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ANTIPSYCHOTIC DRUGS BLOCK LSD-INDUCED DISAGGREGATION OF BRAIN POLYSOMES

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Summary

Intravenous injection of LSD at 10, 25 and 100 µg/kg to young rabbits induces brain specific disaggregation of polysomes to monosomes. Polysomes in the cerebral hemispheres, cerebellum and remaining brain stem are affected. Neurotransmitter receptors are involved since prior injection of the receptor blockers haloperidol, chlorpromazine, propranolol, phentolamine, or pizotyline prevent drug-induced polysome shift. Depression of neuronal activity with sedative levels of ethanol or pentobarbital also eliminates polysome disaggregation.

We have previously reported that the intravenous administration of LSD to young rabbits increases the acetylation of specific brain histones (1), stimulates the ability of isolated brain nuclei to synthesize RNA (2) and induces a transient disaggregation of brain polysomes to monosomes (3). LSD appears to influence brain protein synthesis by decreasing the rate of reinitiation of protein synthesis, an effect which increases with postnatal age and has some dependency on stress and environmental factors (4). Reports have appeared describing the binding of LSD to presumptive serotonin receptors (5-7) and also an effect on dopamine-sensitive adenyl cyclase (8-10). The influence of LSD on macromolecular events in the brain (1-4) could involve the binding of LSD to neurotransmitter receptors. Agents which block specific receptors might be expected to alter the effect of LSD on intracellular brain biochemistry. In this report we demonstrate that certain antipsychotic drugs which block neurotransmitter receptor sites can very effectively inhibit LSD-induced brain polysome disaggregation.

Materials and Methods

D-LSD 25 bitartrate, BOL-148 (2-bromo-D-LSD bitartrate), methysergide bimaleate (1-methyl-D-lysergic acid butanolamide)and Pizotyline were products of Sandoz Pharmaceuticals, Switzerland obtained through the Department of National Health and Welfare, Ottawa, Canada. They were dissolved in 0.9% (W/v) NaCl for intravenous injection. All other drugs were acquired in injectable form. Haldol^{R} (haloperidol) - McNeil Laboratories; Largactil^{R} (chlorpromazine) - Poulenc Ltée; Rogitine^{R} (phentolamine) - Ciba-

Geigy; Inderal^{R} (propranolol) - Ayerst Laboratories; Periactin^{R} (cyproheptadine) - Merck, Sharp & Dohme; Nembutal^{R} (sodium pentobarbital) - Abbott Laboratories; Dilantin^{R} (diphenylhydantoin) - Parke-Davis.

Polysome Isolation

Polysomes were prepared and analyzed as previously described (3). Briefly, brain regions were homogenized in 3-5 volumes of cold TKMC buffer (50mM tris pH 7.4 @ 4°C, 25mM KCl, 5mM Mg $[CH_3COO]_2$ 1mM Cleland's reagent) containing 0.25M sucrose. Post-nuclear supernatant, pooled from 2 centrifugations at 780g for 10 min. was re-spun at 15,000 g for 10 min. This postmitochondrial supernatant was adjusted to a final 1% each of sodium deoxycholate and Triton X-100, layered over 5 ml of 2.0M sucrose and centrifuged at 260,000g for 3 hrs on a Beckman 60Ti rotor. The resultant polysome pellet was dissolved in buffer and analyzed on 15-45% linear sucrose gradients.

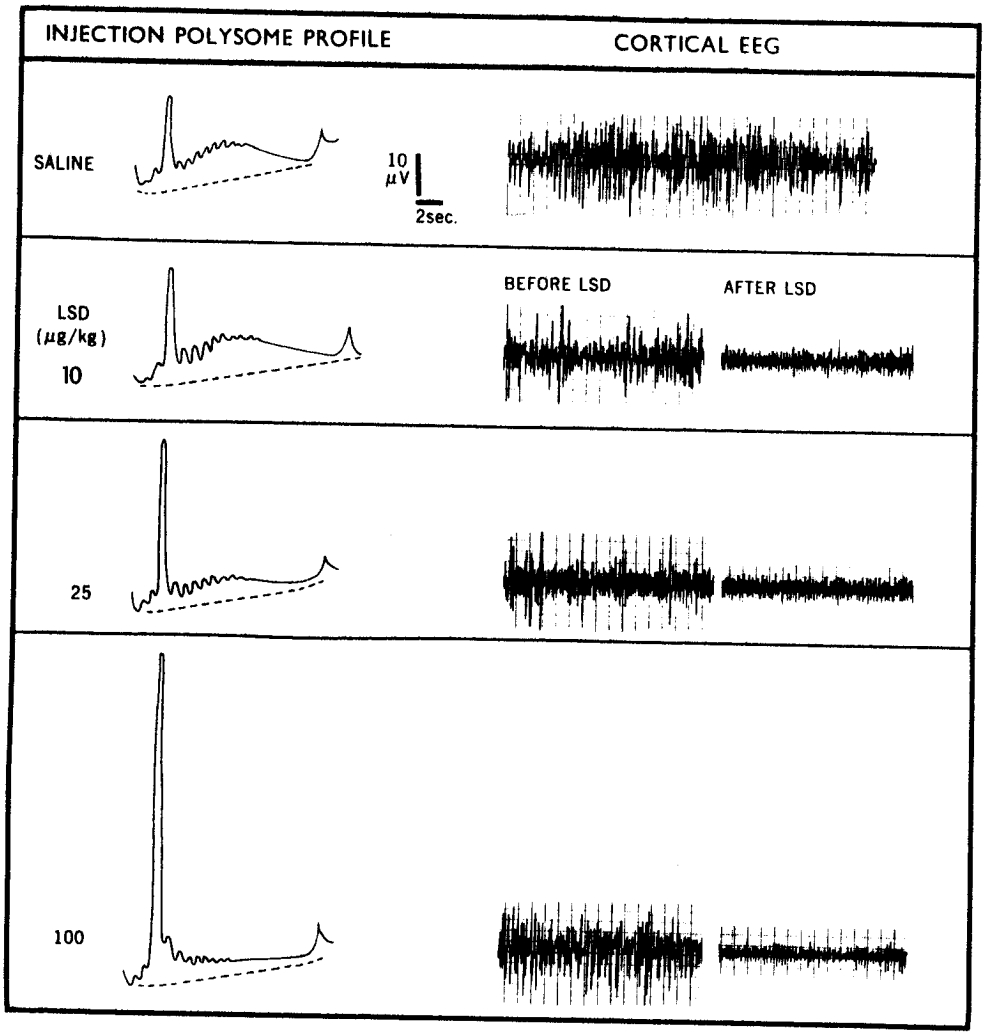
Measurement of EEG's

The rabbits were anesthetized by intravenous injection of 40 mg/kg sodium pentobarbital. They were placed in a stereotaxic apparatus (Kopf Instruments) and the skull was exposed by incision. Periosteum was cleared to reveal the intersection of saggital and coronal sutures. From this marker point burr holes were made down to the surface of the dura 3 1/2 mm right lateral and both 3 1/2 rostral and caudal. A needle was used to puncture the dura and bipolar electrodes, constructed of twisted strands of insulated nichrome wire (0.25 mm diameter) with exposed tips, were positioned in the holes. Screws were secured to the cranium around the electrode assembly and a stainless steel wire (indifferent electrode) was wrapped around them. The assembly was then cemented to the skull. A portion of the indifferent electrode was left exposed for attachment of the ground wire. Recording was done on a Brush Mark 220 strip recorder with the animal in a shielded box.

Results

Figure 1 demonstrates that the degree of brain polysome disaggregation is elevated as the intravenous dosage of LSD is increased from 10, 25 and 100 µg/kg body weight. Allowing for species differences in drug sensitivity the lower dosages are in a range comparable to levels which distort sensory perception in humans (11). The proportion of ribosomes present in polysomes in cerebral hemispheres, cerebellum and remaining brain stem is close to 90% in animals receiving saline injections. After LSD at 100 µg/kg the polysome percent decreases to 65-70% in all regions representing a 200-250% increase in the proportion of monosomes over control values. Our previous results indicate that the drug-induced polysome shift is brain specific and due to decreased reinitiation of protein synthesis rather than RNase degradation of messenger RNA or premature termination (3,4). Cortical EEG's taken immediately after LSD administration show that the drug affects neuronal activity in the rabbit brain (Fig. 1). A definite arousal pattern is indicated at all three dosages by the decrease in EEG amplitude and increase in frequency.

FIG. 1



Cortical bipolar electrodes were implanted under general anesthesia. On the following day the rabbit was immobilized in a holding box for recording EEG's before and after intravenous injection of 10, 25 or 100 μg/kg LSD. The EEG tracings shown were taken 2-5 minutes after LSD administration. After 60 minutes the animal was despatched by cervical dislocation.and polysomes were prepared from three major areas of the brain as described. Representative polysome profiles from brain stem are shown.

TABLE 1

TREATMENT	DOSE (per kg)	EXPERIMENTS	% POLYSOME/TOTAL RIBOSOMES		
			HEMISPHERES	STEM	CEREBELLIUM
SALINE		10	93 ± 0.7	89 ± 0.6	92 ± 0.5
LSD (60 min)	100 µg	14	68 ± 1.6	65 ± 1.9	71 ± 2.8
BOL-148	200 µg	3	89 ± 3.1	88 ± 1.5	92 ± 0.6
HALOPERIDOL	500 µg	7	90 ± 2.1	85 ± 2.1	89 ± 1.4
CHLORPROMAZINE	500 µg	4	82 ± 0.1	79 ± 2.0	86 ± 1.4
	2.5 & 5 mg	1	93 ± 1.6	88 ± 0.7	93 ± 0.7
PHENTOLAMINE	500 µg	5	68 ± 3.3	66 ± 4.2	70 ± 6.5
	2 mg	2	90 ± 2.0	86 ± 3.0	90 ± 1.5
PROPRANOLOL	500 µg	3	78 ± 0.4	78 ± 1.2	84 ± 0.4
	800 µg	2	90 ± 0.5	88 ± 1.0	91 ± 0.5
METHYSERGIDE	2 mg	5	52 ± 9.2	47 ± 9.7	53. ±11.0
CYPROHEPTADINE	2 mg	3	51 ± 5.6	42 ± 4.9	43 ± 7.0
PIZOTYLINE	500 µg	3	89 ± 0.1	83 ± 2.6	88 ± 2.9
SODIUM PENTOBARBITAL	20 mg	4	90 ± 1.5	87 ± 2.1	90 ± 0.8
ETHANOL	4 gm	5	82 ± 3.5	81 ± 3.2	87 ± 2.3
DIPHENYLHYDANTOIN	10 mg	2	88 ± 3.0	87 ± 3.5	91 ± 1.5

Purified polysomes were isolated from three major brain regions as previously described (3). Area under the polysome profile was determined by planimetry. All of the listed drugs except ethanol were given by injection into the marginal ear vein. Ethanol was given intraperitoneally. Receptor blocking drugs were injected 20 to 30 minutes prior to LSD while pentobarbital, ethanol and diphenylhydantoin were given 5-10 minutes before LSD. Animals were left undisturbed in an open observation box until despatched by cervical dislocation 60 minutes after LSD administration. Each value is the mean ± standard error of the indicated number of experiments.

The effect of a number of neuropharmacological agents on the LSD-induced polysome disaggregation is presented in Table 1. These agents were injected in a concentration range (approx. 1-10 mM) assumed sufficient to allow substantial binding to central receptors and in a 3-20 molar excess of LSD. BOL-148, a brominated LSD derivative, binds to the same neural receptors as LSD but it is psychotropically inactive (12). To test a receptor-binding requirement for polysome disaggregation BOL-148 was injected prior to LSD. A complete blockage of the polysome shift was observed. Haloperidol and chlorpromazine are antipsychotic agents which bind to dopamine receptors (13, 14) and are used clinically to treat schizophrenia and LSD overdoses. Both of these agents prevented LSD-induced polysome shifts when administered prior to LSD (Table 1). Haloperidol was found to block disaggregation at as low a dosage as 100 µg/kg (data not shown). The greater effectiveness of haloperidol over chlorpromazine in preventing LSD-induced disaggregation is also seen in its relative clinical potency (13). The results in Table 1 suggest the involvement of dopaminergic receptors in LSD-induced brain polysome disaggregation.

To test whether adrenergic innervation was involved, rabbits were pretreated with the antihypertensive agents phentolamine (α-adrenergic blocker) or propranolol (β-adrenergic blocker). At 500 µg/kg phentolamine had no effect while propranolol partially inhibited the polysome shift. Higher dosages of both agents (2 mg/kg and 800 µm/kg respectively) completely blocked disaggregation.

Electrophysiological (15, 16) and receptor-binding studies (5-7) suggest that LSD interacts with serotonin receptors. The presumptive anti-serotonergic agents methysergide and cyproheptadine when given at 2 mg/kg potentiated LSD-induced polysome disaggregation (Table 1). Pizotyline, a more specific anti-serotonin agent, completely inhibited polysome disaggregation at 500 µg/kg. This agent effectively blocked the polysome shift presumably by preventing binding of LSD to serotonin receptors. A requirement for neuronal activity is suggested by the observation that the drug-induced polysome shift is prevented when conductivity is generally depressed by sedative doses of ethanol, pentobarbital or diphenylhydantoin (Table 1).

Discussion

The involvement of neurotransmitter receptors in regulation of brain protein synthesis has been previously reported. The dopaminergic blockers, haloperidol and pimozide, prevent polysome shifts in the rat brain which are induced by excess L-DOPA (17) and d-amphetamine (18). Our results suggest that a range of neurotransmitter receptors are involved in the disaggregation of brain polysomes which is induced by the intravenous administration of LSD to young rabbits. The polysome shift was seen to be prevented by the prior injection of (a) the dopaminergic antipsychotics haloperidol and chlorpromazine, (b) the adrenergic blockers phentolamine and propranolol and (c) the serotonergic blocker pizotyline. None of the receptor blockers administered alone affected brain polysomes. In particular the non-psychotropic LSD derivative BOL which binds to the same neural receptors as LSD did not alone result in brain polysome disaggregation but blocked the shift when administered prior to LSD.

Recent suggestions that LSD acts on dopamine receptors (8-10) are reinforced by our demonstration that the neuroleptic agents haloperidol and chlorpromazine block LSD-induced brain polysome disaggregation. The specificity of this blockage is shown by the requirement of a much higher dosage of chlorpromazine relative to haloperidol for equal effectiveness thus reflecting their known clinical potencies (13).

Both α and β adrenergic agents when administered in substantial excess by weight relative to the LSD dosage blocked the polysome shift. Ergot alkaloids were the first-known adrenergic blocking agents on peripheral processes such as vasodilation (19). The adrenal medulla may have been affected by the adrenergic receptor blockers leading to an alteration in the stress-like responses that normally occur after LSD (11). Adrenergic mediation appears to be involved in the LSD-polysome response by way of a complex interaction of central and peripheral effects.

There is considerable evidence in the literature that LSD acts on serotonin receptors (5-7, 20, 21). Methysergide and cyproheptadine are serotonin antagonists as defined by peripheral activity. These two agents may not have the same effective 5-HT antagonism in the CNS (20, 22, 23). When administered prior to LSD these two antagonists potentiated brain polysome disaggregation while having no effect alone on polysomes. Bennett and Synder (20) demonstrated [^{3}H]-LSD binding to both an LSD specific site and a separate 5-HT receptor, suggesting that there may be two tryptaminergic receptors. Both methysergide and cyproheptadine by this competitive binding assay (20) had higher affinity for the LSD rather than the 5-HT site. A pharmacological definition of potentiation is that blockage by a competitive antagonist increases the bioavailability of the second incoming agent to act on other sites (24). Thus more LSD might be available after administration of methysergide or cyproheptadine to act on the second 5-HT specific receptor leading to potentiation of the polysome shift. If two tryptaminergic sites are present then it could be assumed that pizotyline blocked the LSD effect by binding to both types preventing any LSD binding. Another consideration is the concept of cooperativity between two associated sites, an example being the greater than additive increase in c-AMP after administration of epinephrine together with adenosine (25). Perhaps some such cooperativity occurs between LSD and methysergide or cyproheptadine.

Our results suggest that a range of biogenic amine neurotransmitter receptors are involved in the mechanism by which LSD affects the protein synthesis apparatus of the brain. General neuronal activity is also a necessary element in the effect as blockage was produced by general anesthetics. The tryptaminergic receptors may exist in at least two forms having differing affinities for LSD. The dopaminergic antipsychotic drugs may have blocked LSD binding to dopamine receptors. Perhaps as is more likely with the adrenergic agents they blocked synaptic transmission along catecholaminergic interneuronal pathways which amplify the effect of the initial binding of LSD to serotonergic receptors. Most significantly it appears that altered synaptic activity propagated throughout the brain following initial selective binding of LSD mediates the effect of the drug on brain protein synthesis.

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