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Endogenous Ligands of a Putative LSD-Serotonin Receptor in the Cerebrospinal Fluid: Higher Level of LSD-Displacing Factors (LDF) in Unmedicated Psychotic Patients

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Abstract. In human cerebrospinal fluid substances were detected that are capable of reversibly displacing the hallucinogen d-lysergic acid diethylamide (LSD) from its high-affinity binding sites in neuronal membranes. The binding sites may represent a specific type of LSD-serotonin receptor protein. The LSD-displacing factors (LDF) occur physiologically in concentrations high enough to interact with the putative LSD-serotonin receptors. The LDF can be separated from proteins and inorganic salts. LDF was found to be clearly different from endogenous indoleamine ligands such as serotonin, being anionic at pH 7.4.

The LDF concentration was assayed in the cerebrospinal fluid of 49 nonpsychotic and of 19 acute psychotic patients before 30 days therapy with the antipsychotic drugs haloperidol or clozapine. LDF concentration was found to be significantly higher ($P < 0.001$) in the group of unmedicated acute psychotic patients (5.55 U/ml) in comparison to the control group (3.56 U/ml). Within this group of acute psychotic patients, a high positive correlation was found between concentration of LDF and clinical improvement ($r = 0.650$). Thus, a nosological subgroup was traced out, characterized by both a higher concentration of LDF and a higher responsiveness to antipsychotic drugs ($P < 0.01$). Ratings of clinical improvement and determinations of the concentration of LDF before drug therapy were performed in a double-blind study. Since antipsychotic drugs act on dopamine receptors and LDF acts on putative serotonin receptors, dopamine and serotonin receptors may both be affected in the psychotic state. A working hypothesis is offered that links the dopamine and the serotonin hypotheses.

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The psychedelic model for biochemical abnormalities in schizophrenia is based on the hypothesis that the illness might be caused by an abnormal chemical metabolite functioning as an endogenous psychotogen. The search for psychedelic methylated metabolites of physiological compounds was stimulated by the transmethylation hypothesis of Osmond and Smythies (1952). Psychedelic substances such as N,N-dimethyltryptamine have, indeed, been detected in schizophrenic patients, but such endogenous hallucinogens occur in concentrations far too low to be effective as hallucinogens and are also detectable in nonschizophrenic patients (Carpenter et al., 1975; Gillin et al., 1976).

As a highly sensitive method for detecting endogenous psychedelic ligands, we have used an in vitro system that measures the binding of ^{3}H-LSD to putative hallucinogen receptor sites in neuronal membrane preparations. High-affinity LSD-serotonin binding sites have been studied extensively in rat brain (Farrow and van Vunakis, 1973; Bennett and Aghajanian, 1974; Bennett and Snyder, 1975) and have been highly purified from pig brain (Mehl and Weber, 1974). It may be expected that the interaction of such hallucinogens as LSD with these binding sites would be the first molecular event for eliciting hallucinations. On the basis of their pronounced pharmacological specificity and high affinity, the sites may be regarded as putative serotonin receptors. However, only some of them, which are characterised by a low affinity for the

nonhallucinogenic 2-bromo-d-LSD, may be considered to represent the actual serotonin-hallucinogen receptor (Mehl, 1975).

In this study we have found that the CSF contains physiological ligands which displace d-LSD from the high-affinity binding sites. The substances have been provisionally named 'LSD-displacing factors' (LDF) until they are chemically identified. We have studied whether the LDF concentration is normal in the CSF of neuroleptic resistant and neuroleptic responsive psychotic patients.

METHODS AND SUBJECTS

Radioligand Receptor Assay. A cerebral membrane fraction (for preparation see below) was incubated with ^{3}H-LSD for assay of LSD-binding to high-affinity sites and for assay of LSD-displacing factors in CSF. The incubation mixtures (final vol: 0.6 ml) contained 1.5 mg of cerebral membrane protein, phosphate-buffered saline (25 mM sodium phosphate, pH 7.4, and 50 mM NaCl) 2 nM ^{3}H-LSD [2-^{3}H (n)-LSD, 21 Ci/mmole, Amersham, Buchler] and 0.15 or 0.3 ml of CSF. The samples were incubated for 60 min at 25° with shaking in a dimly lit room. A 500 µl aliquot of each sample was filtered on Whatman glass fiber discs (GF 82, 26 mm diameter) which had been moistened with 1 ml of phosphate-buffered saline. The filter residue was rinsed with two 10-ml portions of phosphate-buffered saline at 20°. The entire operation of removal of free LSD took less than 20 s. The moist filters were vigorously shaken with 10 ml of Unisolve, a water-miscible scintillation fluid (Koch-Light), and radioactivity was measured in a liquid scintillation counter. Specific LSD-binding was calculated as the difference between total bound ^{3}H-LSD and ^{3}H-LSD bound in the presence of 1 µM unlabelled d-LSD. Nonspecifically bound ^{3}H-LSD amounted to 20–30% of the total bound LSD. A unit of LDF was defined as that amount inhibiting 50% of the specific LSD binding. Less than 300 µl of CSF were used if the inhibition exceeded 70%. The inhibition ratio (Co/Cx) of LSD binding was calculated from LSD binding in the absence (Co) and presence (Cx) of CSF. A calibration curve (inhibition ratio versus U/ml) was constructed for conversion of the inhibition ratio into units per ml. Samples were assayed in duplicate.

Preparation of Cerebral and Synaptosomal Membrane Fractions. Synaptosomal membranes were prepared from cerebral cortex of pig brain by zonal centrifugation (Cotman et al., 1968). For LDF assay a crude cerebral membrane fraction was prepared at 4° as follows: 100 g of cerebral cortex was homogenized in 900 ml of 0.3 M sucrose with a Potter-Elvehjem homogenizer and centrifuged at 11000 g for 30 min. The supernatant was collected and spun at 30000 g for 30 min. The resulting pellets were suspended in distilled water to give a protein concentration of 10 mg/ml. Aliquots were kept at −60° until assay and were used within 1 day after thawing.

Chromatography. The residue from 45 ml of freeze-dried cerebrospinal fluid was redissolved in 3 ml of distilled water and centrifuged at 10000 g for 10 min. The clear supernatant was separated on a 160-ml column of Sephadex G-10 (Pharmacia, Frankfurt) with 25 mM sodium phosphate, 50 mM NaCl, pH 7.4 (elution rate 10 ml/h). The resulting fractions (5 ml) were assayed for electrical conductivity. All fractions were adjusted to the same conductivity as the elution buffer by addition of distilled water. For LDF assay, 50 or 150 µl of the various fractions were used.

Clinical Data. Eighteen of the psychotic patients were inpatients of the Psychiatrische Klinik der Universität München. Fifty patients were inpatients of the Max-Planck-Institut für Psychiatrie. The patients of both institutions were fed on a diet of the composition recommended by the 'Deutsche Ernährungsgesellschaft.' The group of psychotic patients included 14 males and 5 females (mean age 34 ± 13 years; range 19–68 years). Fifteen patients were

Table 1
Data of psychotic patients

No.	Diagnosis	ICD No.	Sex	Age	Improvement[a]
Resistant psychotics:					
1	Schizophrenia simplex	295.0	M	19	1
2	Schizoaffective psychosis	295.7	M	24	1
3	Hebephrenia[b]	295.1	M	27	1
4	Schizoaffective psychosis	295.7	M	21	1
5	Paranoid schizophrenia	295.3	M	42	1
6	Schizoaffective psychosis	295.7	M	24	0
7	Paranoid invol. psychosis	297.1	F	68	0
8	Paranoid schizophrenia	295.3	M	41	1
9	Hebephrenia	295.1	M	21	1
Responsive psychotics:					
1	Paranoid invol. psychosis	297.1	F	51	3
2	Schizoaffective psychosis	295.7	F	26	3
3	Paranoid schizophrenia[c]	295.3	F	36	2
4	Paranoid psychosis	293.0	M	49	3
5	Paranoid schizophrenia	295.3	M	29	2
6	Paranoid schizophrenia[b]	295.3	M	26	2
7	Alcohol hallucinosis	291.2	F	37	3
8	Hebephrenia[b]	295.1	M	27	2
9	Schizophrenia simplex (?)	295.0	M	51	3
10	Schizophrenic episode	295.4	M	22	3

[a] Global rating of clinical improvement during 30 days medication with neuroleptics
[b] Neuroleptic medication in the anamnesis but no neuroleptics for over 1 year before admission
[c] Thioridazine medication was terminated 1 month before the admission

experiencing an acute psychosis for the first time and had apparently never received neuroleptic medication. None of the psychotic patients received psychoactive drugs for at least 1 month before study. The diagnoses (Table 1) were made independently by two experienced psychiatrists according to the ICD classification. On careful medical examination of the psychotic patients and on standard analysis of their body fluids (blood, urine, cerebrospinal fluid) no clinical symptoms of organic brain disease nor of other diseases could be detected except in the two cases of alcohol hallucinosis and arteriosclerosis.

As 'normal controls,' some CSF specimens were included from healthy subjects where no pathologic results had been obtained upon neurologic diagnostic procedures such as myelography and serologic tests for neurolues. In addition, 31 patients were included in a further control group designated 'other patients.' These patients were inpatients of the Max-Planck-Institut für Psychiatrie and had mainly neurologic diagnoses with the following frequencies: brain atrophy 6; lesion of an intervertebral disk 5; polyneuropathy 3; meningo-encephalitis 3; brain tumors (meningiom) 3; multiple sclerosis 2; spinal muscle atrophy 2; other neurologic diagnoses 4; and neuroses 3. The mean age of the 49 patients in the control groups was 45 ± 18 years (SD, range 16–72).

The lumbar puncture was carried out between 9 and 12 a.m. before the patients had received any neuroleptic medication. A sample (2 ml) of CSF was taken at the third intervertebral space for standard analysis and a further portion of 3–4 ml was sampled for LDF assay. The patients had been prepared for over 10 h before puncture by fasting and by the instruction to lie still in bed. The CSF samples were quickly frozen and stored at − 25° in tightly closed containers.

After the puncture, 15 of the psychotic patients were treated with haloperidol (8–14 mg/day) over a period of 30 days. One patient was treated after 3 days of haloperidol with electroconvulsive therapy and then with other neuroleptics. One patient was treated with clozapine after 10 days of haloperidol because of changed symptomatology. Two patients were treated only with clozapine (25–200 mg/day).

Psychopathology was rated by means of the AMP-system (Angst et al., 1969). Scores were calculated using the four symptom groups: thought disorders, delusions, hallucinations, and affective disorders before and after treatment for 1 month with neuroleptics (for exceptions see above). In addition, a global clinical impression rating scale was used. The scales were: 0 = no improvement, 1 = some improvement, 2 = marked improvement, 3 = full remission. A patient was rated as markedly improved when he could be discharged after 30 days and when his score for each of the four symptom groups reached less than half of that measured at the time of hospitalization. For statistical analysis of the LDF level of patients, the Wilcoxon (Mann-Whitney) test was used. When more than two groups were compared, the Kruskal-Wallis test was applied.

RESULTS

Endogenous Ligands of a Putative LSD-Serotonin Receptor Protein. Cerebral membranes purified from pig cerebral cortex contain high-affinity LSD-binding sites with an equilibrium dissociation constant of 2.4 ± 0.6 nM (Mehl, 1975). When such membranes were incubated with ^{3}H-LSD in the presence of cerebrospinal fluid, a decrease in the amount of ^{3}H-LSD bound to these high-affinity sites was observed. The binding of LSD to medium-affinity sites, however, was not influenced by CSF. These medium-affinity sites are unspecific, since they cannot discriminate stereospecifically between the pharmacologically active d-LSD and the inactive l-isomer (Bennett and Snyder, 1975). The unspecific binding at 1 µM LSD was 200 times the amount of LSD specifically taken up at 2 nM. Therefore, very low concentrations of LSD were used for the detection of LDF.

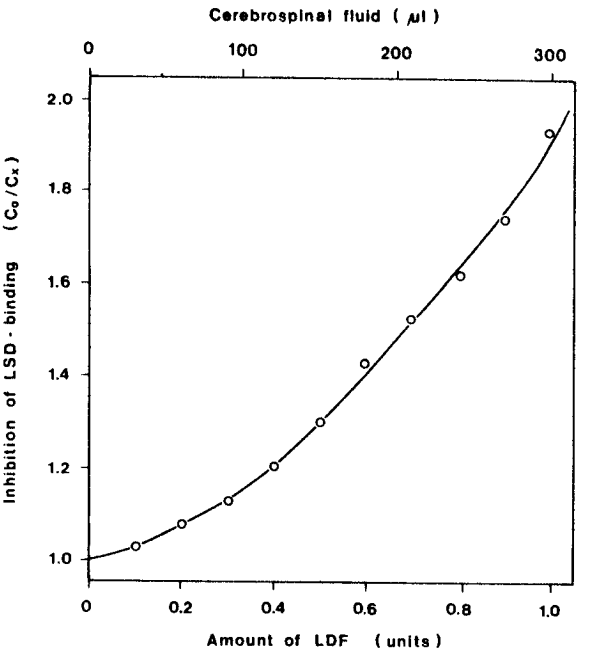

Fig. 1. Determination of LSD-displacing factors (LDF) in cerebrospinal fluid by a ^{3}H-LSD receptor assay. After incubation in absence (Co) of cerebrospinal fluid and in its presence (Cx) the radioactivity of ^{3}H-LSD bound to high-affinity sites of cerebral membranes was measured. The inhibition of LSD binding is expressed as the ratio Co/Cx. Incubation conditions are as described in the text, except that the volume of CSF was varied

The effect of adding different volumes of fresh cerebrospinal fluid to the ^{3}H-LSD receptor assay is shown in Figure 1. The inhibition by LDF was expressed as the ratio of specific LSD-binding in absence (Co) and presence (Cx) of LDF. In 300 µl of original cerebrospinal fluid, the amount of LDF was sufficient to displace 50% of the specifically-bound LSD (Co/Cx = 2) in a final assay volume of 600 µl. In other words, the LDF concentration in CSF is high enough to saturate more than 50% of the high-affinity LSD-serotonin binding proteins in the cerebral cortex. The relationship of LDF inhibition (Co/Cx) relative to LDF concentration (µl of CSF) is not linear, suggesting that the inhibition is noncompetitive. This result was confirmed with partially purified LDF and purified synaptosomal membranes (Fig. 2). The LDF was partially purified by gel filtration (Fig. 3). The preparation obtained was free from

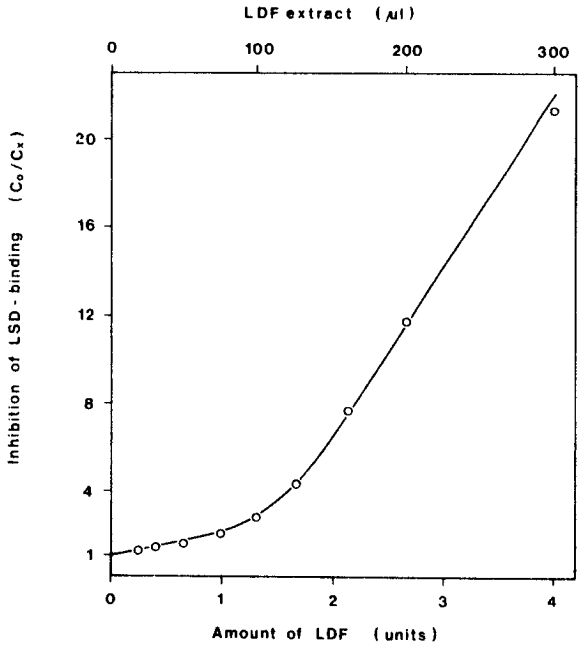

Fig. 2. Determination of the LSD-displacing capacity of a partially purified LDF-fraction in the ^{3}H-LSD receptor assay. The partially purified LDF-fraction was prepared by column chromatography on Sephadex G-10 (Fig. 3, fraction 18) and was incubated with purified synaptosomal membranes. For further conditions see Figure 1 and Methods and Subjects

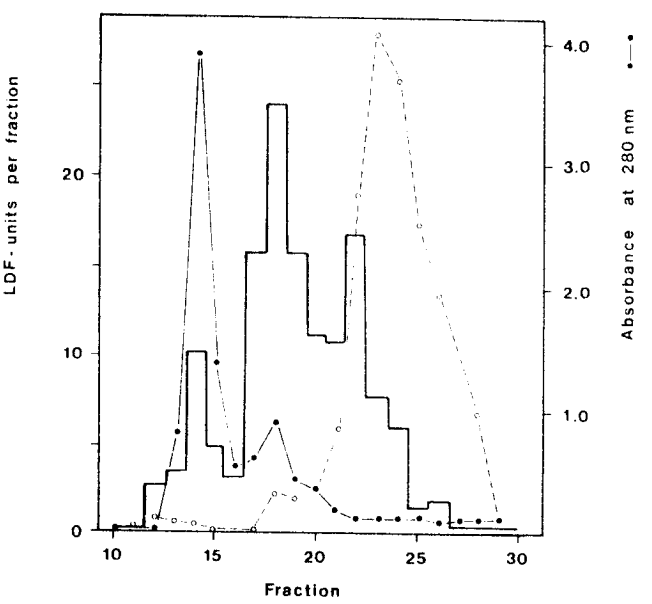

Fig. 3. Separation of cerebrospinal fluid into three LDF fractions by column chromatography on a molecular sieve (Sephadex G-10). ● ● Proteins and other substances, absorbance at 280 nm; LDF-containing fractions, main fraction approx. 500 daltons; ○ inorganic ions and serotonin, relative electrical conductivity

smaller molecules such as inorganic salts contained in the original CSF. By using the buffer-equilibrated LDF fraction, it was possible to maintain the pH and ionic strength at constant values for all samples in the ^{3}H-LSD receptor assay. Specific LSD-binding was inhibited beyond 95% (Co/Cx = 20) when 300 µl of such a partially purified, fourfold concentrated LDF-fraction was used (Fig. 2). The quantitative relationship of LDF-inhibition to LDF-units did not follow a straight line but showed a downward convexity, indicating noncompetitive inhibition type.

By column chromatography on a molecular sieve (Sephadex G-10), freeze-dried cerebrospinal fluid from nonpsychotic patients was separated into 3 LDF fractions (Fig. 3). The minor LDF fraction migrated with the protein peak (fraction 14). The main LDF fraction (fraction 18) penetrated into the gel matrix, which had a nominal molecular exclusion-limit of above 700 daltons. The main LDF fraction (approx. 500 daltons) was clearly separated from the final peak of maximal electrical conductivity (inorganic salts and other small charged molecules, fraction 23). When applied together with cerebrospinal fluid in a test run, radioactive serotonin was eluted with fraction 26. Thus LDF can be separated from proteins, salts, and amines such as serotonin. These results exclude the possibility that changes of pH, ionic strength, and free LSD by binding to CSF macromolecules were responsible for the inhibition of LSD binding.

The LDF in cerebrospinal fluid was tested for ionic groups and stability (Table 2). LDF was not adsorbed by a cation exchanger, in contrast to added serotonin. The LDF was, however, adsorbed to an anion exchanger at pH 7.4. The LDF is stable under conditions of enzyme inactivation such as heating or proteolytic degradation. The LDF inhibition of LSD binding was found to be partially reversible: LSD binding was restored when the LDF was removed by dialysis from neuronal membranes. Instead of a theoretical value of zero, which would be obtained if all LDF were dialysable, the original standard LSD binding capacity was only partially restored as indicated by a residual LDF potency of 0.56.

High LDF Level in Acute Psychotic Patients. The LDF units were assayed in lumbar CSF from 49 nonpsychotic patients and 19 acute psychotic patients (Table 1) who were in a florid psychotic state and had not taken neuroleptics for over a month before their admission. The LDF concentration in the group of psychotic patients was 5.55 ± 1.07 U/ml (mean and SD) and was significantly different ($P < 0.001$) from the LDF

Table 2. On the chemical identification of LDF from cerebrospinal fluid. Tests for ionic groups and chemical stability

Pretreatment of 2 ml samples of CSF before addition to the ^{3}H-LSD receptor assay	Relative LDF-potency of 300 µl samples of CSF	Properties of LDF at pH 7.4
1. LDF without treatment	1.00	–
2. Batch exposure to 1 g of cation exchanger[a] (30 min at 25 °)	1.00	not a cation not an amine
3. Batch exposure to 1 g of anion exchanger[b] (30 min at 25 °)	0.29	anionic group
4. Heat exposure (20 min at 95 °)	1.08 ± 0.06	heat-stable
5. Proteolysis (2 mg of Pronase, 4 h at 37 °, then heating 20 min at 95 °)	1.03	not digestible
6. Dialysis of LDF-inhibited membrane sample (dialysis against 100 vol PBS, 15 h, 2°)	0.56	inhibition is reversible

[a] AG 50 W – X 2, sodium form, 200–325 mesh (Bio-Rad, Lab.)
[b] AG 1 – X 2, acetate form, 200–400 mesh
Assays were done at least in triplicate

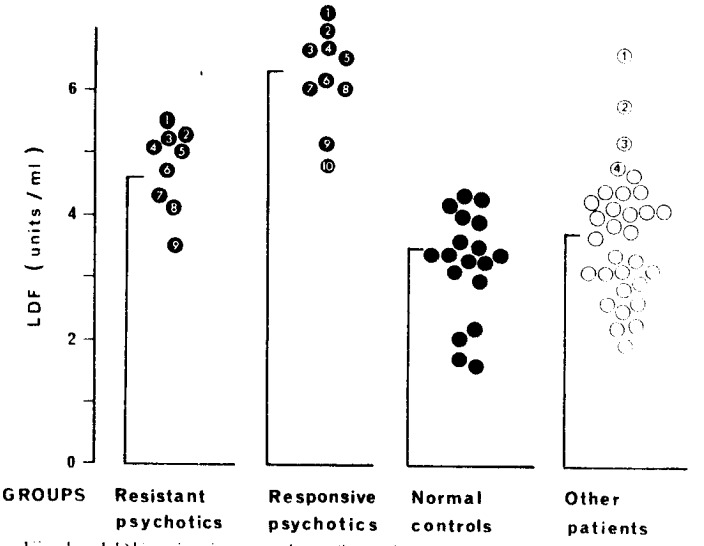

Fig. 4. LDF-units in samples of cerebrospinal fluid in several groups of psychotic and nonpsychotic patients. On the basis of the response to 30 days treatment with neuroleptics, the psychotic patients were divided into a subgroup 'resistant psychotic' ($N = 9$) showing only some response and a subgroup 'responsive psychotic' ($N = 10$) with marked improvement on neuroleptics. 'Normal controls' were patients ($N = 18$) with no psychosis in their history nor any manifest neurological or clinical symptom. The group of 'other patients' included 31 patients with mainly neurological diagnoses. The numbers 1–10 refer to individual patients in the resistant and the responsive groups (see Table 1). The numbers 1–4 in the group 'other patients' refer to three patients with severe lesion of intervertebral disk and one patient (4) with acute encephalomyelitis

level of 3.56 ± 1.01 U/ml in the nonpsychotic group. All the psychotic patients except one were in the same hospital living under similar conditions of social regime and food quality. The patients were rated for improvement by two experienced raters during 30 days of medication with the neuroleptics. A high positive correlation was found ($r = 0.650$) between improvement on neuroleptic medication and the LDF concentration in the psychotic state before starting the medication. Thus, a nosological subgroup was defined characterized by both a higher LDF level in the predrug period and responsiveness to the above-mentioned neuroleptic medication.

On the basis of their improvement during neuroleptic medication, the group of 19 psychotic patients was divided into two subgroups, the neuroleptic resistant psychotics (some or no improvement) and the neuroleptic responsive psychotics (marked improvement or full remission). The responsive group (Fig. 4) had 6.34 ± 0.94 LDF U/ml and was significantly ($P < 0.01$) different from the resistant group (4.74 ± 0.66 LDF U/ml). Both groups achieved similar high scores, when the psychopathological symptoms were rated (thought disorder, delusion, hallucination, affective disturbance, and motor activity). Since in both groups the psychopathological spectrum was similar before therapy, it is unlikely that the higher LDF level in the responsive group was merely due to unspecific excitement by the psychotic state itself. As an apparent exception, patients 9 and 10 of the responsive group showed a marked improvement under neuroleptic therapy in spite of their rather low LDF values. As can be seen (Table 1), the diagnosis was questionable or was 'schizophrenic episode.' Any psychiatric misdiagnosis would of course contaminate the homogeneity of the responsive group and a spontaneous remission while under neuroleptic could then be misinterpreted as a therapeutic effect. Within the group of nonpsychotic controls ($N = 49$), no correlation between age and LDF-content was detectable.

Males ($N = 27$) had 3.93 ± 1.04 LDF U/ml and females ($N = 22$) had only 3.09 ± 0.79 U/ml. The sex difference of LDF concentration was statistically significant ($P < 0.01$). When the LDF units for females ($N = 4$) or for males ($N = 6$) in the responsive psychotic groups were compared to LDF values of the corresponding nonpsychotic sex group, the increase

of LDF in the responsive group of both males and females was statistically significant ($P < 0.001$). Even in the nonpsychotic group, some patients (patients 1–4) had a pathologically high LDF concentration. They were suffering from severe inflammatory processes such as encephalomyelitis or from spinal compression with partial or complete blockade of spinal fluid circulation. Therefore, the LDF-assay of psychotic states is only possible when the cerebrospinal fluid is normal in terms of the content of proteins and cellular elements. The control groups included mostly patients with neurological disorders. In addition to our finding of normal LDF in three neurotic patients, a psychiatric control group (neuroses, affective disorders) would be needed for testing further the diagnostic specificity of higher LDF levels in psychiatric disorders.

DISCUSSION

Endogenous LDF was detected in CSF in concentrations that would be high enough to influence the affinity of the putative LSD-serotonin receptors in vivo. At least under in vitro conditions, more than 50% of the high-affinity LSD-serotonin binding sites were occupied by LDF. Preliminary tests have shown that the cerebrospinal tissue is probably the original source of cerebrospinal LDF. The reversibility of LDF binding, the heat stability and the low molecular weight of LDF preclude the possibility that LDF would be an artifact caused by enzymatic activities destroying LSD or the receptor. In addition, the concentrations of serotonin and 5-hydroxyindoleacetic acid in CSF are far too low to disturb the LDF assay: in normal CSF the concentrations of serotonin and 5-hydroxyindoleacetic acid are 1 ng/ml (Singh et al., 1965) and 30 ng/ml (Post et al. 1975) respectively. Even when added at concentrations ten times higher these substances had no effect on the LDF assay. LDF might represent just one chemical substrate or a group of substances in the CSF. It is therefore an open question as to whether the increase of LDF in neuroleptic responsive psychotic patients would be only a quantitative change or a qualitative one, due to a hypothetical pathological LDF-type. At the present stage we assume that the higher LDF levels might represent a change of serotonergic activity, but not the core defect of acute schizophrenia.

The pharmacological effects of LDF cannot be predicted from the outcome of binding assays. The action of LSD itself is very complex in the CNS: LSD mimicks serotonin in its stimulatory (Andén et al., 1968) or inhibitory (Tebēcis and Di Maria, 1972) antagonist (Boakes et al., 1970). Further information concerning the pharmacology of LDF must await its purification.

Conclusions as to the nosological specificity of the symptom of higher LDF level are not possible at the present stage. Since the LDF increase was found even in a patient with alcohol hallucinosis the biochemical symptom may not be strictly specific for acute schizophrenia. Therefore, studies are now in progress with a psychiatric control group in addition to our neurological group.

Many investigations have shown that neuroleptic responsive patients experienced most of the clinical improvement within a month of neuroleptic medication (N.I.M.H. Collaborative Study Group, 1964; Klein and Davis, 1969). However, we cannot exclude that patients in the 'nonresponsive' group of our study might have shown further improvement if the treatment were prolonged beyond 30 days.

Antipsychotic drugs such as haloperidol can half-maximally displace LSD from high-affinity binding sites at very low concentrations such as 1.0 μM (Bennett and Snyder, 1975). Therefore, only those patients who had not taken neuroleptics over the month before admission, by the criteria of anamnesis, were included in our study. Most of the selected patients were experiencing their first psychosis. The florid psychotic state of the responsive group is the best evidence that, except in one case, their body fluids did not contain neuroleptics in therapeutically effective concentrations. The incidence of LDF increase in eight out of 19 psychotic patients might not reflect the actual frequency distribution of the symptom in acute psychoses. The overexcited and the noncooperative patients were excluded from the study.

Since longitudinal studies with CSF were not performed, it remains to be established if the LDF concentration was high only during psychosis, returning to normal in the remission period. Otherwise, high LDF concentrations could reflect merely characterological traits such as the schizoid-amotivational one. For such longitudinal studies, the assay of the anionic LDF in blood and urine samples must be developed. Separation of several nonspecific LDF factors must be achieved before the LDF assay can be applied to blood and urine specimens for psychiatric research.

In CSF from acute schizophrenic patients, the levels of the serotonin metabolite 5-hydroxyindoleacetic acid have been reported to be normal (see Post et al., 1975) or lowered (Ashcroft et al., 1966). The levels were negatively and significantly correlated with good prognosis type (Bowers, 1975) or with the severity of hallucinations (Post et al., 1975). In agreement with this indication of the possibility of lowered serotonergic activity, some clinical improvement of chronic

schizophrenic patients was achieved by medication with L-tryptophan (Pollin et al., 1961) or 5-hydroxytryptophan (Wyatt et al., 1972). Medication with L-tryptophan was also suitable in the treatment of acute psychoses that occured during L-Dopa therapy of Parkinsonian patients (Birkmayer et al., 1974). When autoptic brain samples from control groups, nonpsychotic Parkinsonian patients, and patients with L-Dopa psychosis were compared, it was established that in the latter group the serotonin content of the nucleus ruber was twice as high as in the control groups (Birkmayer et al., 1974). On the basis of these results, it has been hypothesized that psychotic symptoms might be triggered by the loss of balance between the dopaminergic and the serotonergic system. Further, when the hallucinogenic drug harmaline was given to rats, a disturbance of the cerebral serotonin-dopamine balance was observed (Hassler and Bak, 1969). The mutual interdependence of the two systems has also been studied morphologically. Serotonergic fibres arising from the raphe nuclei make synaptic contacts with dopaminergic cells in the substantia nigra (Parizek et al., 1971).

There is now considerable evidence that neuroleptic drugs may act via blockade of dopamine receptors (Rossum, 1966; Seeman et al., 1975; Creese et al., 1975). However, it may not necessarily be concluded that there is any molecular defect in these receptors in schizophrenia. In addition to the influence on the dopaminergic activity, neuroleptics lead to changes in the serotonin concentration of rat brain (Gey and Pletscher, 1961) and cause a transitory increase of 5-hydroxyindoleacetic acid in the CSF of acute psychotic patients during treatment (Bowers, 1973; Ackenheil et al., 1974; Rüther et al., 1976). The cataleptogenic effect of neuroleptics apparently depends on both dopaminergic and serotonergic activity since block of the serotonin synthesis or destruction of raphe nuclei abolished the effect (Kostowski et al., 1972). Similarly, primarily serotonergic activities such as tryptophan-induced locomotor hyperactivity (Green and Grahame-Smith, 1976) or LSD-elicited behavior in rats (Horita and Hamilton, 1973) need the dopamine system for behavioral manifestation.

Since LDF and neuroleptics bind to putative serotonin and dopamine receptors, the high level of LDF in the neuroleptic responsive group can be regarded as compatible with the hypothesis that the serotonin-dopamine balance may be affected in acute psychosis.

A nosological heterogeneity of schizophrenia had been indicated by familial studies (Kant, 1942; Vaillant, 1962; Stephens and Astrup, 1963; Robins and Guze, 1970) and by different responsiveness to neuroleptic treatment. A substantial group of schizophrenic patients, especially the chronic schizophrenics (Gerlach et al., 1974) responded minimally or not at all (N.I.M.H. Collaborative Study Group, 1964; Davis, 1975, 1976). This subgroup of 'poor prognosis' type could be identified to some limited extent by prognostic psychopathological tests (McCabe et al., 1972; Taylor and Abrams, 1975) and corresponds largely to the dementia praecox described by Emil Kraepelin (1896). The concept of a nosological heterogeneity in schizophrenia is further supported by our finding of two subgroups differing biochemically and pharmacologically in LDF-level and in responsiveness to neuroleptic treatment.

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