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Microdialysis studies on 3,4-methylenedioxyamphetamine and structurally related analogues

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The acute effect of 3,4-methylenedioxyamphetamine (MDA) and three structural analogues on the extracellular concentrations of dopamine (DA) and its major metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the striatum was studied using in vivo microdialysis in awake, freely moving rats. MDA significantly ($P < 0.001$) increased and decreased the extracellular concentrations of DA and its metabolites, respectively, following i.p. administration. Similarly, acute administration of the N-methyl (MDMA) and N-ethyl (MDE) derivatives of MDA significantly ($P < 0.05$) increased the concentration of DA in the striatum. The α -ethyl homologue of MDMA, MBDB, increased the extracellular concentration of DA but significantly ($P < 0.05$) less than MDA, MDMA or MDE. The rank order of potency for these amphetamine derivatives to increase the extracellular concentration of DA was: MDA > MDMA > MDE > MBDB > vehicle. The increase in extracellular DA concentration following a single administration of these compounds was negatively correlated with the level of serotonin (5-HT) and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in the contralateral striatum measured 7 days following drug administration. Thus, extending the alkyl group on the nitrogen or α -carbon of MDA reduces the ability of these compounds both to increase acutely the extracellular concentration of DA and to produce long-lasting depletions of 5-HT in the brain.

Dopamine; 5-HT (5-hydroxytryptamine, serotonin); Microdialysis (in vivo); MDA (methylenedioxyamphetamine); MDMA (3,4-methylenedioxymethamphetamine); Neurotoxicity

1. Introduction

The report by Ricaurte et al. (1985) offered the first evidence that repeated administration of high doses of 3,4-methylenedioxyamphetamine (MDA) produced a long-lasting decrease in the concentrations of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in select forebrain regions in the rat. That initial study was followed by numerous reports which have unequivocally established that single or repeated administration of MDA and structural analogues (i.e. 3,4-methylenedioxymethamphetamine HCl (MDMA), N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine HCl (MBDB), N-ethyl-3,4-methylenedioxyamphetamine HCl (MDE)) decrease tryptophan hydroxylase activity as well as the concentrations of 5-HT and 5-HIAA in the brain of rodents and non-human primates (Stone et al., 1986; Ricaurte et al., 1988; Johnson and Nichols, 1989; Gibb

et al., 1990). Moreover, the decreases in these serotonergic parameters have been linked to 5-HT axon terminal degeneration (O'Hearn et al., 1988).

It has been suggested that excessive release of dopamine (DA) may play a role in the MDMA-induced 5-HT neurotoxicity (Stone et al., 1988; Nash et al., 1990). In this regard, MDA and its N-methyl homologue, MDMA, have been reported to release DA from brain slice preparations (Johnson et al., 1986) as well as to inhibit the uptake of DA into striatal synaptosomes (Steele et al., 1987). Lengthening the methyl substitution on the nitrogen to ethyl (MDE), decreases its potency with respect to DA release from striatal brain slices (Schmidt, 1987). Similarly, increasing the length of the alkyl substitution on the α -carbon of these 3,4-methylenedioxy-phenethylamines diminishes their effect on DA release and uptake (Johnson et al., 1986; Steele et al., 1987). Thus, alkyl substituents longer than methyl on either the nitrogen or α -carbon decrease the ability of these compounds to release neuronal stores of DA as measured in vitro.

MDMA has been reported to increase the extracel-

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lular concentration of DA in the striatum, as measured by *in vivo* voltammetry (Yamamoto and Spanos, 1988) and microdialysis (Hiramatsu and Cho, 1990; Nash, 1990). Moreover, Hiramatsu and Cho (1990) found that the S-(+) isomer of MDMA increased the concentration of DA to a greater extent than the R-(−) isomer. These *in vivo* studies are in agreement with the aforementioned *in vitro* studies and are suggestive that the active isomer of MDMA resembles amphetamine in its ability to increase DA concentrations.

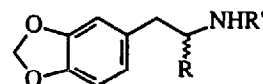
Extension of the nitrogen or α -carbon alkyl reduces the ability of these 3,4-methylenedioxy-phenethylamines to produce long-lasting decreases in the concentrations of 5-HT and 5-HIAA in the brain. For example, a single administration of MDE had no long-term effect on the concentration of 5-HT in the cortex (Schmidt, 1987). In contrast, a single administration of MDA or MDMA has been reported to decrease significantly 5-HT and 5-HIAA content, the number of 5-HT uptake sites and tryptophan hydroxylase activity in several forebrain regions (Stone et al., 1986; Gibb et al., 1990; Nash et al., 1991). Thus, increasing the length of the alkyl substitution on the nitrogen decreases the long-term 5-HT depleting effect of MDA.

The objective of the present study was to determine the effect of nitrogen or α -carbon alkyl substitution in MDA on the drug-induced changes in extracellular concentrations of DA and its major metabolites in the ventrolateral striatum, as measured by *in vivo* microdialysis. In addition, the ability of a single dose of MDA, MDMA, MDE and MBDB to decrease the concentration of 5-HT and 5-HIAA in the striatum 7 days following administration was determined.

2. Materials and methods

2.1. Materials

Racemic 3,4-methylenedioxyamphetamine HCl (MDA), 3,4-methylenedioxymethamphetamine HCl (MDMA) and N-ethyl-3,4-methylenedioxyamphetamine HCl (MDE) were generously provided by the National Institute on Drug Abuse. The racemic mixture of N-methyl-1(1,3-benzodioxol-5-yl)-2-butanamine HCl (MBDB) was synthesized by procedures established in one of our laboratories (Nichols et al., 1986). The structure of these amphetamine derivatives is illustrated in fig. 1. Chloral hydrate and the HPLC standards were purchased from Sigma Chemical Co. (St. Louis, MO). All drugs were dissolved in saline and administered *i.p.* in a volume of 1 ml/kg. The dialysis studies were begun at 09:00 h and drugs were administered between 12:00-13:00 h.



MDA	R = CH ₃ , R' = H
MDMA	R = CH ₃ , R' = CH ₃
MDE	R = CH ₃ , R' = C ₂ H ₅
MBDB	R = C ₂ H ₅ , R' = CH ₃

Fig. 1. Molecular structures of MDA, MDMA, MDE and MBDB.

2.2. Animals

Male Sprague-Dawley rats weighing between 220-260 g were purchased from Zivic Miller (Allison Park, PA) and used in all experiments. Animals were housed two per cage in a temperature controlled room (23 °C) with a 12/12 h lighting schedule (lights on at 06:00 h). Food and water were available *ad libitum*.

2.3. *In vivo* microdialysis

Under chloral hydrate (400 mg/kg *i.v.*) anesthesia, each rat was placed in a stereotaxic frame. A U-shaped dialysis probe (Nash, 1990) was inserted into the ventrolateral striatum using the following coordinates: A: +1.0, L: +3.5, V: −6.5, from bregma, incisor bar −3.3, according to the atlas of Paxinos and Watson (1982). The dialysis probe was secured using dental cement and a skull screw. The dialysis probe was flushed with a modified Ringer solution (mM: KCl 1.7, KH₂PO₄ 1.0, CaCl₂ 1.2, NaCl 136.0, Na₂HPO₄ 6.0 at pH 7.4) and the animals were placed in clear plastic cages. On the following day, the dialysis probe was connected to a Harvard infusion pump (Harvard Instruments, South Natick, MA) set to deliver Ringer solution at a rate of 1.8 μ l/min. Dialysate samples were collected every 30 min. Baseline samples were collected for 2-3 h, after which an equimolar dose (87 μ M/kg; approximately 20 mg/kg) of either MDA, MDMA, MDE, MBDB or vehicle was administered and samples were collected over a 3 h time period. At the end of each study, the rats were returned to their home cage. Each rat was killed 7 days following drug administration and the brain was rapidly removed from the skull. The location of the dialysis probe was confirmed in each rat and the contralateral striatum was removed and rapidly frozen on dry ice. The tissue samples were stored at −80 °C until the time of assay.

2.4. Biochemical measurements

The concentrations of DA and its major metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were determined in dialysate samples using previously established HPLC methods

(Nash, 1990; Johnson et al., 1990). Briefly, each sample (50 μ l) was injected onto a 3 μ m C18 column (Phenomenex, Rancho Palos Verdes, CA) connected to an LC-4B amperometric detector (Bioanalytical Systems, W. Lafayette, IN) equipped with a glassy carbon electrode set at a potential of 0.65 V relative to the Ag/AgCl reference electrode. The mobile phase consisted of 35 mM citric acid, 50 mM sodium acetate, 0.67 mM disodium EDTA, 0.23 mM 1-octanesulfonic acid, 0.1% v/v triethylamine and 5% v/v methanol at pH 4.25.

Post-mortem tissue concentrations of DA, DOPAC, HVA, 5-HT and 5-HIAA were determined using previously reported methods (Nash et al., 1990). Briefly, each sample was homogenized in 1 ml of 0.2 M perchloric acid/0.01% cysteine and spun at $9500 \times g$ for 20 min. The supernatant from each sample (50 μ l) was assayed for monoamines and their metabolites using the HPLC methods described above. The pellets were dissolved with 1.0 N NaOH and the protein content determined according to the method of Lowry et al. (1951).

2.5. Statistical analysis

The effect of MDA, MDMA, MDE, MBDB and vehicle on the concentrations of DA, DOPAC and HVA in the microdialysis studies was analyzed using a repeated measures analysis of variance (ANOVA). The effect of these treatments on post-mortem tissue concentrations of the monoamines was analyzed using a one-way ANOVA. Statistical differences between treatment groups were determined by Scheffe's test. The area under the curve (AUC_{180}) was calculated using Simpson's rule. Linear regression analysis was used to determine the relationship between tissue 5-HT or 5-HIAA content and the AUC_{180} for DA in the dialysis studies. In each case, statistical significance was set at $P < 0.05$.

3. Results

The acute effect of MDA, MDMA, MDE and MBDB (87 μ M/kg i.p.) on the extracellular concentrations of DA, DOPAC and HVA in the striatum is illustrated in fig. 2. All four compounds significantly ($P < 0.05$) increased the extracellular concentration of DA as compared to basal (time = 0) concentrations. The maximal increase in DA concentrations occurred 60 min following drug administration. For MDA, MDMA and MDE, the concentration of DA remained significantly ($P < 0.01$) elevated throughout the 3 h time period (fig. 1). MBDB significantly ($P < 0.05$) increased the extracellular concentration of DA, 60 and 90 min following administration. The rank order of

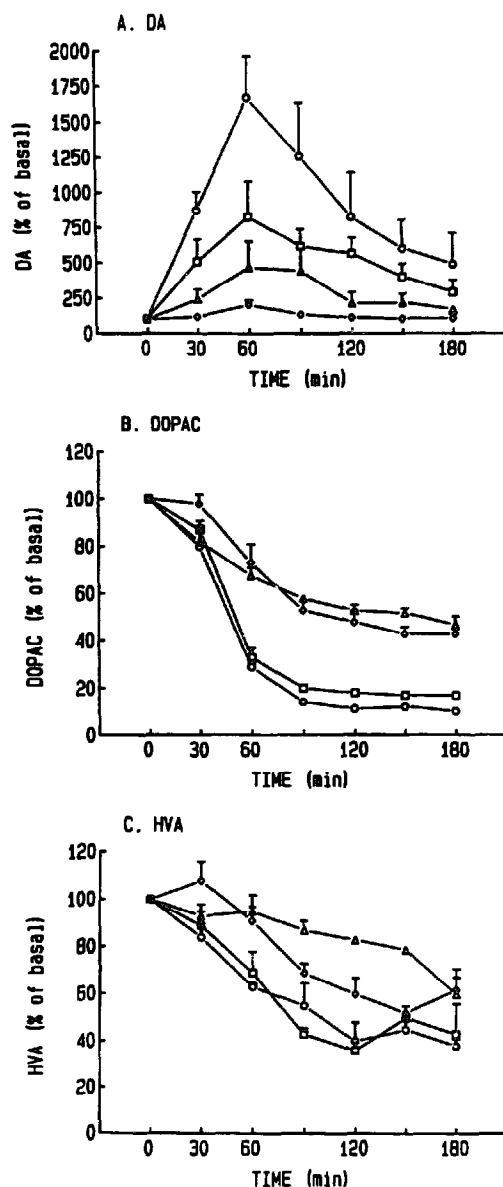


Fig. 2. Effect of MDA, MDMA, MDE and MBDB on the extracellular concentrations of DA (A), DOPAC (B) and HVA (C) in the striatum as measured by in vivo microdialysis. Each value represents the mean $\pm$ S.E. of four rats expressed as the percent of basal concentrations for each rat. Each drug was administered at time 0 and samples were collected at 30 min intervals for 180 min. The treatment groups are represented by the following symbols: MDA (circle), MDMA (square), MDE (triangle) and MBDB (diamond). Basal concentrations of DA, DOPAC and HVA are presented in table 1.

potency for increasing DA concentrations was $MDA > MDMA > MDE > MBDB$. There were no differences between the treatment groups in the basal concentrations of DA and its metabolites (table 1). Vehicle administration had no effect on extracellular concentrations of DA, DOPAC or HVA (data not shown).

All four compounds significantly ($P < 0.01$) decreased the extracellular concentrations of DOPAC

TABLE 1

Summary of the basal concentrations of DA, DOPAC and HVA and the AUC_{180} for each treatment group. Each value represents the mean $\pm$ S.E. of four rats. The AUC_{180} values are the absolute increase in the extracellular concentration of DA over the 180 min time period (pg $\cdot$ 180 min) following drug administration.

Treatment	DA ^a	DOPAC ^a	HVA ^a	AUC_{180} (pg/180 min)
Vehicle	41.3 $\pm$ 7.2	6428 $\pm$ 884	4816 $\pm$ 874	278 $\pm$ 56
MDA	43.8 $\pm$ 7.2	6941 $\pm$ 844	5415 $\pm$ 885	1973 $\pm$ 401 ^b
MDMA	46.5 $\pm$ 4.8	6529 $\pm$ 469	4668 $\pm$ 699	1498 $\pm$ 262 ^b
MDE	50.0 $\pm$ 9.0	5587 $\pm$ 780	5421 $\pm$ 748	746 $\pm$ 99 ^b
MBDB	37.5 $\pm$ 6.1	7330 $\pm$ 545	5082 $\pm$ 274	334 $\pm$ 69

^a pg/30 min. ^b $P < 0.05$ as compared to vehicle-treated rats.

and HVA (fig. 2). There was no difference in the effect of MDA as compared to MDMA with respect to the decrease in the concentrations of the DA metabolites. Similarly, MBDB and MDE produced the same magnitude of effect on HVA and DOPAC. Both MDMA and MDA produced a significantly ($P < 0.05$) greater decrease in the concentrations of HVA and DOPAC when compared to MDE or MBDB.

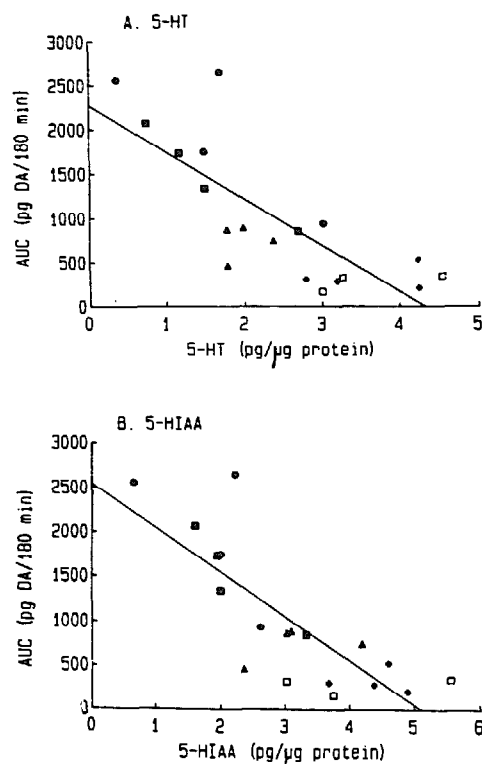


Fig. 3. Correlation between the concentration of either 5-HT (A) or 5-HIAA (B) measured 7 days after drug administration and the acute (3 h) increase AUC_{180} for DA obtained in the microdialysis studies. The treatment groups are represented by the following symbols: MDA (circle), MDMA (square), MDE (triangle) and MBDB (diamond). Vehicle-treated rats are the open squares. The concentrations (pg/ μ g protein) of 5-HT and 5-HIAA were determined 7 days following the administration of drug in the same animals used in the dialysis studies. The AUC_{180} is the absolute (pg/180 min) DA response to the drugs.

The effect of a single administration of MDA, MDMA, MDE, MBDB or vehicle on the concentrations of 5-HT and 5-HIAA in the striatum 7 days following a single dose is presented in table 2. MDA, MDMA and, to a lesser extent, MDE significantly ($P < 0.05$) reduced the concentrations of 5-HT and 5-HIAA in the striatum as compared to vehicle-treated rats. MBDB had no effect on the concentrations of 5-HT or 5-HIAA. Neither MDA nor the other analogues studied had a significant effect on the concentration of DA or its metabolites 7 days following a single dose administration (table 2).

Figure 3 illustrates the correlation between the concentration of 5-HT or 5-HIAA, measured 7 days following drug administration, and the acute (3 h) increase in the extracellular concentration of DA as measured by *in vivo* microdialysis (fig. 2). There was a significant ($P < 0.001$) negative correlation ($r = -0.79$) between the concentration of 5-HT or 5-HIAA and the AUC_{180} for DA (table 1) following the administration of MDA, MDMA, MDE, MBDB or vehicle. That is, the greater the acute (3 h) increase in the extracellular concentration of DA the greater the decrease in the concentrations of 5-HT and 5-HIAA in the striatum 7 days following drug administration.

4. Discussion

MDA and its structural analogues increased the extracellular concentration of DA in the striatum as measured by *in vivo* microdialysis. All four compounds significantly decreased the extracellular concentrations of the DA metabolites, DOPAC and HVA. Addition of an ethyl group onto the amine nitrogen or the α -carbon significantly diminished the ability of these compounds to increase the concentration of DA. These data are consistent with *in vitro* studies of the release of DA from striatal slices as well as the inhibition of synaptosomal DA uptake.

The increase and decrease in the extracellular concentrations of DA and DOPAC or HVA, respectively,

TABLE 2

Effect of MDA, MDMA, MDE and MBDB on the concentration of DA, 5-HT and their metabolites in the striatum 7 days following administration. Each value represents the mean $\pm$ S.E. of four rats. MDA, MDMA, MDE MBDB (87 μ M/kg i.p.) or vehicle was administered 7 days prior to killing.

Treatment	DA	DOPAC	HVA	5-HT	5-HIAA
Vehicle	100.9 $\pm$ 5.3	9.0 $\pm$ 0.5	6.8 $\pm$ 0.8	4.30 $\pm$ 0.77	4.78 $\pm$ 0.85
MDA	96.0 $\pm$ 2.3	8.1 $\pm$ 0.3	5.5 $\pm$ 0.5	1.64 $\pm$ 0.54 ^b	1.87 $\pm$ 0.42 ^b
MDMA	94.2 $\pm$ 5.8	10.1 $\pm$ 0.9	7.6 $\pm$ 0.6	1.53 $\pm$ 0.20 ^b	2.22 $\pm$ 0.38 ^b
MDE	88.8 $\pm$ 6.7	9.8 $\pm$ 0.7	6.9 $\pm$ 0.7	1.99 $\pm$ 0.14 ^b	3.17 $\pm$ 0.38 ^b
MBDB	106.2 $\pm$ 3.8	8.1 $\pm$ 0.8	7.1 $\pm$ 0.7	3.62 $\pm$ 0.26	4.39 $\pm$ 0.26

^a pg/ μ g protein. ^b P < 0.05 as compared to vehicle-treated rats.

produced by MDMA administration in the present study are consistent with the results obtained elsewhere (Hiramatsu and Cho, 1990; Nash, 1990). The prolonged increase in the extracellular concentrations of DA produced by these 3,4-methylenedioxy-phenethylamines could be the result of carrier- and/or vesicle-mediated DA release as well as uptake inhibition. The decrease in the extracellular concentrations of the DA metabolites could be attributed to the inhibition of monoamine oxidase and/or a decrease in the intraneuronal concentration of DA as a result of release and uptake inhibition of DA. Additional studies are necessary to determine the mechanism(s) responsible for the effects observed in the present study.

Previous studies conducted in our laboratories have found that the α -ethyl homologue of p-chloroamphetamine has significantly reduced pharmacological effects (Johnson et al., 1990). Specifically, the release of DA in the striatum and the 5-HT depleting effects of p-chloroamphetamine are significantly attenuated by extension of the α -methyl to an ethyl group. These data are in agreement with the present findings in that MBDB is significantly less potent than MDA or the other analogues with respect to DA release and 5-HT depletion.

It has been suggested that 3,4-methylenedioxy-phenylalkylamines may represent a novel class of pharmacological agents (Nichols et al., 1986). These compounds which include MDA, MDMA, MDE and MBDB do not fit the pharmacological profile of either phenethylamine hallucinogens (i.e. 1-(2,5-dimethoxy-5-iodophenyl)-2-aminopropane; DOI) or psychostimulants such as amphetamine. For example, with the exception of the R(-) enantiomer of MDA, these compounds do not substitute for the potent hallucinogen, LSD, in two lever drug discrimination studies (Nichols et al., 1986). Unlike hallucinogens, the S-(+) isomer of the 3,4-methylenedioxy-phenylalkylamines is more potent than the R(-) isomer in rodent as well as human studies (for review see: Nichols and Glennon, 1984). Thus, these compounds differ in stereoselectivity from phenethylamine hallucinogens.

The fact that S-(+) MDMA is more potent than the

R(-) isomer resembles the stereoselectivity of amphetamine. In this regard the S-(+) isomer of amphetamine will completely substitute for MDMA in drug discrimination studies (Oberlender and Nichols, 1988). However, in animals trained to discriminate amphetamine from saline, MDMA only partially substituted for amphetamine, whereas neither MBDB nor MDE produced amphetamine lever selection (Oberlender and Nichols, 1988; Glennon and Misenheimer, 1989). These results may be related to the differential effect of the compounds on the release of DA as presented in fig. 2. Nevertheless, these data are suggestive that although MDMA and its structural analogues share some similarities with amphetamine, there are significant differences.

MDA, MDMA, MDE and MBDB have nearly equivalent effects on the release of 5-HT and on 5-HT uptake inhibition (Johnson et al., 1986; Steele et al., 1987; Hekmatpanah and Peroutka, 1990). However, these compounds differ in their ability to produce long-lasting decreases in the concentrations of 5-HT and 5-HIAA in brain following single or repeated administration (Schmidt, 1987; Ricaurte et al., 1987; Johnson et al., 1987; Johnson and Nichols 1989). Therefore, it seems unlikely that the release of 5-HT alone can account for the 5-HT depleting effects of these compounds.

Conversely, these structurally related compounds differ in their ability to release DA and block its reuptake (Johnson et al., 1986; Steele et al., 1987). Moreover, in the present study the increase in the extracellular concentration of DA following the acute administration of these compounds was negatively correlated with the concentration of 5-HT or 5-HIAA in the same brain region in the same animal 7 days later. Although these data do not demonstrate a cause-effect relationship, they are supportive of the hypothesis that DA plays a significant role in the 5-HT depleting effects of these compounds (Stone et al., 1988; Nash et al., 1990).

The rank order of potency for these compounds to increase the extracellular concentration of DA parallels their euphoriant properties as well as their abuse

potential. For example, neither MDE nor MBDB produce the degree of euphoria associated with MDMA administration (Braun et al., 1980; Nichols et al., 1986). Based on the results obtained in the present study, it is tempting to speculate that the euphoriant effects of these compounds are mediated, in part, via DA release. A similar hypothesis could be offered regarding the abuse liability of these compounds since, so far, neither MDE nor MBDB appear to have become popular recreational drugs. Clearly, the use of microdialysis may provide the answer to some of these questions.

In summary, alkyl substitutions longer than methyl on the nitrogen or α -carbon of MDA significantly diminish the potency of these compounds with respect to the release of DA in the striatum, as measured by *in vivo* microdialysis. In addition to their attenuated effect on DA release, the ability of these compounds to produce long-lasting depletion of brain 5-HT is also reduced.

Acknowledgements

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