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INHIBITORY EFFECTS OF LYSERGIC ACID DERIVATIVES AND RESERPINE ON 5-HT BINDING TO NERVE ENDING PARTICLES*

R. M. MARCHBANKS†

Cognitive Systems Research Program,
Cornell University, Ithaca, N.Y., U.S.A.

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Abstract—In order to assess the functional significance of a high-affinity binding of 5-HT to nerve ending particles the effects of lysergic acid derivatives and other pharmacological antagonists of 5-HT on the binding have been investigated. The non-hallucinogenic L-lysergic acid diethylamide was found to inhibit the 5-HT binding, whereas the hallucinogenic D-1-acetyl-lysergic acid diethylamide was only a weak inhibitor of the binding. There is therefore no consistent correlation between the hallucinogenic potency of lysergic acid diethylamide derivatives and their ability to inhibit 5-HT binding to nerve ending particles. The action of D-2-brom-lysergic acid diethylamide was found to be erratic, and evidence is presented of its breakdown under mild conditions. Reserpine and some other compounds which release 5-HT from its storage sites *in vivo* were found to be inhibitors of the 5-HT binding. However the 5-HT binding component inhibited by reserpine was shown to be different from that inhibited by D-lysergic acid diethylamide. The functional significance of these two 5-HT binding components present in nerve ending particles is discussed.

THE POSSIBILITY that 5-hydroxytryptamine (5-HT) has a role in the processes of synaptic transmission has been strengthened by the observations that it has an excitatory action on single cerebral cortical neurons,¹ and on single neurons in the brain stem.² In the hippocampus,³ the hypothalamus,⁴ cortex,⁵ and lateral geniculate body,⁶ 5-HT has mainly a depressant effect when applied to single neurons. D-Lysergic acid diethylamide (D-LSD) has an inhibitory effect on the excitatory action of 5-HT,¹ but does not appear to affect the inhibitory effect of 5-HT in the cortex.⁵ By histochemical methods Hillarp, Fuxe and Dahlstrom⁷ have shown that 5-HT is concentrated in the pre-synaptic terminals of certain nerve cells of the lower brain stem and spinal cord. Spinal cord tissue has been shown to release 5-HT on stimulation.⁸

The implication of 5-HT in the processes of synaptic transmission makes it of interest to study the binding of this substance to pre-synaptic nerve terminals isolated by centrifugation procedures.⁹ In a previous study¹⁰ it has been shown that nerve ending particle preparations contain a 5-HT binding component distinguishable

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† Present address: Biochemistry Department, Tennis Court Road, University of Cambridge Cambridge, U.K.

Abbreviations used: 5-HT, 5-hydroxytryptamine; D-LSD, D-lysergic acid diethylamide.

from non-specific binding to protein by its high-affinity for 5-HT and its sensitivity to inhibition by D-LSD. This binding component is present mainly in nerve ending particles, and principally in those from the hypothalamus, rhinencephalon and mid-brain regions. D-LSD as well as being a central and peripheral 5-HT antagonist is also a powerful hallucinogenic agent. In order to explore any possible relationship between the hallucinogenic activity of D-LSD and its inhibition of the high-affinity 5-HT binding component in nerve ending particles the effect of other LSD derivatives and 5-HT antagonists on the binding has been examined. It was also noted in the previous study that there appeared to be at least two components contributing to the high-affinity binding. An attempt has been made to distinguish the functional significance of these two components by examining their susceptibility to inhibition by various pharmacological agents having effects on the 5-HT system.

METHODS

Preparations

A fraction enriched in nerve ending particles was prepared from whole rat brain as described by Whittaker,^{9, 11} except that a fixed angle rotor was used instead of a swing out head. The mitochondrial fraction was first spun in 0.7 M sucrose at 60,000 g for 2 hr to separate the myelin fraction which remained in the supernatant fluid. The pellet was then resuspended in 1.2 M sucrose and respun at 60,000 g for 2 hr. The nerve ending particles remained in the supernatant fluid while the mitochondria were precipitated. The sucrose solution containing nerve ending particles was diluted to 0.32 M and spun at 30,000 g for 30 min to precipitate the fraction which was then dialysed against 500 vol. 0.1 M sodium phosphate buffer at pH 7.3.

Assay procedures

5-HT. The fluorescence at 5,500 Å in 3 N HCl¹² was used to estimate 5-HT after alkaline extraction by the procedure of Bogdanski *et al.*¹³

Thin-layer chromatography. Thin-layer chromatography of D-LSD derivatives was carried out on silica gel G (Brinkmann-Desaga) using methanol as solvent. After preparation the samples were extracted into chloroform and applied to the plate. The spots were visualized by exposure to iodine vapour or examined under u.v. light.

Radioactivity. Radioactivity was measured in a Packard automatic scintillation counter using a dioxane base scintillation medium.¹⁴ Uniformity of relative counting efficiency of standards and experimental tubes was checked by the addition of a known amount of radioactivity ([2-¹⁴C] 5-HT) after the initial radioactivity estimate, and the appropriate correction applied. Where the radioactivity was determined in the presence of appreciable quantities of homogenate (as in the Sephadex method), the homogenate was solubilised in formamide. Treatment of 0.5 ml samples with 2 ml formamide at 50° for 24 hr was found to be a satisfactory procedure. An equivalent amount of homogenate was added just before formamide incubation to the standards which were then exposed to the same treatment. The sp. act. of 5-HT was determined by extracting the sample by the alkaline extraction procedure, and measuring the radioactivity and 5-HT in the extracted sample.

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Measurement of binding. The amount of 5-HT bound at a particular 5-HT concentration was determined as has been described previously.¹⁰ In general experiments were performed in triplicate by both the centrifugation and Sephadex methods. All binding experiments were done in 0.1M sodium phosphate buffer pH 7.3, at 2°. Usually aliquots of brain fractions equivalent to 0.5 g fresh weight of brain were used. 5-HT solutions and inhibitors were made up freshly for each experiment, and standards in triplicate run through the experimental procedure in each case.

Radioactive 5-HT ([2-¹⁴C] 5-HT) was used and the amount of 5-HT bound determined from the bound radioactivity and the sp. act. of the 5-HT. In control experiments it was established that the sp. act. of 5-HT of blanks incubated without homogenate did not differ significantly from that of experimental tubes containing homogenate, thus eliminating the possibility that the bound radioactivity was a degradation product of 5-HT. Measurements of bound radioactivity and bound 5-HT were found to be equivalent.

Centrifugation method. After a 12 hr incubation the macromolecular phase was separated by centrifugation at 60,000 g for 30 min, the supernatant fluid was removed for determination of the equilibrium 5-HT concn. The pellets were rinsed *in situ* with buffer and bound 5-HT determined. The amount of 5-HT lost from the supernatant was found to be equivalent to that found in the pellet, provided the macromolecular phase was completely centrifuged down. This was the case with the nerve ending particle preparation. It was found advantageous to add sodium tungstate (final concn. 3 per cent) just prior to the centrifugation stage. This ensured complete precipitation, and reduced the variability of the relative counting efficiency when the radioactivity in the supernatant was examined. The per cent standard deviation (S.D.) of the amount bound calculated from the loss of 5-HT from the supernatant was ± 9 (12). The per cent S.D. of the amount of 5-HT found in the pellet was ± 7 (12). Since the two measures had approximately equal variability the amount bound was usually calculated from the losses from the supernatant, this being more convenient.

Sephadex method. The cross-linked dextran, Sephadex, was used to obtain a partial separation of macromolecules from small molecules. The following procedure was used: 100 mg. portions of G-50 (coarse) Sephadex were incubated with a sample of homogenate and an aliquot of 5-HT. After incubation (12 hr) a portion of the external fluid was removed by pipetting (a glass wool plug preventing the removal of Sephadex grains) and the total amount of 5-HT estimated in the withdrawn portion.

The amount of 5-HT in the withdrawn portion is composed of all the 5-HT bound to macromolecules and an amount of free 5-HT in solution in the suspending medium. The amount of free 5-HT in solution was determined by running control tubes without added homogenate, and thus by subtracting this amount from the total the amount of bound 5-HT was determined.¹⁰ The per cent S.D. of the amount bound determined by the Sephadex method was ± 18 (12).

Determination of binding constants. The association constant of the 5-HT macromolecular complex was determined by the graphical procedure of Scatchard.¹⁵ Experiments were performed in the high-affinity binding 5-HT concentration range: 0.1 μ M–5 μ M. Inhibitors were characterized by an I_{50} value, defined as that concn. of inhibitor required to reduce the apparent binding constant by one half. I_{50} equals K_i (inhibitor-macromolecule dissociation constant) for cases where only one binding component is present. Otherwise $I_{50} > K_i$, the degree of discrepancy being dependent

on the amount of binding not inhibited by the agent.

Materials. [2-¹⁴C] 5-HT oxalate was purchased from New England Nuclear Corporation. D-LSD (LSD 25), D-2-brom-LSD (BOL 148), L-LSD, D-1-acetyl LSD (ALD 52) tartrates and psilocybin were generously donated by Sandoz Pharmaceuticals. Benanserine (1-benzyl-2-methyl-3-(2-aminoethyl)-5-methoxyindole HCl) (SQ4788) and 2'-(3-dimethylaminopropylthio) cinnamanilide HCl (SQ 10463) were kindly donated by the Squibb Institute for Medical Research. Guanethidine sulphate was given by CIBA, Tofranil HCl by Geigy Pharmaceuticals and *N*-methylpiperidyl benzilate (Ditran) by Lakeside laboratories to all of whom thanks are due. Harmine, reserpine and mescaline hemisulphate were purchased from Calbiochem.

RESULTS

The effects of LSD derivatives and other hallucinogenic drugs on the 5-HT binding in the 5-HT concentration range 0.1 μ M–1.0 μ M were examined in order to see if there was any correlation between inhibition of binding and hallucinogenic activity. The results are shown in Table 1, along with data from other sources indicating the hallucinogenic potency of the drugs.

TABLE 1. EFFECT OF LSD DERIVATIVES AND OTHER HALLUCINOGENIC DRUGS ON 5-HT BINDING TO NERVE ENDING PARTICLES IN THE 5-HT CONCENTRATION RANGE 0.1 μ M–1.0 μ M

Agent	Conc. (μ M)	Effect	I ₅₀ value	Approximate hallucinogenic dose (mg/kg) in humans
D-LSD	1.0	Inhibitory	4×10^{-7}	0.001 ¹⁶
L-LSD	1.0	Inhibitory	4×10^{-7}	Non-hallucinogenic ¹⁶
D-1-acetyl LSD	10	Slightly inhibitory	10^{-5}	0.001 ¹⁶
D-2-brom-LSD (solubilised in water at 2°)	2.0	Not inhibitory		Non-hallucinogenic ¹⁶
D-2-brom-LSD (solubilised by warming in phosphate buffer)	2.0	Inhibitory		
Psilocybin	20	Inhibitory	2×10^{-5}	0.08 ¹⁷
Bufotenin	500	Inhibitory	2×10^{-4}	0.5 ¹⁸
Mescaline	1000	Not Inhibitory		7 ¹⁹
<i>N</i> -methyl piperidyl benzilate	30	Not Inhibitory		0.2 ²⁰

The effect of D-2-brom-LSD was erratic. This variability was traced to the method of solubilisation of the solid material in initial experiments. A stock solution of D-2-brom-LSD was made up in cold glass distilled water; one portion was then incubated with phosphate buffer (0.1 M, pH 7.3) at 50° for 1 hr, the other remained at 2° in distilled water. The two portions were then adjusted so that each contained the same concentration of D-2-brom-LSD (2.0 μ M) and phosphate buffer, and were then tested for their inhibitory action on 5-HT binding in the 5-HT concentration range 0.1 μ M–1.0 μ M.

The sample amount of 5-HT with phosphate. This result in some of the high-affinity between 40° and 50° was destroyed.

An attempt was made to prepare a stock solution of the LSD derivatives in thin layers of silica under the main layer was exposed to the sample with the results for the results for

There was no material was seen from the same *R_F* as D-2-brom-LSD with iodine vapor. It is apparent that the results for

In previous experiments from a 5-HT concentration of 2×10^{-6} M and the component antagonists arise the differences in the constants of the

In Table 2 the results of 0.5 μ M and

The following results affect the binding of 5-HT tartrate (0.1 nM), 5-HT (0.5 mM), toluidine (0.5 mM), isoproterenol (0.5 mM), atropine sulphate (0.5 mM), and nine (0.4 mM).

The inhibitory effect of harmine was 10 times that of serine, *l*-norepinephrine,

The sample that had been solubilised in cold distilled water did not decrease the amount of 5-HT bound. In the presence of D-2-brom-LSD which had been warmed with phosphate buffer the amount of 5-HT bound was 32 per cent of the control. This result indicated that the mild treatment with heat and phosphate ions converted some of the D-2-brom-LSD into a highly effective inhibitor of 5-HT binding to the high-affinity binding component. The temperature optimum for activation was between 40° and 50°. At 100° the inhibitory action of D-2-brom-LSD (and D-LSD) was destroyed.

An attempt was made to characterise this change using thin-layer chromatography. A stock solution of D-2-brom-LSD was prepared and exposed to a variety of treatments. After treatment, 0.1 M sodium phosphate buffer was added to each tube such that the concentration of buffer and LSD derivatives were the same in each sample. The LSD derivatives were then extracted into chloroform and chromatographed on thin layers of silica gel. It can be seen from Fig. 1 that in each case there is a faint spot under the main D-2-brom-LSD spot. The spots are slightly stronger where the sample was exposed to phosphate ions and heat. However, there is a visible spot even when the sample was only exposed to distilled water at 2° so this does not entirely explain the results found with the D-2-brom-LSD inhibition of 5-HT binding.

There was no impurity in the original sample of D-2-brom-LSD because when the material was dissolved directly in chloroform only one spot was found (Fig. 2). It can be seen from Fig. 2 that the faint spot found in degraded D-2-brom-LSD has the same R_F as D-LSD. It should be pointed out that in the figures the spots were visualised with iodine vapour, but that when the chromatograms were viewed under u.v. characteristically 4 spots fluoresced (R values; 1.1, 0.8, 0.63, 0.45. D-2-brom-LSD = 1.0). It is apparent that D-2-brom-LSD is unstable in aqueous solution.

In previous studies it had been observed that the 5-HT binding measured in the 5-HT concn. range 0.1 μ M–1.0 μ M (high-affinity binding) was composed of contributions from at least two components having association constants approximately 2×10^6 and 5×10^5 . It was found that D-LSD only inhibited the 5-HT binding to the component having the higher association constant. The effect of a number of 5-HT antagonists and other agents on the binding in 5-HT concn. ranges selected to emphasise the differences between the two components was investigated in order to try and distinguish them more clearly than was possible by measurements of the association constants of their 5-HT binding.

In Table 2 the inhibition of 5-HT binding by various agents at 5-HT concentrations of 0.5 μ M and 2.5 μ M is shown.

The following agents at the molar concentrations specified in parentheses did not affect the binding of 5-HT at either of the two 5-HT concentrations: Ergotamine tartrate (0.1 mM), ergonovine maleate (0.5 mM), SQ 10463 (0.5 mM), guanethidine (0.5 mM), tofranil (0.5 mM), bulbocapnine HCl (1.0 mM), *l*-epinephrine tartrate (0.5 mM), iso-proterenol (0.5 mM), yohimbine HCl (1.0 mM), acetylcholine HCl (0.1 mM), atropine sulphate (0.5 mM), *d*-tubocurarine Cl (0.5 mM), physostigmine sulphate (0.5 mM), dopamine HCl (1.0 mM), γ -aminobutyric acid (0.5 mM), strychnine (0.4 mM), adenosine triphosphate (0.1 mM).

The inhibitory actions of D-LSD, psilocybin and picrotoxin were not affected when harmine was present in the experimental and control tubes, but the effects of benaserin, *l*-norepinephrine and reserpine were reduced though not eliminated when

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TABLE 2. EFFECT OF INHIBITORS OF THE HIGH-AFFINITY 5-HT BINDING AT DIFFERENT 5-HT CONCENTRATIONS

Inhibitor	Concentration (μ M)	5-HT bound in presence of inhibitor as % controls	
		at 5-HT concn. 0.5 μ M	at 5-HT concn. 2.5 μ M
D-LSD	2.0	60*	103
Psilocybin	50	53*	97
Picrotoxin	1.0	55*	91
Benanserin	500	45*	52*
<i>l</i> -norepinephrine	100	65*	69*
Reserpine	5.0	63*	75*

* Inhibition significant $P < 0.05$ (*t* test).

harmine (5.0 μ M) was added. *d*-Amphetamine (1.0 mM) and α -methyl-*m*-tyrosine (0.05 mM) inhibited 5-HT binding in both the lower and higher 5-HT concentration ranges, but no inhibition was observed when the experiments were done in the presence of harmine.

In Table 2 it can be seen that D-LSD, psilocybin and picrotoxin were effective inhibitors of 5-HT binding only in the lower 5-HT concentration range; reserpine, benanserin and *l*-norepinephrine inhibited in both concentration ranges. This suggested that reserpine, benanserin and *l*-norepinephrine inhibit a different binding component than that inhibited by D-LSD, psilocybin and picrotoxin.

In order to establish this point more thoroughly the inhibitory effect of D-LSD in the presence of a maximally inhibitory concentration of reserpine was examined. If reserpine inhibits a different 5-HT binding component than D-LSD then D-LSD should cause inhibition of binding in the presence of reserpine. Reserpine should also inhibit in the presence of D-LSD.

TABLE 3. EFFECT OF D-LSD AND RESERPINE IN THE PRESENCE OF EACH OTHER ON THE HIGH-AFFINITY 5-HT BINDING TO NERVE ENDING PARTICLES

Inhibitor	5-HT bound in presence of inhibitors as % controls	
	at 5-HT concn. 0.5 μ M	at 5-HT concn. 2.5 μ M
D-LSD (1.0 μ M) in presence of 10 μ M reserpine in experimental and control tubes	49*	93
Reserpine (5.0 μ M) in presence of 2.0 μ M D-LSD in experimental and control tubes	39*	79*

* Inhibition significant $P < 0.05$ (*t* test).

Inhibition of 5-HT binding by D-LSD in the presence of reserpine, and by reserpine in the presence of D-LSD was measured in the 5-HT concentration ranges 0.5 μ M and 2.5 μ M. As Table 3 shows further inhibition by both D-LSD and reserpine in the presence of the other takes place.

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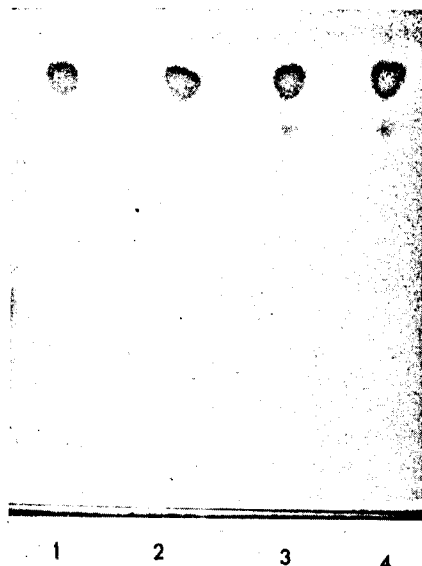


FIG. 1. Thin-layer chromatogram of the effect of warming and phosphate ions on D-2-brom-LSD. 15 μ g incubated for 8 hr in (1) water at 2°, (2) water at 50°, (3) 0.1 M Na⁺ phosphate buffer pH 7.3 at 2°, (4) 0.1 M Na⁺ phosphate buffer pH 7.3 at 50°.

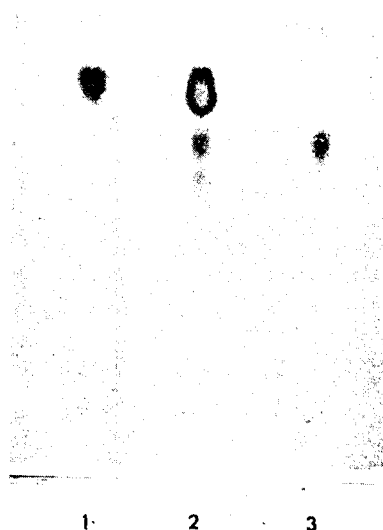


FIG. 2. Thin-layer chromatogram of original and degraded samples of D-2-brom-LSD, and of D-LSD. (1) D-2-brom-LSD dissolved directly in CHCl₃ and 10 μ g spotted. (2) D-2-brom-LSD after 15 hr in phosphate buffer at 2°. 20 μ g spotted by same procedure as in Fig. 1. (3) 10 μ g D-LSD.

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DISCUSSION

D-LSD is an indolic compound and a peripheral antagonist of 5-HT. This observation led to the proposal by Woolley and Shaw²¹ and Gaddum²² that the hallucinogenic activity of this agent was due to its central antagonism to 5-HT function. However, D-2-brom-LSD, an equally powerful peripheral antagonist of 5-HT function is not a hallucinogen. The results in this study suggest that D-2-brom-LSD is not an inhibitor of the high-affinity binding of 5-HT to nerve ending particles, but that it degrades under very mild conditions to a highly active inhibitor of the 5-HT binding. It seems likely that this degradation would occur under the conditions of test for its hallucinogenic potency and many other pharmacological tests. L-LSD, the non-hallucinogenic optical isomer of D-LSD, is an active inhibitor of the 5-HT binding, and the hallucinogenic D-1-acetyl-LSD is a very weak inhibitor of the 5-HT binding. There is therefore no consistent correlation between the inhibitory effects of the LSD derivatives and their hallucinogenic activity. The inhibitory activity of D-LSD derivatives on the 5-HT binding is sensitive to substitution in the 1 and 2 positions of the indole nucleus and not to the conformation at the C₈ position. In contrast hallucinogenic potency is not sensitive to substitution in the 1 position, but is sensitive to the conformation at the C₈ position.

It is noteworthy that the non-indolic psychotomimetics mescaline and *N*-methyl piperidyl benzilate do not inhibit the 5-HT binding, but that within the series D-LSD, psilocybin and bufotenin there is a rough parallel between inhibitory action on the 5-HT binding and psychotomimetic activity. Ability to inhibit the high-affinity 5-HT binding is fairly restricted among 5-HT antagonists. Ergonovine,²³ SQ 10463,²⁴ and ergotamine, tofranil, *l*-epinephrine, isoproterenol and yohimbine have all been reported as peripheral 5-HT antagonists (review: Garattini and Valzelli,²⁵ appendix IV), but they do not inhibit the 5-HT binding. It is not clear to what extent the LSD sensitive 5-HT binding reflects the properties of 5-HT receptors in brain; nonetheless these results suggest that brain receptors are considerably more specific than peripheral receptors.

The fact that the inhibitory effect of D-LSD, psilocybin and picrotoxin could be overcome by raising the 5-HT concentration from 0.5 μ M to 2.5 μ M whereas the inhibitory effect of reserpine, benanserin and *l*-norepinephrine was not overcome suggested that the latter compounds were inhibiting a 5-HT binding component with a lower 5-HT affinity constant than that inhibited by D-LSD. However, this test, although suggestive, does not eliminate the possibility that reserpine, benanserin and *l*-norepinephrine inhibit both the D-LSD inhibited component and the one with the lower affinity constant. This possibility with respect to reserpine was ruled out, since both reserpine and D-LSD exert inhibitory effects in the presence of maximally inhibitory concentrations of the other. D-LSD and reserpine therefore inhibit different 5-HT binding components, the reserpine sensitive binding having a slightly lower 5-HT affinity constant than the D-LSD sensitive binding.

Reserpine,²⁶ *d*-amphetamine,²⁷ and benanserin²⁸ have all been shown to release 5-HT from its storage sites in various tissues. The small 5-HT depleting effect of α -methyl-*m*-tyrosine has also been ascribed to interference with the amino binding sites.²⁹ These compounds were found to inhibit 5-HT binding in both 5-HT concentration ranges, in contrast to D-LSD which does not release 5-HT from its storage sites in brain,³⁰

and only inhibits 5-HT binding in the lower 5-HT concentration range. The high concentration of 5-HT observed histochemically in presynaptic terminals⁷ suggests that a storage site for 5-HT is present in nerve ending particle preparations. The reserpine sensitive 5-HT binding might therefore be related to the 5-HT storage site *in vivo*. However, reserpine and compounds with a similar effect on the 5-HT binding are all to some extent inhibitors of monoamine oxidase (Review: Pletscher, Gey and Burkard⁸¹), and their inhibition of 5-HT binding is reduced by the monoamine oxidase inhibitor harmine. There is a medium-affinity binding component of 5-HT in sub-cellular fractions from brain which was found to be associated with the activity of monoamine oxidase because it was inhibited by harmine.¹⁰ It is possible, therefore, that the inhibition of 5-HT binding by these compounds is a reflection of their activity as monoamine oxidase inhibitors, which would explain the reduction of their inhibitory effect in the presence of harmine. However, the 5-HT concentration range for reserpine inhibition of 5-HT binding is much lower than the 5-HT concentration range to show harmine inhibition of the medium-affinity binding. Moreover, the monoamine oxidase inhibitory effect of reserpine is weak³² compared with its serotonin releasing effect. An alternative explanation is that harmine itself is a releaser of 5-HT from its storage sites and therefore competes with reserpine for this 5-HT binding component, thus reducing its inhibitory action. It has been suggested that harmine releases catecholamines from their peripheral stores³³ but there seems to be no direct evidence that there is an effect of harmine on 5-HT storage *in vivo*, so this can remain no more than a suggestive possibility.

Several putative transmitter compounds were tested for their inhibitory action on the 5-HT binding. Only *l*-norepinephrine had any effect. Reserpine did not inhibit in the presence of *l*-norepinephrine, suggesting that these two compounds compete for the same 5-HT binding component. Interactions between serotonin and catecholamines are known in many physiological situations (Review: Ref. 25, p. 212). In particular reserpine releases both norepinephrine and 5-HT from their storage sites *in vivo*, suggesting that the sites have characteristics in common. The sensitivity of the reserpine sensitive 5-HT binding component to inhibition by *l*-norepinephrine would be explicable on this basis.

The effect of picrotoxin was unexpected; it exerted an inhibitory effect on binding similar to D-LSD both in its potency and its 5-HT concentration dependency. A possible explanation is that the convulsant activity of picrotoxin is due to its competition for a 5-HT receptor site *in vivo* and its inhibition of 5-HT binding reflects this possibility. The convulsant effect of picrotoxin can be antagonised by prior administration of 5-HT.³⁴

Studies of binding phenomena *in vitro* can only be evaluated in the light of pharmacological results from whole tissues and organs. It is not expected that the binding components in isolated nerve ending particles completely replicate the properties of the functional sites *in vivo*, but it has been possible to distinguish two 5-HT binding components in the high-affinity binding to nerve ending particles on the basis of the different pharmacological antagonists of 5-HT which inhibit them.

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