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Formation of 6-Hydroxydopamine in Caudate Nucleus of the Rat Brain After a Single Large Dose of Methylamphetamine[1]

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SEIDEN, L. S. AND G. VOSMER. *Formation of 6-hydroxydopamine in caudate nucleus of the rat brain after a single large dose of methylamphetamine.* PHARMACOL BIOCHEM BEHAV 21(1) 29–31, 1984.—We now report that 6-hydroxydopamine (0.39±0.31 nanograms/mg of tissue at 2 hr) is formed in the rat caudate nucleus after a single injection of methylamphetamine (100 mg/kg). The same dose of methylamphetamine causes approximately 50% depletion of caudate dopamine 2 weeks after the injection. We suggest that the formation of 6-hydroxydopamine from endogenous dopamine is responsible for the neurotoxicity to dopamine terminals seen after methylamphetamine administration.

6-Hydroxydopamine Methylamphetamine Neurotoxicity

METHYLAMPHETAMINE (MA) when administered in large doses or for prolonged periods of time causes irreversible depletion of dopamine in several brain areas in monkeys, rats, guinea pigs and mice [14, 19, 21]. The depletion of dopamine is caused by the loss of dopaminergic nerve terminals as evidenced by a decrease in tyrosine hydroxylase, a decrease in the high affinity reuptake capacity of dopamine terminals, and argyophyllic terminals in the caudate as determined by the method of Fink and Heimer [2] indicating that nerve terminals are degenerating [11,13]. Intraventricular injection of 6-hydroxydopamine (6-OHDA) also causes long-term depletion of dopamine and norepinephrine and is associated with a neuronal degenerative process [4, 9, 18].

We now report that 6-OHDA is formed in the rat caudate nucleus after a single large dose of MA which causes long-term damage to DA-containing cells in the rat caudate nucleus [11, 12, 19, 20]. We suggest that the formation of 6-OHDA from endogenous DA is responsible for the neurotoxicity to DA terminals seen after methylamphetamine administration.

METHOD

Male Sprague-Dawley rats (Harlem Sprague-Dawley, Terre Haute, IN) weighing about 200 grams were used in these studies. They were injected with either saline (controls) or d-methylamphetamine hydrochloride (obtained from the National Institute on Drug Abuse) at a dose of 100 mg/kg (expressed as the salt and prepared at a concentration of 25 mg/ml) of body weight. Rats were sacrificed by decapitation at 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 and 24.0 hours after the MA injection. Four rats were injected with methylamphetamine (100 mg/kg) and four with 0.9% saline. These rats were killed two weeks later to confirm that a single injection of MA has neurotoxic effects. A group of twelve rats were injected intraventricularly with 150 μg of 6-OHDA hydrobromide (Sigma Chemical Co., St. Louis, MO) [8]. The brain was removed from the skull and dissected over ice by the method of Heffner *et al.* [5]. The caudate nucleus was frozen in liquid nitrogen until assay.

Caudate tissue was assayed using high performance reverse phase liquid chromatography with electrochemical detection as described by Kotake *et al.* [7]. The stainless steel column (Alltech Assoc., Deerfield, IL) packed with C18HL resin (5 micron particle size) was eluted at a flow rate of 1 ml/min with an aqueous mobile phase containing: 125 mM citric acid, 125 mM sodium phosphate, 0.01% (w/v) ethylene diamine tetraacetic acid (EDTA) and octyl sodium sulfate (30 mg/l). The pH of the mobile phase was adjusted to 2.5 with phosphoric acid (85%). One hundred microliter aliquots of standard or sample were applied to the column and detected amperometrically with the glassy carbon working electrode maintained at a potential of +0.8 V relative to the reference electrode. Samples were quantified using external standards and peak height ratios. The retention times of 6-OHDA, DA and dihydroxyphenylacetic acid (DOPAC) were 8 min, 13 min and 20 min, respectively. The sensitivity for 6-OHDA was 1.0 ng/2.5 nA.

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TABLE 1

LEVELS OF 6-OHDA, DA AND DOPAC IN RATS TREATED WITH 100 mg/kg METHYLAMPHETAMINE

Treatment	Time of Kill	ng/mg tissue 6-OHDA	DA	DOPAC	DOPAC/DA
Saline N= 8	20 minutes/ post injection	0 0	8.1 ±0.25	0.87 ±0.09	10.6
100 mg/kg N= 8	30 minutes/ post injection	0.20 ±0.17	8.7 0.68	0.72 0.07	10.2
100 mg/kg N= 5	1 hour/ post injection	0.39 0.31	6.1 0.60	0.62 0.07	8.3
100 mg/kg N= 6	2 hour/ post injection	0.24 0.21	6.4 0.84	0.44 0.56	6.8
100 mg/kg N= 5	4 hour/ post injection	0 0	5.7 0.65	0.37 0.08	6.4
100 mg/kg N= 6	8 hour/ post injection	0 0	6.9 0.54	0.33 0.04	4.8
100 mg/kg N= 4	16 hour/ post injection	0 0	6.3 2.6	0.78 0.25	12.4
100 mg/kg N=10	24 hour/ post injection	0 0	4.1 1.2	0.77 0.29	18.8

TABLE 2

6-OHDA MEASUREMENT AFTER IVT INJECTION

Time of Sacrifice	ng/mg tissue 6-OHDA	DA	DOPAC
30 minutes (N=2)	1.48 ± 1.24	13.66 ± 0.510	0.297 ± 0.297
1 hour (N=5)	1.04 ± 0.819	15.41 ± 0.791	0.167 ± 0.113
2 hours (N=3)	0.107 ± 0.046	13.58 ± 1.70	0.116 ± 0.039
4 hours (N=2)	0.079 ± 0.033	15.09 ± 1.15	0.315 ± 0.268

Rats were pretreated with pargyline (50 mg/kg, IP) and DMI (25 mg/kg, IP) 40 minutes prior to IVT injection of a total of 150 μg 6-OHDA.

RESULTS AND DISCUSSION

Rats injected with 100 mg/kg of MA formed 6-OHDA in the caudate nucleus between 0.5 and 2.0 hr after the injection. There was no 6-OHDA found in the control rats (saline injected or rats that were killed 3 hours after the MA injection. DA levels were depleted after the injection and DOPAC levels were reduced (Table 1). The 6-OHDA that was formed after the injection was formed within two hours and there was not a definitive time course to the formation of the 6-OHDA; the amounts detected at each of the 3 time points where it was observed were variable as can be seen from the large standard error of the mean. However, some 6-OHDA was detected in every rat sacrificed between 0.5 and 2 hr. DA levels were reduced due to the MA engendered release of DA, and DOPAC levels fell with time due to the fact that in high doses, MA, like amphetamine (a principle metabolite, is an effective monoamine oxidase inhibitor [3,22]. Rats that were injected with methylamphetamine and allowed to survive two weeks showed depletion of caudate DA (saline treated: 10.4±1.18 nanograms/mg tissue; methamphetamine treated: 5.18±0.63 nanograms/mg tissue). These results are consistent with other studies using slightly different injection regimens [11, 19, 21]. A sample of methylamphetamine (2.5 mg/100 μl or 0.025 mg/μl, the same amount used for rat injection) was analyzed using the procedure described above to determine if the 6-OHDA detected in the brains of animals injected with 100 mg/kg of MA was due to contamination of the MA; no 6-OHDA was found in the methylamphetamine sample.

In the 12 rats injected intraventricularly with 6-hydroxydopamine, the 6-OHDA is detectable in the cau-

date nucleus from 0.5 to 4.0 hr after the IVT injection. While the levels are higher than the levels in the MA injected rats (Table 2), they are of the same order of magnitude and interestingly, the levels of 6-OHDA are as variable after the administration of methylamphetamine (Table 2). Furthermore, according to the mechanism proposed, the 6-OHDA is formed from DA released at the synapse, and the local concentration may be even higher than 6-OHDA injected by the intraventricular route of administration.

Senoh and Witkop [16] and Senoh *et al.* [15] have experimentally shown that DA can be non-enzymatically converted to trihydroxyphenethylamines among which 2,4,5-trihydroxyphenethylamine (i.e., 6-hydroxydopamine) is a possible oxidative metabolite. We hypothesize that the 100 mg/kg dose of MA causes massive release of dopamine from the cytoplasmic bound pool [10]. Since monoamine oxidase is inhibited, the released DA cannot be as rapidly degraded. Therefore, under conditions of increased release accompanied by inactivation of MAO the non-enzymatic formation of the trihydroxyphenethylamines is favored and 6-OHDA is formed. The 6-OHDA so formed is at the synapse and can act like exogenously administered 6-OHDA. The 6-OHDA is taken up into, or formed in the DA cell and causes neural damage. The fact that external administration of 6-OHDA as well as 6-OHDA formed from DA are of the same order of magnitude after the administration of MA adds further weight to the argument that the formed 6-OHDA seen after MA administration is reponsible for the neurotoxicity.

Parkinson's disease as well as other diseases of the central nervous system are believed to involve altered DA neurotransmitter function [6], but the etiology of the neurotoxicity is not well understood [1]. The idea that neurotoxicity or abnormal metabolites of transmitters might be responsible for dysfunction in the central nervous system has been proposed [17]. The data presented in this report is the first demonstration that a toxic metabolite of DA is formed after drug administration.

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