Life Sciences, Vol. 23, pp. 593-598

# HETEROGENEITY OF LSD-DISPLACING FACTORS AND MULTIPLE TYPES OF HIGH AFFINITY LSD-BINDING SITES

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## Summary

Heterogeneity of high affinity LSD-binding sites was confirmed by displacement studies with 2-bromo(+)-LSD and with apamin, a peptide neurotoxin. In line with the concept of multiple binding sites, a number of fractions of putative endogenous ligands could be separated from rat brain extract. The LSD-displacing ß-fraction was not detectable in tissues lacking high affinity LSD-binding sites. High affinity dopamine- and serotonin-binding was differentially affected by the ß-fraction.

In the peripheral nervous system, the antagonistic action of LSD at some serotonin receptors has been shown, but the central receptors involved in the hallucinogenic effect are not definitely known. Experimental results point to the possibility that indoleamines other than serotonin, such as 5-methoxytryptamine (1) or tryptamine (2) represent the functional ligand at the hallucination-eliciting LSD receptor. Some direct (3) or indirect (4) effects were also indicated at dopamine receptors. Results obtained with binding assays between [$^3$H]LSD and brain fractions did not allow conclusive identification of the LSD receptor since a heterogeneity of the high affinity binding sites was observed both with intact neuronal membranes (5) and with solubilized purified LSD-binding proteins after affinity chromatography (6).

In human cerebrospinal fluid, unidentified substances were detected that are capable of reversibly displacing LSD from its high affinity binding sites (7). The substances were different from serotonin, tryptamine and 5-methoxytryptamine and were provisionally named LSD-displacing factors. Higher levels of LSD-displacing factors were found in a subgroup of acutely psychotic patients who showed a higher responsiveness to neuroleptic therapy.

In this article we report studies on the specificity of association of LSD-displacing factors with specific organs from the rat. In particular we have attempted to elucidate which factor might be present in the cerebrum but not in the cerebellum where only one type of high affinity binding site is present (Kaiser and Mehl, submitted for publication).

## Materials and Methods

Preparation of tissue extracts and column chromatography. After decapitation, organs from adult Sprague-Dawley rats were washed with 0.25 M sucrose solution, and were extracted at 4°C by homogenizing 20 g of tissue with 180 ml of dist. water. After centrifuging at 150,000 x g for 90 min, the supernatant was heated at 95°C for 30 min. After removal of the protein precipitate by centrifugation, the supernatants were concentrated to 8 ml under reduced pressure, adjusted to pH 7.0 with 1 M $NH_4OH$, and clarified by low-speed centrifugation.

The extracts were fractionated on 120-ml columns of Sephadex G-10 (Pharmacia, Frankfurt) using 0.1 M ammonium acetate, pH 7.0, containing 0.002% $NaN_3$. The columns were eluted at a rate of 8 ml/hour at 20°C. Fractions 1-60 consisted of 2 ml each, and fractions 61-95 of 12 ml each. For assay of LSD-displacing capacity, 300 µl of the eluted fractions were tested in the LSD-binding assay and were compared to standard binding with 300 µl of ammonium acetate buffer. For the extraction of LSD-displacing factors from beef brain, the centrifugation was replaced by a filtration step after adjusting the homogenate to pH 4.5 with 1 M acetic acid.

Assay of binding sites. A microsomal $P_3$-fraction was prepared from porcine cerebral cortex (7) and was incubated with 2 nM radioactive ligand for 60 min at 25°C, using 1.5 mg of protein and 25 mM potassium phosphate buffer, pH 7.0, in a total volume of 600 µl. Free and bound ligand were separated by filtration and by washing with ice-cold buffer as described (7). The following labelled drugs were obtained from Amersham Buchler, Braunschweig: 2-[$^3$H(n)]LSD, 21 Ci/mmol; 5-hydroxy[G-$^3$H]tryptamine creatinine sulfate, 10.7 Ci/mmol; [ethylamine-1,2-$^3$H]dopamine hydrochloride, 10 Ci/mmol. [$^3$H]Spiroperidol (9 Ci/mmol) was a gift of Janssen Pharmaceutica, Beerse. For binding studies with apamin (Serva, Heidelberg), the $P_3$ fraction was prewashed by centrifugation with dist. water and with 50 mM potassium phosphate, pH 7.0 (2 ml per g of brain), and was dialyzed twice against 400 ml of phosphate buffer in the cold before assay.

## Results and Discussion

In binding studies, a putative hallucinogenic LSD receptor should be characterized by different affinities for LSD and for 2-bromo-LSD, since the latter is devoid of hallucinogenic activity. In binding assays with both substances, different binding curves were obtained (Fig. 1) indicating at least two types of high affinity LSD-binding sites in synaptosomal membranes. One type was characterized by a medium affinity ($IC_{50}$ = 200 nM) for 2-bromo-LSD. The heterogeneity of binding sites became obvious when a neurotoxin from bee venom was tested. Apamin is a centrally active peptide (8) eliciting hyperthermia and hyperreflexia as also observed after LSD. We found that only a small subpopulation (less than 10%) of the high affinity LSD-binding sites was blocked by apamin with an $IC_{50}$-value of 1 nM (Fig. 1), whereas the bulk of high affinity sites was blocked with an $IC_{50}$-value of 100 µM. Medium affinity LSD binding was not affected. In agreement with the results above, a significant inhibition of labelling of up to 10% of the high affinity [$^3$H]LSD-binding sites was found when apamin

(3 µmol/kg) was given to mice subcutaneously, 3 hours before they were killed (data not shown).

The heterogeneity of high affinity binding sites was also reflected by a heterogeneity of LSD-displacing factors in tissue extracts. After heat-denaturation, the tissue extracts were fractionated by gel chromatography. The eluted fractions were assayed for their inhibitory capacity by testing the interference with binding of [$^3$H]LSD to a microsomal fraction from porcine cerebral cortex. Typical chromatographic separations are shown in Fig. 2. In addition to the LSD-displacing macromolecular fraction E in the void volume (fraction 25), whole cerebrum extract contained the low-molecular weight α-fraction and the ß-fraction (fraction 32-38). Inorganic salts were eluted with fraction 40-45. Authentic [$^3$H]dopamine was co-eluted with the δ-fraction of LSD-displacing factors (fraction 61). Authentic [$^3$H]serotonin and [$^{14}$C]tryptamine were co-eluted with the γ-fraction (fraction 78-90). In extracts of the large intestine, all LSD-displacing fractions isographic with the cerebral fractions were detectable except the δ-fraction which was also not found in extracts from autoptic human brain (9). In contrast, the LSD-displacing capacity of spleen and cerebellum extract was isographic only with the E and α-fraction. This indication of a tissue-specific distribution of LSD-displacing ß- and δ-fraction in brain but not in cerebellum is interesting in the light of the result that one type of high affinity binding site of rat brain ($K_D$ = 1.6 nM, 14 nmol/kg brain) was not detectable

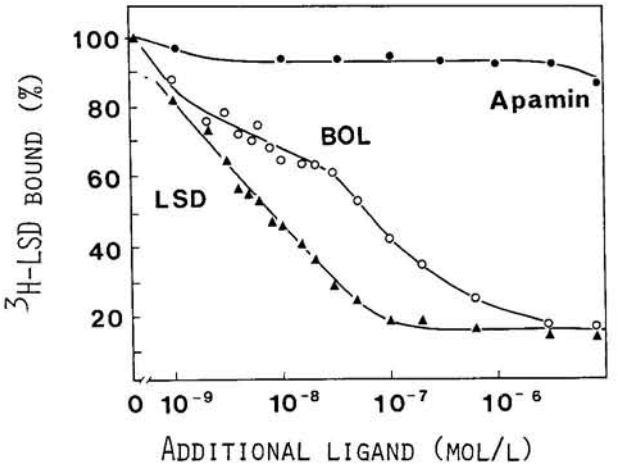


FIG. 1

Heterogeneity of high affinity [$^3$H]LSD binding sites: Differential or partial blockade by apamin and by 2-bromo (+)-LSD. Apamin was studied under standard conditions. Unlabelled (+)-LSD and its 2-bromo derivative were preincubated with a preparation (7) of synaptosomal plasma membrane for 30 min, before the final addition of 2 nM[$^3$H]LSD. All assays were performed in triplicate.

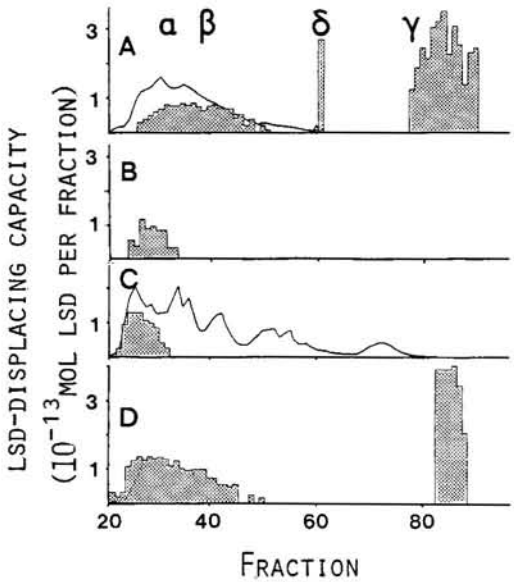


FIG. 2

Chromatographic separation of LSD-displacing factors in crude extracts from brain (A), cerebellum (B), spleen (C) and large intestine (D) of the rat. The LSD-displacing capacity was assayed with 300-µl samples of the eluted fractions, and was calculated as the difference between the cpm of [$^3$H]LSD bound in the absence and presence of the eluted fractions. Curve = absorbance at 280 nm.

TABLE I

Effects of the crude LSD-displacing ß-Fraction from Beef Brain on High- and Medium-affinity binding of [$^3$H]Dopamine, Serotonin, and Spiroperidol

| [$^3$H]Ligand added (2nM) | [$^3$H]Ligands bound to $P_3$-fraction (cpm/mg of protein) | |
|---|---|---|
| | Standard binding | Addition of ß-fraction |
| [$^3$H]Dopamine | 830 ± 66 | *540 ± 46 |
| +1µM dopamine | 560 ± 55 | *375 ± 36 |
| [$^3$H]Serotonin | 985 ± 95 | 930 ± 81 |
| +1µM serotonin | 247 ± 17 | *194 ± 9 |
| [$^3$H]Spiroperidol | 675 ± 67 | 685 ± 32 |
| +1µM(+)-butaclamol | 215 ± 26 | 225 ± 30 |

All samples contained 10 µM pargyline and 0.01% ascorbic acid
Mean ± S.E. from 9 or 10 assays
*Different from standard binding at P < .001 (Student t-test)

in the cerebellum but was replaced by another type ($K_D$ = 21 nM, 20 nmol/kg tissue). In addition, since a high affinity binding site ($K_D$ = 2.2 nM, 3.4 nmol/kg) was also detectable in the longitudinal muscle of the guinea pig ileum, but not in the spleen, the ß-fraction is a candidate for the role of an endogenous ligand of the high affinity binding site.

As a control for specificity, the crude ß-fraction from beef brain was tested further with other high affinity binding systems using labelled serotonin, dopamine and spiroperidol at 2 nM and 1 µM (medium affinity sites). High affinity binding of spiroperidol (10) and serotonin (11) was not affected by the ß-fraction (Table I). A statistically significant inhibition was found in the case of medium affinity serotonin binding. High and medium affinity dopamine-binding (12) were both inhibited. At present we are trying to separate LSD-displacing and dopamine-displacing factors in the ß-fraction. Further studies are necessary to test whether LSD-displacing factors do indeed resemble a functional ligand of the LSD receptors, as was established for the endogenous opioid peptides in relation to morphine receptors (13).

## Acknowledgements

The authors wish to express their gratitude to Mrs. Jutta Redemann and Mrs. Lisa Guiard for excellent technical assistance. We thank Dr. M. Reddington for correction of the English text and are indebted to the Deutsche Forschungsgemeinschaft for support. - Parts of this work were prepared by B. S. for a doctoral thesis.

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