*J. Physiol., Paris, 1981, 77,* 351-362
Symposium The Serotoninergic Neuron
CNRS, Marseille, July 9-11, 1980

# Serotoninergic receptors in brain tissue : properties and identification of various $^3$H-ligand binding sites *in vitro*

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SUMMARY :

*In vitro* binding studies to serotoninergic receptors were performed using $^3$H-LSD, $^3$H-5-HT and $^3$H-spiperone. An overview is given on findings using these three ligands with respect to the following : (1) Localization of specific binding sites, *i.e.*, in various animal species, the regional distribution in the brain and periphery, the subcellular and cellular distribution. (2) Properties of the binding sites, *i.e.*, influence of the composition of the assay medium, binding kinetic properties, receptor regulation *in vivo*. (3) Identity of the binding sites, *i.e.*, differences between sites for various $^3$H-ligands, pharmacological specificity of the membranous binding sites, chemical composition of the macromolecular complex constituting the binding site. (4) Function of the receptor.

Binding affinities of 44 compounds were measured in binding assays using $^3$H-spiperone and $^3$H-LSD with rat frontal cortex membrane preparations and using $^3$H-5-HT and $^3$H-LSD with rat hippocampal membrane preparations.

A significant correlation between binding affinities for $^3$H-LSD and $^3$H-spiperone binding sites in the frontal cortex was found. Binding to these sites correlates with potencies of compounds to antagonize 5-HT-induced contraction in isolated rat caudal arteries and also with potencies of compounds to antagonize tryptamine-induced clonic seizures in rats.

There was a very significant correlation between binding affinities for $^3$H-LSD and $^3$H-5-HT labelled sites in the hippocampus. These hippocampal binding sites are different from those for $^3$H-LSD and $^3$H-spiperone in the frontal cortex. Binding to $^3$H-5-HT or $^3$H-LSD sites in the hippocampus could not be related to a pharmacological effect.

*Key-words :* Review. Serotoninergic receptors. *In vitro* binding. $^3$H-5-hydroxytryptamine. $^3$H-LSD. $^3$H-spiperone.

## INTRODUCTION

The biochemical study of specific binding sites on membranes for neurotransmitters or drugs, and the relationship between such sites and pharmacologically or physiologically defined receptors, became possible through the availability of highly radioactively labelled compounds, known as labelled ligands. This allowed detection of high affinity binding to membranes in suspension. *In vitro* binding studies were much facilitated by the empirical finding that labelled membranes were retained through adsorption on glass-fibre filters after vacuum filtration, thus providing a fast and simple technique for separating « bound » and « free » radioactivity (BENNETT and AGHAJANIAN, 1974). Two main criteria were imposed to define specific binding : saturability of the binding of the $^3$H-ligand at nanomolar concentrations, and specific inhibition of membrane labelling by unlabelled pharmacological or physiological agents for the envisaged receptor. The portion of the binding inhibited by excess of an unlabelled drug is called specific binding, the remaining membrane labelling represents non-specific binding.

In biochemical receptor research several goals are pursued. For the present review, we have considered four major aspects :

I. *Localization of the specific binding sites :* in various animal species ; regional distribution in the brain and the periphery ; subcellular and cellular distribution.

II. *Properties of the binding sites :* influence of the composition of the assay medium ; binding kinetic properties ; receptor regulation *in vivo*.

III. *Identity of the binding sites :* differences between sites for various $^3$H-ligands ; pharmacological specificity of the membranous binding sites ; chemical composition of the macromolecular complex constituting the binding site.

IV. *Function of the receptors.*

Serotoninergic receptors on membranes were first studied by biochemical means using $^3$H-lysergic acid diethylamide (LSD) to label specific binding sites. Using an equilibrium dialysis method, FARROW and VAN VUNAKIS (1972, 1973) demonstrated saturable and stereospecific d-LSD binding in rat cerebral cortex. The occurrence of high affinity stereospecific LSD binding sites in several rat brain areas was confirmed by BENNETT and AGHAJANIAN (1974) using the rapid filtration method. Ever since, membranous serotoninergic receptors have been widely studied using $^3$H-LSD, $^3$H-5-

* Reçu en première lecture le 10 juillet 1980, en deuxième lecture le 14 août 1980.

hydroxytryptamine (5-HT) and $^3$H-spiperone, mainly in brain tissue of various mammals, but also in peripheral tissues, lower animal species and cell cultures.

The present review is focussed on the investigation of brain serotoninergic receptors. An attempt is made to summarize the relevant literature, to indicate problems and gaps in our knowledge with respect to the four major points mentioned above.

The review is supplemented with some original work, recently performed in our laboratories, relating binding affinities of drugs for sites labelled by $^3$H-5-HT, $^3$H-LSD and $^3$H-spiperone in rat frontal cortex and hippocampus with the potencies of the drugs in *in vivo* and *in vitro* pharmacological tests.

## METHODOLOGY

In general, a similar technique was used by most researchers. Fresh or frozen brain tissue is homogenized and a particular membrane fraction is prepared by centrifugation techniques. The washed, and occasionally preincubated membrane fraction is suspended in high dilution (about 1 mg protein/ml) in a given assay medium at physiological pH. Membrane suspensions are incubated with nanomolar concentrations of a $^3$H-ligand in the absence or the presence of unlabelled drugs. When maximal binding is reached, the incubation mixture is rapidly filtered under vacuum through glass fibre filters and rinsed. Radioactivity on the filters is counted in a liquid scintillation counter. Specific $^3$H-LSD and $^3$H-5-HT binding was usually defined by the portion inhibited using excess unlabelled LSD or 5-HT respectively. Specific $^3$H-spiperone binding was defined by inhibition using unlabelled (+)-butaclamol or cinanserine. Binding data are analysed according to transformations of the law of mass action or first and second order rate equations (see SEGEL, 1975). Besides the binding assay, various other techniques are used in receptor research such as centrifugation, cell-lesion, enzyme treatment, solubilization and purification.

## I. — LOCALIZATION

The regional distribution in the brain of specific $^3$H-LSD and $^3$H-5-HT binding was studied in rats (BENNETT and AGHAJANIAN, 1974 ; BENNETT and SNYDER, 1976 ; FILLION *et al.*, 1976 ; NELSON *et al.*, 1978) and in monkeys (BENNETT and SNYDER, 1975), stereospecific $^3$H-spiperone binding was studied in rat brain (LADURON *et al.*, 1978). The $^3$H-5-HT binding was most enriched in the hippocampus and the striatum and less was found in cerebral cortex areas. In contrast, $^3$H-LSD binding was most enriched in the cerebral cortex and also occurred in high amounts in the striatum. In the latter area, however, $^3$H-LSD was shown to label dopaminergic receptors (BURT *et al.*, 1976). Serotoninergic receptors labelled with $^3$H-spiperone were most abundant in rat frontal cortex (LEYSEN and LADURON, 1977 ; LEYSEN *et al.*, 1978 *b*). Similar binding sites could be detected in the limbic areas (nucleus accumbens and tuberculum olfactorium) provided that binding to dopaminergic receptors was prevented by the addition of an excess of a selective dopamine antagonist (domperidone) or dopamine agonist (2-(N,N-dipropyl)amino-5,6-dihydroxytetralin or ADTN) (LEYSEN *et al.*, 1979 ; QUIK and IVERSEN, 1979). It thus appeared that the regional distribution of specific binding sites for the various $^3$H-ligands used for labelling serotoninergic receptors was different and did not correspond to the content of endogenous 5-HT nor to the distribution of 5-HT uptake sites in the brain.

Investigation of the subcellular distribution of the specific binding sites by diffential centrifugation revealed the highest enrichment in the microsomal fraction for all three ligands (BENNETT and SNYDER, 1975, 1976 ; LEYSEN *et al.*, 1978 *a*). However, by discontinuous gradient centrifugation of lysed mitochondrial fractions, $^3$H-5-HT and $^3$H-LSD binding were mostly found in the synaptosome ghost membrane fraction (BENNETT and SNYDER, 1975, 1976 ; FILLION *et al.*, 1978).

Cellular distribution of binding sites was investigated following various kinds of lesions in rat brain. Lesions of the mid-brain raphe nuclei by 5,6-dihydroxytryptamine did not produce a change in the maximal number of $^3$H-LSD or $^3$H-5-HT binding sites in the cortex (BENNETT and AGHAJANIAN, 1974 ; BENNETT and SNYDER, 1975, 1976) or the forebrain (FILLION *et al.*, 1978 ; SEGAWA *et al.*, 1979). However, after 5,7-dihydroxytryptamine lesions, $^3$H-5-HT binding sites were reported to be 40 % increased in the hippocampus and 25 % in the striatum. However, the latter appeared not to be a significant change (NELSON *et al.*, 1978). The $K_D$-values seemed not to be significantly altered in any of the studies. 5-HT uptake sites were always completely abolished, indicating that 5-HT neurons had been sufficiently lesioned. It was concluded that $^3$H-LSD and $^3$H-5-HT binding sites seemed not to occur on presynaptic membranes of these neurons. Following kainic acid lesions in rat striatum, high affinity $^3$H-5-HT binding disappeared and low affinity binding became apparent (FILLION *et al.*, 1979). Therefore, high affinity $^3$H-5-HT binding sites were assumed to be localized on striatal inter-neurons. Following 6-hydroxydopamine lesions of the nigrostriatal pathway, $^3$H-5-HT binding sites in the striatum remained unchanged, indicating that the sites were not localized on dopaminergic neurons (REISINE *et al.*, 1979). Effects of brain lesions on $^3$H-spiperone

binding to brain serotoninergic receptors have not yet been reported so far.

## II. — PROPERTIES

### A) In vitro *binding properties.*

Binding properties can be considerably influenced by assay conditions. In particular, $^3$H-5-HT binding seemed to be sensitive to the ionic composition of the medium. The specific binding showed a 20-35 % increase in 4 mM $Ca^{++}$ and a 30 % reduction in high concentrations of $Na^+$ and $K^+$ (BENNETT and SNYDER, 1975, 1976 ; NELSON *et al.*, 1978). $^3$H-LSD and $^3$H-spiperone binding were much less affected. However, we recently observed that in the presence of physiological concentrations of $Na^+$ (120 mM), $K^+$ (5 mM), $Mg^{++}$ (1 mM) and $Ca^{++}$ (2 mM) and of 10 µM pargyline and 0.1 % ascorbic acid, the non-specific binding of $^3$H-5-HT, $^3$H-LSD and $^3$H-spiperone was considerably reduced and specific binding of $^3$H-5-HT and $^3$H-LSD apparently increased with respect to the bindings measured in Tris buffer not containing these ions. This was not observed by others (GRIPENBERG and JANSSON, 1978). The effects of nucleotides on the *in vitro* binding is not clear. NELSON reported that 2 mM ATP with and without 2 mM $Mg^{++}$ decreased $^3$H-5-HT binding in rat forebrain by 50-70 % (NELSON *et al.*, 1978), whereas PEROUTKA did not find any effect of 1 mM ATP, ADP, AMP and GMP on $^3$H-5-HT, $^3$H-LSD and $^3$H-spiperone binding in rat frontal cortex (PEROUTKA *et al.*, 1979). In contrast, 1 mM GTP and GDP reduced $^3$H-5-HT binding by 40 % and $^3$H-LSD binding by 20 %, whereas $^3$H-spiperone binding was not affected. The guanine nucleotides appeared to decrease the apparent binding affinity of agonists without changing the number of binding sites (PEROUTKA *et al.*, 1979).

Even more confusing are the numerous reports on the investigation of binding affinities of the $^3$H-ligands. For $^3$H-5-HT, $K_D$-values, derived from Scatchard analysis, varied between 1.4 and 8 nM for high affinity binding, and occasionally, a concomitant lower affinity site ($K_D$ = 30 nM) was observed (FILLION *et al.*, 1978 ; SEGAWA *et al.*, 1979). The $K_D$-values of $^3$H-LSD ranged between 4 and 10 nM. Curved Scatchard plots were observed for $^3$H-spiperone binding in rat frontal cortex microsomal fraction ($K_D$-values : 0.6 and 1.4 nM), whereas plots were straight using the mitochondrial fraction ($K_D$ = 1.3 nM) (LEYSEN *et al.*, 1978 *a*). Using a total membrane fraction, PEROUTKA found a $K_D$-value of 0.7 nM (PEROUTKA and SNYDER, 1979). The various studies reported in the literature were performed using tissue from different species, different brain areas and subcellular fractions and variable assay conditions. This renders the comparison and the evaluation of the data nearly impossible. NELSON claimed that extensive washing and short preincubation of the membranes was necessary to remove endogenous 5-HT, resulting in a 4-fold increase in the apparent binding affinity of $^3$H-5-HT (NELSON *et al.*, 1978). We have recently noticed that $K_D$-values vary considerably amongst several batches of $^3$H-5-HT, even when impurities in the $^3$H-ligand are beyond the detection limit of a thin layer chromatography analysis. Therefore, variations in $K_D$-values are difficult to interpret and cannot be taken as an indication for variations in binding sites.

Association and dissociation rates at 37 °C appeared to be fast ($t_{1/2} \leq 2$ min) for all $^3$H-ligands, and cooperative effects of binding were not apparent in dissociation studies using rat cortex tissue (BENNETT and SNYDER, 1976 ; LEYSEN and GOMMEREN, 1978).

When studying the inhibition of $^3$H-ligand binding by unlabelled drugs, various anomalies may be encountered. For the inhibition of $^3$H-spiperone binding by various 5-HT antagonists and agonists, it was observed that 60 % of the binding was stereospecifically inhibited by (+)-butaclamol, and that this part of the binding apparently involved serotoninergic receptors. However, when using unlabelled spiperone, an additional 20 % of the binding was inhibited with high affinity. This part of the binding appeared to be related to the spirodecanone moiety of the molecule and showed chemical specificity, but lacked a relationship with a pharmacological effect of the compounds (LEYSEN and GOMMEREN, 1978 ; HOWLETT *et al.*, 1979). These findings demonstrate the importance of an accurate definition of specific binding and the danger of using the unlabelled ligand. Indeed, membranes may accidentally contain high affinity and saturable binding sites which specifically bind certain structural moieties, but which are not related to a pharmacological or physiological receptor.

Furthermore, it has been observed that inhibition of $^3$H-LSD binding by unlabelled 5-HT and vice versa, produced shallow inhibition isotherms or slopes in Hill plots of less than unity (BENNETT and SNYDER, 1975 ; FILLION *et al.*, 1978). Others noted that the inhibition of $^3$H-5-HT binding by quipazine, yielded a slope < 1 in the Hill plot, whereas inhibition by antagonists such as metergoline, cinanserine, pizotifen yielded a slope = 1 (NELSON *et al.*, 1978). One parsimonious interpretation was that interactions between various compounds were not always competitive. However, a systematic study of the type of inhibition between various compounds using various $^3$H-ligands in different assay conditions has not been carried out. In the case of the binding of dopamine agonists and antagonists to dopaminergic receptors on striatal membranes, it has recently been argued that physicochemical events at the surface of the membrane micelles may cause anomalous binding patterns (LEYSEN and GOMMEREN, 1980). In particular, when using

compounds of different physicochemical nature, such as water-soluble agonists and lipophilic antagonists, different surface effects may be provoked. Prior to the interaction with the binding sites, compounds with different physicochemical properties will differentially interact with the surface of the membrane micelles and already be non-competitive at this level. With these assumptions, it may be argued that observations of irregular binding patterns are not necessarily an indication of the involvement of multiple sites. Surface phenomena always occur during interactions between compounds in solution and suspended micelles (either membrane micelles or detergent micelles which contain « solubilized » receptors). This is often overlooked in *in vitro* binding studies. Since they may considerably influence the binding, they should be taken into account when analyzing binding data.

B) In vivo *regulation of the binding sites.*

The ontogenetic development of $^3$H-5-HT binding sites was investigated in rat cerebral cortex areas and superior collicus (UZBECOV *et al.*, 1979). Area specific decline in the number of receptors from birth to adulthood was noticed. However, in these studies optimal binding was measured at pH 8.5-9.0, which was different from all other studies. The instability of the $^3$H-5-HT in these conditions might cause problems for measuring specific binding.

The effect of various drug treatments on $^3$H-5-HT binding in rat brain has been investigated by several authors ; data are summarized in Table I. Experimental protocols differed widely, findings were unclear and irreproducible either in different brain areas or in different laboratories. If anything, drugs which enhance the endogenous 5-HT concentration in the synaptic cleft tend to decrease the number of 5-HT sites, without changing the $K_D$-values. Repeated administration of LSD decreased both the $B_{max}$ and $K_D$-values. Metergoline seemed to increase $B_{max}$ in some brain areas after prolonged treatment. After a single administration of methiothepin, $B_{max}$ was increased 3-6 days afterwards. The 5-HT depletor, p-chlorophenylalanine, seemed to increase the $B_{max}$-value considerably after prolonged treatment. Effects of drug treatment on $^3$H-LSD and $^3$H-spiperone binding to serotoninergic receptors have not yet been reported, except for monoamine oxidase inhibitors, which do not seem to affect the binding. However, after treatment with monoamine reuptake blockers, variations in adrenergic receptors were reported along with the variations in $^3$H-5-HT binding sites (references in Table I).

## III. — IDENTITY

To identify binding sites with a neurotransmitter receptor, the sites labelled by various $^3$H-agonists and $^3$H-antagonists have to be compared. In addition, the specificity and binding affinity of a wide variety of compounds have to be investigated. As mentioned before, $^3$H-ligands thus far used to label serotoninergic receptors are : $^3$H-5-HT, $^3$H-LSD and $^3$H-spiperone. $^3$H-Methiothepine appeared unsuitable because of its too high non-specific binding (NELSON *et al.*, 1979). Binding of $^3$H-5-HT has mainly been studied in forebrain, cortex and hippocampus. Binding of $^3$H-LSD and $^3$H-spiperone to serotoninergic receptors had to be studied in cortical areas, since in brain areas rich in dopaminergic receptors such as the striatum and the mesolimbic areas, these compounds also label dopaminergic receptors with high affinity. As mentioned above, the latter binding can be prevented by adding, in excess, a selective unlabelled dopamine receptor blocker, but this has only been occasionally applied (WHITAKER and SEEMAN, 1978 ; LEYSEN *et al.*, 1979).

Sites labelled with $^3$H-5-HT in the cerebral cortex, caudate or forebrain exhibited a very high selectivity towards structural derivatives of 5-HT. The 5-hydroxyl group appeared necessary for a binding affinity at nanomolar concentrations. Besides these, some ergot derivatives also showed high binding affinity, such as metergoline, ergotamine and d-LSD. The binding sites appeared stereoselective since d-LSD was much more potent than the l-enantiomer. 5-HT antagonists lacking an indole nucleus, such as cyproheptadine, mianserin and pizotifen, showed moderate binding affinity. Harmaline derivatives and 5-hydroxyindole acetic acid, although containing an indole nucleus, did not occupy the sites at micromolar concentrations and neither did neurotransmitters other than 5-HT (BENNETT and SNYDER, 1976 ; FILLION *et al.*, 1978 ; NELSON *et al.*, 1978 ; WHITAKER and SEEMAN, 1978 *b*). Sites labelled by $^3$H-LSD in the cerebral cortex displayed a different drug selectivity. Various 5-HT antagonists were potent competitors, whereas 5-HT derivatives were less potent. However, 5-HT showed a higher binding affinity than other neurotransmitters (BENNETT and SNYDER, 1976 ; FILLION *et al.*, 1978). Neither $^3$H-5-HT nor $^3$H-LSD sites seemed pharmacologically related to 5-HT uptake sites (NELSON *et al.*, 1978) and antidepressants revealed only weak binding affinity (TANG and SEEMAN, 1980). Binding affinities of compounds for sites labelled by $^3$H-spiperone in rat frontal cortex correlated significantly with binding affinities for $^3$H-LSD labelled sites in the same area. Besides 5-HT antagonists, neuroleptics with antiserotoninergic properties bound to these sites at nanomolar concentrations. The binding affinities correlated significantly with the potencies of the drugs to antagonize tryptamine-induced clonic seizures in rats *in vivo* (LEYSEN and LADURON, 1977 ; LEYSEN *et al.*, 1978 *b*). Hence, sites labelled by the agonist $^3$H-5-HT and by the antagonist $^3$H-spiperone, seemed to differ in

TABLE I. — *Effect of various* in vivo *drug treatments of rats on specific* $^3$*H-5-HT binding in brain areas.*

| Drug | Treatment: Dose mpk, daily | Treatment: Duration treatment | Treatment: Duration drug free | $^3$H-5-HT binding*: Brain area | $^3$H-5-HT binding*: $B_{max}$ | $^3$H-5-HT binding*: $K_D$ | Reference |
|---|---|---|---|---|---|---|---|
| *5-HT releasing agent* | | | | | | | |
| d-fenfluramine | 2.5 (2 ×) I.P. | 14 d | 5 d | Diencephalon | ↓ | — | SAMANIN *et al.*, 1980 |
| | | id. | id. | Other areas | ∿ | — | id. |
| | | 28 d | id. | Diencephalon | ↓ | — | id. |
| | | id. | id. | Cortex | ↓ | — | id. |
| | | id. | id. | Brain stem | ∿ | — | id. |
| | | id. | id. | Striatum | ∿ | — | id. |
| | | id. | id. | Hippocampus | ∿ | — | id. |
| *Mono-amine reuptake blockers* | | | | | | | |
| nisoxetin + fluoxetin** | 10 + 10 (1 ×) | 21 d | 48 h | Frontal cortex | ↓ | — | MAGGI *et al.*, 1980 |
| | | id. | id. | Hippocampus | ∿ | — | id. |
| fluoxetin | 10 (2 ×) I.P. | 4 d | 24 h | Cortex | ∿ | ∿ | SAVAGE *et al.*, 1979 |
| chlorimipramine | 10 (2 ×) I.P. | 4 d | 24 h | Cortex | ∿ | ∿ | id. |
| desmethylimipramine | 6 (1 ×) I.P. | 1, 4, 6, 12 w | 24 h | Cortex | ∿ | ∿ | BERGSTROM and KELLAR, 1979 |
| | | 2 w | id. | Cortex | ↓ | ∿ | id. |
| various tricyclics | 10 (2 ×) I.P. | 3 w | 0.5-3 h | Forebrain high affin. | ↓↓ | ∿ | SEGAWA *et al.*, 1979 |
| | | | | Forebrain low affin. | ↓↓ | ∿ | |
| | 30 (1 ×) I.P. | acute | 0.5 h | Forebrain | ∿ | ∿ | id. |
| *MAO inhibitors* | | | | | | | |
| (A) clorgyline | 1 (1 ×) I.P. | 4 d | 24 h | Cortex | ↓ | ∿ | SAVAGE *et al.*, 1979 |
| (B) deprenyl | 1 (1 ×) I.P. | 4 d | 24 h | Cortex | ∿ | ∿ | id. |
| 5-HT depletor | | | | | | | |
| p-chlorophenyl-alanine | 300 (1 ×) | acute | 2 d | Forebrain | ∿ | ↓↓ | STEIGRAD *et al.*, 1978 |
| | 3 inj. | 6-8 d intervals | 2 d | Forebrain | ↑ | ∿ | id. |
| | 100 (1 ×) | 3 d | 1 d | Forebrain | ∿ | ↓↓ | id. |
| | | 6 d | 1 d | Forebrain | ∿ | ↓↓ | id. |
| | | 9 d | 1 d | Forebrain | ∿ | ↓ | id. |
| | | 12 d | 1 d | Forebrain | ↑ | ↓ | id. |
| *Receptor agonist/antagonist* | | | | | | | |
| LSD | 0.1 (4 ×) I.P. | 4 d | 24 h | Forebrain | ↓ | ↓ | TRULSON and JACOBS, 1979 |
| | | | | Brain stem | ↓ | ↓ | id. |
| bromo LDS | 0.1 (4 ×) I.P. | 4 d | 24 h | Forebrain | ∿ | ∿ | id. |
| metergoline | 1 (2 ×) I.P. | 14 d | 5 d | Various areas | ∿ | — | SAMANIN *et al.*, 1980 |
| | | 28 d | id. | Cortex | ↑↑ | — | id. |
| | | id. | id. | Striatum | ↑ | — | id. |
| | | id. | id. | Hippocampus | ↑ | — | id. |
| | | id. | id. | Diencephalon | ∿ | — | id. |
| | | id. | id. | Brain stem | ∿ | — | id. |
| methiothepine | 20 (1 ×) I.P. | acute | 1 h | Forebrain | ↓ | — | NELSON *et al.*, 1979 |
| | | id. | 1 d | Forebrain | ∿ | — | id. |
| | | id. | 2 d | Forebrain | ∿ | — | id. |
| | | id. | 3 d | Forebrain | ↑ | — | id. |
| | | id. | 3 d | Hippocampus | ↑ | ∿ | id. |
| | | id. | 6 d | Forebrain | ↑ | — | id. |

* — No information ; ∿ no change ; ↑ increase ; ↓ decrease ; ↑ : < 50 % ; ↑↑ :50-100 % ; ↑↑↑ : > 100 % of controls.
** Separate administration of the drugs caused no change in $^3$H-5-HT binding in that study.

both distribution in the brain (see above) and in drug specificity. The fact that it concerns two distinct sites was recently emphasized by PEROUTKA and SNYDER, who designated 5-HT-1 receptors the sites labelled by $^3$H-5-HT which could be inhibited by 300 nM unlabelled 5-HT, and 5-HT-2 receptors the sites labelled by $^3$H-spiperone which could be inhibited by 30 nM unlabelled spiperone (PEROUTKA and SNYDER, 1979). In these studies using rat frontal cortex, they found an approximately equal amount of both types of binding sites : 9.2 and 8.5 pmoles/g tissue. The latter apparently only concerned part of the $^3$H-spiperone binding sites since we found a total amount of 21.9 pmoles/g tissue (LADURON *et al.*, 1978) and curved Scatchard plots (LEYSEN *et al.*, 1978 *a* ; PEDIGO *et al.*, 1978). PEROUTKA showed that in rat frontal cortex, $^3$H-LSD labelled both the $^3$H-spiperone and the $^3$H-5-HT sites (PEROUTKA and SNYDER, 1979).

While the antagonist sites can be related to a pharmacological effect, this has not been the case yet for

the agonist sites. Because of apparent regional variations, and a lack of sufficient data in the literature, we investigated relative binding affinities of a large series of compounds for both $^3$H-spiperone and $^3$H-LSD sites in rat frontal cortex and for both $^3$H-5-HT and $^3$H-LSD sites in rat hippocampus. The $IC_{50}$-values and methodological specifications are given in Table II. As reported before, a significant correlation was found between binding affinities for $^3$H-spiperone and $^3$H-LSD labelled sites in the frontal cortex (Fig. 1). However, in the hippocampus, binding affinities for the $^3$H-LSD sites correlated significantly with binding affinities for the $^3$H-

TABLE II. — *Relative binding affinities ($pIC_{50}$, M)* of various compounds for $^3$H-ligand binding sites in rat brain areas.*

| Compound (abbreviation) | Frontal cortex | | Hippocampus | |
|---|---|---|---|---|
| | $^3$H-spiperone | $^3$H-LSD | $^3$H-5-HT | $^3$H-LSD |
| Serotonin (5-HT) | 5.5 ± 0.1 | 6.1 ± 0.1 | 8.0 ± 0.6 | 7.8 ± 0.1 |
| Bufotenin (BUFO) | 5.8 ± 0.1 | 6.2 ± 0.07 | 7.1 ± 0.2 | 7.2 ± 0.3 |
| Tryptamine (TRYP) | 4.78 ± 0.07 | 5.3 ± 0.1 | 6.6 ± 0.2 | 5.9 ± 0.5 |
| Apomorphine (APO) | 5.0 ± 0.1 | 5.5 ± 0.1 | 5.2 ± 0.2 | 5 |
| 2(N,N-dipropyl)amino-5,6-dihydroxytetralin (TETRAL) | 4.1 ± 0.1 | 4.4 ± 0.1 | < 5 | < 5 |
| Dopamine (DA) | 3.6 ± 0.1 | 3.7 ± 0.1 | 4.6 ± 0.5 | < 5 |
| Noradrenaline (NA) | — | — | 3.6 ± 0.2 | < 5 |
| Acetylcholine (ACh) | — | — | < 4 | < 3 |
| Ergotamine (ERGOT) | 7.4 ± 0.1 | 7.9 ± 0.1 | 8.1 ± 0.4 | 8.7 ± 0.1 |
| Lysergic acid diethylamide (LSD) | 7.6 ± 0.1 | 8.3 ± 0.1 | 7.6 ± 0.2 | 8.0 ± 0.1 |
| Metergoline (METER) | 8.4 ± 0.2 | 8.6 ± 0.1 | 7.6 ± 0.2 | 7.8 ± 0.2 |
| Bromocryptine (BRCR) | 7.4 ± 0.1 | — | 7.1 ± 0.2 | 7.6 ± 0.3 |
| Methysergide (METHYS) | 7.45 ± 0.05 | 8.1 ± 0.05 | 6.9 ± 0.01 | 7.2 ± 0.2 |
| Cyproheptadine (CYPRO) | 7.7 ± 0.1 | 7.55 ± 0.05 | 6.1 ± 0.1 | 6.2 ± 0.1 |
| Pizotifen (PIZOT) | 7.7 ± 0.1 | 7.7 ± 0.1 | 5.7 ± 0.2 | 5.4 ± 0.2 |
| Mianserin (MIAN) | 7.4 ± 0.1 | 7.35 ± 0.05 | 5.9 ± 0.4 | 5.8 ± 0.1 |
| Cinanserin (CINAN) | 6.9 ± 0.05 | 6.95 ± 0.05 | 5.4 ± 0.1 | 5.1 ± 0.2 |
| Ketotifen (KETOT) | 5.0 ± 0.2 | 4.7 ± 0.2 | 5.0 ± 0.2 | 5.0 ± 0.2 |
| Spiperone (SPIP) | 8.45 ± 0.05 | 8.2 ± 0.01 | 6.7 ± 0.2 | 7.12 ± 0.2 |
| Methiothepine (METIT) | 8.23 ± 0.07 | 7.95 ± 0.05 | 7.1 ± 0.4 | 7.3 ± 0.3 |
| Propericiazine (PROPE) | 8.1 ± 0.1 | 7.2 ± 0.1 | 5.2 ± 0.2 | 5.6 ± 0.2 |
| Chlorprothixene (CPTX) | 8.0 ± 0.05 | 7.61 ± 0.09 | 6.0 ± 0.1 | 6.3 ± 0.1 |
| Pipamperone (PIPAM) | 7.79 ± 0.06 | 7.57 ± 0.07 | 5.2 ± 0.2 | 5.6 ± 0.2 |
| Clothiapine (CLOTHIA) | 7.74 ± 0.08 | 7.4 ± 0.1 | — | — |
| Benperidol (BENPE) | 7.7 ± 0.05 | 7.6 ± 0.1 | 6.6 ± 0.4 | 7.0 ± 0.3 |
| Milenperone (MILEN) | 7.4 ± 0.1 | 7.6 ± 0.1 | 6.1 ± 0.2 | 6.4 ± 0.1 |
| Azaperone (AZAP) | 7.45 ± 0.06 | 7.5 ± 0.1 | 6.9 ± 0.5 | 6.8 ± 0.5 |
| Flupenthixol (FPTX) | 7.4 ± 0.05 | 6.75 ± 0.05 | 5.0 ± 0.2 | 5.6 ± 0.2 |
| Clozapine (CLOZ) | 7.32 ± 0.09 | 7.08 ± 0.07 | 5.4 ± 0.2 | 6.2 ± 0.2 |
| Chlorpromazine (CPZ) | 7.21 ± 0.07 | 6.6 ± 0.1 | 5.4 ± 0.2 | 5.65 ± 0.2 |
| (+)-butaclamol ((+)-BUT) | 7.0 ± 0.1 | 7.38 ± 0.06 | 5.5 ± 0.2 | 6.6 ± 0.2 |
| Haloperidol (HAL) | 6.83 ± 0.07 | 6.15 ± 0.05 | 5.0 ± 0.2 | 5.5 ± 0.2 |
| Bromperidol (BROMPE) | 6.4 ± 0.2 | — | 5.1 ± 0.2 | 5.6 ± 0.2 |
| Domperidone (DOMP) | 6.4 ± 0.2 | — | 5.0 ± 0.2 | 5.5 ± 0.2 |
| Oxiperomide (OXIP) | 6.05 ± 0.05 | 6.35 ± 0.05 | 6.9 ± 0.2 | 7.1 ± 0.2 |
| (—)-butaclamol | < 6 | < 6 | < 5 | — |
| Astimizole (ASTI) | 6.8 ± 0.2 | — | 5.0 ± 0.2 | 5.8 ± 0.2 |
| Oxatomide (OXAT) | 7.2 ± 0.2 | 6.0 ± 0.2 | 5.0 ± 0.2 | 5.8 ± 0.2 |
| Doxepin (DOXE) | 7.1 ± 0.1 | 6.7 ± 0.2 | 5.2 ± 0.2 | 5.7 ± 0.2 |
| Phenoxybenzamine (PHBZ) | — | — | 5.7 ± 0.5 | 5.8 ± 0.2 |
| Phentolamine (PHENT) | < 6 | — | 5.0 ± 0.2 | 5.6 ± 0.2 |
| Alprenolol (ALP) | < 6 | — | 5.3 ± 0.2 | 6.5 ± 0.2 |
| Atropine | 4.5 | — | < 4 | < 5 |
| Assay conditions | | | | |
| $^3$H-ligand concentration | 2 nM | 2 nM | 3 nM | 3 nM |
| non-specific binding | 2 μM (+)-BUT | 1 μM LSD | 2 μM LSD | 2 μM 5-HT |
| total membrane fraction | 25 mg/2.2 ml | 25 mg/2.2 ml | 25 mg/2.2 ml | 25 mg/2.2 ml |
| buffer | Tris-HCl 50 mM, pH 7.6 Salts** | Tris-HCl 50 mM, pH 7.4 — | Tris-HCl 50 mM, pH 7.6 Salts** | Tris-HCl 50 mM, pH 7.6 Salts** |
| incubation temperature, time | 37 °C, 10 min | 37 °C, 10 min | 37 °C, 10 min | 37 °C, 10 min |

* $pIC_{50}$-values are the means of at least 3 independent determinations in duplicate, using 5 drug concentrations per inhibition curve. $IC_{50}$ (nM) = $10^{9-pIC\ (M)}$, *i.e.*, the drug concentration inhibited 50 % of the specific binding of the labelled ligand.

** Salts : NaCl 120 mM, KCl 5 mM, $CaCl_2$ 2 mM, $MgCl_2$ 1 mM, pargyline 10 μM, ascorbic acid 0.1 %.

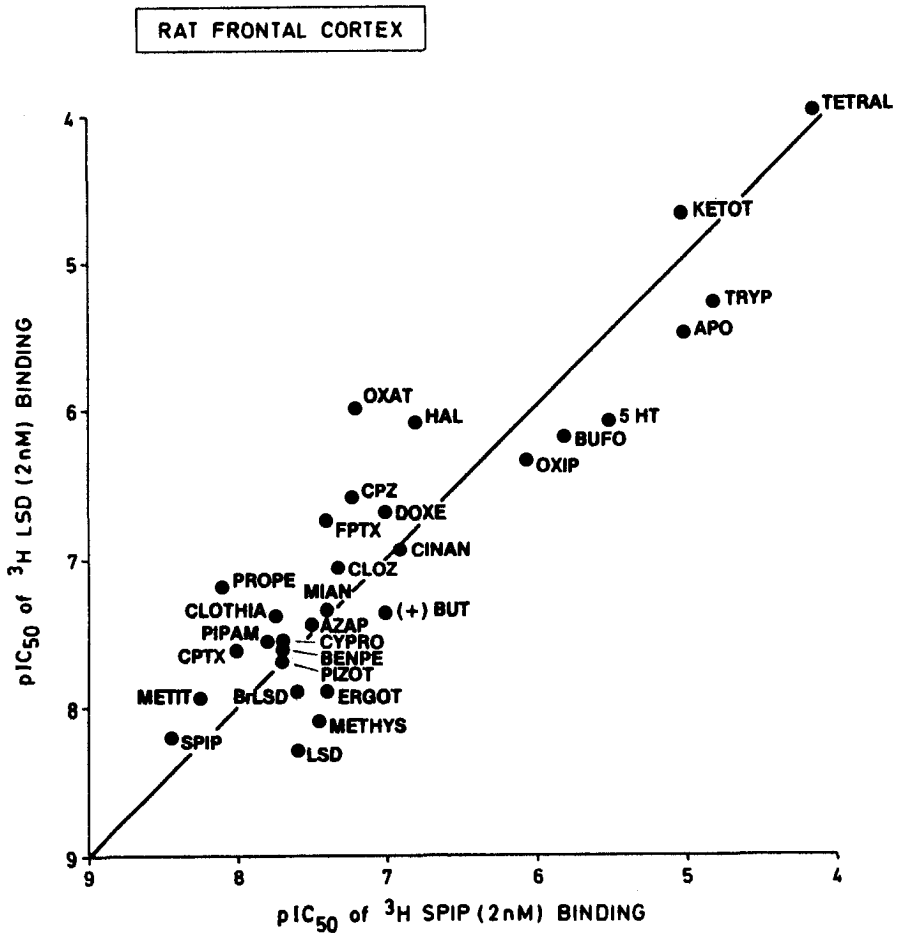


FIG. 1. — *Correlation between relative binding affinities of drugs ($pIC_{50}$, M) for $^3$H-spiperone and $^3$H-LSD labelled sites in rat frontal cortex.*

Spearman rank correlation coefficient : r = .87, n = 32, p < .001. Compounds and assay conditions are indicated in Table II.

5-HT sites (Fig. 2). As indicated in Fig. 2, the most active compounds in these tests were compounds with an indole nucleus such as 5-HT and ergot derivatives. There was no correlation between binding affinities for $^3$H-spiperone sites in the frontal cortex and $^3$H-5-HT sites in the hippocampus (Fig. 3). From these results it appears that in the frontal cortex the sites labelled by $^3$H-LSD at 2 nM show the overall binding characteristics of the $^3$H-spiperone sites, indicating that in these conditions « 5-HT-2 receptors » should be more involved. In the hippocampus, $^3$H-spiperone sites were hardly measurable, but $^3$H-5-HT sites were present in high amounts. Since $^3$H-LSD is able to label these sites too, the hippocampal $^3$H-LSD binding sites are similar to the $^3$H-5-HT sites. As shown in Figs. 4 and 5, binding affinities for $^3$H-spiperone sites in the frontal cortex correlated significantly with the drug potencies to antagonize tryptamine-induced clonic seizures in rats (NIEMEGEERS and JANSSEN, 1979) and with the potencies to antagonize 5-HT-induced contractions in rat caudal artery *in vitro* (VAN NUETEN and VANHOUTTE, 1980). It is also shown that such correlations were not found with the binding affinities for the $^3$H-5-HT sites in the hippocampus. Potencies of drugs to antagonize 5-HT-induced contractions in the rat fundus *in vitro* (VAN NUETEN and VANHOUTTE, 1980) did not correlate with binding affinities either in the frontal cortex or in the hippocampus. According to these observations, the sites labelled by $^3$H-spiperone and $^3$H-LSD in the frontal cortex could be related to « pharmacological receptors », since these sites show binding properties which can be correlated with 5-HT mediated pharmacological effects. However, both the role and the significance of these sites in the brain remains to be clarified. On the other hand, the $^3$H-5-HT binding sites were suggested to be coupled to a 5-HT sensitive adenylate cyclase (FILLION *et al.*, 1979). This remains, however, to be proven. So far, the binding characteristics of the $^3$H-5-HT labelled sites cannot be related to a pharmacological or physiological effect of 5-HT and therefore cannot truly be called « receptors ». Extreme care has to be taken in the identification of « binding sites » with receptors. As discussed above, membranes may contain structural recognition sites which are saturable and which may bind certain chemical structures with high affinity, with no relation whatsoever to pharmacological or physiological

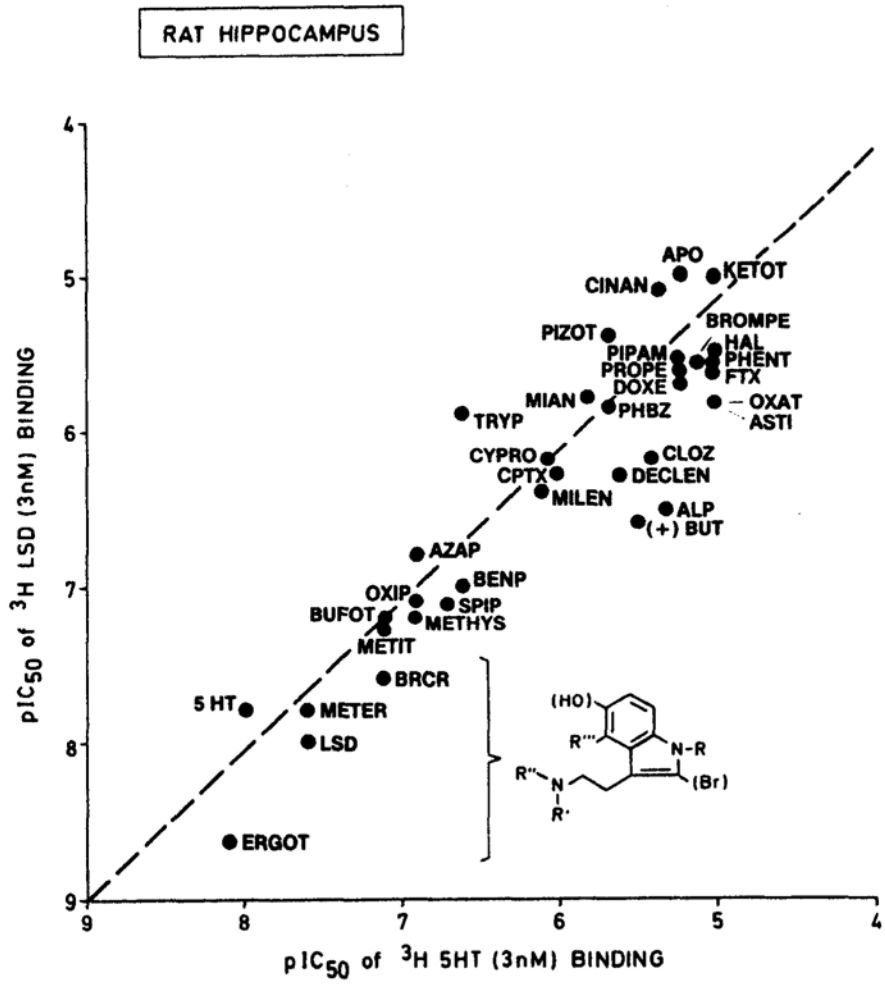


FIG. 2. — *Correlation between relative binding affinities of drugs ($pIC_{50}$, M) for $^3$H-5-HT and $^3$H-LSD labelled sites in rat hippocampus.*

Spearman rank correlation coefficient : r = .86, n = 36, p < .001. Compounds and assay conditions are indicated in Table II.

effects. Since so far, high binding affinity to the 5-HT labelled sites seemed to be restricted to certain indole derivatives, the possibility of the involvement of structural recognition sites cannot be disregarded.

The chemical nature of the macromolecules constituting the binding site in the membrane has been investigated by treating the membranes with various reagents, enzymes and detergents (BENNETT and SNYDER, 1976). A summary of the findings is given in Table III. Further attempts to identify the « binding »

TABLE III. — *Effect of various membrane treatments on specific binding in rat cortex* (BENNETT and SNYDER, 1976).

↓ : decrease ; ↑ : increase ; ↓ < 50 % ; ↓↓ : 50 % ; ↓↓↓ : > 50 %.

| | $^3$H-5-HT | $^3$H-LSD | Important for binding | Not important for binding |
|---|---|---|---|---|
| Sulfhydryl reagents | ↓↓ | ↓↓ | SH | |
| Carboxyl reagents | ↓↓↓ | ↓↓ | COOH | |
| Tryptophane reagents | ↓ | ↓ | Integral protein | |
| Trypsin TPCK | ↓↓ | ↓↓ | | |
| α-chymotrypsin | ↓↓ | ↓ | | |
| Phospholipase A | ↓↓↓ | ↓↓↓ | Fatty acids | |
| Phospholipase D | ↑ | ↑ | | — Polar « heads » of phospholipids |
| Neuraminidase | ↑ | ↑ | | — Sialic acid |
| Triton X 100 | ↓↓↓ | ↓↓↓ | Membrane structure | |
| Deoxycholate | ↓↓↓ | ↓↓↓ | | |
| Tween | ↓↓ | ↓↓ | | |

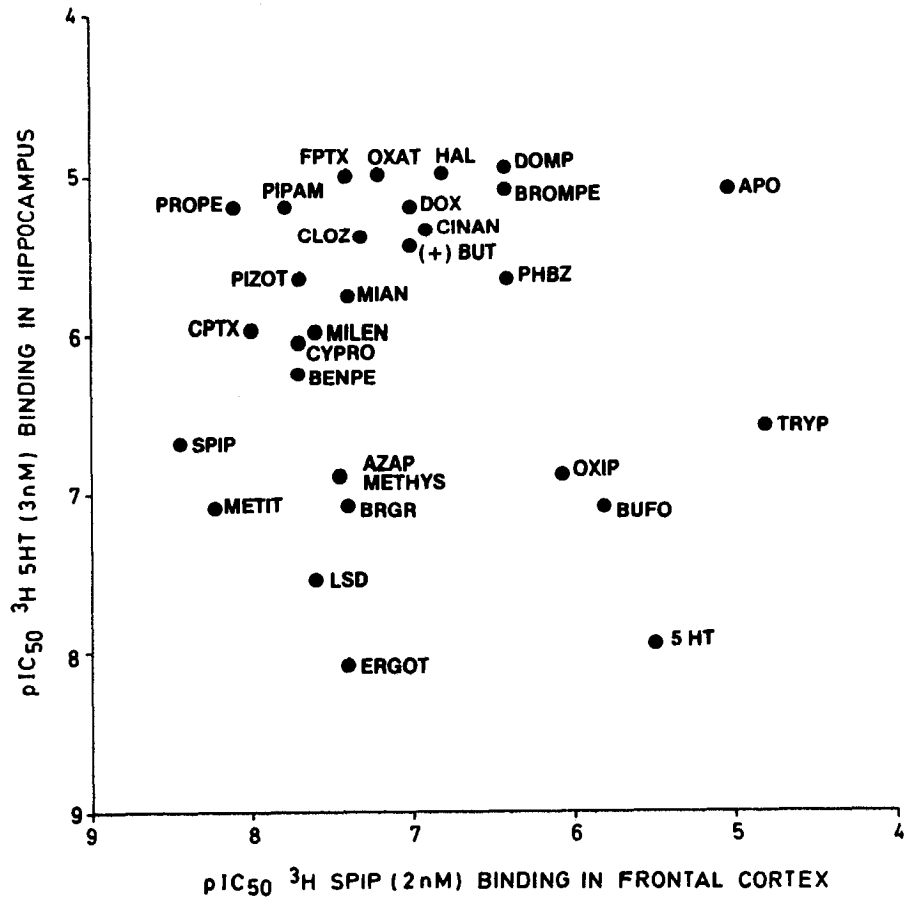


FIG. 3. — *Correlation between relative binding affinities of drugs ($pIC_{50}$, M) for $^3$H-spiperone sites in rat frontal cortex and $^3$H-5-HT sites in rat hippocampus.*

Spearman rank correlation coefficient : r = .33, n = 35, p > .01. Compounds and assay conditions are indicated in Table II.

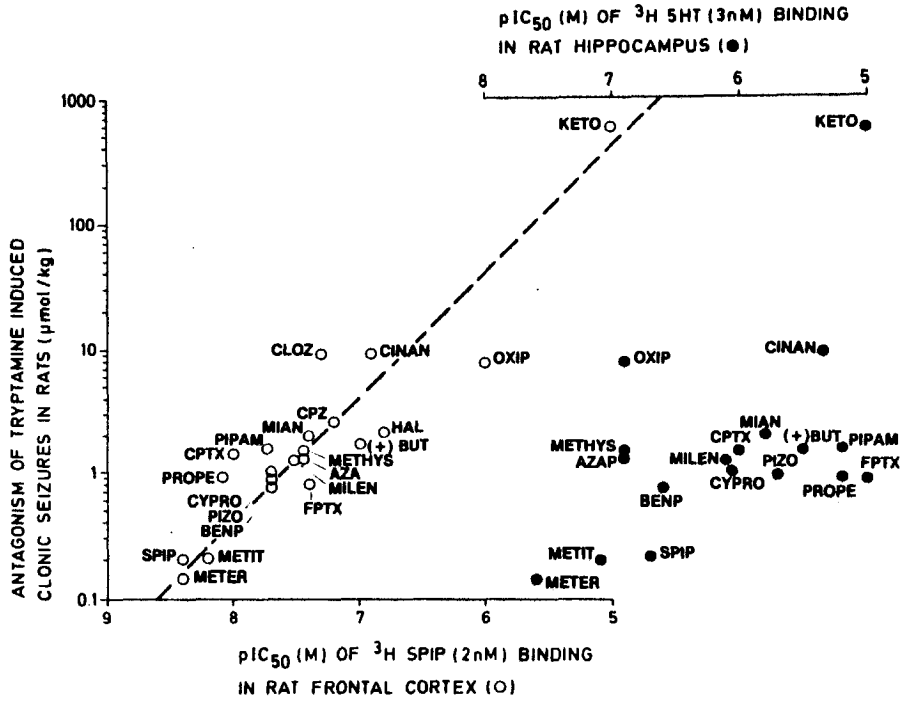


FIG. 4. — *Correlation between potencies of drugs to antagonize tryptamine-induced clonic seizures in rats* in vivo *(NIEMEGEERS and JANSSEN, 1979) and relative binding affinities of drugs ($pIC_{50}$, M) for $^3$H-spiperone sites in rat frontal cortex (o) or for $^3$H-5-HT sites in rat hippocampus (•).*

Spearman rank correlation coefficient for $^3$H-spiperone : r = − .87, n = 22, p < .001 ; for $^3$H-5-HT : r = − .51, n = 22, p > .01. Compounds and assay conditions are indicated in Table II.

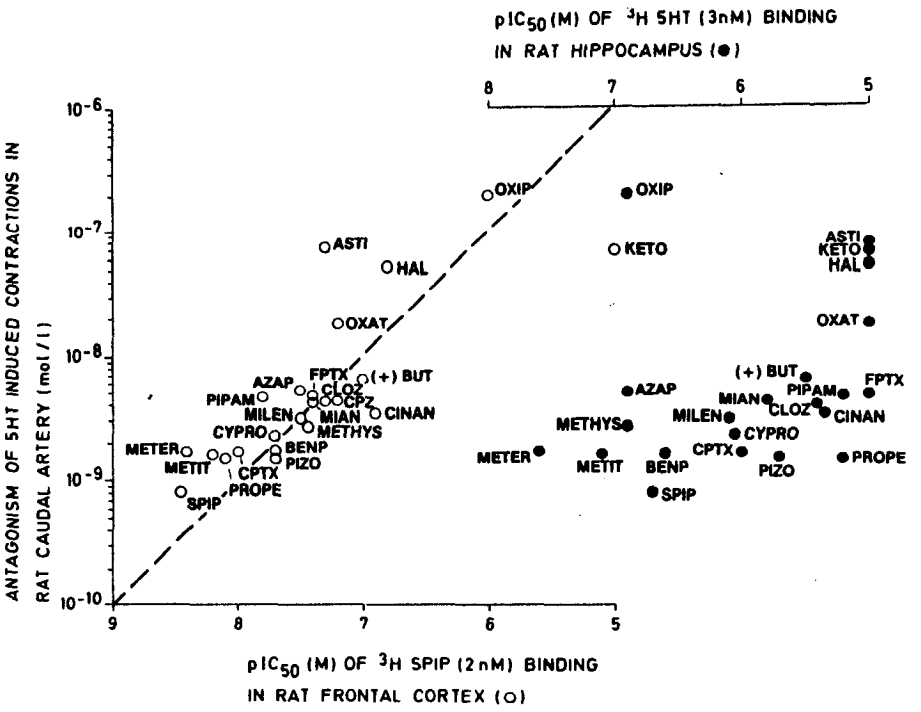


FIG. 5. — *Correlation between potencies of drugs to antagonize 5-HT-induced contractions in rat caudal artery* in vitro *(VAN NUETEN and VANHOUTTE, 1980) and relative binding affinities of drugs ($pIC_{50}$, M) for $^3$H-spiperone sites in rat frontal cortex (o), or for $^3$H-5-HT sites in rat hippocampus (•).*

Spearman rank correlation coefficient for $^3$H-spiperone : r = − .82, n = 24, p < .001 ; for $^3$H-5-HT : r = − .41, n = 24, p > .01. Compounds and assay conditions are indicated in Table II.

macromolecules have not been reported yet. The still elusive goal of the chemical composition of the receptor can only be achieved after solubilization and purification of the binding macromolecule.

## IV. — FUNCTION

The function of the binding sites on membranes in brain tissue, and their relation to the action of 5-HT, is unknown and represents a vast and complicated field for future research. To be investigated are *e.g.* the immediate effects on the membrane or cell of receptor triggering. Examples of reactions which may be initiated are : (1) phospholipid turnover, known to play a role in various receptor systems, *e.g.*, phosphatidyl inositol-phosphatidic acid turnover (MICHELL, 1979), phospholipid methylation (STRITTMATTER *et al.*, 1979), acylation-deacylation (SCHREY and RUBIN, 1979), etc. ; (2) receptor coupling with enzymes, *e.g.*, adenylate cyclase as described for the β-receptor system (STROSBERG, 1980) ; (3) receptor coupling with ion channels, etc.

The *in vivo* behavioural effects resulting from triggering serotoninergic receptors in various brain areas remain to be explored. *In vivo* receptor binding experiments have shown that $^3$H-LSD accumulates specifically in certain brain areas. In the cortex, it was shown that labelling could be selectively displaced by serotonin agonists and antagonists (DUCHEMIN *et al.*, 1979). A combinaton of the *in vivo* binding technique using selective agents and careful scoring of pharmacological effects might be an interesting tool in clarifying the issues. A rewarding goal would be to extend our knowledge regarding the involvement of receptor alterations in diseased states. Preliminary studies in this area indicate a decrease in LSD binding sites, but unchanged 5-HT binding sites in schizophrenia (BENNETT *et al.*, 1979) and decreased LSD and spiperone binding sites in Huntington's disease (ENNA *et al.*, 1976 ; REISINE *et al.*, 1977). However, much more research is needed to find out the relevance and significance of these receptor changes in the etiology of mental diseases.

## CONCLUSION

Measurements of *in vitro* binding to membranes require little skill and simple technical equipment. The subject is fashionable and many isolated experiments are being reported. However, the biochemistry and physicochemistry of ligand interactions with membranes are complicated and comprise many unknown and therefore uncontrollable phenomena. Assay conditions may considerably influence membrane conformations, and have to be carefully checked and standardized to make observations reproducible. Binding patterns should be critically interpreted, taking into account possible anomalies caused by surface events on membrane micelles or by degradation of unstable $^3$H-ligands. Since « receptor » is a physiologically or pharmacologically defined concept, a « binding site »

should only be identified with a given receptor when the relationship with physiological or pharmacological effects can be made. Finally, receptor events are only the start of a chain of reactions resulting in a response. When trying to understand receptor functions and regulations, not only the site of attachment, but the whole system has to be studied. This review has shown that the knowledge about serotoninergic receptors is rather limited. In order to resolve the intriguing problem of what the features and functions of serotoninergic receptors are, extensive and carefully designed further research is necessary.

ACKNOWLEDGEMENT

Part of this work was supported by a grant from I.W.O.N.L. The author sincerely thanks Drs. C.J.E. NIEMEGEERS and J.M. VAN NUETEN (Dept. of Pharmacology, Janssen Pharmaceutica) for providing data of *in vivo* and *in vitro* pharmacological tests.
Thanks are due to Dr. J.P. TOLLENAERE and D. ASHTON for their help in preparing the manuscript.

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