REVERSAL OF AMPHETAMINE INDUCED POLYSOME DISSOCIATION BY NEUROLEPTIC AGENTS IN RAT BRAIN

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Received May 10,1977

SUMMARY: Administration of d-amphetamine to rats causes the dissociation of brain polysomes in a dose-dependent manner. Further, the dose of d-amphetamine required to induce a stereotypic state in rats coincides with the dose needed to cause polysome dissociation. The enantiomeric form, i.e. l-amphetamine, is ineffective in inducing both the behavioral and biochemical changes even at a dose as high as 30 mg/kg. Clinically potent neuroleptics such as haloperidol and chlorpromazine can effectively reverse the polysome dissociation as well as the behavioral changes induced by a near toxic dose of d-amphetamine (15 mg/kg).

INTRODUCTION. It has been shown that amphetamine, a potent sympathomimetic drug which induces a stereotypic state in animals (1) can induce a psychotic state in humans that is often compared to paranoid schizophrenia (2-4). It is also well known that neuroleptic drugs afford protection against the development of stereotypies although the chemical basis of action of these drugs is not clearly understood. The literature is replete with data suggesting that the mechanisms of action of the neuroleptic agents may somehow involve the post-synaptic blockade of dopamine receptors, possibly through the inhibition of function of adenylate cyclase, thus decreasing the level of cyclic AMP (5-9). Recent experiments by several independent groups (10, 11), however, suggest the involvement of additional mechanisms of sites of neuroleptic action. Results reported in this paper are supportive of this latter possibility.

We as well as Moskowitz et al (12-14) have independently shown that in vivo administration of d-amphetamine causes dissociation of brain polysomes in rats. Our result also indicates that the extent of polysome dissociation is dose dependent with almost complete dissociation occurring at a near toxic dose of 15 mg/kg (12). It has also

ISSN 0006-291X

been demonstrated that d-amphetamine inhibits in vitro protein synthesis directed by synthetic (13) as well as natural mRNA (15). In an attempt to elucidate sites as well as mechanisms of action of antipsychotic drugs, we have tested the ability of antipsychotic agents to reverse the brain polyribosome dissociation in amphetamine induced stereotypic rats.

MATERIALS AND METHODS: Charles River CD male rats weighing between 250 and 300 g were used. They were individually housed at room temperature and were maintained on a 12:12 hour (7 a.m.-7 p.m.) light-dark schedule with Purina laboratory chow and water continuously available.

d-Amphetamine sulfate (Smith, Kline and French) was administered intraperitoneally in the dose range of 3-15 mg/kg as required. l-Amphetamine sulfate (Smith, Kline and French) was also administered in a similar manner to d-amphetamine; however, the dosage schedule ranged up to 30 mg/kg. The three neuroleptics, chlorpromazine (Smith, Kline and French), haloperidol (McNeil) and thioridazine (Sandoz) were administered intraperitoneally 15-30 minutes after administration of d- or l-amphetamine. When the animals reached a quiescent state after injection of any of the neuroleptics (usually in about 15 to 30 minutes, or roughly 30-45 minutes after the injection of amphetamines), the animals were anesthetized by butyrolactone (0.8 ml/100 gm) and decapitated. Their brains were removed, washed with cold 0.9% NaCl and used for preparing the polysomes.

Brains were homogenized in a buffer containing 25 mM Tris-HCl (pH 7.5), 5 mM Mg $(CH_3COO)_2$, 5 mM 2-mercapto ethanol, 100 mM KCl and 10% sucrose(w/v), and polysomes were pelleted through 2 M sucrose as described before (12). Polysomes were suspended in a buffer containing 25 mM Tris-HCl (pH 7.5), 5 mM Mg $(CH_3COO)_2$, 100 mM KCl and 5 mM 2-mercapto ethanol and used for velocity sedimentation in sucrose gradients. One to two ml polysome samples containing 6.0 to 12.0 A_{260} units were layered on 10-35% linear sucrose gradients of 29 ml volume containing 25 mM Tris-HCl (pH 7.5), 100 mM KCl and 3 mM MgU_2. Centrifugation was carried out in an SW 25 rotor of a Beckman L2-50 ultracentrifuge at 76,000 x g for 3 hours (3^{o}-5^{o} C.). Gradients were fractionated using a model UA-5 Isco density gradient fractionator monitored at 254 nm.

RESULTS AND DISCUSSION: The sedimentation profiles of polysomes from control and stereotypic rat brains have been presented in Fig. 1. It seems clear that most of the fast sedimenting polymeric particles seen in the control preparation are dissociated into monomers and subunits after administration of 15 mg/kg d-amphetamine. The extent of dissociation is dose dependent (12) and the percentage of 260 nm absorbing material in the region of monomeric 80 S and subunits ranges from 25% in control to over 75% in rats administered 15 mg/kg d-amphetamine (12). To determine if the polysome dis-

SEDIMENTATION PATTERNS OF CONTROL AND AMPHETAMINE TREATED RAT BRAIN POLYSOMES

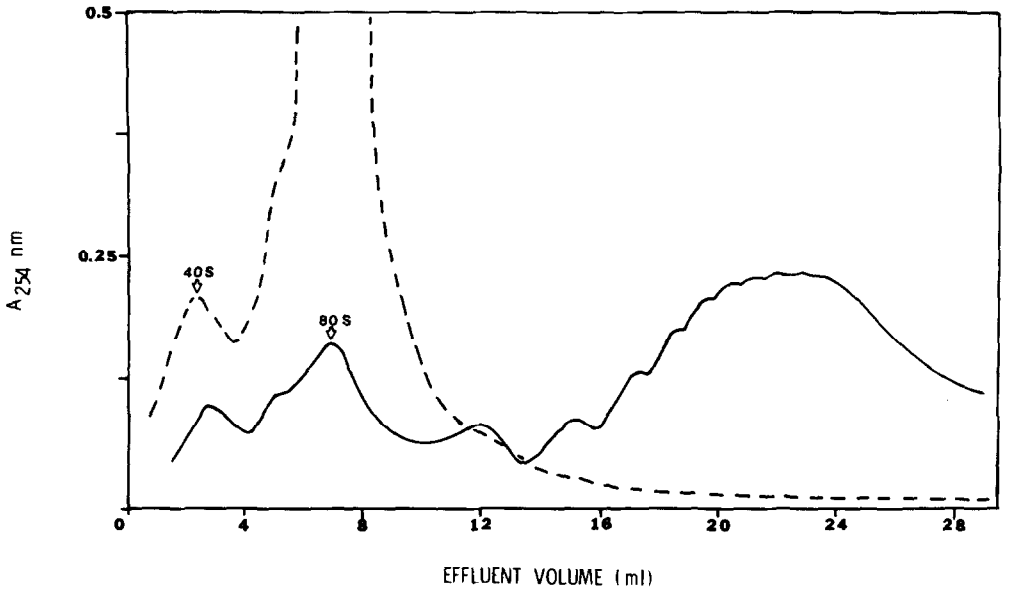

Fig. 1. Dissociation of rat brain polysomes by d-amphetamine. Amphetamine was administered intraperitoneally at a dose rate of 15 mg/kg. Control rats were injected with equal volumes of sterile saline (1-2 ml). Polysomes were isolated from brain and analyzed on 10-35% linear sucrose gradients as described in MATERIALS AND METHODS. Control rats (———); rats injected with 15 mg/kg d-amphetamine (------).

sociation is related to stereotypy, we have tested over 300 animals with varied doses of d-amphetamine. In over 90% of the cases, rats exhibiting stereotypic behavior (sniffing, wire biting and walking backwards) showed dissociated brain polysomes. These two events appear to have good correlation and it is possible to predict the state of brain polysomes on the basis of behavioral observations with a high degree of accuracy.

The results on correlation between stereotypy and polysome dissociation were further supported by experiments using l-amphetamine. It has been shown that d-amphetamine is several-fold more potent than the l-form in inducing stereotypic behavior (16). It is interesting to note that l-amphetamine, even at 30 mg/kg level, has almost no effect on the brain polysome profile, while a dose as low as 8.0 mg/kg of d-

Table 1. In vivo effects of d- and l-forms of amphetamine on brain polysomes

Form of Amphetamine	Dose (mg/kg)	Number of rats tested	Polymeric particles %
None (Control)	0.0	> 50	72.50 ± 5.0
d-Amphetamine	12.0	22	45.60 ± 12.0
	15.0	18	36.25 ± 6.0
l-Amphetamine	15.0	5	75.6 ± 7.3
	30.0	6	74.8 ± 8.2

Polysomes were isolated from rats administered d- and l-forms of amphetamine as indicated and analyzed on sucrose gradients as described in Fig. 1. The area under 40 S/60 S subunits and 80 S monomeric region were measured and compared with the total area on the optical density scans to calculate the % of polymeric particles in the ribonucleoprotein preparations (see Ref. 12).

amphetamine results in a marked dissociation (Table 1). These results agree with earlier findings on the relative ineffectiveness of l-amphetamine to induce neuropsychosis (17). It is, therefore, reasonable to conclude that the effects of d-amphetamine on brain polysomes may involve specific neurobiological action rather than nonspecific or indirect effects.

In order to verify further if the observed dissociation of brain polysomes by amphetamine is indeed related to psychosis, we tested the effects of some well-known antipsychotic agents such as chlorpromazine and haloperidol. The relative proportions of monomeric and polymeric particles in the ribonucleoprotein preparation have been used as the criteria to determine the degree of protection. In control rats it has been shown (see Table 1) that roughly 75% of the total isolate exists in the polymeric form. Based on this and the statistical analysis of degree of dissociation as a function of dose rate

Table 2. Effects of psychotropic drugs on amphetamine-induced polysome dissociation

Drug Used	Number of animals tested	Number of animals having dissociated polysomes	% animals protected
None (Amphetamine Control)	18	18	0
Chlorpromazine	23	4	82.6
Haloperidol	23	7	69.6
Thioridazine	7	5	28.6

Experimental details were as described in Fig. 1 and Table 1. Chlorpromazine (10-12 mg/kg), haloperidol (15 mg/kg), and thioridazine (25 mg/kg) were injected intraperitoneally 15-30 min. after the administration of 15 mg/kg d-amphetamine. On the basis of statistical analysis reported previously (Ref. 12, 13), ribonucleoprotein preparations showing > 68% of absorbance in > 110 S region are considered intact polysomes.

reported earlier (12, 13), ribonucleoprotein preparations containing up to 32% of the material in the monomeric 80 S and the subunits have been considered as relatively intact or undissociated polysomes. Using this criterion, almost 100% of the animals administered 15 mg/kg amphetamine exhibit polysome dissociation. As shown in Table 2, both of the neuroleptic agents appear to provide a high degree of protection against polysome dissociation by d-amphetamine. The generality of this protective effect was tested using thioridazine, a neuroleptic whose structure is that of a phenothiazine but containing a side chain which is different from other phenothiazines. As expected, thioridazine exhibits far lower activity in reversing polysome dissociation than the two neuroleptic agents described above (see Table 2).

Although not shown here, the degree of protection by all three drugs is dose dependent. Optimal protection of polysomes as shown in Table 2 by chlorpromazine re-

quires a dose rate of about 10-12.5 mg/kg. In the case of haloperidol, a maximum protection of about 69% is achieved at a dose of 15 mg/kg. The least active psychotropic agent, thioridazine, exhibits a maximum of 28% protection at a dose rate of 25 mg/kg. It should also be pointed out that all three neuroleptics provided a significantly higher degree of protection when administered 15-30 minutes before amphetamine treatment.

It seems clear from the data presented here that despite the considerably greater clinical potency in human schizophrenics as well as in animals of the neuroleptic haloperidol (18), there is considerably less reversal of polysome dissociation by this potent butyrophenone than by chlorpromazine (Table 2). Thus the biochemical potencies of these agents as described in this paper do not agree with the clinical potencies of the drugs. Our results on polysome protection by haloperidol and chlorpromazine, however, agree well with the recently reported pharmacological test involving affinities of various antischizophrenic drugs for dopamine binding sites (10). These investigators found that clinical efficacy of different classes of neuroleptics correlates well with their ability to inhibit the binding of haloperidol to dopamine receptors in mammalian brain. While it has been shown that butyrophenones as a class are less effective than chlorpromazine in inhibiting the action of dopamine sensitive adenylate cyclase, clinically they are considerably more potent. This is challenging to the current hypothesis that the potency of neuroleptics relates to their blocking action of the post-synaptic dopamine receptors. Recent work by Roufogalis et al (11) has clearly established the involvement of mechanisms other than dopamine receptors associated with adenylate cyclase.

It is, therefore, possible that the action of amphetamine may involve more than one site and the biochemical, pharmacological tests used might reflect only a part of the mechanism. The reversal of polysome dissociation appears to agree well with this interpretation.

Results reported in this paper clearly establish the following: (1) Doses of the enantiomeric form of amphetamine inactive in inducing stereotypic behavior have no effect on the brain polysome; (2) The dissociation of brain polysomes by d-amphetamine is reversed by clinically potent antipsychotic agents. Although the exact mechanism involved in the dissociation of brain polysomes by amphetamine and the reversal by neuroleptic agents is not known, our results appear to suggest that the reported effects of amphetamine on polysomes (12-14) may be related to psychosis and stereotypic behavior. In conclusion, the effects on brain polysomes might present a simple biochemical method to test the antipsychotic activities of various neuroleptics.

ACKNOWLEDGMENT: This research was supported by U.S. Veterans Administration Project No. 9391-01.

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