Amphetamine induces depletion of dopamine and loss of dopamine uptake sites in caudate

Article abstract—Long-lasting depletion of dopamine and concomitant loss of dopamine uptake sites follow repeated administration of methylamphetamine to rats. We found similar effects after similar treatment with *d*-amphetamine, but not after treatment with methylphenidate. Methylphenidate also failed to produce long-term depletion of regional catecholamine levels in rhesus monkeys. These long-lasting alterations of the dopaminergic system suggest that amphetamines or their metabolites have toxic interactions with dopaminergic neurons, which do not occur with methylphenidate.

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Repeated administration of high doses of methylamphetamine results in long-lasting changes in regional brain catecholamine levels.[1-3] Rhesus monkeys showed depletion of dopamine in the caudate nucleus and depletion of norepinephrine in frontal cortex and midbrain as long a 6 months after the last injection of the drug.[1] Methylamphetamine caused similar depletion of striatal dopamine in rats and guinea pigs.[2] In the rat, these depletions were dose-related, lasted for at least 8 weeks after the injection period, and were accompanied by loss of striatal dopamine high-affinity uptake sites.[3]

D-amphetamine and methylphenidate, like methylamphetamine, are used to treat minimal brain dysfunction and obesity, and all are subject to abuse.[4-7] We attempted to determine whether repeated administration of these stimulants also results in long-lasting catecholamine depletion and concomitant loss of high-affinity uptake sites.

Methods. Forty-six male Sprague-Dawley rats (Holtzman Co.), weighing approximately 250 gm, were housed individually with ad libitum access to food (Teklad Co.) and water. Colony room lights were automatically turned on at 0600 hours and off at 1800 hours. Temperature was maintained at 22 ± 1° C.

The rats were divided randomly into three groups. The first group received subcutaneous injections of *d*-amphetamine sulfate dissolved in physiologic saline at a dose of 25 mg per kilogram. Drug injections were 1 ml per kilogram. Injections were given twice daily at 0800 and 1700 hours for a total daily dose of 50 mg per kilogram. The second group received subcutaneous injections of methylphenidate hydrochloride dissolved in physiologic saline in the same dose and volume. The third group received an equal volume of the vehicle solution. Injections continued for 30 days, and rats were killed 2 weeks after the last injection.

After the rats were killed, brains were removed quickly and dissected on ice. The hypothalamus was lifted free after a shallow circumferential incision, and then the telencephalon, midbrain, pons medulla, and caudate nucleus were dissected as described previously.[2] Brain parts were stored in liquid nitrogen until assayed. The concentration of catecholamines in the rats' brain tissue was determined by high-performance liquid chromatography with electrochemical detection and alumina adsorption, with subsequent flurometric assay.[8-10]

Four adult rhesus monkeys, two male and two female, were also used. They were housed individually with ad libitum access to food (Purina Monkey Chow) and water. Colony room lights were automatically turned on at 0600 hours and off at 1800 hours. Temperature was maintained at 22 ± 1° C.

One male and one female monkey received intramuscular injections of methylphenidate hydrochloride dissolved in physiologic saline. The initial dose was 1 mg per kilogram per day, given in two injections at 0700 and 1800 hours. The dose was gradually increased over 6 months until a final dose of 96 mg per kilogram per day was given during the last week. These monkeys were killed 4 weeks after the last injection, and the other two monkeys served as controls.

The monkey brains were dissected to yield samples of frontal cortex, hypothalamus, pons medulla, midbrain, and caudate nucleus as described.[1] Brain parts were stored in liquid nitrogen until assayed. The concentration of catecholamines in monkey brain tissue was determined by Dowex cation exchange chromatography and fluorometric assay.[11]

Studies of uptake of 3H-dopamine were conducted on two groups of six rats treated for 4 days with 100 mg per kilogram per day of either *d*-amphetamine sulfate or methylphenidate hydrochloride; a third group received the same volume of the vehicle, physiologic saline. Rats were killed and assayed 2 to 3 weeks after the last injection. The uptake of 3H-dopamine was measured by the method of Snyder and Coyle[12] with minor modifications described elsewhere.[3]

Results. Caudate dopamine content in rats treated with *d*-amphetamine was significantly depleted to 56% of the content in control rats. There was no significant difference in caudate dopamine

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Table 1. Regional catecholamine levels in rats receiving subcutaneous *d*-amphetamine sulfate (*d*-AMPH) or methylphenidate hydrochloride (MP) at a dose of 50.0 mg per kilogram per day for 30 days

	Caudate DA (μg/g)	Telencephalon NE (μg/g)	Midbrain NE (μg/g)	Pons medulla NE (μg/g)	Hypothalamus NE (μg/g)
Vehicle	9.91 ± 0.82	0.40 ± 0.02	0.64 ± 0.03	0.53 ± 0.01	2.02 ± 0.19
d-AMPH (50 mg/kg/d)	5.53 ± 0.85*	0.45 ± 0.02	0.66 ± 0.04	0.59 ± 0.02	2.02 ± 0.14
MP (50 mg/kg/d)	9.66 ± 1.44	0.40 ± 0.04	0.60 ± 0.06	0.55 ± 0.04	2.11 ± 0.19

Values reported are μg per gram tissue ± standard error of the mean. Rats were killed 2 weeks after the last injection. DA = dopamine; NE = norepinephrine.
* < 0.05 versus vehicle groups (Student t test).

Table 2. Regional catecholamine levels of rhesus monkeys receiving methylphenidate (MP) for a period of 6 months

	Dopamine				
	Caudate	Pons medulla	Midbrain	Hypothalamus	Frontal cortex
Control 1	9.95	0.03	0.13	0.16	0.16
Control 2	8.87	0.03	0.14	0.31	0.08
MP 3	8.37	0.09	0.37	0.22	0.11
MP 4	9.89	0.01	0.08	0.01	0.04
	Norepinephrine				
	Caudate	Pons medulla	Midbrain	Hypothalamus	Frontal cortex
Control 1	0.18	0.43	0.56	1.76	0.23
Control 2	0.04	0.49	0.54	3.09	0.31
MP 3	0.16	0.60	0.75	2.13	0.18
MP 4	0.13	0.53	0.80	2.97	0.29

The dose was initially 1.0 mg per kilogram per day and was increased to a final dose of 96.0 mg per kilogram per day, which was given for 1 week. Monkeys were killed 4 weeks after the last injection. Values reported are μg per gram tissue. DA = dopamine; NE = norepinephrine.

content of methylphenidate-treated rats and controls, nor of norepinephrine levels in any brain regions of rats treated with either *d*-amphetamine or methylphenidate and controls (table 1). There was no significant depletion of either dopamine or norepinephrine in any of the brain regions of the monkeys treated with methylphenidate (table 2).

Rats treated for 4 days with 100 mg per kilogram per day of *d*-amphetamine sulfate displayed fewer (decreased V_{max}) caudate dopamine uptake sites 2 to 3 weeks later. The density of dopamine-uptake sites was decreased to 45% of the density in controls. The affinity (Km) of residual sites was not altered by the drug treatment. Rats treated with 100 mg per kilogram per day of methylphenidate hydrochloride showed no change in either the V_{max} or Km of caudate dopamine uptake sites (figure).

Discussion. This study extended the finding of long-lasting depletion of striatal dopamine and concomitant loss of dopaminergic uptake sites after repeated administration of methylamphetamine[1-3] to *d*-amphetamine. In rats and guinea pigs we found no long-lasting depletions of norepinephrine in any brain region after administration of methylamphetamine or *d*-amphetamine. In addition, no long-lasting catecholamine-system changes were observed after repeated administration of high doses of methylphenidate to rats or rhesus monkeys.

Any comparison between two drugs' effects must take into account the differences in relative po-

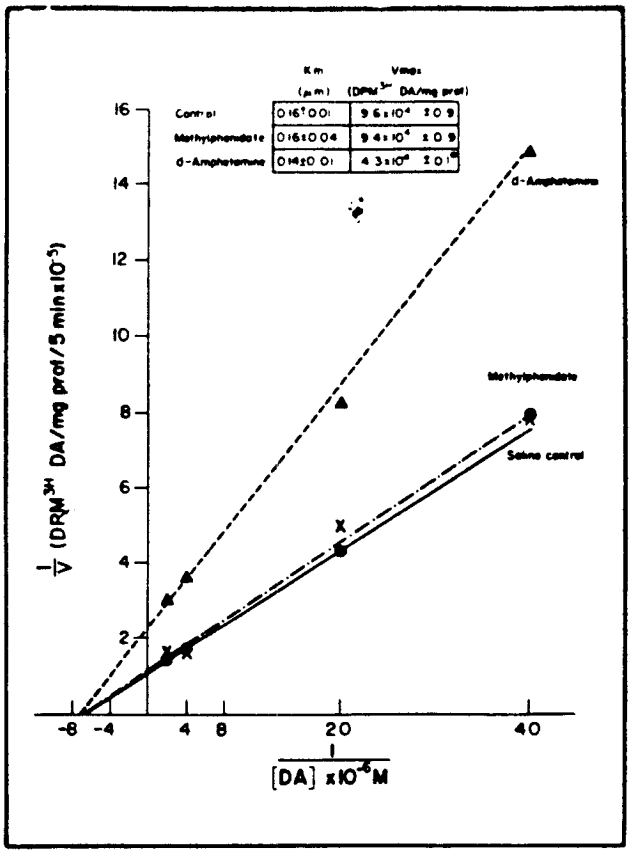

Figure. Lineweaver-Burk plot of 3H-dopamine (DA) accumulation by striatal homogenates prepared from rats treated with either d-amphetamine sulfate or methylphenidate hydrochloride 2 weeks prior to sacrifice.

tency. Moore, Chiueh, and Zeldes[13] found that amphetamine was 10 times more potent than methylphenidate in releasing dopamine. Pearl and Seiden[14] found that amphetamine was four times more potent than methylphenidate in inducing behavioral effects. However, toxicity data from the present study indicated that the failure of methylphenidate to cause long-lasting depletion of catecholamines was not due to an improperly low dosage. The rat lethality rate induced by methylphenidate was approximately 47% (7 of 15); with *d*-amphetamine, it was only 33% (4 of 12). (In a previous study of 50 mg per kilogram per day of methylamphetamine for 30 days, approximately 50% of the rats died.) Also, the highest daily dose of methylphenidate administered to rhesus monkeys (which did not cause any long-lasting change in the catecholaminergic system) was four times greater than the daily dose of methylamphetamine reported to cause long-lasting depletion.

Therefore, these doses of methylphenidate were comparable to doses of methylamphetamine that cause long-lasting disruption of the catecholamine system. However, methylphenidate did not cause any of these long-lasting alterations in rats or rhesus monkeys. Consequently, this study indicates that amphetamines or their metabolites have toxic properties on catecholaminergic neurons, which are not found with methylphenidate.

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