg kg^{-1}) following vehicle or antagonist drugs or combinations of drugs are shown in Figure 4. Although in vehicle experiments the response to MDMA was biphasic, antagonist drugs revealed two components to the pressor response: for simplicity we can look at the initial peak pressor response (20–30 s: plotted at 30 s in Figure 4), the 1 min response, and the maximum depressor response.

Initial pressor response to MDMA (5 mg kg^{-1}) in anaesthetized rats The initial pressor response to MDMA (5 mg kg^{-1}) was significantly reduced by the α_2-adrenoceptor antagonist methoxyidazoxan (0.1 mg kg^{-1}) and cocaine (10 mg kg^{-1}) but not cocaine (1 mg kg^{-1}) (Figures 4 and 5). Combinations of methoxyidazoxan 0.1 mg kg^{-1} and cocaine (1 or 10 mg kg^{-1}) also reduced the pressor response to MDMA, although surprisingly methoxyidazoxan 1 mg kg^{-1} with cocaine (10 mg kg^{-1}) had no effect (Figure 5). Methiothepin (0.1 mg kg^{-1}) markedly reduced the pressor response to MDMA. Methiothepin (0.1 mg kg^{-1}) in combination with methoxyidazoxan (0.1 mg kg^{-1}) reduced the peak pressor response to 10.2 ± 3.1 mmHg (n = 5) (data not shown), which was not significantly different from the response after methiothepin alone. However, combinations of WAY 100635, GR 55562 and BRL 15572 did not significantly affect the pressor response, suggesting that the action of methiothepin does not involve 5-HT_1-receptors. Prazosin (0.1 mg kg^{-1}) or ritanserin (1 mg kg^{-1}) had no effect when given alone, but the combination of ritanserin and prazosin significantly reduced the initial pressor response (Figures 4 and 5).

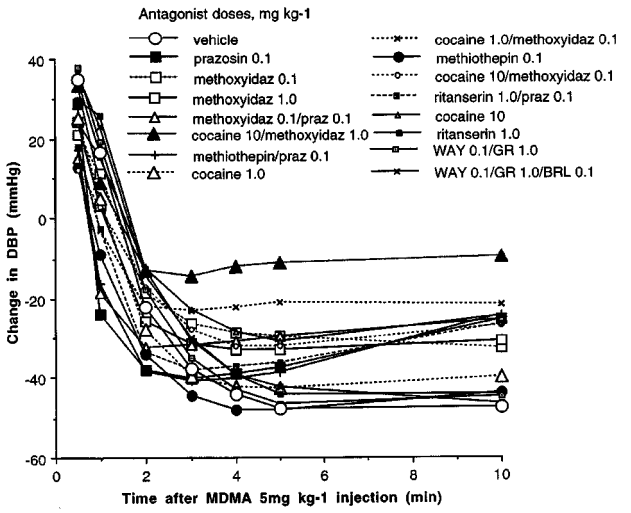

Figure 4 Time course of the change in diastolic blood pressure (DBP) produced by MDMA (5 mg kg^{-1}) in the anaesthetized rat following vehicle or test antagonists, alone or in combination. Abbreviations: BRL, BRL 15572; GR, GR 55562; praz, prazosin; methoxyidaz, methoxyidazoxan; WAY, WAY 100635. For clarity, error bars are omitted from this Figure but are included in Figures 5–7.

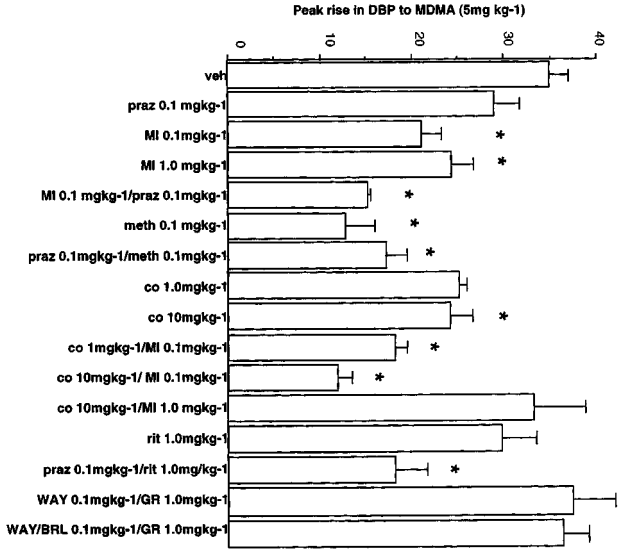

Figure 5 Initial pressor response, measured as increase in diastolic blood pressure (DBP), in the anaesthetized rat to MDMA (5 mg kg^{-1}) following vehicle, prazosin, methoxyidazoxan, methiothepin, cocaine, ritanserin, or combinations of drugs. Abbreviations: veh, vehicle; BRL, BRL 15572; GR, GR 55562; praz, prazosin; MI, methoxyidazoxan; meth, methiothepin; co, cocaine; rit, ritanserin; WAY, WAY 100635. Numbers following drug names indicate dose in mg kg^{-1}. Vertical bars represent s.e.mean from at least four experiments (for n values see Table 1). Asterisks denote the significance of difference of effects of MDMA following test drugs from effects of MDMA in vehicle experiments (Analysis of Variance and Dunnett's test: P < 0.05). Data taken from Figure 4.

Response to MDMA (5 mg kg^{-1}) at 1 min in anaesthetized rats In vehicle experiments, the response to MDMA (5 mg kg^{-1}) at 1 min was a pressor response of 16.7 ± 3.2 mmHg (n = 15). Prazosin (0.1 mg kg^{-1}), alone or

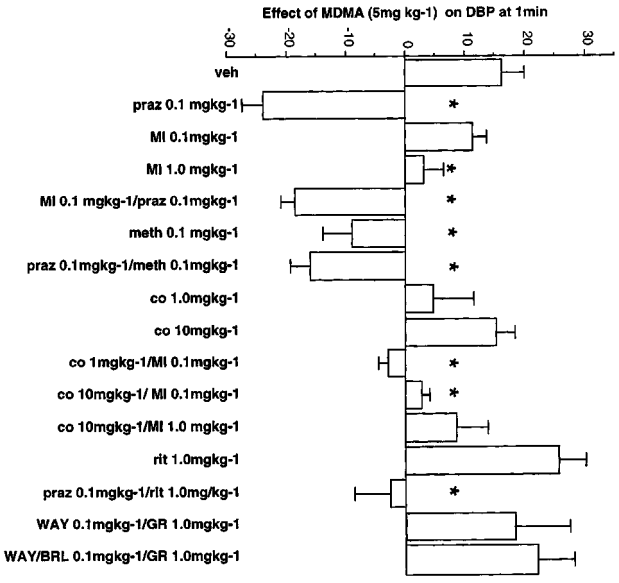

Figure 6 The diastolic blood pressure (DBP) response to MDMA in the anaesthetized rat 1 min after injection, following vehicle, prazosin, methoxyidazoxan, methiothepin, cocaine, ritanserin, or combinations of drugs. Abbreviations: veh, vehicle; BRL, BRL 15572; GR, GR 55562; praz, prazosin; MI, methoxyidazoxan; meth, methiothepin; co, cocaine; rit, ritanserin; WAY, WAY 100635. Numbers following drug names indicate dose in mg kg^{-1}. Vertical bars represent s.e.mean from at least four experiments (for *n* values see Table 1). Asterisks denote the significance of difference of effects of MDMA following test drugs from effects of MDMA in vehicle experiments (Analysis of Variance and Dunnett's test: $P<0.05$). Data taken from Figure 4.

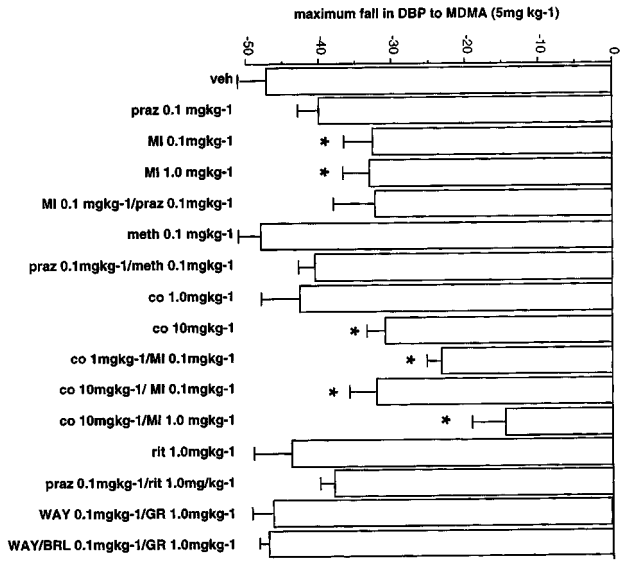

Figure 7 Maximum depressor response, measured as diastolic blood pressure (DBP), in the anaesthetized rat to MDMA (5 mg kg^{-1}), following vehicle, prazosin, methoxyidazoxan, methiothepin, cocaine, ritanserin, or combinations of drugs. Abbreviations: veh, vehicle; BRL, BRL 15572; GR, GR 55562; praz, prazosin; MI, methoxyidazoxan; meth, methiothepin; co, cocaine; rit, ritanserin; WAY, WAY 100635. Numbers following drug names indicate dose in mg kg^{-1}. Vertical bars represent s.e.mean from at least four experiments (for *n* values see Table 1). Asterisks denote the significance of difference of effects of MDMA following test drugs from effects of MDMA in vehicle experiments (Analysis of Variance and Dunnett's test: $P<0.05$). Data taken from Figure 4.

in combination, reversed this 1 min response to a depressor response of approximately 20 mmHg (Figures 4 and 6). Methiothepin (0.1 mg kg^{-1}) and methoxyidazoxan (1.0 mg kg^{-1}) and the combination of cocaine (1.0 or 10 mg kg^{-1}) and methoxyidazoxan (0.1 mg kg^{-1}) also significantly reduced or reversed the 1 min response to a depressor response. The effects of other drugs and combinations were non-significant.

Depressor response to MDMA (5 mg kg^{-1}) in anaesthetized rats The sustained depressor response to MDMA (5 mg kg^{-1}) was significantly reduced by the α_2-adrenoceptor antagonist methoxyidazoxan (0.1 mg kg^{-1}) (Figures 4 and 7). Methoxyidazoxan 1 mg kg^{-1} had no more effect than methoxyidazoxan 0.1 mg kg^{-1} (Figure 7). Cocaine 10 mg kg^{-1} had no more effect than methoxyidazoxan 0.1 mg kg^{-1} (Figure 7). Cocaine 10 mg kg^{-1} but not cocaine 1 mg kg^{-1} significantly reduced the depressor response to MDMA (Figure 7). The combination of methoxyidazoxan (0.1 or 1 mg kg^{-1}) and cocaine (1 or 10 mg kg^{-1}) greatly reduced the depressor response to MDMA (5 mg kg^{-1}). Prazosin, methiothepin or ritanserin or the combinations of the 5-HT_1 receptor antagonists (WAY 100635, GR 555623 and BRL 15572) had no significant effect on the maximum depressor response (Figures 4 and 7). However, when responses to MDMA (5 mg kg^{-1}) were examined at 10 min after injection, all drugs and combinations significantly reduced the depressor response except for methiothepin, ritanserin, the combinations of the 5-HT_1 receptor antagonists, and cocaine (1 mg kg^{-1}) (Figure 4).

Heart rate in anaesthetized rats In anaesthetized rats, resting heart rate was 389 ± 3 min^{-1} ($n=124$). In separate vehicle experiments, MDMA (1, 5 and 20 mg kg^{-1}) raised heart rate by 77 ± 4 ($n=3$), 74 ± 8 ($n=15$) and 85 ± 12 min^{-1} ($n=4$). Of the antagonist combinations employed, prazosin (0.1 mg kg^{-1}), cocaine (1 mg kg^{-1}), and methoxyidazoxan (0.1 mg kg^{-1}) alone or in combination with cocaine, significantly reduced the tachycardia to MDMA (5 mg kg^{-1}). However, all except cocaine (1 mg kg^{-1}) significantly raised resting heart rate. Cocaine (1 mg kg^{-1}) significantly reduced the tachycardia to MDMA (5 mg kg^{-1}) to 35 ± 9 min^{-1} ($n=6$, $P<0.05$).

Discussion

We have previously shown that MDMA has two actions in the rat atrium and vas deferens: the well-known indirect actions to displace noradrenaline from nerve terminals and a direct agonist action on prejunctional α_2-adrenoceptors on nerve terminals to inhibit neurotransmitter release (Lavelle *et al*., 1999). While the former action of MDMA is well documented, the latter action as an α_2-adrenoceptor agonist is novel. Since α_2-adrenoceptor agonists have major actions affecting blood pressure by central and peripheral actions, we have examined the vascular actions of MDMA in the anaesthetized rat. MDMA (5 mg kg^{-1}) was chosen as the test dose because it produced a biphasic effect on DBP. MDMA (1 mg kg^{-1}) produced only a pressor response, although the depressor component could be revealed in the

presence of prazosin. MDMA (20 mg kg^{-1}) produced a biphasic response, but the depressor component developed much more slowly.

The following antagonist drugs were employed: the α_1-adrenoceptor antagonist prazosin, the α_2-adrenoceptor antagonist methoxyidazoxan (the α_2-adrenoceptor antagonist yohimbine was employed in some studies in the pithed rat), the non-selective 5-HT receptor antagonist methiothepin (0.1 mg kg^{-1}) (Bradley *et al*., 1986; Docherty, 1988), the 5-HT_{1A} receptor antagonist WAY 100635 (0.1 mg kg^{-1}) (Saxena *et al*., 1998) the 5-HT_{1B} receptor antagonist GR 55562 (1 mg kg^{-1}) (MacLean *et al*., 1996), the 5-HT_{1D} receptor antagonist BRL 15572 (0.1 mg kg^{-1}) (Saxena *et al*., 1998), the 5-HT_2 receptor antagonist ritanserin, the noradrenaline re-uptake blocker cocaine. However, studies in the pithed rat revealed that methiothepin (0.1 mg kg^{-1}) and methoxyidazoxan (1 mg kg^{-1}) had significant antagonist actions at α_1-adrenoceptors, and were approximately 10 and 100 times less potent than prazosin, respectively.

Studies in the pithed rat demonstrated peripheral vasoconstrictor actions of MDMA (1 and 5 mg kg^{-1}). Studies with prazosin suggested that the predominant response is α_1-adrenoceptor mediated, but since prazosin did not abolish the response to MDMA (1 mg kg^{-1}), the response cannot be exclusively α_1-adrenoceptor mediated (compare effects of prazosin against phenylephrine in Figure 1a). Even yohimbine/prazosin/methiothepin or ritanserin/prazosin/methoxyidazoxan in combination did not completely block the pressor response to MDMA (5 mg kg^{-1}). However, since yohimbine/prazosin/methiothepin or ritanserin/prazosin/methoxyidazoxan had significantly greater effects than prazosin alone, it is likely that α_2-adrenoceptors and/or 5-HT_2 receptors are also involved in pressor responses to MDMA. Results obtained with ritanserin suggest that any 5-HT_2 component is relatively weak: the major actions of methiothepin are presumably at α_1-adrenoceptors. The effects of yohimbine/prazosin (α_1-adrenoceptor and α_2-adrenoceptor antagonism) or methiothepin (5-HT receptor and α_1-adrenoceptor antagonism) were not significantly greater than the effects of prazosin alone: hence, it is difficult to distinguish which of these secondary responses is more important.

Since MDMA is likely to have peripheral actions both to inhibit pressor nerve responses by action at prejunctional α_2-adrenoceptors and to produce direct pressor responses by action at postjunctional α_2-adrenoceptors, we wished to investigate which action would predominate. In pithed rat experiments in which MDMA was given either between intermittent pressor nerve stimulation or during continuous pressor nerve stimulation to raise DBP to levels found in the anaesthetized rat, there was no clear evidence for prejunctional inhibition of the nerve-evoked response. This may suggest that the major peripheral action of MDMA is pressor, so that any depressor actions found in the anaesthetized rat are likely to be central in origin.

In the anaesthetized rat, effects of drugs on baseline blood pressure must first be considered. Drugs which increase baseline blood pressure are likely to reduce pressor responses to test agents, and drugs which decrease baseline blood pressure are likely to decrease depressor responses to test agents, purely by effects on baseline blood pressure. However, these effects are difficult to quantify, but the possibility that changes in baseline blood pressure affected response to MDMA must always be considered. It can be assumed that the falls in baseline DBP produced by prazosin, alone or in combination, and by methiothepin are due to α_1-adrenoceptor blockade. Methoxyidazoxan (1 mg kg^{-1}) also reduced DBP, presumably due to α_1-adrenoceptor blockade but this did not reach significance (except in combination with cocaine). In contrast, methoxyidazoxan (0.1 mg kg^{-1}) tended to increase DBP, although this did not reach significance (except in combination with cocaine): an increase in DBP may be due to central or peripheral α_2-adrenoceptor antagonism: idazoxan is reported to raise blood pressure in man (Brown *et al*., 1985) (see also heart rate effects).

In the anaesthetized rat, MDMA had biphasic actions with three components: a pressor response with two components (initial peak and a secondary response at 1 min) and a sustained depressor response. Clearly, drugs may interact with all three components, making interpretation difficult.

By analogy with α_2-adrenoceptor agonists such as clonidine or xylazine, it might be expected that the initial pressor response to MDMA is due to action at peripheral postjunctional α_1- and α_2-adrenoceptors (Haeusler, 1974; Docherty & McGrath, 1980), and, given the pithed rat results obtained with MDMA, particularly α_1-adrenoceptors. In the anaesthetized rat, the initial pressor response was markedly reduced by the non-selective 5-HT receptor antagonist methiothepin but not by selective 5-HT_1-receptor antagonists in combination, suggesting that these actions involve 5-HT_2-receptors (or α_1-adrenoceptors). Methoxyidazoxan, alone or in combination with cocaine, also reduced the pressor response. Prazosin or the 5-HT_2 receptor antagonist ritanserin were ineffective when given alone, but produced a significant reduction in the pressor response in combination. This may suggest that the initial pressor response to MDMA involves a combination of α_2- and possibly α_1-adrenoceptors and/or 5-HT_2 receptors, presumably located peripherally on vascular smooth muscle: the actions of methiothepin may involve both α_1-adrenoceptors and 5-HT_2 receptors. It should be noted that, whereas in the pithed rat, pressor responses to MDMA were largely α_1-adrenoceptor mediated, the α_2-adrenoceptor response was dominant, with possibly α_1-adrenoceptor and 5-HT_2 components, in the anaesthetized rat: differences may be due to the very low level of resting vasoconstriction present in the pithed rat.

The response to MDMA (5 mg kg^{-1}) at 1 min was reversed from a pressor response to a depressor response by drugs with α_1-adrenoceptor actions: prazosin, alone or in combination, methiothepin and methoxyidazoxan (1 mg kg^{-1}). This suggests that α_1-adrenoceptors dominate the pressor component at 1 min, so that the presumed α_2-adrenoceptor/5-HT_2 components appear to be relatively short-lived. This response now more closely resembles the response in the pithed rat.

Methoxyidazoxan (0.1 mg kg^{-1}) or cocaine (10 mg kg^{-1}) significantly reduced the maximum depressor response to MDMA, and the combination of the two agents caused a very marked reduction. Methiothepin, ritanserin, selective 5-HT_1 receptor antagonists and cocaine (1 mg kg^{-1}) failed to affect the maximum depressor response to MDMA, nor did they affect the time course of the depressor response (see Figure 4). Although prazosin, alone or in combination, did not affect the maximum depressor response to MDMA,

prazosin and combinations affected the time course of the depressor response so that there was significant recovery by 10 min (Figure 4). However, we cannot rule out the possibility that this was influenced by the fact that prazosin and combinations significantly lowered resting DBP. Hence, we can at least say that the depressor actions of MDMA involve activation predominantly of α_2-adrenoceptors. These α_2-adrenoceptors are presumably located centrally (Haeusler, 1974). There may be other components to the depressor response to MDMA, given the large residual response remaining even following methoxyidazoxan and cocaine, but these do not seem to involve 5-HT_1 or 5-HT_2 receptors.

We wished to investigate whether the actions of MDMA involved displacement of noradrenaline or direct activation of α_2-adrenoceptor, or both, by investigating the interaction with the noradrenaline re-uptake blocker cocaine and with receptor antagonists. Cocaine (1 mg kg^{-1}) did not affect any parameter, and cocaine (10 mg kg^{-1}) reduced only the peak pressor and maximum depressor responses. Hence, marked block of noradrenaline re-uptake with high doses of cocaine does produce some effects, but much more marked effects can be obtained to the combination of methoxyidazoxan and cocaine. Since our previous results show major actions of MDMA at all three subtypes of α_2-adrenoceptor (Lavelle *et al*., 1999), it would appear that the depressor response involves direct stimulation of α_2-adrenoceptors by MDMA and indirect actions by displacement of noradrenaline. For the response at 1 min, the results are even clearer: cocaine had no effect on this α_1-adrenoceptor mediated pressor response, suggesting that actions of MDMA are predominantly direct.

Effects of MDMA on heart rate were also examined in anaesthetized rats: MDMA (1, 5 and 20 mg kg^{-1}) produced similar rises in heart rate, although the duration of action was greatest for the highest dose. The heart rate reached (475 – 500 min^{-1}) is near the maximum obtainable in an anaesthetized rat: hence, antagonists which raise basal heart rate are likely to reduce the tachycardiac response to MDMA. Prazosin and methiothepin, alone or in combination, significantly lowered DBP, and therefore the significant tachycardia to these agents is likely to be baroreflex in origin. Methoxyidazoxan produced a significant tachycardia (alone or in combination with cocaine) without producing a significant fall in DBP; this action may be due to antagonist actions at peripheral prejunctional α_2-adrenoceptors on cardiac nerves and/or to central α_2-adrenoceptor actions. Indeed, α_2-adrenoceptor antagonists produce tachycardia in man (e.g. Schafers *et al*., 1992). Of the antagonists employed, only prazosin (0.1 mg kg^{-1}), methoxyidazoxan (0.1 mg kg^{-1}), and cocaine (1 mg kg^{-1}), significantly reduced the tachycardia to MDMA, and of these only cocaine did not significantly affect resting heart rate. This might suggest that cocaine at this dose reduced the tachycardia to MDMA by preventing displacement of noradrenaline. However, cocaine (10 mg kg^{-1}) did not significantly reduce the tachycardia to MDMA. Perhaps, cardiac actions of MDMA are not easy to investigate using this protocol.

In man, cardiovascular actions of MDMA have not been widely studied, but it has been reported generally to produce a rise in blood pressure (Grob *et al*., 1996; Vollenweider *et al*., 1998). However, Downing (1986) reported an initial rise in blood pressure, but also a tendency for blood pressure to fall below baseline after several hours. Most studies were not carried out under controlled conditions in which α_2-adrenoceptor actions could be investigated. It is likely that MDMA in recreational doses has effects in man similar to the 1 mg kg^{-1} dose reported here for the rat: dominant pressor actions which mask the α_2-adrenoceptor mediated depressor actions. Further information is needed to elucidate the effects of MDMA on blood pressure in man.

Finally, do our findings that MDMA has significant effects as an agonist at α_2-adrenoceptors impact on the recreational abuse and toxic effects of MDMA? Certainly, such actions have not been widely considered in reviewing the effects of MDMA in man: serotonergic mechanisms are assumed to predominate. However, it is feasible that α_2-adrenoceptor agonism contributes to the central abusive and peripheral cardiovascular and autonomic side effects of MDMA. For instance, it has been reported that MDMA decreases firing rates of serotonergic and noradrenergic but not dopaminergic neurones in the rat dorsal and median raphe (Piercey *et al*., 1990): an action at prejunctional α_2-adrenoceptors is likely. Actions of MDMA at postjunctional α_2-adrenoceptor centrally and on vascular and non-vascular smooth muscle may also be considered. Further research is needed to elucidate these effects.

In conclusion, in anaesthetized rats, MDMA has significant α_2-adrenoceptor agonist actions which contribute to the depressor response in anaesthetized rats; these α_2-adrenoceptors are presumably located centrally. The initial transient pressor response may involve actions at α_2- and possibly α_1-adrenoceptors and 5-HT_2 receptors, which are presumably located peripherally.

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