Blockade of Striatal 5-Hydroxytryptamine_2 Receptors Reduces the Increase in Extracellular Concentrations of Dopamine Produced by the Amphetamine Analogue 3,4-Methylenedioxymethamphetamine

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Abstract: 5-Hydroxytryptamine_2 (5-HT_2) receptor antagonists have been shown to interfere with the stimulation of striatal dopamine synthesis and release produced by the amphetamine analogue 3,4-methylenedioxymethamphetamine (MDMA). To localize the receptors responsible for the attenuation of MDMA-induced release, 5-HT_2 receptor antagonists were infused via the microdialysis probe directly into the brains of awake, freely moving rats before the systemic administration of MDMA. Intrastriatal infusions of the selective 5-HT_2 antagonist MDL 100,907 produced a concentration-dependent inhibition of MDMA-induced dopamine release. Similar results were observed with intrastriatal infusions of the 5-HT_3 antagonist amperozide. In contrast, infusion of MDL 100,907 into the midbrain region near the dopaminergic cell bodies was without effect on the MDMA-induced elevation of extracellular dopamine in the ipsilateral striatum. Neither antagonist attenuated basal transmitter efflux nor the MDMA-stimulated release of [3H]dopamine from striatal slices in vitro indicating that the in vivo effect of the antagonists was not due to inhibition of the dopamine uptake carrier. Intrastriatal infusion of tetrodotoxin reduced both basal and MDMA-stimulated dopamine efflux and eliminated the effect of intrastriatal MDL 100,907. The results indicate that 5-HT_2 receptors located in the striatum augment the release of dopamine produced by high doses of MDMA. Furthermore, these 5-HT_2 receptors appear to be located on nondopaminergic elements of the striatum. Key Words: Amphetamine—Dopamine—3,4-Methylenedioxymethamphetamine—Microdialysis—Serotonin_2 receptors. J. Neurochem. 62, 1382–1389 (1994).

Interactions between the serotonergic and dopaminergic systems of the brain have been described in numerous investigations. Several recent studies have focused specifically on the role of 5-hydroxytryptamine_2 (5-HT_2) receptors in the regulation of forebrain dopaminergic function and the potential contribution of 5-HT_2 receptor blockade to antipsychotic activity (Goldstein et al., 1989; Meltzer et al., 1989; Ugedo et al., 1989; Saller et al., 1990; Gudelsky et al., 1992; Ichikawa and Meltzer, 1992; Rivest and Marsden, 1992; Sorensen et al., 1992). The amphetamine analogue 3,4-methylenedioxymethamphetamine (MDMA) has proved to be a useful tool in the study of this interaction. MDMA is a potent releaser of both 5-HT and dopamine, acutely producing wide-ranging and often dramatic neurochemical effects on both transmitter systems (Johnson et al., 1986; Schmidt et al., 1987). In addition to its effects on release, administration of MDMA to rats produces acute increases in striatal dopamine synthesis (Nash et al., 1990) and a resultant increase in tissue concentration of the transmitter (Schmidt et al., 1986). In contrast to the changes in the dopaminergic system, terminal field concentrations of 5-HT fall precipitously after MDMA due to a rapid inactivation of tryptophan hydroxylase and a simultaneous increase in 5-HT efflux (Stone et al., 1986, 1987; Schmidt and Taylor, 1987). The relationship between the release of 5-HT by MDMA and its effects on the dopaminergic system was first clarified in a study by Nash et al. (1990) demonstrating that the stimulation of dopamine synthesis produced by MDMA was dependent on 5-HT_2 receptor activation. In this study, pretreatment with the 5-HT_2/C antagonist ketanserin prevented MDMA-stimulated dihydroxyphenylalanine (DOPA) accumulation in rats treated with the L-aromatic amino acid decarboxylase inhibitor, NSD 1015. In a subsequent microdialysis study, ketanserin also was shown to attenuate MDMA-induced dopamine release in vivo (Nash, 1990).
We have used the potent 5-HT<sub>2</sub> receptor antagonist MDL 100,907 to further extend these findings. MDL 100,907 is an α-phenyl-4-piperidinemethanol with a 300-fold preference for binding at the 5-HT<sub>2</sub> receptor over closely related sites such as the 5-HT<sub>1c</sub>, α<sub>1</sub>-adrenergic, or D<sub>2</sub> dopamine receptor (Dudley et al., 1990). In vivo, MDL 100,907 is a potent antagonist of both MDMA-stimulated dopamine synthesis and release (Schmidt et al., 1992). The activity of MDL 100,907 against both responses to MDMA specifically implicates the 5-HT<sub>2</sub> subtype as the 5-HT receptor responsible for this phenomenon. In related studies, MDL 100,907 was also shown to be a potent antagonist of both the amphetamine-induced decrease in A<sub>d</sub> dopaminergic neuron firing and of amphetamine-stimulated locomotor activity, suggesting that the drug has potential antipsychotic activity (Sorenson et al., 1993).

The diphenylbutylpiperazine amperozide is another selective 5-HT<sub>2</sub> antagonist reported to have potential as an antipsychotic agent. Like MDL 100,907, amperozide reduces locomotor activity produced by amphetamine (Gustafsson and Christensson, 1990) as well as cocaine (Kimura et al., 1993). An important element in defining the mechanism responsible for the attenuation of MDMA-stimulated dopamine synthesis and release by 5-HT<sub>2</sub> antagonists is the location of the specific 5-HT<sub>2</sub> receptors involved in this effect. In the present study we have attempted to answer this question by examining the effects of central infusions of 5-HT<sub>2</sub> antagonists on MDMA-induced dopamine release in vivo.

**MATERIALS AND METHODS**

**Microdialysis**

Microdialysis probes were made based on a concentric design described previously (Schmidt et al., 1992). Most of the experiments were performed using Spectra/Por hollow-fiber membranes to prepare either a 4-mm (striatum) or 2-mm (nigral) length of sealed dialysis tubing at the tip of the probe. The last set of experiments involving tetrodotoxin (TTX) infusions used probes made with AN 69-HOSPAL membranes (CGH Medical Inc., Lakewood, CO, U.S.A.). The larger diameter of these fibers required substituting 25-gauge stainless steel tubing (Small Parts, Inc., Miami Lakes, FL, U.S.A.) for the 26-gauge tubing used previously.

All procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the in-house animal care committee. Probes were implanted stereotaxically in the right hemisphere of Sprague-Dawley rats (250–300 g) under pentobarbital anesthesia. Coordinates relative to bregma were 1 (A/P), 2.5 (L/M), and −8.0 (V/D) mm from the top of the skull for implantation into the striatum and −4.8 (A/P), 1.5 (L/M), −7.8 (V/D) mm for midbrain probes (Paxinos and Watson, 1986). All coordinates were verified by prior dye injection studies. For the midbrain implantations, these coordinates placed the tip of the microdialysis probe at the dorsal surface of the substantia nigra zona compacta. A small machine screw was also partially inserted into the skull to serve as an anchor for each probe. Dental acrylic was used to secure the probe to the screw and the skull.

All experiments were performed 1 day after probe implantation. Each implanted rat was tethered in a circular 12-inch Plexiglas container within a lighted and soundproof isolation chamber. The chambers were ventilated by means of a small baffled fan. A two-channel liquid swivel was used to connect both inlet and outlet lines to the probe. Four-channel liquid swivels were used for studies in which two probes were simultaneously perfused. A Harvard 22 pump (Harvard Apparatus, Inc., South Natick, MA, U.S.A.) was used to perfuse the probe at 2 µl/min with artificial CSF containing (mM): NaCl 147.0, KCl 4.0, CaCl<sub>2</sub> 2.3, and MgCl<sub>2</sub> 0.9, pH 6.3 (Kuczenski and Segal, 1989). The dialysate was collected into tubes containing 10 µl of 0.5 M perchloric acid using a refrigerated microfraction collector (Carnegie Medicin, Stockholm, Sweden). The probes were perfused for a period of 30 min before sample collection. A collection period of 20 min was used for all experiments.

MDMA-HCl was administered in saline as the free base. MDL 100,907 was initially dissolved in dimethyl sulfoxide at 10 mM before dilution in artificial CSF. Amperozide and TTX were dissolved directly in artificial CSF. Infusions of the 5-HT<sub>2</sub> antagonists were timed to allow 50 min of exposure to the drug before MDMA administration. For the TTX experiments, both MDL 100,907 and TTX were infused 110 min before MDMA administration. Dialysis was discontinued 4 h after MDMA administration and samples were frozen at −70°C until assayed. All animals were killed 1 week later and the site of probe placement was verified visually.

Dopamine, dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were determined by HPLC with electrochemical detection. Thirty-two microliters of the dialysate was injected directly onto a 3-mm × 8-cm Pecosphere C18 column (Perkin-Elmer, Norwalk, CT, U.S.A.). The column was eluted with a mobile phase containing 0.15 M NaH<sub>2</sub>PO<sub>4</sub>, 0.15 mM sodium dodecyl sulfate, 0.10 mM EDTA, and 20% methanol, at pH 3.5. The flow rate was maintained at 1.0 ml/min using a Waters 600E multisolvant delivery system (Waters Chromatography Division, Millipore Corporation, Milford, MA, U.S.A.). Detection was by means of a Waters 460 electrochemical detector set to a potential of +0.65 V. Individual components in the samples were integrated and quantitated by comparison to external standards using Waters baseline 810 chromatography software. No corrections for recovery were made.

**Calculations**

Dopamine efflux for each animal was converted to the percentage of basal release using the first 1 h of collection to calculate a mean basal value. The percent basal release at each time point was then averaged for all animals within a treatment group. The data are presented as the mean percent basal release ± SEM. Total release over the first 3 h after MDMA administration was calculated from the area under the curve (AUC<sub>300</sub>) for each animal after subtraction of the basal release. The results were averaged for each group and compared by ANOVA followed by a Student–Newman–Keuls multiple comparison test using SigmaStat (Jandel Scientific, San Rafael, CA, U.S.A.). A value of p < 0.05 was accepted as indicating a significant difference between groups.

**In vitro release studies**

All superfusion experiments were conducted using slices of rat striata preloaded with [3H]dopamine. Slices (250...
Figure 1 shows the results of experiments in which the first series of experiments (Figs. I and 3) with an

concentration of DOPAC and HVA were 2,628 ± 328 and incubated for 10 min at 37°C in Krebs-Ringer bicar-

and r.

tative analysis was initiated. One-half of the chambers served as controls and the other half were continuously exposed to

x 250 µm) were prepared using a Mcllwain tissue chopper and incubated for 10 min at 37°C in Krebs--Ringer bicar-

imate the point at which the antagonist reached the probe and the second indicates the time of the MDMA injection. Both concentra-

ions of MDL 100,907 produced a significant inhibition of MDMA-stimulated release (p < 0.05). Each point is the mean ± SEM for n values of 7, 4, and 5 for MDMA alone, 0.1 µM MDL 100,907 plus MDMA, and 1.0 µM MDL 100,907 plus MDMA, respectively.

FIG. 1. Concentration-dependent effect of intrastral MDL 100,907 on the release of striatal dopamine produced by MDMA administration (20 mg/kg s.c.). MDL 100,907 was infused via the microdialysis probe directly into the striatum. The first arrow indicates the point at which the antagonist reached the probe and the second indicates the time of the MDMA injection. Both concentrations of MDL 100,907 produced a significant inhibition of MDMA-stimulated release (p < 0.05). Each point is the mean ± SEM for n values of 7, 4, and 5 for MDMA alone, 0.1 µM MDL 100,907 plus MDMA, and 1.0 µM MDL 100,907 plus MDMA, respectively.

through the microdialysis probe at 0.1 or 1.0 µM. Absolute concentrations of dopamine in the dialysate (not corrected for recovery) were 5.2 ± 0.8 pg/32 µl (n = 16) before MDMA administration. Systemic admin-

stration of MDMA (20 mg/kg s.c.) produced a massive and sustained increase in extracellular concentra-

ions of dopamine that peaked 80 min after drug administration at ~2,000% of basal levels. Infusions of MDL 100,907 were timed such that the drug reached the microdialysis probe 50 min before the administra-

of MDMA. Although infusion of MDL 100,907 had no effect on basal dopamine efflux, local application of the antagonist produced a concentration-de-

pendent reduction in the effect of MDMA on extracellular transmitter concentrations (p < 0.05 for both: concentrations of MDL 100,907). This effect was most apparent during the early or peak phase of dopamine release. Although extracellular dopamine concentrations remained elevated above baseline throughout the 4-h period of collection, the concentra-

ions declined most rapidly in the MDMA alone group such that all three treatment groups were indistin-

guishable by the end of the experiment.

In contrast to the effect of MDL 100,907 on dopamine release, the antagonist had no effect on MDMA-induced changes in extracellular concentrations of the dopamine metabolites DOPAC and HVA. Basal concentrations of DOPAC and HVA were 2,628 ± 328 pg/32 µl (n = 15) and 2,690 ± 301 pg/32 µl (n = 16), respectively. Both metabolites declined in concentra-

ation by ≥50% after MDMA administration regardless of the presence of MDL 100,907 (Fig. 2).

The effect of intrastral infusion of MDL 100,907 was duplicated using the selective 5-HT3 antagonist:

amperozide. Infusion of 1 µM amperozide produced a reduction in MDMA-stimulated dopamine efflux almost identical to that observed at the same concentra-

of MDL 100,907 (Fig. 3). Amperozide was without effect on the MDMA-induced changes in DOPAC and HVA (data not shown).

A second potential site of serotonergic regulation of the striatal dopamine system is at the level of the mid-

brain dopaminergic nuclei. To determine if blockade of 5-HT3 receptors in this region would have any ef-

fect on MDMA-induced increases in striatal dopamine release, rats were implanted with microdialysis probes in both the midbrain and ipsilateral striatum. The location of the midbrain probe was selected to position the dialysis membrane immediately dorsal to the substantia nigra zona compacta without physically disturbing the nucleus. This probe was then used to infuse MDL 100,907 into the cell body region while extracellular concentrations of dopamine and its metabolites were determined simultaneously in the striatum. Figure 4 illustrates the results from these experiments. The effect of MDMA (20 mg/kg s.c.) on dopamine efflux was very similar to that observed in the first series of experiments (Figs. 1 and 3) with an ~20-fold increase in extracellular transmitter con-

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endopine of the dopamine uptake carrier (Schmidt et al., 1987). Therefore, such activity on the part of MDL 100,907 or amperozide could explain their ability to inhibit MDMA-induced dopamine release in vivo. This possibility was addressed by examining the effect of both agents on the concentration-response for MDMA-induced efflux of [3H]dopamine from striatal slices in vitro. Striatal slices preloaded with [3H]dopamine were superfused with KRB alone or KRB plus 1.0 μM antagonist during exposure to a series of concentrations of MDMA. Neither agent inhibited the efflux of [3H]dopamine elicited by MDMA (Fig. 5).

A final series of experiments was undertaken to determine if the 5-HT₂ receptors of interest were located on the dopaminergic terminals of the striatum. If concentrations. However, infusion of MDL 100,907 (1.0 μM) into the region of the brain containing the dopaminergic cell bodies had no effect on MDMA-stimulated dopamine release in the striatum. Midbrain infusion of MDL 100,907 was also without effect on the MDMA-induced decreases in extracellular concentrations of striatal DOPAC and HVA (not shown).

MDMA-induced dopamine release is sensitive to inhibitors of the dopamine uptake carrier (Schmidt et al., 1987). Therefore, such activity on the part of MDL 100,907 or amperozide could explain their ability to inhibit MDMA-induced dopamine release in vivo. This possibility was addressed by examining the effect of both agents on the concentration-response for MDMA-induced efflux of [3H]dopamine from striatal slices in vitro. Striatal slices preloaded with [3H]dopamine were superfused with KRB alone or KRB plus 1.0 μM antagonist during exposure to a series of concentrations of MDMA. Neither agent inhibited the efflux of [3H]dopamine elicited by MDMA (Fig. 5).

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![Image](https://via.placeholder.com/150)

**FIG. 2.** Effect of intrastratial infusion of MDL 100,907 on the MDMA-induced changes in striatal concentrations of dopamine metabolites. See Fig. 1 for experimental details.

![Image](https://via.placeholder.com/150)

**FIG. 3.** Attenuation of MDMA-induced dopamine release during the intrastratial infusion of amperozide. The experimental procedures are identical to those described for Fig. 1. MDMA-induced dopamine release was significantly reduced in the presence of the antagonist (p < 0.05). The MDMA control data are the same as those shown in Fig. 1. n = 4 for the amperozide plus MDMA group.

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**FIG. 4.** Effect of midbrain infusion of MDL 100,907 on the release of striatal dopamine by MDMA (20 mg/kg s.c.). Dialysis probes were implanted in both the midbrain and striatum. The antagonist was infused directly into the midbrain via the probe. Other experimental conditions are the same as those described in the legend to Fig. 1. The MDMA control curve is the mean of four rats, whereas n = 3 for the MDL 100,907 plus MDMA curve.

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efflux to ~1,000% of the original baseline regardless of the presence of MDL 100,907. Infusion of TTX also produced a rapid decline in striatal concentrations of DOPAC and HVA, which decreased further after the administration of MDMA. Coinfusion of MDL 100,907 had no effect on these changes (not shown).

DISCUSSION

The results of this study indicate that 5-HT₂ receptors located in the rat striatum play an important role in augmenting the dopamine release produced by the indirect agonist MDMA. Direct intrastriatal infusion of two unrelated 5-HT₂ receptor antagonists caused a significant reduction in the efflux of endogenous dopamine produced by systemic administration of MDMA. In contrast, intranigral infusion of an antagonist was without effect on MDMA-induced release.

Both MDL 100,907 and amperozide are under consideration as potential antipsychotic agents based on their ability to antagonize “dopamine-dependent” behaviors (Christensson and Bjork, 1990; Sorensen et al., 1993). Amperozide has been reported to show some antipsychotic efficacy in an initial open trial (Axelsson et al., 1991). Although it possesses somewhat less affinity at the 5-HT₂ receptor than does MDL 100,907 (Kᵢ = 17 vs. 0.39 nM; Dudley et al., 1990; Svartengren and Simonsson, 1990), amperozide does discriminate between the 5-HT₂ and 5-HT₁c receptor to a similar extent (Roth et al., 1992). The selectivity and specificity of amperozide and MDL 100,907 make these agents ideal tools for examining the physiology of 5-HT₂ receptors in the CNS. Identifying the anatomical and biochemical sites at which such agents interact with the dopamine system can provide a first step toward understanding the mechanism of their antipsychotic activity.

Intrastratal administration of either agent reduced the increase in extracellular dopamine concentrations produced by MDMA. Although it is difficult to estimate the regional concentrations of each agent attained in the brain tissue surrounding the probe, in general, the recovery across the Spectra/Per membranes used in these probes is <10%. Considering this limit on drug diffusion and given the effect of infusions of MDL 100,907 at concentrations as low as 0.1 μM, the results strongly support the claim that the inhibition of release produced by these agents is due to the blockade of 5-HT₂ receptors. Although neither MDL 100,907 nor amperozide completely prevented MDMA-stimulated dopamine efflux, this is not surprising given the results of similar experiments with α-methyl-p-tyrosine. The latter agent directly inhibits dopamine synthesis yet also fails to completely block MDMA-induced dopamine release (Schmidt et al., 1992; Brodkin et al., 1993). In addition, MDL 100,907 does not completely prevent dopamine synthesis. We have previously demonstrated that MDL 100,907 has no effect on basal dopamine synthesis.

FIG. 6. Effect of TTX coinfusion on the inhibition of MDMA-induced dopamine release by intrastriatal infusion of MDL 100,907. The effect of TTX on basal dopamine concentrations is shown using an expanded scale in the inset. Infusion of TTX alone (n = 4) or TTX plus MDL 100,907 (n = 6) began 110 min before MDMA administration (20 mg/kg s.c.). Other experimental details are similar to those described for Fig. 1.

FIG. 5. Release of [3H]dopamine by MDMA from superfused striatal slices in the absence and presence of MDL 100,907 (A) or amperozide (B). Slices were given consecutive 4-min pulses of MDMA at increasing concentrations during continuous exposure to the antagonists. Fractional release above baseline is plotted as a function of the MDMA concentration. Each point is the mean ± SEM for n = 8.
but only prevents the activation of synthesis produced by MDMA (Schmidt et al., 1992). Autoradiographic localization of 5-HT₂ receptors and 5-HT₂ receptor mRNA has demonstrated significant receptor expression in the striatum (Appel et al., 1990; Mengod et al., 1990). In contrast, both approaches indicate little or no expression of 5-HT₂ receptors in the substantia nigra. There is, however, electrophysiological evidence suggesting 5-HT₂ receptors may play a role in the regulation of dopaminergic activity at the cell body level (Nedergaard et al., 1988). It therefore appeared reasonable to consider the possibility that a functionally significant number of 5-HT₂ receptors might exist in this region and that these receptors may be responsible for the effect of 5-HT₂ receptor blockade on MDMA-induced dopamine release.

The results from the intranigral infusion experiments address two issues. The failure of 5-HT₂ receptor blockade in the midbrain to alter MDMA-induced dopamine efflux in the striatum indicates that it is unlikely that the receptors in the cell body region are responsible for the attenuation of MDMA-induced release observed with systemic administration of 5-HT₂ antagonists. The results also indicate that diffusion of MDL 100,907 within the brain during dialysis is restricted and probably limited to the immediate vicinity of the probe.

Although the concentrations of the 5-HT₂ antagonists achieved in the brain during infusion were quite low, the direct application of the antagonist to striatal tissue could result in nonreceptor-mediated effects. The reported activity of amperozide as a dopamine uptake inhibitor (Erikkson and Christensson, 1990) suggests the possibility that interference with the dopamine carrier could underlie the inhibition of MDMA-induced release observed here. Interference with the dopamine uptake carrier has been shown to block MDMA-induced dopamine release both in vitro (Schmidt et al., 1987) and in vivo (Nash and Brodkin, 1991a). Indeed, direct intrastriatal infusions of high concentrations of amperozide have been shown to block amphetamine-induced dopamine release by inhibition of the dopamine uptake carrier. However, concentrations of amperozide of ≥25 μM were required to produce this effect (Yamamoto and Meltzer, 1992). These concentrations of amperozide also produced a significant increase in the basal efflux of striatal dopamine. The concentration of amperozide (1.0 μM) used in the present study is less than the no-effect concentration used by Yamamoto and Meltzer (1992) and was without effect on basal efflux.

To address this question further, we examined the effect of both amperozide and MDL 100,907 on the MDMA-induced efflux of [3H]dopamine from superfused striatal slices. Both antagonists were tested at 1.0 μM, the highest concentration perfused through the probes, for their ability to shift the concentration-response curve for MDMA. Neither agent significantly attenuated MDMA-induced release under these conditions. An increase in efflux was observed with MDL 100,907 at 30 μM MDMA; however, this is the opposite response from that expected with an inhibitor of the dopamine uptake carrier. It is also contrary to the results observed in vivo. Therefore, it appears unlikely that the effect of either MDL 100,907 or amperozide on MDMA-induced dopamine release described in the present study can be attributed to interference with the interaction of MDMA and the dopamine uptake carrier.

Recently, Nash and Brodkin (1991a) examined the effects of a number of pharmacological agents on the release of dopamine produced by intrastriatal infusion of MDMA. These authors reported that the systemic administration of the dopamine uptake blockers mazindol or GBR 12909 attenuated the dopamine efflux elicited by infusion of 10 μM MDMA through the dialysis probe. The 5-HT uptake inhibitor fluoxetine also caused a small but significant reduction in the release produced by MDMA. In contrast to the result reported in the present study, systemic administration of the 5-HT₂ antagonist ketanserin produced only a small attenuation of release, which did not reach statistical significance. Beyond transposing the routes of agonist and antagonist administration, there are a number of important differences between these two studies. The most important of these variables may be the concentration of MDMA in the striatum and the duration of drug exposure. Our hypothesis regarding the mechanism by which 5-HT₂ antagonists inhibit MDMA-induced release is based upon the concept that the carrier-dependent release produced by MDMA is dependent on the continuous availability of a small pool of newly synthesized transmitter. By interfering with the stimulation of synthesis, 5-HT₂ receptor antagonists effectively eliminate the pool of transmitter available for release (Schmidt et al., 1991). This is consistent with the ability of the tyrosine hydroxylase inhibitor α-methyl-p-tyrosine to reduce MDMA-induced dopamine release by >70% when administered only 20 min before MDMA (Schmidt et al., 1992), although studies have shown this treatment reduces tissue stores of striatal dopamine by <20% at 1 h (Soares-da Silva and Garrett, 1990). In the study by Nash and Brodkin (1991a), intrastriatal infusions of 10 μM MDMA produced a fivefold increase in dopamine release, which lasted 30 min. Systemic administration of MDMA at a dose of 20 mg/kg has been reported to result in a brain drug concentration of 165 μM (Zaczek et al., 1989) and in the present study increased striatal dopamine concentrations above baseline for >180 min with a peak release of 18- to 20-fold above baseline. It therefore appears reasonable that the small amount of release produced by direct infusion of MDMA would not require the same degree of support from the biosynthetic machinery of the dopaminergic terminal. Under these conditions, 5-HT₂ re-
receptor blockade might be expected to have little effect on small increases in dopamine release. Indeed, 5-HT₂ antagonists are more effective at inhibiting the behavioral effects of high doses of MDMA compared with low doses (J. H. Kehne, personal communication). Based on these considerations, the failure of ketanserin to inhibit the release of dopamine produced by the infusion of low concentrations of MDMA is not at variance with our conclusion that 5-HT₂ receptor antagonists interfere with MDMA-stimulated dopamine release by blocking the action of 5-HT at receptors in the striatum.

The final series of experiments included in the present study sought to determine if the location of the 5-HT₂ receptors of interest was presynaptic with respect to the dopaminergic terminals. This was examined by the coinfusion of TTX into the striatum with MDL 100,907. By blocking voltage-dependent sodium channels, TTX interferes with axonal conduction and interrupts electrical activity in the tissue (Narahashi, 1974). MDMA-induced transmitter release is nonexcocytotic and therefore occurs in the absence of cell firing. Thus, in the presence of TTX and MDMA, 5-HT release and 5-HT₂ receptor activation will still occur. If the 5-HT₂ receptors are located on the dopaminergic terminal, the effect of 5-HT₂ receptor stimulation on MDMA-induced dopamine release should be unaffected by the presence of TTX. If they are located elsewhere in the striatum, it is likely that the effect of 5-HT₂ receptor activation is communicated to the dopaminergic terminal by an alteration in the firing rate of that postsynaptic element. This feedback could not occur in the presence of TTX and would be manifested as a loss of the effect of MDL 100,907. The results conform to the latter scenario in that intrastriatal infusion of MDL 100,907 had no effect on MDMA-induced dopamine release in the presence of TTX. The results are therefore consistent with the proposal that the 5-HT₂ receptors responsible for the effects of MDL 100,907 and related antagonists on MDMA-induced dopamine efflux are located on nondopaminergic sites in the striatum.

There is at least one caveat to this interpretation of the TTX data. Interrupting electrical activity in the striatum with TTX reduces basal dopamine efflux as reported in numerous studies previously (Westerink et al., 1987; Osborne et al., 1990; Santiago and Westerink, 1990). Although carrierr-mediated release by agents such as amphetamine or MDMA still occurs, the increase in striatal dopamine efflux produced by these agents has been shown to be attenuated by TTX (Westerink et al., 1987; Nash and Brodkin, 1991b). Examination of the data in Fig. 6 shows that in the presence of TTX, MDMA increased extracellular dopamine concentration by only 10-fold, whereas in the absence of TTX, this value was between 18- and 20-fold (see Figs. 1 and 4). Thus, TTX alone reduced MDMA-stimulated dopamine efflux to the same extent as MDL 100,907. Although the mechanism responsible for this effect of TTX is not clear, one potential explanation is that the reduction in depolarization-induced Ca²⁺ influx slows the rate of dopamine synthesis. Tyrosine hydroxylase is normally activated by a Ca²⁺/calmodulin-dependent phosphorylation, which is subject to changes in cell firing and Ca²⁺ influx (Roth et al., 1987). A reduction in the rate of terminal depolarization could decrease the level of dopamine synthesis and thereby affect the pool of transmitter available for carrier-dependent release. Such an effect of TTX on synthesis could essentially mask any effect of 5-HT₂ receptor blockade on MDMA-induced release. Further work will be required to eliminate this possibility.

In conclusion, the results indicate that the activation of 5-HT₂ receptors on nondopaminergic elements of the striatum augments the dopamine release produced by high doses of the amphetamine analogue MDMA. Further studies are required to determine the precise neuronal location of these receptors and the mechanism through which they influence dopaminergic function. However, the nondopaminergic location of these receptors suggests that striatal 5-HT₂ receptors could mediate both dopamine-dependent and independent actions of MDL 100,907 and related agents on basal ganglia function.

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