Lysergic Acid Diethylamide (LSD) Binding to Deoxyribonucleic Acid (DNA) (33203)

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A number of workers have reported recently that lysergic acid diethylamide (LSD), in addition to its central nervous system effect, causes chromosomal breaks both *in vitro* and *in vitro* (1,2). Although there remains some disagreement about the significance of these initial observations (3), the results suggested the possibility that this drug may interact with some chromosomal component. A study of the interactions between LSD and such maeromolecules as DNA may also be relevant to the psychotomimetic actions of such drugs. Accordingly, purified DNA has been examined for its ability to bind LSD.

Materials and Methods. Spectra of solutions of D-LSD, L-LSD, and D,L-2-Br-LSD containing varying concentrations of DNA were recorded with a Cary model 15 spectrophotometer. From these experiments an extrapolated value was obtained for the absorption of the fully bound drug and the extent of binding calculated for a variety of drug concentrations. Thus, using the general method of Klotz *et al.* (4) calculations were made of the intrinsic equilibrium constant and the number of binding sites on the polymer. Fluorescence measurements employed the Aminco Bowman spectrophotofluorometer. In the course of the latter experiments it was found that prolonged irradiation of the drug at 310 mμ resulted in its photo destruction. Caution was exercised to avoid such changes during each experiment. The nature of this process will be the subject of a subsequent paper.

Results. The absorbance spectrum of LSD is presented in Fig. 1. As shown, native calf thymus DNA added to the drug solution resulted in a progressive decrease in absorbance at 310 mμ.

Figure 2 shows a similar effect of DNA on the fluorescence emission spectrum of the drug. Based on UV spectra the extrapolated value for the intrinsic dissociation constant for binding of both D- and L-LSD was approximately 5×10^{-4} M and the extrapolated maximum binding ratio was 1 drug molecule per base moiety in the DNA chain. Experiments with similar results were also performed using the biologically ineffective analogue D,L-2-Br-LSD. Thus, binding to DNA would appear to be a general property of this group of drugs. In similar experiments, yeast RNA in a final concentration of 3.4×10^{-4} M did not cause a significant depression in the spectrum of 1×10^{-4} M LSD. It would appear therefore that the binding process is specific for DNA.

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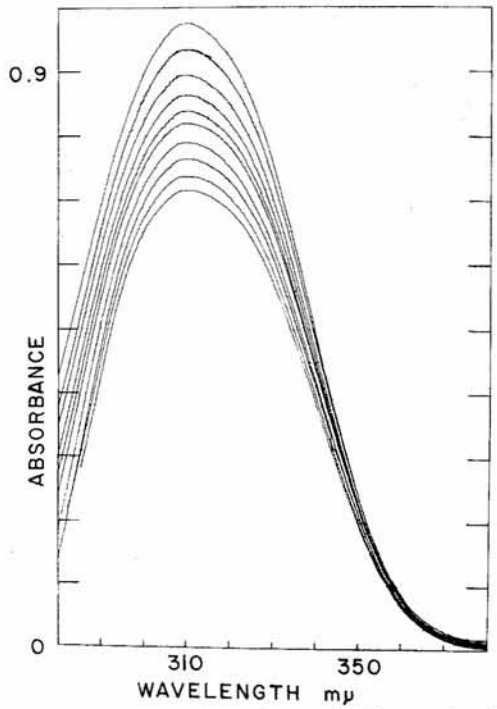

FIG. 1. Effect of calf thymus DNA on the absorption spectrum of D-LSD at 26°C. Reaction mixture consisted of 1×10^{-4} *M* D-LSD in 0.01 *M* PO_4 buffer, pH 6.1, in top curve, using a light path of 1 cm. The progressive depression of the curve resulted from addition of increments of DNA from 0.86 to 7.9×10^{-4} *M*, dissolved in the same buffer.

The role of DNA tertiary structure was also evaluated. At pH 3, where DNA is nonhelical, no spectral alteration of the drug resulted from the addition of DNA. Similarly, DNA which had been heated at 100° for a period of 30 min and cooled in ice was much less effective than native DNA in depressing the spectrum of LSD. Thus, it would appear that LSD binds primarily to helical DNA.

The addition of 3.4×10^{-3} *M* $MgCl_2$ to the drug–DNA mixture reduced the spectral change produced by the DNA. This effect of $MgCl_2$ could be due either to its effect on the DNA helix or could interfere with an electrostatic attraction between the DNA-phosphate and the drug. However, at pH 8.5, where the drug should not be protonated, binding was also demonstrated suggesting that charge attraction is not an important aspect of binding.

These preliminary results showing that LSD can bind to this class of macromolecues provide an interesting model for consideration, although the relationship of this *in vitro* binding process to the biological effects of LSD would be conjecture at this point. Two obstacles must be considered however: the lack of difference in binding of the active and inactive optical isomers; and the concentrations of the drug employed. These are not sufficient reasons, however, to dismiss such macromolecular binding as an important phenomenon. First, drug specificity in this instance may be based on some factor in addition to stereospecificity of the receptor site. For example, if the different

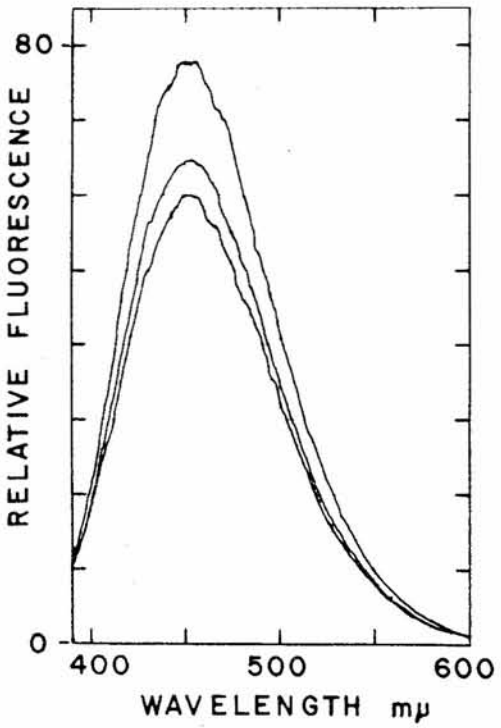

FIG. 2. Effect of calf thymus DNA on fluorescence emission of D-LSD at 26°C. Reaction mixture contained 1×10^{-6} *M*, D-LSD in 0.01 *M* PO_4 buffer, pH 6.1, in top curve. The depression of the emission shown in the 2 lower curves resulted from 1.76 and 8.80×10^{-4} *M* DNA. An activation wavelength of 310 μ was used in these experiments.

drugs are handled differently by the organism, they may be delivered to receptor sites at grossly different rates. Secondly, the usual considerations of drug concentration ignors the role of receptor site concentrations. In the event the receptor site concentration is high (as it most certainly is for DNA within the nucleus) significant binding may occur even with very low drug concentrations.

Summary. Addition of DNA (but not RNA) to solutions of lysergic acid diethylamide resulted in a depression of the absorption and fluorescence spectra of the drug. The K_d for D-LSD:DNA binding, calculated from spectral changes, was 5×10^{-4} M, and each nucleotide residue was a potential binding site. Binding did not occur at pH 3 (where DNA is nonhelical), and was decreased by prior heating of the DNA or by adding $MgCl_2$ at a concentration of 3.7×10^{-3} *M*.

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Comparison of Sensitivities of Immunodiffusion Methods* (33204)

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Double diffusion in agar is widely used to study precipitating antigen–antibody systems (1). Although many different methods are used, few data have been published comparing their sensitivities in detecting antigen and antibody (2). The study by Finger and Kabat (3) appears to be the only one in which the sensitivity of some of these techniques was determined by quantitative immunochemical methods. Since then, new methods have been described about which comparative information is not available. In this study we compared quantitatively some currently used double-diffusion methods.

Materials and Methods. Antigens. Crystalline human serum albumin (HSA) was purchased from Mann Research Laboratories, Inc. (New York). Human γ-globulin (HGG) was a gift from the American Red Cross through the courtesy of Dr. James H. Pert. The concentration of these antigens was determined by Markham micro-Kjeldahl analysis (4) and by their absorption at 277 mμ in a Beckman DU-2 spectrophotometer.

Antisera. Rabbits were immunized by toe pad and repeated subcutaneous injections of HSA and HGG in Freund's complete adjuvant (Difco). Because of reports indicating the importance of complement (C′) in double-diffusion analysis in agar with haptenic antigens (5), the first component of C′ was removed from anti-HSA by dialysis against 0.001 *M* phosphate buffer (pH 7.4) containing 0.004 *M* sodium chloride as described by Nelson (6). Fresh normal rabbit serum contained 22 C'_{50} units/ml; rabbit serum dialyzed against this solution contained only C'_{50} units/ml when analyzed by quantitative C′ fixation (4). The antibody content of the antisera was determined by the quantitative precipitin method (4). Anti-HSA contained 772 μg of N/ml and anti-HGG contained 125 μg of N/ml.

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