Biochemical Pharmacology, Vol. 52, pp. 1271–1277, 1996.

ISSN 0006-2952/96/$15.00 + 0.00
PII S0006-2952(96)00482-0

Studies on the Mechanism of *p*-Chloroamphetamine Neurotoxicity

Jon E. Sprague,§ Michael P. Johnson,† Christopher J. Schmidt† and David E. Nichols‡*

*THE DEPARTMENT OF MEDICINAL CHEMISTRY AND MOLECULAR PHARMACOLOGY, PURDUE UNIVERSITY, WEST LAFAYETTE, IN 47907; AND †HOECHST MARION ROUSSEL, CINCINNATI, OH 45215

ABSTRACT. Studies were conducted to investigate the sensitivity of *p*-chloroamphetamine (PCA)-induced neurochemical changes to various pharmacological manipulations known to block the neurochemical effects of 3,4-methylenedioxymethamphetamine (MDMA). The monoamine oxidase-B (MAO-B) inhibitor L-deprenyl (2 mg/kg) given 4 hr before a nonneurotoxic dose of PCA (2 mg/kg) was shown not to alter the amount of [^{3}H]paroxetine bound to serotonin (5-HT) uptake sites 7 days after treatment. L-Deprenyl 4 hr before a neurotoxic dose of PCA (10 mg/kg) did not change the acute hyperthermia. Further, neither L-deprenyl nor another selective MAO-B inhibitor, MDL-72,974 (1.25 mg/kg), given 30 min before or daily for 4 days before a single dose of PCA attenuated or potentiated the decrease in the number of [^{3}H]paroxetine binding sites measured 7 days after PCA treatment. The combination of the MAO-A inhibitor clorgyline (2.5 mg/kg) or a nonspecific dose of L-deprenyl (10 mg/kg) with the selective 5-HT releasing agent 5,6-methylenedioxy-2-aminoindan did not lead to changes in the levels of 5-HT, 5-hydroxyindoleacetic acid or dopamine 7 days after treatment. Finally, the 5-HT$_{2A}$ receptor antagonist MDL-11,939 (5 mg/kg) did not protect against the neurotoxicity of PCA. By comparing the present work with previous studies of MDMA, these results can be interpreted to suggest that the mechanism of the neurotoxicity induced by PCA is not identical to that induced by MDMA. The relationship of these results to the neurotoxicity induced by MDMA is also discussed. BIOCHEM PHARMACOL 52;8:1271–1277, 1996.

KEY WORDS. *p*-chloroamphetamine; 3,4-methylenedioxymethamphetamine; monoamine oxidase; deprenyl; neurotoxicity; serotonin

PCA[‖] is a substituted amphetamine derivative that appears to be very similar to MDMA (Fig. 1) in its behavioral, pharmacological, and neurotoxic effects. In two-lever drug discrimination studies, PCA fully substituted in MDMA-trained rats [1]. PCA also induces locomotor hyperactivity in rats with a concurrent decrease in investigatory behavior that is similar to that produced by MDMA [2].

The short-term neurochemical effects of PCA are also very similar to those of MDMA. *In vitro* studies have shown that PCA releases both 5-HT and DA from synaptosomes [3], results similar to those from studies conducted with MDMA [4, 5]. Brain 5-HT and 5-HIAA are acutely depleted following both PCA [6, 7] and MDMA [5, 8] treatment. PCA also induces a rapid acute increase in brain DA levels [9] as does MDMA [10]. *In vivo* microdialysis studies indicate that both PCA and MDMA induce a large increase of extracellular DA in the caudate nucleus [1, 10].

PCA-induced neurotoxicity also appears similar to that seen following MDMA. PCA induces a persistent decrease in 5-HT and 5-HIAA [6, 7], tryptophan hydroxylase activity, and in the number of 5-HT uptake sites [11, 12]. Molliver *et al.* [13] demonstrated that PCA destroys fine 5-HT axons originating from the dorsal raphe nucleus, findings that parallel studies with MDMA.

Certain agents that protect against MDMA-induced neurotoxicity also protect against PCA-induced neurotoxicity. For example, 5-HT uptake inhibitors block the long-term effects of PCA even if given up to 8 hr after PCA [14–16] and attenuate MDMA-induced neurotoxicity, given up to 6 hr after treatment [5]. All of these similarities might lead to the hypothesis that the mechanisms by which these two substituted amphetamines produce these responses are identical.

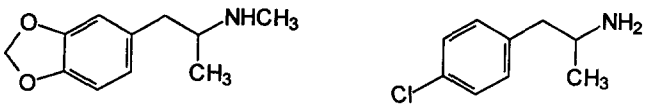
Despite all the similarities between the effects of MDMA and PCA, however, there are differences that suggest that these two agents do not have identical mechanisms of action. For example, a 5 mg/kg dose of PCA has been shown to result in a 70% reduction in 5-HT and 5-HIAA levels in the hippocampus, whereas a 4-fold larger (20 mg/kg) dose of MDMA leads only to a 30% reduction in these same pa-

§ Present address: Department of Pharmaceutical and Biomedical Sciences, Ohio Northern University, Ada, OH 45810.

‡ Corresponding author: David E. Nichols, Ph.D., Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907. Tel. (317) 494-1461; FAX (317) 494-6790.

‖ *Abbreviations:* PCA, *p*-chloroamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; 5-HT, serotonin, 5-HIAA, 5-hydroxyindoleacetic acid; DA, dopamine; MDAI, 5,6-methylenedioxy-2-aminoindan; and MAO, monoamine oxidase.

Received 29 January 1996; accepted 7 May 1996.



MDMA **PCA**

FIG. 1. Structures of MDMA and PCA.

rameters in the hippocampus [17]. In the same study, chlormethiazole, a γ-aminobutyric acid$_A$ ($GABA_A$) agonist and MK-801, and *N*-methyl-D-aspartate (NMDA) receptor antagonist, were shown to protect against MDMA-induced 5-HT neurotoxicity, but not against PCA-induced neurotoxicity. Colado and Green [17] concluded that the mechanisms by which these substituted amphetamines produce 5-HT neurotoxicity were not identical.

Recently, Benmansour and Brunswick [18] demonstrated that L-deprenyl, an MAO-B inhibitor potentiates the neurotoxicity of a nonneurotoxic dose of PCA. This report is in contrast to our finding of attenuation of MDMA-induced neurotoxicity by L-deprenyl [19, 20]. Thus, the experiments in this report were designed to compare and contrast the effects of MAO-B inhibition on the toxicity of PCA at both toxic and nonneurotoxic doses to identify possible reasons for this discrepancy. The present data suggest that the toxicity of PCA is not dependent on the excessive deamination of DA within the 5-HT terminal, as we have speculated may be the case with MDMA [19, 20].

MATERIALS AND METHODS

Animals and Drug Administration

Male Sprague–Dawley rats (Harlan Inc., Indianapolis, IN) weighing 175–199 g were used in all studies. Animals were housed individually. Room temperature was maintained at 22–24° with a 14/10 light/dark schedule. Food and water were provided *ad lib.*

In the first series of experiments, eighteen animals were divided into three treatment groups: saline, PCA (2 mg/kg, s.c.), or L-deprenyl (2 mg/kg, i.p.) 4 hr before PCA. Seven days after treatment, the striatum and frontal cortex were dissected out and assayed for numbers of [^{3}H]paroxetine binding sites.

In the second series of experiments, the effect of L-deprenyl (2 mg/kg, i.p.) on the hyperthermia induced by PCA (10 mg/kg, s.c.) was examined. Eighteen animals were divided into three treatment groups: (1) saline, (2) PCA, or (3) L-deprenyl given 30 min before PCA. Rectal temperatures were then monitored hourly for 4 hr in a room maintained at 24°.

In the third series of experiments, twenty-four animals were allocated randomly to four treatment groups. Rats were injected with saline, the selective MAO-A inhibitor clorgyline (2.5 mg/kg, i.p.), or a nonspecific dose of L-deprenyl (10 mg/kg, i.p.). Fifteen minutes after these treatments, the animals were treated with the selective serotonin releasing agent MDAI (20 mg/kg, s.c.). A single dose of MDAI has been shown to lack serotonergic neurotoxicity [21], while multiple doses do produce slight long-term deficits in serotonin markers [22]. We speculated that clorgyline or L-deprenyl might potentiate the weak neurotoxicity of MDAI, similar to the results reported with a low dose of PCA by Benmansour and Brunswick [18]. Seven days after these treatments, the frontal cortex and striatum were removed and assayed for 5-HT, 5-HIAA, and DA.

In the fourth series of experiments, the effect of L-deprenyl on the PCA-induced decrease in the number of [^{3}H]paroxetine binding sites was investigated. Again, eighteen animals were divided into three treatment groups of saline, PCA (10 mg/kg, s.c.), or L-deprenyl (2 mg/kg, i.p.) 30 min before PCA. Seven days after treatment, the animals were decapitated and the striatum, hippocampus, and frontal cortex were removed. These regions were then assayed for the number of [^{3}H]paroxetine binding sites.

In the fifth set of experiments, eighteen animals were divided into three treatment groups. Animals received saline, PCA (10 mg/kg, s.c.), or the selective MAO-B inhibitor MDL-72,974 (1.25 mg/kg, I.p.) 1 hr before PCA. Seven days after treatment, the animals were processed as in the other experiments.

In the final series of experiments, the 5-HT_{2A} receptor antagonist MDL-11,939 [23] was tested for its effects on neurotoxicity induced by PCA (2.5 mg/kg, s.c.). Fifteen animals were divided into three treatment groups: saline, PCA, or the combination of PCA and MDL-11,939 (5 mg/kg). Seven days after treatment, the animals were killed, and the frontal cortex and striatum were assayed for 5-HT levels.

[^{3}H]Paroxetine Labeling of the 5-HT Uptake Protein

A modified procedure of Marcusson *et al.* [24] was employed to measure [^{3}H]paroxetine binding sites. Since it has been reported previously that only B_{max} and not the K_d value is altered after MDMA treatment [25], it is possible to estimate the number of 5-HT uptake sites with a single saturating (1 nM) concentration of [^{3}H]paroxetine. Nonspecific binding was determined with 1 μM fluoxetine. Brain samples were thawed, weighed, and homogenized in 5 mL of Tris buffer (Tris–HCl, 50 mM; NaCl, 120 mM: KCl, 5 mM) with a Brinkmann polytron (setting 6, 2 × 20 sec). The homogenates were centrifuged at 30,000 *g* for 10 min with an intermittent buffer wash and were then resuspended in the same volume of assay buffer. Incubations were initiated by the addition of 150 μL of tissue homogenate to 1.50 mL of Tris buffer containing [^{3}H]paroxetine to give a total volume of 1.65 mL. The final [^{3}H]paroxetine concentration was 1 nM. The tubes were then incubated for 1 hr at 24° before adding 4 mL of ice-cold buffer and filtration with a Brandel Cell Harvestor through Whatman GF/C filters. The tubes and filters were then washed twice with 4 mL of ice-cold buffer, and the filters were placed in 10 mL of scintillation counting fluid. The vials were sealed and allowed to stand overnight before counting.

HPLC

HPLC with electrochemical detection (EC) was used to determine biogenic amines and their precursor and metabolite levels. The mobile phase consisted of 50 mM NaH_2PO_4, 30 mM citric acid, 0.1 mM Na_2EDTA, 0.34% sodium octyl sulfate, and 23–25% (v/v) methanol. Tissue samples were prepared by sonicating the weighed brain area from one hemisphere in 0.5 mL of 0.4 N $HClO_4$ containing 0.05% Na_2EDTA and 0.1% $Na_2S_2O_5$; tissues were sonicated for 15 sec.

The HPLC-EC system consisted of the following equipment: a refrigerated autosampler (TosoHaas, Philadelphia, PA), a model 400 EG&G Princeton electrochemical detector (Princeton, NJ), and a Brownlee C18 reverse phase analytical column (Anspec, Ann Arbor, MI). A series dual electrode cell set at $E_1 = -200$ and $E_2 = 850$ mV versus the Ag/AgCl reference electrode was used. The levels of monoamines and their metabolites were determined from standard curves using the Dynamax Methods Manager software (Rainin, Woburn, MA).

Temperature Measurements

Rectal temperatures were measured using a digital thermometer (CMA/150 Carnegie Medicin, Stockholm, Sweden). The probe was lubricated with K-Y jelly and inserted approximately 5 cm into the rectum. Temperature readings were taken after the probe had been inserted for 80 sec. Body temperatures were monitored hourly for 4 hr after drug treatment. Measurements were performed at an environmental temperature of 24°.

Drugs

[^{3}H]Paroxetine was purchased from New England Nuclear (Boston, MA) at a specific activity of 20.5 Ci/mmol. Fluoxetine hydrochloride was a gift of Eli Lilly & Co. (Indianapolis, IN). L-Deprenyl was purchased from Research Biochemicals International (Natick, MA). PCA was synthesized in our laboratory following standard methods. MDL-72,974 and MDL-11,939 were gifts from Marion Merrell Dow (Cincinnati, OH).

Statistical Analysis

Raw data were analyzed for significant differences by ANOVA with a Student–Newman–Kuels post-hoc test. For graphical presentations, except for the hyperthermia data, raw data and standard errors were converted to percentages. When comparison between only two groups was made, a Student's *t*-test was utilized. Significance was set at $P \leq 0.05$. All measurements were based on tissue wet weight.

RESULTS

Effects of L-Deprenyl on [^{3}H]Paroxetine Binding Following a Non-neurotoxic Dose of PCA

Figure 2 presents the results of the study of the effect of L-deprenyl on the neurotoxic effect of low dose PCA. The

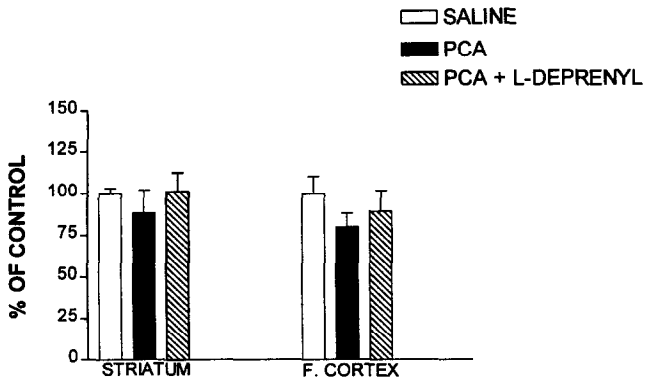


FIG. 2. Effects of PCA (2 mg/kg) and L-deprenyl (2 mg/kg) on [^{3}H]paroxetine binding sites 7 days after treatment. Saline control values were: striatal uptake sites ([^{3}H]paroxetine bound), 28.3 ± 1.0 fmol/mg wet wt; and frontal cortex, 19.9 ± 2.1 fmol/mg wet wt. Each value is the mean ± SEM for 6 animals.

results indicate that in neither brain region examined was there a significant decrease in the amount of [^{3}H]paroxetine bound. These results are at variance with those of Benmansour and Brunswick [18], who reported a decrease in the number of 5-HT uptake sites in the caudate putamen following treatment with a similar dosing regimen of PCA and L-deprenyl.

Effects of L-Deprenyl on PCA-Induced Hyperthermia

PCA (10 mg/kg) resulted in a prolonged elevation in rectal temperature lasting more than 4 hr after treatment. L-Deprenyl was not able to reduce this increase in body temperature (Fig. 3). In this same study, all the PCA-treated animals died within 24 hr whereas the PCA + L-deprenyl-treated animals all survived.

Effects of L-Deprenyl and Clorgyline on the Neurochemical Response to MDAI

Both clorgyline and L-deprenyl failed to alter the neurochemical effects induced by MDAI using 5-HT and

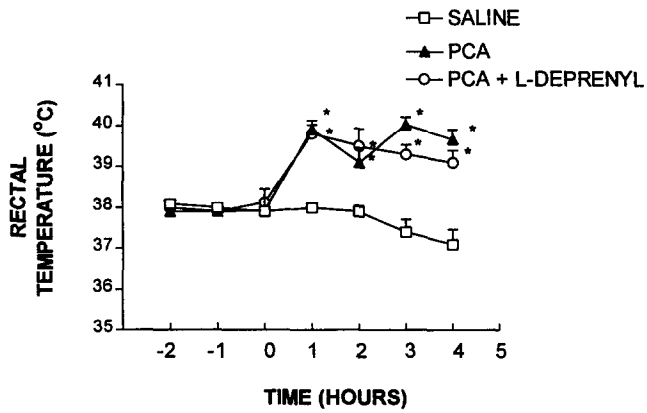


FIG. 3. Effect of PCA (10 mg/kg) and L-deprenyl (2 mg/kg) 30 min before PCA on rectal temperatures. All drug treatments were given at time zero. Key: (*) significant difference ($P \leq 0.05$) from saline control. Each value is the mean ± SEM for 6 animals.

5-HIAA levels as markers. DA was increased significantly in the striatum by both clorgyline and L-deprenyl (47 and 38%, respectively) 7 days after treatment (Fig. 4).

Effects of L-Deprenyl on [^{3}H]Paroxetine Binding Following a Neurotoxic Dose of PCA

L-Deprenyl given 30 min before PCA was ineffective at reversing the long-term decrease in the number of [^{3}H]paroxetine binding sites (Fig. 5). PCA alone induced a 50–60% decrease in the amount of [^{3}H]paroxetine bound in all three brain regions. L-Deprenyl + PCA resulted in approximately the same percentage decrease as PCA alone. This same pattern of response was seen when L-deprenyl was given 4 hr or for 4 consecutive days before PCA (data not shown).

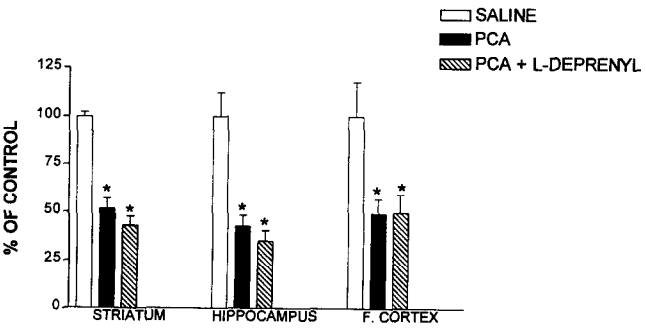


FIG. 5. Effect of PCA (10 mg/kg) and L-deprenyl (2 mg/kg) 30 min before PCA on [^{3}H]paroxetine binding sites 7 days after treatment. Saline control values were: striatum uptake sites ([^{3}H]paroxetine bound), 31.0 ± 0.7 fmol/mg wet wt; hippocampus, 13.2 ± 1.6 fmol/mg wet wt; and cortex, 17.3 ± 3.1 fmol/mg wet wt. Key: (*) significantly different ($P \leq 0.05$) from saline control. Each value is the mean ± SEM for 6 animals.

Treatment of the animals with the MAO-B inhibitor MDL-72,974 1 hr before PCA resulted in a pattern of response similar to that seen with four daily doses of L-deprenyl before PCA (Fig. 6). That is, PCA alone resulted in a significant decrease in the amount of [^{3}H]paroxetine binding in all three regions studied, ranging from a 50% decrease in the frontal cortex to an 80% decrease in the striatum. PCA plus MDL-72,974 gave similar reductions in the frontal cortex and striatum, but appeared to potentiate the effects of PCA in the hippocampus. However, none of these changes were significantly different from PCA alone.

The long-term effects of MDMA on neurochemical parameters of serotonergic function have been shown to be

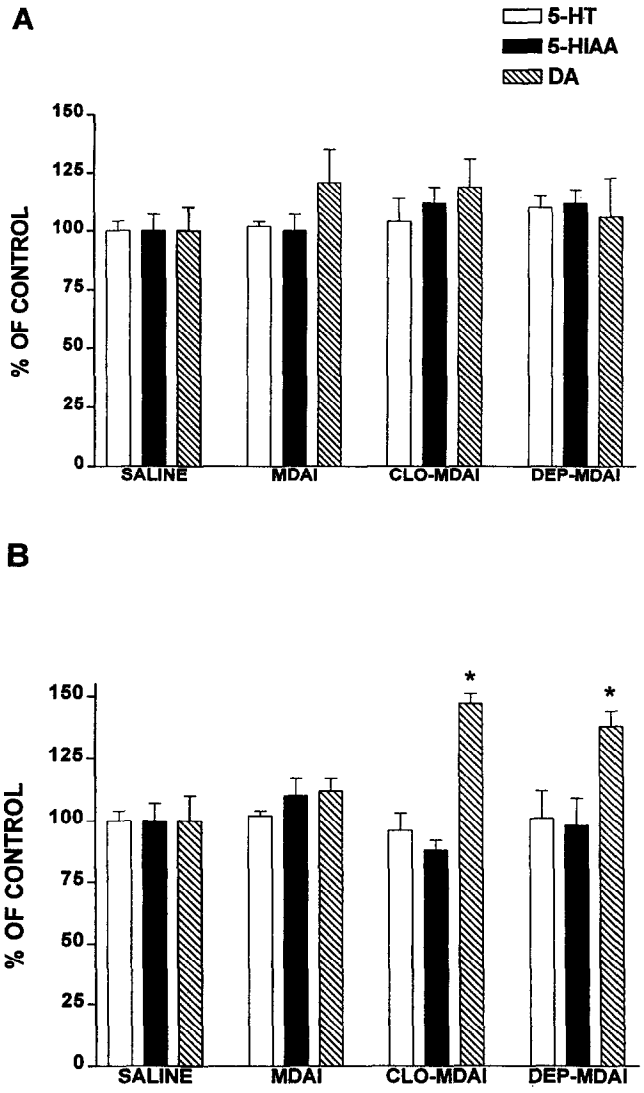


FIG. 4. Effects of clorgyline (2.5 mg/kg) and L-deprenyl (10 mg/kg) on the neurochemical effects of MDAI (20 mg/kg) 1 week after treatment. Saline control values were: (A) frontal cortex: 5-HT, 332 ± 14; 5-HIAA, 229 ± 16; and DA, 36 ± 4 pg/mg wet wt; (B) striatum: 5-HT, 535 ± 32; 5-HIAA, 694 ± 39; and DA, 16,708 ± 115 pg/mg wet wt. Key: (*) significantly different ($P \leq 0.05$) from saline control. Each value is the mean ± SEM for 6 animals.

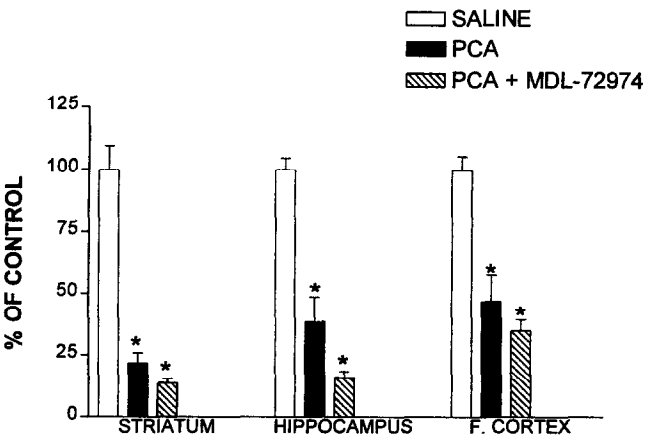


FIG. 6. Effects of PCA (10 mg/kg) and MDL-72,974 (1.25 mg/kg) 1 hr before PCA on [^{3}H]paroxetine binding sites 7 days after treatment. Saline control values were: striatum uptake sites ([^{3}H]paroxetine bound), 24.8 ± 2.3 fmol/mg wet wt; hippocampus, 17.1 ± 0.8 fmol/mg wet wt; and cortex, 20.5 ± 1.1 fmol/mg wet wt. Key: (*) significantly different ($P \leq 0.05$) from saline control. Each value is the mean ± SEM for 6 animals.

sensitive to 5-HT_{2A} receptor blockade [23, 26, 27]. However, the 5-HT_{2A} receptor antagonist MDL-11,939 failed to alter the serotonergic neurotoxicity of a low dose of PCA in the cortex and only provided partial protection in the striatum (Fig. 7).

DISCUSSION

Current data suggest that the selective serotonergic neurotoxicity of MDMA results from a sequence of events. MDMA induces a rapid release of 5-HT [28–30] and DA [10]. The 5-HT that is released activates the 5-HT_{2A} receptor, resulting in an increase in DA synthesis [23, 26, 27]. Several researchers have shown that DA can be transported into 5-HT terminals [20, 31, 32]. Once inside the terminal, the DA is deaminated by MAO-B located within the 5-HT terminal [33]. Support for this latter hypothesis is generated from data showing that the inhibition of MAO-B with L-deprenyl or MDL-72,974 protects against the neurotoxicity induced by MDMA in the striatum [19, 20]. We hypothesized at that time that the same or similar events might be induced by PCA. However, the results of the present study suggest that this is probably not the case, because MAO-B inhibition with either L-deprenyl or MDL-72,974 failed to protect against the neurotoxicity induced by PCA.

In contrast to the results of Benmansour and Brunswick [18], in our studies L-deprenyl produced no change in the effect of low dose PCA (2 mg/kg) on the number of 5-HT uptake sites. Furthermore, PCA (10 mg/kg) produced the anticipated hyperthermic response seen following administration of many substituted amphetamines. Benmansour and Brunswick [18] reported that L-deprenyl potentiated the neurotoxicity of low dose PCA (2 mg/kg) as measured by a decrease in the number of [^{3}H]cyanoimipramine binding sites 7 days after treatment. In the same study it was also reported that this dose of PCA produced significant hypothermia 1 hr post-treatment at an ambient temperature of 18°. This finding contrasts with the results of Colado and Green [17], who showed that PCA (2.5 mg/kg) caused no change in rectal temperature for up to 2 hr post-treatment. Although substituted amphetamines have been shown to both increase and decrease body temperature, decreases in body temperature are usually only observed when the ambient temperature is low (e.g. 10°) [34]. In the present study, a neurotoxic dose of PCA (10 mg/kg) at an ambient temperature of 24° resulted in a significant *elevation* of body temperature (+2.0°) that was not affected by 2 mg/kg of L-deprenyl.

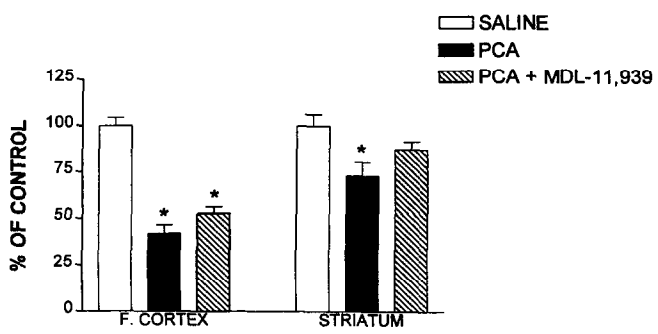


FIG. 7. Effects of MDL-11,939 (5 mg/kg, s.c.) on the serotonergic toxicity of PCA (2.5 mg/kg) as indicated by 5-HT levels. Saline control values were 344 ± 14.6 and 428 ± 26 pg/mg wet weight for the frontal cortex and the striatum, respectively. Key: (*) significantly different ($P \leq 0.05$) from saline control. Each value is the mean ± SEM for 5 animals.

There are several investigations of the effects of substituted amphetamines on body temperature. Yehuda and Wurtman [35] showed that *d*-amphetamine induces a hyperthermia at ambient temperatures between 20 and 37°. This same study indicated that *d*-amphetamine produces hypothermia at ambient temperatures between 4 and 10°. Pawlowski [36] showed that PCA (6.5 mg/kg) results in a hyperthermic response that is maintained for at least 150 min in a 28° room. Schmidt *et al.* [37] showed that MDMA (20 mg/kg) produces a hyperthermic response for 3 hr post-injection. Gordon *et al.* [34] studied in detail the effects of MDMA on body temperature. Their report showed that MDMA (30 mg/kg) produces hyperthermia at an ambient temperature of 30° but no change in core temperature at 20°. The same dose of MDMA produces a hypothermic response when the ambient temperature is lowered to 10°. Gordon *et al.* [34] further showed that the hyperthermia produced by MDMA (20 mg/kg) is maintained for up to 6 hr after treatment at 25°. Recently, several laboratories have shown that agents that decrease core temperature can protect against the neurotoxicity of substituted amphetamines [38, 39]. Therefore, the report by Benmansour and Brunswick [18] showing that PCA (2 mg/kg) produced hypothermia for only 1 hr in a room maintained at 18° is somewhat divergent from the previous literature. Furthermore, the ability of L-deprenyl to produce hypothermia and to potentiate the neurotoxicity of PCA is also in disagreement with the previous literature. The present results suggest that L-deprenyl neither potentiates nor protects against the neurotoxicity of PCA. L-Deprenyl and MDL-72,974 also failed to reverse the neurotoxicity of PCA (10 mg/kg), in contrast to what we observed with a single dose of MDMA [19, 20]. However, L-deprenyl and MDL-72,974 were unable to protect against the neurotoxicity of multiple doses of MDMA (unpublished data).

We also attempted to potentiate the selective serotonergic neurotoxicity of MDAI with either a nonspecific dose of L-deprenyl (10 mg/kg) or with the MAO-A inhibitor clorgyline (2 mg/kg). Again, in contrast to the results of Benmansour and Brunswick [18] with PCA, we were unable to potentiate the neurotoxicity of this substituted amphetamine analogue.

Previous reports have shown that antagonism of the 5-HT_{2A} receptor could attenuate the increase in DA synthesis [23, 27] and the serotonergic neurotoxicity [21, 31] induced by MDMA. Our present results indicate that an-

tagonism of the 5-HT_{2A} receptor with MDL-11,939 was unable to protect against the neurotoxicity of PCA in the frontal cortex. The results of these studies would suggest that the selective serotonergic neurotoxic effects induced by PCA and MDMA are not produced by identical mechanisms.

In support of this hypothesis, Colado *et al.* [40] showed that the $GABA_A$ agonist, chlormethiazole and the NMDA antagonist MK-801 are able to protect against MDMA-induced 5-HT neurotoxicity, but not against PCA-induced neurotoxicity. Colado *et al.* [40] also concluded that the mechanism by which these substituted amphetamines produce 5-HT neurotoxicity is not identical. Unlike MDMA, Colado *et al.* [40] showed that PCA does not alter DA metabolism. These latter results coupled with the present studies suggest that the difference in the two compounds could be due to differences in their effects on the dopaminergic system.

References

1. Johnson MP, Huang X, Oberlander R, Nash JF and Nichols DE, Behavioral, biochemical and neurotoxicological actions of the α-ethyl homologue of *p*-chloroamphetamine. *Eur J Pharmacol* **191:** 1–10, 1990.
2. Callaway CW, Johnson MP, Gold LH, Nichols DE and Geyer MA, Amphetamine derivatives induce locomotor hyperactivity by acting as indirect serotonin agonists. *Psychopharmacology (Berlin)* **104:** 293–301, 1991.
3. Ross SB, Interactions between reserpine and various compounds on the accumulation of [^{14}C]-5-hydroxytryptamine and [^{3}H[-noradrenaline in homogenates from rat hypothalamus. *Biochem Pharmacol* **28:** 1085–1088, 1979.
4. Johnson MP, Hoffman AJ and Nichols DE, Effects of the enantiomers of MDA, MDMA and related analogues on [^{3}H]serotonin and [^{3}H]dopamine release from superfused rat brain slices. *Eur J Pharmacol* **132:** 269–276, 1986.
5. Schmidt CJ, Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine. *J Pharmacol Exp Ther* **240:** 1–7, 1987.
6. Fuller RW and Snoddy HH, Long-term effects of 4-chloroamphetamine on brain 5-hydroxyindole metabolism in rats. *Neuropharmacology* **13:** 85–90, 1974.
7. Steranka LR and Sanders-Bush E, Long-term reduction of brain serotonin by *p*-chloroamphetamine: Effects of inducers and inhibitors of drug metabolism. *J Pharmacol Exp Ther* **206:** 460–467, 1978.
8. Commins DL, Vosmer G, Virus RM, Woolverton WL, Schuster CR and Seiden LS, Biochemical and histological evidence that methylenedioxymethamphetamine (MDMA) is toxic to neurons in the rat brain. *J Pharmacol Exp Ther* **241:** 338–345, 1987.
9. Leonard BE, Acute and chronic effects of 4-chloroamphetamine on monoamine metabolism in the rat brain. *Psychopharmacology (Berlin)* **46:** 11–18, 1976.
10. Nash JF, Ketanserin pretreatment attenuates MDMA-induced DA release in the striatum as measured by *in vivo* microdialysis. *Life Sci* **47:** 2401–2408, 1990.
11. Ricaurte GA, Schuster CR and Seiden LS, Long-term effects of repeated methylamphetamine administration on dopamine and serotonin neurons in the rat brain: A regional study. *Brain Res* **193:** 153–163, 1980.
12. Sekerke HJ, Smith HE, Bushing JA and Sanders-Bush E, Correlation between brain levels and biochemical effects of the optical isomers of *p*-chloroamphetamine, *J Pharmacol Exp Ther* **193:** 835–844, 1973.
13. Molliver ME, Berger UV, Mamounas LA, Molliver DC, O'Hearn E and Wilson MA, Neurotoxicity of MDMA and related compounds: Anatomic studies. *Ann NY Acad Sci* **600:** 640–664, 1990.
14. Fuller RW, Perry KW and Molloy BB, Effects on 3-(*p*-trifluoromethylphenoxy)*N*-methyl-3-phenylpropylamine on the depletion of brain serotonin by 4-chloroamphetamine. *J Pharmacol Exp Ther* **193:** 796–803, 1975.
15. Fuller RW, Perry KW and Molloy BB, Reversible and irreversible phases of serotonin depletion by 4-chloroamphetamine. *Eur J Pharmacol* **33:** 119–124, 1975.
16. Fuller RW, Snoddy HD, Perry KW, Bymaster FP and Wong DT, Importance of duration of drug action in the antagonism of *p*-chloroamphetamine depletion of brain serotonin–Comparison of fluoxetine and chlorimipramine. *Biochem Pharmacol* **27:** 193–198, 1978.
17. Colado MI and Green AR, A study of the mechanism of MDMA ('Ecstasy')-induced neurotoxicity of 5-HT neurones using chlormethiazole, dizocilpine and other protective compounds. *Br J Pharmacol* **111:** 131–136, 1994.
18. Benmansour S and Brunswick DJ, The MAO-B inhibitor deprenyl, but not the MAO-A inhibitor clorgyline, potentiates the neurotoxicity of *p*-chloroamphetamine. *Brain Res* **650:** 305–312, 1994.
19. Sprague JE and Nichols DE, Inhibition of MAO-B protects against MDMA-induced neurotoxicity in the striatum. *Psychopharmacology (Berlin)* **118:** 357–359, 1995.
20. Sprague JE and Nichols DE, The monoamine oxidase-B inhibitor L-deprenyl protects against 3,4-methylenedioxymethamphetamine-induced lipid peroxidation and long term serotonergic deficits. *J Pharmacol Exp Ther* **273:** 667–673, 1995.
21. Nichols DE, Brewster WK, Johnson MP, Oberlender R and Riggs RM, Nonneurotoxic tetralin and indan analogues of 3,4-(methylenedioxy)amphetamine. *J Med Chem* **33:** 703–710, 1990.
22. Johnson MP, Pharmacological characterization of 3,4-methylenedioxymethamphetamine analogues: Corollaries to mechanisms of behavioral and neurotoxic activity. *Ph.D. Thesis*. Purdue University, West Lafayette, IN, 1991.
23. Schmidt CJ, Fadayel GM, Sullivan CK and Taylor VL, 5-HT2 receptors exert a state-dependent regulation of dopaminergic function: Studies with MDL 100,907 and the amphetamine analogue, 3,4-methylenedioxymethamphetamine. *Eur J Pharmacol* **223:** 65–74, 1992.
24. Marcusson JO, Bergstrom M, Eriksson K and Ross SB, Characterization of [^{3}H]paroxetine binding in the rat brain. *J Neurochem* **50:** 1783–1790, 1988.
25. Battaglia G, Yeh SY, O'Hearn E, Molliver ME, Kuhar MJ and De Souza EB, 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: Quantification of neurodegeneration by measurement of [^{3}H]paroxetine-labeled serotonin uptake sites. *J Pharmacol Exp Ther* **242:**911–916, 1987.
26. Huang X and Nichols DE, 5-HT_2 receptor-mediated potentiation of dopamine synthesis and central serotonergic deficits. *Eur J Pharmacol* **238:** 291–296, 1993.
27. Nash JF, Meltzer HY and Gudelsky GA, Effects of 3,4-methylenedioxymethamphetamine on 3,4-dihydroxyphenylalanine accumulation in the striatum and nucleus accumbens. *J Neurochem* **54:** 1062–1067, 1990.
28. Nichols DE, Lloyd DH, Hoffman AJ, Nichols MB and Yim GKW, Effects of certain hallucinogenic amphetamine analogues on the release of [^{3}H]serotonin from rat brain synaptosomes. *J Med Chem* **25:** 530–535, 1982.
29. Schmidt CJ, Levin JA and Lovenberg W, *In vitro* and *in vivo* neurochemical effects of methylenedioxymethamphetamine

on striatal monoaminergic systems in the rat brain. *Biochem Pharmacol* **36:** 747–755, 1987.
30. Steele TD, Nichols DE and Yim GKW, Stereochemical effects of 3,4-methylenedioxymethamphetamine (MDMA) and related amphetamine derivatives on inhibition of uptake of [^{3}H]-monoamines into synaptosomes from different regions of the rat brain. *Biochem Pharmacol* **36:** 2297–2303, 1987.
31. Schmidt CJ and Lovenberg W, *In vitro* demonstration of DA uptake by neostriatal serotonergic neurons of the rat. *Neurosci Lett* **59:** 9–14, 1985.
32. Waldmeier PC, Displacement of striatal 5-hydroxytryptamine by dopamine released from endogenous stores. *J Pharm Pharmacol* **37:** 58–60, 1985.
33. Westlund KN, Denney RM, Kochersperger LM, Rose RM and Abell CW, Distinct monoamine oxidase A and B populations in primate brain. *Science* **230:** 181–183, 1985.
34. Gordon CJ, Watkinson WP, O'Callaghan JP and Miller DB, Effects of 3,4-methylenedioxymethamphetamine on autonomic thermoregulatory responses of the rat. *Pharmacol Biochem Behav* **38:** 339–344, 1991.
35. Yehuda S and Wurtman RJ, The effects of D-amphetamine and related drugs on colonic temperature of rats kept at various ambient temperatures. *Life Sci.* **11:** 851–859, 1972.
36. Pawlowski L, Different action of 5-hydroxytryptamine (5-HT) uptake inhibitors on fenfluramine- but not *p*-chloroamphetamine-induced hyperthermia in rats. *J Pharm Pharmacol* **3:** 538–540, 1981.
37. Schmidt CJ, Black CK and Taylor VL, Antagonism of the neurotoxicity due to a single administration of 3,4-methylenedioxymethamphetamine. *Eur J Pharmacol* **181:** 59–70, 1990.
38. Che S, Gibb JW, Hanson GR and Johnson M, The effect of body temperature on the rapid decrease in tryptophan hydroxylase activity induced by 3,4-methylenedioxymethamphetamine. *Soc Neurosci Abstr* **20:** 1624, 1994.
39. Malberg JE, Malis RW, Sabol KE and Seiden LS, Drugs that protect against MDMA-induced serotonin neurotoxicity have different effects on body temperature in the rat when administered in combination with MDMA. *Soc Neurosci Abstr* **20:** 290, 1994.
40. Colado MI, Murray TK and Green AR, 5-HT loss in rat brain following 3,4-methylenedioxymethamphetamine (MDMA), *p*-chloroamphetamine and fenfluramine administration and effects of chlormethiazole and dizocilpine. *Br J Pharmacol* **108:** 583–589, 1993.