

Tritiated LSD Binding in Frontal Cortex in Schizophrenia

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• It has been reported that the binding of tritiated LSD (at 2 or 4 nm) to frontal cortex is reduced in schizophrenia, a finding that has been interpreted as a reduction in the number of serotonin receptors. The present study, however, reveals in a Scatchard analysis of tritiated LSD binding in frontal cortex in the brains of 13 schizophrenic patients that there was no decrease in binding by comparison with eight control brains. Quantities of neuroleptic remaining in the brain after death cannot be readily washed out and could have led to the previous report of reduced LSD binding. A decrease in affinity of LSD binding sites consistent with this possibility has been demonstrated in chlorpromazine-treated rats. In the brains of five patients who had probably been neuroleptic-free for the year before death, tritiated LSD binding was significantly increased. This result needs to be replicated in larger samples.

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In animal studies, the binding characteristics of CNS receptors have been shown to adapt to drugs and other treatments.^{1,2} In Huntington's chorea, tritiated D-LSD binding has been reported to be reduced,³ and tritiated neuroleptic binding has been found to be increased in schizophrenia.^{4,5} This increase in tritiated neuroleptic binding is present in caudate nucleus, putamen, and nucleus accumbens septi⁶; but in some studies,⁶ its presence may have been obscured by neuroleptic drug concentrations remaining in the brain after death.⁷

Recently, a decrease in postsynaptic serotonin (5-HT) receptor binding in the frontal cortex of schizophrenics has been suggested on the basis of an observation that tritiated D-LSD binding is decreased.⁸ However, although LSD was introduced as a label for serotonergic sites,^{9,10} more recent work¹¹ has indicated that it also labels dopaminergic and, to a lesser extent, adrenergic and histaminergic sites. In addition to the nonspecificity of the label,

another problem is the presence in the tissue of residual neuroleptic, which interferes with ligand binding.⁷

In view of these difficulties, it seems that a decrease in serotonin receptors in the brain in schizophrenia has not been definitely established. In the present study, tritiated LSD binding in the frontal cortex in schizophrenia has been reinvestigated with saturation (Scatchard) analysis, which has the potential for revealing effects of residual drugs.⁷

METHODS

Brains from schizophrenic and control patients were obtained from a variety of hospitals and were matched for age, sex, and time to autopsy, as described in an earlier study (Table 1).⁵ Diagnosis of schizophrenia was established by application of the Present State Examination Syndrome Checklist¹² to the case notes. Patients were included only if there was evidence of the presence of nuclear (Schneiderian first-rank) symptoms and if the Feighner et al¹³ criteria for schizophrenia were also satisfied. The brains of schizophrenics were further divided into two groups; in one group the schizophrenics had received neuroleptic medication, and in the other they had received no medication for at least one year prior to death, according to all available medical and nursing notes.

Cortical samples (Brodmann's areas 4, 10, and 11) were taken from frozen whole brains, and a homogenate was prepared as described previously.⁵ The final protein concentrations ranged from 1.2 to 2.2 mg/mL.

Table 1.—Characteristics of Subjects and Cortical Samples Used in Tritiated D-LSD Binding Study*

Subjects	N	Sex	Age, yr	Mean ± SEM	
				Death to Morgue, hr	Death to PM,† hr
Controls	8	4 F, 4 M	70.5 ± 4.9	2.2 ± 0.36	44.9 ± 5.0
Schizophrenics					
Total	13	5 F, 8 M	69.1 ± 3.1	2.9 ± 0.46	39.8 ± 4.8
Medicated	8	3 F, 5 M	68.4 ± 2.8	2.9 ± 0.60	46.6 ± 8.2
Unmedicated	5	2 F, 3 M	70.0 ± 7.4	2.9 ± 1.20	22.4 ± 9.1

*None of the differences was significant.

†PM indicates postmortem examination.

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Binding assays were performed by incubating for a half hour (at 22 °C) the following aliquots: 0.2 mL of tritiated D-LSD (final concentration from 0.8nM to 10nM); 0.2 mL of buffer or buffer containing nonradioactive D-LSD (final concentration, 1,000nM); and 0.2 mL of brain homogenate.

After incubation, a 0.5-mL aliquot was filtered and washed with 5 mL of tris buffer (concentration, 50nM; pH, 7.4). Radioactivity on the filter was determined using a triton-toluene scintillant.

Specific binding was defined as the difference between the tritiated D-LSD bound in the presence of buffer alone and in the presence of buffer containing nonradioactive D-LSD. Specific binding was determined in triplicate at five different concentrations of tritiated D-LSD and was analyzed for maximum binding capacity (B_{max}) and the dissociation constant (K_D) by the technique described by Scatchard.¹⁴

Following this initial study, an animal study of the effects of residual neuroleptic on tritiated D-LSD binding appeared to be necessary. This was performed as follows. Six male rats were given 75 mg/kg/day of chlorpromazine hydrochloride in their drinking water for one week, while six control rats were given ordinary drinking water. The rats were decapitated, and crude homogenates were prepared from frontal cortex. The binding characteristics of tritiated LSD were determined by saturation (Scatchard) analysis using the technique described for human samples.

RESULTS

The number of tritiated D-LSD binding sites (assessed as B_{max}) was not decreased in the brains of schizophrenics by comparison with the brains of controls (Table 2). In the group of five unmedicated patients there was a significant increase by comparison with controls ($P < .01$) or medicated patients ($P < .02$). The mean K_D was found to be 3.45nM in the brains of schizophrenics and 4.21nM in the brains of controls.

In the rat study of residual neuroleptic effects on tritiated D-LSD binding, there was a significant increase ($P < .05$) in the K_D of binding in the treated rats by comparison with the controls (Table 3). The B_{max} values were not decreased.

COMMENT

Our findings conflict with those of an earlier report that tritiated LSD binding in frontal cortex is reduced in schizophrenia. Possible explanations for the discrepancy are as follows.

Areas of Cortex Sampled

Bennett et al⁸ examined samples of Brodmann's areas 6, 8 to 11, and 44 to 47, while we included samples from areas 4, 10, and 11. Thus, both studies included areas of orbital and lateral frontal cortex. Too little information on the distribution and characteristics of tritiated LSD binding in human brain is available to determine whether differences in the other areas sampled could account for the discrepancies in the two studies.

Differences in Collection and Storage of Brains

As can be seen from Table 1, the brains of schizophrenics and controls were closely matched for the times between death and arrival in the morgue and death to postmortem examination. In the previous study,⁸ there was a significantly ($P < .001$) longer time from death to postmortem examination in the brains of schizophrenics (34.9 hours) as compared with the brains of controls (13.0 hours), and the time from death to morgue was nonsignificantly longer for the schizophrenics (3.1 hours) compared with the controls (1.3 hours). Whether these differences are relevant to the discrepancies in the findings is not clear since no systematic data on the decay of tritiated LSD in human brain at room temperature or at 4 °C are available, although a slow decrease in high-affinity 5-HT binding at 22 °C has been described in the rat.¹⁵

Table 2.—Values for Tritiated D-LSD in Frontal Cortex Samples*

Subjects	N	Mean $\pm$ 1 SEM	
		B_{max}	K_D
Controls	8	368.3 $\pm$ 36.9	4.21 $\pm$ 0.79
Schizophrenics			
Total	13	478.5 $\pm$ 46.5	3.45 $\pm$ 0.43
Medicated	8	394.6 $\pm$ 39.4	2.87 $\pm$ 0.55
Unmedicated	5	600.0 $\pm$ 76.2†	4.39 $\pm$ 0.46

*The values for B_{max} (maximum binding capacity) are femtomoles per milligram of protein; the values for K_D (the dissociation constant) are nanomolar.

†The value for the unmedicated schizophrenics is significantly different from that of the controls ($P < .01$) and the medicated schizophrenics ($P < .02$). All other differences are nonsignificant.

Table 3.—Tritiated D-LSD Binding in Cortex of Chlorpromazine-treated and Control Rats*

	N	Mean $\pm$ 1 SEM	
		K_D	B_{max}
Control	6	5.33 $\pm$ 0.99	140.01 $\pm$ 32.04
Treated	6	8.52 $\pm$ 0.64	198.53 $\pm$ 32.61

*Chlorpromazine was given in drinking water for one week at a dose of 75 mg/kg/day. K_D and B_{max} and their values are as explained in Table 2. The K_D values for the groups were significantly different at $P < .05$. There was no significant difference between the B_{max} values.

Age Differences Between Subjects and Controls

In the present study there was no difference between the age of the brains of schizophrenics and the brains of controls. In the previous study,⁸ the brains of schizophrenics were significantly older in two of the three studies, and overall the schizophrenic patients were significantly ($P < .01$) older at death than the controls (schizophrenics' mean age, 58.7 $\pm$ 5.6 [SEM] years; controls' mean age, 42.6 $\pm$ 3.4 years). This may be relevant since a decrease in affinity of 5-HT binding has been reported with increasing age in human postmortem tissue.¹⁶ However, no data on the effect of age on LSD binding are available, and the previous authors⁸ provide reasons why they consider this age difference an unlikely cause of their results.

Effects of Drugs

Previous neuroleptic medication can affect ligand binding in the brain after death. This has already been demonstrated in the case of spiroperidol binding, where an increase in the K_D is observed in patients who have been treated with neuroleptics before death. This change has been replicated in animal experiments. It has the effect that at low ligand concentrations there is an apparent decrease of receptor numbers that is due to competitive inhibition by residual neuroleptics. However, no change is observed in B_{max} values obtained from a saturation analysis.

In the present experiments, an increase in the K_D for LSD was observed following chlorpromazine administration in experiments on rats. That residual neuroleptic could account for the previous observation of low tritiated LSD binding in the brain of schizophrenics is suggested by the facts that (1) in these authors' data there was a trend toward tritiated LSD binding values being lower in drug-treated than in non-drug-treated patients,⁸ (Table 5) and

(2) the differences observed between patients and controls were greater when lower concentrations of tritiated LSD were used (ie, 2nM in studies 2 and 3 in contrast to 4nM in study 1). The authors claim that the washing procedure they adopted would have eliminated the effects of residual neuroleptic drug, but this does not answer this point since it has been found that repeated washings do not readily eliminate the effects of residual neuroleptic drug.⁷

However, against the view that the previously reported reduction in tritiated LSD binding in the brain of schizophrenics is entirely attributable to effects of neuroleptic drugs are two findings. First, the authors reported a significant reduction in six patients who apparently had been drug-free before death (although premortem drug treatment is not always readily established). Second, in the present study, the K_D was not increased in patients who were taking neuroleptic drugs by comparison either with controls or with a group of five patients who apparently were drug-free for the year before death. Thus, the effects of neuroleptic drugs on tritiated LSD binding may not be as straightforward as was just suggested.

Whatever the explanation of the decrease in tritiated LSD binding observed by Bennett et al,⁸ this seems

unlikely to reflect a change in numbers of 5-HT receptors. In their own study, tritiated 5-HT binding was unchanged in frontal cortex in schizophrenia, and in a report on the brain samples included in the present study, both tritiated 5-HT and tritiated LSD binding were found unchanged in corpus striatum.¹⁷

In brains of patients who probably had not received neuroleptic medication for the year before death, a significant increase ($P < .01$) in cortical tritiated D-LSD binding was observed. However, in view of the small sample size ($N = 5$) and the fact that an increase in B_{max} was not found in medicated schizophrenics, this may be a chance finding. If replicable and related to the disease process, that increase in tritiated LSD binding in the brain of schizophrenics should also not necessarily be interpreted as an increase in numbers of 5-HT receptors. LSD also binds to dopaminergic and, to a lesser extent, α -adrenergic and histaminergic receptors in cortex. Further studies of these receptors in frontal cortex and other areas of the brain of schizophrenics are indicated.

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