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Neutralization of LSD by Active Immunization

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Abstract. Mice immunized with a lysergic acid derivatized protein showed subsequent resistance to the effects of intravenously administered LSD as measured by the poke and rearing tests. Mice immunized with a tryptophyl conjugated protein displayed similar refractoriness relative to normal control mice. Thus, significant immunological cross reactivity as expressed by neutralization, was evident due to the common indole moiety of the two haptenic groups studied.

Key words: LSD — Tryptophan — Immune Neutralization.

The development of immunochemical reagents for immunoassays and immunotherapy of hallucinogenic drugs is an area of critical need. The potential of applying immunological systems to drug problems was demonstrated by Spector and Parker (1970) with their development of a sensitive radioimmunoassay for the detection of morphine. Recently, the production and characterization of antibodies to D-lysergic acid was reported (Van Vunakis *et al.*, 1971). However, it has not been shown that specific antibodies can neutralize the psychological effects of drugs. To establish the potential of antibodies as a neutralizing agent, the comparative effects of D-lysergic acid diethylamide (LSD) was studied in normal and immunized mice.

Methods

Hallucinogenic effects of LSD were assayed by the poke test (Cohen and Wakeley, 1968), the rearing test (Dandiya *et al.*, 1969), and the depth perception test (Walk and Gibson, 1961). The poke test apparatus consisted of a 40 × 49 cm wooden platform containing twenty equally spaced holes (3 cm diameter) mounted on four legs, 48 cm in height. Individual mice were placed in the center (origin) of the grid and their exploratory movements measured for 2 min. An activity score was amassed for each mouse based on a cumulative total of the times a mouse poked his head into a hole, the number of holes explored, and the frequency with which he looked over the edge of the platform. The rearing test was originally described by Dandiya *et al.* (1969), and is defined as the number of times

* Supported by a grant from the Pharmaceutical Manufacturers Association Foundation, Inc.

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a mouse stands on its hind legs during a 2-min period. The depth perception test is a visual height discrimination test. The apparatus consisted of a hollow enclosed wooden box with a floor of glass (25×30 cm). Four supporting legs (45 cm high) were placed at each corner. Patterned material was placed directly under the glass on the shallow side and on the floor below. Illumination was supplied by indirect fluorescent lighting directly under the shallow side. This minimized reflection of the lights from the glass. The mice were placed on an elevated (3.7 cm) central platform, from where they descended to the optically shallow or deep side in a 2-min assay period.

Lysergic acid diethylamide (LSD) was supplied by the Food and Drug Administration, U.S. Public Health Service.

Immunogens were prepared as follows: L-tryptophan (Pierce Chemical Co., Rockford, Ill.) and lysergic acid (LSA, Grade II, Sigma Chemical Co., St. Louis, Mo.) were conjugated to rabbit gamma globulin (RGG) or rabbit serum albumin (RSA) by the carbodiimide reaction (Sheehan and Hess, 1955; Sheehan *et al.*, 1956). Equal weights of the haptens and 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-p-toluenesulfonate (Aldrich Chemical Co., Milwaukee, Wis.) were incubated at pH 8.5 for 10 min and then added to an equal weight of protein (20 mg/ml). After incubating for 24 h at room temperature, the reactants were extensively dialyzed against 0.01 M, phosphate buffer, pH 7.5 at 5°. Tryptophyl groups per mole of protein were determined by the spectrophotometric method of Edelhoch (1967) and dry weight analysis of the proteins. Validity of the Edelhoch method was verified by derivatization of protein with ^{3}H-L-tryptophan (sp. act. 4.0 c/mole, Schwarz BioResearch, Inc., Orangeburg, N.Y.) diluted with unlabeled tryptophan to a known specific activity. In a control experiment, 15.4 tryptophyl groups were determined by the radioactive measurement and 14.5 by the Edelhoch method per mole of RGG (M.W. = 155000). Lysergic acid groups were determined by dry weight analysis of the protein and absorbance of the lysergyl group at 310 nm (E_m = 8300).

Mice were immunized intraperitoneally with the appropriate immunogens emulsified in complete Freund's adjuvant. Prior to injection, a test for emulsion was made by placing a drop of the mixture in distilled water. All mice were boosted with equivalent amounts of immunogen by the same route of immunization.

Results

Measurements of LSD Effects in Normal Mice

Albino mice of both sexes, weighing 30—35 g, were tested individually in all assays in the normal and hallucinogenic state. All mice were injected intravenously (tail vein). Normal mice, or mice injected with buffer,

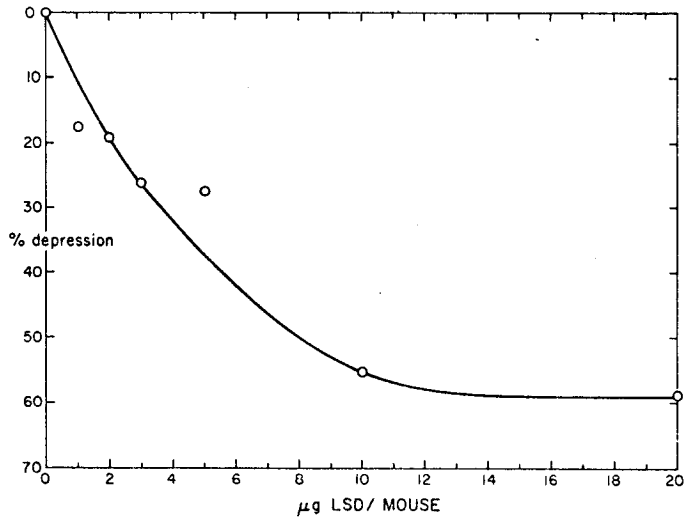

Fig. 1. Dose response curve relating percent depression in the poke test of mice receiving 0.1 ml LSD intravenously. Mice were tested within 20 min after receiving the drug

scored a total of 45—50 points in the poke test and reared 15—16 times in a 2-min period. Normal mice selected the shallow side about 82% of the time in the depth perception test. Fig. 1 represents a dose-response curve for LSD administered intravenously (0.1 ml) in normal mice as assayed in the poke test. All mice were tested within 20 min after intravenous injection. An activity depression of 55% was measured at 10 μg of LSD per mouse and 20 μg depressed only an additional 2—3%. Thus, based on these results and the amount of LSD available, 10 μg of LSD per mouse was chosen as the test dosage. Mice receiving 10 μg of LSD reared on an average of 3—4 times or a 77.5% depression. These mice selected the optically shallow side 65% of the time in the depth perception test.

Measurements of LSD Effects in Immunized Mice

Immunized mice were studied to determine the neutralizing ability of antibodies elicited by active immunization on the hallucinogenic effect of LSD. Mice were divided into four groups: Group 1 consisted of 11 non-immunized mice; 10 mice in Group 2 were immunized with the lysergic acid-rabbit gamma globulin (LSA-RGG) immunogen; Group 3, 10 mice received the tryptophan-rabbit gamma globulin (TRYP-RGG) conjugate; and Group 4, 14 mice were not immunized and received an intravenous injection of buffer (0.1 ml, 0.05 M, phosphate pH 8.0) in place of LSD at

Table 1 A. *Comparative per*
and normal mice after

Group No.	Immunogen
1	None
2	LSA-RGG
3	TRYP-RGG
4[a]	None

[a] All groups received
[b] Value normalized as

Treatments
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[a] Degrees of freedom.
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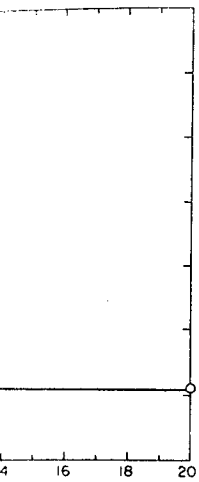

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Table 1 A. *Comparative percent depression in the poke and rearing assays with immune and normal mice after intravenous administration of 10 μg of LSD per mouse*

Group No.	Immunogen	Poke test		Rearing test	
		Mean score	Percent change	Mean score	Percent change
1	None	21.2	55.4	3.6	76.6
2	LSA-RGG	32.2	32.4	5.8	62.3
3	TRYP-RGG	33.3	29.9	7.9	48.7
4[a]	None	47.6[b]	0	15.4[b]	0

[a] All groups received 10 μg of LSD except Group 4.
[b] Value normalized as 100% for calculation of percent depression.

Table 1 B. *Analysis of variance*

	Poke test		Rearing test	
	df[a]	MS[b]	*df*	MS
Treatments	4	1155.5[c]	4	399.3[c]
Groups 1—3 vs. 4	1	3663.9[c]	1	1501.2[c]
Groups 1 vs. 2—3	1	874.8[d]	1	60.0
Groups 2 vs. 3	1	6.0	1	22.0
Error	49	116.7	61	51.8

[a] Degrees of freedom.
[b] Mean square.
[c] Level of significance = 0.001.
[d] Level of significance = 0.01.

the time of testing. Group 3 was included to explore the degree of cross reactivity between the tryptophyl and lysergyl groups due to the common structural indole moiety.

Mice were injected with 300—400 μg of each immunogen emulsified in complete Freunds adjuvant. Groups 2 and 3 were boosted 25—30 days after the primary immunization and assayed 5—10 days after the secondary immunization. Table 1 shows the effects of the intravenous injection of 10 μg LSD per mouse in Groups 1, 2, and 3. The data shows that Groups 2 and 3 were not depressed by LSD to the same degree as the non-immunized Group 1. Results also indicate that anti-tryptophan antibodies neutralized the effects of LSD to an equal or greater degree than anti-LSA antibodies. Differences cited may be a function of the relative amounts of antibody produced, the relative affinities or class of immunoglobulins elicited.

In the depth perception test the shallow side of descent was selected about 50% of the time by the mice in Groups 1, 2 and 3 indicating no depth perceptibility. Thus, data from the depth perception test, with respect to neutralization of the hallucinogenic state, did not correlate

with the poke and rearing tests. Mice in Group 4 selected the shallow side with the same frequency (85%) as previously tested non-immunized mice.

Analysis of Variance

An analysis of variance (ANOVA) of the poke test data (Table 1B) indicated that there is significant difference among the groups, and in particular, Groups 1 through 3 are significantly different from Group 4, and Group 1 is significantly different from Groups 2 and 3. Similarly, ANOVA of the rearing test data (Table 1B) indicated that there is significant difference among the groups, and, in particular, that Groups 1 through 3 are significantly different from Group 4. Although a dose-response curve for the rearing assay was not formulated, the index of correlation between the two assays is very high, $r = 0.97$. This correlation between the two assays indicates that the rearing assay compares very favorably with the poke assay in terms of measuring the effects of LSD.

Measurement of Antibody Response

Mice were randomly assayed for anti-lysergic acid or anti-tryptophyl activity by injection of 100 μg (0.1 ml) of the test antigens (i.e. lysergyl-RSA or tryptophyl-RSA) and buffer intradermally on the shaved ventral surface. Each mouse was immediately injected intravenously (tail vein) with 0.05 ml of 0.5% Evans blue in physiological saline and the injection site scored within 15—30 min. Immunized mice showed positive reactions with lysergyl and tryptophyl-RSA. Positive reactions were also noted with RGG and carbodiimide treated-RSA. A buffer control was negative in all mice tested. Several mice from each group were bled by rupturing (capillary tube) the ophthalmic venous plexus (McVeigh and Voss, 1969). Pooled sera were tested by the capillary precipitin test with LSA and tryptophyl conjugated test antigens. Results obtained were in agreement with the skin test.

Discussion

Results of these experiments indicate that certain effects of LSD can be measured by appropriate test systems. Reductions in exploratory activity (i.e. poke test) and ability to perceive depth by LSD in the mice tested, is consistent with results obtained by Cohen and Wakeley (1968). Data obtained from the rearing test seemed to substantiate the results of the poke test. The dose response curve (Fig. 1) suggests that although 10 μg LSD per mouse should not be considered optimum, it did give near maximum depression. Since 10 μg of LSD per mouse is not on the plateau phase of the dose response curve, animal variation can have significant effect on the results. However, analysis of variance calculations support the significance of the data and suggest that animal variation could not account for the results. The data indicate that the three assays vary as

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measurements of immune neutralization. The most significant neutralization was measured in the poke test. Results from the rearing test show marginal neutralization, while significant differences were not observed in the depth perception test. It is conceivable that these assays are different in what they measure or, alternatively, they vary in sensitivity as assays for immune neutralization.

These experiments indicate that mice producing anti-tryptophan and anti-lysergic acid antibodies are relatively resistant to these measurable effects of LSD. Cross neutralization suggests significant serological cross reactivity. This cross reactivity between the antibodies was verified by the PCA and precipitation analyses. The common indole moiety is considered the structural basis of this cross reactivity. Table 1 A indicates that the hallucinogenic effect was not totally suppressed by antibodies as elicited in these experiments. Although the reason for the lack of total drug neutralization is not obvious, it may relate to the simulation of a reversible hapten:anti-hapten reaction (i.e. LSD:anti-LSA) necessitating that a certain percentage of hapten (relative to the binding constant of the serum antibody) is free (unbound) at any point in time. However, it is clear that the levels of immune neutralization reported are significant and suggest that further studies into the interaction of antibodies and hallucinogenic drugs are warranted.

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