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BRIEF COMMUNICATION

Pargyline Increases 6-Hydroxydopamine Levels in the Neostriatum of Methamphetamine-Treated Rats

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MAREK, G. J., G. VOSMER AND L. S. SEIDEN. *Pargyline increases 6-hydroxydopamine levels in the neostriatum of methamphetamine-treated rats.* PHARMACOL BIOCHEM BEHAV **36**(1) 187–190, 1990.—Neostriatal 6-hydroxydopamine (6-OHDA) was detected in 6 of 13 rats pretreated 2 or 4 hr earlier with methamphetamine (MA; 100 mg/kg, SC) and pargyline (25 mg/kg, IP, 30 min before MA injection). Neostriatal 6-OHDA was detected in 2 of 16 rats treated 2 or 4 hr earlier with MA. These results suggest that pargyline pretreatment may enhance formation of 6-OHDA from endogenous stores of dopamine (DA) following MA administration. Alternatively, these results suggest that pargyline pretreatment may protect endogenously formed 6-OHDA from oxidative deamination by monoamine oxidase. Enhancement of MA-induced neostriatal 6-OHDA levels may be the mechanism by which pargyline enhances the long-term neurotoxic effects of MA upon dopaminergic nerve terminals. These observations support the hypothesis that MA toxicity to DA-containing fibers is caused by the conversion of released DA into 6-OHDA.

Methamphetamine Pargyline 6-Hydroxydopamine Neurotoxicity

ADMINISTRATION of N-methyl-B-phenylisopropylamine (methamphetamine, MA) produces neurotoxic effects on brain dopaminergic and serotonergic neurons as evidenced by long-term dopamine (DA) and serotonin (5-hydroxytryptamine; 5-HT) depletions, decreased tyrosine hydroxylase and tryptophan hydroxylase activity, decreased DA and 5-HT high-affinity uptake sites in rats, cats, guinea pigs and primates (7, 15, 19, 24, 25). In addition, MA produces degenerating axons and terminals in the neostriatum of rats (16) as revealed by silver-impregnation of degenerating nerve fibers (4). Wagner *et al.* (26) provided evidence in experiments involving the tyrosine hydroxylase inhibitor alpha-methyl-p-tyrosine (AMT) and the disruptor of monoamine storage granules reserpine suggesting that either DA or a DA metabolite is directly responsible for the neurotoxicity seen following MA administration. In addition, inhibition of MAO with pargyline enhanced the long-term DA depletions induced by MA administration (8). Consistent with the above work, 6-OHDA has been detected in the rat brain following administration of a single 100 mg/kg dose of MA (20).

The purpose of the present experiments was to study the mechanism by which pargyline enhances MA-induced neurotoxicity. The present experiment attempted to measure 6-OHDA in rats treated with MA both with and without a 30-min pargyline pretreatment. We chose to measure 6-OHDA at 2 and 4 hr following MA administration as in preliminary work with pargyline and MA administration, we observed much higher 6-OHDA levels in a pargyline/MA-treated rat that survived 3½ hr than we have previously observed in any MA-treated rat. The results of the present experiment suggest that pargyline increased levels of 6-OHDA in MA-treated rats.

METHOD

Animals

Subjects for this study were male Sprague-Dawley rats (Harlan Sprague-Dawley, Terre Haute, IN) weighing 280 (± 10) g when the drug treatments were initiated. Rats were housed singly in wire-mesh cages with free access to laboratory chow (Teklad 4% Rat Diet) and water. Ambient temperature was maintained at 22 ± 1°C. Fluorescent lighting was automatically turned on at

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06.00 hr and off at 20.00 hr.

Drug Treatment

In the present experiment, groups of 6–8 rats were injected with either pargyline (25 mg/kg, IP) or physiological saline in a volume of 1 ml/kg. Thirty min later these same groups of rats were injected with either MA (100 mg/kg, SC) or physiological saline in a volume of 4 ml/kg. These rats were then killed by decapitation either 2 or 4 hr following MA administration for determination of neostriatal 6-OHDA, DA and DOPAC levels using reverse-phase ion-pair high performance liquid chromatography with electrochemical detection. Three of the eight rats in the 4-hr pargyline-MA group died and were not included in the data analysis.

Regional Brain Dissection

The neostriatum was obtained as described previously (6) except that the neostriatal sample was also taken from the two slices anterior to the reference blade instead of only the slice immediately anterior to the reference blade. The tissue samples were wrapped immediately in aluminum foil and stored in liquid nitrogen.

DA and 5-HT Level Determinations

Concentrations of DA, DOPAC and 6-OHDA were determined by reverse-phase ion-pair high performance liquid chromatography with electrochemical detection as follows. RSIL C-18 HL columns (4.6 mm i.d. by 25 cm, Alltech Assoc.) were eluted at a flow rate of 1 ml per minute with a mobile phase containing 0.27 mM EDTA, 0.09 M citric acid, 0.005 M sodium phosphate, 0.09 mM octyl sodium sulfate and 1% methanol. The potential of the working electrode was 0.8 V relative to the Ag/AgCl reference electrode. The neostriatal samples were sonicated in 400 μl 0.4 N PCA with 0.05% EDTA and centrifuged 10 min at 20,000 × g immediately prior to assay by HPLC-EC. One hundred microliters of supernatant was injected onto the HPLC column. DA, DOPAC and 6-OHDA were quantified with an integrator (Shimadzu, CR5A chromatopac). Retention times were as follows: 6-OHDA, 16 min; DOPAC, 23 min; DA 27 min. The sensitivity of the assay for 6-OHDA was 0.1 ng/100 μl with the minimum amount of 6-OHDA detected in 100 μl of supernatant being 0.2 ng/100 μl.

Statistical Analysis

The significance of differences between group means was assessed with the Student *t*-test (27). Significance was accepted at the $p<0.05$ level. The incidence of 6-OHDA formation was analyzed using the nonparametric Fisher exact probability test (21).

Drugs and Materials

Methamphetamine was supplied by the National Institute of Drug Abuse (Bethesda, MD); pargyline hydrochloride, dopamine hydrochloride, dihydroxyphenylacetic acid (Sigma Chemical Co., St. Louis, MO); 6-hydroxydopamine (Regis Chemical Co., Morton Grove, IL) were purchased from their suppliers.

RESULTS

Administration of a single 100 mg/kg dose of MA resulted in neostriatal 6-OHDA formation in only 2 of 16 rats compared to 0 of 6 saline-treated rats ($p=0.519$), irrespective of the MA pretreatment time (Table 1). Pretreatment with pargyline (25 mg/kg, IP) 30 min prior to MA resulted in 6-OHDA formation in 6 of 13 rats which tended to be significantly greater than the incidence of 6-OHDA formation in the saline-saline group ($p=0.063$) and the MA-saline group ($p=0.052$). The incidence of 6-OHDA formation was significantly greater in the MA-pargyline group ($p=0.010$) than in the pargyline-saline group (0 of 12 rats).

TABLE 1

NEOSTRIATAL 6-HYDROXYDOPAMINE, DOPAMINE AND DIHYDROXYPHENYLACETIC ACID IN RATS TREATED WITH METHAMPHETAMINE (MA, 100 mg/kg) OR SALINE AND PARGYLINE (25 mg/kg) OR SALINE

Group	N	6-OHDA	DA	DOPAC
Sal-sal	6	0.00	10.54 ± 0.86	1.82 ± 0.32
Pargyline-sal (2 hr)	6	0.00	†14.00 ± 0.66	†0.72 ± 0.18
Pargyline-sal (4 hr)	6	0.00	*13.91 ± 0.70	‡0.34 ± 0.07
Sal-MA (2 hr)	8	0.00	‡6.45 ± 0.49	†0.73 ± 0.05
Sal-MA (4 hr)	8	[a]0.038 ± 0.026	‡4.53 ± 0.14	‡0.46 ± 0.05
Pargyline-MA (2 hr)	8	[b]0.178 ± 0.089	‡7.09 ± 0.32	‡¶#0.36 ± 0.05
Pargyline-MA (4 hr)	5	[c]0.101 ± 0.046	†‡6.43 ± 0.51	‡§0.20 ± 0.05

Either pargyline (25 mg/kg, IP) or saline were given in a 1 ml/kg volume 30 min prior to an injection of either MA (100 mg/kg, SC) or saline in a 4 ml/kg volume. Rats were killed by decapitation either 2 or 4 hr following the second injection. All values expressed in ng/mg tissue (mean ± SEM).

*$p<0.05$ vs. sal-sal; †$p<0.01$ vs. sal-sal; ‡$p<0.001$ vs. sal-sal; §$p<0.01$ vs. sal-MA; ¶$p<0.001$ vs. sal-MA; #$p<0.05$ vs. parg-sal 2 hr.

[a]6-OHDA detected in 2 out of 8 saline-MA 4-hr rats (.124, .182); [b]6-OHDA detected in 3 out of 8 pargyline-MA 2-hr rats (.442, .390, .595); [c]6-OHDA detected in 3 out of 5 pargyline-MA 4-hr rats (.131, .130, .244).

Pargyline (25 mg/kg, IP), by itself, significantly increased neostriatal DA and decreased neostriatal DOPAC with both the 2½-hr and 4½-hr pretreatment times (Table 1). MA (100 mg/kg, SC), by itself, significantly depleted neostriatal DA and DOPAC with both the 2-hr and 4-hr pretreatment times. Administration of pargyline ½ hr before MA antagonized the decrease in neostriatal DA at the 4-hr pretreatment time and further enhanced the decrease in neostriatal DOPAC at both the 2-hr and the 4-hr pretreatment times.

DISCUSSION

MA and amphetamine exert long-term neurotoxicity upon dopaminergic neurons (5, 7, 16, 18, 23, 25). Pretreatment with doses of pargyline that block both MAO-A and MAO-B enhanced the long-term DA depletions engendered by MA administration (8). Previously, 6-OHDA has been measured in the rat neostriatum following administration of a single 100 mg/kg dose of MA (20). The present demonstration that pargyline pretreatment increased the detection of 6-OHDA formation in the rat neostriatum suggests that pargyline enhanced MA's neurotoxic effects on dopaminergic neurons by increasing the incidence and/or quantity of MA-induced 6-OHDA formation.

There are several mechanisms by which pargyline may have increased the incidence and/or quantity of MA-induced 6-OHDA formation. First, pargyline may have interfered with MA metabolism. Less than 2% of amphetamine administered in the rat is excreted in the urine as an oxidative deaminated produced while 74% of amphetamine administered is excreted in the urine as

amphetamine or p-hydroxyamphetamine (22). In the present experiment, MA and amphetamine levels were not measured to rule out the possibility that pargyline may have inhibited the p-hydroxylation of amphetamine (3). Second, pargyline may have increased the detection of 6-OHDA by protecting 6-OHDA from oxidative deamination. Third, pargyline may have increased the detection of 6-OHDA by enhancing formation of the neurotoxin 6-OHDA from endogenous stores of DA by preventing oxidative deamination of DA itself. Fourth, a combination of the above factors may be at work to bring about the present results.

Previous work has suggested that metabolism of DA is involved in the neurotoxic effects of MA upon dopaminergic neurons. Inhibition of tyrosine hydroxylase with alpha-methyl-p-tyrosine (AMT) antagonized long-term MA-induced depletions (25). AMT also prevents MA-engendered 5-HT toxicity but the putative toxin appears to be 5,6-dihydroxytryptamine (5,6-DHT) which is synthesized from 5-HT. The manner in which DA enters this reaction is not clearly understood.

Disruption of monoamine storage vesicles with reserpine enhanced the long-term MA-induced neostriatal DA depletions (25). These findings are consistent with the concept that the amphetamines release DA from an AMT sensitive, newly synthesized cytoplasmic transmitter pool (9, 14, 18). Reserpine would increase intracellular levels of "free" DA (2). This distribution of DA in the cell is important in light of MA inhibition of MAO which would further increase levels of DA available for release. This line of evidence is consistent with the third hypothesis that pargyline may increase 6-OHDA formation by preventing oxidative metabolism of "free" cytoplasmic DA.

It has been suggested that under conditions of MAO inhibition and DA release caused by MA administration, that 6-OHDA can form in the presynaptic terminal or in the synaptic cleft. Amphetamine is known to be a weak reversible inhibitor of MAO whereas pargyline is known to be a potent irreversible inhibitor of MAO (12,13). Enhanced DOPAC depletions in the MA-pargyline groups vs. the MA-saline groups suggest that administration of pargyline increases inhibition of MAO in MA-treated rats. These pargyline experiments support the hypothesis that metabolism of DA is involved in the neurotoxic effects of MA. Recent work with DA uptake inhibitors provide additional support that DA metabolism (formation of 6-OHDA from endogenous stores of DA) is important for MA-induced neurotoxicity upon dopaminergic neurons (5, 10, 11, 23).

In other work, it has been claimed that in vivo formation of 6-OHDA from released DA is unlikely to explain the neurotoxic effects of amphetamine and MA (17). A number of problems complicate that interpretation. First, Rollema and his colleagues did not show that the doses of MA or amphetamine they administered caused long-term neurotoxic effects upon dopaminergic neurons. Second, these investigators used an IP route of administration vs. a SC route employed by Seiden and Vosmer (20) and the route of administration is known to have important pharmacokinetic effects (1). Third, the Rollema *et al.* study employed a small n for the groups receiving MA or amphetamine. Recent work in this laboratory has noted that the variability and low incidence in which 6-OHDA is detected in MA-treated rats appears to correlate to the variability and low incidence in which DA levels are dramatically reduced several weeks following a single 100 mg/kg MA dose (10,11).

In summary, pargyline increased the detection of 6-OHDA in MA-treated rats. This finding is consistent with the previous demonstration that pargyline enhances the long-term DA-depleting action of MA (8). Converging evidence with reserpine, alpha methyl-p-tyrosine, pargyline and DA uptake inhibitors all provide support for the hypothesis that formation of 6-OHDA mediates the neurotoxic effect of MA upon dopaminergic nerve fibers.

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