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METHYLENEDIOXYMETHAMPHETAMINE-INDUCED INHIBITION OF NEURONAL FIRING IN THE NUCLEUS ACCUMBENS IS MEDIATED BY BOTH SEROTONIN AND DOPAMINE

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Abstract—Methylenedioxymethamphetamine (MDMA) is a mood-altering, legally restricted drug that has been reported to inhibit glutamate-evoked firing of cells in the nucleus accumbens. This study used extracellular recording combined with microiontophoresis to examine whether the inhibitory effect of MDMA on neuronal firing in the nucleus accumbens is mediated by serotonin and/or dopamine. Serotonin and serotonin agonists with relative selectivity for the receptor subtypes 5-HT_{1A}, 5-HT_{1B}, 5-$HT_{2A/2C}$ and 5-HT_3 all significantly ($P < 0.01$) inhibited glutamate-evoked firing of cells in the nucleus accumbens compared to the effects of an acidic saline control solution (30–60 nA, 60 s ejection currents for all). The current (dose)-dependent inhibition produced by the serotonin agonists did not differ significantly from the inhibition produced by MDMA except for the 5-HT_{1A} agonist 8-hydroxy-(2-di-*n*-propylamino)tetralin, which inhibited glutamate-evoked firing significantly more than MDMA or any of the other serotonin agonists. At the highest ejection current tested (60 nA, 60 s), glutamate-evoked firing was inhibited by MDMA in 94% of tested cells, by serotonin in 80% of tested cells and by the serotonin receptor subtype agonists in 95–100% of the tested cells. In addition to being mimicked by serotonin and serotonin agonists, MDMA-induced inhibition of glutamate-evoked firing in the nucleus accumbens was partially blocked by the serotonin antagonists ketanserin (100% of tested cells), methysergide (80% of tested cells), methiothepin (100% of tested cells) and WAY100135 (100% of tested cells). Furthermore, application of the serotonin uptake blocker fluoxetine, which prevents MDMA-induced serotonin release, also significantly attenuated MDMA-induced inhibition of glutamate-evoked firing in all of the cells that were tested. These observations suggest that MDMA-induced inhibition of nucleus accumbens cell firing is at least partially mediated by serotonin. Depletion of dopamine by pretreatment with the neurotoxin 6-hydroxydopamine and the synthesis inhibitor α-methyl-*p*-tyrosine blocked the inhibition of glutamate-evoked firing produced by MDMA applied with low ejection currents (30–40 nA, 60 s). However, this dopamine depletion had no effect on inhibition of glutamate-evoked firing produced by serotonin ejected with low or high currents (20–60 nA, 60 s).

These results suggest that both dopamine release and an intermediate step of MDMA-induced serotonin release are necessary for the inhibitory effects of MDMA on neuronal excitability in the nucleus accumbens. The dopamine- and serotonin-mediated inhibitory effects of MDMA on glutamate-evoked firing of nucleus accumbens cells may play a role in the mood-altering properties of this increasingly popular drug. Copyright © 1996 IBRO. Published by Elsevier Science Ltd.

Key words: MDMA, serotonin, dopamine, drug abuse, nucleus accumbens, ventral striatum.

Methylenedioxymethamphetamine (MDMA) or ''ecstasy'' is an amphetamine derivative that induces elevated mood, increased energy and heightened alertness, as well as peripheral sympathomimetic symptoms in humans.[53,64] A variety of evidence suggests that the CNS stimulant and euphoric properties of MDMA are likely to be mediated by alterations in monoaminergic neurotransmission in the brain. Acute administration of MDMA increases extracellular levels of both dopamine (DA) and serotonin (5-hydroxytryptamine, 5-HT) in several forebrain and brainstem regions.[3,14,19,20,27,41,43,61,73,77] Systemically or locally applied MDMA alters neuronal activity in the brain, decreasing the excitability of serotonergic cells in the dorsal raphe nucleus,[63] dopaminergic cells in the ventral tegmental area,[28,35] and neurons in the prefrontal cortex, nucleus accumbens and caudate–putamen that are innervated by serotonergic and dopaminergic terminals.[46,47,73,74]

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Abbreviations: AMPT, α-methyl-*p*-tyrosine; CGS12066B, 7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrrolo(1,2-a) quinoxaline; CPBG, 1-(*m*-chlorphenyl)-biguanide; DA, dopamine; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; 5-HT, serotonin (5-hydroxytryptamine); MDMA, methylenedioxymethamphetamine; 2m5-HT, 2-methyl-5-hydroxytryptamine; 6-OHDA, 6-hydroxydopamine; 8-OH-DPAT, 8-hydroxy-(2-di-*n*-propylamino)tetralin; WAY100135, *n*-tert-butyl-3-4-(2-methoxyphenyl)piperazin-1-yl-2-phenylpropanamide dihydrochloride).

The effects of MDMA on neurotransmission within the nucleus accumbens are of particular interest because dopaminergic neurotransmission in this forebrain region has been implicated in the rewarding properties of cocaine and several other drugs of abuse.[13,32]

Locally applied MDMA inhibits glutamate-evoked firing of most cells in the nucleus accumbens,[73] and increases extracellular levels of DA and 5-HT in the nucleus accumbens and adjacent regions of the ventral striatum as measured by *in vivo* dialysis or *in vivo* voltammetry.[41,73,77] The inhibitory effect of MDMA on neuronal firing in the nucleus accumbens appears to be at least partially mediated by DA. Locally applied DA and DA agonists inhibit firing of most cells in the nucleus accumbens,[71,76] and the MDMA-induced inhibition of accumbens cell firing is partially blocked by the dopaminergic D_1 antagonist SCH39166.[73]

The role of 5-HT in MDMA-induced inhibition of neuronal firing in the nucleus accumbens is less clear. Although glutamate-evoked firing of most accumbens cells is inhibited by microiontophoretic application of 5-HT, MDMA-induced inhibition of accumbens cells is not attenuated by intravenous administration of the 5-HT antagonist methysergide.[73] Furthermore, 5-HT increases glutamate-evoked firing of a subpopulation of nucleus accumbens cells,[70,72] and has been reported to produce a direct, 5-HT_2 receptor-mediated depolarization of neurons in the nucleus accumbens in brain slice preparations.[44] Multiple receptor subtypes for 5-HT are present in the nucleus accumbens, including high densities of 5-HT_4 receptors,[25] intermediate densities of 5-HT_{2A} (formerly 5-HT_2), 5-HT_{2C} (formerly 5-HT_{1C}) and 5-HT_{1B} receptors,[37,38,51,52] moderate to low densities of 5-HT_3 receptors,[1,30] and low or sparse densities of 5-HT_{1A} receptors.[29] These receptor subtypes may mediate differential and potentially opposite direct and indirect effects on neuronal excitability. For example, it is well established that 5-HT or 5-HT agonists applied locally in the nucleus accumbens or the striatum induce an increase in extracellular DA.[2,5,9,17,2448] Thus, 5-HT may have an indirect inhibitory effect on nucleus accumbens neuronal excitability by releasing DA, in addition to having a direct depolarizing effect on some of the cells in the nucleus.

In the present study, relatively selective agonists and antagonists for 5-HT receptor subtypes were employed to determine (i) whether agonists that are relatively selective for different receptor subtypes may have differential effects on nucleus accumbens neuronal excitability *in vivo* and (ii) whether any of the 5-HT receptor subtype antagonists might attenuate the effects of MDMA on nucleus accumbens neuronal excitability. In addition, 5-HT was tested in a group of rats that were depleted of DA using the neurotoxin 6-hydroxydopamine (6-OHDA) combined with a synthesis inhibitor to determine whether 5-HT has effects on neuronal excitability in the nucleus accumbens that are independent of 5-HT-induced DA release.

EXPERIMENTAL PROCEDURES

Animals

Adult male Sprague–Dawley rats (250–350 g) were obtained from Simonsen Breeding Laboratories (Gilroy, CA, U.S.A.). The animals were housed in a temperature- and humidity-controlled environment with food and water available *ad libitum*. Recordings were made from the core region of the nucleus accumbens of 62 rats.

Materials

The 5-HT agonists used were: 8-hydroxy-(2-di-*n*-propylamino)tetralin (8-OH-DPAT), a selective agonist for 5-HT_{1A} receptors;[40,67] 7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrrolo(1,2-a)quinoxaline (CGS12066B), an agonist with 17-times more affinity for 5-HT_{1B} than 5-HT_{1A} receptors and minimal affinity for adrenoreceptors or DA receptors;[42] 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a selective agonist for 5-$HT_{2A,2C}$ receptors;[18,67] 1-(*m*-chlorphenyl)-biguanide (CPBG), a selective agonist for 5-HT_3 receptors;[33] and 2-methyl-5-hydroxytryptamine (2m5-HT), an agonist with relatively high selectivity for 5-HT_3 receptors.[23,33]

The 5-HT antagonists used were: *n*-tert-butyl-3-4-(2-methoxyphenyl)piperazin-1-yl-2-phenylpropanamide dihydrochloride) (WAY100135), a selective 5-HT_{1A} receptor antagonist;[15,59] ketanserin, a selective 5-$HT_{2A,2C}$ receptor antagonist that also has affinity for adrenoceptors;[26,67] methysergide, a non-selective 5-HT antagonist with higher affinity for 5-HT_2 receptors than for 5-HT_1 receptors;[54] and methiothepin, a non-selective 5-HT antagonist with higher affinity for 5-HT_1 receptors than methysergide.[34]

Fluoxetine was employed as a specific blocker of 5-HT uptake.[75] 6-OHDA, a specific catecholaminergic neurotoxin,[65] and α-methyl-*p*-tyrosine (AMPT), an inhibitor of catecholamine synthesis, were administered to deplete DA in a group of six rats.

(±)-3,4-MDMA was provided by the National Institute on Drug Abuse (Bethesda, MD, U.S.A.), ketanserin was a gift from Janssen Pharmaceutica Research Laboratories, fluoxetine was a gift from Eli Lilly and Company, methysergide was a gift from Sandoz Pharmaceuticals and WAY100135 was a gift from Wyeth Research (U.K.) Ltd. DOI, CPBG, 2m5-HT, 8-OH-DPAT and methiothepin were purchased from Research Biochemicals Inc. All other chemicals were purchased from Sigma Chemical Co.

Neuronal recording

Rats were anesthetized with urethane (1.25 g/kg, i.p., with i.v. supplements as needed). The trachea was intubated to assist respiration and the right femoral vein was cannulated for intravenous drug administration. The skull overlying the nucleus accumbens was removed, leaving intact a narrow midline strip of bone overlying the sinus. The head was fixed in a Kopf stereotaxic headholder, the meninges were cut and the electrode was inserted into the brain 10.0–11.0 mm anterior to the interaural line and 1.1–2.0 mm lateral to the midline, and advanced 5.7–7.2 mm below the surface of the cortex.[50] All recordings were made from the nucleus accumbens in the right hemisphere.

Seven-barreled glass microdot microelectrodes with tip diameters of 7–10 μm were used for single-cell extracellular recording and iontophoretic application of drugs. The center barrel and one side-barrel were filed with 4 M NaCl for recording and automatic current balance. Other barrels contained 0.2 M monosodium-L-glutamate, pH 6.5, to drive quiescent cells; 2% Pontamine Sky Blue in 0.5 M

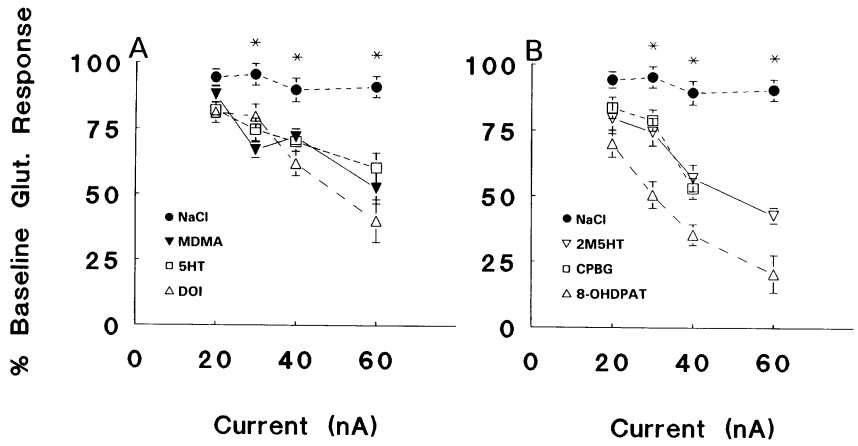

Fig. 1. Microiontophoretic application (20–60 nA, 60 s) of MDMA, 5-HT and 5-HT receptor subtype agonists all significantly inhibited glutamate-evoked firing of neurons in the core region of the nucleus accumbens. Means and S.E.M. are plotted. (A) The effects of MDMA, 5-HT and the 5-$HT_{2A,2C}$ receptor agonist DOI are compared to effects of the acidic saline control solution (NaCl) applied with equivalent ejection currents. MDMA was tested on 57 cells in 28 rats, 5-HT on 44 cells in nine rats and DOI on 35 cells in 10 rats. (B) Effects of agonists that are relatively selective for 5-HT_3 receptors (2m5-HT and CPBG) and 5-HT_{1A} receptors (8-OH-DPAT) were also compared to effects of the control solution (NaCl). 2m5-HT was tested on 13 cells in five rats, CPBG on 27 cells in seven rats and 8-OH-DPAT on 18 cells in six rats. *Significant difference between the acidic saline control solution (NaCl) and the test drugs, $P < 0.01$.

sodium acetate to mark the position of the electrode tip; and some combination of (±)-3,4-MDMA hydrochloride, 0.01 M, pH 5–6; 5-HT creatinine sulfate, 0.04 M, pH 4.5; DA hydrochloride, 0.8 M, pH 5; DOI hydrochloride, 0.03 M, pH 4–6; CPBG hydrochloride, 0.025 M, pH 5; 2m5-HT maleate, 0.025 M, pH 5–6; 8-OH-DPAT hydrobromide, 0.025 M, pH 5–6; CGS12066B maleate, 0.001 M, pH 5; ketanserin tartrate, 0.01 M, pH 4; methiothepin mesylate, 0.01 M, pH 4.5; WAY100135 hydrochloride, 0.005 M, pH 4.5; methysergide maleate, 0.004 M, pH 4; fluoxetine, 0.01 M, pH 4.5; and acidic saline (NaCl), 0.01 M, brought to pH 4 with hydrochloric acid for pH control. Drugs were made up in deionized water except for DOI, which was dissolved in saline and brought to pH 4–6 with NaOH or HCl. Solutions were frozen in aliquots suitable for one experiment and used within 6 h of thawing. A five-channel Medical Systems microiontophoresis system was used to eject and retain the drugs in the microelectrode barrels. Retaining currents were +10 nA for glutamate and −10 nA for all other solutions except the dye, which received no retaining current. Glutamate and the dye were ejected as anions, all other drugs were ejected as cations.

Cells in the core region of the nucleus accumbens were driven at stable, relatively low firing rates by cycled 10-s pulses of glutamate delivered every 20 s throughout the recording session. Neuronal activity was monitored with a Tektronix oscilloscope and audio monitor and stored on videocassette tape. Single units were isolated with a Neurolog window discriminator, and spikes from single units were counted with a pulse integrator. Cumulative counts of spikes from individual cells were displayed with a Grass (curvilinear) oscillograph. Glutamate ejection currents which produced a stable baseline response were established empirically for each cell. Following establishment of a glutamate response that remained stable for at least 3 min, effects of MDMA, 5-HT or 5-HT agonists were tested. No more than two test drugs were applied to a single cell. Test drugs were applied for 60 s at increasing current intensities (doses), with at least 3 min separating each application. Cells were excluded from the experiment if the action potential amplitude or shape changed during the test drug ejection period. Current–response curves were generated for each test drug for each cell by comparing the average number of spikes that occurred during the drug ejection period produced by the 60-s application of each current intensity to the average number of spikes that occurred in the 2-min baseline period which preceded drug ejection. A change of 15% or more from the baseline glutamate response was considered to be significant. This value was selected because it was greater than the inhibition that was produced occasionally by ejections of the acidic saline control solution (see Fig. 1). Effects of MDMA and 5-HT agonists on glutamate-evoked firing were compared to the effects of the acidic saline control solution using two-way, repeated measures analysis of variance, with Tukey *post hoc* comparisons of means as appropriate.

To test whether 5-HT antagonists could block MDMA effects on glutamate-evoked firing, MDMA was applied for a 60-s period during the third minute of a 5-min application of the antagonist. Following offset of antagonist application, MDMA tests were repeated every 3 min until the MDMA response returned to pre-antagonist levels. Paired, two-tailed *t*-tests were used to compare MDMA effects prior to antagonist ejection to MDMA effects during antagonist ejection. Effects of DA depletion on nucleus accumbens cell responses to 5-HT and MDMA were tested using animals that had been pretreated with the neurotoxin 6-OHDA. Responses to 5-HT and MDMA were compared in 6-OHDA-pretreated and vehicle-pretreated control rats using two-way, repeated measures analysis of variance.

At the end of a recording session for a cell, the position of the electrode was marked by application of Pontamine Sky Blue dye (30 μA negative current for 12 min). Brains were removed at the end of the experiment, fixed in formalin, sectioned at 50 μm and counterstained with Cresyl Violet. Locations of dye spots for recording sites and needle tracts for i.c.v. injections were determined histologically.

Dopamine depletion

Six rats received i.c.v. injections of the neurotoxin 6-OHDA and six control rats received i.c.v. injections of the vehicle. The rats were preteated with i.p. injections of desipramine hydrochloride (25 mg/kg) to prevent uptake of the neurotoxin into noradrenergic terminals. Thirty minutes

later, the rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and burr holes were made in the skull over the right lateral ventricle. A Hamilton syringe with a 30-gauge needle was used to slowly infuse 5 µl of 6-OHDA (100 mg free base in 0.1% ascorbic acid in 0.9% NaCl) into the right lateral ventricle, or 5 µl of the ascorbic acid vehicle alone. The scalp was sutured and xylocaine jelly was applied to the sutured region. Rats were fed a soft mash of ground rat chow and apple sauce for one week following surgery. Nucleus accumbens recording sessions were conducted 12–16 days following the i.c.v. injection. On the day of recording, rats in the group that received the 6-OHDA injections were given an injection of the tyrosine hydroxylase inhibitor AMPT (300 mg/kg, i.p.), followed 2 h later by a second injection (200 mg/kg, i.p.). This combination of 6-OHDA and AMPT treatment has been reported to reduce ipsilateral striatal DA by 99%.[22] Recording sessions began 90 min after the second AMPT injection and continued for no more than 5 h. Effects of both MDMA and 5-HT on glutamate-evoked responding were tested for all cells, and the MDMA dosage series was administered prior to the 5-HT series.

RESULTS

Methylenedioxymethamphetamine, serotonin and serotonin agonist effects on neuronal firing

All of the recording sites were located in the core region of the nucleus accumbens in a rostrocaudal extent from 11.2 to 10.0 mm anterior to the interaural line.[50] No spontaneously firing cells were encountered in the nucleus accumbens of these urethane-anesthetized rats, so all of the cells were driven by cycled pulses of glutamate. Microiontophoretic application of MDMA, 5-HT and all of the 5-HT receptor agonists that were tested produced current-dependent inhibitions of glutamate-evoked firing of the cells (Fig. 1A, B). Equivalent ejection currents applied to the acidic saline control solution (NaCl) did not significantly inhibit glutamate-evoked firing of the cells (Fig. 1A, B). The NaCl data are repeated in both parts of Fig. 1 for ease of visual comparison with the test drugs. A two-way repeated measures analysis of variance with Tukey *post hoc* comparisons of means indicated that glutamate-evoked responding was significantly inhibited ($P < 0.01$) by all of the test drugs compared to the control solution (NaCl) for all ejection currents except the lowest (20 nA). Even at the 20 nA current intensity, all of the test drugs except MDMA (Fig. 1A) and CPBG (Fig. 1B) inhibited glutamate-evoked firing significantly more than the acidic saline control solution applied with a 20 nA current ($P < 0.01$).

The agonists with high affinity for the 5-HT_3 receptors 2m5-HT and CPBG produced markedly similar inhibitions of glutamate-evoked firing (Fig. 1B), although CPBG was not tested with the highest ejection current (60 nA). The inhibition produced by these 5-HT_3 agonists did not differ significantly from the inhibition produced by MDMA, 5-HT, the 5-$HT_{2A,2C}$ agonist DOI (Fig. 1) or the 5-HT_{1B} agonist CGS12066B (data not shown). However, the 5-HT_{1A} agonist 8-OH-DPAT (Fig. 1B) was significantly more inhibitory than all the other test drugs when applied with the 30, 40 and 60 nA ejection currents ($P < 0.05$).

Examples of individual neuronal responses to 5-HT and four 5-HT agonists are shown in Fig. 2; the effect of MDMA is illustrated for a cell in Fig. 3. The peak of each histogram in these records indicates the cumulative number of spikes that were evoked during each 20-s cycle (glutamate on 10 s, off 10 s). The flat peaks of most of the histograms reflect the fact that the cells were usually silent between successive applications of glutamate. Applications of 5-HT (Fig. 2A), the 5-HT agonists (Fig. 2B–E) and MDMA (Fig. 3) all significantly decreased glutamate-evoked firing of these nucleus accumbens cells ($\geq$15% decrease from baseline responding). However, the latency to onset of inhibition produced by MDMA was longer than for 5-HT or 5-HT agonists for most of the cells that were tested (Fig. 3 compared to Fig. 2). Glutamate-evoked firing was significantly increased by MDMA (40 nA, 60 s) for only one nucleus accumbens cell, and neither 5-HT nor any of the 5-HT agonists had a significant excitatory effect ($\geq$15% increase from baseline) on any cell that was tested (Table 1). As indicated in Table 1, the relatively selective 5-HT_{1B} agonist CGS12066B, which is not illustrated in Fig. 1 and Fig. 2, also significantly inhibited glutamate-evoked firing of nearly every cell that was tested.

Some cells failed to be significantly affected by 5-HT, MDMA or 5-HT agonists applied at 40 nA for 60 s (Table 1). However, at the highest current tested (60 nA, 60 s), MDMA, 5-HT and all of the selective 5-HT receptor subtype agonists significantly inhibited firing of 94%, 80% and 95–100% of tested cells, respectively. Only 8-OH-DPAT decreased glutamate-evoked firing in 100% of the cells when applied with the 40 nA ejection current (Table 1). 8-OH-DPAT also significantly inhibited glutamate-evoked firing of 100% of the cells that were tested with the 30 nA current and 81% of the cells that were tested with the 20 nA current.

Fluoxetine and serotonin antagonist effects on methylenedioxymethamphetamine-induced inhibition of firing

Microiontophoretic application of fluoxetine, a 5-HT reuptake blocker that prevents MDMA-induced 5-HT release, attenuated the inhibitory effect of MDMA on glutamate-evoked firing of nucleus accumbens cells when applied with low ejection currents that did not alter baseline firing rates of the cells. As illustrated in Fig. 3, fluoxetine (FLX) applied at 5 nA for 7 min prevented most of the inhibition produced by MDMA alone (50 nA, 60 s). The inhibitory effect of MDMA was fully restored for this cell by 8 min following the offset of fluoxetine ejection (Fig. 3). To determine the statistical significance of the fluoxetine effect, inhibition produced by MDMA (40 or 50 nA, 60 s) alone and in the presence of fluoxetine (5 or 10 nA, 5 min) was compared in

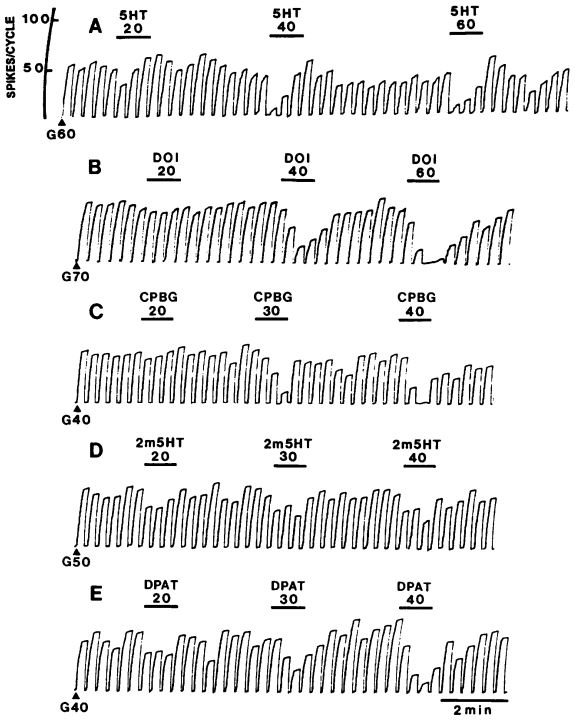

Fig. 2. Examples of inhibitory effects of 5-HT (A) and four 5-HT receptor subtype agonists (B–E) on glutamate-evoked firing of five different cells in the nucleus accumbens core. In E, DPAT is 8-OH-DPAT. Cells were driven by 10-s pulses of glutamate (G) delivered in 20-s cycles (10 s on, 10 s off throughout the recording). Ejection current intensities (nA) for glutamate are indicated by the numbers below each record on the left. Durations of 5-HT agonist application are indicated by the lines above the records and current intensity is indicated by the numbers. Onset of each glutamate application reset the pen (curvilinear) that recorded cumulative action potentials. The peak of each histogram indicates the number of spikes that occurred during each 20-s cycle. The flat top of most of the histograms indicates that most cells were quiescent in the 10-s period between successive applications of glutamate.

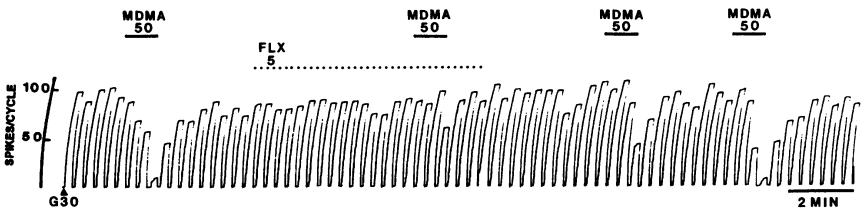

Fig. 3. Microiontophoretic application of MDMA (50 nA, 60 s) produced a delayed inhibition of glutamate-evoked firing of this neuron in the core of the nucleus accumbens. The inhibition was markedly attenuated by application of the selective 5-HT uptake blocker fluoxetine (FLX). The inhibitory effects of MDMA application gradually reappeared following the offset of fluoxetine ejection. This cell was driven by glutamate (G) applied with 30 nA ejection currents for 10 s every 20 s. Periods of MDMA ejection are indicated by solid lines above the record and the period of fluoxetine ejection is indicated by the dotted line. Onset of each glutamate application reset the pen (curvilinear) that recorded cumulative action potentials.

eight cells (Table 2). Fluoxetine significantly attenuated the MDMA-induced inhibition of glutamate-evoked firing of all of these cells ($P < 0.05$, paired *t*-test, two-tailed).

5-HT antagonists also attenuated the inhibitory effect of MDMA on glutamate-evoked firing of nucleus accumbens cells. Examples of effects of the relatively selective 5-$HT_{2A,2C}$ antagonist ketanserin, the non-selective 5-HT antagonist with high affinity for 5-HT_1 receptors methiothepin and the highly selective 5-HT_{1A} antagonist WAY100135 on MDMA-induced inhibition of glutamate-evoked firing of three nucleus accumbens cells are shown in Fig. 4. Ketanserin and WAY100135 attenuated MDMA-induced inhibition at currents that did not alter the basal firing rate of the cells. Methiothepin alone tended to decrease glutamate-evoked firing at ejection currents that could attenuate the inhibitory effects of MDMA (Fig. 4). However, all three of these antagonists and the non-selective 5-HT

Table 1. Nucleus accumbens cells affected by methylenedioxymethamphetamine,serotonin and serotonin agonists

Drug*	*n*	Cells inhibited (%)	Cells excited (%)	Cells with <15% change (%)
MDMA	57	77	2	21
5-HT	44	75	0	25
DOI	35	89	0	11
CPBG	27	96	0	4
2m5-HT	13	92	0	8
CGS12066B	12	92	0	8
8-OH-DPAT	18	100	0	0

*Ejection current: 40 nA, 60 s.

antagonist methysergide significantly attenuated the inhibitory effects of MDMA as indicated by paired, two-tailed *t*-tests (Table 2). The inhibitory effect of MDMA was attenuated by at least 15% in all of eight cells by fluoxetine, all of nine cells by methiothepin, eight of 10 cells by methysergide, all of eight cells by ketanserin and all of 10 cells by WAY100135. Although the 5-HT_{1A} antagonist WAY100135 markedly attenuated the inhibitory effect of MDMA (Fig. 4, Table 2), it did not significantly alter the inhibitory effect of DA (Fig. 5, Table 2). When applied with the same ejection current (5 nA, 5 min) that significantly attenuated the inhibition of glutamate-evoked firing that was produced by MDMA, WAY100135 had no effect on the inhibition produced by DA (Fig. 5 compared to Fig. 4). The non-selective 5-HT antagonist methysergide attenuated the inhibitory effects of the $5\text{-HT}_{2A,2C}$ agonist DOI in nine of 10 cells, in addition to attenuating the inhibitory effects of MDMA on glutamate-evoked firing (Table 2).

Dopamine depletion effects

The extent to which 5-HT inhibition of glutamate-evoked firing might be mediated by 5-HT-induced DA release was examined by testing the effects of 5-HT in rats that had been depleted of DA. Pretreatment with the catecholamine neurotoxin 6-OHDA and the catecholamine synthesis inhibitor AMPT did not alter 5-HT-induced inhibition of glutamate-evoked firing in the nucleus accumbens (Fig. 6A, 6-OHDA-PreT.) compared to effects in the vehicle-pretreated group (Fig. 6A, Vehic.-PreT.). However, the inhibitory effects of MDMA on glutamate-evoked firing were markedly decreased for the same cells in the 6-OHDA-pretreated group compared to inhibition produced in the control animals (Fig. 6B). A two-way, repeated measures analysis of variance comparing the effects of MDMA in the two groups indicated that the groups and current main effects and the interaction were all significant ($P < 0.01$). *Post hoc* comparisons of means indicated that MDMA was significantly less inhibitory in the 6-OHDA-pretreated group compared to the vehicle-pretreated group for both the 30 and 40 nA currents ($P < 0.01$). Indeed, the mean inhibition produced by the 40 nA, 60 s current of MDMA in the 6-OHDA-pretreated group (90 ± 5.3%) was equivalent to the effect produced by ejection of the acidic saline (NaCl) control solution (89.7 ± 4.5%) in untreated animals (Fig. 1). Inhibition produced by MDMA (40 nA, 60 s) in the vehicle-pretreated group (70.1 ± 3.7%) was similar to inhibition produced in untreated animals (71.9 ± 2.7%; Fig. 1A). At the highest current of MDMA tested (60 nA, 60 s), MDMA-induced inhibition was equivalent in the DA-depleted and the control animals. MDMA and 5-HT were tested on 23 cells and 15 cells, respectively, from the six rats in the 6-OHDA-pretreated group, and 27 and 25 cells, respectively, from the six rats in the vehicle-pretreated group. The baseline responding prior to MDMA or 5-HT application did not differ for the two pretreatment groups (49.1 ± 2.7 spikes/cycle for the 6-OHDA group and 49.4 ± 1.8 spikes/cycle for the vehicle control group). The intensity of glutamate current required to produce these equivalent baseline firing rates also did not differ significantly for the two groups (40.2 ± 2.7 nA for the 6-OHDA group and 43.1 ± 1.6 nA for the vehicle control group).

DISCUSSION

Microiontophoretic application of MDMA inhibited glutamate-evoked firing of most nucleus accumbens cells, as has been observed previously in urethane-anesthetized rats.[73,74] This inhibition was mimicked by microiontophoretic application of 5-HT, a monoamine that is released from serotonergic terminals in the accumbens and the dorsal striatum by MDMA.[14,19,61,73] Other laboratories have also reported that locally applied 5-HT inhibits glutamate-evoked firing of most nucleus accumbens cells.[70,72] However, a subpopulation of nucleus accumbens cells that has been reported to be excited by 5-HT in chloral hydrate-anesthetized rats[70,72] was not found in the present study. Neither 5-HT nor any of the 5-HT agonists significantly increased glutamate-evoked firing of any nucleus accumbens cell. This failure to find any excitatory effects of 5-HT in the urethane-anesthetized rats probably reflects differences in the degree of CNS depression produced by the two anesthetics. Most of the cells that have previously been reported to be excited by 5-HT in chloral hydrate-anesthetized rats were spontaneously active,[70,72] whereas all of the cells in the urethane-anesthetized rats required glutamate for activation.

Serotonin receptor subtype agonists

All of the agonists for 5-HT receptor subtypes that were tested also inhibited glutamate-evoked firing of the neurons in the nucleus accumbens core. Two of these agonists were highly selective, 8-OH-DPAT for

Table 2. Attenuation of methylenedioxymethamphetamine-induced inhibition of nucleus accumbens cells by serotonin reuptake and serotonin receptor blockers

Blocker†	*n*	% of baseline glutamate response MDMA only‡	MDMA + blocker
Fluoxetine	8	49.4 ± 3.7	73.3 ± 7.1*
Methiothepin	9	51.0 ± 6.7	75.5 ± 8.1**
Methysergide	10	57.0 ± 4.2	80.7 ± 6.1**
Ketanserin	8	64.8 ± 4.7	81.7 ± 5.0*
WAY100135	10	57.9 ± 4.0	82.3 ± 4.3**
	DA§ only	DA + blocker	
WAY100135	5	49.9 ± 8.0	55.3 ± 7.5
	DOI only	DOI + blocker	
Methysergide	10	59.5 ± 4.9	73.9 ± 4.3**

†Blockers (5–10 nA, 5 min).
‡MDMA (30–50 A, 60 s).
§DA (30 nA, 60 s); DOI (30–40 nA, 60 s).
*$P < 0.05$.
**$P < 0.01$.

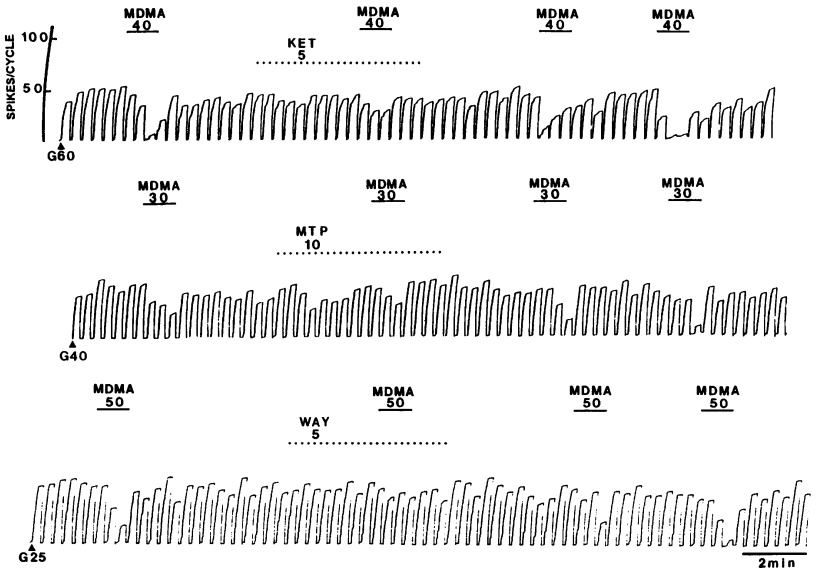

Fig. 4. The inhibitory effects of MDMA on glutamate-evoked firing were attenuated by microiontophoretic application of 5-HT antagonists and gradually reappeared following offset of antagonist application. MDMA ejection periods are indicated by the solid lines above the records. 5-HT antagonist applications are indicated by the dotted lines. Records from three different cells in the nucleus accumbens core are shown. (Top) The 5-$HT_{2A,2C}$ antagonist ketanserin (KET), applied with a 5 nA ejection current, markedly attenuated MDMA-induced inhibition. (Middle) The non-selective 5-HT antagonist methiothepin (MTP) alone produced some inhibition, but never the less attenuated the inhibition produced by MDMA application. (Bottom) The 5-HT_{1A} antagonist WAY100135 (WAY), applied with a 5 nA ejection current, abolished the inhibitory effect of MDMA on this cell. Cells were driven by cycled pulses of glutamate (G) delivered for 10 s every 20 s at ejection currents indicated by the numbers below each record. Onset of each glutamate application reset the pen that recorded cumulative action potentials. The peak of each histogram indicates the number of spikes that were produced during each 20-s cycle.

5-HT_{1A} receptors and DOI for both 5-HT_{2A} and 5-HT_{2C} receptors.[67] The latter two receptors have recently been renamed. The 5-HT_{2A} receptor was previously the 5-HT_2 receptor and the 5-HT_{2C} receptor was formerly referred to as the 5-HT_{1C} receptor.[33] Receptor autoradiography and *in situ* hybridization studies have revealed significant densities of 5-HT_{2C} (5-HT_{1C}) receptor binding and high to intermediate levels of 5-HT_{2C} mRNA expression in the nucleus accumbens.[37,56] Intermediate levels of 5-HT_{2A} binding sites and receptor mRNA expression were also identified in the nucleus accumbens.[38,52,56] Local application of the 5-$HT_{2A,2C}$ agonist DOI, which has very weak or no affinity for dopaminergic or cholinergic receptors,[67] inhibited glutamate-evoked firing of nucleus accumbens cells. Although DOI has some affinity for α_1 and α_2 adrenergic receptors,[67] the DOI inhibition of glutamate-evoked

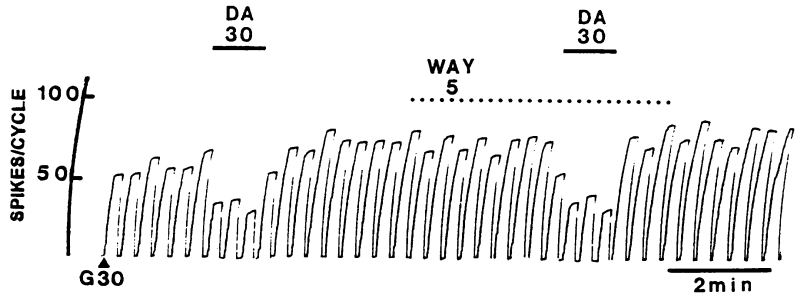

Fig. 5. The inhibitory effect of DA on glutamate-evoked firing of this cell in the nucleus accumbens core was not attenuated by application of the 5-HT_{1A} antagonist WAY100135 (WAY). Periods of DA ejection are indicated by the solid lines above the record and the period of WAY100135 ejection is indicated by the dotted line. Glutamate (G) was ejected (30 nA) for 10 s every 20 s throughout the recording.

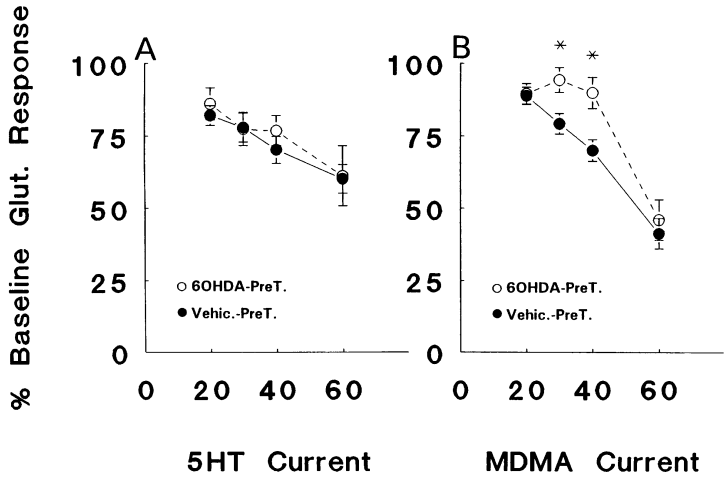

Fig. 6. Depletion of DA by pretreatment with the neurotoxin 6-OHDA and the synthesis inhibitor AMPT (6-OHDA-PreT. group) did not alter inhibition of glutamate-evoked firing produced by 5-HT (A), but significantly reduced inhibition produced by MDMA (B) compared to effects on cells in vehicle-pretreated control animals (Vehic.-PreT. group). For the six rats in the 6-OHDA-pretreated group, MDMA was tested on 23 cells and 5-HT was also tested on 15 of the same cells. For the six rats in the vehicle-pretreated group, MDMA was tested on 27 cells and 5-HT was also tested on 25 of the same cells. Means and S.E.M. are plotted. *Significant differences between the 6-OHDA- and the vehicle-pretreated groups, $P < 0.01$.

firing was significantly attenuated by methysergide, a non-selective 5-HT antagonist that does not have significant affinity for adrenoreceptors. Therefore, DOI appeared to inhibit glutamate-evoked firing of nucleus accumbens cells by interacting with 5-HT_{2A} and/or 5-HT_{2C} receptors. The inhibitory effect of DOI and 5-HT was presumably indirect, because bath application of 5-HT has been reported to produce a tetrodotoxin-insensitive depolarization of nucleus accumbens cells in brain slices that was attenuated by the 5-$HT_{2A,2C}$ antagonist ketanserin.[44] The intracellular recording study found no evidence for direct hyperpolarization of accumbens cells by 5-HT *in vitro*. However, it is not yet known how 5-HT and selective 5-HT receptor subtype agonists affect glutamate-mediated synaptic potentials in nucleus accumbens cells *in vitro*, the comparison that would be more appropriate for the glutamate-evoked firing that was measured in the present study. Microiontophoretically applied 5-HT or synaptically released 5-HT may indirectly inhibit glutamate-evoked firing of nucleus accumbens cells by modulating release of DA and/or other neurotransmitters from presynaptic terminals in the nucleus. The anatomical framework for indirect mechanisms of action for 5-HT in the accumbens is known to exist. Ultrastructural studies have identified 5-HT-immunoreactive terminals in the nucleus accumbens that are in apposition to axons and terminals that contain tyrosine hydroxylase (presumably DA terminals), and in apposition to unlabeled terminals that contain unidentified neurotransmitters.[66] Furthermore, DA release in the nucleus accumbens that was induced by local infusion of 5-HT was markedly attenuated by local infusion of a selective 5-HT_2 antagonist and by a selective 5-HT_3 antagonist.[48] Thus, the inhibition of glutamate-evoked firing by the 5-$HT_{2A,2C}$ agonist DOI that was observed in the present study may have been mediated by DOI-induced release of DA from presynaptic terminals.

This inhibitory effect of 5-HT and 5-HT agonists on glutamate-evoked firing of nucleus accumbens cells is of interest because the nucleus accumbens receives major excitatory glutaminergic inputs from the prefrontal cortex and the hippocampus.[6,16,21,69]

The selective 5-HT_{1A} agonist 8-OH-DPAT[67] had a powerful inhibitory effect on glutamate-evoked firing of the nucleus accumbens cells. The magnitude of this effect was surprising since 5-HT_{1A} receptors, although present, are sparse in the nucleus accumbens.[29,55] These receptors may be especially important for affecting neurotransmission in the cholinergic interneurons in the accumbens. Rada *et al.*[57] have proposed that 5-HT inhibits the cholinergic interneurons via 5-HT_{1A} receptors, because local infusion of 8-OH-DPAT into the accumbens through a dialysis probe significantly decreased extracellular acetylcholine levels. The cholinergic interneurons in the accumbens are large in size, though small in number.[39] Therefore, it is possible that they constituted a sizeable proportion of the population of cells that were recorded in the present study.

The nucleus accumbens contains moderate densities of 5-HT_{1B} binding sites[51] and moderate to high levels of 5-HT_{1B} receptor mRNA expression.[7,68] The locomotor activation produced in rats by MDMA has been reported to be mimicked by the relatively selective 5-HT_{1B} agonist RU24969.[58] However, RU24969 also has high affinity for 5-HT_{1A} and 5-HT_{2C} receptors, and moderate affinity for adrenoceptors and DA receptors as well.[67] Therefore, CGS12066B, which has 17 times more affinity for 5-HT_{1B} receptors than for 5-HT_{1A} receptors, and minimal affinity for adrenoceptors and DA receptors,[42] was used in the present study. This drug also mimicked the inhibitory effect of MDMA on glutamate-evoked firing of nucleus accumbens cells. Potent and selective agonists for 5-HT_3 receptors are not available.[67] However, 2m5-HT and CPBG have often been used as 5-HT_3 agonists.[23,33] Although 2m5-HT has as much affinity for 5-HT_1 and 5-HT_{2C} receptors as for 5-HT_3 receptors, it has weak or no affinity for adrenoceptors or DA receptors.[67] CPBG has weak or no affinity for 5-HT receptors other than 5-HT_3, but it has almost equal affinity for α_2 adrenoceptors as for 5-HT_3 receptors.[67] Both 2m5-HT and CPBG significantly inhibited glutamate-evoked firing of nearly every accumbens cell that was tested. Thus, these 5-HT_3 agonists had equivalent effects whether they had any affinity for adrenoceptors or not. Similarly to the 5-$HT_{2A,2C}$ agonist, the 5-HT_3 agonists may have inhibited glutamate-evoked firing of nucleus accumbens cells at least partially by releasing DA from presynaptic terminals. Infusion of a 5-HT_3 agonist directly into the nucleus accumbens via a dialysis probe has been demonstrated to produce a large increase in extracellular DA in the accumbens.[9] The multiplicity of 5-HT receptor subtype agonists that were found to inhibit glutamate-evoked firing of nucleus accumbens cells in the present study undoubtedly reflects the fact that 5-HT can interact with multiple receptor subtypes to alter the excitability of the cells directly and also indirectly by modulating release of neurotransmitters such as DA[9,48] and acetylcholine.[57] We were unable to identify a 5-HT agonist that had significant excitatory effects on glutamate-evoked firing of nucleus accumbens cells. High 5-HT_4 receptor densities[25] and high levels of mRNA for 5-HT_6 receptors have recently been found in the nucleus accumbens,[60] but possible functional effects for these receptors in the accumbens have yet to be determined.

Serotonin uptake blocker and serotonin receptor antagonists

The inhibitory effect of MDMA on neuronal firing in the accumbens was significantly attenuated by application of the 5-HT uptake inhibitor fluoxetine prior to the onset of MDMA ejection. This observation suggests that the inhibitory effect of MDMA was mediated by 5-HT release, because fluoxetine pretreatment is known to block MDMA-induced 5-HT release in brain tissue.[3,14,20] Pretreatment with fluoxetine also blocked the inhibitory effect of MDMA on firing of neurons in the medial prefrontal cortex[46] and in the dorsal raphe nucleus,[63] suggesting that MDMA alters neurotransmission by releasing 5-HT in those brain areas as well. As further evidence that MDMA inhibits neuronal excitability in the nucleus accumbens via 5-HT release, the inhibitory effect of MDMA was also significantly decreased by the relatively selective 5-$HT_{2A,2C}$ antagonist ketanserin, the highly selective 5-HT_{1A} antagonist WAY100135 and the non-selective 5-HT antagonists methiothepin and methysergide in the present study. Systemic application of methysergide failed to attenuate MDMA-induced inhibition of neuronal firing in the nucleus accumbens in a previous study in our laboratory,[73] but local application by microiontophoresis was effective in the present study. 5-HT antagonists have also been reported to block MDMA's effects on neuronal firing in the prefrontal cortex[47] and its effects on behavior.[4,8]

Dopamine-mediated effects

Although the inhibitory effects of MDMA on neuronal firing in the nucleus accumbens were mimicked by 5-HT and 5-HT agonists and significantly attenuated by fluoxetine and by 5-HT antagonists, both the MDMA and the 5-HT agonist effects may have been mediated indirectly by DA release. Several agonists for 5-HT receptors, including 5-HT itself, have been demonstrated to release DA in the accumbens and in the dorsal striatum when infused locally through the dialysis probe *in vivo*.[2,9,48] Co-perfusion with 5-HT antagonists attenuated this 5-HT-induced DA release. Furthermore,

pretreatment with the selective 5-HT uptake blocker fluoxetine produced a small but significant decrease in MDMA-induced DA release in the striatum *in vivo*,[31,41] suggesting that some of the DA release was dependent upon MDMA-induced 5-HT release. In synaptosome preparations and brain slices, MDMA was demonstrated to be a more potent releaser of 5-HT than of DA,[27,61] but dialysis studies *in vivo* found that locally applied MDMA produced far greater increases in extracellular DA than 5-HT in both the nucleus accumbens and the dorsal striatum.[19,73] Pretreatment with selective DA uptake blockers markedly reduced MDMA-induced DA release *in vitro*[14,31] and *in vivo*,[41] suggesting that MDMA releases DA by interacting directly with the DA transporter and also indirectly by interacting with the 5-HT transporter to release 5-HT, which in turn stimulates DA release.

Inhibitory effects of DA on glutamate-evoked responding of nucleus accumbens cells are well known.[71,72,73] Simultaneous application of MDMA and DA by microiontophoresis has been shown to have additive inhibitory effects on accumbens cells, as did simultaneous application of 5-HT and MDMA.[73] The inhibitory effect of MDMA on neuronal firing in the nucleus accumbens was significantly attenuated by the selective dopaminergic D_1 receptor antagonist SCH39166 and by simultaneous depletion of both 5-HT and DA with synthesis inhibitors.[73] These findings suggest that at least some of the inhibitory effect of MDMA on glutamate-evoked firing in the accumbens is mediated by DA. The ability of 5-HT antagonists to attenuate the inhibitory effect of MDMA that was demonstrated in the present study suggests that 5-HT receptors also mediate some of the inhibitory effects of MDMA. Most 5-HT receptor antagonists have some affinity for DA receptors.[67] However, in the present study, the 5-HT_{1A} antagonist WAY100135 significantly attenuated MDMA-induced inhibition of nucleus accumbens cells when applied at currents that had no effect on DA-induced inhibition of the cells. The possibility remains that WAY100135 and/or the other 5-HT antagonists used may have attenuated the inhibitory effect of MDMA by interacting with 5-HT receptors to prevent 5-HT-induced DA release. In the striatum, local infusions of 5-HT_2 antagonists were found to prevent increases in extracellular concentrations of DA that were produced by MDMA.[62] To our knowledge, the effects of other 5-HT receptor subtype antagonists on MDMA-induced DA release have not yet been tested. However, 5-HT-induced increases in DA levels in dialysate samples from the nucleus accumbens have been shown to be significantly attenuated by antagonists with high affinities for 5-HT_1, 5-HT_2 and 5-HT_3 receptors.[48]

To determine whether 5-HT might have any effects on nucleus accumbens neuronal firing that were not mediated by 5-HT-induced DA release, 5-HT was tested in animals that had been pretreated with a DA neurotoxin (6-OHDA) and a synthesis inhibitor (AMPT). This pretreatment failed to attenuate the inhibitory effect of 5-HT on glutamate-evoked firing of nucleus accumbens cells, indicating that 5-HT receptors can mediate the inhibition independently of DA release. In the same cells, the inhibitory effect of MDMA was completely blocked by DA depletion, except for the highest current of MDMA that was tested. This highest current may have released residual DA or may have released sufficient 5-HT to produce a direct 5-HT-mediated inhibition or a 5-HT-induced alteration of other neurotransmitters[49] that was independent of 5-HT-induced DA release. These results suggest that the inhibitory effects of low currents of MDMA on glutamate-evoked firing of nucleus accumbens cells are dependent upon DA release. However, 5-HT antagonists significantly attenuated the inhibitory effects of low currents of MDMA without necessarily attenuating the inhibitory effects of DA. Therefore, an intermediate step of MDMA-induced 5-HT release also appears to be necessary for MDMA-induced inhibition of glutamate-evoked firing of neurons in the core region of the nucleus accumbens.

CONCLUSIONS

The results of this study suggest that MDMA-induced inhibition of glutamate-evoked firing of cells in the nucleus accumbens is dependent upon both DA and 5-HT release and is mediated by both DA and 5-HT receptors. It will be important to determine the physiological relevance of this finding by determining whether MDMA also inhibits excitatory synaptic inputs to the accumbens cells. It is becoming increasingly important to understand how MDMA affects neurotransmission in the brain because use of this drug appears to be increasingly popular in both the United States and Europe,[10,12,36] and reports of psychopathology ranging from depression to panic attacks and psychosis in MDMA users are becoming more frequent.[11,36,45]

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