Reprinted from
Proc. Nat. Acad. Sci. USA
Vol. 72, No. 3, pp. 834–836, March 1975

d-Amphetamine Disaggregates Brain Polysomes Via a Dopaminergic Mechanism

(dopamine receptors/brain protein synthesis/L-dopa/catecholamines/brain development)

MICHAEL A. MOSKOWITZ, BETTE F. WEISS, LOY D. LYTLE, HAMISH N. MUNRO, AND RICHARD J. WURTMAN

Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Mass. 02139

Contributed by Hamish N. Munro, December 11, 1974

ABSTRACT **Brain polysomes are disaggregated in rats given moderate to large doses of *d*-amphetamine sulfate; this response is rapid in onset, lasts for at least 4–6 hr, and varies with the age of the animal. Pretreatment with a dopamine receptor blocking agent, haloperidol or pimozide, blocks the amphetamine-induced disaggregation.**

Rats treated with large single doses of L-dopa (a catecholamine precursor) exhibit a major disaggregation of brain polysomes (1) and a parallel decrease in the net rate of brain protein synthesis (2, 3). The L-dopa-induced disaggregation of brain polysomes is suppressed in animals pretreated with drugs that block the conversion of dopa to dopamine, or with drugs that block dopamine receptors (4, 5). Hence, the drug-induced polysome disaggregation may result from a direct action of dopamine on a receptor that controls the intracellular protein-synthetic apparatus.

Amphetamine, a sympathomimetic agent, acts on the central nervous system to affect locomotor activity (6–9), appetite (10), and thermoregulation (11–14); in high doses, it causes a psychotic reaction in humans that resembles paranoid schizophrenia (15). Catecholamine-containing brain neurons appear to mediate many of the behavioral and physiological effects of this drug (16). Relatively little information exists regarding effects of amphetamine on brain protein synthesis. We now report that large doses of *d*-amphetamine sulfate disaggregate brain polysomes in rats, and that this effect, like that of L-dopa, appears to be mediated by dopamine receptors.

MATERIALS AND METHODS

Male albino rats of various ages (Charles River Laboratories, Wilmington, Mass.) were exposed to light from 9 a.m. to 9 p.m. and given free access to food and water. All animals were maintained at ambient temperatures ranging from 20–22°. Animals less than 26 days of age remained with the dam in litters of eight pups; older animals were caged in groups of four for 1 day prior to an experiment. All injections were administered intraperitoneally.

Polysomes were prepared as described (1, 4). Briefly, the brains were removed, pooled two per sample, minced, and homogenized in an ice-cold 0.25 M sucrose medium containing 0.05 M Tris·HCl, 0.10 M KCl, and 0.012 M $MgCl_2$, at pH 7.6. The homogenates were then centrifuged for 20 min at 21,000 $\times$ *g;* the post-mitochondrial supernatants were treated with sodium deoxycholate to a concentration of 1% and then layered over discontinuous sucrose gradients (0.5 M, 2.0 M). After this centrifugation, the pellets of ribosomes were again suspended and applied to a 10–40% continuous sucrose gradient. Absorbance profiles were recorded at 260 nm. The percentage of polysomes in the profile represents the fraction of total area attributable to polyribosomes (1, 4, 5).

RESULTS

In the first experiment, 26-day-old animals received 0.9% (w/v) saline vehicle (1 ml/kg) or *d*-amphetamine sulfate (1.5, 10, 15, or 50 mg/kg, salt weight); they were decapitated 1 hr later. Doses of 10 mg/kg of *d*-amphetamine or larger disaggregated the brain polysomes; the *magnitude* of disaggregation, however, was not dose-related (the percentages of total ribosomes represented by polysomes were: 72% with vehicle, 38% with 10 mg/kg, 40% with 15 mg/kg, and 37% with 50 mg/kg). Disaggregation was significantly different from the control 30 min after the drug was administered, and after 1 hr it had reached its maximal effect; the response persisted for at least 4–6 hr (Table 1).

To determine whether sensitivity to disaggregation by amphetamine might be age-related, 15 mg/kg of amphetamine were injected into rats 7, 21, 26, 40, or 70 days of age. Although this dose produced significant polysome disaggregation in animals 26 days of age or older, the brain polysome profiles of animals 21 days or younger showed no change (Table 2). However, larger doses of amphetamine (50 mg/kg) disaggregated brain polysomes in 7- to 9-day-old pups.

Since catecholamine-containing brain neurons appear to mediate many of the behavioral and physiological effects of amphetamine (16), we examined the extent to which pretreatment with drugs known to block brain dopamine receptors affects the amphetamine-induced disaggregation. Rats 26 days of age were pretreated with haloperidol (20 mg/kg in 1

TABLE 1. *Disaggregation of brain polysomes in rats receiving d-amphetamine sulfate*

Time after injection	Percent polysomes
Control	74.6 ± 2.9
30 min	44.0 ± 6.8*
1 hr	34.7 ± 4.1*
2 hr	45.7 ± 3.3*
4 hr	58.6 ± 1.2*
6 hr	67.2 ± 1.5

Twenty-six-day-old male albino rats were injected intraperitoneally with saline (control group: killed 1 hr after injection) or 15.0 mg/kg of *d*-amphetamine sulfate, and killed after various intervals. Data represent means ± standard errors.

* $P < 0.01$ differs from controls.

TABLE 2. *Effects of d-amphetamine sulfate on brain polysome aggregation in rats of different ages*

Age (days)	Percent polysomes		
	Saline	Amphetamine (15 mg/kg)	Amphetamine (50 mg/kg)
7	80.3 ± 0.1	72.6 ± 2.4	48.6 ± 2.1*
21	77.2 ± 2.5	67.3 ± 2.0	
26	74.2 ± 1.4	40.0 ± 3.7*	37.3 ± 5.0*
40	71.7 ± 0.9	38.0 ± 8.0*	
70	69.7 ± 0.9	44.0 ± 8.8*	

Male albino rats were injected with saline vehicle or *d*-amphetamine sulfate (15 mg/kg or 50 mg/kg), and killed 1 hr later. Data represent means ± standard errors.

* $P < 0.01$ differs from controls.

ml) or with its diluent (50 mM citric acid). Other rats 40 days of age were pretreated with pimozide (25 mg/kg in 1 ml) or with its vehicle (0.1% methylcellulose). Thirty minutes later, half of each group received 15 mg/kg of amphetamine or the saline vehicle. The animals were killed after 1 hr, and the brain polysome profiles were obtained. Neither haloperidol nor pimozide alone modified polysome aggregation; however, administration of either receptor blocker substantially inhibited the amphetamine-induced disaggregation (Table 3).

TABLE 3. *Effects of haloperidol or pimozide on polysome disaggregation induced by d-amphetamine sulfate*

Treatment	Percent polysomes
Haloperidol*	
Vehicle + saline	75.7 ± 2.1
Haloperidol + saline	73.3 ± 1.0
Vehicle + amphetamine	43.7 ± 2.7†
Haloperidol + amphetamine	72.6 ± 2.6
Pimozide‡	
Vehicle + saline	67.9 ± 2.0
Pimozide + saline	68.0 ± 1.2
Vehicle + amphetamine	44.6 ± 2.6†
Pimozide + amphetamine	64.3 ± 1.8

* Twenty-six-day-old male albino rats were injected with vehicle (50 mM citric acid) or 20 mg/kg of haloperidol, and 30 min later, with 0.9% saline or 15 mg/kg of *d*-amphetamine sulfate. Animals were killed 1 hr after the second injection.

† $P < 0.01$ differs from control.

‡ Forty-day-old albino rats were injected with vehicle (0.1% methylcellulose) or pimozide (25 mg/kg), and 30 min later, with 0.9% saline or 15 mg/kg of *d*-amphetamine sulfate. Animals were killed 1 hr after the second injection.

DISCUSSION

Many of the acute behavioral and neurochemical effects of amphetamine apparently result from its actions on catecholamine-containing brain neurons (16). Thus, the release of brain catecholamines into synapses may mediate the amphetamine-induced stereotypy (17, 18), locomotor hyperactivity (6–9), cerebral glycogenolysis (19, 20), and body temperature changes observed in hot and cold environments (11–14). The biochemical mechanisms by which amphetamine may increase intrasynaptic catecholamines include: (*a*) the enhanced release of catecholamines from storage sites; (*b*) the blockade of re-uptake mechanisms; (*c*) the inhibition of monoamine oxidase; and (*d*) the apparent replacement of the catecholamines in storage granules by the amphetamine metabolite *para*-hydroxynorephedrine (21–23). Catecholamine release may also be a factor in toxic responses that occur with very large doses of amphetamine; pretreatment with drugs that block catecholamine receptors (e.g., propranolol, haloperidol, or chloropromazine) may reduce the mortality or the increase in plasma lactic acid concentration that follows massive doses of amphetamine (24).

The present study shows that polyribosomes in rat brain disaggregate shortly after animals receive large doses of *d*-amphetamine sulfate (10 mg of sulfate per kg or more). This response persists for 4–6 hr; the effective dose varies with the age of the animal. Pretreatment of rats with haloperidol or pimozide, two drugs known to block brain dopamine receptors (25, 26), also blocks amphetamine-induced polysome disaggregation, suggesting that this effect of amphetamine is mediated by dopamine receptors. This evidence can be interpreted to mean either (*a*) that amphetamine releases dopamine into synapses and the receptor blocking agents prevent its action on post-synaptic receptors, or (*b*) that amphetamine has a direct, intracellular action on the protein-synthetic apparatus, which is also prevented by the receptor blockers.

Many parallels exist between the effects of *d*-amphetamine and L-dopa on brain protein synthesis. The disaggregation of brain polyribosomes induced by both drugs occurs within 1 hr of their administration, persists for relatively short periods (i.e., 2 hr for L-dopa and 6 hr for amphetamine), and can be blocked by pretreatment with the dopamine receptor blocking agents, haloperidol and pimozide. On the other hand, while adolescent and mature rats showed brain polysome disaggregation even at low doses of *d*-amphetamine, infant rats treated before weaning were relatively resistant to the amphetamine-induced disaggregation. These age-dependent changes may reflect ontogenetic changes in synaptogenesis or in the protein-synthetic mechanisms within individual brain cells. The relative insensitivity of infant rats to amphetamine treatment contrasts with their heightened sensitivity to dopa-induced disaggregation (1).

L-Dopa-induced disaggregation of brain polysomes is accompanied by decreased incorporation of [^{14}C]lysine and [^{3}H]leucine into brain microtubular protein and total brain protein (2, 3). Since the administration of amphetamine also disaggregates brain polysomes, it might be anticipated that amphetamine also can disrupt brain protein synthesis. Studies elsewhere have shown that pretreating animals with large doses of amphetamine reduces the incorporation of amino acids into brain protein *in vitro* (27).

The time course of the amphetamine-induced polysome disaggregation coincides with those of numerous other behavioral and physiological effects of amphetamine. However, the dosages necessary to achieve polysome disaggregation in whole rat brains are several times greater than those needed to produce some behavioral and physiological effects. It is, of course, possible that considerably lower doses of amphetamine might significantly reduce protein synthesis within particular brain regions and that such effects might underlie some of the behavioral, physiological, or toxic effects of this drug.

These studies were supported in part by USPHS Grants NS-10459 and MH-25050. M.A.M. is supported by a postdoctoral fellowship from the Foundation's Fund for Research in Psychi-

atry. *d*-Amphetamine sulfate was generously supplied by Smith, Kline & French, Philadelphia, Pa. We acknowledge the excellent editorial assistance of Ms. Susanna Fein.

1. Weiss, B. F., Munro, H. N. & Wurtman, R. J. (1971) "L-Dopa: Disaggregation of brain polysomes and elevation of brain tryptophan," *Science* **173,** 833–835.
2. Roel, L. E., Schwartz, S. A., Weiss, B. F., Munro, H. N. & Wurtman, R. J. (1974) "*In vivo* inhibition of rat brain protein synthesis by L-dopa," *J. Neurochem.* **23,** 233–239.
3. Munro, H. N., Roel, L. E. & Wurtman, R. J. (1973) "Inhibition of brain protein synthesis by doses of L-dopa that disaggregate brain polyribosomes," *J. Neural Trans.* **34,** 321–323.
4. Weiss, B. F., Munro, H. N., Ordonez, L. A. & Wurtman, R. J. (1972) "Dopamine: Mediator of brain polysome disaggregation after L-dopa," *Science* **177,** 613–616.
5. Weiss, B. F., Roel, L. E., Munro, H. N. & Wurtman, R. J. (1974) in *Aromatic Amino Acids in the Brain* (Ciba Foundation Symposium 22, Elsevier, London), pp. 325–332.
6. Adler, M. W. (1961) "Changes in sensitivity to amphetamine in rats with chronic brain lesions," *J. Pharmacol. Exp. Ther.* **134,** 214–221.
7. Smith, C. B. (1965) "Effects of *d*-amphetamine upon brain amine content and locomotor activity of mice," *J. Pharmacol. Exp. Ther.* **147,** 96–99.
8. Dingell, J. V., Owens, M. L., Norvich, M. R. & Sulser, F. (1967) "On the role of norepinephrine biosynthesis in the central action of amphetamine," *Life Sci.* **6,** 1155–1162.
9. Scheel-Kruger, J. (1971) "Comparative studies of various amphetamine analogues demonstrating different interactions with the metabolism of the catecholamines in the brain," *Eur. J. Pharmacol.* **74,** 47–59.
10. Lesses, M. F. & Meyerson, A. (1937) "Human autonomic pharmacology," *N. Engl. J. Med.* **218,** 119–121.
11. Dolfini, E., Angel, A. R. D., Garattini, S. & Valzelli, L. (1970) "Brain catecholamine release by dextroamphetamine in three strains of mice," *Eur. J. Pharmacol.* **9,** 333–336.
12. Dolfini, E., Tansella, M., Valzelli, L. & Garattini, S. (1969) "Further studies on the interaction between desipramine and amphetamine," *Eur. J. Pharmacol.* **5,** 185–190.
13. Simovni, J. & Szentoyogy, D. (1959) "The effect of actedron (phenylisopropylamine) and thyroxine on the body temperature," *Arch. Intern. Pharmacodyn.* **80,** 1–13.
14. Yehuda, S. & Wurtman, R. J. (1972) "Release of brain dopamine as the probable mechanism for the hypothermic effect of *d*-amphetamine," *Nature* **240,** 477–478.
15. Kalant, D. J. (1973) *The Amphetamines: Toxicity and Addiction* (Charles C Thomas, Springfield, Ill.).
16. Costa, E. & Garattini, S., eds. (1970) *Amphetamines and Related Compounds* (Raven Press, New York).
17. Randrup, A. & Munkvad, I. (1970) in *Amphetamines and Related Compounds,* eds. Costa, E. & Garattini, S. (Raven Press, New York), pp. 695–713.
18. Randrup, A. & Scheel-Kruger, J. (1966) "Diethyldithiocarbamate and amphetamine stereotyped behavior," *J. Pharm. Pharmacol.* **18,** 752–755.
19. Hutchins, D. A. & Rogers, K. J. (1973) "Effect of depletion of cerebral monoamines on the concentration of glycogen and on amphetamine-induced glycogenolysis in the brain," *Brit. J. Pharmacol.* **48,** 19–29.
20. Rogers, K. J. & Hutchins, D. A. (1972) "Studies on the relation of chemical structure to glycogenolytic activity in the brain," *Eur. J. Pharmacol.* **20,** 97–103.
21. Rutledge, C. O. (1970) "The mechanisms by which amphetamine inhibits oxidative deamination of norepinephrine in the brain," *J. Pharmacol. Exp. Ther.* **171,** 188–195.
22. Glowinski, J. & Axelrod, J. (1965) "Effect of drugs on the uptake, release, and metabolism of [^{3}H]norepinephrine in the rat brain," *J. Pharmacol. Exp. Ther.* **149,** 43–49.
23. Brodie, B. B., Cho, A. K. & Gessa, G. L. (1970) in *Amphetamines and Related Compounds,* eds. Costa, E. & Garattini, S. (Raven Press, New York), pp. 217–230.
24. Davis, W. M., Logston, D. G. & Hikenbottom, J. P. (1974) "Antagonism of acute amphetamine intoxication by haloperidol and propranolol," *Toxicol. Appl. Pharmacol.* **29,** 397–403.
25. Carlsson, A. & Lindqvist, M. (1963) "Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain," *Acta Pharmacol. Toxicol.* **20,** 140–144.
26. Anden, N. E., Dahlstrom, A., Fuxe, K. & Larsson, K. (1966) "Functional role of the nigro-neostriatal dopamine neurons," *Acta Pharmacol. Toxicol.* **24,** 263–274.
27. Loh, H., Hitzemann, R. & Stolman, S. (1973) "Effect of *d*-amphetamine on brain protein synthesis," *Toxicol. Appl. Pharmacol.* **26,** 476–481.