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METHAMPHETAMINE-INDUCED CHANGES IN BRAIN CATECHOLAMINES IN RATS AND GUINEA PIGS*

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Summary

Repeated administration of methamphetamine was found to cause long-term changes in caudate dopamine levels in the rat and guinea pig. Methamphetamine was administered twice a day for thirty days. Two weeks following the last injection, the animals were killed and brains assayed for catecholamine content. These long-term depletions of dopamine, when combined with similar observations previously reported in rhesus monkeys, indicate a species generality of the effects of methamphetamine on caudate dopamine levels.

Introduction

It was previously reported by Seiden *et al.* [1] that long-term administration of high doses of *d*-methamphetamine to rhesus monkeys depleted norepinephrine in the frontal cortex and midbrain and dopamine in the caudate nucleus. These depletions are most likely irreversible since they are found to remain as long as twelve months past the repeated injection period. The purposes of this study were to further explore long-term changes in catecholamine levels in two non-primate species and to explore the possible role of metabolites in producing long-term changes by examining the rat and the guinea pig which metabolize methamphetamine in different ways [2, 3]. It is now reported that high doses of *d*-methamphetamine given subcutaneously over a thirty-day period cause prolonged dopamine depletions in the caudate nucleus of rats and guinea pigs suggesting the drug has long-lasting effects when given in this dosage regimen.

Methods

Thirty-six male Sprague–Dawley rats were housed individually with free access to food and water. All rats weighed approximately 300 g at the

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start of the experiment. Colony room lights were automatically turned on at 0600 hours and off at 1800 hours. The temperature was maintained at 22 ± 1 °C.

Rats were given subcutaneous injections of *d*-methamphetamine hydrochloride (n = 26) or the saline vehicle (n = 10) twice a day at 0800 and 1700 hours. The initial dose given to three rats was 25 mg/kg per day. It was found that most rats could tolerate higher doses with subcutaneous injections and therefore the dose for 23 additional rats was 50 mg/kg per day*. Injections continued for 30 days but if any rat showed extreme pulmonary congestion, the dose was decreased by 50%. Sweetened condensed milk was given to the methamphetamine-treated rats as a dietary supplement to overcome the weight loss engendered by the anorexic effects of methamphetamine.

Eleven male guinea pigs (weighing approximately 450 g at the start of the experiment) were also injected subcutaneously with *d*-methamphetamine hydrochloride twice a day at 0800 and 1700 hours for thirty days. Four guinea pigs received saline injections for the same period. It was found through pilot work that guinea pigs could not tolerate initial doses as high as rats. Therefore, the initial dose given was 6 mg/kg per day. As the guinea pigs became tolerant to a given dose, as evidenced by weight measure and observation, the dose was increased by increments of 6 mg/kg until a dose of 30 mg/kg per day was given for the last 16 days**.

Following the thirty days of injection, all animals were maintained without injections for two weeks and then killed by decapitation. Their brains were removed and dissected over ice. The telencephalon, midbrain, caudate nucleus and pons–medulla were dissected as previously described [4, 5], and were stored in liquid nitrogen until assayed. The concentration of catecholamines in tissue was determined by ion-exchange chromatography and fluorometric assay [6 - 8].

The animals displayed severe pulmonary congestion, weight loss, and self-mutilation while treated with methamphetamine. Twelve rats and five guinea pigs died while on the drug regimen.

Results

A significant difference was found in the caudate dopamine levels between treated and control groups in both rats and guinea pigs. The methamphetamine treated rats in the 25 and 50 mg/kg per day groups had 25% and 60% depletions, respectively, as compared to vehicle animals. The overall

*The methamphetamine hydrochloride was dissolved in physiological saline at 25 mg/ml. Injection volumes were 1 ml/kg or 2 ml/kg for the 25 or 50 mg/kg per day treatments. One-half the daily injection dose was given at 0800 hours and the other half at 1700 hours.

**The same solution concentrations were used with adjusted volumes. Dose schedule: 6 mg/kg per day for two days; 12 mg/kg per day for four days; 18 mg/kg per day for six days; 24 mg/kg per day for two days; 30 mg/kg per day for 16 days.

analysis of variance was significant, as were the individual comparisons between vehicle and 50 mg/kg per day and 25 *vs.* 50 mg/kg per day treatments (Table 1). Caudate dopamine levels were 51% lower in the methamphetamine-injected guinea pigs than in the vehicle-treated animals* (Table 2).

TABLE 1

Regional brain catecholamine levels in rats receiving subcutaneous methamphetamine hydrochloride (*d*-Meth·HCl) for thirty days, for a total of 25 or 50 mg/kg per day. Values reported are group means in μg/g tissue ± standard error of the mean. Rats were killed two weeks after the last injection. DA = dopamine; NE = norepinephrine.

	n	Caudate DA (μg/g)	Telencephalon NE (μg/g)	Midbrain NE (μg/g)	Pons–medulla NE (μg/g)
Vehicle	10	8.67 ± 0.67	0.34 ± 0.02	0.69 ± 0.04	0.50 ± 0.03
25 mg/kg per day *d*-Meth·HCl	8	6.44 ± 0.88	0.29 ± 0.04	0.71 ± 0.05	0.45 ± 0.05
50 mg/kg per day *d*-Meth·HCl	11	3.47 ± 0.41	0.35 ± 0.03	0.66 ± 0.07	0.50 ± 0.03

TABLE 2

Regional brain catecholamine levels in guinea pigs receiving subcutaneous methamphetamine hydrochloride (*d*-Meth·HCl) for thirty days. Values reported are group means in μg/g tissue ± standard error of the mean. Guinea pigs were killed two weeks after the last injection. DA = dopamine; NE = norepinephrine.

	n	Caudate DA (μg/g)	Telencephalon NE (μg/g)	Midbrain NE (μg/g)	Pons-medulla NE (μg/g)
Vehicle	4	9.83 ± 0.27	0.33 ± 0.03	0.50 ± 0.10	0.30 ± 0.02
6-30 mg/kg per day *d*-Meth·HCl	6	4.83 ± 0.66	0.33 ± 0.03	0.42 ± 0.08	0.35 ± 0.05

Discussion

The lack of long-term changes in norepinephrine levels in the rodent species examined which were present in the monkey may be indicative of species variations. This may reflect either differences in the metabolism of the methamphetamine or differences in the sensitivity of the noradrenergic system to methamphetamine. The rat metabolizes methamphetamine to *p*-hydroxynorephedrine (which has been shown to displace catecholamines from neuronal pools) while the guinea pig metabolizes methamphetamine to

*Rats caudate dopamine: overall $F = 4, 41$, d.f. = 2, 21, $p < 0.05$. Individual Students *t*-tests: vehicle *vs.* 50 mg/kg per day = 6.7, d.f. = 20; 25 *vs.* 50 mg/kg per day = 3.26, d.f. = 13, $p < 0.01$. Guinea pig caudate dopamine: Students *t*-test = 5.06, d.f. = 9, $p < 0.01$.

benzoic acid [2, 3]. An alternative explanation might be related to the greater depletions of catecholamines observed in the monkey compared with rodents. Norepinephrine depletions in rhesus monkeys given methamphetamine reached 33 - 52% while the dopamine depletions reached about 75%. The average depletion of dopamine in the rodent was only 45%. If higher dopamine depletions had been attained in the rodents either by increasing the dose or duration of the regimen, the norepinephrine levels may have also been affected. It is also possible that changes in the norepinephrine levels of the frontal cortex were not observed in rats due to the fact that, in the rat, the frontal cortex was assayed with the telencephalon, while in the monkey it was assayed separately.

There is some evidence from these data that the dopamine depletions may be dose-dependent; rats receiving the highest total dose of methamphetamine had the lowest caudate dopamine levels. The 45% depletions of dopamine in the caudate are similar to those reported by Lewander [2] in a study in which guinea pigs received methamphetamine for 18 days and were killed four hours after the last injection.

The present report extends the previously reported finding that the methamphetamine causes dopamine depletions in the rhesus monkey to two other species suggesting possible neurotoxic effects. In the rhesus monkey these effects persisted as long as twelve months after drug administration was discontinued, leading to the tentative conclusion that the changes are permanent. Although the presented data in rodents is only for two weeks post drug, it would appear that changes in the rodent may also be permanent. The generality of these preliminary findings of dopamine depletions raises serious concern about the long-term adverse effects of repeated methamphetamine administration by humans.

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