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DRUG ANTAGONISM AND REVERSIBILITY OF THE BINDING OF INDOLEAMINES IN BRAIN

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The specificity of the binding of serotonin to brain preparations was investigated with various competitive agents. A probable relationship between their structure and their capability of displacement was suggested. Bufotenine and morphine displaced serotonin (0.5×10^{-5} M) binding to synaptic membranes 87 and 49% respectively. Dissociation constants of the binding of 5-HT and tryptamine to synaptic membranes, and displacement constants of certain drugs were determined. The binding of 5-HT and tryptamine to calf brain preparations was also investigated by equilibrium dialysis, in order to determine affinity constants and reversibility of the binding. Differences were noted in the specificity of binding sites for serotonin and tryptamine, suggesting a different binding site for tryptamine. Extrapolations of Scatchard plots were used for determination of the constants. A characteristic low dissociation constant was found for 5-HT in synaptic membranes ($K \sim 1 \times 10^{-6}$ M). Probably, the binding macromolecule (receptor?) is a proteolipid.

Serotonin Tryptamine Binding sites Bufotenine Morphine

1. Introduction

During the past few years it was shown that biologically active substances may bind to specific sites of tissue macromolecules (receptor structures) in a reversible manner, and this binding may produce conformational changes and important physiological effects (Changeux et al., 1967).

Serotonin (5-hydroxytryptamine, 5-HT) was found to play an important role in the central nervous system, but its main role is still unknown. It was suggested (Marchbanks, 1966) that high affinity binding of serotonin was associated with nerve ending particles, medium affinity binding with the enzyme monoamine oxidase, and low affinity binding was omnipresent.

Gaddum and Picarelli (1957) have proposed two types of serotonergic receptors in guinea pig ileum, the M-receptors and D-receptors which may be blocked by morphine and dibenzyline or LSD respectively.

Alivisatos et al. (1971) have reported that the interaction of serotonin with specific binding sites in the central nervous system may involve Schiff base formation between the ethylamine residue of 5-HT and an activated carbonyl residue at the binding site. The reversible imine formed may be stabilized by reduction with a mild reducing agent, e.g., sodium borohydride.

Considering some criteria which characterize receptors for 5-HT in situ (Alivisatos and Seth, 1971) (e.g., chemical structure necessary for its binding, reversibility, high affinity constants, in situ displacement by pharmacological agents) we have investigated in the present paper the effect of various compounds on the attachment of indoleamines to brain tissues.

Equilibrium dialysis was used to establish

the degree of affinity and reversibility of the binding of indoleamines to bovine brain preparations.

2. Materials and methods

Reagents used in the present work were purchased from the following commercial sources: 5-hydroxy-(side chain-2-^{14}C)-tryptamine creatinine sulfate (specific activity, 55 Ci/mole) from Amersham/Searle Corp.; tryptamine bissuccinate [2-^{14}C] (side chain label), specific activity, 47 Ci/mole, from New England Nuclear Corp., 5-hydroxytryptamine creatinine sulfate (5-HT) and tryptamine · HCl [3-(2-aminoethyl)-indole · HCl] from Sigma Chem. Company, U.S.A. Other reagents used in this study were of the highest commercially available quality.

Mouse and calf brain tissue was used in our studies. Calf thalamus homogenates were used for the preparation of synaptosomes according to Whittaker (1959). After osmotic disruption of the synaptosome fraction, subfractionation of this fraction in discontinuous sucrose gradients was performed according to De Robertis et al. (1962) and Azcurra and De Robertis (1967). Membrane particles were used for binding experiments.

Brain preparations were preincubated in 0.1 M bicarbonate buffer, pH 8.9, 0.1 M sucrose, 0.7 mM pargyline for 20 min at 30°C, to eliminate monoamine oxidase activity. Subsequently, the samples were incubated with radioactive serotonin or tryptamine in presence or absence of displacing agents for 10 min at 30°C in a final volume of 1 ml. After incubation, the samples were treated with sodium borohydride (5 mg in 0.1 ml ethylene glycol monomethyl ether) where indicated and incubation was continued for 30 min with shaking. The samples were then acidified with 10% trichloroacetic acid and washed with 10 ml of the same acid 5 times. The washed precipitates were dissolved in hydroxide of hyamine 10-X, transferred into vials with scintillation fluid, and counted in a Packard Tri-Carb liquid scintillation counter. Radioactivity measurements and protein determinations were done as described previously (Alivisatos et al., 1970).

The dissociation constants of 5-HT and tryptamine, as well as some displacement constants were determined by measuring the binding as a function of concentration and plotting the data graphically in double-reciprocal form as described by Lineweaver and Burk (1934). At least 5 different concentrations were used for each determination of the dissociation constant. The displacement constant K_i (dissociation constant of inhibitor—macromolecule complex) was determined from graphic analysis of data on displacement of the binding, stabilized by sodium borohydride, at 2 or 3 concentrations of the displacing agent.

Besides the sodium borohydride methodology, we also used equilibrium dialysis for the investigation of the affinity of the indoleaminergic attachment. Equilibrium dialysis was performed according to the method used by Eldefrawi et al. (1971) and O'Brien et al. (1970).

1 ml of bovine brain homogenate (4 mg protein) or 1 M sucrose layer membrane fraction (1 mg protein) was placed into a segment of 1/4 inch dialysis tubing, tied at both ends and suspended in 50 ml of [^{14}C]-serotonin or [^{14}C]-tryptamine in Krebs—Ringer solution pH 7.4 or pH 9 (Dawson et al., 1959) which contained 0.4 mM pargyline and 0.85 mM $CaCl_2$. The samples were shaken for 20 hr at 4°C. Subsequently, aliquots from the bath and the inside of the bag were counted in a Packard Tri-Carb liquid Scintillation counter. The difference between radioactivity of the inside fluid and an equal volume of the outside fluid, represented the amount of ligand bound. Each dialysis experiment was done with duplicate or triplicate samples. Scatchard plots were used for the determination of the dissociation constants of serotonin and tryptamine (Klotz et al., 1948; Klotz, 1953).

The reversibility of the binding of 5-HT and tryptamine to calf thalamus preparations

was determined by performing dialysis of 6 equal samples at pH 7.4 and pH 9.0 for 20 hr. The degree of binding was determined from 3 bags (controls). The other 3 bags were transferred to ligand-free Ringer solution and dialyzed for 24 hr at 4°C. Subsequently, the last procedure was repeated and dialysis continued for 24 hr. The final counts were compared to the counts of the controls and the reversibility was computed from the sharp decrease of the binding of the radioactive ligand after 48 hr of dialysis. To determine the approximate nature of the binding macromolecule, the effect of treatment with trypsin and phospholipase C was explored. Calf thalamus homogenates were incubated in presence and absence of phospholipase C at pH 7.4 (5 mM $CaCl_2$) or trypsin at pH 8 (10 mM $CaCl_2$) at 37°C for 1 hr. After incubation the ice cooled samples were placed into dialysis bags, which were immersed in baths, containing [^{14}C]-serotonin in Ringer solution pH 7.4 and dialyzed for 20 hr at 4°C. Binding of [^{14}C]-serotonin was determined as above.

3. Results

The degree of displacement of the binding of [^{14}C]-serotonin to mouse brain homogenates by various compounds is shown in table 1. As evident from this table, substances used for displacement studies were of differ-

TABLE 1

EFFECT OF VARIOUS AGENTS ON THE BINDING OF SEROTONIN TO BRAIN HOMOGENATES

Brain homogenates of untreated white Swiss, male mice were preincubated in 0.5 mM pargyline for 20 min. After preincubation the samples were incubated 10 min at 30°C with [^{14}C]-serotonin, 2×10^{-6} M, specific activity 56 Ci/mole in presence and absence of displacing agents. Subsequently, incubation was continued in presence and absence of sodium borohydride for 30 min. The washed precipitates were counted in a TriCarb scintillator. The percentage of displacement was calculated from the decrease of the binding, stabilized by borohydride, in the presence of displacing agents as compared to the stabilized binding of incubations having no displacing agents. Incubation at 0°C was done to prevent the rapid oxidation of ortho hydroxyl groups. Values are the means ± standard deviations of 3 experiments performed in duplicates. Samples used in each of the 3 experiments derived from pooled homogenates, obtained from a group of ten mice. Significance of difference from controls: $p < 0.001$, except for salsolinol: $p < 0.01$ and for tetrahydropapaveroline: $p < 0.5$, for d-tubocurarine: $p < 0.1$ and for β-phenylethylamine: $p < 0.01$.

Displacing agent	Binding **Δ dpm/g brain	% displacement
None	102207 ± 2438	0
D-Lysergic acid diethylamide (d-LSD-25)	81837 ± 4538	20
1-Methyl-d-lysergic acid butanolamide (methysergide)	82646 ± 1538	19
β-Phenylethylamine	88016 ± 3534	14
Tyramine	81204 ± 5899	21
5-Hydroxy-N,N-dimethyltryptamine (bufotenine)	44971 ± 3823	56
3-[(Dimethylamino)-methyl]-indole (gramine)	101696 ± 4068	0
Morphine sulfate	64390 ± 2761	37
D-Tubocurarine	83215 ± 10415	19
None *	15234 ± 1613	0
1-Methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol) *	8265 ± 695	46
Tetrahydropapaveroline *	12443 ± 2715	18

* Incubation at 0.

** ($NaBH_4$ present minus $NaBH_4$ absent).

TABLE 2

Effect of various agents on the binding of serotonin and tryptamine to calf brain synaptic membranes.

Membranous material, obtained from 1.2 M sucrose discontinuous gradients of calf thalamus synaptosomes was incubated 10 min at 30°C with [^{14}C]-serotonin or [^{14}C]-tryptamine in presence and absence of displacing agents, in 0.1 M bicarbonate buffer pH 8.9, 0.7 mM pargyline. Subsequently, incubation was continued in presence and absence of $NaBH_4$ for 30 min. After incubation the samples were washed 5 times with ten per cent trichloroacetic acid and counted in a Tri-Carb liquid scintillator. Specific radioactivity of [^{14}C]-serotonin and [^{14}C]-tryptamine was 13.7 Ci/mole. The percentage of displacement was calculated from the decrease of the binding, stabilized by sodium borohydride in presence of competitors as compared to the stabilized binding in absence of them. The dissociation constants and displacement constants were determined by double reciprocal graphs, plotting the binding stabilized by $NaBH_4$ as a function of the concentrations of indoleamines in presence and absence of the antagonists. Values are the means ± standard deviations of 6 experiments. Significance of difference from controls: $p < 0.001$.

Concentration (mole/liter)		Binding * Δ dpm/mg protein	Displacement constants (M)	% Displacement
Displacing agent	Indoleamine			
	Serotonin			
None	0.5×10^{-5}	401 ± 48		0
None	1.0×10^{-5}	586 ± 58		0
5-Hydroxy-N,N-dimethyl tryptamine (bufotenine)			0.8×10^{-4}	
2.5×10^{-4}	0.5×10^{-5}	52 ± 5		87
2.5×10^{-4}	1.0×10^{-5}	82 ± 9		86
N,N-Dimethyltryptamine				
2.5×10^{-4}	0.5×10^{-5}	481 ± 72		0
2.5×10^{-4}	1.0×10^{-5}	703 ± 126		0
Morphine			4.2×10^{-4}	
5.0×10^{-4}	0.5×10^{-5}	204 ± 24		49
5.0×10^{-4}	1.0×10^{-5}	336 ± 44		43
Chlorpromazine			8.9×10^{-4}	
5.0×10^{-4}	1.0×10^{-5}	404 ± 68		31
	Tryptamine			
None	0.5×10^{-5}	488 ± 43		0
None	1.0×10^{-5}	830 ± 47		0
5-Hydroxy-N,N-dimethyl-tryptamine (bufotenine)			2.0×10^{-4}	
2.5×10^{-4}	0.5×10^{-5}	244 ± 24		50
2.5×10^{-4}	1.0×10^{-5}	440 ± 26		47
N,N-Dimethyltryptamine			1.3×10^{-4}	
2.5×10^{-4}	0.5×10^{-5}	190 ± 28		61
2.5×10^{-4}	1.0×10^{-5}	333 ± 46		60

* $NaBH_4$ present minus $NaBH_4$ absent.

ent chemical structure and pharmacological effect.

Results presented in table 2 show a correlation between chemical structure of the psychotomimetic drugs bufotenine and N,N-dimethyltryptamine (DMT) and their capability to displace serotonin or tryptamine from their binding to synaptic membranes.

Scatchard plots of the binding of 5-HT or tryptamine in presence and absence of the drugs mentioned in table 2 are shown in figs. 1 and 2.

The amount of binding was plotted as a function of the concentrations of the indoleamines. The association constants of 5-HT and tryptamine, determined from extrapola-

tion of these plots, reflect on the higher affinity of 5-HT for nerve ending membranes, compared to that of tryptamine. As can be seen from fig. 1, DMT increases to some extent the binding of 5-HT, stabilized by sodium borohydride.

We also used equilibrium dialysis to study the binding of 5-HT and tryptamine to bovine thalamus preparations, being primarily concerned with the investigation of high affinity sites of the binding. We established (table 3) the presence of a high affinity site in homogenates for 5-HT, concentration range (1×10^{-7} to 5×10^{-6} M), and a low one for the concentration range (5×10^{-6} to 1×10^{-4} M) at pH 7.4 and 9.0. For tryptamine concentrations (1×10^{-7} to 1×10^{-5} M) we have only the evidence of a medium affinity site. Other binding sites could not be estimated. The synaptic membranes (1 M sucrose layer) exhibited a high affinity site for 5-HT, concentration range (5×10^{-8} to 5×10^{-6} M). We could not determine, however, a low affinity site in the membranes.

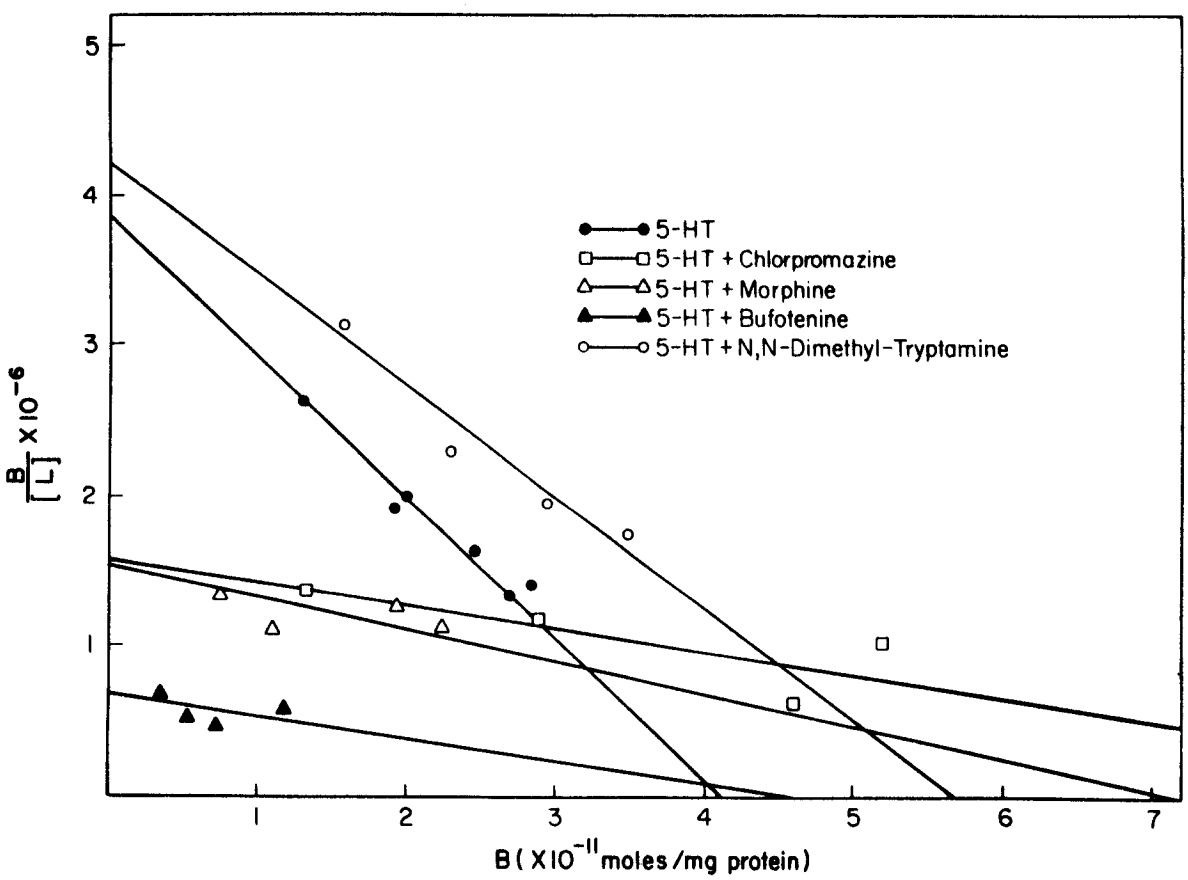

Fig. 1. Scatchard plots of the binding of [^{14}C]-serotonin to bovine thalamus postsynaptic membranes in absence and presence of chlorpromazine (5×10^{-4} M), morphine (5×10^{-4} M) bufotenine (1×10^{-4} M) and N,N-dimethyltryptamine (2.5×10^{-4} M). B = binding of 5-HT, stabilized by $NaBH_4$, [L] = molar concentration of 5-HT. Values are the means of 3 experiments. Approximate —K association of 5-HT = 0.9×10^5 M.

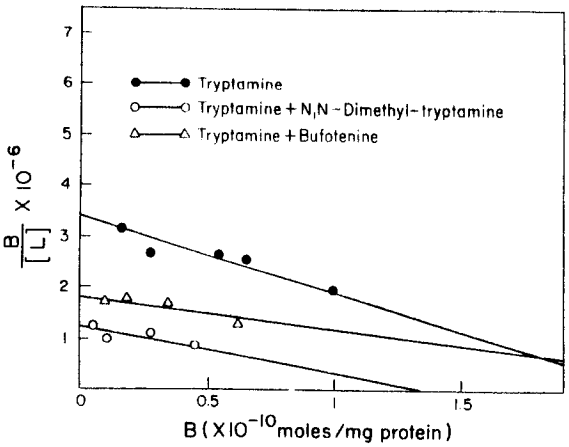

Fig. 2. Scatchard plots of the binding of [^{14}C]-tryptamine to bovine thalamus postsynaptic membranes in absence and presence of bufotenine (1×10^{-4} M) and N,N-dimethyltryptamine (2.5×10^{-4} M), B = binding of tryptamine, stabilized by $NaBH_4$, [L] = molar concentration of tryptamine. Values are the means of three experiments. Approximate —K association of tryptamine = 1.5×10^4 M.

Results in table 3 indicate that the binding affinity of 5-HT and tryptamine is greater at pH 9 than at pH 7.4.

In fig. 3 the demonstration of two slopes in the case of 5-HT indicates two binding sites in bovine homogenates, one of high affinity, the other of low affinity. The Scatchard plot for the binding of tryptamine, however, shows only one binding site. These results also reveal the implication of the 5-hydroxyl group of 5-HT in contributing to the binding of the indoleamine.

In order to determine the chemical nature of the binding macromolecule, we explored the effects of trypsin and phospholipase C on the binding of 5-HT to bovine thalamus homogenates, using the equilibrium dialysis method. It was found that after phospholipase C or trypsin digestion the binding of 5-HT was decreased 38 and 40%, respectively.

TABLE 3

Binding constants and concentration of binding sites of 5-hydroxytryptamine and tryptamine in calf thalamus preparations.

Equilibrium dialysis of calf thalamus homogenates was performed at pH 7.4 and 9.0, using 4 mg protein, whereas 1 mg protein was used in the case of the 1 M sucrose layer membrane fraction. Equilibrium dialysis and reversibility studies were performed as described in the text. The dissociation constants and maximal binding of the ligands were computed from Scatchard plots. Values are the means of 3—4 experiments for reversibility determinations. Points of the Scatchard plots are either from 2 or 3 experiments. Concentrations of the binding sites of the ligands are equal to the intercepts on the abscissa in the Scatchard plots, expressing the maximal binding with a characteristic dissociation constant of the ligand's molecules to a certain binding site (see fig. 3) and (Eldefrawi et al., 1971).

Ligand	Tissue preparation	Dissociation constant, K(M)		Concentration of binding sites $\times 10^{-7}$ moles/g tissue		Observed reversibility (%)	
		pH 7.4	pH 9.0	pH 7.4	pH 9.0	pH 7.4	pH 9.0
5-Hydroxy-tryptamine	Calf thalamus homogenate	$K_1 = 3.00 \times 10^{-6}$ $K_2 = 1.28 \times 10^{-4}$	$K_1 = 0.80 \times 10^{-6}$ $K_2 = 2.17 \times 10^{-5}$	0.10 1.67	0.13 2.80	75	85
Tryptamine	Calf thalamus homogenate	$K = 1.33 \times 10^{-5}$	$K = 1.20 \times 10^{-5}$	1.80	2.20	96	93
5-Hydroxy-tryptamine	1 M sucrose layer membrane fraction	$K_1 = 1.00 \times 10^{-6}$		0.00035		76	

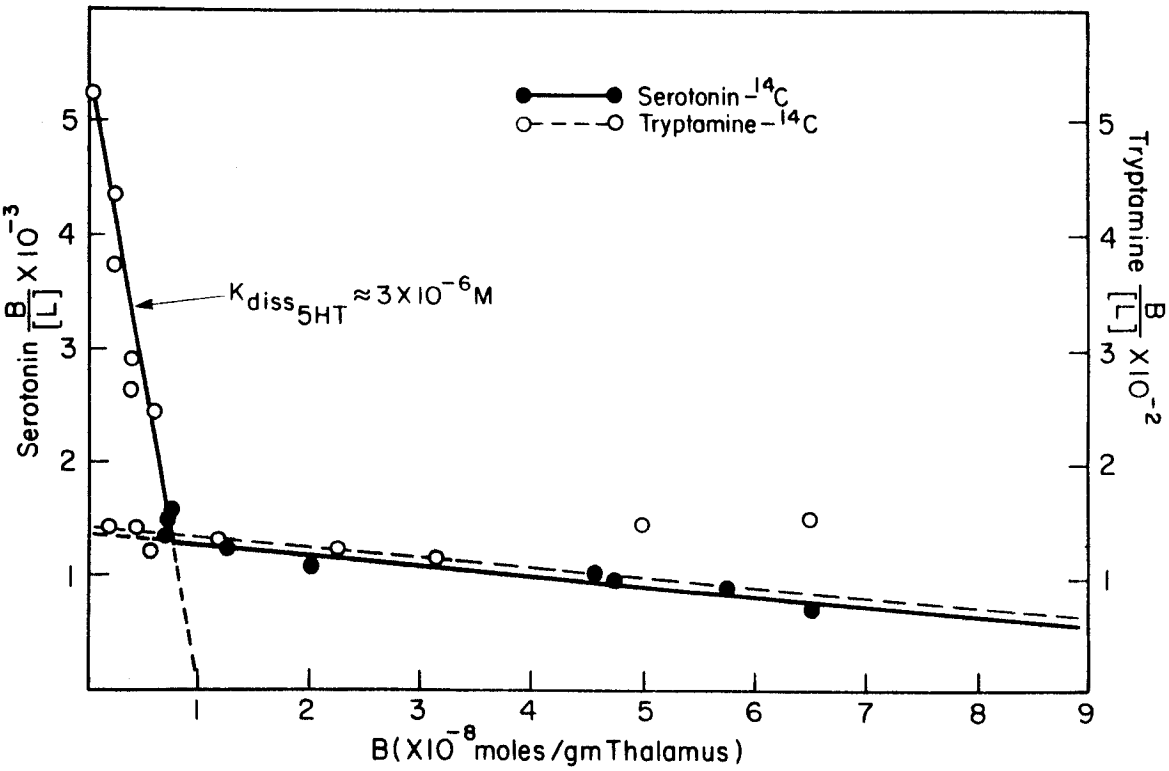

Fig. 3. Scatchard plots of the binding of [^{14}C]-serotonin and [^{14}C]-tryptamine to calf thalamus homogenates. Points are either from 2 or 3 experiments, [L] is molar concentration of ligands, B is amount bound in 10^{-8} moles/g thalamus. Binding of ligands was studied by equilibrium dialysis as described in the text.

4. Discussion

We attempted to elucidate the specificity and reversibility of the binding of 5-HT in the central nervous system by studying the displacement capability of various drugs upon 5-HT binding to brain preparations. The various displacing agents have certain structural features, similar to 5-HT, such as the indole nucleus, or any other unsaturated, resonating ring structures, electron releasing functional groups like the phenolic hydroxyl or a halogen, a primary ethylamine moiety, or secondary and tertiary amine structures.

We tried, therefore, in our studies to establish any structure—displacement relationship, comparing our blocking results with known pharmacological data of 5-HT antagonism. Drugs attach themselves reversibly in the CNS by weak covalent bonds, electrostatic attractions and repulsions (Alivisatos et al., 1971), hydrogen bonds, hydrophobic interactions, charge transfer complexing, van der Waals forces, surface adsorption and other types of binding (free energy of formation of each individual bond approx. —2 to —7 kcal/mole).

As stated in our previous work (Alivisatos et al., 1971) the 3-ethylamine residue of 5-HT, which is able to form a Schiff base, and the 5-hydroxyl are essential for the strong attachment of the agonist to its binding site, the phenolic OH increasing considerably the firmness of the 5-HT binding. In addition, the indole ring of 5-HT is only weakly attached to the binding macromolecule, due to hydrophobic segregation and charge transfer complexing.

In the present work, a few generalizations

may possibly be deducted in reference to structure—displacement relationship. As indicated in table 1, various drugs, used in same concentrations, exhibited different displacement capabilities. LSD-25, a powerful stimulant of the central nervous system, is structurally related to 5-HT, having an indolethylamine moiety as part of its bulky, hydrophobic structure which may cause steric hindrance. The N-substituted heterocyclic, saturated ring has two asymmetric carbons, carbon 5 and carbon 8; only d-LSD has psychotomimetic activity. The 20% displacement achieved by d-LSD is rather modest compared to its powerful, competitive antagonism, exerted on the contractile effects of 5-HT on isolated rat uterus. Less specific and more feeble is its action on guinea pig ileum (Gaddum and Picarelli, 1957). Bennett and Aghajanian (1974) postulated the existence of a specific d-LSD binding site associated with a 5-HT receptor and an unspecific one in the CNS. Our results may indicate the possibility that d-LSD binds, besides to 5-HT binding sites, also to unspecific sites.

Boakes et al. (1970) found that LSD-25 antagonized the 5-HT excitation of neurons in the brain stem of the cat. Andén et al. (1968) suggested that LSD-25 interacted with 5-HT receptors of postsynaptic membranes by competing with 5-HT for common receptors (D-receptors). The LSD-25 congener, the N^1-methylated compound, methysergide, has like LSD-25 only a moderate displacement effect (19%). It inhibits, however, strongly the vasoconstrictor and pressor effects of 5-HT and its action on various smooth muscles.

β-Phenylethylamine, the parent compound of the sympathomimetic amines, possesses an unsubstituted benzene ring and an aliphatic side chain, ethylamine. Its small, probable effect of 5-HT antagonism may be due to the action of the primary amine, which is also able to form a reversible Schiff base. Antagonism by tyramine (4-hydroxyphenylethylamine) is bigger because of its phenolic OH group. Tyramine is known to have indirect actions by releasing norepinephrine, which antagonizes the effects of 5-HT on the isolated colon of rat (Garattini and Valzelli, 1965). Both amines are vasoconstrictor and CNS stimulating agents like 5-HT. Bufotenine, which is the N-dimethylated congener of 5-HT, having all three structural features of the latter compound, namely, the indole ring, the 5-OH and an ethylamine side chain, proved to be the most effective antagonist of 5-HT, displacing it 56%. As at the specific binding site of 5-HT or tryptamine there may be two juxtaposed groups, one negatively charged (e.g., a $-COO^-$) and a carbonyl residue, the indolethylamine side chain could either bind as salt or as a Schiff base (resonant alternatives). Bufotenine would presumably exert its antagonistic effect mainly by salt formation with a $-COO^-$ group and by its 5-OH attachment. Bufotenine has also been reported to produce psychotomimetic effects in man (Fabing and Hawkins, 1956). Gramine, an indole with a N-dimethylated methylamine side chain, lacks the phenolic OH. Also, the lack of an additional methylene group accounts for weaker accommodation of the side chain and therefore for a smaller binding affinity. It did not displace 5-HT. 5-Benzyloxygramine, however, antagonizes the action of 5-HT on rat uterus (D-receptors) strongly, having only a small effect on M-receptors (Gaddum and Picarelli, 1957). Statistical evaluation of results fail to show significance of the displacement effects of d-tubocurarine and tetrahydropapaveroline.

The alkaloid morphine has a complex ring system which includes a furan ring, a cyclohexane ring and the N-methyl-γ-phenylpiperidine. Morphine having one phenolic, 3-OH and one alcoholic 6-OH group in its phenantrene nucleus and a tertiary amine in its piperidine ring, possesses similar structural elements to 5-HT, displacing it considerably (37%). Morphine blocks the stimulatory effects of 5-HT on the inferior mesenteric ganglion of the cat (Gyermek and Bindler, 1962) and on the isolated guinea pig ileum (Gaddum and Picarelli, 1957). 5-HT may be involved in the development of morphine tolerance and

physical dependence.

Salsolinol, a condensation product of dopamine with acetaldehyde, has a benzene ring with two ortho OH groups and a heterocyclic ring, forming a secondary amine. This structural relationship with 5-HT seems to cause a significant decrease of the latter binding, 46%. Tetrahydroisoquinoline alkaloids of the salsolinol type have both central nervous system depressant or stimulant actions, and various effects on smooth muscle like 5-HT (Yamanaka et al., 1970).

The significant decrease of the binding of 5-HT in the presence of morphine and salsolinol indicates an interaction of these drugs with serotonergic binding sites in the central nervous system. The competition between 5-HT and these alkaloids for common CNS binding sites is consistent with the postulated suggestions by Collier (1966) that development of tolerance to opiates may depend on the number of receptors, reacting with these substances. Furthermore, the biosynthesis of salsolinol was demonstrated recently (Sandler et al., 1973) and might have also a bearing on the relation of this compound with 5-HT in the CNS. As evident from results in table 2, bufotenine due to its phenolic OH displaces considerably the binding of serotonin, as well as of tryptamine while N,N-dimethyltryptamine, lacking a hydroxyl group does not displace the firm binding of 5-HT. However, its presence diminishes the less firm binding of tryptamine, the latter binding being probably connected with other receptors. That tryptamine has another binding site than 5-HT was also deducted from equilibrium dialysis studies which show for tryptamine only one binding site of medium affinity in contrast to 5-HT for which we demonstrated also a high affinity binding site. As the existence of endogenous tryptamine in the brain was confirmed, this amine might have functions of its own in the CNS. Smythies et al. (1972) suggested that N,N-dimethyltryptamine and LSD-25 act on the 5-HT receptors in the brain. The implication of the short acting psychological effect of DMT is that the drug is rapidly metabolized in the body (Szara, 1956). Bufotenine and DMT were reported to mimic as well as block the effects of 5-HT on rat brainstem neurons (Bradley and Briggs, 1974).

Furthermore, morphine and chlorpromazine block the binding sites of 5-HT in membrane particles. Chlorpromazine (CPZ) was reported to have 5-HT blocking activity (Gyermek, 1966). CPZ has a three-ringed bulky structure in which two benzene rings are linked by a sulfur and a nitrogen. Chloro-substitution at position 2 increases the electron donor activity of the phenothiazine which itself has strong charge-transfer properties. At position 10, the substituent is a N-dimethyl-propylamine; the ring nitrogen forms a tertiary amine. All these structural features probably contribute to its antagonistic activity. CPZ is known to undergo extensive metabolic breakdown by liver enzymes. Posner et al. (1962) have reported on the pharmacological activity of CPZ metabolites. CPZ sulfoxide was the least active. Monodemethylation and hydroxylation resulted in active compounds. The N-oxide, hydroxy and monomethyl metabolites, possessing electron-releasing substituents (CH_3 or OH) should be capable of blocking the 5-HT binding sites (charge transfer complexing). The more polar compounds, formed by conjugation and sulfoxidation, having electron-withdrawing groups should have weaker antagonistic effects. They also would have difficulty in entering the CNS and have access to the 5-HT binding sites. In our studies, however, we used thalamus membrane particles. Metabolic changes of CPZ with such particles, to our knowledge, have not yet been reported.

As evident from table 2 the displacement constants give to some extent a quantitative index of the displacement capability of the agents used.

In our binding experiments we were primarily concerned with the biochemical aspect of these two phase chemical reactions. In order to promote these reactions in our model, in vitro experiments, we used higher concen-

trations of 5-HT and of its antagonists. It is often necessary to use higher concentrations in decompartmentalized in vitro systems for demonstrative purposes (Alivisatos and Denstedt, 1951).

Equilibrium dialysis studies show as expected (pH 9 is closer to the 9.6 pK-value of the aliphatic amine) that the higher pH favors Schiff base formation (table 3). Also, the reversibility of the binding would be due to a reversible imine formation, and therefore, greater in the case of tryptamine where the binding is mainly due to the aliphatic amine. From the results presented it is obvious that besides a non-specific binding (high dissociation constant) observed in homogenates, there is a much tighter one. This specific, high affinity site is localized at the membranous level. The dissociation constant of the binding of 5-HT to plasmalemal membranes at pH 7.4 points to a high affinity of 5-HT to its binding site. A high affinity binding is characteristic for a reversible neurotransmitter receptor complex (Cuatrecasas, 1974).

Preliminary results suggest a phospho-lipid-protein nature of the macromolecule involved in the binding of 5-HT. As reported previously (Arora et al., 1973) the binding material, associated with high affinity binding, was extractable with chloroform—methanol (2 : 1), confirming the postulate that it may be a proteolipid.

From the results above we can conclude that there is a membrane localized macromolecule of proteo-lipid structure to which 5-HT binds reversibly. Specific drugs compete with serotonin for this site of binding.

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References

Alivisatos, S.G.A. and O.F. Denstedt, 1951, Lactic dehydrogenase and DPNase activity of blood, Science 114, 281.

Alivisatos, S.G.A., A.D. Papaphilis, F. Ungar and P.K. Seth, 1970, Chemical nature of binding of serotonin in the central nervous system, Nature 226, 455.

Alivisatos, S.G.A. and P.K. Seth, 1971, Current approaches in the study of receptors in the CNS, in: Methods in Neurochemistry, Vol. 1, ed. R. Fried (M. Dekker, Inc., New York) p. 205.

Alivisatos, S.G.A., F. Ungar, P.K. Seth, L.P. Levitt, A.J. Geroulis and T. Meyer, 1971, Receptors: Localization and specificity of binding of serotonin in the central nervous system, Science 177, 809.

Andén, N.-E., H. Corrodi, K. Fuxe and T. Hökfelt, 1968, Evidence for a central 5-hydroxytryptamine receptor stimulation by lysergic acid diethylamine, Brit. J. Pharmacol. 34, 1.

Arora, R., A.J. Geroulis, B. Tabakoff, F. Ungar and S.G.A. Alivisatos, 1973, Serotonergic receptors from brain: Further purification and characteristics, Federation Proc. 32, 695.

Azcurra, J.M. and E. De Robertis, 1967, Binding of dimethyl-^{14}C-d-tubocurarine methyl-^{14}C-hexamethonium, and ^{3}H-alloferine by isolated synaptic membranes of brain cortex, Intern. J. Neuropharmacol. 6, 15.

Bennett, J.L. and G.K. Aghajanian, 1974, D-LSD binding to brain homogenates: Possible relationship to serotonin receptors, Life Sci. 15, 1935.

Boakes, R.J., P.B. Bradley, I. Briggs and A. Dray, 1970, Antagonism of 5-hydroxytryptamine by LSD 25 in the central nervous system: A possible neuronal basis for the actions of LSD 25, Brit. J. Pharmacol. 40, 202.

Bradley, P.B. and I. Briggs, 1974, Actions of serotonin and related substances on single neurons in the brain, in: Advances in Biochemical Psychopharmacology, Serotonin: New Vistas, Vol. 10, eds. E. Costa, G.L. Gessa and M. Sandler (Raven Press, New York) p. 159.

Changeux, J.R., J. Thiery, Y. Tung and C. Kittle, 1967, On the cooperativity of biological membranes, Proc. Nat. Acad. Sci., U.S.A. 57, 335.

Collier, H.O.J., 1966, Tolerance, physical dependence and receptors. A theory of the genesis of tolerance and physical dependence through drug-induced changes in the number of receptors, Advan. Drug Res. 3, 171.

Cuatrecasas, P., 1974, Membrane receptors, in: Annual Review of Biochemistry, Vol. 43, eds. E.E. Snell, P.D. Boyer, A. Meister, C.C. Richardson (Annual Reviews, Inc., Palo Alto, California) p. 169.

Dawson, R.M.S., D.C. Elliot, W.H. Elliot and K.M. Jones, 1959, Buffers and physiological media, in: Data for Biochemical Research (Oxford Univ. Press, Oxford) p. 208.

De Robertis, E., A. Pellegrino de Iraldi, G. Rodriguez de Lores Arnaiz and L. Salganicoff, 1962, Cholinergic and non-cholinergic nerve endings in rat brain-I: Isolation and subcellular distribution of acetylcholine and acetylcholinesterase, J. Neurochem. 9, 23.

Eldefrawi, M.E., A.T. Eldefrawi and R.D. O'Brien, 1971, Binding sites for cholinergic ligands in a particulate fraction of electrophorus electroplax, Proc. Nat. Acad. Sci., U.S.A. 68, 1047.

Fabing, H.D. and J.R. Hawkins, 1956, Intravenous bufotenine injection in the human being, Science 123, 886.

Gaddum, J.H. and Z.P. Picarelli, 1957, Two kinds of tryptamine receptor, Brit. J. Pharmacol. 12, 323.

Garattini, S. and L. Valzelli, 1965, Serotonin, (Elsevier Publishing Company, Amsterdam—New York) p. 89.

Gyermek, L., 1966, Drugs which antagonize 5-hydroxytryptamine and related indolealkylamines, in: Handbook of Experimental Pharmacology, Vol. XIX, 5-Hydroxytryptamine and Related Indolealkylamines, ed. V. Erspanner, (Springer-Verlag, New York) p. 471.

Gyermek, L. and E. Bindler, 1962, Blockade of the ganglionic stimulant action of 5-HT, J. Pharmacol. Exptl. Therap. 135, 344.

Klotz, J.M., 1953, Protein interactions, in: The Proteins: Chemistry, Biological Activity, and Methods, Vol. 1, eds. H. Neurath and K. Bailey (Academic Press, New York) p. 27.

Klotz, J.M., H. Triwush and F.M. Walker, 1948, The binding of organic ions by proteins. Competition phenomena and denaturation effects, J. Amer. Chem. Soc. 70, 2935.

Lineweaver, H. and D. Burk, 1934, The determination of enzyme dissociation constants, J. Amer. Chem. Soc. 56, 658.

Marchbanks, R.M., 1966, Serotonin binding to nerve ending particles and other preparations from rat brain, J. Neurochem. 13, 1481.

O'Brien, R.D., L.P. Gilmour and M.E. Eldefrawi, 1970, A muscarone-binding material in electroplax and its relation to the acetylcholine receptor, II. Dialysis assay, Proc. Nat. Acad. Sci., U.S.A. 65, 438.

Posner, H.S., E. Hearst, W.L. Taylor and G.J. Cosmides, 1962, Model metabolites of chlorpromazine and promazine: Relative activities in some pharmacological and behavioral tests, J. Pharmacol. Exptl. Therap. 137, 84.

Sandler, M., S.B. Carter, K.R. Hunter and G.M. Stern, 1973, Tetrahydroisoquinoline alkaloids: in vivo metabolites of L-dopa in man, Nature 241, 439.

Smythies, J.R., J.M. Beaten, F. Bennington and R.D. Morin, 1972, The design of some new compounds to block psychotomimetic drugs, European. J. Pharmacol. 17, 270.

Szara, S., 1956, Dimethyltryptamine: Its metabolism in man; the relation of its psychotic effect to the serotonin metabolism, Experienta 12, 441.

Whittaker, V.P., 1959, The isolation and characterization of acetylcholine-containing particles from brain, Biochem. J. 72, 694.

Yamanaka, Y., M.J. Walsh and V.E. Davis, 1970, Salsolinol, an alkaloid derivative of dopamine formed in vitro during alcohol metabolism, Nature 227, 1143.