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D-LSD BINDING TO BRAIN HOMOGENATES:

POSSIBLE RELATIONSHIP TO SEROTONIN RECEPTORS

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SUMMARY

Using a rapid filtration technique we find stereospecific high-affinity d-LSD binding (4×10^{-9}M, half-saturation) in brain fractions from a number of subcortical as well as cortical brain regions. Among the putative neurotransmitters tested only serotonin effectively displaces d-LSD from this specific binding site. Moreover, only the serotonin-displaceable component of binding is saturable in a high-affinity range. No change is observed in specific d-LSD binding in forebrain homogenates from rats in which ascending serotonergic pathways are destroyed by lesions in the raphe nucleus. We conclude that a vast majority of the d-LSD binding sites may be associated with a postsynaptic serotonin receptor rather than a presynaptic receptor associated with serotonergic (raphe) inputs.

By equilibrium dialysis Farrow and Vunakis (1,2) found saturable high affinity d-LSD (d-lysergic acid diethylamide) binding in subcellular fractions from cerebral cortex but not other rat brain areas. The bound d-LSD was displaced by serotonin (5-hydroxytryptamine; 5-HT) and certain hallucinogenic drugs. The fact that binding occurs only in the cortex is puzzling since d-LSD alters the physiological and biochemical activity of certain neurons in the sub-cortex. For example, d-LSD has a potent inhibitory effect on 5-HT neurons in the midbrain dorsal raphe nucleus (3,4). This inhibition is thought to initiate a reduction in the turnover of this amine in the forebrain (5,6,7,8). In addition, Diab et al. (9) demonstrated, by autoradiography, ^{3}H-LSD binding to dorsal raphe and other subcortical neurons. Using a slight modification of the rapid filtration technique, as described by Pert and Snyder (10), we have found that stereospecific, saturable, high-affinity

binding of d-LSD occurs in brain fractions from subcortical brain fractions as well as the cerebral cortex.

METHODS

Male Sprague-Dawley rats, 7-12 weeks old, were decapitated and brains homogenized at 4° in 6 volumes of a modified Krebs-Ringer solution (buffered in .05M Tris at a pH of 7.4) by 15 strokes of a motor driven teflon pestle. The homogenate was then diluted 17 fold with the modified Krebs solution. Conditions for measuring binding consisted of: 1) a 3 ml aliquot of the brain homogenate placed in a 20.0 ml beaker, kept at 4°, containing 0.1 ml of the modified Krebs solution and varying concentrations of d-LSD (2-^{3}H(N); 14.1 Ci/mmole; New England Nuclear), or 2) all of the above plus .1 mM or 1.0 mM 5-HT. Samples were incubated at 37° for 15 minutes then cooled to 4° and filtered under reduced pressure through Whatman glass-fiber circles (GF-B) in Buchner Funnels. The beakers were rinsed with 4.0 ml of cold Krebs solution. The filters were washed with the rinse solution and then twice more with 5.0 ml of cold Krebs solution. Immediately after each sample had been filtered a rubber stopper was placed on top of the Buchner funnel. Since brain homogenates do not metabolize d-LSD (14) the filters were removed and placed directly into counting vials containing 2.0 ml 10% sodium dodecyl sulfate and shaken for 30 minutes. Protein was measured by the method of Lowry et al. (11) with bovine serum albumin as a standard. Dissection of brain regions and lesions of the midbrain raphe nucleus were carried out according to Kuhar et al. (12). The concentration (13) of d-LSD in the midbrain 5 minutes after i.v. administration of ^{3}H-d-LSD (7.5 µg/kg) was determined as follows: ^{3}H-d-LSD was extracted from homogenates of the midbrain by the method of Axelrod et al. (14). The amount of ^{3}H-d-LSD was determined by liquid scintillation spectrometry.

RESULTS

General.

Using the filtration method we observed binding of d-LSD in brain homo-

genates. Coincubation with 5-HT, a putative neurotransmitter which is structurally related to d-LSD, suppressed the amount of bound d-LSD. Saturable binding of d-LSD was observed with increasing d-LSD concentration when the amount of d-LSD bound in the presence of high concentrations of 5-HT (.1 or 1.0 mM) was subtracted from the amount bound in the absence of 5-HT. Saturable binding was half-maximal at 4×10^{-9} molar and is termed "specific" binding (Fig. 1) since it represents that fraction of the bound d-LSD that could be displaced by 5-HT. The bound d-LSD which could not be displaced by 5-HT increased linearly with increasing d-LSD concentration. This non-saturable non-5-HT displaceable fraction of the bound d-LSD is termed "non-specific" binding (Fig. 1). Specific binding of d-LSD reaches equilibrium by 15 minutes and is linear with brain tissue protein over the range of .1 to 1.0 mg of protein/ml. Specific binding is optimal between the pH range 6.5 to 7.4, but non-specific binding increases with decreasing pH. We find that preincubation of the homogenate at pH 3, as described by Farrow and Van Vunakis (2), produces a precipitate which retards filtration. Specific binding is reduced by 90% when brain homogenates are preincubated at 80° for 15 minutes. At 4° specific binding is reduced to 18% of the 37° values. d-LSD binding is displaced by non-radioactive d-LSD with half-maximal displacement at 5 to 6×10^{-9} molar. Preliminary results indicate that specific d-LSD binding is predominately associated with the synaptosomal fractions isolated from cortical and subcortical regions.

Distribution of d-LSD Binding Sites in Brain.

Since the high affinity, saturable component of d-LSD binding is displaceable by 5-HT, we examined the distribution of this binding in brain regions with high or low levels of serotonergic (5-HT) input. Presumably, if d-LSD alters the physiology or serotonergic transmission, d-LSD binding should occur in regions of the brain known to contain serotonergic input. Consistent with this expectation, we found specific d-LSD binding in areas,

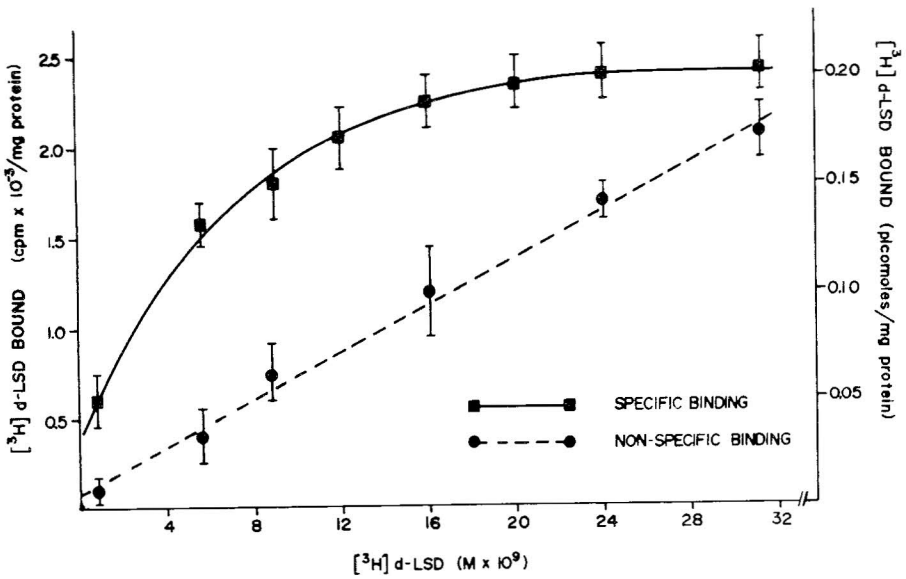

FIG. 1

Binding of ^{3}H-d-LSD to brain homogenates. There is a saturable, 5-HT displaceable component (———) and a non-saturable, non-5-HT displaceable component (--------). Bracketed vertical lines represent standard errors of the means. Each point represents the mean of five to ten experiments.

both cortical and subcortical (e.g. striatum and diencephalon) known to receive a 5-HT input, but not in the cerebellum which has little or no 5-HT input (Fig. 2)(15,16).

Physiological Correlates of d-LSD Binding.

To investigate the correlation between specific d-LSD binding in vitro and concentrations of the drug in vivo which produce physiological effects, we selected a dosage known to effect the firing of serotonergic neurons. There is nearly a complete inhibition of serotonergic (midbrain raphe) neurons lasting approximately 5 minutes after intravenous administration of 7.5 μg/kg of d-LSD (free base) (17). We therefore measured the concentration of d-LSD in the midbrain 5 minutes after this dose of the drug. We found that the concentration of d-LSD in the midbrain under these conditions (assuming uniform distribution) is approximately 2 x 10^{-8}M. This

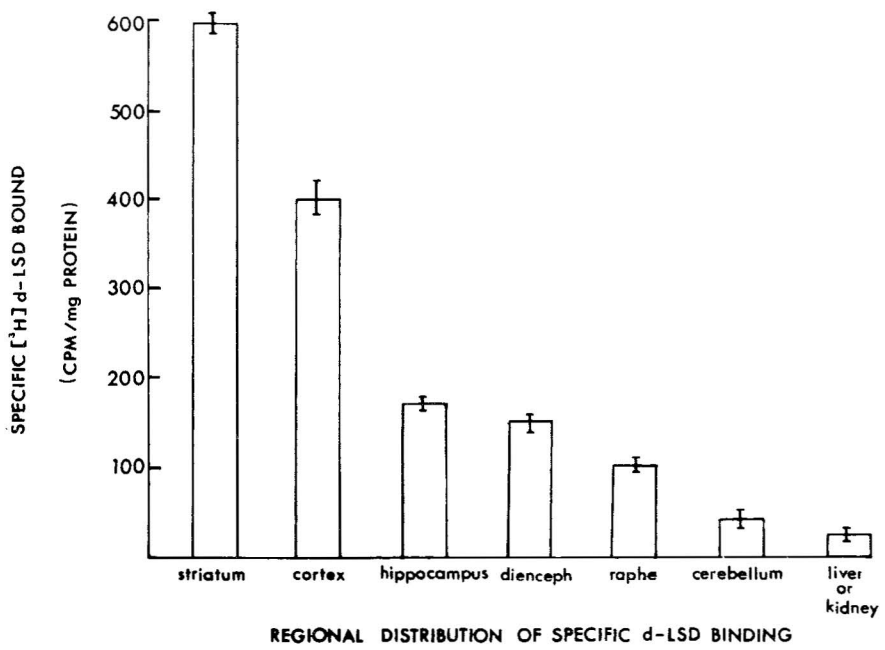

FIG. 2

The amount of ^{3}H-d-LSD bound, at a concentration of 4 nM, by homogenates from various brain regions and organs. Bracketed vertical lines represent standard errors of the means of five to ten experiments.

concentration corresponds to the in vitro concentration at which specific d-LSD binding sites are approaching saturation (Fig. 1).

d-LSD Binding in Brain Homogenates from Raphe Lesioned Rats.

To determine if high-affinity d-LSD binding is directly associated with the 5-HT input to the forebrain we destroyed this input by placing a lesion in the midbrain raphe nuclei. Such a lesion results in the degeneration of the serotonergic input to the forebrain as judged by measures in whole tissue and homogenates (12,24). We found no change in specific d-LSD binding in forebrain homogenates from rats with lesions in the raphe nuclei.

Effects of Drugs on d-LSD Binding.

Finally, we examined the ability of various drugs to decrease specific d-LSD binding (Table 1). Among the putative neurotransmitters tested, 5-HT is the most effective in reducing specific d-LSD binding. Methysergide, a compound which is structurally similar to LSD, and DMT a hallucinogen structurally related to 5-HT, are also effective in displacing d-LSD. All tricyclics tested are effective in reducing d-LSD binding. DOM is approximately 10 times more effective in reducing d-LSD binding than mescaline. L-LSD, the non-hallucinogenic LSD isomer, is approximately 10,000 times less effective than d-LSD. This correlates with the fact that l-LSD has no effect on the firing rate of serotonergic neurons (18).

DISCUSSION

Our results demonstrate the presence of "specific" high affinity d-LSD binding sites in crude homogenates and synaptosomal fractions of the brain. These "specific" high affinity d-LSD binding sites were distinguished from "non-specific" d-LSD binding sites by the fact that 5-HT could displace the bound d-LSD from the "specific" sites but not from the "non-specific" sites. "Specific" d-LSD binding was observed in crude homogenates as well as synaptosomal fractions of the subcortex and cortex. At low concentrations of d-LSD (4 nM) only 20 to 25% of the d-LSD molecules were associated with the "non-specific" binding sites in crude homogenates. This percentage drops to between 5 and 10% in synaptosomal fractions and in homogenates which contain a high concentration of "specific" d-LSD binding sites, e.g., the striatum. In addition "non-specific" binding did not saturate when placed in the presence of various concentrations (1 to 32 nM) of d-LSD. Thus, this "non-specific" component of the bound d-LSD has no apparent function, but it should be accounted for in d-LSD binding studies since at high d-LSD concentrations (32 nM) it represents a significant fraction of the total amount of bound d-LSD.

There is one major difference between our results and previously

TABLE 1

RELATIVE POTENCIES OF DRUGS IN REDUCING STEREOSPECIFIC [^{3}H]-d-LSD BINDING TO RAT BRAIN HOMOGENATES*

DRUG	ED_{50} (molar)	NO EFFECT AT .1 mM
d-LSD	6×10^{-9}	
Methysergide	1×10^{-7}	
Serotonin	2×10^{-7}	GABA
N, N-Dimethyltryptamine	3×10^{-7}	Norepinephrine
Promethazine and Chlorpromazine	1×10^{-7}	Glutamate Histamine
Chlorimipramine	5×10^{-7}	Carbachol
Imipramine	1×10^{-6}	Iproniazid
Desimipramine	3×10^{-6}	Pargyline
Cyproheptadine	4×10^{-6}	
dl-DOM (4-methyl-2,5-dimethoxyamphetamine)	5×10^{-6}	
Mescaline (3,4,5-trimethoxy-phenylethylamine)	4×10^{-5}	
Reserpine	4×10^{-5}	
l-LSD	7×10^{-5}	
Dopamine	3×10^{-4}	

*Three concentrations of each drug, in triplicate, were incubated for 15 minutes at 37° in the presence of (^{3}H)-d-LSD (4 nM). Percent inhibition of control specific (^{3}H)-d-LSD binding was computed and plotted as a function of drug concentration on log-probit paper.

published reports (1,2). We found specific high-affinity d-LSD binding sites in subcortical as well as cortical regions of the brain while Farrow and Van Vunakis observed binding only in cortical regions. The presence of specific d-LSD binding sites within the subcortex is significant since the physiological and biochemical activity of the serotonergic neurons in the subcortex

are known to be significantly altered by d-LSD (3,4,5,6,7,8). A possible reason as to why Farrow and Van Vunakis did not observe d-LSD binding in the subcortex could be to the different methods employed, i.e., equilibrium dialysis vs. rapid filtration. Since there are marked differences between the concentrations of d-LSD binding sites in various regions of the brain, it is unlikely that d-LSD is binding to elements that are randomly distributed throughout the brain such as glia or blood vessels.

Our results with drugs that alter d-LSD binding are, in many respects, similar to the results obtained by Farrow and Van Vunakis; e.g. we both observed the effectiveness of 5-HT (compared to other putative neurotransmitters) in displacing d-LSD from its high-affinity binding site. Drugs structurally related to LSD showed considerable variation in their potencies. Methysergide, a drug known to interact with peripheral (19) and central 5-HT receptors (20) is approximately 1000 times more potent than l-LSD. The ineffectiveness of l-LSD demonstrates that the binding site for LSD is highly stereospecific for the d-isomer. Marchbanks reported that the binding of ^{14}C-5-HT (1×10^{-6}M) to nerve ending particles was effectively inhibited by both d and l-LSD (4×10^{-7}M)(25). Thus, this 5-HT binding site is different from our d-LSD binding site since it showed no stereospecificity towards the two isomers of LSD. The phenothiazines were the most potent of the tricyclics tested. However, promethazine, which has no known antipsychotic activity, is as effective as chlorpromazine. The potency of the antidepressant tricyclics correlates with their potency to block uptake of 5-HT (21). However, these compounds have no known 5-HT receptor actions. The fact that DOM is 10 times more effective than mescaline in displacing d-LSD correlates with the fact that DOM is a more potent hallucinogen (22).

Since 5-HT is the most effective putative neurotransmitter in displacing specifically bound d-LSD it is possible that the macromolecule which binds this drug is a 5-HT receptor. Recent evidence indicates that there are least two 5-HT receptors, one located on raphe neurons (presynaptic receptor) the

other on neurons which receive a 5-HT input (postsynaptic receptor)(23). Since raphe lesions specifically destroy the serotonergic input to the forebrain (11), and since specific d-LSD binding is not altered in this region after the raphe had been lesioned, we conclude that if d-LSD is binding to a 5-HT receptor this receptor is predominately associated with the postsynaptic membrane. On the other hand, the presynaptic 5-HT receptor on raphe neurons are the most sensitive to d-LSD in terms of physiological response (23). It is therefore significant that the concentration of d-LSD in the midbrain, at a time when raphe cell firing is just at the point of total inhibition, is the same range as when d-LSD binding sites are approaching saturation.

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