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Psychopharmacology 59, 1 – 5 (1978)

Psychopharmacology

High-Affinity ^{3}H-Serotonin Binding to Caudate: Inhibition by Hallucinogens and Serotoninergic Drugs

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Abstract. The specific binding of ^{3}H-serotonin to calf caudate homogenate was studied. The dissociation constant was 2 nM and the number of specific sites was 14 fmoles/mg protein. Of many drugs tested, inhibition of specific ^{3}H-serotonin binding occurred almost exclusively with serotonin agonists and antagonists. The concentrations for 50% inhibition of ^{3}H-serotonin binding by serotonergic agonists follow: bufotenin, 6 nM; 5-methoxytryptamine, 12 nM; psilocin, 35 nM; dimethyltryptamine, 220 nM; and tryptamine, 270 nM. The concentrations for the antagonists were: LSD 9.5 nM; methysergide 16 nM and metergoline 25 nM.

Key words: Hallucinogens – LSD – Receptors – Psychotomimetics

In order to examine the role of serotonin receptors in the pharmacological actions of drugs, it is desirable to have a radio-receptor assay with a low dissociation constant (i.e., high affinity) for ^{3}H-serotonin. Earlier studies on the binding of ^{3}H-serotonin reported dissociation constants of 500 µM (Marchbanks, 1967) and 9.7 nM (rat striatum; Bennett and Snyder, 1976). More recently, Fillion et al. (1976) described a distinct set of synaptosomal high-affinity binding sites for ^{3}H-serotonin with a dissociation constant of 1.1 nM. Because Fillion et al. tested only a few drugs on this high-affinity site, and since a wide variety of drugs have recently been proposed as directly affecting serotoninergic systems (quipazine, Hong et al., 1969; metergoline, Sastry and Phillis, 1977; methaqualone, Boggan, 1977; ketamine, Azzaro and Smith, 1977; fenfluramine, Neckers et al., 1976; morphine, Burks and Dafny, 1977; propranolol, Weinstock et al., 1971; phentolamine, Carroll et al., 1977), we decided to characterize this high-affinity site by testing various serotoninergic drugs on the specific binding of ^{3}H-serotonin to this site.

Materials and Methods

Preparation of Membranes. Calf brains were obtained fresh from the Canada Packers Hunisett plant (Toronto). The caudates were removed within 2 h after death, pooled, sliced into small cubes, and suspended in buffer at an approximate concentration of 50 mg of wet weight per ml of buffer. The buffer contained 15 mM Tris HCl (pH 7.4), 5 mM Na_2 EDTA, 1.1 mM ascorbate and 12.5 µM nialamide. A preliminary crude homogenate of the suspension was made with a glass homogenizer with a teflon piston (0.13 – 0.18 mm clearance); this piston, rotating at 500 rpm, was passed up and down 20 times. The crude homogenate was incubated at 37° for 60 min, and then stored in 5-ml aliquots at – 20° C for future use. Before using, the samples were thawed, resuspended with an additional 5 ml of buffer, and centrifuged at 39000 × g for 15 min at 4°. The supernatant was discarded, and the pellet resuspended in 15 ml of buffer, using a ground glass homogenizer for 5 up-and-down strokes. This suspension was then homogenized by a Polytron homogenizer (Brinkmann Instrument Co.) at a setting of 7 (full range = 10) for 10 s, using a PT-10 homogenizer probe, and a 50 ml polycarbonate tube to contain the suspension. This resulted in a final protein concentration of 0.2 mg per tube. Because the membrane homogenate was made up immediately prior to use, it was not necessary to store it on ice.

The Specific Binding of ^{3}H-Serotonin. The [^{3}H]-serotonin binding assays were done using 12 × 75 mm glass test tubes, in which the following aliquots were placed (using Eppendorf Brinkmann pipettes with polypropylene tips): 0.1 ml of serotonin oxalate (final concentration of 100 nM) or 0.1 ml of buffer; 0.1 ml of buffer with or without different concentrations of various drug competitors; 0.2 ml of [^{3}H]-serotonin (25.6 Ci/mmole; New England Nuclear Corp., Boston; final concentration of 1.0 nM); 0.2 ml of brain homogenate. Each determination was done in quintuplicate, with five tubes containing serotonin, and five containing buffer. After the tubes were incubated for 30 min[1] at room temperature (22° C), an aliquot of 0.5 ml was removed from each tube, and filtered, by vacuum, through a glass fibre filter (GF/B, Whatman, 24 mm diameter) using a Millipore stainless steel mesh support for the filter. The filter was then washed with 5 ml of buffer at room temperature. The wash was delivered by gravity from a syringe re-pipette in under

[1] Maximum specific binding occurred within 20 min with no further increase up to 1 h incubation

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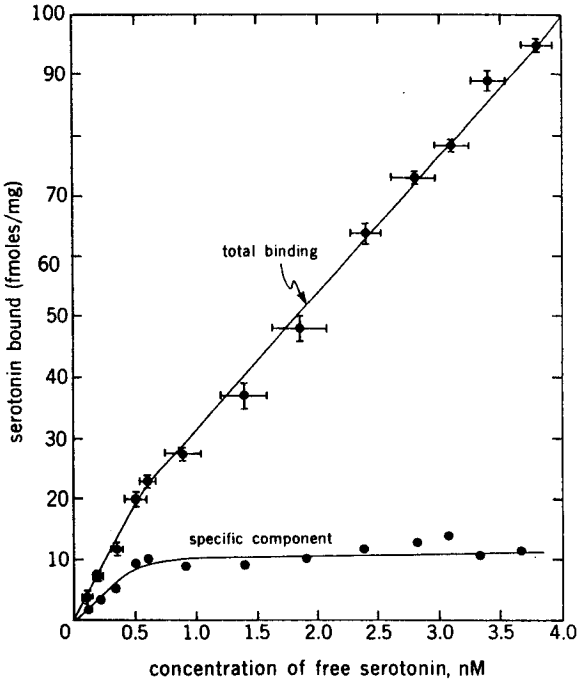


Fig. 1. Adsorption isotherm (22° C) of ^{3}H-serotonin to crude homogenate of calf caudate nucleus. Specific binding of ^{3}H-serotonin is total bound minus that occurring in presence of 100 nM serotonin. The high-affinity sites were detected in the 0.5 – 2 nM region. *Vertical bars:* SEM. *Horizontal bars:* range of concentrations tested. *Each point:* average of 4 or 5 experiments, each in sextuplicate

2 s. The filter was placed into a liquid scintillation vial, 9 ml of aquasol (New England Nuclear Corp.) was added, and the filters monitored for ^{3}H (after 6 h, during which time the filters became translucent). Specific binding of serotonin was defined as the total amount bound (in the absence of an excess concentration of non-radioactive serotonin) minus that bound in the presence of 100 nM non-radioactive serotonin.

Drugs. All drugs used in this study were HCl salts, except amphetamine sulphate, atropine sulphate, dihydroergotamine tartrate, sodium diphenylhydantoin, methysergide hydrogenmaleate, morphine sulphate, adrenochrome semicarbazine, bufotenine monooxalate hydrate, 5,6-dihydroxytryptamine creatinine sulphate, p-chlorophenylalanine methyl ester, tranylcypromine sulphate, mescaline sulphate, and sodium phenobarbital.

Results

The adsorption of ^{3}H-serotonin to calf caudate homogenate is shown in Figure 1, and the data indicate specific and nonspecific ^{3}H-serotonin binding sites. The high-affinity sites were investigated separately by using a range of ^{3}H-serotonin concentrations, and these results are graphed in the form of a Scatchard analysis in Figure 2. The dissociation constant was 2 nM and the number of specific sites was 14 fmoles/mg protein.

The effects of various drugs, including serotoninergic agonists and antagonists, on the specific binding of

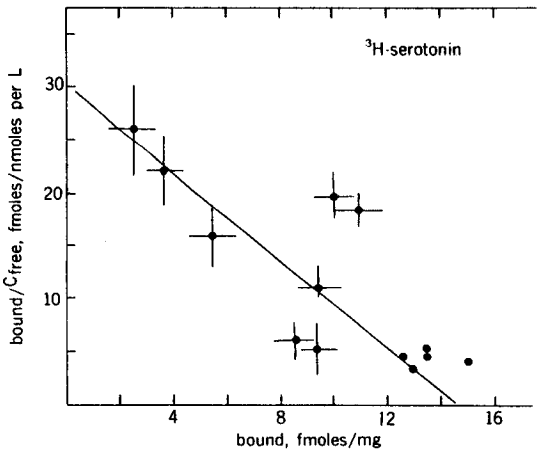


Fig. 2. Scatchard analysis of data in Figure 1, indicating that dissociation constant (K_D) for high affinity sites for ^{3}H-serotonin was 2 nM. The number of specific sites was 14 fmoles/mg protein. *Vertical and horizontal bars:* range of experimental values

^{3}H-serotonin is shown in Figure 3 and 4, and Table 1. Figures 3 and 4 indicate that the slopes for inhibition of binding are generally similar but not identical. The data in Figure 4 and Table 1 indicate that strong inhibition of binding was observed in those serotonin agonists that had at least one (but not two) polar groups substituted on the benzyl portion of the indole. With the exception of serotonin itself, none of the other neurotransmitters or their metabolites were active in competing versus ^{3}H-serotonin. Those drugs listed in Table 1 as having IC_{50} values over 1000 nM generally produced 10 – 30% inhibition of binding at 1000 nM; higher concentrations were not tested.

Discussion

The caudate was chosen for study here in order to compare results with those of Bennett and Snyder (1976), who found abundant serotonin sites under different experimental conditions, and in order to search for conditions that would selectively reveal serotonin sites despite the presence of other different types of receptors in the caudate. The sites described herein may differ from such sites in the cortex.

The binding sites for ^{3}H-serotonin were of high-affinity ($K_D = 2$ nM) and were highly specific for the binding of serotonin and its analogues. There was no interaction with the serotonin receptor by several general pharmacological groups of drugs, such as the anticonvulsants (diphenylhydantoin, diazepam), antidepressants (imipramine, nialamide, tranylcypromine), antihistamines (promethazine), or sedative-hypnotics (methaqualone, phenobarbital, meprobamate); there was a low potency displacement of serotonin by the

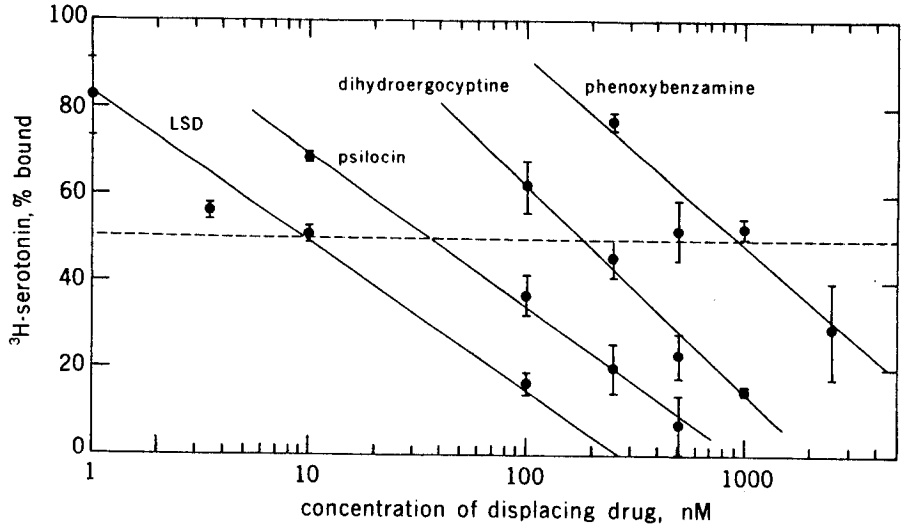


Fig. 3
Examples of various drugs inhibiting specific binding of ^{3}H-serotonin. *Vertical bars:* SEM

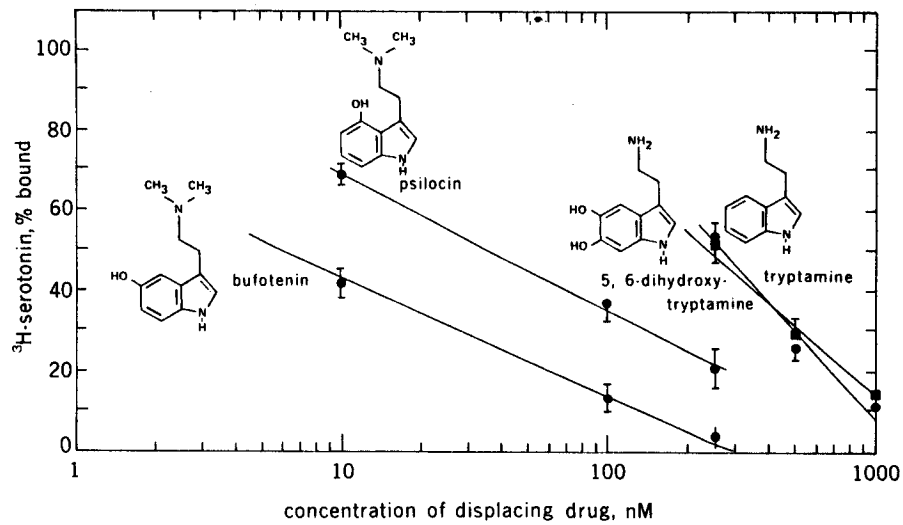


Fig. 4
Drugs having one hydroxyl in the 5-position on indole were potent in inhibiting specific binding of ^{3}H-serotonin; the IC_{50} (concentration for 50% inhibition) values for serotonin itself was 1.5 nM and that for bufotenin was 6 nM. Psilocin, having $-OH$ in 4-position was weaker, and tryptamine, without any indole $-OH$, was very weak. Dihydroxylated indoles, such as 5,6-dihydroxytryptamine, was also very weak. *Vertical bars:* SEM

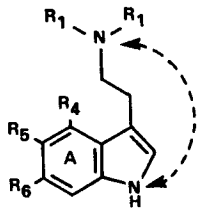
neuroleptics chlorpromazine and haloperidol, and a more potent displacement by the neuroleptic, butaclamol. These results do not, however, necessarily indicate involvement of the serotonergic receptor with neuroleptic activity (Whitaker and Seeman, 1977).

The sites labelled were probably similar to those detected by Fillion et al. (1976), on the basis of similar K_D and comparable inhibition by tryptamine and dimethyltryptamine. Although lysergic acid diethylamide and bufotenin inhibited ^{3}H-serotonin binding in this study as well as in the one by Bennett and Snyder (1976), it is unlikely that the sites in this study are the same as those studied by Bennett and Snyder (or Middlemiss et al., 1977). This is because there were other drugs that inhibited rather differently (e.g., psilocin, dimethyltryptamine) in these two studies. The assay of Bennett and Snyder, furthermore, used a sevenfold higher concentration of ^{3}H-serotonin (7 nM) and a 100-fold higher concentration of nonradioactive serotonin (10000 nM), all of which could have meant labelling of different set of receptors.

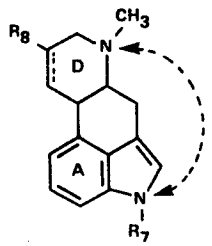
On the basis of binding potencies of the tested drugs, several observations of gross structure-activity relationships can be made. The presence of an hydroxy or methoxy group on position five of the benzene portion of the indole nucleus is necessary for activity equivalent to serotonin, while hydroxylation of the four position decreases potency to at least 1/10, and the lack of an hydroxy group altogether decreases potency to 1/100 (see tryptamine, psilocin, and serotonin).

The ethylamine side chain is necessary for binding activity, as can seen from the lack of inhibition by indole. Dimethylation of the nitrogen has little effect (tryptamine vs. dimethyltryptamine), however, and N-

Table 1. Effect of drugs on ^{3}H-serotonin binding



R_1	R_4	R_5	R_6
H	H	OH	H
CH_3	H	OH	H
CH_3	H	CH_3O	H
CH_3	OH	H	H
H	H	OH	OH
CH_3	H	H	H
H	H	H	H



	IC_{50} (nM)
Neuroleptics, others	
(+)-Butaclamol	120
Chlorpromazine	1000
Haloperidol	1000
Reserpine	1000
Phenoxybenzamine	900
Serotoninergic drugs, etc.	
Tryptophan	NE
5-Hydroxy, 3-indolacetic acid	NE
Indole	NE
Melatonin	NE
Serotonin uptake blockers	
Chlorimipramine	NE
Fluoxetine	NE
Imipramine	1000
Serotonin agonists	
Serotonin	2.5
Bufotenin	6
5-Methoxy, dimethyltryptamine	12
Psilocin	35
5,6-Dihydroxytryptamine	230
Dimethyltryptamine	220
Tryptamine	270
Quipazine	620
Serotonin antagonists	
LSD	9.5
Dihydroergotamine	8
Methysergide	16
Metergoline	25
Dihydroergocryptine	180
Mianserin	230

Effects of various serotoninergic drugs, agonists and antagonists, on specific binding of ^{3}H-serotonin (1 nM) to calf striatal homogenate. IC_{50} values are drug concentrations that inhibited specific binding by 50%. Strong agonist activity occurs in those drugs which have at least one (but not two) of the polar groups substituted on the A ring. D ring is saturated in dihydroergots; R_7 is methyl group in methysergide; R_8 is extensively substituted in all antagonists. Arc drawn between N atoms indicates probable critical distance (6.3 A; Lovell and Freedman, 1976) between sites necessary for attachment to serotonin receptor. The following drugs at 10^{-6} M inhibited binding by only 10–20%: (±)-propranolol, phentolamine, promethazine, apomorphine, and atropine. The following had no effect at 10^{-6} M: acetylcholine, dopamine, gamma-amino-butyric acid, noradrenaline, adrenochrome, histamine, tyramine, homovanillic acid, carbachol, clonidine, cocaine, deanol, diazepam, amphetamine, diphenylhydantoin, fenfluramine, harmaline, ibogaine, isoprenaline, ketamine, meprobamate, mescaline, methaqualone, methylphenidate. morphine, naloxone, nialamide, nicotine, pemoline, p-chlorophenylalanine, phenobarbital, and tranylcypromine
NE = no effect

acetylation appears to inactivate the molecule (5-methoxy-N,N-dimethyl-tryptamine vs. melatonin). The drugs that have an ergot nucleus, and which have significant activity (metergoline, methysergide, dihydroergotamine, dihydroergocryptine, LSD), hold the position of the side chain very differently from that position held in either ibogaine or harmaline, which have little or no activity at all.

The assay was very reproducible, and could quickly give the binding characteristics of a drug tested. According to the results of this study, therefore, fenfluramine, morphine, ketamine, methaqualone, and phentolamine do not have their effect on serotonergic systems due to any significant binding to central serotonin receptors. This does not, however, eliminate the possibility of active metabolites.

Acknowledgements. We thank Mrs. Joan Dumas, Mrs. Anna Banaszuk, and Mrs. M. Chau-Wong for their excellent assistance. We are grateful to Dr. R. A. Graham of the Health Protection Branch, Department of National Health and Welfare, Canada, for supplying us with DMT, psilocin, and LSD. The following drugs were also kindly donated: butaclamol HCl (Ayerst Research Laboratories); chlorimipramine HCl and imipramine HCl (Ciba-Geigy Canada Ltd.); fenfluramine (A. H. Robins Co.); fluoxetine (Lilly Research Laboratories); metergoline (Farmitalia); mianserin (Organon Labs); quipazine (Miles Laboratories, Inc.); amd methaqualone (Merck Frosst Laboratories). The Canada Packers Hunisett plant (Mr. J. Milkovich) generously donated calf brains. The work was supported by the Ontario Mental Health Foundation and the Medical Research Council of Canada.

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Received October 5, 1977; Final Version January 18, 1978