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The 5-HT_2 receptor antagonist, MDL 28,133A, disrupts the serotonergic–dopaminergic interaction mediating the neurochemical effects of 3,4-methylenedioxymethamphetamine

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The selective 5-HT_2 receptor antagonist MDL 28,133A dose dependently blocked the long-term deficits in rat brain 5-HT concentrations produced by the substituted amphetamine analogue 3,4-methylenedioxymethamphetamine (MDMA). This protective effect of MDL 28,133A could be abolished by coadministration of the dopamine precursor, L-dihydroxyphenylalanine (L-DOPA). Electrophysiological experiments demonstrated that the ability of MDL 28,133A to block the MDMA-induced slowing of A9 dopaminergic neurons was also sensitive to L-DOPA administration. Both sets of experiments suggest an interaction of MDL 28,133A at the level of dopamine synthesis. Consistent with this explanation, MDL 28,133A antagonized the MDMA-induced stimulation of dopamine synthesis in vivo. MDMA-induced 5-HT release did not reduce the firing rate of dopaminergic neurons as assessed by dopamine depletion following synthesis inhibition with α-methyl-p-tyrosine (α-MPT). This indicates that the effect of 5-HT_2 receptor antagonists on MDMA-induced dopamine synthesis is not due simply to the removal of an inhibitory serotonergic input followed by an increase in dopamine cell firing and autoreceptor activation. MDL 28,133A was also shown to be without effect on the sensitivity of terminal dopamine autoreceptors. The results are consistent with the hypothesis that 5-HT_2 receptors are permissive for the stimulation of dopamine synthesis necessary to support MDMA-induced transmitter efflux.

MDMA (3,4-methylenedioxymethamphetamine); Amphetamine; 5-HT_2 receptors; Dopamine neurons

1. Introduction

Administration of a single high dose of the amphetamine analogue, 3,4-methylenedioxymethamphetamine (MDMA), to laboratory animals results in a selective and persistent decrease in central serotonin (5-hydroxytryptamine, 5-HT) concentrations (Schmidt et al., 1986; Stone et al., 1986). Other neurochemical markers of serotonergic function such as 5-HT uptake and tryptophan hydroxylase activity show corresponding reductions (Schmidt, 1987). The loss of 5-HT uptake sites as measured by ligand binding (Battaglia et al., 1987) and the associated argyrophilia (Commins et al., 1987) support the suggestion that these long-term neurochemical changes reflect the degeneration of serotonergic nerve terminals.

A number of the investigations into the mechanism responsible for this loss of 5-HT nerve terminals have focussed on the role of dopamine as a key mediator of this process. MDMA has been shown to release dopamine as well as 5-HT through the same Ca^{2+}-independent, carrier-mediated process characterized for amphetamine (Schmidt et al., 1987; Hekmatpanah and Peroutka, 1990). Interference with this dopamine efflux by blockade of the dopamine uptake carrier, chemical lesioning of the mesencephalic dopamine systems or inhibition of dopamine synthesis all prevent the serotonergic degeneration produced by MDMA (Stone et al., 1988; Schmidt et al., 1990a).

5-HT release was also implicated in this process by the observation that coadministration of selective 5-HT_2 receptor antagonists would prevent MDMA-induced serotonergic deficits (Nash et al., 1990; Schmidt and Kehne, 1990; Schmidt et al., 1990a,b). Further investigation indicated that the protective effect of the 5-HT_2 receptor antagonists are also ultimately mediated by the dopamine system. Interactions between these two monoaminergic neurotransmitter systems have been described in numerous studies. In general, these studies suggest that the serotonergic system exerts an in-

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hibitory influence on the dopaminergic system (Mabry and Campbell, 1973; Fibiger and Miller, 1977; Dray et al., 1978). Although not entirely consistent with this view, recent studies on the interaction of 5-HT_2 receptor antagonists with MDMA suggest that 5-HT_2 receptor activation may be required for dopaminergic function under some conditions. Nash et al. (1990) first demonstrated that the 5-HT_2 receptor antagonist ketanserin could inhibit the acute stimulation of dopamine synthesis produced by MDMA as well as prevent the long-term neurochemical effects of MDMA. Ketanserin also partially reduced MDMA-elicited dopamine release in vivo as measured by microdialysis (Nash, 1990). If 5-HT_2 receptor antagonists prevent the long-term effects of MDMA by this interference with stimulated dopamine synthesis and release, we reasoned that this protection should be sensitive to administration of the dopamine precursor, L-dihydroxyphenylalanine (L-DOPA). L-DOPA administration effectively bypasses the rate limiting step in dopamine synthesis. This hypothesis was tested in experiments using the selective 5-HT_2 receptor antagonists, MDL 11,939 and ritanserin. We have reported that the administration of L-DOPA simultaneously with MDMA nullifies the ability of the 5-HT_2 receptor antagonists to block the serotonergic deficits produced by MDMA (Schmidt et al., 1991).

Although the results of these studies are consistent with a role for 5-HT_2 receptors in the control of stimulated dopamine synthesis, further studies are necessary to confirm this hypothesis. To this end, we have studied the effects of a new and highly selective 5-HT_2 receptor antagonist, MDL 28,133A (Carr et al., 1991); on the acute electrophysiological and neurochemical alteration of dopaminergic function produced by MDMA. The effects of MDL 28,133A on the long-term neurochemical consequences of MDMA administration described above were also examined.

2. Materials and methods

2.1. Neurochemical studies

Male Sprague–Dawley rats (200–300 g) were maintained on a 12-h light and dark cycle and allowed free access to food and water. Long-term neurochemical studies were conducted only after animals had been on site for 1 week. The timing of individual experiments, routes of drug administration and doses are provided in the Results section. At the termination of each experiment the animals were killed by decapitation and their brains were rapidly dissected on ice. Samples of cortex, hippocampus and striatum were removed and immediately frozen on dry ice. Tissue samples were stored at −70°C until assayed.

Tissue concentrations of 5-HT, DOPA and dopamine were determined by high performance liquid chromatography with electrochemical detection. Samples were homogenized in 0.1 M monochloroacetic acid containing 0.1 mM EDTA at pH 3.0. After centrifugation at 4°C (15 min, 30000 × *g*) the samples were injected onto a 250 × 4 mm, 5 μm Lichrospher 100 RP-18 cartridge column (EM Separations, Gibbstown, NJ) and eluted with a mobile phase containing (mM): NaH_2PO_4 150, EDTA 0.10 and PIC-B7 5 mM (Waters, Milford, MA) at pH 3.0. The methanol concentration was varied from 1 to 5% to modify separation. A Coulochem 5100A electrochemical detector (ESA Inc., Bedford, MA) set to a potential of +0.4 V was used for detection. Components were quantified by comparison to a curve generated from external standards. 5-HT concentrations in the hippocampus and cortex were corrected for recovery by comparison to an internal standard (N-acetylserotonin). Recovery in the striatal samples was greater than 90% and did not require correction.

Neurochemical results were analyzed for statistical significance using the Microstat-II program (Ecosoft, Inc. Indianapolis, IN). Following an ANOVA, differences between groups were determined using the least significant difference test with a level of $P < 0.05$ being accepted as significant.

2.2. Electrophysiological measurements

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and then secured in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) with the incisor bar tilted −3.8 mm. Body temperature was maintained within normal limits (36–37°C) by means of a homeothermic heating pad. The scalp and periosteum were reflected and a 1 mm section of the skull overlying the A9 region was removed. Coordinates, determined from the atlas of Paxinos and Watson (1986), were 5.2–5.6 mm posterior and 2–2.4 mm lateral to bregma.

Tungsten microelectrodes (1–3 MΩ) were used for the extracellular recording of dopamine neurons. Electrodes were lowered into the A9 region (6.0–8.0 mm below the dural surface), one dopamine neuron was isolated for each animal in the study and its firing rate was recorded. Dopamine neurons were identified using previously established criteria for midbrain dopamine neurons (Bunney et al., 1973; Grace and Bunney, 1983), i.e. a slow regular or slow decremental bursting pattern of spontaneous activity with a firing rate between 1 and 10 Hz; a biphasic or triphasic waveform with a predominant positive phase often accompanied by a notch or shoulder; a wide action potential duration (greater than 2 ms) and a characteristic low pitch when monitored on audio. Electrode potentials were passed

through a high impedance amplifier, band pass filtered (200 Hz–4 kHz and monitored with a digitizing oscilloscope (Tektronix, Inc., Beaverton, OR). The firing rate was recorded, analyzed, and stored utilizing the data acquisition program, Brainwave Discovery (Brainwave Systems, Broomfield, CO). After observing and recording a baseline firing rate for at least 10 min, drugs were administered as described in the Results.

Data from individual neurons were normalized to basal firing rates so that the averages could be expressed as a percentage of the control firing rate. Statistical evaluations were then made using Student's t-test with paired observations. The level of significance was set at $P < 0.05$.

2.3. Drugs

Drugs for all studies were administered in saline as the salt with the exception of MDMA, α-methyl-p-tyrosine (α-MPT) and L-DOPA which were administered as the free base. Saline was used as the vehicle control. MDL 28,133A required sonication for 1–2 min to insure solubility. MDMA-HCl was provided by the National Institute on Drug Abuse. MDL 28,133A (1-(4-fluorophenyl)-2-[4[(4-methanesulfonamido-phenyl)-carbonyl]-1-piperidinyl]-ethanone hydrochloride) was synthesized at the Marion Merrell Dow Research Institute (Cincinnati, OH). Apomorphine, L-DOPA methyl ester, NSD 1015, α-MPT methyl ester were purchased from Sigma Chemical Co. (St. Louis, MO) and haloperidol was purchased from Research Biochemicals (Natrick, MA). Carbidopa was the generous gift of Merck, Sharp and Dohme Research Laboratories (Rahway, NJ).

3. Results

3.1. Reversal of MDMA-induced 5-HT deficits by MDL 28,133A

Figure 1 shows the effect of MDL 28,133A on the MDMA-induced reduction in forebrain 5-HT concentrations 1 week after drug administration. The 30 mg/kg s.c. dose of MDMA reduced 5-HT in the cortex and hippocampus by greater than 60% with a characteristically smaller effect in the striatum (approximately 40% reduction). Coadministration of the 5-HT_2 receptor antagonist, MDL 28,133A reversed the reduction in 5-HT concentrations measured in all three regions of the forebrain. Significant antagonism of the MDMA effect was observed with doses of MDL 28,133A as low as 0.1 mg/kg and a complete reversal of the MDMA effect was observed in all regions.

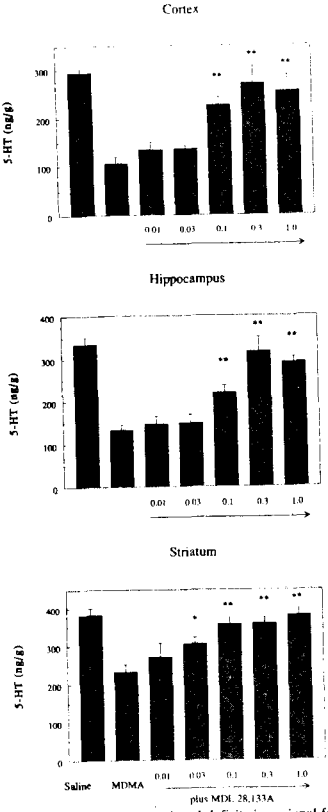

Fig. 1. Antagonism of MDMA-induced deficits in regional forebrain 5-HT concentrations by the selective 5-HT_2 antagonist, MDL 28,133A. MDMA (30 mg/kg) and the antagonist were administered simultaneously by the s.c. route 1 week prior to killing. MDMA produced significant depletions in 5-HT concentrations in all three regions ($P < 0.01$). All values represent the mean ± S.E.M. (*, **, $P < 0.05, 0.01$, respectively, compared to MDMA).

3.2. Effect of simultaneous administration of L-DOPA

The effect of MDMA (30 mg/kg s.c.) on cortical, hippocampal and striatal 5-HT concentrations 1 week after drug administration is shown again in fig. 2. As already demonstrated, coadministration of MDL 28,133A (1 mg/kg s.c.) with MDMA significantly attenuated the transmitter deficits ($P < 0.05$), although a complete reversal of MDMA's effect was not observed

in this experiment. Also shown is the effect of coadministration of L-DOPA with MDMA and MDL 28,133A, 30 min following pretreatment with the peripheral decarboxylase inhibitor carbidopa (25 mg/kg i.p.). In the presence of the dopamine precursor, MDMA again produced deficits in 5-HT concentrations similar to those observed in the absence of the 5-HT_2 receptor antagonist.

3.3. Effect of MDL 28,133A on the MDMA-induced slowing of A9 dopaminergic neurons

The effect of MDL 28,133A on the reduction in A9 cell firing rates following MDMA administration was examined as an additional endpoint for MDMA-induced dopamine release. Figure 3 contains firing rate histograms for three A9 dopaminergic neurons exposed to three different treatment regimens. Basal firing rates were routinely recorded for 20 min prior to the administration of any drugs. Panel A shows the effect of MDMA (15 mg/kg i.v.) on A9 firing. A high dose of 15 mg/kg was selected for these studies to be consistent with the high doses used in the long-term neurochemical studies. This dose of MDMA produced a rapid and dramatic slowing of the cell firing rate. The effect of MDL 28,133A (1 mg/kg i.v.) administration 20 min prior to MDMA is shown in panel B. The presence of the antagonist completely prevented the effect of MDMA on cell firing but did not block the effect of the direct dopamine receptor antagonist, apomorphine (25 μg/kg, i.v.). In panel C, L-DOPA (100 mg/kg i.v.) was coadministered with the 5-HT_2 receptor antagonist 30 min following pretreatment with carbidopa (25 mg/kg i.p.). Administration of the dopamine precursor completely restored the effect of MDMA on cell firing in the presence of the 5-HT_2 receptor antagonist.

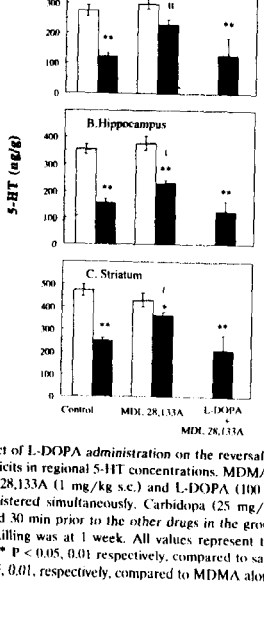

Fig. 2. Effect of L-DOPA administration on the reversal of MDMA-induced deficits in regional 5-HT concentrations. MDMA (30 mg/kg s.c.), MDL 28,133A (1 mg/kg s.c.) and L-DOPA (100 mg/kg i.p.) were administered simultaneously. Carbidopa (25 mg/kg i.p.) was administered 30 min prior to the other drugs in the group receiving L-DOPA. Killing was at 1 week. All values represent the means + S.E.M. (*,** $P < 0.05, 0.01$ respectively, compared to saline; †,†† $P < 0.05, 0.01$, respectively, compared to MDMA alone.)

Figure 4 summarizes the data from the electrophysiological experiments. The 15 mg/kg dose of MDMA reduced A9 firing to 25% of the control rate. This effect was significantly attenuated in animals pretreated with MDL 28,133A ($P < 0.01$) such that rates declined to only 93% of the control values following MDMA administration. MDL 28,133A alone did produce a small elevation in the basal firing rate although this did not reach the level of statistical significance in these experiments. Administration of the dopamine precursor restored the ability of MDMA to slow cell firing even in the presence of the 5-HT_2 receptor antagonist ($P < 0.01$). Interestingly, L-DOPA itself did not alter the basal firing rate of the cells nor did it affect the extent of slowing produced by MDMA.

3.4. Effect of MDL 28,133A on MDMA-stimulated dopamine synthesis

The ability of L-DOPA administration to prevent the reversal of MDMA's neurochemical and electrophysiological effects by MDL 28,133A suggests a requirement for 5-HT_2 receptors in the activation of dopamine synthesis and release. To examine this question more directly, the effect of MDL 28,133A on the MDMA-stimulated accumulation of DOPA was determined in rats following decarboxylase inhibition. NSD 1015 (100 mg/kg i.p.) was administered 30 min prior to killing. Administration of MDMA (20 mg/kg s.c.) 30 min prior to the NSD 1015 produced a small but significant increase in DOPA accumulation as shown in fig. 5. Pretreatment with MDL 28,133A 30 min prior to MDMA blocked this stimulation with a U-shaped dose–response curve. The results shown in fig. 5 are the mean results from two experiments in which essentially identical results were observed. The lowest dose of MDL 28,133A tested, (0.01 mg/kg), completely prevented the MDMA-induced stimulation of dopamine synthesis. This effect was lost at intermediate doses of 0.1 and 0.3 but was observed again at the highest dose (1 mg/kg) of the antagonist.

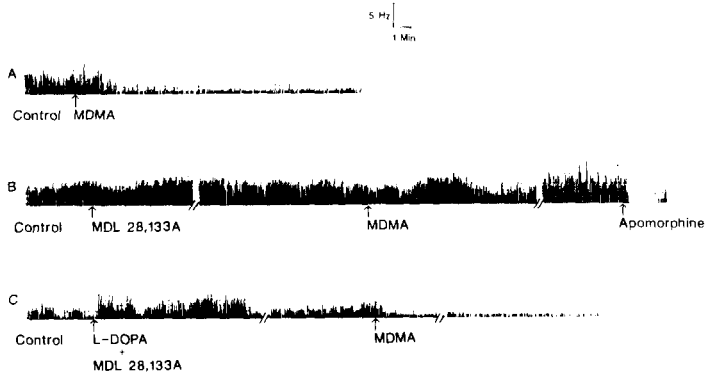

Fig. 3. Data from individual experiments showing the effect of MDL 28,133A with or without L-DOPA on the MDMA-induced slowing of a single A9 dopamine neuron. Panel (A) is a strip chart recording of the firing rate of a single neuron treated with MDMA (15 mg/kg i.v.). Panel (B) illustrates the firing rate of a single neuron exposed to MDL 28,133A (0.2 mg/kg i.v.) 20 min prior to MDMA. Panel (C) is a cell pretreated with both MDL 28,133A and L-DOPA (100 mg/kg i.v.) before the MDMA challenge. Carbidopa (25 mg/kg i.p.) was administered 30 min prior to L-DOPA. All values represent the means ± S.E.M.

3.5. MDMA-induced changes in dopamine turnover in the presence of α-MPT

To examine the role of MDMA-induced 5-HT release in the control of dopamine turnover, the effect of MDMA on the rate of dopamine depletion following the administration of α-MPT was determined. Preliminary experiments indicated that a 2 h treatment period with MPT (200 mg/kg i.p.) reduced striatal dopamine concentrations by 40–50%. As shown in fig. 6, pharmacological treatments modifying the firing rate of the neurons can alter this rate of dopamine depletion. The increase in dopaminergic cell firing produced by haloperidol (0.1 mg/kg s.c.) results in a significant reduction in striatal dopamine concentrations compared to that observed with MPT alone ($P < 0.05$).

MDMA (20 mg/kg s.c.) administered simultaneously with MPT and again at 1 h had no effect on the rate of striatal dopamine depletion. The combination of MDL 28,133A and MDMA was similarly without

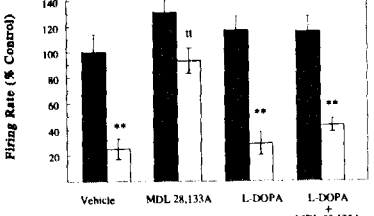

Fig. 4. Mean data illustrating the effect of MDL 28,133A with or without L-DOPA on the MDMA-induced slowing of A9 dopamine neurons. Control bars represent pretreatment with either no drug (vehicle), MDL 28,133A (0.2 mg/kg i.v.) or MDL 28,133A with L-DOPA (100 mg/kg i.v.). The effect of L-DOPA alone is also shown. MDMA-labeled bars represent treatment with MDMA (15 mg/kg i.v.) 20 min after the appropriate control condition. All bars represent the means + S.E.M. of between five and eight neurons as a percentage of their control. (** $P < 0.01$ compared to vehicle control; tt $P < 0.01$ compared to MDMA alone.)

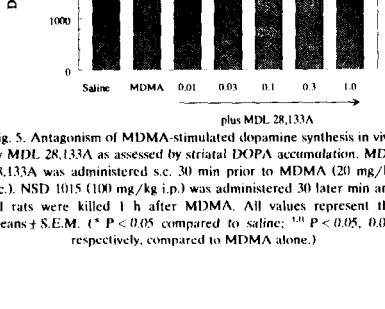

Fig. 5. Antagonism of MDMA-stimulated dopamine synthesis in vivo by MDL 28,133A as assessed by striatal DOPA accumulation. MDL 28,133A was administered s.c. 30 min prior to MDMA (20 mg/kg s.c.). NSD 1015 (100 mg/kg i.p.) was administered 30 later min and all rats were killed 1 h after MDMA. All values represent the means ± S.E.M. (* $P < 0.05$ compared to saline; tt $P < 0.05$, 0.01, respectively, compared to MDMA alone.)

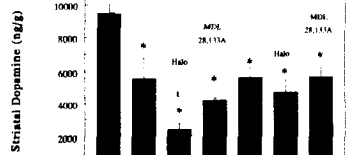

Fig. 6. Drug-induced alterations in dopamine turnover as assessed by the depletion of striatal dopamine concentrations after tyrosine hydroxylase inhibition with α-MPT. All rats were killed 2 h after α-MPT administration (200 mg/kg i.p.). Haloperidol (0.1 mg/kg s.c.) was administered simultaneously with α-MPT. MDL 28,133A (1 mg/kg s.c.) and MDMA (20 mg/kg s.c.) were given with α-MPT and again 1 h prior to killing. All values represent the means ± S.E.M. (* $P < 0.05$ compared to saline; † $P < 0.05$ compared to α-MPT alone.)

effect. When given alone, the 5-HT_2 receptor antagonist produced a slight but nonsignificant increase in the rate of dopamine depletion. Interestingly, MDMA did appear to reverse the effect of haloperidol in this model.

3.6. Effect of MDL 28,133A on striatal dopamine autoreceptor function

Alterations in dopamine autoreceptor function could account for the effect of 5-HT_2 receptor blockade on the MDMA-induced slowing of A9 dopamine neurons. To determine if MDL 28,133A did reduce striatal autoreceptor activity, the effect of the antagonist on the apomorphine-induced inhibition of DOPA accumulation was examined in γ-hydroxybutyrolactone (GBL)-treated rats. MDL 28,133A (1.0 mg/kg s.c.), apomorphine (0.1 or 1.0 mg/kg i.p.) and GBL (750 mg/kg i.p.) preceded NSD 1015 (100 mg/kg i.p.) by 30, 10 and 5 min, respectively. Killing was 30 min following NSD 1015 administration. Although significant inhibition of DOPA accumulation was observed only at the high dose of apomorphine, MDL 28,133A did not appear to modify the effect of the agonist at either dose nor did the antagonist alone affect the accumulation of DOPA produced by GBL (table 1).

TABLE 1

Effect of MDL 28,133A on the inhibition of GBL-induced DOPA accumulation by apomorphine in NSD 1015-treated rats. NSD 1015 (100 mg/kg i.p.) was administered 30 min prior to killing. MDL 28,133A (1 mg/kg s.c.), apomorphine, and GBL (750 mg/kg i.p.) preceded the decarboxylase inhibitor by 30, 10 and 5 min, respectively. Values are provided as the means ± S.E.M. (* $P < 0.05$ compared to control by ANOVA with least significant difference test).

	DOPA (ng/g)	
	Saline (%)	MDL 28,133A (%)
Control	4340 ± 546 (100)	4098 ± 512 (94.2)
Apomorphine, 0.1 mg/kg	3026 ± 561 (69.6)	3200 ± 534 (73.6)
Apomorphine, 1.0 mg/kg	1979 ± 2.99 (45.5) *	2562 ± 482 (58.9) *

4. Discussion

In this study we have made a detailed examination of the effects of 5-HT_2 receptor blockade on the neurochemical effects of MDMA. These experiments were performed using the novel 5-HT_2 receptor antagonist, MDL 28,133A (Carr et al., 1991). Consistent with our previous results (Schmidt and Kehne, 1990) and those of Nash (1990), blockade of 5-HT_2 receptors with MDL 28,133A antagonized the long-term serotonergic deficits produced by MDMA. MDL 28,133A dose dependently reversed the depletions produced 1 week after 30 mg/kg of MDMA at doses as low as 0.1 mg/kg. The potency of this effect and the specificity of MDL 28,133A indicates such protection is mediated by blockade of 5-HT_2 receptors and not some other nonspecific effect of the drug. We have also shown this effect of 5-HT_2 receptor antagonists to be stereoselective using the optical isomers of the 5-HT_2 antagonist, MDL 11,939, further supporting this claim (Schmidt et al., 1991). The postulated role of dopamine release in the long-term neurochemical effects of MDMA and the ability of 5-HT_2 receptor antagonists to prevent these effects suggest that an interaction between 5-HT_2 receptors and the dopaminergic system may be an important component of the long-term neurochemical effects of MDMA.

The reversal of the protective effects of MDL 28,133A and other 5-HT_2 receptor receptor antagonists by L-DOPA (Schmidt et al., 1991) suggests that this interaction with the dopaminergic system may occur at the level of transmitter synthesis. Interference with dopamine synthesis is consistent with protection against the long-term effects of MDMA as MPT has been shown to be protective in this model (Stone et al., 1988; Schmidt et al., 1990a). However, since the role of dopamine in the serotonergic deficits produced by MDMA is itself hypothetical, the effect of MDMA on the slowing of A9 dopaminergic neurons was examined as an additional indicator of MDMA-induced dopamine release.

MDMA reduces the firing rate of A9 dopaminergic neurons by a mechanism sensitive to synthesis inhibition with MPT (Kelland et al., 1989). This is similar to results observed with amphetamine (Bunney and Aghajanian, 1975) and suggests that dopamine released from

a newly synthesized pool is responsible for this inhibitory effect. Thus, the effect of 5-HT_2 receptor antagonists in this system also suggests an alteration of drug-induced dopamine release. Several recent studies have reported that selective 5-HT_2 receptor antagonists can also block amphetamine-induced slowing of dopamine cell firing rates (Goldstein et al., 1989; Sorensen et al., 1992). In the A10 region, the effect of amphetamine on cell firing could be prevented by pretreatment with either ritanserin or MDL 28,133A and completely reinstated by the administration of L-DOPA (Sorensen et al., 1992). These results and those described here indicate the mechanism of action of the 5-HT_2 receptor antagonists is similar in both mesolimbic (A10) and nigrostriatal (A9) dopaminergic pathways.

Both the neurochemical and electrophysiological results are compatible with an effect of 5-HT_2 receptor antagonists on dopamine synthesis. Furthermore, this explanation is consistent with the reported ability of PCPA pretreatment or i.c.v. 5,7-dihydroxytryptamine to reduce the potency of MDMA for decreasing nigrostriatal cell firing rates (Kelland et al., 1989). We have reported a similar effect of 5-HT depletion with PCPA on the amphetamine-induced slowing of A10 dopaminergic neurons (Sorensen et al., 1992).

The remaining experiments described in this study further tested the validity of this hypothesis and considered alternative explanations for observed effects of the 5-HT_2 receptor antagonists. Nash et al. (1990) previously reported that the 5-HT_2 antagonist, ketanserin, can block MDMA-stimulated DOPA accumulation as well as the long-term 5-HT depletions. As predicted, MDL 28,133A also antagonized the in vivo increase in dopamine synthesis occurring in response to MDMA. Although a U-shaped dose–response curve was observed, the results suggest that over time, the net effect of the antagonist would be a reduction in the size of the newly synthesized pool of dopamine. Since MDMA releases transmitter from the newly synthesized pool, an immediate consequence of such an effect would be a reduction in MDMA-induced dopamine release. In support of this, microdialysis studies with ketanserin have shown that the antagonist does partially reduce the release of dopamine produced by MDMA in vivo (Nash, 1990).

The serotonergic input to the midbrain dopaminergic systems is believed to be largely inhibitory with respect to the firing rate (Fibiger and Miller, 1977). Blockade of this input by the 5-HT_2 receptor antagonist ritanserin has been shown to increase the firing rate of both A9 and A10 neurons (Ugedo et al., 1990). The small stimulatory effect of MDL 28,133A observed here is in agreement with these results. Although the effect of MDL 28,133A on basal firing rates was small, the 5-HT releasing action of MDMA (Johnson et al., 1986; Schmidt et al, 1987) would significantly increase serotonergic input to the midbrain and could thereby increase the relative effect of a 5-HT_2 receptor antagonist. A potential outcome of the ability of MDL 28,133A to maintain a near normal firing rate in the presence of MDMA is that the amount of dopamine release occurring in the striatum would be greater than that produced by MDMA alone. Although this is inconsistent with the effect of 5-HT_2 receptor antagonists on the long-term effect of MDMA, the resultant increase in striatal autoreceptor activation could produce the observed inhibition of MDMA-stimulated dopamine synthesis.

To determine if a 5-HT_2 receptor antagonist did increase MDMA-induced dopamine release in vivo or if MDMA-induced 5-HT release did directly inhibit dopamine cell firing, the rate of dopamine turnover was examined neurochemically by measuring dopamine concentrations following MPT administration as described by Andén et al. (1967, 1971). In the absence of synthesis, the rate of dopamine depletion is a function of the rate of neuronal activity as demonstrated by the ability of haloperidol to accelerate the depletion of transmitter. Amphetamine-like releasing agents would be expected to have little effect in this model due to the lack of a newly synthesized and hence readily releasable pool of transmitter (Miller and Shore, 1982). However, in contrast to amphetamine, MDMA is a very potent releaser of 5-HT as well as dopamine (Johnson et al., 1986; Schmidt et al., 1987) hence any effect of 5-HT on dopamine turnover should be apparent. Yet there was no indication that the administration of even two doses of MDMA could modify the rate of dopamine depletion in the MPT model nor did MDL 28,133A produce any further effect. These neurochemical results demonstrate that the changes observed in the electrophysiological experiments with MDMA or MDMA plus MDL 28,133A do not occur in the presence of MPT, or alternatively, in the absence of continuous dopamine synthesis. Therefore, if MDMA-induced 5-HT release does modify the firing rate of dopaminergic neurons it must do so through an initial effect on dopamine synthesis with the subsequent enhancement of transmitter release. It follows that the 5-HT_2 receptor antagonist effect on firing rate is secondary to an alteration in synthesis as well.

It is worth noting that the ability of MDMA to reverse the effect of haloperidol on dopamine depletion in the MPT model is similar to that observed for amphetamine by Miller and Shore (1982). This suggests that like amphetamine, MDMA may cause some redistribution of the intraneuronal pool of transmitter. How this may relate to the other effects of MDMA described here is not clear.

The ability of 5-HT_2 receptor antagonists to negate the effects of MDMA or amphetamine on dopamine

cell firing rate could also be attributed to an alteration in the sensitivity of the dopamine autoreceptor. A dramatic reduction in autoreceptor sensitivity would have functional effects like that of a D_2 receptor antagonist. Neuroleptics such as haloperidol do reverse the inhibitory effect of amphetamine on cell firing in the A9 and A10 regions by blocking the activation of inhibitory autoreceptors and polysynaptic feedback loops (Bunney and Aghajanian, 1976; Wang, 1981). This question was examined neurochemically by determining if MDL 28,133A altered the apomorphine-induced inhibition of DOPA accumulation following the administration of GBL to NSD 1015 treated rats. The effect of apomorphine in this model is dependent upon dopamine autoreceptor sensitivity as shown by the blockade produced by D_2 receptor antagonists (Walters and Roth, 1976). 5-HT_2 receptor blockade with MDL 28,133A had no apparent effect on the sensitivity of striatal DOPA accumulation to apomorphine. Although this does not address the possibility that changes in somatodendritic autoreceptors are involved in the effects of the 5-HT_2 receptor antagonists described here, the ability of apomorphine to inhibit dopamine cell firing in the presence of MDL 28,133A indicates these receptors are still functional. Therefore the results suggest that 5-HT_2 receptor antagonists probably do not produce a marked change in dopamine autoreceptor sensitivity. Full dose–response studies of the effect of apomorphine on firing rate of nigrostriatal neurons are now being conducted in the absence and the presence of 5-HT_2 receptor antagonists to address this question more completely.

In summary, the most satisfactory explanation of these results is that 5-HT_2 receptors play a required role in the phasic support of dopamine synthesis under conditions of enhanced transmitter efflux. Both amphetamine and MDMA produce such conditions. In the presence of a 5-HT_2 receptor antagonist, transmitter synthesis appears to be unable to keep pace with the transmitter efflux produced by agents such as MDMA. The result is a reduction in MDMA-induced dopamine release such as that observed in the microdialysis studies described by Nash (1990). It is this reduction in dopamine release which is responsible for the ability of 5-HT_2 receptor antagonist to block the long-term neurochemical effects of MDMA as well as its effect on the firing rate of A9 dopaminergic neurons.

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