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Microdialysis Studies on 3,4-Methylenedioxymethamphetamine-Induced Dopamine Release: Effect of Dopamine Uptake Inhibitors[1]

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ABSTRACT

The effect of dopamine (DA) and serotonin (5-HT) uptake inhibitors on 3,4-methylenedioxymethamphetamine (MDMA)-induced increase in DA efflux was studied using *in vivo* microdialysis. MDMA was infused directly into the anterolateral striatum *via* the dialysis probe. The local administration of MDMA produced a dose- and time-dependent increase in the extracellular concentration of DA in the striatum. Peripheral administration of the DA uptake blockers, mazindol (5 mg/kg, i.p.) or GBR 12909 (10 mg/kg, i.p.), produced a slight but significant increase in the extracellular concentration of DA. Moreover, pretreatment with either mazindol or GBR 12909 30 min before the infusion of MDMA (10 μM) significantly attenuated the MDMA-induced increase in the extracellular concentration of DA. Pretreatment with fluoxetine (10 mg/kg, i.p.), a 5-HT uptake blocker, 30 min before the infusion of MDMA produced a slight but significant inhibition of MDMA-induced increase in DA concentration. In contrast, pretreatment with the 5-$HT_{2/1C}$ antagonist, ketanserin (3 mg/kg, i.p.), had no significant effect on the increase in DA concentration produced by the local administration of MDMA. These data are suggestive that MDMA increases the concentration of DA in the striatum, in part, *via* a carrier-mediated mechanism which is largely independent of its effects on 5-HT release.

It is well documented that single or repeated high-dose administration of MDMA and related compounds produce long-lasting decreases in serotonergic markers in the brain of rodents and nonhuman primates (for review see McKenna and Peroutka, 1990). The evidence supporting this observation includes decreases in the following parameters: 1) the concentrations of 5-HT and its major metabolite, 5-HIAA, in several forebrain regions (Stone *et al.*, 1986; Schmidt, 1987); 2) the activity of tryptophan hydroxylase the rate-limiting enzyme in the synthesis of 5-HT (Stone *et al.*, 1986); 3) the number of 5-HT uptake sites as estimated by [^{3}H]paroxetine binding (Battaglia *et al.*, 1987, 1988); and 4) the density of fine axon terminals as determined by immunocytochemical studies (O'Hearn *et al.*, 1988). Collectively, these results are suggestive that administration of MDMA selectively damages 5-HT axon terminals in the brain.

A large number of studies have investigated the neurochemical events which occur after MDMA administration. However, only a few studies have examined the mechanism(s) by which MDMA damages 5-HT axon terminals. On the basis of earlier work using methamphetamine (Schmidt *et al.*, 1985), Stone and colleagues (1988) demonstrated that the ability of MDMA to produce long-lasting changes in 5-HT content and tryptophan hydroxylase activity was mediated, in part, *via* dopaminergic mechanisms. That is, depletion of brain DA content with either α-methylparatyrosine or reserpine significantly attenuated the long-term depleting effects of MDMA on brain 5-HT and 5-HIAA concentrations. In addition, lesions of the substantia nigra with 6-OH-DA blocked the neurotoxic effects of MDMA in the striatum. Moreover, Schmidt *et al.* (1990b) reported that 6-OH-DA lesions of the substantia nigra not only attenuate the 5-HT-depleting effects of MDMA in the striatum, but also in other forebrain regions such as the frontal cortex and hippocampus, which are believed to contain a much lower concentration of DA. These results are supportive of the view that DA plays a role in the long-term neurotoxic effects of MDMA.

All of the treatment effects described above deplete brain tissue DA concentrations. However, Stone *et al.* (1988) reported that pretreatment with the DA uptake inhibitor, GBR 12909

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ABBREVIATIONS: 6-OH-DA, 6-hydroxydopamine; MDMA, 3,4-methylenedioxymethamphetamine; DA, dopamine; 5-HT, serotonin; HPLC, high performance liquid chromatography; GBR 12909, 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine; 5-HIAA, 5-hydroxyindoleacetic acid; ANOVA, analysis of variance; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid.

blocked MDMA-induced decreases in 5-HT and 5-HIAA content as well as tryptophan hydroxylase activity in the striatum. Because GBR 12909 does not deplete brain DA content but does protect against MDMA-induced neurotoxicity, these data are suggestive that DA tissue depletion is not necessary for protection against the long-term neurotoxic effects of MDMA. It is not known exactly how DA uptake blockade inhibits MDMA-induced 5-HT depletion.

Both *in vitro* and *in vivo* studies have found that MDMA increases DA efflux. For example, MDMA and structurally related compounds produce a concentration-dependent increase in the release of [^{3}H]DA from striatal slices (Johnson *et al.*, 1986; Schmidt *et al.*, 1987). In addition, MDMA inhibits [^{3}H]DA uptake into striatal synaptosomes (Steele *et al.*, 1987). Schechter (1986) reported that MDMA may be acting as an indirect DA agonist based on drug discrimination studies. Although post-mortem neurochemical studies revealed slight increases in the concentration of DA following the acute administration of MDMA (Stone *et al.*, 1987), the results obtained by Yamamoto and Spanos (1988) were the first to provide direct evidence that MDMA administration increases dopaminergic neurotransmission in the striatum and nucleus accumbens as measured by *in vivo* voltametry. These results were confirmed and extended using *in vivo* microdialysis (Hiramatsu and Cho, 1990; Nash, 1990). Thus, peripheral administration of MDMA produces a dose-dependent increase in the extracellular concentration of DA in the brain.

Support for the hypothesis that MDMA-induced increase in DA activity and, in particular, release is involved in long-term 5-HT depletion has been generated in our laboratory. Initially, we reported that pretreatment with the 5-HT$_2$ antagonist, ketanserin, significantly inhibited MDMA-induced 5-HT depletion as well as the decrease in the number of 5-HT uptake sites (Nash *et al.*, 1990, 1991). Moreover, pretreatment with ketanserin significantly attenuated MDMA-induced activation of tyrosine hydroxylase (Nash *et al.*, 1990) and the increase in extracellular DA concentration in the striatum as measured by *in vivo* microdialysis (Nash, 1990). These results have been confirmed and extended using more selective 5-HT$_2$ antagonists (Schmidt *et al.*, 1990a). These data are also supportive of the hypothesis that DA is involved in the 5-HT-depleting effects of MDMA.

Given the hypothetical role of DA in the 5-HT neurotoxic effect of MDMA, the present studies were designed to investigate the mechanism by which local administration of MDMA increases the release of DA from terminal regions within the anterolateral striatum using a pharmacological approach. MDMA was administered directly into the striatum *via* the microdialysis probe and the concentration of DA in the dialysate was determined. The ability of the DA uptake blockers, mazindol and GBR 12909, to inhibit MDMA-induced DA release was determined. In addition, the role of 5-HT in this response to the localized infusion of MDMA was determined.

Materials and Methods

Animals. Male Sprague-Dawley rats weighing 225 to 300 g were purchased from Zivic Miller Laboratory (Allison Park, PA) and used in all experiments. Animals were housed two per cage in a temperature-controlled room (23°C) with a 12/12 hr lighting schedule (lights on at 6:00 A.M.). Food and water were available *ad libitum*.

Drugs. The racemic mixture of MDMA hydrochloride was generously provided by the National Institute on Drug Abuse (Rockville, MD). Chloral hydrate and the HPLC standards were purchased from Sigma Chemical Co. (St. Louis, MO). GBR 12909 HCl was purchased from Research Biochemicals Inc. (Natick, MA). The following drugs were generously provided by the manufacturers: ketanserin tartrate (Janssen Pharmaceutica, Beerse, Belgium), fluoxetine HCl (Eli Lilly Co., Indianapolis, IN) and mazindol (Sandoz, East Hanover, NJ). MDMA was dissolved in a modified Ringer's solution (see experimental procedure) in concentrations ranging from 1 to 10,000 μM of the free base and administered *via* the dialysis probe. Mazindol and GBR 12909 were dissolved in 0.1% acetic acid and administered i.p. Ketanserin was dissolved in 0.01 M tartaric acid and fluoxetine was dissolved in saline. Both compounds were administered i.p.

Experimental procedure. Under chloral hydrate (400 mg/kg, i.v.) anesthesia, each rat was placed in a stereotaxic frame. A loop-shaped dialysis probe (Nash, 1990) was implanted into the anterolateral striatum (A: 1.0, L: 3.5, V: −6.5, from bregma) according to the atlas of Paxinos and Watson (1982). The probe was secured using a skull screw and dental cement and flushed with a modified Ringer's solution (NaCl 145 mM, Na_2PO_4 6 mM, KCl 1.7 mM, KH_2PO_4 1.0 mM, $CaCl_2$ 1.2 mM at pH 7.4). The animals were placed in clear plastic cages with food and water available *ad libitum*.

On the following day, the dialysis probe was connected to a zero dead-volume liquid switch which was attached to an infusion pump set to deliver Ringer's solution at a rate of 1.7 μl/min. Base-line samples were collected every 30 min over a 2- to 3-hr time period. MDMA (1–10,000 μM) was diluted in Ringer's solution and infused for 30 min after the last base-line sample. Dialysate samples were collected for 2 hr every 30 min after the termination of MDMA infusion. In the drug interaction studies, MDMA (10 μM) was infused for 30 min after the last base-line sample and again 90 min later. GBR 12909 (10 mg/kg), mazindol (5 mg/kg), fluoxetine (10 mg/kg), ketanserin (3 mg/kg) or saline was administered i.p. 30 min before the second infusion of MDMA. The dose of each drug was selected on the basis of preliminary results and previously published studies (Heikkila *et al.*, 1977; Schmidt 1987; Stone *et al.*, 1988; Nash, 1990). Dialysate samples were collected for 90 min after the second infusion of MDMA. At the end of the study, each rat was sacrificed by decapitation and the brain removed from the skull. The location of the dialysis probe was confirmed in each rat.

Dialysis probes. A loop-shaped dialysis probe was constructed as previously described (Nash, 1990). The dialysis membrane extended 2.5 mm beyond the tip of the 22-gauge cannula. The dead volume of the probes was calculated. The percent recovery of DA, DOPAC and HVA using these dialysis probes was 8, 15 and 17%, respectively, at a flow rate of 1.7 μl/min at room temperature (23°C).

Biochemical measurements. The concentrations of DA, DOPAC and HVA were determined in dialysate samples using previously reported HPLC procedures (Nash, 1990; Nash and Nichols, 1991). Briefly, each sample (50 μl) was injected onto a 3-micron C18 column (Phenomenex; Rancho Palos Verdes, CA) connected to an LC-4B amperometric detector (Bioanalytical Systems, W. Lafayette, IN) equipped with a glassy carbon electrode set at a potential of 0.65 V relative to the Ag/AgCl reference electrode. A Hewlett-Packard (HP 3396A) integrator was used to quantitate the concentrations of DA and its metabolites in the dialysate samples.

Statistical analysis. The data were analyzed using paired *t* tests and repeated measures ANOVA as indicated. In general, the increase in DA efflux produced by the first infusion of MDMA was compared to the increase in DA concentration produced by the second infusion of MDMA using a paired *t* test. Differences between treatment groups were analyzed using a one-way ANOVA. Comparisons between individual means were done by Scheffe's *post hoc* test. In all cases, treatment effects were considered statistically significant at $P < .05$.

Results

The effect of the local infusion of MDMA on the extracellular concentration of DA is illustrated in figure 1, A and B. MDMA produced a concentration-dependent increase in DA efflux in

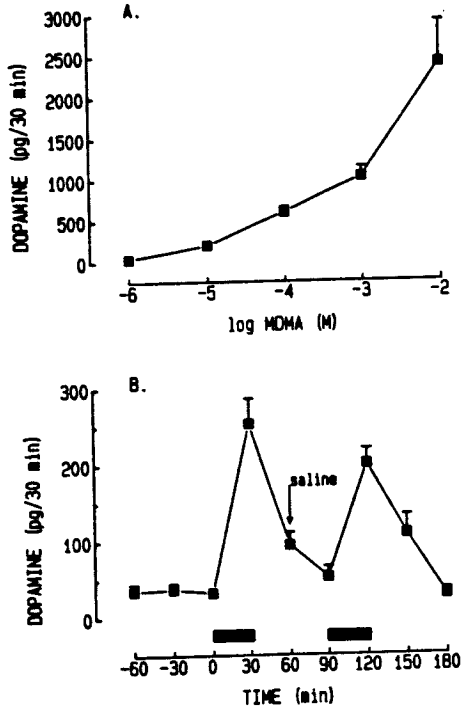

Fig. 1. Effect of different concentrations (A) or repeated administration (B) of MDMA on the extracellular concentration of DA in the anterolateral striatum. In figure 1A, MDMA (1–10,000 μM) was infused directly into the ventrolateral striatum for 30 min. Each value is the maximum increase (pg/30 min) in the DA concentration obtained during drug infusion. The values are the mean ± S.E. of three to four rats. In 1B, MDMA (10 μM) was infused directly into the striatum *via* the dialysis probe for 30 min at the 0- to 30-min and 90- to 120-min time points as indicated by the black bar. Each value is the mean ± S.E. of 10 rats. The extracellular concentration of DA was increased significantly ($P < .001$) during both 30-min periods of drug infusion. The DA concentration returned to base-line levels within 60 min after cessation of drug administration. Saline was administered 30 min before the second infusion of MDMA.

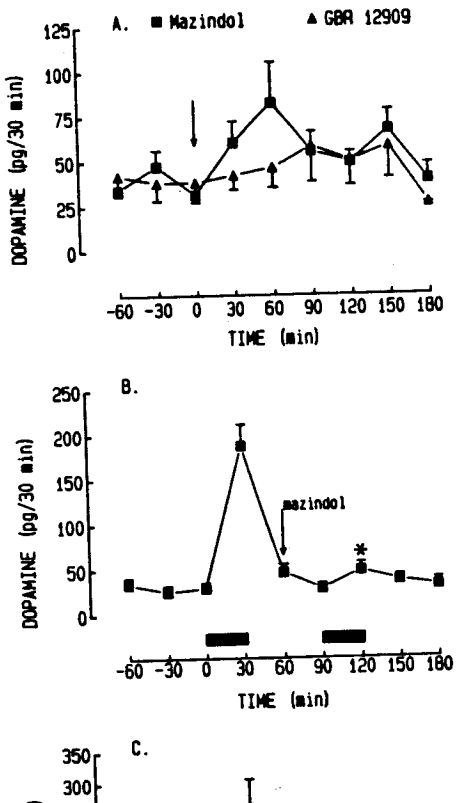

Fig. 2. Effect of mazindol and GBR 12909, alone, and before the infusion of MDMA on the extracellular concentration of DA in the striatum. Each value represents the mean ± S.E. of five rats. Mazindol (5 mg/kg) or GBR 12909 (10 mg/kg) were administered at time = 0 min as indicated by the arrow (A). In B and C, MDMA (10 μM) was infused directly into the striatum *via* the dialysis probe for 30 min at the 0- to 30 min and 90- to 120 min time points as indicated by the solid black bar. Mazindol (5 mg/kg) or GBR 12909 (10 mg/kg) were administered 30 min before the infusion of MDMA as indicated by the arrow (B and C, respectively). The asterisk indicates a significant ($P < .02$) decrease as compared to the first infusion of MDMA.

the anterolateral striatum during the 30 min of drug infusion (fig. 1A). The administration of 1 μM MDMA had no effect on the extracellular concentration of DA; however, after the infusion of 10 μM MDMA, the extracellular concentration of DA was increased significantly ($P < .01$) 5 to 7 times the base-line concentration (43 ± 9 pg/30 min). This response was consistent and approximated the effect on the extracellular concentration of DA produced by the peripheral (i.p.) administration of MDMA 10 mg/kg (Hiramatsu and Cho, 1990; Nash, 1990). After termination of the drug infusion, DA concentrations returned to base-line levels within 60 min. The local infusion of MDMA produced a slight decrease in the extracellular concentrations of DOPAC and HVA (data not shown).

The effect of repeated infusion of MDMA (10 μM) on the extracellular concentration of DA in the anterolateral striatum is illustrated in figure 1B. MDMA was infused into the striatum *via* the dialysis probe at 0 to 30 min and again at 90 to 120 min. The extracellular concentration of DA was increased significantly ($P < .01$) during both time periods. There was no significant difference between the first and second infusion with respect to the increase in extracellular DA concentrations. The concentration of DA returned to base-line levels within 60 min after termination of the infusion of MDMA into the probe.

The effect of the DA uptake inhibitors, mazindol and GBR 12909, on MDMA-induced DA release is illustrated in figure 2, A, B and C. Pretreatment with mazindol (5 mg/kg) 30 min before the infusion of MDMA (10 μM) significantly ($P < .02$) attenuated MDMA-induced DA release as presented in figure 2B. Similarly, pretreatment with GBR 12909 (10 mg/kg) significantly ($P < .03$) inhibited the increase in the extracellular concentration of DA produced by the local administration of MDMA (10 μM) as illustrated in figure 2C. Both mazindol and, to a lesser extent, GBR 12909 produced a significant ($P < .05$) increase in the extracellular concentration of DA as compared to the predrug base-line DA concentration (fig. 2A).

The effect of the 5-HT uptake inhibitor, fluoxetine (10 mg/kg), on the extracellular concentration of DA is presented in figure 3A. Fluoxetine had no effect on the extracellular concentration of DA following administration (data not shown). Pre-

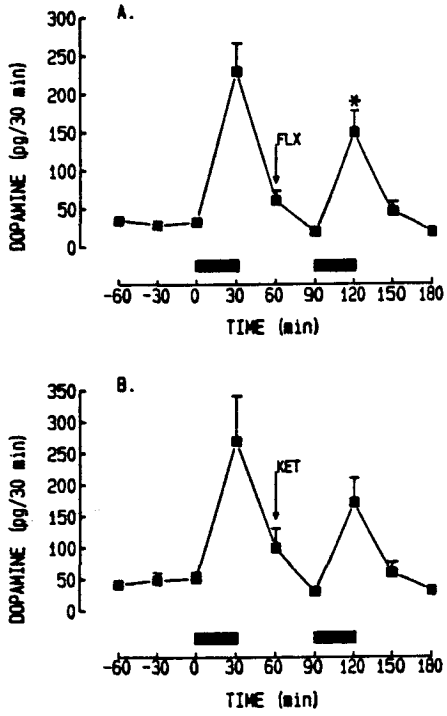

Fig. 3. Effect of fluoxetine (A) or ketanserin (B) on MDMA-induced increase in the extracellular concentration of DA in the striatum. Each value represents the mean ± S.E. of five rats. MDMA (10 μM) was infused directly into the striatum *via* the dialysis probe for 30 min at the times indicated by the solid black bar. Fluoxetine (10 mg/kg) or ketanserin (3 mg/kg) was injected 30 min before the second infusion of MDMA as indicated by the arrow (A and B, respectively). The asterisk represents a significant ($P < .05$) decrease as compared to the first infusion of MDMA.

treatment with fluoxetine produced a slight but significant ($P < .05$) inhibition of the MDMA-induced increase in extracellular DA concentration compared to the first infusion of MDMA (fig. 3A).

Finally, the effect of ketanserin (3 mg/kg), the 5-$HT_{2/1C}$ antagonist, on the extracellular concentration of DA after the local infusion of MDMA is illustrated in figure 3B. Pretreatment with ketanserin did not significantly ($P < .1$) decrease the MDMA-induced increase in the extracellular concentration of DA. Consistent with previous studies (Nash, 1990), ketanserin (3 mg/kg) had no significant effect on the extracellular concentration of DA (data not shown).

For clarity and comparison, these data are summarized in table 1. After the first infusion of MDMA (10 μM), there was no significant difference between the treatment groups with respect to the increase in extracellular DA concentration produced by MDMA administration as evaluated using a one-way ANOVA. The second infusion of MDMA (10 μM) was inhibited significantly by mazindol, GBR 12909 and, to a lesser extent, fluoxetine, compared to the saline + MDMA treatment group. Mazindol and GBR 12909 produced a significantly ($P < .05$) greater inhibition of MDMA-induced DA release as compared to fluoxetine.

Discussion

The use of microdialysis techniques to study the neurochemical properties of drugs such as MDMA has several unique advantages. The drug can be applied directly to the site of action which eliminates pharmacokinetic effects as well as the effect on other brain regions. For example, in the present study, MDMA was infused directly into the striatum, greatly reducing its direct effects on DA cell bodies located in the midbrain or other neurotransmitter systems located outside the anterolateral striatum (*i.e.*, dorsal raphe, locus coeruleus, *etc.*). In addition, the repeated infusion of MDMA in the same animal reduces interanimal variability. The present study utilized this approach in an attempt to further understand the effect of MDMA on dopaminergic systems in the brain.

TABLE 1

Effects of pharmacological manipulations on the increase in the extracellular concentration of DA produced by the local infusion of MDMA (10 μM) into the striatum[a]

Treatment	DA	% Decrease
	pg/30 min	
MDMA	253 ± 33	15 ± 16
Saline + MDMA	200 ± 20	
MDMA	202 ± 28	74 ± 10
Mazindol + MDMA	48 ± 9**	
MDMA	259 ± 46	58 ± 12
GBR 12909 + MDMA	108 ± 24**	
MDMA	229 ± 37	36 ± 5
Fluoxetine + MDMA	149 ± 28*	
MDMA	269 ± 57	33 ± 8
Ketanserin + MDMA	171 ± 39	

[a] Each value represents the mean ± S.E. of 4 to 10 rats. The first infusion of MDMA (10 μM) was followed by a second infusion 90 min later. The drugs were administered 30 min before the second infusion of MDMA.
* Significant ($P < .05$) decrease compared to control value.
** Significant ($P < .01$) decrease compared to control value.

MDMA produced a dose-dependent increase in the extracellular concentration of DA when infused into the striatum *via* the dialysis probe as illustrated in figure 1. Similar results have been obtained after the local infusion of amphetamine into the striatum. For example, Nomikos *et al.* (1990) found that the local infusion of *d*-amphetamine (1–1000 μM) produced a dose-dependent increase in the concentration of DA in the striatum. The mechanism by which amphetamine increases the release of DA has been studied extensively using *in vitro* and *in vivo* techniques. In striatal synaptosomes, amphetamine has been shown to increase the release of DA by a facilitated exchange mechanism which utilizes the DA uptake carrier (Raiteri *et al.*, 1979; Fischer and Cho, 1979; Liang and Rutledge, 1982). Recent *in vivo* dialysis studies have found that amphetamine increases the release of DA which is completely blocked by the DA uptake inhibitor, nomifensine (Westerink *et al.*, 1987b; Butcher *et al.*, 1988; Hurd and Ungerstedt, 1989). Given the structural similarities between MDMA and amphetamine coupled with the ability of DA uptake inhibitors to attenuate their effects on DA release, it is reasonable to suggest that the local administration of MDMA also increases the release of DA *via* a facilitated exchange mechanism as discussed below.

Pretreatment with mazindol or GBR 12909 significantly inhibited the increase in the extracellular concentration of DA produced by the local administration of MDMA. Mazindol has been reported to be a potent inhibitor of both norepinephrine and DA uptake (Koe, 1976; Fuller *et al.*, 1978; Sugrue *et al.*, 1977). In addition, mazindol is also a weak 5-HT uptake inhibitor (Heikkila *et al.*, 1977). GBR 12909, on the other hand, is a selective inhibitor of DA uptake and has very little affinity for 5-HT or norepinephrine uptake sites (Heikkila and Manzino,

1984; Andersen, 1987). The ability of either mazindol or GBR 12909 to significantly inhibit MDMA-induced increase in the extracellular concentration of DA is supportive of the hypothesis that MDMA releases DA *via* its interaction with the DA uptake carrier located on axon terminals in the anterolateral striatum. It is unlikely that the DA uptake blockers inhibit MDMA transport into the axon terminal, resulting in an inhibition of its effect because MDMA is relatively lipid soluble and has been reported to enter striatal synaptosomes through passive diffusion (Zaczek *et al.*, 1990).

Both mazindol and GBR 12909 produced a slight but significant increase in the extracellular concentration of DA (fig. 2). Previous studies have found that the peripheral administration of GBR 12909 produced a similar increase in DA concentrations in the striatum (Westerink *et al.*, 1987a). The increase in DA concentrations observed after the administration of mazindol is further evidence that DA uptake blockers produce small increases in the extracellular concentration of DA. The slight increase in DA concentration produced by the second infusion of MDMA (fig. 2C) in the GBR 12909-pretreated rats is most likely the result of an incomplete inhibition of the effect of MDMA on DA release. Full dose-response studies are needed to clarify this relationship as well as the potency of the uptake inhibitors used in this study.

The peripheral administration of mazindol has been reported to block the 5-HT-depleting effects of *p*-chloromethamphetamine (Carruba *et al.*, 1977) and *p*-chloroamphetamine (Fuller *et al.*, 1978). More recently, Stone *et al.* (1988) have found that the administration of GBR 12909 blocked MDMA-induced 5-HT depletion. The results obtained in the present study provide direct evidence that DA uptake blockers such as mazindol and GBR 12909 inhibit MDMA-induced DA release *in vivo*. Therefore, it is possible that the ability of DA uptake blockers to attenuate the long-term depletion of 5-HT produced by the administration of MDMA is the result of their inhibition of DA release. This explanation is consistent with the hypothesis that excessive DA release is involved in the 5-HT neurotoxic effects of MDMA.

Fluoxetine, the specific 5-HT uptake inhibitor, has been found to inhibit the short-term (3–6 h) reduction in 5-HT content as well as the long-term (>7 days) 5-HT neurotoxic effects of MDMA (Schmidt, 1987). The mechanism(s) responsible for this effect are not entirely clear. Based on *in vitro* and *in vivo* studies, it has been suggested that fluoxetine may block the uptake of MDMA into 5-HT axon terminals, the carrier-mediated release of 5-HT or both (Schmidt, 1987; Hekmatpanah and Peroutka, 1990; Bradberry *et al.*, 1990). Alternatively, fluoxetine has been found to block the uptake of DA into striatal synaptosomes (Schmidt and Lovenberg, 1985). In the present studies, pretreatment with fluoxetine produced a slight but significant ($P < .05$) decrease in MDMA-induced release of DA in the striatum. It is possible that fluoxetine administration blocks the increase in 5-HT release produced by the local infusion of MDMA, which might otherwise stimulate the release of DA. However, this interaction seems unlikely based on a large body of evidence which is supportive of the hypothesis that 5-HT inhibits, rather than stimulates, DA release from terminal regions in the striatum (for review see Soubrie *et al.*, 1984). Regardless, it is unlikely that the small inhibitory effect of fluoxetine on the local release of DA produced by the local infusion of MDMA is the mechanism by which 5-HT uptake inhibitors block the long-term 5-HT neurotoxic effects of MDMA.

As stated in the introduction, previous studies in this laboratory have found that the 5-$HT_{2/1C}$ antagonist, ketanserin, inhibits long-term decreases in 5-HT and 5-HIAA content as well as [^{3}H]paroxetine binding after a single administration of MDMA (Nash *et al.*, 1990, 1991). In addition, ketanserin pretreatment significantly attenuated the increase in the extracellular concentration of DA in the striatum as measured by *in vivo* microdialysis after the peripheral administration of MDMA (Nash, 1990). Therefore, it was of interest to determine the effect of ketanserin on the release of DA produced by the local administration of MDMA. In the present study, pretreatment with ketanserin had a slight but nonsignificant effect on the increase in DA produced by the local infusion of MDMA into the striatum *via* the dialysis probe. These data are suggestive that the site(s) at which ketanserin inhibits MDMA-induced DA release are not located in the striatum. Thus, when MDMA is administered peripherally, it must interact with ketanserin-sensitive sites located outside of the terminal regions within the striatum to increase DA efflux. These "sites" may be 5-HT_2 or 5-HT_{1C} receptors or, perhaps, the recently described nonserotonergic ketanserin binding sites (Leysen *et al.*, 1987; Dewar *et al.*, 1990). Although further studies are needed to clarify this point, the results obtained in the present studies are suggestive that the interaction between MDMA and ketanserin is not in the striatal terminal regions, where the nonserotonergic ketanserin binding sites are located (Leysen *et al.*, 1988).

The mechanism by which single or repeated administration of MDMA, and related phenethylamines such as methamphetamine, damages 5-HT axon terminals remains unsolved. The most popular hypothesis, to date, suggests that the administration of high doses of MDMA produces a massive and prolonged release of DA which may be taken up into 5-HT axon terminals and oxidized to form 6-hydroxydopamine (Marek *et al.*, 1990), quinones, or both, resulting in long-term axon terminal damage. However, this hypothesis fails to explain why MDMA depletes 5-HT in areas of the brain other than the striatum which are not richly innervated by DA (*i.e.*, hippocampus).

In sum, the results obtained in the present studies are direct evidence that MDMA increases the release of DA from terminal regions in the anterolateral striatum *via* a carrier-mediated DA uptake site. The local effect of MDMA on the release of DA appears to be largely independent of its effect on 5-HT uptake or receptor mechanisms. The relationship between MDMA-induced DA release and its long-term 5-HT neurotoxic effects requires further study.

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