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5-HT_2 receptors exert a state-dependent regulation of dopaminergic function: studies with MDL 100,907 and the amphetamine analogue, 3,4-methylenedioxymethamphetamine

Christopher J. Schmidt, Gina M. Fadayel, Christine K. Sullivan and Vicki L. Taylor

Marion Merrell Dow Research Institute, Cincinnati, OH, USA

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The highly selective 5-HT_2 receptor antagonist, MDL 100,907, was used to explore the role of serotonin in the stimulation of dopaminergic function produced by the amphetamine analogue 3,4-methylenedioxymethamphetamine (MDMA). MDL 100,907 blocked MDMA-stimulated dopamine synthesis in vivo without affecting basal synthesis. The long-term deficits in 5-HT concentrations believed to be a consequence of MDMA-induced dopamine release were also blocked by MDL 100,907 over the same dose range. In vivo microdialysis confirmed that 5-HT_2 receptor blockade with MDL 100,907 attenuated MDMA-induced increases in extracellular concentrations of striatal dopamine. In contrast to its effect on MDMA-induced synthesis, MDL 100,907 did not alter dopamine synthesis stimulated by haloperidol or reserpine. In vivo dopamine release produced by haloperidol was also unaffected by MDL 100,907. The results suggest a permissive role for 5-HT_2 receptors in the activation of the dopamine system which occurs during states of high serotonergic activity or during conditions of elevated dopamine efflux with high D_2 receptor occupancy.

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

1. Introduction

Recent studies using the ring-substituted amphetamine analogue 3,4-methylenedioxymethamphetamine (MDMA) have provided evidence of a significant interaction between the ascending serotonergic and midbrain dopaminergic systems of the brain. As a unique central stimulant, MDMA is perhaps best known as a drug of abuse (Peroutka, 1990) but in addition this agent possesses a number of behavioral and biochemical properties that have made it a useful research tool. One such neurochemical effect is the ability of high doses of MDMA to produce evidence of selective serotonergic nerve terminal degeneration in laboratory animals (Schmidt, 1987) including primates (Ricaurte et al., 1988; Kleven et al., 1989). The administration of a single high dose of MDMA to rats produces characteristic deficits in a number of neurochemical indices of serotonergic function within one week. Most prominant among these changes is the depletion of 5-HT in all major terminal fields of the ascending serotonergic system (Stone et al., 1986; Schmidt, 1987; O'Hearn et al., 1988). This is accompanied by a decline in the activity of tryptophan hydroxylase (Stone et al., 1986) and a loss in the number of high affinity 5-HT uptake sites (Battaglia et al., 1987; Schmidt, 1987). Evidence of degenerating nerve terminals and axons has also been obtained from histochemical studies (Commins et al., 1987). Results from a number of laboratories indicate that this neurodegenerative effect of MDMA is dependent upon MDMA-induced dopamine release. Although the precise mechanism underlying the requirement is unknown, agents which interfere with the release produced by MDMA can prevent or attenuate its effects on the serotonergic system. Such agents include the dopamine uptake inhibitor GBR 12909 (Stone et al., 1988) and the dopamine synthesis inhibitor α-methyl-p-tyrosine (Stone et al., 1988; Schmidt et al., 1990b). Prior chemical lesioning of the midbrain dopamine systems with 6-hydroxydopamine also prevents the neurotoxicity of MDMA (Stone et al., 1988; Schmidt et al., 1990a). Less direct but equally compelling evidence comes from

Correspondence to: C.J. Schmidt, Marion Merrell Dow Research Institute, 2210 E. Galbraith Road, Cincinnati, OH 45215, USA.

studies demonstrating a correlation between the potency of MDMA analogues for producing serotonergic deficits and releasing dopamine in vitro (Schmidt et al., 1987) and in vivo (Nash and Nichols, 1991).

The observation that selective 5-HT_2 receptor antagonists could prevent the long-term effects of MDMA on the serotonergic system seemed at first inconsistent with the proposed role of dopamine in this response (Schmidt and Kehne, 1990). However, studies by Nash et al. (1990) indicated that this protective effect of 5-HT_2 receptor antagonists may itself occur via alterations in the dopaminergic system. These authors showed that the 5-HT_2 antagonist ketanserin could block both the acute stimulation of dopamine synthesis produced by MDMA as well as the serotonergic deficits measured one week later. A subsequent study demonstrated that ketanserin could attenuate the release of dopamine produced by high doses of MDMA in vivo (Nash, 1990). The link between these acute effects of the 5-HT_2 antagonists and their ability to prevent the long-term neurochemical effects of MDMA was established in experiments showing that the protective effect of the 5-HT_2 antagonist, MDL 11,939, was prevented if dopamine synthesis was maintained by the administration of L-dihydroxyphenylalanine (Schmidt et al., 1991). These studies lead to the hypothesis that the activation of 5-HT_2 receptors is necessary for the stimulation of dopamine synthesis necessary to support MDMA-induced dopamine release.

Although numerous biochemical and behavioral studies have suggested that the serotonergic system may somehow regulate dopaminergic function, the neurochemical mechanism(s) involved in this interaction has eluded description. This may be due to the existence of a number of different serotonergic influences with each being mediated by any one of the several unique 5-HT receptor subtypes now known to be present in the CNS. However, the studies just described clearly suggest that one such serotonergic influence occurs at the level of dopamine synthesis and that this effect is mediated by 5-HT_2 receptors.

We have recently begun to examine this interaction using a number of novel and highly selective 5-HT_2 antagonists. The phenyl piperadine, MDL 100,907, has subnanomolar affinity for the 5-HT_2 receptor and possesses a 300-fold selectivity for this site over either the closely related 5-HT_{1C} or α_1-adrenoceptors (Carr et al., 1991). Considering the selectivity of this agent for the 5-HT_2 receptor over the 5-HT_{1C} site, we have used MDL 100,907 to confirm and extend some of these earlier studies on the effects of 5-HT_2 receptor blockade on dopaminergic function. MDMA-induced dopamine synthesis and release were examined in vivo as was the antagonist's effect on the long-term serotonergic deficits produced by MDMA. The effect of MDL 100,907 on the stimulation of dopamine synthesis by reserpine or haloperidol was also examined as was its effect on haloperidol-induced dopamine release in vivo.

2. Materials and methods

2.1. Ex vivo neurochemical experiments

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, dihydroxyphenylalanine (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 improve the 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 (LSD) test with a level of $P < 0.05$ being accepted as significant.

2.2. In vivo release experiments

2.2.1. Probe design

Microdialysis probes were made using a concentric design. Each probe consisted of a 15 mm 26 gauge stainless steel tube connected to PE20 tubing which served as the inlet. The outlet consisted of a fused silica capillary (75 μm i.d., 150 o.d., Polymicro Tech-

nologies, Phoenix, AZ) passed through the wall of the PE20, threaded down through the stainless steel tubing and into a 4 mm length of sealed dialysis tubing (Spectro/Por hollow fibers, molecular weight cutoff 6000, Spectrum Medical Industries, Los Angeles, CA). A piece of PE10 was attached to the silica capillary where it exits the probe. All junctions were glued using 2-ton epoxy (Devon Bearings, Inc., Brooklyn Hts., OH).

2.2.2. Surgery

Probes were implanted stereotaxically in the right striatum of Sprague-Dawley rats (250–300 g) under pentobarbital anesthesia. Coordinates relative to bregma were 1, 2.5 and −8.0 mm from the top of the skull. A small machine screw was also partially inserted into the skull to serve as an anchor for the probe. Dental acrylic was used to secure the probe to the screw and the skull.

2.2.3. Microdialysis

All experiments were performed 1 day after probe implantation. Each implanted rat was tethered in a circular 12-inch plexiglass container to allow freedom of movement. The containers were placed inside lighted and sound-proof environmental chambers. 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. A Harvard 22 pump (Harvard Apparatus, Inc., South Natick, MA) was used to perfuse the probe at 2 μl/min with artificial cerebrospinal fluid (CSF) containing (mM): NaCl 147.0, KCl 4.0, $CaCl_2$ 2.3 and $MgCl_2$ 0.9, pH 6.3. The dialysate was collected into tubes containing 10 μl of 0.5 M perchloric acid using a refrigerated microfraction collector (Carnegie Medicin, Stockholm, Sweden). A collection period of 20 min was used for all experiments.

MDMA (20 mg/kg s.c.) was administered after 2 h of basal dialysates were collected. In seven of seventeen animals, MDL 100,907 (1 mg/kg s.c.) was administered 20 min prior to MDMA. 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 implantation was verified visually.

2.2.4. Chromatography

Dopamine, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were determined by high performance liquid chromatography with electrochemical detection. Thirty-two microliters of the dialysate were directly injected onto a 3 μm × 3 cm Pecosphere C-18 column (Perkin-Elmer, Norwalk, CT). The column was eluted with a mobile phase containing 0.15 M NaH_2PO_4, 0.15 mM sodium dodecylsulfate, 0.10 mM EDTA and 11% methanol at pH 3.0. The flow rate was maintained at 0.7 ml/min using a Waters 600E multisolvent delivery system (Waters Chromatography Division, Millipore Corporation, Milford, MA). 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.

2.2.5. Calculations

Dopamine efflux for each animal was converted to the percent of basal release using the first 2 h of collection to calculate a mean basal value. The percent basal release at each time point was then averaged for all animals in the MDMA or MDMA plus MDL 100,907 groups. The data are presented as the mean percent basal release ±S.E.M. Total release over the 3 h period following MDMA administration was calculated from the area under the curve (AUC_{180}) for each animal after subtraction of the basal release. The results were averaged for each group and compared by the two-tailed Student's t-test.

2.3. Drugs

MDL 100,907 was administered as a suspension in Tween-80. All other drugs were administered in saline. Doses refer to the salt with the exception of MDMA-HCl and α-methyl-p-tyrosine methyl ester which were given as the free base. MDL 100,907 (R(+)-α-(2,3-dimethoxyphenyl)-1-[2(4-fluorophenylethyl)]-4-piperidinemethanol) was synthesized at the Marion Merrell Dow Research Institute (Cincinnati, OH). MDMA hydrochioride was provided by the National Institute on Drug Abuse (Washington, DC). Reserpine and NSD 1015 were purchased from Sigma Chemical Co. (St. Louis, MO). Haloperidol was purchased from RBI (Natrick, MA).

3. Results

3.1. Inhibition of MDMA-stimulated dopamine synthesis in vivo by MDL 100,907

The administration of MDMA (20 mg/kg s.c.) produced an acute rise in the rate of striatal dopamine synthesis at 1 h as illustrated in fig. 1. The administration of MDL 100,907 at doses of 0.01 to 1 mg/kg s.c. 30 min prior to MDMA completely prevented the increased accumulation of striatal DOPA at all doses tested. In contrast to the effect on stimulated transmitter synthesis, 5-HT_2 receptor blockade with MDL 100,907 did not affect the basal rate of dopamine synthesis.

3.2. Antagonism of MDMA-induced serotonergic deficits by MDL 100,907

As shown in fig. 2, 1 week after the administration of MDMA (30 mg/kg s.c.), 5-HT concentrations in the cortex, hippocampus and striatum had been reduced to 35, 40 and 61% of control, respectively. The higher dose of MDMA was used in these experiments to produce a larger depletion of 5-HT than is typically seen with a 20 mg/kg dose. The effect of the simultaneous administration of MDL 100,907 by the s.c. route over the same dose range used in the DOPA accumulation experiments is also shown. The 5-HT_2 receptor antagonist dose dependently reversed the depletion produced by MDMA in the cortex and hippocampus. A flattened response was observed in the striatum although even the lowest dose of 10 μg/kg MDL 100,907 produced a significant effect. All three brain areas showed a complete reversal of this long-term effect of MDMA following MDL 100,907 administration.

3.3. Antagonism of MDMA-induced dopamine release in vivo by MDL 100,907

In vivo microdialysis in awake freely moving rats was used to assess the effect of 5-HT_2 receptor blockade on the release of striatal dopamine by MDMA. The effect of direct inhibition of dopamine synthesis with the tyrosine hydroxylase inhibitor, α-methyl-p-tyrosine (α-MPT), on MDMA-induced dopamine release was also examined for comparative purposes.

Figure 3A and B show the increase in extracellular dopamine concentrations in the striatum following the injection of MDMA (20 mg/kg s.c.). Transmitter levels

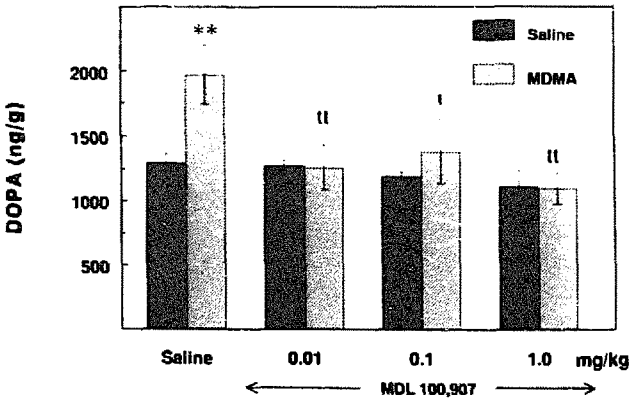

Fig. 1. 5-HT_2 receptor blockade with MDL 100,907 prevents MDMA-induced stimulation of striatal dopamine synthesis in vivo. Rats were pretreated with MDL 100,907 30 min prior to MDMA (20 mg/kg s.c.) and 60 min prior to NSD 1015 (100 mg/kg i.p.). All animals were killed at 90 min. Data are provided as the mean of five animals ± S.E.M. ** $P < 0.01$ compared to saline; t, tt $P < 0.05$, 0.01 compared to MDMA, respectively, by ANOVA-LSD.

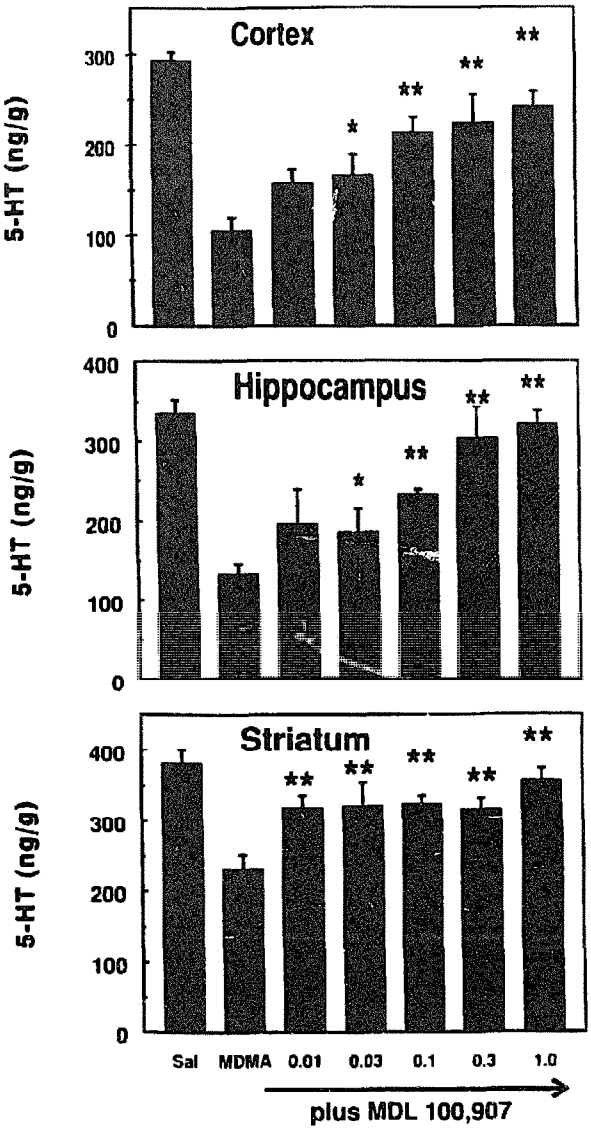

Fig. 2. MDL 100,907 dose dependently prevents the MDMA-induced serotonergic deficits measured 1 week after drug administration. Rats were given MDMA (30 mg/kg s.c.) with vehicle or MDL 100,907 simultaneously 1 week prior to being killed. Data are presented as the mean of five or more animals ± S.E.M. *-** $P < 0.05$, 0.01 compared to MDMA alone by ANOVA-LSD.

began to increase approximately 40 min after drug administration and peaked at approximately 1400% of control at 100 min. Transmitter levels remained elevated above baseline for the entire 4 h of collection following MDMA administration.

The administration of α-MPT (200 mg/kg i.p.) 20 min prior to MDMA significantly attenuated the stimulated increase in extracellular dopamine (fig. 3A). Based on the area under the curve for the 180 min immediately following drug administration (AUC_{180}), α-MPT reduced extracellular levels of the transmitter by 72% ($P < 0.01$).

The effect of MDL 100,907 (1 mg/kg s.c.) administered 20 min prior to MDMA on extracellular

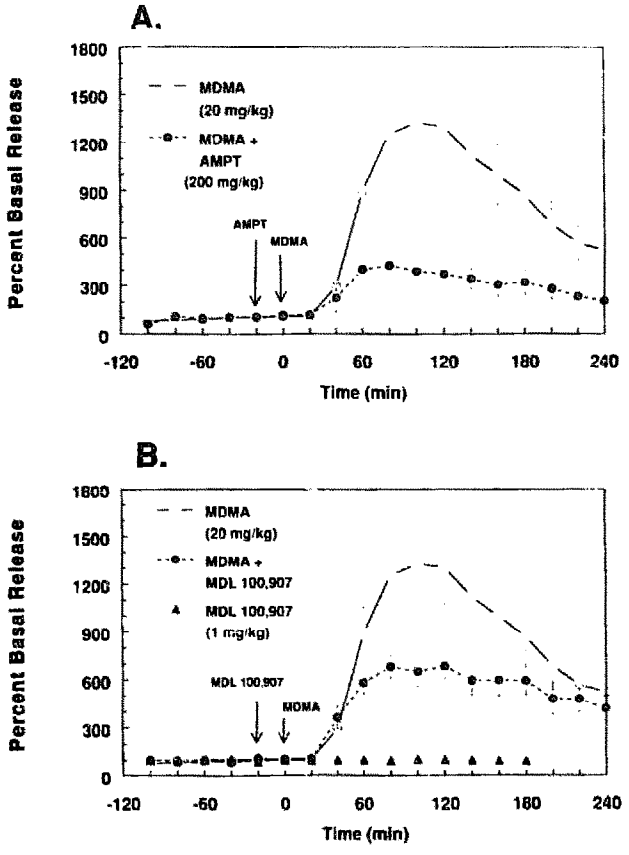

Fig. 3. Attenuation of MDMA-induced dopamine release in vivo in the rat striatum by (A) α-MPT or (B) MDL 100,907. Rats were implanted stereotaxically 24 h prior to dialysis. Fractions were collected every 20 min using a flowrate of 2 μl per min. Data are presented as the mean percent change ± S.E.M. over basal release which was calculated as the average of the first four fractions. Basal efflux typically ranged from 5 to 10 pg of dopamine/32 μl of dialysate without correction for recovery. The n for each curve was MDMA (10), α-MPT (3), MDMA plus MDL 100,907 (7) and MDL 100,907 alone (6). MDMA and MDL 100,907 were administered by the s.c. route while α-MPT was administered i.p.

dopamine concentrations is shown in fig. 3B. The 5-HT_2 antagonist also produced a significant reduction in the MDMA-induced increase in extracellular concentrations of dopamine. The primary effect appeared to be a blunting of the peak release produced by MDMA. Release occurring both in the absence and presence of the 5-HT_2 antagonist was again similar by the termination of the experiment. AUC_{180} calculations show a 46% decrease in stimulated transmitter efflux ($P < 0.05$). MDL 100,907 alone was without effect on baseline concentrations of striatal dopamine. Figure 4A and B show the effect of MDMA on the in vivo levels of DOPAC and HVA in the absence and presence of MDL 100,907. Striatal concentrations of both dopamine metabolites began declining within 40 min of MDMA administration. In contrast to the pattern of MDMA-induced changes observed for dopamine concentrations, DOPAC and HVA levels declined to approximately 20% of control where they remained for the balance of the experiment. The administration of MDL 100,907 was without effect on basal levels of both metabolites and did not alter the effect of MDMA on concentrations of DOPAC. However, the MDMA-induced decline in striatal HVA concentrations was enhanced to a small but significant extent in the presence of MDL 100,907 ($AUC_{180} = 82.1\%$ of MDMA alone, $P < 0.01$).

3.4. Effect of 5-HT_2 receptor blockade with MDL 100,907 on the stimulation of dopamine synthesis produced by reserpine or haloperidol

Both reserpine and haloperidol are known to increase dopamine synthesis in vivo. Since the mechanism(s) for this activation differs from that of MDMA, the effect of MDL 100,907 on the stimulation of

TABLE 1

Effect of 5-HT_2 receptor blockade with MDL 100,907 on the stimulation of striatal dopamine synthesis by haloperidol or reserpine. Haloperidol and reserpine were administered 60 min prior to NSD 1015 (100 mg/kg i.p.) and 90 min prior to being killed.

	Striatal DOPA (ng/g tissue) (% saline control)			
	Control	Haloperidol (0.5 mg/kg i.p.)	Control	Reserpine (5.0 mg/kg i.p.)
Saline	1354 ± 78 (100 ± 6%)	4761 ± 585 (352 ± 43%) [b]	1388 ± 66 (100 ± 5%)	2882 ± 496 (208 ± 36%) [a]
MDL 100,907				
0.01 mg/kg	1266 ± 99 (94 ± 7%)	3310 ± 892 (244 ± 66%) [a]	1334 ± 55 (96 ± 4%)	3417 ± 701 (246 ± 51%) [b]
0.10 mg/kg	807 ± 157 (60 ± 12%)	5084 ± 349 (375 ± 26%) [b]	–	–
1.0 mg/kg	1400 ± 12 (103 ± 8%)	5145 ± 1328 (380 ± 98%) [b]	1309 ± 120 (94 ± 9%)	3491 ± 561 (252 ± 7%) [b]

[a] $P < 0.05$, [b] $P < 0.01$.

dopamine synthesis produced by each of these agents was also examined (table 1). Reserpine (5 mg/kg i.p.) approximately doubled the rate of striatal DOPA accumulation 90 min following drug administration ($P < 0.05$). The simultaneous administration of MDL 100,907 at doses of 0.01 and 1.0 mg/kg (s.c) had no effect on this accelerated rate of dopamine synthesis. Haloperidol (0.5 mg/kg i.p.) produced an even larger increase in dopamine synthesis at the same time point (352% of control). MDL 100,907 was also ineffective at altering the haloperidol-stimulated accumulation of DOPA.

3.5. Effect of MDL 100,907 on the haloperidol-induced increase in striatal dopamine release in vivo

Haloperidol (0.5 mg/kg s.c.) produced a modest increase in the extracellular concentration of dopamine in the striatum (fig. 5). Transmitter levels rose to approximately 150% of basal within 60 to 80 min of drug administration and remained stable for the duration of the experiment. The administration of MDL 100,907 (1 mg/kg s.c.) 20 min prior to haloperidol had no effect on this rate of transmitter efflux. The haloperidol-induced increase in the concentration of both DOPAC and HVA was also unaffected by the 5-HT_2 antagonist (not shown).

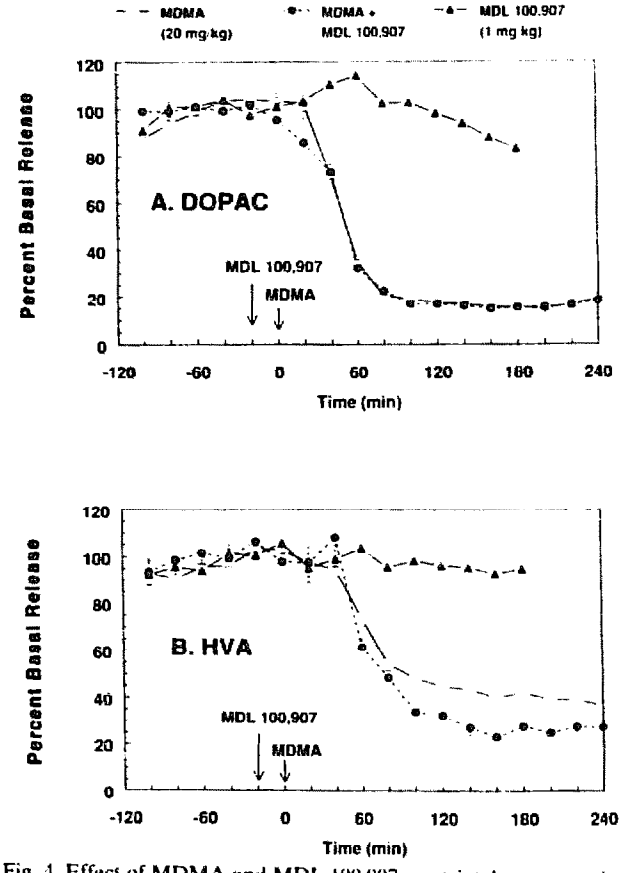

Fig. 4. Effect of MDMA and MDL 100,907 on striatal concentrations of DOPAC and HVA as measured by microdialysis. Basal concentrations of DOPAC and HVA ranged from 2 to 5 ng/32 μl of dialysate. See fig. 3 for additional details.

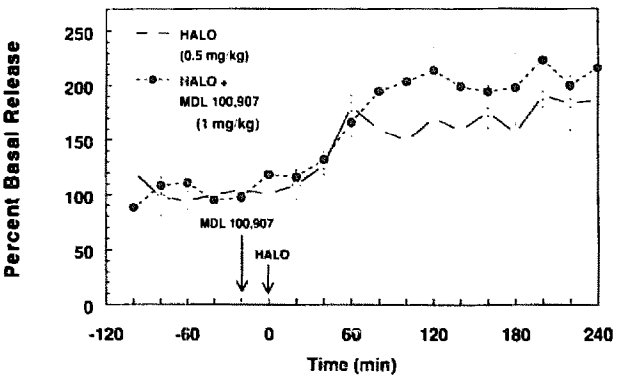

Fig. 5. Lack of effect of 5-HT_2 receptor blockade on the increase in striatal dopamine concentrations produced by haloperidol (0.5 mg/kg s.c.) in vivo. The n values for haloperidol and haloperidol plus MDL 100,907 were 4 and 5, respectively. See fig. 3 for additional details.

4. Discussion

Serotonergic regulation of the dopamine system is a well-accepted concept. The majority of pharmacological and behavioral studies of this interaction suggest that 5-HT exerts an inhibitory influence on dopaminergic activity (Fibiger and Miller, 1977; Dray et al., 1978; Tsai, 1989; Ugedo et al., 1989). For example, chemical depletion or lesions of the serotonergic system have been shown to increase dopamine turnover (Giambalvo and Snodgrass, 1978) as well as potentiate the stimulant properties of amphetamine (Mabry and Campbell, 1973; Segal, 1976). However, there are reports suggesting that the serotonergic system can have a stimulatory effect on some aspects of dopaminergic activity. De Simoni et al. (1987) have reported that electrical stimulation of the dorsal raphe nucleus increases dopamine metabolism in the striatum without increasing transmitter release. Direct infusion of 5-HT into the ventral tegmental area (VTA) reportedly has a similar effect on dopamine metabolism in the nucleus accumbens (Guan and McBride, 1989). The present study describes conditions under which specific serotonergic input also appears to have a positive modulatory effect on dopaminergic activity and neurotransmission.

Our results support the hypothesis that 5-HT_2 receptors can regulate dopamine synthesis under very specific conditions and that interference with this regulation can have functional consequences. These consequences include an inhibition of transmitter release produced by the amphetamine analogue MDMA and antagonism of the serotonergic deficits produced as a result of that release. The high selectivity of MDL 100,907 and its potency for antagonism of these actions

of MDMA is convincing evidence that these effects are mediated specifically by 5-HT_2 receptors.

Previous studies of the effect of 5-HT_2 antagonists on dopamine synthesis induced by MDMA have used less specific antagonists such as ketanserin (Nash et al., 1990) or MDL 28,133A (Schmidt et al., in press). In addition to its high affinity for the 5-HT_2 receptor ($K_i = 3.2$ nM), ketanserin has relatively high affinity for both the α_1-adrenoceptor ($K_i = 6.7$ nM, Dudley et al., 1988) and the so-called 'ketanserin-binding site' associated with the synaptic vesicles of monoaminergic neurons (Leysen et al., 1987). Ketanserin also has significant affinity for the 5-HT_{1C} receptor (Sanders-Bush and Breeding, 1988; Van Wijngaarden et al., 1990). While MDL 28,133A distinguishes between subtypes of the 5-HT receptor, the compound also has high nanomolar affinity for the D_2 dopamine site (Carr et al., 1991). Furthermore, in a previous study, MDL 28,133A generated an unexplained U-shaped dose-response curve for the inhibition of MDMA stimulated dopamine synthesis (Schmidt et al., in press). In comparison, MDL 100,907 has subnanomolar affinity for the 5-HT_2 receptor ($K_i = 0.36$ nM) with minimum 300-fold lower affinity for all other neurotransmitter receptors examined, including the 5-HT_{1C} subtype (K_i = 105 nM, Carr et al., 1991). The complete blockade of MDMA-induced dopamine synthesis by MDL 100,907 at doses as low as 10 μg/kg (fig. 1) identifies the 5-HT_2 receptor as the subtype involved in mediating this effect on dopaminergic function.

These data for MDL 100,907 do not preclude the existance of an interaction between other 5-HT receptor subtypes and the dopamine system. Indeed, numerous studies have reported on the effects of 5-HT_{1A} and 5-HT_3 ligands on dopaminergic function (e.g. Invernizzi et al., 1988; Carboni et al., 1989). However, we have previously shown that the serotonergic deficits produced by MDMA are not blocked by antagonists of either 5-HT_{1A} or 5-HT_3 receptors (Schmidt et al., 1990a) which would further support our conclusion that the effects observed in the present study are due exclusively to the blockade of 5-HT_2 receptors.

A majority of the available data supports the hypothesis that the ability of MDMA to produce serotonergic nerve terminal degeneration is dependent on the dopamine release produced by the amphetamine analog. MDMA-induced dopamine release has been characterized both in vitro (Johnson et al., 1986; Schmidt et al., 1987) and in vivo (Hiramatsu and Cho, 1990; Nash, 1990; Gough et al., 1991). Like other amphetamines, MDMA is believed to release dopamine from the nonvesicular or newly synthesized pool of transmitter by a carrier-mediated process (Schmidt et al., 1987). Thus, the synthesis inhibitor, α-MPT, has the rapid effect on MDMA-induced dopamine release demonstrated here (fig. 3A) and antagonizes the serotonergic deficits produced by MDMA (Stone et al., 1988; Schmidt et al., 1990a). Like α-MPT, MDL 100,907 is an effective antagonist of both MDMA-induced 5-HT deficits and MDMA-induced transmitter release. A 20-min pretreatment with MDL 100,907 was sufficient to reduce extracellular concentration of dopamine (fig. 3B) and its extraneuronal metabolite, HVA (fig. 4B), following MDMA administration. This effect occurs at a dose of MDL 100,907 shown to block MDMA-stimulated dopamine synthesis in vivo. The results of this study are therefore consistent with both the proposed role for the dopaminergic system in the long-term effects of MDMA and with a role for 5-HT_2 receptors in the regulation of dopaminergic function.

The apparent requirement for 5-HT_2 receptor activation in the stimulation of dopamine synthesis produced by MDMA is not observed for all initiators of dopamine synthesis. In contrast to its potent antagonism of MDMA-stimulated dopamine synthesis, MDL 100,907 was without effect on the more robust stimulation of synthesis produced by either reserpine or haloperidol (table 1). The modest haloperidol-induced increase in extracellular concentrations of dopamine was also unaffected by MDL 100,907 (fig. 5). The most obvious difference between MDMA and these agents is the mechanism by which they stimulate activity in the nigrostriatal pathway. Increases in dopamine synthesis due to amphetamines such as MDMA occur in response to the carrier-mediated release of transmitter (Connor and Kuczenski, 1986). Although not entirely clear, this stimulation of synthesis may be due to disinhibition of tyrosine hydroxylase following the release of newly synthesized dopamine and the inhibition of reuptake (Maura and Raiteri, 1982). The effects of reserpine and haloperidol are less directly related to release and more a function of an increase in impulse flow resulting from the reduction in receptor-mediated feedback inhibition. For reserpine, this is due to dopamine depletion (German et al., 1981) while with haloperidol, direct D_2 receptor blockade is responsible (Carlsson et al., 1972). Presynaptic receptor vacancy in the striatum probably contributes to the enhanced synthesis produced by such agents (Walters and Roth, 1976; Commissiong et al., 1990). In contrast to the effects of reserpine and haloperidol, high doses of MDMA significantly decrease the firing rate of nigrostriatal neurons presumably by increasing D_2 receptor activation (Kelland et al., 1989; Matthews et al., 1989; Schmidt et al., in press). However, a decrease in firing rate does not appear to be the critical determinant of serotonergic involvement. The γ-aminobutyric acid-mimetic, γ-butyrolactone (GBL), stimulates dopamine synthesis by inhibiting dopamine cell firing and transmitter release. The resultant reduction in occupancy of the terminal autoreceptors is believed to relieve a tonic feedback inhibition of synthesis (Walters and Roth,

1976). Yet the stimulation of dopamine synthesis produced by GBL is unaffected by the 5-HT_2 antagonist, MDL 28,133A (Schmidt et al., in press). The remaining interpretation of these observations is that 5-HT_2 receptor stimulation is not involved in those increases in dopamine synthesis which occur due to the elimination of dopamine receptor-mediated feedback inhibition. This would be true if the effects of the 5-HT_2 antagonists on dopaminergic function are mediated by changes in dopamine autoreceptor activity. Although we were previously unable to find any evidence that the 5-HT_2 receptor antagonist, MDL 28,133A, altered the ability of apomorphine to inhibit GBL-stimulated dopamine synthesis, the interpretation of such experiments is complicated by the fact that GBL may reduce serotonergic tone to the point that the addition of the 5-HT_2 antagonist is superfluous. Furthermore, experiments using the GBL model only consider those dopamine autoreceptors coupled to transmitter synthesis. Those autoreceptors linked to transmitter release at the terminals as well as the somatodendritic receptors regulating firing rate must also be considered as potential sites of serotonergic regulation.

The effect of each of the activators of dopamine synthesis on serotonergic tone is an additional consideration. MDMA should increase 5-HT_2 receptor activation by virtue of its potent 5-HT releasing action (Johnson et al., 1986; Schmidt et al., 1987). In contrast, reserpine depletes 5-HT and should reduce 5-HT release while haloperidol is reported to be without effect on the firing rate of dorsal raphe neurons (Gallager and Aghajanian, 1976). Thus the extent of 5-HT_2 receptor activation is likely to be higher following MDMA administration than after either reserpine or haloperidol. Leysen and Pauwels (1990) have proposed that the 5-HT_2 receptor is seldom activated under normal physiological conditions. This is consistent with the lack of effect of 5-HT_2 receptor antagonists on either basal dopamine synthesis or release (Nash et al., 1990; Nash, 1990; Schmidt et al., in press). Hence the regulation of dopamine synthesis mediated by 5-HT_2 receptors is likely to be a phasic rather than a tonic effect which becomes significant only during states of high serotonergic and dopaminergic transmission. The requirement for both serotonergic and dopaminergic activity to be elevated simultaneously may explain the ability of electrical stimulation of the DRN to increase dopamine metabolism without increasing release (De Simoni et al., 1987). Serotonergic activation of dopamine synthesis in the absence of an increased demand for dopamine release would merely result in the metabolism of the excess transmitter.

An important question is what other situations may produce such a state of neuronal activity? Currently, there is evidence accumulating that an imbalance of serotonergic and dopaminergic activity may underlie some symptoms of schizophrenia (Meltzer, 1991). 5-HT_2 receptors are reportedly reduced in number in the brains of schizophrenics (Mita et al., 1986) which may suggest enhanced activity at these receptors. This is consistent with the reduced neuroendocrine response of schizophrenics to the 5-HT_2/5-HT_{1C} agonist MK 212 and reports of elevated 5-hydroxyindoleacetic acid in the CSF of schizophrenics (Meltzer, 1991). The improved efficacy of clozapine over typical antipsychotic agents has been attributed to its antagonism of 5-HT_2 receptors (Meltzer, 1989; Meltzer et al., 1989).

Thus the ability of 5-HT_2 antagonists to regulate phasic increases in dopaminergic activity may underlie their potential use in the treatment of schizophrenia. MDL 100,907 has potent effects in a number of animal models believed to be predictive of antipsychotic activity including inhibition of amphetamine-induced locomotion and reversal of the slowing of A10 dopaminergic neurons produced by amphetamine (Sorensen et al., 1992). Chronic treatment of rats with MDL 100,907 also produces a selective reduction in the activity of A10 dopaminergic neurons (Sorensen et al., 1992).

In conclusion, pharmacological blockade of 5-HT_2 receptors with the highly selective antagonist, MDL 100,907 prevents the activation of dopamine synthesis necessary to support dopamine release stimulated by the amphetamine analogue, MDMA. Such treatment also blocks the long-term MDMA-induced deficits in serotonin concentrations believed to be due to dopamine release. The stimulation of dopamine synthesis produced by the elimination of receptor-mediated feedback inhibition is not altered by 5-HT_2 blockade. The results suggest that the serotonergic regulation of dopamine synthesis is conditional with respect to ongoing dopaminergic and serotonergic activity.

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