In vitro Effect of d-Lysergic Acid Diethylamide on Immunoglobulin Synthesis

by

Edward W. Voss, Jr.
James E. Babb
Peyton Metzel
Jeffrey L. Winkelhake

Department of Microbiology
University of Illinois
Urbana, Illinois 61801

Received November 8, 1972

Summary: Rabbit anti-fluorescyl antibody producing lymphoid cells incubated *in vitro* with LSD do not secrete the 7S form of immunoglobulin. The low molecular weight extracellular labeled material shows no measurable anti-fluorescyl antibody activity. Results indicate that during a short incubation period LSD interferes with tryptophan incorporation into antibody protein.

The hallucinogenic and physiological effects of d-lysergic acid diethylamide (LSD) are well characterized (1), but definable biochemical effects remain conjecture. At the molecular level, LSD:nucleic acid interactions have received considerable attention (2, 3). However, LSD induced chromosome damage and related carcinogenic, mutagenic, or tetragenic properties are unproven scientifically (4). Information available describing the influence of psychotomimetic drugs on protein biosynthesis is minimal. Certain drug induced physiological changes, such as reduction of tryptophan and tyrosine levels in serum have been reported (5). These measurable effects between LSD (a structural analogue of indole) and tryptophan suggest possible interference with 1) metabolic pathways, 2) protein biosynthesis, or 3) both metabolism and amino acid incorporation into proteins. Results reported below on the *in vitro* effect of LSD on anti-fluorescyl antibody producing lymphoid cells suggest that the drug alters immunoglobulin biosynthesis by interferring with tryptophan incorporation.

Adult albino rabbits were immunized with 5.0 mg fluorescyl conjugated

porcine gamma globulin (Fl_{23} PGG) emulsified in complete Freunds adjuvant (6). Rabbits received footpad and intrascapular injections on days 0, 45 and 200. Five to 10 days after the final immunization, the hyperimmune rabbits were bled, sacrificed, and the spleens and popliteal lymph nodes removed surgically. Cells were separated from the capsular material (7), filtered through a wire mesh filter, and the suspension washed 3-4 times in Minimum Essential Medium (MEM, Grand Island Biological Co.). Centrifugations were performed in a refrigerated International Centrifuge (PR-2 Model) at 2500 rpm for 10 min. After washing, cells (0.1 ml packed volume aliquots) were incubated in the following reactions: A) 1.0 ml MEM minus leucine, B) and C) 1.0 ml MEM minus leucine and tryptophan. To reactions A, B, and C was added 50 µl of ^{3}H leucine (spec. act. 52 Ci/mmol, Schwarz/Mann). Reactions B and C also received 50 µl or approximately 10 µg of ^{14}C tryptophan (spec. act. 29 mc/mmol, Schwarz/Mann). Purity of ^{14}C tryptophan was verified by silica gel chromatography in chloroform:ethanol:acetic acid (18:10:2, R_F=0.65). To reaction C was added varying amounts (0.1-10.0 µg) of LSD (supplied as a tartrate salt by the National Institute of Mental Health). Reactions were carried out in duplicate and incubated in a CO_2 incubator (20% CO_2 and 10% air, Wedco model 2-17B) at 37° for 16-18 hrs. All reactions were run in duplicate. Cell viability was measured by trypan blue (0.4% in 0.15 M saline) staining as previously described (8). Equal cell viability was measured in all reactions (i.e. A, B and C) over the 16-18 hour period. After incubation, the cells were pelleted by centrifugation, and the extracellular supernates harvested. Supernates were dialyzed against repeated changes of 4 liter volumes of 0.05 M K-phosphate buffer, pH 8.0 for 48 hrs at 5°. Pre-immunization and antiserum samples, obtained at 14 and 60 days post-primary immunization, were tested for anti-fluorescyl activity by capillary precipitin tests with fluorescyl substituted bovine serum albumin (Fl_{10} BSA) at 100, 500, and 1,000 µg/ml. Quantitative precipitin tests (9) of the 60 day antisera indicated precipitating antibody levels

of 2.1 to 6.2 mg/ml. Results of similar tests performed on the hyperimmune antiserum samples obtained at the time of sacrifice, indicated high levels of anti-fluorescyl antibodies.

After dialysis, the extracellular supernates (Reactions A and B) were analysed for radioactive label incorporation into antibody protein. Trichloroacetic acid (5%) precipitation indicated that 95-100% of the nondialyzable material was precipitable. Sucrose gradient analysis (Fig. 1A); specific precipitations (85-90%) by goat anti-rabbit gamma globulin antiserum; precipitation in 50% ammonium sulfate (80-90%) and isoelectric focusing (Fig 1B) indicated the extracellular labeled material in reactions A and B to be 80-90% immunoglobulin. The total amount of ^{3}H leucine incorporation per 0.1 ml cells in reactions A and B was nearly identical. This comparison indicates that when the tryptophan content (10 µg/ml) in MEM was replaced by ^{14}C-tryptophan, protein synthesis in terms of radioactive leucine incorporation was not altered. Comparative incorporation levels in reactions B and C indicate that the addition of LSD resulted in a depression of ^{14}C tryptophan incorporation into secreted protein as evidenced by increased ^{3}H leu/^{14}C tryp ratios (Table 1). Since the total ^{3}H leucine incorporation between reactions B and C was similar, the cells were synthesizing and secreting approximately equal amounts of labeled protein. Listed in Table 1 are results from 5 representative rabbits from 20 similar experiments. Ratios measured in reaction B are considered averages, defining a heterogeneous extracellular protein population. However, since the total protein synthesized was quantitatively similar, ratio changes are significant and not attributable to reduction or elimination of a specific component within the heterogenous population. Equivalent cell viability (60-70%) between reactions B and C at 18 hrs. eliminates the possibility of selective lethality by LSD resulting in ratio changes.

Comparison and analysis of the extracellular material from reaction B on sucrose gradient and isoelectric focusing (Fig. 1A) shows that the

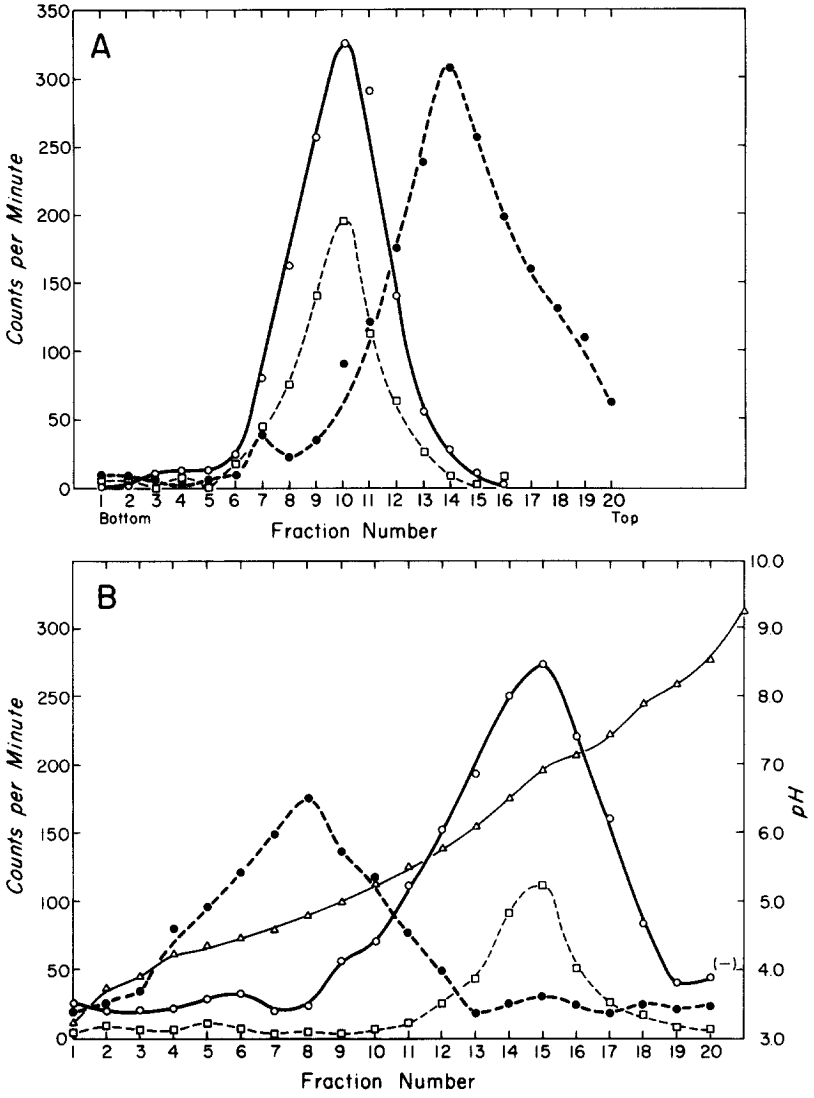

Fig. 1A Sucrose density gradient profile of purified ^{125}I rabbit anti-DNP IgG antibody (□ -- □) and extracellular material from reactions B (0——0) and C (●——●). Experiments were performed on 10-40% gradients in 0.05 M K-phosphate buffer, pH 8.0. Centrifugation was performed in a Beckman Spinco Model L centrifuge with a SW-39 rotor at 35,000 rpm for 16 hr.

Fig. 1B Isoelectric profiles in pH 3-10 gradient at 300 V for 72 hr of materials indicated in 1A. The pH gradient is indicated by △——△.

Table 1

Effects of LSD on *in vitro* Incorporation of Labeled Amino Acids into Hyperimmune Anti-fluorescyl Producing Cells

Rabbit No.	Cells	Reaction	LSD (μg/ml)	$^{3}H/^{14}C$ [a]	% Change [b]
17	Spleen	B	----	8.5 ± 1.0	----
		C	10.0	11.2 ± 0.8	+ 32
18	Spleen	B	----	7.8 ± 0.9	----
		C	2.5	8.4 ± 0.6	+ 7.5
		C	5.0	9.2 ± 0.7	+ 18.0
	Node	B	----	5.5 ± 0.5	----
		C	2.5	14.2 ± 1.1	+ 158
20	Spleen	B	----	9.4 ± 0.5	----
		C	2.5	10.6 ± 0.6	+ 12.6
		C	10.0	13.5 ± 0.6	+ 43.6
	Node	B	----	5.1 ± 0.5	----
		C	2.5	6.8 ± 0.6	+ 33.2
		C	10.0	7.4 ± 0.6	+ 45.0
21	Spleen	B	----	4.9 ± 0.6	----
		C	5.0	7.7 ± 0.6	+ 57.0
		C	10.0	8.4 ± 0.7	+ 71.0
27	Node	B	----	3.9	----
		C	10.0	10.7	+ 175

(a) Range in ratio values ± the mean value. Duplicates not available for Rabbit 27.

(b) Based on average values.

secreted material coincides in molecular weight and isoelectric point to (^{125}I) purified rabbit anti-hapten IgG antibodies. Extracellular material from reaction C sedimented as lower molecular weight than IgG antibody (M.W. 150,000) and had a significantly lower pI value on a pH 3-10 ampholyte gradient (Fig. 1B). Immunoadsorption (10) of the extracellular material from reaction C indicated little or no adsorbable anti-fluorescyl antibody. Sucrose gradient analysis and isoelectric focusing revealed that little or no 7S antibody was being secreted from cells incubated in the presence of LSD. Reaction C extracellular material was not precipitable by 50% ammonium sulfate or goat anti-rabbit gamma globulin antiserum. Upon the addition of 100 µg of L-tryptophan in reaction C, 80% of the extracellular material was precipitable by 50% ammonium sulfate and identifiable as IgG by precipitation with goat anti-rabbit gamma globulin antiserum. Addition of equal amounts of L-tyrosine did not restore the secretion of 7S molecules.

These results indicate that LSD has a significant effect on antibody synthesis. Similar results have been obtained *in vitro* with hyperimmune anti-2,4 dinitrophenyl and anti-bovine serum albumin synthesizing lymphoid cells. Thus, the effects of LSD on immunoglobulin biosynthesis are not peculiar to the anti-fluorescyl system, and the interference with tryptophan incorporation appears to be a general phenomenon.

Preliminary results indicate that when d lysergic acid (LSA) is incubated with antibody producing cells under similar conditions, an *in vitro* effect similar to that of LSD is noted. These results suggest that the indole alkaloid LSD (and its analogue LSA) interferes with tryptophan incorporation into antibody protein during *de novo* synthesis. Studies are currently in progress to determine if: a) LSD interferes with tryptophan incorporation and results in peptide termination. Presumably, these peptides are not precipitable by 50% ammonium sulfate or anti-gamma globulin antisera; or b) if LSD is modified and incorporated in place of tryptophan residues.

References and Notes

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11. Supported by PHS research grant MH 20302 and Pharmaceutical Manufacturers Association.