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PHARMACOLOGICAL CHARACTERIZATION OF ^{3}H-LSD BINDING SITES IN MOUSE BRAIN IN VIVO

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Summary

In mice receiving i.v. low doses of ^{3}H-LSD the accumulation of radioactivity in brain appears to reflect a selective binding to high affinity sites as indicated by the heterogenous regional distribution (paralleling that observed in in vitro binding studies) and by the saturable character of the process (ED_{50} around 30µg.kg^{-1} in cerebral cortex).
The identity of the binding sites was assessed in various regions by administration of agonists or antagonists of different neurotransmitters. In cortex specific accumulation of ^{3}H-LSD was easily prevented by administration of serotonin antagonists (cyproheptadine, methysergide, methiothepin) or by tryptamine derivatives (psilocin, psilocybin, dimethyltryptamine) and 5-hydroxytryptophan + pargyline. Among neuroleptics some prevented ^{3}H-LSD binding (spiperone, haloperidol) whereas 1mg.kg^{-1} pimozide was ineffective. In addition a large variety of agents (adrenergic, cholinergic, morphine) were ineffective. These data suggest a selective binding to cortical serotonin receptors.

Since the discovery that LSD decreases brain serotonin (5-HT) metabolism (1) and produces a profound depressant action on 5-HT containing midbrain raphe neurons (2,3), most authors speculate that the prominent mechanism of action of LSD involves 5-HT receptors. More recently, direct ^{3}H-LSD binding studies with membrane preparations from rat and bovine brain (4-7) have also provided a strong support for this view.

Besides the information obtained from in vitro binding studies, the development of methods to study cerebral receptors in the living animal potentially presents several advantages i) it avoids possible artifacts resulting from the preparation of membranes and hence helps to confirm in vitro findings and possibly to reveal changes in receptor properties lost during in vitro manipulation, ii) the potency of various ligands assessed in vivo reflects not only their affinity but also their biodisposability and is therefore a better reflection of their activity, iii) such studies are preliminary to the autoradiographic localization of receptors. While ^{3}H-ligands suitable for in vivo studies have been described for catecholamine (8-10) and opiate (11,12) receptors the work of Bennett and Aghajanian (16), reporting that ^{3}H-LSD distributed heterogenously in rat brain, suggested that this agent could be used to label 5-HT receptors in vivo. We have therefore determined the best experimental conditions to reveal a "specific", i.e. saturable binding of ^{3}H-LSD in the brain of the living mouse and have characterized the binding sites pharmacologically

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by administration of various classes of psychotropic agents. No attempt was made in this study to eliminate the radioactivity due to non-specific binding by preparing membrane fractions, in order to avoid redistribution or other artifacts.

Materials and Methods

Male swiss albino mice (18-20g) (Lessieux, France) were housed in a well ventilated room at 24°C and under artificial illumination. They were injected with ^{3}H-LSD (50µCi.kg^{-1}, 1µg.kg^{-1}) in the tail vein and were killed at various time-intervals thereafter. Various brain areas were dissected and homogenized into 0.05M Tris-HCl buffer, pH 7.4. The radioactivity was determined in aliquots by liquid scintillation spectrometry in P.P.O-P.O.P.O.P-Toluene-Triton X 100 (16.5g-0.45g-21-11) at a counting efficiency of 32%. No attempt was made to identify the ^{3}H-products present in brain, but the work of Bennett and Aghajanian (16) suggests that the radioactivity consists entirely of unchanged ^{3}H-LSD. The tissue weight was estimated by measuring the protein content using the method of Lowry et al. (13). Results were expressed as fmoles. mg^{-1} of protein, taking into account the specific activity of ^{3}H-LSD. Concentration-response curves were fitted graphically or by employing the computer method of Parker and Waud (14).

^{3}H-d-LSD was purchased from the Radiochemical Centre, Amersham (16.3Ci. mmole^{-1}). Psilocybin, Psilocin, d-LSD, Apomorphine, Methysergide were obtained from Sandoz. Dimethyltryptamine, Bufotenine were obtained from Sigma. Tryptophan, Histidine were obtained from Merck. Other drugs were supplied by Lebrun (Haloperidol, Pimozide), Houdé (Atropine, Scopolamine), Geigy (Chlorimipramine, Desimipramine), Glaxo (Salbutamol). Cyproheptadine, Cinanserin and Mianserin were generously provided by Dr. M. Hamon (Laboratoire de Neurobiologie, Collège de France, Paris).

Results

Time-course of ^{3}H-LSD accumulation in different areas of mouse brain :

Two minutes after administration of a low dose (1µg.kg^{-1}) of ^{3}H-LSD substantial levels of radioactivity can be detected in brain. The maximal levels are found between 15-60 minutes with relatively large regional differences : the highest accumulation occurs in cortex and the lowest in cerebellum. After 1h while the level in cerebellum remains almost constant, that in other regions slowly declines and tends to reach the cerebellar level after 4h (Fig. 1).

When a relatively high dose of non-radioactive d-LSD (0.5mg.kg^{-1}) is administered together with ^{3}H-LSD (1µg.kg^{-1}) the radioactivity accumulated into cerebellum 1h later is not significantly affected whereas in other brain areas, it is reduced to the cerebellar level (Fig. 2A) ; in contrast after 4h the levels of radioactivity are much less affected by the administration of non-radioactive LSD (Fig. 2B). Hence the cerebellar level in each animal seems to provide an estimation of a non-saturable component of ^{3}H-LSD accumulation in brain regions and, therefore, "specific" accumulation of ^{3}H-LSD has been estimated by substracting this value from the total radioactivity. Defined in this way specific accumulation at 60 min represents 46% of the total in cortex, 33% in striatum and less than 25% in other brain regions.

Pharmacological characterization of specific ^{3}H-LSD accumulation in mouse cortex :

Because of the relatively high percentage of specific accumulation in cortex, this region was selected for the in vivo pharmacological characterization of LSD binding sites.

The in vivo saturation kinetics were first determined : half-maximal specific accumulation occurs at a dose as low as 30 $\pm$ 5µg.kg^{-1} whereas maximal specific accumulation is achieved for about 100µg.kg^{-1} and corresponds to a ma-

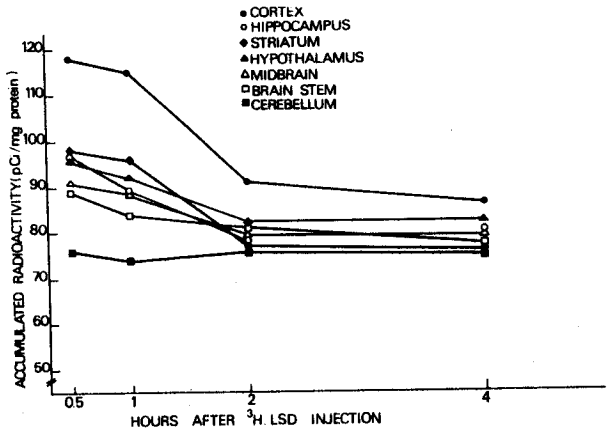

FIG. 1

Regional Accumulation of Radioactivity in Various Regions of Mouse Brain as a Function of Time After Administration of ^{3}H-LSD

Groups of 10-20 mice received 50μCi.kg^{-1}, i.e. 1μg.kg^{-1} of ^{3}H-LSD. Means of data varying less than 10%.

ximal capacity of 210 ± 12fmoles.mg protein^{-1} ; the Hill coefficient (nH = 1.2 ± 0.15) does not differ significantly from unit (analysis of the data of Fig. 3 according to Parker and Waud (14).

The prevention of specific ^{3}H-LSD accumulation in mouse cortex by 5-HT agonists and antagonists as well as by other classes of psychotropic agents simultaneously administered with 1μg.kg^{-1}of ^{3}H-LSD is reported in Fig. 4 and Tables I and II.

Drugs considered as 5-HT antagonists are the most potent agents in preventing specific ^{3}H-LSD accumulation (Table II). Cyproheptadine and methysergide lead to inhibition-dose curves parallel to that of LSD with ID_{50} of less than 0.1mg.kg^{-1} but the maximal inhibition is apparently inferior to that produced by LSD (Fig. 4). Cinanserin and mianserin are somewhat less potent. Among tryptamine derivatives only dimethyltryptamine, psilocin and psilocybin significantly inhibit the specific accumulation of ^{3}H-LSD (Table I). Interestingly the dose-inhibition curve for dimethyltryptamine is more spread out than that of LSD or 5-HT antagonists and the ID_{50} is of 2.5mg.kg^{-1} (Fig. 4). Quipazine elicits a significant inhibition but the latter is apparently not dose-related (Table III) ; it must however be stressed that the increase in non-saturable accumulation (in cerebellum) elicited by this agent renders difficult the interpretation. A combination of 5-HTP with a MAO inhibitor also reduces significantly the specific accumulation of ^{3}H-LSD whereas tryptophan, DOPA and L-histidine are ineffective. Among a series of drugs interfering with the action of various neurotransmitters, only neuroleptics are found effective although significant differences exist between miscellaneous compounds (Table III).

Discussion

The main finding of the present study is that ^{3}H-LSD can be used to label

TABLE I

Effects of Various Tryptamine Derivatives on Selective ^{3}H-LSD Accumulation in Mouse Cortex

TREATMENTS	DOSE $mg.kg^{-1}$	SELECTIVE ^{3}H-LSD ACCUMULATION $pCi.mg\ protein^{-1}$	DIFFERENCE
Controls	-	56 ± 5	-
Tryptamine	1.0	58 ± 7	N.S.
N-Methyl tryptamine	1.0	54 ± 4	N.S.
	4.0	67 ± 9	N.S.
N,N'-Dimethyl-tryptamine	1.0	41 ± 3	-27%*
	4.0	33 ± 3	-41%**
Psilocin	1.0	21 ± 4	-62%**
Psilocybin	1.0	31 ± 1	-45%**
5-Methoxydimethyl tryptamine	4.0	48 ± 2	N.S.
5-Methoxy-tryptamine	4.0	60 ± 3	N.S.
Bufotenine	4.0	48 ± 3	N.S.

*$p<0.05$; **$p<0.001$. All drugs administered i.v. simultaneously with ^{3}H-LSD ($50\mu Ci \simeq 1\mu g.kg^{-1}$) and mice killed 1h later. Selective accumulation represents the difference between ^{3}H-LSD levels in cortex and in cerebellum. The latter is not significantly modified by any drug treatment. Means ± S.E.M. of 5-15 experiments.

TABLE II

Effects of Serotonin Antagonists ($1mg.kg^{-1}$) on Selective ^{3}H-LSD Accumulation in Mouse Cortex

DRUGS	SELECTIVE ^{3}H-LSD ACCUMULATION ($pCi.mg\ protein^{-1}$)	DIFFERENCE
Controls	59 ± 5	-
Methysergide	22 ± 2	-63%**
Cyproheptadine	20 ± 2	-66%**
Cinanserin	38 ± 1	-36%*
Mianserin	41 ± 3	-31%*
Methiothepin	19 ± 5	-68%**

*$p<0.005$; **$p<0.001$ as compared to controls. Means ± S.E.M. of 5-13 experiments. All drugs administered i.v. simultaneously with ^{3}H-LSD ($50\mu Ci.kg \simeq 1\mu g.kg^{-1}$) and mice killed 1h later. Selective accumulation represents the difference between ^{3}H-LSD levels in cortex and cerebellum.

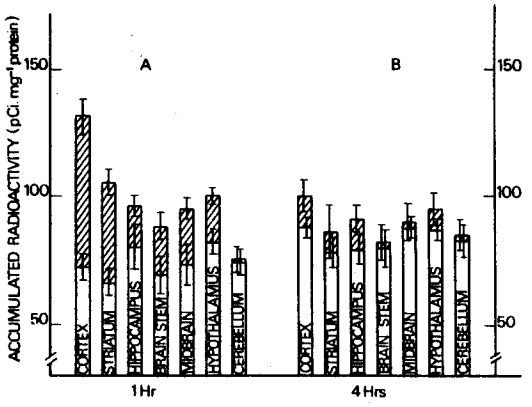

Fig. 2

Saturable Accumulation of Radioactivity in Various Regions of Mouse Brain

Groups of 5 mice were killed 1h (A) or 4h (B) following the administration of ^{3}H-LSD (50µCi, i.e. 1µg.kg^{-1}) either alone or simultaneously with 0.5mg.kg^{-1} LSD. The shaded area in the columns represents the difference between levels in the two groups of animals.

in vivo the 5-HT receptors already characterized by various in vitro binding studies using the same ^{3}H-ligand. In fact striking similarities as well as small interesting differences can be noted in the data obtained by the two approaches.

At a given time after administration of a small dose (1µg.kg^{-1}) of ^{3}H-LSD the accumulation of radioactivity in brain regions seems to reflect at least two processes : a non-saturable accumulation which appears to be of the same magnitude in all brain regions and a saturable accumulation, presumably due to binding to receptor sites, which appears to be almost absent in a region like the cerebellum characterized by a low 5-HT content and a negligible number of binding sites for ^{3}H-5-HT or ^{3}H-LSD (4-7, 15). Hence, administration of non-radioactive LSD at a relatively high dosage reduces the accumulation of radioactivity in all brain regions to the level found in cerebellum, whereas the latter is not significantly affected. This observation justifies the estimation of a "specific accumulation" in the various regions by substracting from the total level the level found in the cerebellum of the same animal.

Defined in this way specific accumulation of ^{3}H-LSD varies markedly among brain regions and this regional variation is closely parallel to that reported by others using in vitro binding techniques (4-7, 15). The ratio of "specific" to total accumulation was the highest in cortex where it represented about 50% making this region particularly suitable for a pharmacological characterization of the postulated binding sites.

A typical dose-saturation curve could be obtained in this region which compares rather favourably with corresponding in vitro data and allowed the application of classical methods (Scatchard or Hill plots) used to analyze the latter. The half-maximal saturation occurs for a dose (ED_{50}) of 30µg.kg^{-1} and

TABLE III

Effect of Various Drugs on Selective ^{3}H-LSD Accumulation in Mouse Cortex

DRUGS	DOSE ($mg.kg^{-1}$)	SELECTIVE ^{3}H-LSD ACCUMULATION ($pCi.mg\ protein^{-1}$)	DIFFERENCE
Controls	-	57 ± 5	-
LSD	1	13 ± 2	-77%**
Tryptophan + Pargyline	100 60	62 ± 7	N.S.
5-Hydroxytryptophan + Pargyline	100 60	19 ± 7	-67%**
L-DOPA + Pargyline	100 60	50 ± 2	N.S.
Quipazine	0.5	28 ± 3	-51%***
	1	35 ± 10	-36%*
	3	31 ± 4	-46%**
Haloperidol	1	34 ± 4	-40%***
Spiperone	1	23 ± 5	-60%***
Pimozide	1	61 ± 9	N.S.
Levopromazine	10	30 ± 4	-47%**

*$p<0.05$; **$p<0.005$; ***$p<0.001$ as compared to controls. Means ± S.E.M. of 5-20 experiments. Pargyline, chlorimipramine, desimipramine, L-histidine administered i.p. 2h before the i.v. injection of ^{3}H-LSD ($50\mu Ci.kg^{-1}$; $1\mu g.kg^{-1}$). Tryptophan, 5-hydroxytryptophan, L-DOPA, pimozide, scopolamine, atropine administered 1h before ^{3}H-LSD. All other drugs administered i.v. simultaneously with ^{3}H-LSD and mice killed 1h later. Selective accumulation of ^{3}H-LSD represents the difference between levels in cortex and cerebellum (151 ± 9 and 93 ± 7pCi.mg protein^{-1}, respectively in controls). The levels in cerebellum were not significantly modified by the drug treatments except after $0.5mg.kg^{-1}$ quipazine (+65%) or $1mg.kg^{-1}$ quipazine (+80%).
The following drug treatments had no effect : scopolamine ($2.5mg.kg^{-1}$), atropine ($5mg.kg^{-1}$), morphine ($25mg.kg^{-1}$), chlorimipramine ($40mg.kg^{-1}$), desimipramine ($40mg.kg^{-1}$), propranolol ($1mg.kg^{-1}$), salbutamol ($0.3mg.kg^{-1}$), L-histidine ($500mg.kg^{-1}$).

saturation corresponds to a capacity of 210fmoles.mg protein^{-1}. While the ED_{50} cannot easily be compared with the Kd of ^{3}H-LSD for its binding sites in vitro because, in vivo, the concentration of free LSD in the vicinity of its binding sites is not known, it can be noted however that this value is in close agreement with the ED_{50} of LSD as regard one of its most characteristic central actions, i.e. the inhibition of the firing of serotoninergic neurons in the dorsal raphe nucleus (16). The total number of apparent ^{3}H-LSD binding sites determined in vivo is in the same range as that determined in vitro in rat cortex (6,7) or as the number of high affinity binding sites for ^{3}H-5-HT in mouse cortex (17), i.e. 150fmoles.mg protein^{-1}. Furthermore when the in vivo saturation curve is submitted to Hill plot analysis (14) the calculated nH coefficient (1.2 ± 0.15) is the same as in in vitro studies (18).

That the specific accumulation of ^{3}H-LSD in mouse cortex reflects the binding to 5-HT receptors is suggested by the inhibition elicited by various serotoninergic agents (Fig. 4 and Tables I and II) contrasting with the lack

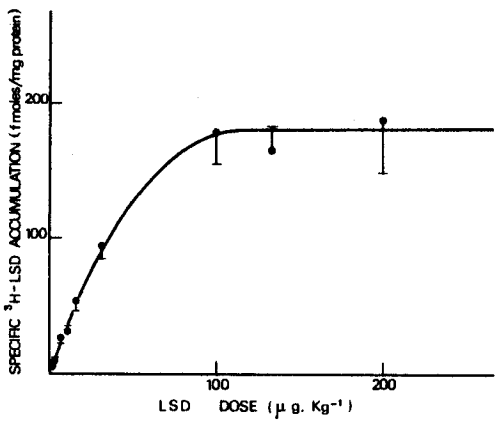

Fig. 3

Specific Accumulation of ^{3}H-LSD in the Cortex of Mice Receiving Increasing Doses of d-LSD

Groups of 5-10 mice killed 1h after the administration of ^{3}H-LSD (50μCi.kg^{-1}) together with non-radioactive d-LSD in increasing dosage. The specific accumulation represents the difference between the levels of ^{3}H-radioactivity in cortex and cerebellum.

of effects of miscellaneous non-serotoninergic agents. The most effective are agents generally considered as 5-HT antagonists like methysergide or cyproheptadine, with ED_{50} around 50μg.kg^{-1} and inhibition curves parallel to that of LSD. However the somewhat lower maximal inhibition observed with a high dosage of methysergide or cyproheptadine as compared to LSD might reflect an heterogeneity of ^{3}H-LSD binding sites. The protection afforded by 5-HT agonists presents striking similarities with that observed in in vitro studies (4,5). First, relatively high doses are required as compared to those of antagonists ; this would explain that increasing the endogenous 5-HT formation by 5-HTP administration is capable of inhibiting ^{3}H-LSD specific accumulation, whereas administration of tryptophan or reuptake inhibitors is not. Secondly, the inhibition curve for an agonist like dimethyltryptamine is not parallel to those of LSD and 5-HT antagonists ; a similar stretched out inhibition curve is found for 5-HT as regard ^{3}H-LSD binding in vitro (5) as well as for apomorphine, a dopamine agonist, as regard the binding of ^{3}H-pimozide in vivo (8,9). These observations could reflect either a difference in the subsites of the receptor involved in the binding of agonists and antagonists or the existence of several classes of receptors or possibly of two conformations of the same receptor.

The inhibition of ^{3}H-LSD specific accumulation elicited by neuroleptics deserves some comments. It was observed for relatively large doses of these agents : for instance the ED_{50} of haloperidol was 700μg.kg^{-1} to be compared to 70μg.kg^{-1} as regard the inhibition of in vivo binding of ^{3}H-pimozide, a dopaminergic ligand (8). Several in vitro studies have recently shown that various neuroleptics recognize with significant affinities 5-HT (19,20) and LSD (6,20) binding sites in brain. Although complete dose-inhibition curves were not esta-

blished for all agents, data from Table III show clear differences in the potencies of the various compounds, the most potent being spiperone and the lesser one pimozide, results which agree with those of in vitro studies (20).

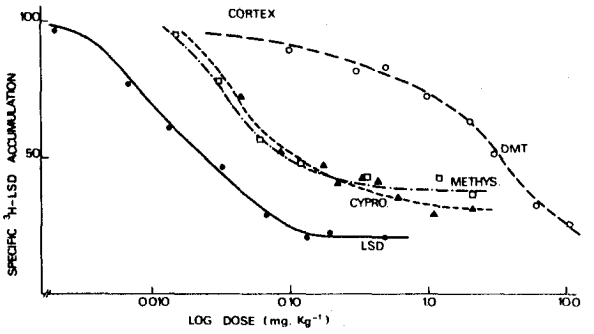

Fig. 4

Inhibition of the Specific Accumulation of ^{3}H-LSD in Mouse Cortex by Administration of Serotoninergic Agents

Groups of 5-10 mice killed 1h after the administration of ^{3}H-LSD (50µCi.kg^{-1}) simultaneously with the various agents. The specific accumulation represents the difference between the levels of ^{3}H-radioactivity in cortex and cerebellum. Means of data varying less than 10%.

On the other hand, and rather interestingly, the respective potencies of various tryptamine derivatives in vivo did not appear to correlate well with their potencies to inhibit ^{3}H-LSD binding in vitro. For instance psilocybin was much more potent than bufotenine or 5-methoxydimethyltryptamine in vivo whereas it was 3 times less in vitro (4). The apparent discrepancy can probably be explained by a better availability of the most active compounds (or their active metabolites) in the vicinity of cerebral receptors in vivo. It is interesting to note that the inhibition of ^{3}H-LSD binding in vivo reflects much better the hallucinogenic potency of various tryptamine compounds (4,5, 21-24) than the in vitro data. This suggests that the in vivo test represents a more reliable test to predict the potency of various agents to occupy 5-HT receptors in brain.

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