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(±)-3,4-Methylenedioxymethamphetamine-induced changes in the basal activity and pharmacological responsiveness of nigrostriatal dopamine neurons

M.D. Kelland, A.S. Freeman and L.A. Chiodo

Laboratory of Neurophysiology, Center for Cell Biology, Sinai Research Institute and the Cellular and Clinical Neurobiology Program, Department of Psychiatry, Wayne State University School of Medicine, Detroit, MI 48235, U.S.A.

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The present study examined the effects of methylenedioxymethamphetamine (MDMA) on the basal activity and pharmacological responsiveness of rat nigrostriatal dopamine (DA) neurons. Under standard in vivo extracellular single-unit recording conditions, acute MDMA administered alone (i.v.) inhibited the firing rate of nigrostriatal DA neurons in a dose-dependent fashion. The potency of MDMA to elicit this inhibition was significantly reduced following depletion of either serotonin or DA. Acute MDMA pretreatments (10 mg/kg i.v., 90 s) also profoundly enhanced the sensitivity of nigrostriatal DA neurons to the rate-inhibitory effects of the D-2 DA receptor agonist quinpirole but not apomorphine. It has previously been demonstrated that the ability of quinpirole and apomorphine to inhibit nigrostriatal DA neuronal activity is dependent on the basal firing rate of the cell. Both acute MDMA and a single dose of MDMA (15 mg/kg i.p.) one week prior eliminated the rate dependency of quinpirole- and apomorphine-induced inhibition of the firing rate of these cells. These data suggest that, although MDMA is known to be a serotonergic neurotoxin, this compound may also exert direct functional effects on the nigrostriatal DA system.

MDMA (3,4-methylenedioxymethamphetamine); Apomorphine; Quinpirole; Midbrain dopamine neurons; Antidromic activation; (Electrophysiology)

1. Introduction

(±)-3,4-Methylenedioxymethamphetamine (MDMA; Ecstasy), a novel psychotropic agent related to both amphetamine and mescaline, has recently been demonstrated to exhibit neurotoxicity for brain serotonin (5-HT) systems (Commins et al., 1987; Johnson et al., 1988; Schmidt, 1987; Stone et al., 1986). MDMA both depletes telencephalic 5-HT and its metabolites and depresses the specific activity of tryptophan hydroxylase, the rate-limiting enzyme in the synthesis of 5-HT (Johnson et al., 1988; Stone et al., 1986). In addition, Commins et al. (1987) have reported histological verification of degenerating 5-HT axon terminals in the rat neostriatum following repeated administration of MDMA. In contrast, MDMA does not appear to be neurotoxic to either dopamine (DA) (Commins et al., 1987; Johnson et al., 1988; Stone et al., 1986) or norepinephrine-containing neurons (Commins et al., 1987). Brain DA levels have, however, been reported to be altered in response to MDMA administration. Stone et al. (1986) first reported that acute administration of MDMA significantly raised DA concentrations in the neostriatum, and that both acute and repeated (five sequential doses at 6 h intervals) administration of MDMA significantly raised neostriatal levels of the DA metabolite homo-

Correspondence to: M.D. Kelland, Center for Cell Biology, Sinai Research Institute, 6767 W. Outer Dr., Detroit, MI 48235, U.S.A.

vanillic acid (see also Johnson et al., 1988). Utilizing in vivo voltammetric techniques, Yamamoto and Spanos (1988) demonstrated the ability of acute MDMA to increase extracellular neostriatal DA concentrations in awake, behaving rats. MDMA has also been shown to decrease neostriatal levels of the DA metabolite dihydroxyphenylacetic acid (Schmidt et al., 1987; Yamamoto and Spanos, 1988). In contrast to acute MDMA, repeated administration of this compound lowers DA levels in the neostriatum (Commins et al., 1987; Johnson et al., 1988; Stone et al., 1986). These effects might not be related to alterations in the biosynthetic pathway for DA, as the rate-limiting enzyme tyrosine hydroxylase was not affected by any of the MDMA protocols used to date (Johnson et al., 1988; Stone et al., 1986).

While these biochemical and histological analyses have advanced our understanding of the central effects of MDMA, corresponding electrophysiological examinations of the effects of this compound are lacking. Given the observation that both acute and chronic MDMA alter neostriatal DA and DA metabolite levels, we have begun to examine the effects of MDMA on the basal activity of nigrostriatal DA neurons and the responsiveness of these neurons to the selective D-2 DA receptor agonist quinpirole and to the mixed D-1/D-2 DA receptor agonist apomorphine. Both quinpirole and apomorphine inhibit the firing of nigrostriatal DA neurons in a dose-dependent fashion (Kelland et al., 1988a; 1989), presumably via actions on impulse-regulating somatodendritic autoreceptors (Aghajanian and Bunney, 1977; Baring et al., 1980; Innis and Aghajanian, 1987; White and Wang, 1984a). In addition, we have further examined the ability of MDMA to influence the basal firing activity of nigrostriatal DA neurons following depletions of either 5-HT or DA.

2. Materials and methods

2.1. Surgical and recording procedures

Male Sprague-Dawley rats (Hilltop, 200-300 g) were anesthetized with chloral hydrate (400 mg/kg i.p.) and then placed in a stereotaxic apparatus (Activational Systems, Inc., SAS-1450). A lateral tail vein was cannulated for i.v. administration of drugs. The skull overlying the substantia nigra (area A9 of Dahlström and Fuxe, 1964) (coordinates relative to bregma: posterior 4.3-6.3 mm, lateral 0.0-3.0 mm; Paxinos and Watson, 1986) was removed and extracellular neuronal signals were sampled through a single barrel glass micropipette filled with 2 M NaCl containing 2% pontamine sky blue dye (in vitro impedances at 135 Hz, 3-6 MΩ). DA-containing neurons located within the substantia nigra (zona compacta division) were identified on the basis of previously described extracellular electrophysiological criteria (Bunney et al., 1973; Guyenet and Aghajanian, 1978; for review see Chiodo, 1988). Throughout the course of this study special attention was paid to the differential responsiveness of slowly firing (< 4 spikes/s) as compared to rapidly firing (> 4 spikes/s) nigrostriatal DA neurons (see Kelland et al., 1988a; 1989).

All DA neurons included for analysis were shown to be nigrostriatal DA neurons by means of antidromic activation (Guyenet and Aghajanian, 1978). Thus, concentric electrodes (SNE-100, Rhodes Medical Instruments) were stereotaxically placed into the head of the caudate nucleus (from bregma: P 0.3 mm, L 3.2 mm) and constant-current square-wave pulses (0.1-3.0 mA, 500 μs duration) were delivered by a digital pulse generator and coupled stimulus-isolation unit (World Precision Instruments, Anapulse Stimulator 301-T). Neurons were considered antidromically activated when the following criteria were met: (1) one antidromic spike elicited per stimulus; (2) a fixed latency for the stimulus-induced antidromic spikes; (3) the antidromic spike followed stimulation frequencies of up to 50 Hz; (4) collision occurred between spontaneously occurring action potentials and stimulus-elicited antidromic spikes (Lipski, 1981).

2.2. Drug administration

MDMA HCl (NIDA), quinpirole HCl (Lilly) and apomorphine HCl (Sigma) were freshly dissolved in 0.9% saline. Injectable haloperidol (McNeil) was diluted in 0.9% saline. Following isola-

tion and identification of nigrostriatal DA cell unit activity, the effects of MDMA alone on the basal firing rate of these neurons were examined. MDMA was administered in cumulative, exponentially increasing doses (0.5, 1.0, 2.0...64 mg/kg, every 90 s) via a lateral tail vein. In the next set of experiments, the ability of either acute MDMA or a single dose of MDMA administered one week prior to alter the pharmacological responsiveness of nigrostriatal DA neurons to either quinpirole- or apomorphine-induced inhibition of cell firing was tested. Acute experiments were performed by administering a bolus injection of either MDMA (1, 5 or 10 mg/kg i.v.) or an equal volume of saline 90 s prior to cumulative, exponentially increasing doses of quinpirole (1-128 μg/kg i.v., every 90 s) or apomorphine (0.5-256 μg/kg i.v., every 90 s). Additional animals received either one injection of MDMA (15 mg/kg i.p.) or an equal volume of saline seven days prior to the recording session in which sensitivities to quinpirole and apomorphine were determined as above.

Both the DA synthesis inhibitor α-methyl-para-tyrosine (AMPT, Sigma) and the 5-HT synthesis inhibitor para-chlorophenylalanine (PCPA, Sigma), as their methyl esters, were dissolved in 0.9% saline. AMPT (250 mg/kg i.p., each injection) was administered both 4 and 2 h prior to administration of MDMA (for dose-response examination, as above). PCPA (300 mg/kg i.p., each injection) was administered on three consecutive days prior to administration of MDMA. The 5-HT-selective neurotoxin 5,7-dihydroxytryptamine creatinine sulfate (5,7-DHT, Sigma) was prepared by dissolving 412 μg of 5,7-DHT in 20 μl of 0.9% saline with 0.05% ascorbic acid, and then administered i.c.v. Animals receiving 5,7-DHT were pretreated (45 min) with desipramine HCl (30 mg/kg; Sigma; to prevent uptake of 5,7-DHT into norepinephrine-containing neurons) dissolved in 0.9% saline. At least 10 days were allowed for neuronal degeneration to occur prior to recording sessions.

2.3. Biochemical measurements

After completion of the dose-response measures, some animals were decapitated and the neostriata were bilaterally dissected (from the overlying cortex and more medial and ventral structures) over ice. Immediately after being dissected free, the tissue was frozen on dry ice and then stored at −70 °C until analyzed. Tissue samples (individual neostriata) were disrupted in 400 μl of 0.1 N perchloric acid, 20 μl of this sample was removed for subsequent Bradford protein analysis and the remainder was microfuged for 2 min at 14000 rpm. The supernatant (350 μl) was transfered to an Eppendorf tube containing 35 μl Tris-buffered saline (pH 11) and 25 mg of alumina and then rapidly mixed (pH approximately 8.5). Endogenous catecholamines are removed by absorption to the alumina. The samples were then microfuged for 2 min, and the indole-containing supernatant was removed and re-acidified with 50 μl 1.0 N perchloric acid. The catecholamine-containing pellet was washed with distilled water, the water was then removed and the catecholamines were eluted with 200 μl 0.4 N perchloric acid.

Levels of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and DA were determined by reversed phase liquid chromatography with electrochemical detection. The mobile phase contained: 17.5% methanol, 0.1 M NaH_2PO_4, 0.2 mM octane sulfonic acid, 0.3% H_3PO_4 and 0.00375% EDTA (pH approximately 2.8).

2.4. Data analysis

Dose-response curves were analysed by comparing the average firing rate during the last 40 s after each dose to the original basal rate. The ED_{50} value for the inhibitory effect of each compound on each cell was determined by log probit analysis (Finney, 1971). Group ED_{50} data are reported as the geometric mean ± 95% confidence intervals (C.I.; the antilog value of the mean log ED_{50} ± 95% C.I.; Sokal and Rohlf, 1969). Overall statistical significance between groups was determined by a repeated measures, factorial analysis of variance (Winer, 1971). Statistical analysis of scatter plots of the relationship between basal firing rate and ED_{50} involved simple linear regression (following log transformation of the data) followed by t-tests for parallelism between appropriate regression lines (Tallarida and Murray,

1981). Comparisons between basal firing rate and the firing rate after bolus MDMA pretreatment (prior to DA agonist administration) were performed with a paired t-test. Biogenic amine levels (control vs. treatment) were compared with Student's t-test.

A PDP-11/23 digital computer was used to perform interspike interval and burst analyses on individual neurons. A total of 500 action potentials was recorded as the baseline for each cell. A burst was considered to have been initiated when an interspike interval of 80 ms or less was encountered and terminated by the occurrence of an interspike interval of at least 160 ms. Neurons were defined as exhibiting burst-firing activity only if at least two three-spike bursts occurred. These parameters have been previously employed when examining subpopulations of DA neurons (Chiodo et al., 1984; Clark and Chiodo, 1988; Grace and Bunney, 1984; Kelland et al., 1989). Comparisons between groups (control vs. treatment) were performed with Student's t-test.

3. Results

3.1. MDMA dose-response effects

MDMA alone (cumulative injections) inhibited the firing of nigrostriatal DA neurons (ED_{50} = 4.2 ± 0.3 mg/kg, n = 10) in a dose-dependent fashion, achieving total inhibition in 8 of 10 cells (figs. 1 and 2). The ability of MDMA to inhibit nigrostriatal DA neurons was not dependent on the basal firing rate (r = 0.02, n = 10; fig. 2, inset B). Haloperidol (0.2 mg/kg; typically administered after a 30 s period during which no action potentials were observed) fully reversed the MDMA-induced inhibition in two animals, partially reversed the effects in four animals, and was without effect in four animals. In a few cases, haloperidol was not administered until approximately 6 min after the confirmation of complete inhibition in order to observe the recovery of the cell. In each of these instances, the cell did not significantly recover from the effects of MDMA within this time period. Bolus injections of 1, 5 or 10 mg/kg of MDMA also inhibited nigrostriatal DA cell firing in a dose-dependent fashion (21.2

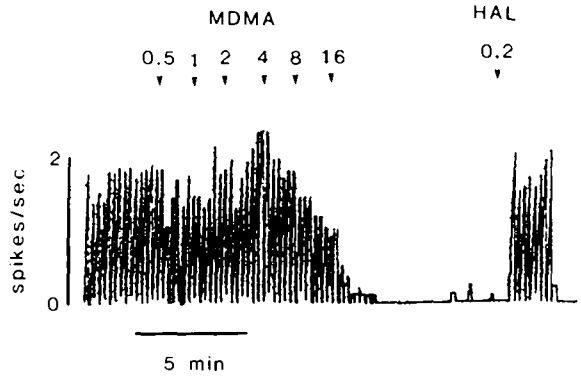

Fig. 1. Cumulative rate histogram demonstrates the ability of MDMA to inhibit the firing of a nigrostriatal DA neuron (complete inhibition achieved at 16 mg/kg). Note that the cell did not recover over a period of approximately 6 min, and that this was one of two cells on which the effects of MDMA were fully reversed by haloperidol (HAL).

± 4.8, 22.0 ± 5.5 and 49.3 ± 7.8% inhibition compared to baseline, respectively, n = 20 in each group; fig. 2, inset A), with the 10 mg/kg dose completely inhibiting the firing of 4 out of 20 cells. There was no significant difference in the degree of inhibition caused by corresponding doses of MDMA administered either in cumulative or bolus injections.

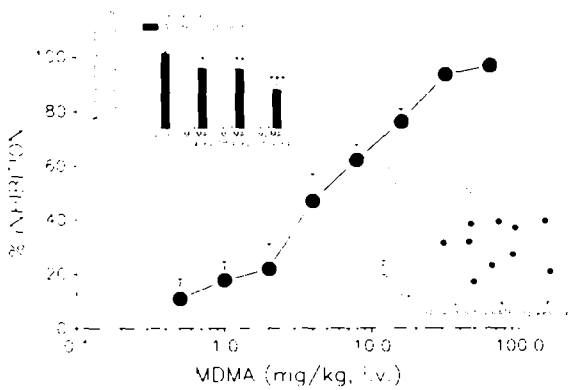

Fig. 2. Dose-response curve illustrates the ability of cumulative administration of MDMA to dose dependently inhibit the firing of nigrostriatal DA neurons. Insets: (A) bar histograms demonstrate the ability of bolus injections of MDMA to inhibit the firing of nigrostriatal DA neurons (each group is compared to its own control); (B) a scatter plot reveals that there is no significant correlation between the basal firing rate of a nigrostriatal DA neuron and the subsequent potency of MDMA to inhibit that cell. * t_{18} = 5.5, P < 0.01; ** t_{18} = 4.3, P < 0.01; *** t_{18} = 6.5, P < 0.01.

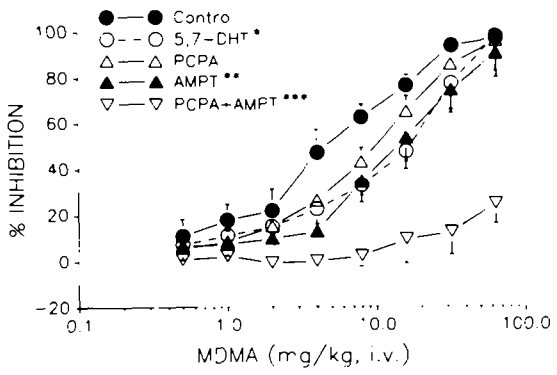

Fig. 3. Cumulative dose-response curves demonstrate the ability of compounds which deplete brain concentrations of either 5-HT (5,7-DHT and PCPA) or DA (AMPT) to reduce the potency of MDMA-induced inhibition of nigrostriatal DA neuronal firing. Note, however, that only the combined treatment eliminates the ability of MDMA to inhibit nigrostriatal DA cell firing. * $F(1,18) = 4.6$, $P < 0.05$; ** $F(1,18) = 8.1$, $P < 0.05$; *** $F(1,18) = 30.1$, $P < 0.01$.

Following depletion of brain 5-HT, the potency of MDMA to inhibit nigrostriatal DA cell firing was reduced. This effect was significant following 5,7-DHT-induced lesions (88% 5-HT depletion) as compared to control (ED_{50}s = 12.4 ± 0.3 and 4.2 ± 0.3 mg/kg, respectively; n = 10 in each group; fig. 3), but only approached significance following PCPA-induced 5-HT depletion (79% depletion; $ED_{50} = 10.5 \pm 0.2$; n = 10; fig. 3). Following depletion of brain DA by AMPT (85%), the potency of MDMA to inhibit nigrostriatal DA cell firing was likewise significantly reduced ($ED_{50} = 15.1 \pm 0.2$; n = 10; fig. 3). A combined pretreatment of both PCPA and AMPT (n = 10) eliminated the ability of MDMA to inhibit nigrostriatal DA cell firing (fig. 3).

3.2. Acute MDMA effects on DA agonist-induced inhibition

Based on the firing rate after pretreatment with MDMA, neither the 1 nor 5 mg/kg doses of MDMA significantly altered the subsequent quinpirole-induced inhibition of nigrostriatal DA neuronal firing as compared to saline-treated control animals (ED_{50}s = 4.6 ± 0.3, 3.5 ± 0.3, 4.8 ± 0.2 μg/kg, respectively, n = 10 in each group; fig. 4A). A higher dose of MDMA (10 mg/kg), however, profoundly increased the sensitivity of these cells to quinpirole ($ED_{50} = 1.1 \pm 0.1$ μg/kg; $F(1,14) = 24.6$, $P < 0.01$ compared to control, n = 7; fig. 4A). One cell was completely inhibited by quinpirole at a dose of 1 μg/kg and the remaining neurons were completely inhibited at 2 μg/kg.

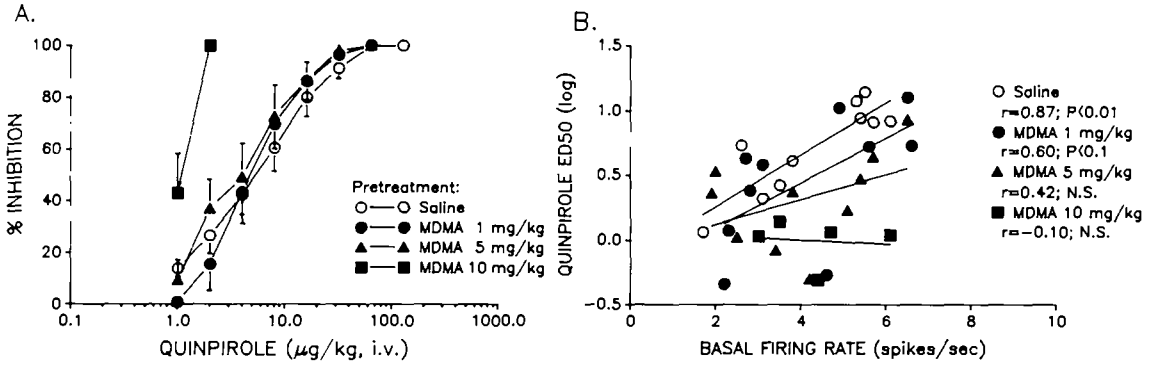

Fig. 4. Effects of acute MDMA pretreatments on the responsiveness of nigrostriatal DA neurons to quinpirole. (A) Cumulative dose-response curves demonstrate the increased sensitivity of nigrostriatal DA neurons to quinpirole following a high dose MDMA pretreatment (as compared to saline-treated controls), but not after low dose MDMA pretreatment. These data are based on the firing rate obtained after the pretreatment dose. (B) Scatter plots derived from the comparison of basal firing rate and quinpirole ED_{50}s following acute pretreatment with either saline or MDMA. Only the control group exhibits a significant positive correlation between these measures, although the 1 mg/kg MDMA pretreatment group was nearly significant. The shift in the slopes between the control and 10 mg/kg MDMA groups was significant. Only five animals from the high dose MDMA group could be used for this analysis, since the remaining cells were either completely inhibited by the MDMA pretreatment or were completely inhibited by the first quinpirole dose.

MDMA also eliminated the rate dependency of quinpirole-induced inhibition of nigrostriatal DA neurons in a dose-dependent fashion, though the only significant difference between slopes of regression lines obtained from the scatter plots was that for the 10 mg/kg MDMA group as compared to control ($t_{11} = 2.83$, $P < 0.05$; fig. 4B). Figure 5 shows individual firing rate histograms which illustrate these effects of MDMA on the responsiveness of nigrostriatal DA neurons to quinpirole. It is important to note that the cells used in this group (quinpirole following 10 mg/kg MDMA) excluded those cells completely inhibited by MDMA itself (the remaining cells were inhibited only 18.2 ± 7.0% by the MDMA pretreatment).

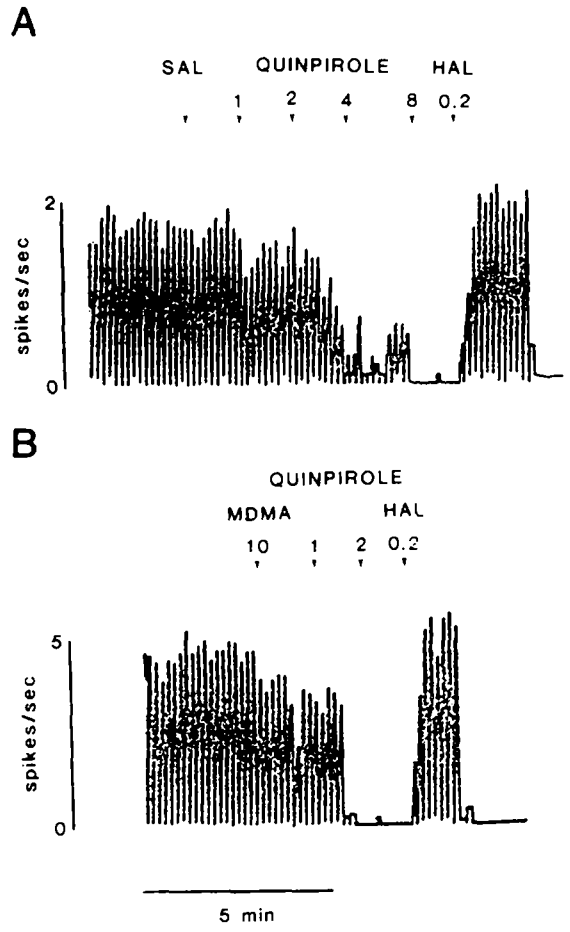

Fig. 5. Cumulative rate histograms demonstrate the acute MDMA-induced enhancement of DA neuronal sensitivity to quinpirole. (A) Response of a slow nigrostriatal DA neuron to quinpirole in a control animal (saline-pretreated; SAL). Complete inhibition was achieved at a typical dose (8 μg/kg), and subsequently reversed by haloperidol (HAL). (B) Response of a fast cell to both MDMA and subsequent quinpirole administration. Note the partial effect of MDMA (17% inhibition) followed by the marked sensitivity to quinpirole (complete inhibition at 2 μg/kg). Note that, given the rate-dependent responsiveness of nigrostriatal DA neurons to quinpirole in control animals, a cell firing at this basal rate would normally require significantly higher doses of quinpirole than the cell above to effect inhibition.

In contrast to the ability of high dose MDMA pretreatment to increase the sensitivity of nigrostriatal DA neurons to quinpirole, neither the 1, 5 nor 10 mg/kg pretreatment doses of MDMA significantly altered the subsequent apomorphine-induced inhibition of nigrostriatal DA neuronal firing as compared to saline-treated control animals (ED_{50}s = 5.0 ± 0.4, 5.5 ± 0.4, 2.8 ± 0.6 and 5.4 ± 0.3, respectively, n = 10 in each group; fig. 6A). However, similar to the results obtained with quinpirole, these acute MDMA pretreatments did eliminate the rate dependency of apomorphine-induced inhibition of nigrostriatal DA neurons in a dose-dependent fashion (fig. 6B). In this instance, the slope of the regression line obtained from the scatter plot for the 10 mg/kg MDMA group was significantly different from both the control ($t_{14} = 4.64$, $P < 0.01$) and 1 mg/kg MDMA ($t_{14} = 4.03$, $P < 0.01$) groups. No other slope comparisons proved significant.

3.3. Long-term MDMA effects on DA agonist-induced inhibition

During the week following MDMA administration (15 mg/kg i.p.), all animals appeared healthy and gained weight at a normal rate (295.7 ± 3.8-323.8 ± 4.5 g) compared to saline-treated control animals (251.1 ± 4.3-308.0 ± 5.0 g). Analysis of the basal activity of nigrostriatal DA neurons one week after either saline or MDMA revealed no significant differences (table 1). Likewise, there was no apparent difference between the ability of quinpirole to inhibit nigrostriatal DA cell firing in animals treated one week prior with either MDMA ($ED_{50} = 2.6 \pm 0.3$ μg/kg, n = 10) or saline ($ED_{50} = 3.1 \pm 0.2$ μg/kg, n = 10) when all cells were considered (fig. 7A). However, when the ability of

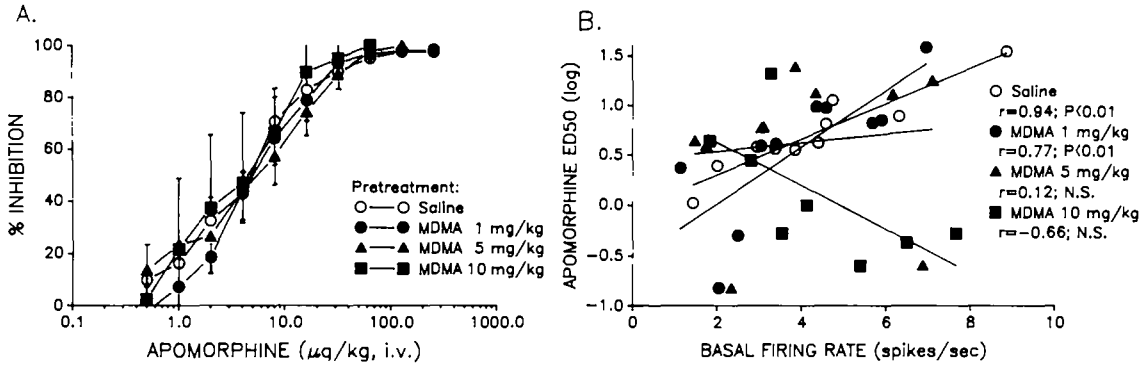

Fig. 6. Effects of acute MDMA pretreatments on the responsiveness of nigrostriatal DA neurons to apomorphine. (A) Cumulative dose-response curves demonstrate no changes in the sensitivity of nigrostriatal DA neurons to quinpirole following MDMA pretreatment (as compared to saline-treated controls). These data are based on the firing rate obtained after the pretreatment dose. (B) Scatter plots derived from the comparison of basal firing rate and apomorphine ED_{50}s following acute pretreatment with either saline or MDMA. The control and 1 mg/kg MDMA groups exhibited significant positive correlations between these measures. The shift in the slopes between the control and 10 mg/kg MDMA groups was significant, as was the slope shift between the 1 and 10 mg/kg MDMA groups. Only eight animals from the high dose MDMA group could be used for this analysis, since one of the remaining cells was completely inhibited by the MDMA pretreatment and the other cell was completely inhibited by the first apomorphine dose.

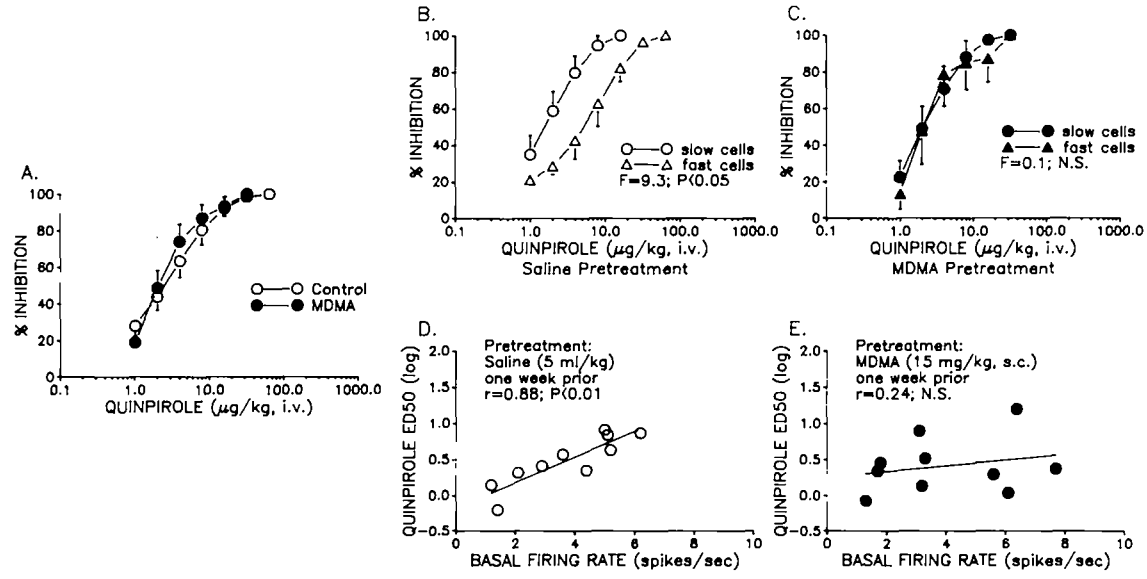

Fig. 7. The effects of a single dose of MDMA administered one week prior on the rate-dependent inhibition of nigrostriatal DA neuronal activity by quinpirole. (A) Quinpirole dose-response curves illustrate that when all nigrostriatal DA neurons were grouped together, there was no apparent influence of MDMA treatment (15 mg/kg i.p., one week prior) on the responsiveness of these cells. (B) Quinpirole dose-response curves from nigrostriatal DA cells sampled in saline-treated control animals. To generate the curves, cells were separated into slow (< four spikes/s) and fast (> four spikes/s) cell groups. There was a statistically significant difference between these groups (overall effect, repeated measures ANOVA). (C) Following the MDMA treatment, there was no significant difference between the quinpirole ED_{50} for slow vs. fast cells. (D) A scatter plot obtained from saline-treated control animals shows the rate-dependent sensitivity of nigrostriatal DA neurons to quinpirole-induced inhibition of spontaneous firing rate; i.e. faster cells required higher doses of quinpirole to effect 50% inhibition. (E) A scatter plot obtained from the MDMA-treated animals shows a lack of rate-dependent sensitivity of nigrostriatal DA neurons to quinpirole-induced inhibition of spontaneous firing rate. The slope of the regression line is significantly different from that shown in (D).

TABLE 1

Effects of MDMA on the basal activity of nigrostriatal dopamine neurons. The effects of a single dose of MDMA administered one week prior on the basal activity of midbrain nigrostriatal DA neurons are compared to saline-treated control animals. The data are presented as means ± S.E.M. The interspike interval was calculated only for spikes occurring within bursts. None of the differences achieved statistical significance (Student's t-test or, for the percentage of bursting cells, Chi-square).

	Saline	MDMA
All cells	N = 10	N = 10
Firing rate (per s)	3.9 ± 0.4	4.0 ± 0.7
Conduction velocity (m/s)	0.51 ± 0.02	0.54 ± 0.03
% spikes in bursts	8 ± 3	17 ± 6
Bursting cells	40 %	50 %
No. bursts	35.7 ± 12.9	49.6 ± 14.3
% spikes in bursts	21 ± 8	30 ± 10
Spikes per burst	2.7 ± 0.1	2.8 ± 0.2
Interspike interval (ms)	76.8 ± 7.6	74.4 ± 9.6
Interburst interval (s)	3.8 ± 1.1	7.4 ± 5.0
Postburst inhibition (ms)	340.8 ± 48.2	468.4 ± 162.3

quinpirole to inhibit either slowly or rapidly firing nigrostriatal DA neurons was considered, an effect of MDMA on the responsiveness of these neurons to quinpirole was revealed. Thus, in control animals there was a significant difference between the dose-response curves for slow as compared to fast cells (ED_{50}s = 1.8 ± 0.3 and 5.2 ± 0.3 μg/kg, respectively, n = 5 in each group, $F(1,8) = 9.22$, $P < 0.05$; fig. 7B). In contrast, there was no difference between the dose-response curves for slow (n = 6) and fast (n = 4) cells in MDMA-treated animals (ED_{50}s = 2.4 ± 0.3 and 3.0 ± 0.7 μg/kg, respectively; fig. 7C). The individual changes in these dose-response curves between groups (i.e. slow vs. slow or fast vs. fast comparisons) did not achieve significance. Further analysis of the scatter plots comparing basal firing rate with ED_{50} revealed a significant positive correlation for saline-treated animals (n = 10, r = 0.88, $P < 0.01$; fig. 7D) but not for MDMA-treated animals (n = 10, r = 0.24, not significant; fig. 7E). A test for parallelism between the regression lines revealed a significant shift in the slope ($t_{16} = 2.15$, $P < 0.05$). In a similar fashion, MDMA administered one week prior eliminated the rate-dependent inhibition of nigrostriatal DA neuronal firing by apomorphine (data not shown).

3.4. Biochemical analysis of biogenic amine levels

Pretreatment with either 5,7-DHT or PCPA resulted in significant depletions of both neostriatal 5-HT and 5-HIAA (table 2). MDMA administered one week prior (15 mg/kg i.p.) caused a non-significant decrease in neostriatal 5-HT and a non-significant increase in 5-HIAA (table 2). Pretreatment with AMPT resulted in a significant depletion of neostriatal DA (table 2).

4. Discussion

These data show that systemically administered MDMA elicited a dose-dependent inhibition of

TABLE 2

The effects of MDMA, PCPA, 5,7-DHT and AMPT on neostriatal biogenic amine levels (ng/mg of protein ± S.E.M.). The values in parentheses represent the percent depletion as compared to control. The control values for 5-HT and 5-HIAA represent pooled data, for statistical analysis each group was compared to its corresponding control animals.

	Biogenic amine levels				
	Control	MDMA	PCPA	5,7-DHT	AMPT
5-HT	14.5 ± 3.7	12.8 ± 3.3 (12%)	3.1 ± 0.9 [a] (79%)	1.7 ± 0.4 [b] (88%)	-
5-HIAA	13.0 ± 1.9	14.3 ± 2.1 (−10%)	3.2 ± 0.2 [c] (75%)	1.3 ± 0.2 [d] (90%)	-
DA	132.1 ± 13.6	-	-	-	20.4 ± 2.8 [e] (85%)

[a] $t_{18} = 2.7$; $P < 0.05$. [b] $t_{18} = 4.8$; $P < 0.01$. [c] $t_{18} = 3.1$; $P < 0.01$. [d] $t_{18} = 4.9$; $P < 0.01$. [e] $t_{18} = 8.1$; $P < 0.01$.

the basal firing rate of nigrostriatal DA neurons, and that both acute MDMA and a single dose of MDMA administered one week prior were able to alter the responsiveness of these neurons to both quinpirole and apomorphine. MDMA administered one week prior eliminated the rate-dependent responsiveness of nigrostriatal DA neurons to quinpirole and apomorphine, whereas an acute, high dose (10 mg/kg) MDMA pretreatment was able to both eliminate the rate-dependent responsiveness of nigrostriatal DA cells to these compounds and increase the overall sensitivity of these cells to quinpirole.

Although both MDMA and d-amphetamine inhibit nigrostriatal DA neuronal firing, numerous differences between their effects on DA function suggest that their mechanisms of action may be dissimilar. For example, d-amphetamine-induced inhibition of nigrostriatal DA and mesoaccumbens DA neuronal firing rate is dependent on the basal firing rate of the cell (Kelland et al., 1989; White and Wang, 1984b), whereas MDMA-induced inhibition is not. In addition, haloperidol, which potently reverses the firing rate inhibition produced by d-amphetamine, fully reversed the effects of MDMA on only 2/10 cells and had no effect on 4/10 cells. Furthermore, in contrast to MDMA, d-amphetamine either decreases (Kuczenski, 1986; Zetterström et al., 1986) or does not alter (Westerink and Korf, 1976) neostriatal homovanillic acid levels. However, like MDMA, d-amphetamine reduces neostriatal dihydroxyphenylacetic acid levels (Bunney et al., 1973; Kuczenski, 1986; Nielsen et al., 1983; Parker and Cubeddu, 1986; Westerink and Korf, 1976; Zetterström et al., 1986). MDMA and d-amphetamine are also similar in that MDMA has now been shown to release dopamine in vitro in neostriatal slices (Johnson et al., 1986; Schmidt et al., 1987). In addition, the results of the present study show that depletion of brain DA reduced the potency of MDMA to inhibit nigrostriatal DA neuronal activity. Thus, it appears that MDMA-induced release of DA plays a significant role in the ability of MDMA to alter DA neuronal activity.

One alternative means by which MDMA inhibits the basal activity of nigrostriatal DA neurons may be through an acute release of 5-HT. Our results have demonstrated that depletion of brain 5-HT also reduces the potency of MDMA to inhibit nigrostriatal DA neuronal activity. MDMA has been reported to potently release 5-HT in vitro from both hippocampal (Johnson et al., 1986) and neostriatal slices (Schmidt et al., 1987). Although direct release of 5-HT onto the cell bodies of nigrostriatal DA neurons might be hypothesized as the most likely means by which MDMA could inhibit nigrostriatal DA neuronal firing, it has been demonstrated that direct intranigral application of 5-HT has only weak effects on DA neuronal activity (Aghajanian and Bunney, 1974). However, 5-HT does appear to exert modulatory influences on nigral DA neurons via synaptic connections on distal dendrites (Nedergaard et al., 1988). 5-HT also appears to modulate glutamate neurotransmission in the substantia nigra (Aghajanian and Bunney, 1974), but at present it is not known whether 5-HT exerts modulatory effects on DA somatodendritic autoreceptors. Thus, 5-HT projections to the neostriatum might contribute to the observed effects of MDMA.

The observation that acute MDMA was able to increase the sensitivity of nigrostriatal DA neurons to quinpirole suggests that this compound may exert a modulatory influence on nigrostriatal DA neurons in addition to its more obvious inhibitory effects. In order to control for the possibility that the effects of these two compounds were merely additive, the dose-response measures and statistical analysis were based on the firing rate obtained after the MDMA pretreatment. If MDMA does elicit an acute release of 5-HT in vivo, then MDMA could be hypothesized to block glutamate-induced excitatory modulation of nigrostriatal DA neurons. Subsequently, nigrostriatal DA neurons might be more sensitive to either quinpirole or other inhibitory influences. This proposal that 'turning up' the raphe-nigral 5-HT input would facilitate nigrostriatal DA neuronal inhibition is the converse of Bunney and DeRiemer's (1982) suggestion regarding the ability of clonidine to influence the activity of nigral DA neurons. However, we are unable to provide an explanation as to why no increase in the sensitivity to apomorphine was observed.

Either repeated or single, high-dose administra-

tion of MDMA is capable of inducing a long-lasting depletion of 5-HT throughout the brain (Commins et al., 1987; Schmidt, 1987). We have previously demonstrated that pretreatment with the 5-HT-depleting agents 5,7-DHT and PCPA eliminates the rate-dependent responsiveness of nigrostriatal DA neurons to quinpirole (Kelland et al., 1988b). Although the MDMA pretreatment used in the present study (15 mg/kg, one week prior) induced only a non-significant (12%) depletion of neostriatal 5-HT, the responsiveness of nigrostriatal DA neurons to either quinpirole- or apomorphine-induced inhibition of firing rate was no longer rate-dependent. It has been proposed that fast-firing DA cells possess fewer somatodendritic autoreceptors and that this explains the rate-dependent nature of direct-acting DA agonist-induced inhibition of midbrain DA neurons (Chiodo et al., 1984; White and Wang, 1984b). The 5-HT depletion data suggest that, whereas somatodendritic autoreceptors may be involved in directly mediating the inhibition itself, afferent regulation by 5-HT is essential for the occurrence and/or maintenance of the rate-dependent nature of that inhibition.

It is important to note that acute MDMA pretreatment also eliminated the rate-dependent responsiveness of nigrostriatal DA neurons. Since MDMA has been shown to acutely increase 5-HT release (Johnson et al., 1986; Schmidt et al., 1987), this result would seem to contradict the above data. Perhaps some critical level of 5-HT must be maintained in order for this neurotransmitter to properly modulate the functioning of midbrain DA systems. It has also been recently demonstrated that although chloral hydrate anesthesia enhances the ability of both quinpirole and apomorphine to inhibit the firing of nigrostriatal DA neurons, the rate-dependent responsiveness of these cells is not significantly altered. However, d-amphetamine-induced inhibition of nigrostriatal DA neuronal firing is rate-dependent only in chloral hydrate-anesthetized animals (Kelland et al., 1989).

The observations that both acute MDMA and MDMA administered one week prior can alter the activity and/or pharmacological responsiveness of nigrostriatal DA neurons may provide some explanation for the subtle alterations observed in neostriatal DA and DA metabolite levels following MDMA administration. The ability of either neostriatal DA depletion or 5-HT depletion to reduce the ability of MDMA to inhibit the firing of nigrostriatal DA neurons further suggests that MDMA influences both of these neurotransmitter systems.

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