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ORIGINAL INVESTIGATION

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Multiple 5-HT receptors are involved in the effects of acute MDMA treatment: studies on locomotor activity and responding for conditioned reinforcement

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Abstract *Rationale:* Responding for conditioned reinforcement is increased by the dopamine releasing agent amphetamine, but reduced by drugs that enhance serotonin (5-HT) function. The amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) releases both monoamines. *Objectives:* The primary purpose of this study was to examine the effects of MDMA on responding for conditioned reinforcement as well as on locomotor activity. The roles of several 5-HT receptor sub-types in mediating these behavioural effects of MDMA were also examined. *Methods:* Locomotion was measured in photocell activity monitors. For conditioned reinforcement experiments thirsty rats learned to associate a conditioned stimulus (CS) with water in operant chambers. Subsequently, two response levers were available; responding on one lever delivered the CS, while responding on the second lever had no consequences. Drug effects on this operant response were measured. *Results:* MDMA dose-dependently increased locomotion but reduced responding for conditioned reinforcement. This latter effect differs from that induced by amphetamine, which potentiates conditioned reinforcement responding. The stimulant effect of MDMA was attenuated by GR127935 and ketanserin, indicating facilitatory roles of 5-HT_{1B} and 5-HT_{2A} receptors in mediating this effect. The 5-HT_{2C} antagonist SB242084 enhanced the stimulant effect of MDMA. Only SB242084 attenuated the suppressant effect of MDMA on responding for conditioned reinforcement. *Conclusions:* The results show that 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors play a facilitatory role in mediating the stimulant effect of MDMA, whereas 5-HT_{2C} receptors are inhibitory. Activation of 5-HT_{2C} receptors also contributes to the deficit in operant responding. Multiple 5-HT receptor sub-types appear to contribute to the behavioural effects of MDMA.

Keywords Serotonin · 5-HT receptor · Dopamine · Conditioned reinforcement locomotor activity · 3,4-Methylenedioxymethamphetamine (MDMA, Ecstasy)

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Introduction

3,4-Methylenedioxymethamphetamine (MDMA; Ecstasy) is an amphetamine derivative that releases both serotonin (5-hydroxytryptamine; 5-HT) and dopamine (see reviews by Green et al. 1995; White et al. 1996; Bankson and Cunningham 2001). MDMA induces a variety of behavioural effects, including stimulation of locomotor activity (e.g. Gold et al. 1988, 1989; Callaway et al. 1990; McCreary et al. 1999). MDMA also has reinforcing and rewarding effects as determined using self-administration (Beardsley et al. 1986; Lamb and Griffiths 1987), brain stimulation (Lin et al. 1997) and conditioned place preference techniques (Marona-Lewicki et al. 1996). While amphetamine shares these properties (e.g. Pickens and Harris 1968; Kelly et al. 1975; Spyraki et al. 1982; Gallistel and Karras 1984), a considerable body of evidence shows that MDMA and amphetamine differ in terms of their behavioural effects and their underlying neurochemical mechanisms. This supports the proposal that MDMA represents a different class of drug from psychomotor stimulants (Nichols et al. 1986).

Drug discrimination studies indicate that MDMA and d-amphetamine have differing stimulus properties. Rats readily discriminate MDMA from amphetamine in a

three-choice discrimination procedure (Goodwin and Baker 2000), while in standard two-choice discrimination tests generalization between MDMA and amphetamine is not reliable (Oberlender and Nichols 1988; Schecter 1989; Baker and Makhay 1996). Behavioural analyses have shown that the locomotor activation induced by MDMA is qualitatively different from that induced by amphetamine (Gold et al. 1988; Callaway et al. 1990). Pharmacological and lesioning studies demonstrate that the locomotor stimulant effect of MDMA is mediated in large part by 5-HT release (Callaway et al. 1990; Kehne et al. 1996), whereas DA (Kelly et al. 1975), but not 5-HT (Callaway et al. 1990; Fletcher et al. 1999), release mediates the stimulant effect of amphetamine. In humans, the subjective effects of MDMA are distinct from those of amphetamine (Solowij et al. 1992). These subjective effects of MDMA are blocked by the selective serotonin re-uptake inhibitor citalopram (Liechti et al. 2000a), and the 5-HT_2 receptor antagonist ketanserin (Liechti et al. 2000b). Thus, both animal and human work indicates that the expression of some behavioural effects of MDMA is mediated in part by elevated 5-HT neurotransmission. However, it cannot be ignored that MDMA also releases dopamine (Nash and Nichols 1991; Kankaanpaa et al. 1998). In rats, the locomotor stimulant effect of MDMA is attenuated by dopamine-depleting lesions of the nucleus accumbens (Gold et al. 1989) and by dopamine receptor antagonists (Kehne et al. 1996), while in humans, subjective effects of MDMA are blocked by haloperidol (Liechti and Vollenweider 2000). Thus, the dopamine-releasing effects of MDMA may also contribute to the expression of behavioural and subjective effects of MDMA.

A characteristic effect of psychomotor stimulants is their ability to enhance incentive motivation. This effect is clearly seen in studies in which a neutral stimulus is paired with a primary reinforcer such as water or food. Subsequently rats learn to respond on a lever delivering that stimulus, now termed a conditioned reinforcer (CR). Amphetamine potentiates responding for conditioned reinforcement via increased release of dopamine (Robbins et al. 1983; Taylor and Robbins 1984). In contrast, we have found that *d*-fenfluramine reduces responding for conditioned reinforcement and attenuates the potentiating effect of amphetamine (Fletcher 1995). This effect of fenfluramine is blocked by the non-selective 5-HT receptor antagonist metergoline indicating that the action of *d*-fenfluramine is probably mediated by 5-HT release. Thus, 5-HT and dopamine seem to have opposing effects on incentive motivation.

Incentive motivational processes play a major role in the acquisition and maintenance of drug-taking behaviour (Robinson and Berridge 2001). Given the widespread recreational use of MDMA (Hegadoren et al. 1999), the primary objective of these experiments was to determine the effects of MDMA on incentive motivational processes as measured by operant responding for a conditioned reinforcer. In particular we were interested in determining whether MDMA would exert a behavioural effect consistent with elevated 5-HT function, namely a suppression of responding for conditioned reinforcement or an effect consistent with dopamine release, namely increased responding for conditioned reinforcement. Following the initial finding of reduced responding, we attempted to determine whether this effect of MDMA was mediated by specific 5-HT receptor subtypes. To this end, we investigated the effects of the 5-$HT_{1B/1D}$ antagonist GR127935 (Skingle et al. 1995), ketanserin, an antagonist that discriminates 5-HT_{2A} from other 5-HT receptor sub-types (Barnes and Sharp 1999), and the 5-HT_{2C} receptor antagonist SB242084 (Bromidge et al. 1997; Kennett et al. 1997) on the response suppressant effects of MDMA. These receptors have all been implicated in the expression of MDMA-induced locomotor activity (Rempel et al. 1993; Kehne et al. 1996; McCreary et al. 1999; Scearce-Levie et al. 1999; Bankson and Cunningham 2001). Parallel experiments also examined the effects of these antagonists on locomotor activity in order to determine effective doses, as well as to permit an analysis of the relationship between changes in locomotor activity and responding for conditioned reinforcement following MDMA treatment. During the course of these experiments the 5-HT_{2C} receptor antagonist SB242084 was found to have prominent effects on MDMA-induced behaviour. Therefore, additional experiments were conducted to determine any possible pharmacokinetic interaction between SB242084 and MDMA, as well as the effects of SB242084 on responding for conditioned reinforcement in rats treated with the 5-HT_{2C} receptor agonist Ro60-0175 (Martin et al. 1998).

Materials and methods

Subjects

Adult male Sprague-Dawley rats (Charles River, Quebec) weighing 280–320 g at the beginning of each study were used. They were housed in clear plastic, rectangular, solid-bottomed cages; rats used for locomotor activity tests were housed in pairs, while those used in conditioned reinforcement studies were housed singly. The housing room was maintained on a 12-h light/dark cycle (lights on at 0800 hours) and at a temperature of 22±2°C. Animals used in locomotor activity tests had food and water available freely at all times. For rats in the conditioned reinforcement studies, access to water was restricted as detailed below. All training and testing was conducted during the light phase. Experimental procedures and manipulations conformed to the guidelines laid down by the Canadian Council on Animal Care and were approved by the CAMH Animal Care Committee.

Locomotor activity

Tests of locomotor activity were conducted in four clear Plexiglas activity chambers (Med Associates Inc., St Albans, Vt., USA) measuring 17 in long, 17 in wide, and 12 in high. An array of 16×16 photodetectors, spaced 1 in apart and positioned 1 in above the floor of the chamber was used to detect locomotor activity. The software allowed a distinction to be made between repetitive interruptions of the same photobeam and interruptions of adjacent photobeams. This latter measure was used as an index of ambulatory activity. All rats were first habituated to the apparatus

by placing them in the activity chambers for 1 h on 3 consecutive days. On test days, rats were placed in the activity chamber for a 30-min habituation period. Initially the effects of different doses of MDMA on activity were determined. Here rats were injected with MDMA or saline immediately after the habituation period. In the antagonist studies, rats were injected with the appropriate antagonist or its vehicle after the habituation period and left in the activity monitors. Thirty minutes later, MDMA or its vehicle were administered. In all experiments activity was measured for 75 min beginning immediately after the MDMA or vehicle injection.

To determine the dose-response function to MDMA rats (n=6) were injected with 1.25, 2.5 or 5 mg/kg MDMA or saline. For antagonist studies separate groups of rats were used to test the effects of 3 mg/kg GR127935 (n=8), 1 mg/kg ketanserin (n=8), 0.5 mg/kg SB242084 (n=12) and 1 mg/kg SB242084 (n=12) on the locomotor activity induced by 2.5 mg/kg MDMA. In all experiments, each rat was tested 4 times, at all doses of MDMA or saline, or following each combination of antagonist or vehicle or MDMA and saline. The order of treatments was randomised, with approximately equal numbers of animals tested under the same treatment condition on the test days.

Conditioned reinforcement

Testing was conducted in six operant-chambers. Each chamber contained two retractable response levers, 4.5 cm wide and 7 cm above the floor of the chamber. The centres of the levers were positioned 6.5 cm to either side of a central recessed water dish 3 cm above the floor. A solenoid-operated water dispenser delivered water to this dish. A red stimulus light was located above each lever, and a Sonalert was positioned behind one of the lights. Each chamber was illuminated by a house light. All chambers were housed in sound-attenuating cabinets equipped with ventilating fans.

Throughout these experiments, rats were allowed access to water for 2 h per day between 3 and 5 p.m. There were three main phases to these experiments. The first habituation phase consisted of the subjects being placed in the operant boxes with the house light on, and approximately 2 ml of water in the dish. The following day, conditioning began. During this phase, the levers were retracted, and the animals were trained to associate a compound stimulus with the delivery of 0.05 ml water. The compound stimulus consisted of a 5-s period of the house-light off and both red stimulus lights on. During the last 0.5 s of this 5-s period, a tone (2900 Hz at approximately 80 dB) sounded and the water was delivered. This stimulus occurred 30 times, on a random time (RT) 30 s schedule. Conditioning was carried out at one session per day for 14 days, and each session lasted on average 15 min. On day 15, the rats were placed in the boxes with both levers present. Pressing the left lever delivered the conditioned stimulus described above, according to a random ratio (RR) 2 schedule; no water was delivered. Pressing the right lever had no programmed consequences. This session lasted until the animal had responded 10 times on the active lever. The purpose of this session was to ensure that all animals had experience with the levers present, and that they had sampled the active lever prior to testing. Testing was carried out during the final third phase, and was conducted in 40-min sessions. During testing sessions both levers were present, and a response on the left lever delivered the conditioned stimulus (now the conditioned reinforcer) on a RR2 schedule; responses on the right lever had no programmed consequence.

In the initial experiment rats (n=6) were injected with 1.25, 2.5 or 5 mg/kg MDMA or saline. For antagonist studies separate groups of rats were used to test the effects of 1 mg/kg GR127935 (n=9), 3 mg/kg GR127935 (n=9), 1 mg/kg ketanserin (n=9), 3 mg/kg ketanserin (n=9), 0.5 mg/kg SB242084 (n=12) and 1 mg/kg SB242084 (n=12) on responding for conditioned reinforcement in rats treated with 2.5 mg/kg MDMA or saline. The MDMA or saline was injected 15 min before testing; antagonists were injected 30 min prior to MDMA or vehicle. In all experiments each rat was tested 4 times, at all doses of MDMA or saline, or following each combination of antagonist or vehicle or MDMA and saline. The order of treatments was randomised with approximately equal numbers of animals tested under the same treatment condition on the test days. In a final experiment rats (n=10) were tested following the four combinations of 1 mg/kg SB242084 or its vehicle followed 30 min later by 1 mg/kg Ro60-0175 or saline (injected SC) 15 min prior to testing.

Measurement of MDMA levels in brain following SB242084

Two groups of rats (n=6 per group) were used. One group was treated with 1 mg/kg SB242084 followed 30 min later by 2.5 mg/kg MDMA. The other group received saline and MDMA. Thirty minutes after MDMA, rats were decapitated; the brains were removed and frozen on dry ice. Brains were packaged in dry ice and shipped to Edmonton overnight. Immediately on arrival they were stored at –80°C. Brain samples were analyzed for levels of MDMA using extraction under basic conditions followed by derivatization with pentafluorobenzoyl chloride and analysis on a gas chromatograph equipped with a nitrogen-phosphorus detector. Briefly, the procedure involved homogenization in ice-cold 0.1 N perchloric acid (containing 0.343 mM EDTA and 50 µM ascorbic acid), centrifugation to remove protein, and retention and basification of the supernatant. The MDMA was extracted from the supernatant by shaking with ethyl acetate; after centrifugation to separate the phases, the organic phase was retained and taken to dryness under a stream of nitrogen. The residue was reacted with pentafluorobenzoyl chloride in toluene; following a wash with saturated sodium tetraborate solution, the toluene phase was retained and a portion injected on a gas chromatograph equipped with a fused silica capillary column (HP5, 30 m), a nitrogen-phosphorus detector and a printer/integrator.

Drugs and injections

MDMA [(±)-3,4-methylenedioxymethamphetamine HCl; NIDA Drug Supply Program] was dissolved in 0.9% saline and injected SC. Ketanserin tartrate (Sigma-RBI, Oakville, Ontario, Canada) was dissolved in 0.9% saline and injected IP. GR127935 2'-methyl-4'-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methylpiperazin-1-yl)-phenyl]-amide) hydrochloride monohydrate (Glaxo Wellcome, UK) was dissolved in distilled water heated to approximately 70°C; this was allowed to cool to room temperature before injection via the IP route. Ro60-0175 [(S)-2-(6-chloro-5-fluoro-indol-1-yl)-1-methylethylamine HCl; F. Hoffmann-La Roche Ltd, Basel, Switzerland] was dissolved in 0.9% saline and administered by SC injection. SB242084 (6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbamoyl] indoline) was synthesised in the Department of Chemistry, Vernalis Research Ltd, Wokingham, UK. SB242084 was prepared in 0.9% saline solution containing 8% 2,4-hydroxypropyl-β-cyclodextrin (Sigma-RBI, Oakville, Ontario, Canada) and 25 mM citric acid and was injected by the IP route. All drug doses are expressed in terms of the salt.

Statistics

Data were analysed by one-, two- or three-way analysis of variance. Post-hoc tests were made using either Dunnett's test for comparisons against a control mean or Tukey's test for pairwise comparisons.

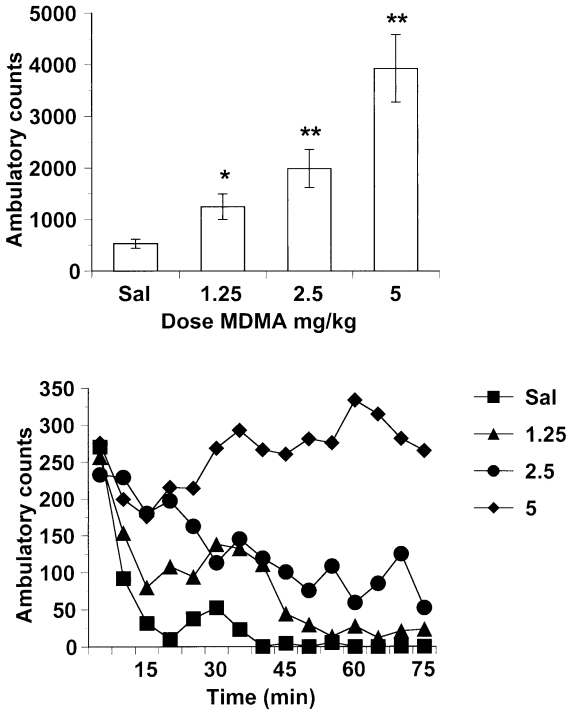

Fig. 1 The *upper panel* demonstrates the increase in locomotor activity induced by increasing doses of MDMA, measured over the 75-min test period. The *lower panel* depicts the time course of this activation. *P<0.05, **P<0.01 compared to Sal

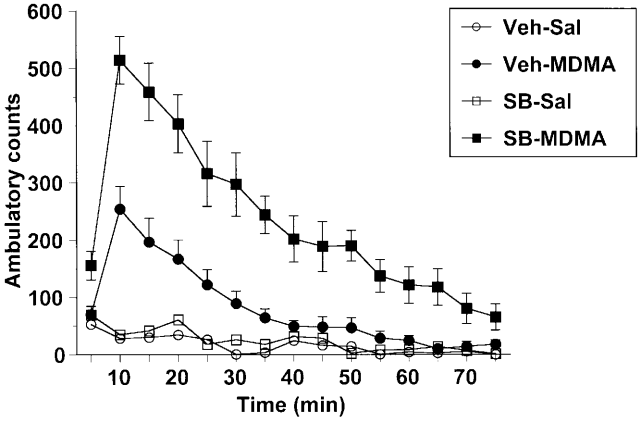

Fig. 2 The potentiating effect of 1 mg/kg SB242084 on the locomotor activity induced by 2.5 mg/kg MDMA. Points represent the mean (±SEM) number of ambulatory counts recorded in 5-min bins for 12 rats. Significant potentiation of the effects of MDMA by SB242084 was found at all time intervals (P<0.05)

Results

Locomotor activity studies

Figure 1 shows that MDMA elicited a dose-dependent increase in the total amount of locomotion measured over the 75-min test period [F(3,15)=18.21, P<0.001], with effects significantly different from saline at all doses (Dunnett's test, P<0.05). Analysis of the time course data showed that the increase in activity induced by 2.5 and 5 mg/kg MDMA was sustained for the duration of the test period.

Table 1 shows the effects of the various antagonists on the activity induced by MDMA. For GR127935, analysis of variance demonstrated a significant interaction between the antagonist and MDMA treatments [F(1,7)= 10.98, P<0.001]. Post-hoc tests showed that MDMA treatment increased activity relative to vehicle treatment, and that GR127935 attenuated this response. A significant interaction between ketanserin and MDMA treatments was also observed [F(1,7)=18.00, P<0.001]. Again MDMA increased activity and this effect was completely reversed by 1 mg/kg ketanserin. In both experiments the antagonist alone did not alter activity levels. Both 0.5 and 1 mg/kg SB242084 appeared to enhance MDMA-induced activity an observation confirmed by significant antagonist×MDMA interactions [F(1,11)=23.82 and 59.04, respectively, P<0.002]. Figure 2 illustrates the time course of the effect of 1 mg/kg SB242084 on MDMA-stimulated activity. The response recorded under SB242084 and MDMA treatment was significantly greater than that recorded under MDMA treatment alone at all time points. An identical pattern of results was obtained with the lower dose of 0.5 mg/kg SB242084.

Brain levels of MDMA after SB242084

Thirty minutes after injection of 2.5 mg/kg MDMA brain levels of MDMA were 2457±105 ng/g tissue. In rats pretreated with 1 mg/kg SB242084 MDMA levels were 2622±220 ng/g tissue. This difference was not significant [t(10)=0.67, P>0.5].

Table 1 Effects of various 5-HT receptor antagonists on the locomotor stimulant effect of 2.5 mg/kg MDMA. Numbers represent the mean and SEM number of ambulatory counts recorded over a 75-min period. In all experiments, scores counts recorded following Veh-MDMA were significantly higher than those obtained with Veh-Sal treatment

	n	Veh Sal	Veh MDMA	Antagonist Sal	Antagonist MDMA
Ketanserin 1 mg/kg	9	412.5 (89.6)	1325.6 (146.7)	356.0 (52.3)	531.5** (106.6)
GR127935 3 mg/kg	9	160.2 (42.4)	1156.9 (151.9)	260.3 (70.9)	789.5** (73.4)
SB242084 0.5 mg/kg	12	283.8 (66.3)	960.1 (103.1)	498.4 (115.7)	3569.4** (566.7)
SB242084 1 mg/kg	12	239.1 (48.1)	1200.2 (197.3)	365.2 (86.9)	3492.3** (405.7)

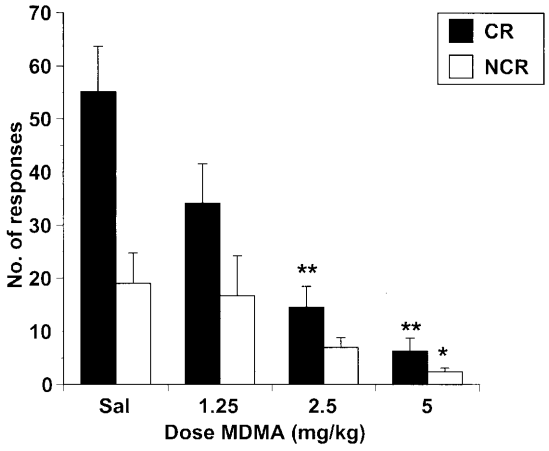

Fig. 3 MDMA suppressed responding in the test of conditioned reinforcement (n=6). *P<0.05, **P<0.01 compared to saline – same lever condition. *CR* denotes responding on the lever delivering conditioned reinforcement; *NCR* denotes responding on the inactive lever

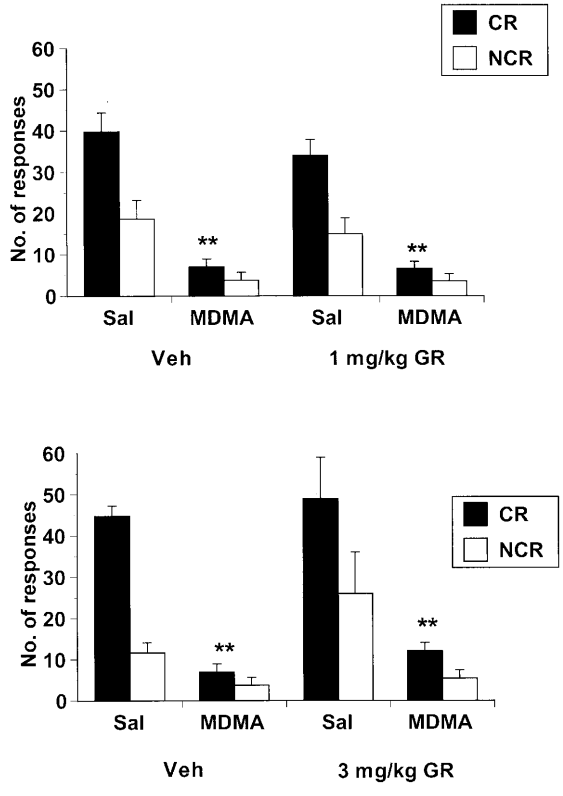

Fig. 4 The effects of pretreatment with 1 mg/kg (n=9) and 3 mg/kg (n=9) GR127935 on the reduction in responding induced by 2.5 mg/kg MDMA. Values represent mean+1 SEM number of responses. *CR* denotes responding on the lever delivering conditioned reinforcement; *NCR* denotes responding on the inactive lever. **P<0.01 compared to vehicle-saline – same lever condition

Conditioned reinforcement

The effects of increasing doses of MDMA on responding for conditioned reinforcement are shown in Fig. 3. Rats showed higher responding on the conditioned reinforcement lever [F(1,6)=30.43, P<0.01] and MDMA generally reduced responding [F(3,18)=13.51, P<0.001]. A significant interaction between dose and lever was found [F(3,18)=10.97, P<0.001]. The interaction reflects the fact that under saline treatment rats showed a clear preference for the conditioned reinforcement versus the inactive lever, but under MDMA treatment this preference was attenuated. However, it should be noted that for the highest dose of MDMA responding was significantly reduced on both levers.

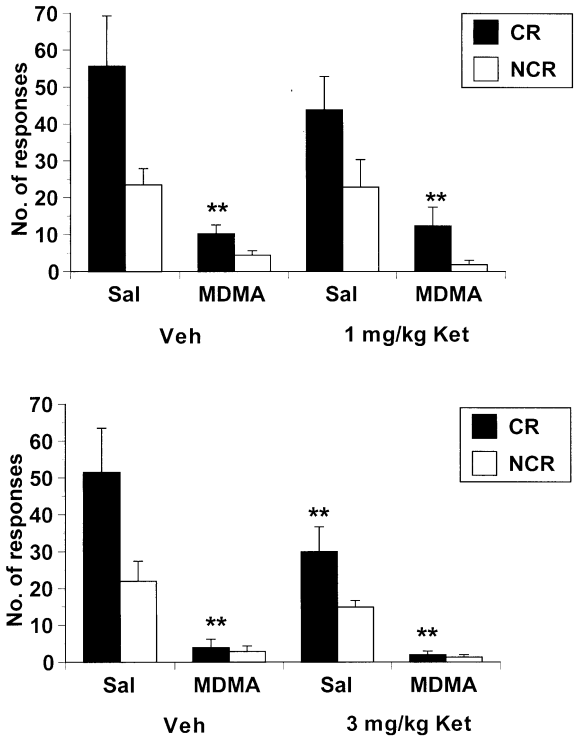

Fig. 5 Effects of pre-treatment with 1 mg/kg (n=9) and 3 mg/kg (n=9) ketanserin on the reduction in responding induced by 2.5 mg/kg MDMA. Values represent mean+1 SEM number of responses. *CR* denotes responding on the lever delivering conditioned reinforcement; *NCR* denotes responding on the inactive lever. **P<0.01 compared to vehicle-saline – same lever condition

The effects of 1 and 3 mg/kg GR127935 on the suppressant effects of 2.5 mg/kg MDMA are shown in Fig. 4. In both experiments responding was significantly higher on the conditioned reinforcement lever [F(1,8)= 45.08 and 14.52, respectively, P<0.01], and responding was reduced by MDMA [F(1,8)=12.56 and 20.97, respectively, P<0.01]. Significant MDMA×Lever interactions [F(1,8)=15.5 and 18.53 respectively, P<0.005] revealed that the effect of MDMA was most pronounced on the conditioned reinforcement lever. None of the interaction terms involving GR127935 pretreatment or the main effect of GR127935 were significant. Thus, GR127935 did not alter the effect of MDMA.

The effects of 1 and 3 mg/kg ketanserin on the suppressant effects of 2.5 mg/kg MDMA are shown in Fig. 5. In both experiments responding was significantly higher on the conditioned reinforcement lever [F(1,8)=

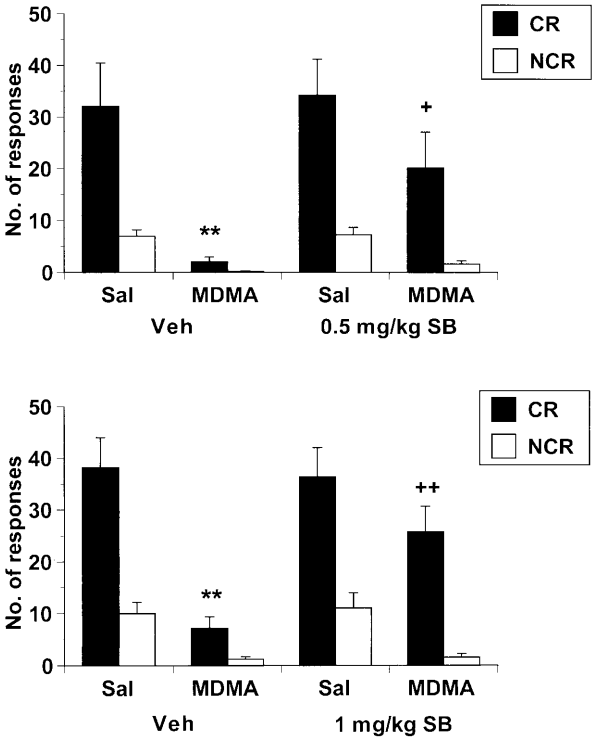

Fig. 6 Effects of pre-treatment with 0.5 mg/kg (n=12) and 1 mg/kg (n=12) SB242084 on the reduction in responding induced by 2.5 mg/kg MDMA. Values represent mean+1 SEM number of responses. *CR* denotes responding on the lever delivering conditioned reinforcement; *NCR* denotes responding on the inactive lever. **P<0.01 compared to vehicle-saline – same lever condition. +P<0.05, ++P<0.01 compared to Veh-MDMA – same lever condition

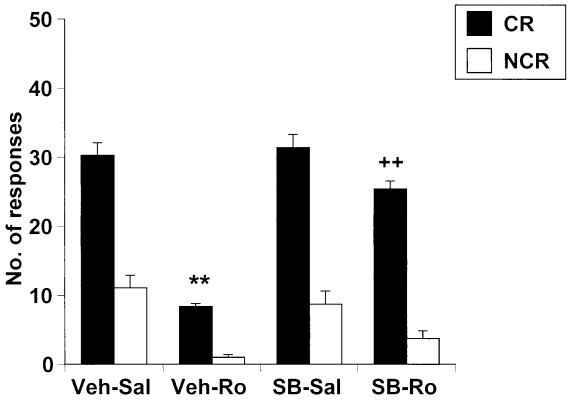

Fig. 7 The effects of pre-treatment with 1 mg/kg SB242084 on the reduction in responding induced by 1 mg/kg Ro60-0175 (n=10). Values represent mean+1 SEM number of responses. *CR* denotes responding on the lever delivering conditioned reinforcement; *NCR* denotes responding on the inactive lever. **P<0.01 compared to vehicle-saline – same lever condition. ++P<0.01 compared to vehicle-Ro60-0175 – same lever condition

12.08 and 11.38 respectively, P<0.01], and responding was reduced by MDMA [F(1,8)=29.17 and 26.06, respectively, P<0.001]. Significant MDMA×lever interactions [F(1,8)=4.9 and 7.79, respectively, P<0.05] revealed that the effect of MDMA was most pronounced on the conditioned reinforcement lever. A significant main effect of ketanserin was found at the 3 mg/kg dose [F(1,8)=6.92, P<0.03], presumably reflecting the fact that this dose reduced responding for conditioned reinforcement in its own right. Ketanserin did not alter the effect of MDMA.

The effects of 0.5 and 1 mg/kg SB242084 are shown in Fig. 6. As in the previous experiments, significant main effects of MDMA [F(1,10)=57.9; F(1,11)=20.8; both P<0.001], lever [F(1,10)=23.28; F(1,11)=92.67; both P<0.0001], and MDMA×lever interactions [F(1,10)= 18.32; F(1,11)=11.17; both P<0.01] were found at the two dose levels of SB242084. A significant three-way interaction between SB242084 pretreatment, MDMA treatment and lever was found only in the experiment involving 1 mg/kg SB242084 [F(1,11)=8.61, P<0.02]. This occurred because the MDMA×SB242084 interaction was significant for responses on the conditioned reinforcement lever [F(1,11)=6.43, P<0.03], but not the inactive lever [F(1,11)=0.1, P>0.7]. Post-hoc tests confirmed that both doses of SB242084 significantly reversed the response suppressant effect of MDMA.

Figure 7 shows that Ro60-0175 reduced responding for conditioned reinforcement [F(1,9)=23.82, P<0.001]. Although none of the interaction terms involving SB242084 were significant, post hoc testing confirmed that the reduction in responding on the conditioned reinforcement lever induced by Ro60-0175 was significantly reversed by SB242084.

Discussion

MDMA reduced responding for conditioned reinforcement at doses that increased locomotor activity. Both GR127935 and ketanserin failed to reverse the effects of MDMA on responding for conditioned reinforcement but attenuated the stimulant effect of MDMA. Conversely, SB242084 reversed the deficit in responding for conditioned reinforcement, while at the same time enhancing the stimulant action of MDMA. The results of these receptor antagonist studies indicate that the suppression of responding for conditioned reinforcement induced by MDMA is not a result of hyperactivity interfering with the operant response. Clearly there is no obvious relationship between altered motor activity on the one hand, and either reduced or enhanced responding for conditioned reinforcement on the other. The action of MDMA to reduce responding further differentiates this drug from amphetamine, which potentiates responding for conditioned reinforcement (Taylor and Robbins 1984). The present results indicate that MDMA does not enhance incentive motivation, and in fact may decrease this process.

The locomotor activating effects of MDMA were blocked by ketanserin and attenuated by GR127935. These results are consistent with previous reports

(Kehne et al. 1996; McCreary et al. 1999; Scearce-Levie et al. 1999) and demonstrate that activation of 5-HT_{2A} and 5-HT_{1B} receptors by released 5-HT is involved in mediating hyperactivity induced by MDMA. In contrast, the 5-HT_{2C} receptor antagonist SB242084 potentiated the effects of MDMA. The pattern of activity in rats treated with SB242084 plus MDMA indicated an increase in the intensity and duration of the effect of MDMA. MDMA is metabolised by cytochrome P450 enzymes (Cho and Kumagi 1994) and some compounds similar in structure to SB242084 inhibit the activity of these enzymes (Bromidge et al. 1997). Although the affinity of SB242084 for some of these enzymes is quite low (Bromidge et al. 1997), it is still important to rule out a possible pharmacokinetic interaction between the drugs as an explanation for these effects. The finding that brain levels of MDMA were not altered by SB242084 suggests that the interaction between SB242084 and MDMA is a true pharmacological one, based on neurochemical substrates, rather than a pharmacokinetic action. Thus, the effect of SB242084 to enhance the locomotor effect of MDMA implies that 5-HT_{2C} receptor stimulation, consequent to 5-HT release by MDMA, exerts an inhibitory role on the expression of MDMA-stimulated locomotion.

The potentiation of the effects of MDMA on locomotion induced by SB242084 may involve an interaction between 5-HT and dopamine systems since 5-HT_{2C} receptors modulate the activity of midbrain dopamine neurons (Di Matteo et al. 2001). The 5-HT_{2C} receptor agonist Ro60-0175 inhibits the firing activity of these neurons leading to reduced extracellular levels of dopamine in terminal areas including the nucleus accumbens (Di Matteo et al. 2000). SB242084 increases the firing rate of these same dopamine neurons (Di Matteo et al. 1999). Eberle-Wang et al. (1997) demonstrated the presence of 5-HT_{2C} mRNA within inhibitory GABA-ergic interneurons that make direct synaptic contact with A9 and A10 dopaminergic cell bodies. Thus, it is possible that blockade of 5-HT_{2C} receptors in the VTA by SB242084 reduces GABA-ergic inhibitory input to these dopaminergic neurons. This could then facilitate dopamine release induced by MDMA leading to increased locomotor activation. Experiments using in vivo microdialysis would be needed to test this possibility.

Superficially, MDMA resembles the 5-HT releaser d-fenfluramine in terms of its effect on responding for conditioned reinforcement (Fletcher 1995). It has been questioned whether 5-HT release is responsible for mediating some of the behavioural effects of fenfluramine (Callaway and Geyer 1994). However, the fact that the 5-HT receptor antagonist metergoline completely blocked the effects of fenfluramine on responding for conditioned reinforcement strongly implicates 5-HT release as an important mechanism involved in this effect. In the case of MDMA it seems highly unlikely that the reduction in responding for conditioned reinforcement results from dopamine release, since this would be expected to enhance responding (Robbins et al. 1983; Taylor and Robbins 1984). Thus, it is reasonable to postulate that 5-HT release may underlie the action of MDMA to suppress responding for conditioned reinforcement. This is further supported by results of studies using microdialysis which indicate a higher proportionate release of 5-HT compared to dopamine by MDMA (Nash and Nichols 1991; Kankaanpaa et al. 1998). As discussed below, the results of the 5-HT receptor antagonist studies also support a serotonergic-based mechanism, involving 5-HT_{2C} receptors, as underlying the effect of MDMA on responding for conditioned reinforcement.

At a dose that altered the expression of hyperactivity GR127935 failed to alter MDMA-induced suppression of responding for conditioned reinforcement, suggesting that 5-HT_{1B} receptors are not involved in mediating this effect of MDMA. Similarly, the effect of MDMA was not altered by 1 mg/kg ketanserin, a dose that fully blocks the 5-HT_{2A} receptor-mediated head-twitch response (Darmani et al. 1990), while a higher dose of ketanserin suppressed responding in its own right. Thus, 5-HT_{2A} receptors are apparently not involved in mediating the reduction in responding induced by MDMA. In a previous experiment the 5-$HT_{1A/1B}$ agonist RU24969 reduced responding for conditioned reinforcement and this effect was blocked by GR127935 but not the 5-HT_{1A} receptor antagonist WAY100935 (Fletcher and Korth 1999a). Therefore the lack of effect of GR127935 on the MDMA-induced disruption of responding for conditioned reinforcement is surprising. Differences in the pharmacological actions of RU24969 and MDMA could account for the discrepant effects of GR127935. In the case of the receptor agonist RU24969 a restricted action localised to the 5-HT_{1B} receptor would be expected. Following treatment with MDMA, released 5-HT would have the potential to interact with multiple 5-HT receptors. Pretreatment with the selective 5-HT_{1B} antagonist would obviously prevent 5-HT from activating this receptor but would not adversely impact the ability of 5-HT to act through its other receptor sub-types. Thus, it is possible that an action on any of these other 5-HT receptor sub-types could still mediate the suppressant effect of MDMA on responding for conditioned reinforcement.

One such 5-HT receptor sub-type involved in mediating this effect of MDMA is the 5-HT_{2C} receptor since SB242084 reversed the response suppressant effect of MDMA. Further evidence demonstrating the importance of 5-HT_{2C} receptors is the finding that the 5-HT_{2C} agonist Ro60-0175 reduced this behaviour, an effect that was also reversed by SB242084. Again, as outlined above, 5-HT_{2C} receptor-mediated inhibition of mesolimbic dopamine function could be one mechanism involved in disrupting responding for conditioned reinforcement induced by Ro60-0175. The disruption of responding for conditioned reinforcement by DA receptor antagonists (Cador et al. 1991; Fletcher and Higgins 1997) gives this potential mechanism additional plausibility. However, it is difficult to conclude that 5-HT_{2C} mediated inhibition of dopamine is solely responsible for the effect of

MDMA given that MDMA clearly enhances dopamine release in the absence of 5-HT_{2C} receptor blockade (Kankaanpaa et al. 1998; Nash and Nichols 1991). Thus, an action of 5-HT, mediated by 5-HT_{2C} receptors, that is independent of reduced dopamine function could also contribute to the reduction of responding for conditioned reinforcement induced by MDMA.

Amphetamine reliably potentiates responding for conditioned reinforcement by increasing dopamine release (Taylor and Robbins 1984). Given the dopamine releasing effects of MDMA it is reasonable to question why SB242084 only leads to a restoration of baseline responding in MDMA-treated rats, and not to a potentiation of responding. At least two possible explanations could be advanced to address this question. Firstly, it may be that MDMA, in the presence of SB242084, does not produce a sufficient increase in dopamine levels to enhance this behaviour. Such an explanation has been used to account for the failure of morphine, which also indirectly increases extracellular levels of DA, to increase responding for conditioned reinforcement (Robbins et al. 1983; Cunningham and Kelley 1992). The second possibility is that in MDMA-treated rats 5-HT_{2C} receptor blockade unmasks an inhibitory role of 5-HT_{1B} receptors that serves to dampen the influence of elevated dopamine function. In support of this possibility, the selective 5-HT_{1B} agonist CP93,129 blocks the potentiating effect of amphetamine on responding for conditioned reinforcement without significantly altering basal levels of responding for conditioned reinforcement (Fletcher and Korth 1999b). Whatever the explanation, it is apparent that at least as far as responding for conditioned reinforcement is concerned removal of an inhibitory influence of 5-HT_{2C} receptors is not sufficient to reveal a significant, or selective, dopaminergic action for MDMA in altering responding for conditioned reinforcement.

Stimulation of 5-HT_{2C} receptors reduces responding for a variety of reinforcers including food, cocaine (Grottick et al. 2000), and nicotine (Grottick et al. 2001). The present results now show that 5-HT_{2C} receptor activation, as induced directly by a 5-HT_{2C} receptor agonist or indirectly via MDMA-induced release of 5-HT, also attenuates responding for a conditioned reward. It is apparent then that 5-HT_{2C} receptor stimulation alters a variety of reinforced behaviours. It seems unlikely that this is due to simple motor impairment since in tests of food- and cocaine- maintained responding rats treated with Ro60-0175 were capable of responding far in excess of responding observed in the present test of conditioned reinforcement (Grottick et al. 2000). Additionally, 5-HT_{2C} receptor stimulation reduces consummatory behaviours, including feeding (Clifton et al. 2000) and ethanol intake (Tomkins et al. 2002). One implication of this body of work is that the 5-HT_{2C} receptor appears to exert a wide-ranging inhibition of motivational processes in general.

In summary, treatment with MDMA induces locomotor activation that is mediated in part by 5-HT_{1B} and 5-HT_{2A} receptors; at the same time the expression of this response is seemingly restrained by 5-HT_{2C} receptor activation. MDMA reduces responding for a conditioned reinforcer primarily via an inhibitory action on 5-HT_{2C} receptors, which may be reflective of a generalised action on motivational systems. Removal of this inhibitory action is not, however, sufficient to reveal a prominent dopaminergic action of MDMA, perhaps because of residual actions on 5-HT_{1B} receptors. Overall, the results indicate that MDMA has a broad spectrum of behavioural effects and that multiple 5-HT receptors contribute to the expression of these effects in both an excitatory and inhibitory fashion.

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