*Biochemical Pharmacology*, Vol. 33, No. 6, pp. 833–839, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00
Pergamon Press Ltd.

# CRITERIA FOR RECEPTOR SITES IN BINDING STUDIES

PIERRE M. LADURON
Department of Biochemical Pharmacology, Janssen Pharmaceutica, B-2340 Beerse, Belgium

**Abstract**—The study of binding is not an easy task especially because of the difficulty of interpreting the results in terms of binding on specific receptor sites. The problem is not new; what is new is the increasing amount of fanciful interpretation that such a technique has generated. The tendency to interpret anomalous or intriguing results in terms of new receptor subtypes seems to have reached its peak. The need to apply severe criteria becomes imperative before concluding that a binding site may be called a receptor site. Some pitfalls in binding studies will be discussed.

Binding studies are becoming legion particularly since the introduction of the filtration technique and the advent of highly labelled ligands. The binding assays are easy, perhaps too easy to perform while the interpretation of the results remains much more complicated and has been the source of numerous controversies. The reason for this is relatively simple: there are no biochemical criteria to assess the binding specificity i.e. to ascertain that a binding site really corresponds to a receptor site. In contrast to this, the identification of a reaction product in enzymology is generally sufficient to demonstrate the nature of the enzymatic reaction. Kinetic analysis, Scatchard or Hill plots, saturability and mostly reversibility remain insufficient parameters to prove the identity of a binding site to a receptor site.

As pointed out elsewhere [1] when one starts any binding study, one must keep in mind the two following postulates: first, there is no equivalence between a binding site and a receptor site; numerous ligands label sites not related to receptors. The second postulate or postulate of receptor homogeneity supposes that the receptor sites are homogenous, thus identical in all parts of the body. This is a working hypothesis but, I think, quite fruitful, because it can force us to examine more carefully the reasons of some intriguing or anomalous results rather than to be lured into the trap of the multiple site concept. Indeed binding studies have generated an impressive number of "receptor subtypes" which generally correspond to displaceable binding sites completely unrelated to physiological receptors.

In this paper, I would like to try to define what we call, in neurotransmission, the receptor concept which will be opposed to the acceptor site concept. Then we will present the criteria that make a binding site a receptor site and last but not least we will discuss some major pitfalls in binding studies. In one of his last papers, Zenon Bacq [2] recalled this story of the French composer Maurice Ravel who asked his colleague Georges Auric to write with him a treatise of orchestration illustrated by a lot of examples showing what one has to avoid; Bacq thought this was a good idea since error is often more instructive than success, when one can understand its genesis and put a finger on the wrong steps.

## HOW TO DEFINE A RECEPTOR?

The word receptor is now used by many scientists with so many different meanings that it is practically impossible to cover in one definition the entire biological processes in which receptors seem to be involved; even the nicotinic receptor markedly differs from the progesterone receptor. However, it is beyond doubt that such a term has been misused in numerous cases; for instance the so-called LDL receptors should be regarded as acceptor sites instead of being called receptors; indeed there is no signal transmission into the interior of the cell through the binding site. The internalization of a molecule is not necessarily receptor mediated; peroxydase does not bind to specific receptors. In the early twentieth century John Newport Langley [3] provided the first definition of a receptor. Studying the effects of nicotine and curare on muscle contraction, Langley came to the conclusion that both must compete at the same "receptive substance" or receptor. The normal function of this receptive substance is thus to transmit a stimulus from the nerve to the muscle. Hence, according to such a definition, the receptor is a site of competition for agonist and antagonist and the stimulus produced by the agonist through a mechanism not yet elucidated leads to a physiological response. As shown in Fig. 1 a receptor involves thus three processes while an acceptor site is only a binding site without signal transmission and thus without physiological response. Often the increase of cyclic AMP production by neurotransmitters is regarded as a physiological response which can be easily measured in test tubes. In fact, it is a biochemical effect which needs to be related to physiological responses; just because an enzyme (adenylate cyclase is such an enzyme) is stimulated or inhibited by a neurotransmitter does not prove that such a process is involved in neurotransmission. If the opposite was true, one also might consider tyrosine hydroxylase as a receptor because neurotransmitters as dopamine and noradrenaline inhibit the enzyme.

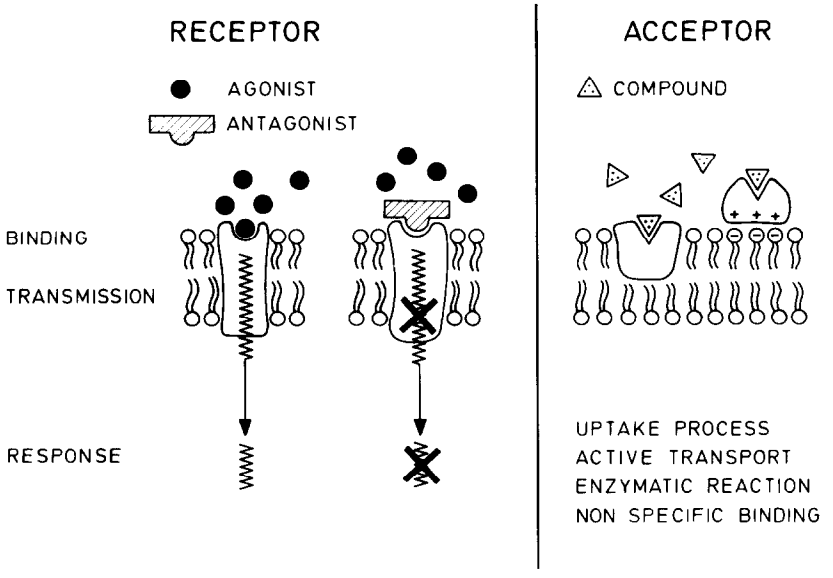



Fig. 1. Schematic representation of receptor site and acceptor site.

Obviously the physiological or the pharmacological response is essential for defining a receptor; the binding studies require a multidisciplinary approach. The problem is not new; the pioneers in the receptor fields who were working at the isolation of the cholinergic receptor protein from fish electric organ were faced with the same difficulties; as quoted by Changeux [4]: "Another crucial requirement was to ascertain whether at all stages of 'reduction' of the system, the isolated structures were physiologically meaningful. In other words, for each step (binding on membranes, solubilization, purification, isolation of subunits etc. . . .) biochemical and physiological (pharmacological) experiments had to be carried out in a parallel and systematic manner."

The receptors involved in neurotransmission are endowed with low affinity for the endogenous neurotransmitters which, however, are released in high amounts into the synaptic cleft. In contrast, hormones bind with high affinity to their receptors but their blood concentration is quite low. Similarly, the antagonists of endogenous neurotransmitters behave like a hormone, low blood level, but high affinity for the receptor. This explains why potent antagonists are generally used as ligands for labelling neurotransmitter receptors.

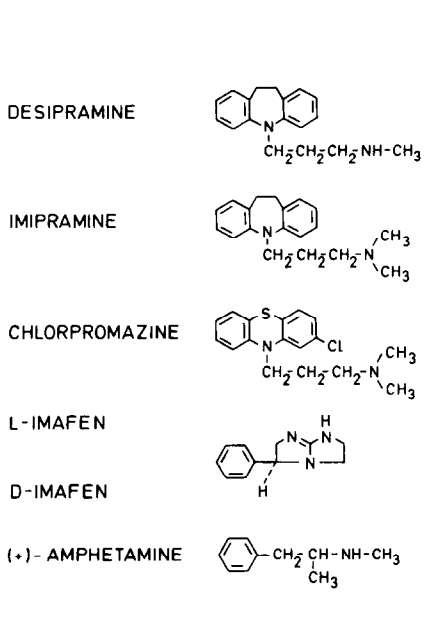
Table 1. Specificity criteria of receptor sites

1. Drug displacement (agonists and antagonists belonging to different chemical and pharmacological classes).
2. Correlation between drug affinity *in vitro* and pharmacological potency *in vivo*.
3. Regional distribution or tissue specificity.
4. Subcellular distribution.
5. Stereospecificity.
6. Saturability.
7. Reversibility.
8. High affinity.

## SPECIFICITY CRITERIA FOR RECEPTOR SITES

Recently [5] we presented the specificity criteria in a different order to that listed in Table 1. At that time, the biochemical criteria (high affinity, reversibility, stereospecificity, saturability . . .) were first described according to the classical view [6]. As these criteria are far from being decisive—they are also valid for acceptor sites—we are now presenting these criteria in a new order to further insist on what we believe to be the most important and more decisive criteria, drug displacement and correlation with pharmacological activity. Only when these points are fulfilled may a binding site be called a receptor site.

Drug displacement consists of determining in the *in vitro* binding assay the affinity of various drugs (agonists and antagonists) belonging to different pharmacological classes but also belonging to different chemical classes of drug but having the same pharmacological properties as the labelled ligand. The first thing to do when one sets up a binding assay, certainly before performing a Scatchard analysis, is to examine carefully the criterion of drug displacement. When $^3$H-cimetidine was reported to label histamine $H_2$ receptors [7, 8] we decided to look at the binding; a displacement curve was performed the first day with unlabelled cimetidine, metiamide and burimamide. The second day we included ranitidine as displacer; surprisingly this compound that is known to be about 10 times more active than cimetidine in pharmacological tests, did not readily compete with $^3$H-cimetidine in the binding assay. These two-day experiments were sufficient to convince us that $^3$H-cimetidine did not label a receptor site. More extensive studies have now clearly shown that $^3$H-cimetidine only binds on an imidazole recognition site while $^3$H-ranitidine binds to a furane recognition site, both being completely unrelated to histamine $H_2$ receptors [9–11]. When a ligand becomes so de-



| | $IC_{50}$ (nM) | |
|---|---|---|
| | $^3$H-DMI BINDING | NA UPTAKE |
| DESIPRAMINE | 30 | 20 |
| IMIPRAMINE | 360 | 790 |
| CHLORPROMAZINE | 130 | 1,800 |
| L-IMAFEN | 700 | 30 |
| D-IMAFEN | 1,400 | 2 |
| (+)-AMPHETAMINE | 12,000 | 70 |

Fig. 2. Effects of various drugs upon $^3$H-desipramine binding and noradrenaline uptake.

void of any physiological meaning, there is always somebody who describes a new receptor subtype, the putative clonidine-sensitive $H_2$ receptor [12]. Recently, $^3$H-tiotidine was found to be a more specific ligand to label $H_2$ receptors [13].

Another example illustrating the need of using drugs belonging to different chemical series is given by the $^3$H-desipramine binding. Figure 2 shows the $IC_{50}$ values for 6 compounds in $^3$H-desipramine binding and noradrenaline uptake. The drugs may be divided into 3 subgroups according to whether the compound is equiactive in both systems or that it is more or less active in the binding than in the uptake. Imipramine and desipramine belong to the first group; this means that their $IC_{50}$ values are practically identical in both tests. In contrast, chlorpromazine is more active in the $^3$H-desipramine binding than in the NA uptake; it is even more potent than imipramine in $^3$H-desipramine binding although it is not an antidepressant drug. In the third group, one finds drugs which are much more active in the NA uptake; for instance the D-enantiomer of imafen is about 1000 times more potent in the uptake than in the binding; this is quite surprising since imafen was found to be approximately equipotent to desipramine as an antagonist of ptosis in the reserpine test [14]. The stereospecificity for both imafen enantiomers observed in NA uptake was not found in the binding assay. Such a discrepancy between both *in vitro* tests was already observed for amphetamine which was found to be 200 times more active as NA reuptake blocker than as displacer of $^3$H-desipramine [15]. Therefore one may conclude that the $^3$H-desipramine binding is not specifically related to noradrenaline uptake as reported previously by different groups [15–17], but simply reveals recognition sites for tricyclic drugs. Our recent fractionation studies are compatible with such a lack of specificity [18]. Similarly, $^3$H-tetrahydrotrazodone which was reported as an antagonist ligand for the serotonin$_1$ binding sites, is certainly not specific for serotonin binding sites, since noradrenaline was much more active than serotonin [19].

In fact, one of the greatest mistakes in binding studies is to believe that the displaceable binding is specific thus related to receptor sites. Displacement curves must be interpreted with caution; if binding sites are displaceable with atropine or dexetimide but not with levitimide in the nanomolar range, there is no doubt about the muscarinic nature of these binding sites. In contrast, when the $IC_{50}$ values of atropine and dexetimide are about $10^{-6}$ M [20–22], they certainly do not reveal muscarinic receptor sites.

As quoted before, there is no receptor without a physiological or a pharmacological response. When the drug displacement characterization has been achieved *in vitro* one has to try to correlate the drug affinity found in the binding assay with the drug potency obtained in pharmacological tests performed either *in vivo* or *in vitro*. It is likely that the latter will give rise to the nicest correlation, because both are carried out *in vitro*. A good example of such a correlation has been recently reported between $^3$H-ketanserin binding on serotonin $S_2$ receptors and serotonin-induced vasoconstriction in rat caudal artery [23]. Surprisingly, when the pharmacological test was performed *in vivo* such as tryptamine-induced clonic seizures or mescaline-induced head twitches, the correlation remains highly significant [23], a fact which is not necessarily expected owing to different pharmacokinetic properties and an uneven brain distribution of the tested drugs. The same was true for the dopamine receptor ($D_2$ sites); more than 17 pharmacological, behavioural and biochemical parameters nicely correlate with the $IC_{50}$ values of at least 20 compounds obtained in the *in vitro* $^3$H-haloperidol

Table 2. Potencies of imipramine in various biochemical models *in vitro*

| | $IC_{50}$ values (nM) | |
|---|---|---|
| $^3$H-Noradrenaline uptake | 46* | 20† |
| $^3$H-5-HT uptake | 550* | 240† |
| $^3$H-Pyrilamine binding ($H_1$) | 63 | |
| $^3$H-WB 4101 binding ($\alpha_1$) | 300 | |
| $^3$H-Spiperone binding ($S_2$) | 100 | |

* Taken from ref. 24.
† Taken from ref. 25.

binding assay [cf. ref. 1]. On the contrary, there was a complete lack of correlation for the $D_1$ sites (dopamine-sensitive adenylate cyclase) and for the $D_3$ sites, the latter being an acceptor site for catechol derivatives. Therefore one may conclude that alone the $D_2$ site is the physiological receptor site of dopamine.

It should be pointed out that correlations must be interpreted with caution; sometimes a correlation may be falsely positive because too small a number of drugs was tested or when there are two clusters of compounds one active ($IC_{50}$ values between 10 and 100 nM) and the other inactive ($IC_{50}$: 1000 nM and higher). Nevertheless it is beyond doubt that the correlations are extremely useful and even essential to decide whether or not a binding site may be called a receptor site.

Regional distribution is particularly evident for dopamine and serotonin receptors which are specifically located in a few brain regions. When the number of binding sites is as high in the liver as in the brain, one may certainly not conclude, as authors recently did [19], that these sites are related to serotonin $S_1$ sites.

## SOME PITFALLS IN BINDING STUDIES

It is no exaggeration to state that all the problems encountered in binding studies turn essentially around one fundamental question: how is it possible to distinguish specific binding, i.e. binding related to receptor sites, from non specific binding in which acceptor or recognition sites are involved? This is the greatest difficulty when interpreting *in vitro* binding studies. In fact one has to take into account numerous different factors such as the ligand itself (specificity and affinity), the heterogeneity of the material used (membrane, intact cells, etc. . . .), the proportion or the balance between displaceable and non displaceable binding, the experimental conditions (temperature, buffer, ions, etc. . . .), the physiological relevance of "specific" binding etc. . . . Let us examine some of these points.

*Lack of ligand specificity.* As a rule, a ligand is supposed to specifically label a given receptor site; sometimes it can label two different receptor sites—this is the case for spiperone—but, here the specificity can be achieved by using selective displacer drugs or simply different brain regions. However, numerous ligands are not selective enough; chlorpromazine for instance binds to five different receptor sites. A good example of non selective ligand is $^3$H-imipramine; Table 2 shows the affinity of imipramine for noradrenaline and 5HT uptake, histamine $H_1$ and serotonin $S_2$ receptor sites. Surprisingly, imipramine was more active in noradrenaline uptake, $H_1$ and $S_2$ sites than as a 5HT reuptake blocker. Consequently $^3$H-imipramine would not be expected to specifically label 5HT uptake sites. In fact many points support this view; first, pyrilamine and phentolamine were found to be potent displacers in the $^3$H-imipramine binding [26]. Similarly zimelidine, a potent serotonin reuptake blocker was poorly active on $^3$H-imipramine binding to human platelets, even much less active than pyrilamine and chlorpromazine [27] which, as far as I know are not antidepressant drugs. Secondly, there is a large piece of evidence showing that the 5HT uptake and $^3$H-imipramine binding sites are not the same molecular entity. In patients suffering from alcoholic cirrhosis, the uptake of 5HT into platelets was found to be markedly reduced whereas the binding of $^3$H-imipramine was unchanged as compared to control volunteers [28].

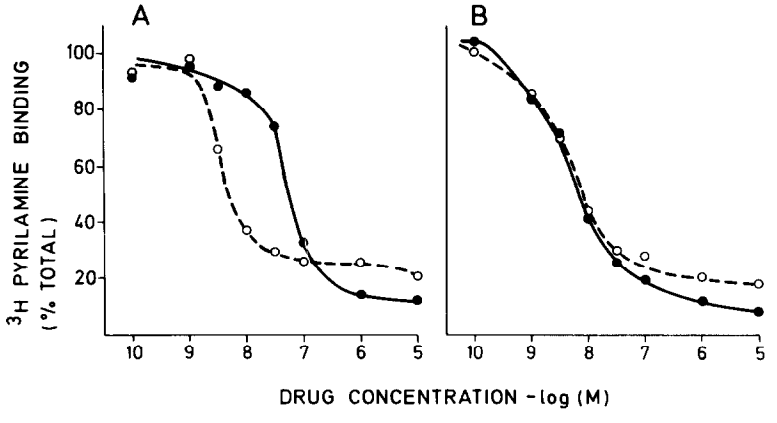



Fig. 3. Dose–response inhibition of astemizole (A) and pyrilamine (B) in the $^3$H-pyrilamine binding assay performed under standard conditions (1.1 ml) (●—●) or under conditions where the volume was brought to 10 ml (○---○) [cf. ref. 51].

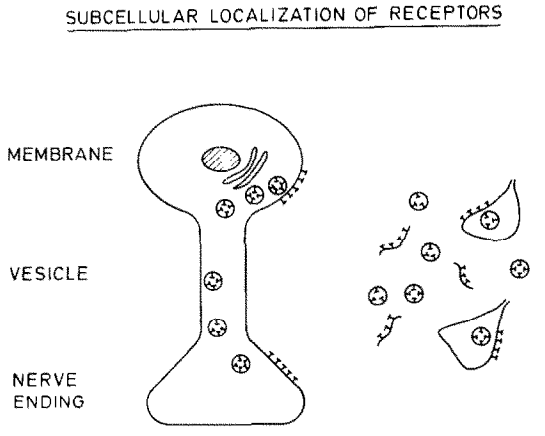



Fig. 4. Scheme illustrating the possible multiple localization of receptor sites: upon the external membrane of nerve terminals, in small vesicles located or not within nerve endings and upon membrane fragments. After homogenization, the receptors are distributed in different membrane-structures.

Such discrepancy between the binding and uptake was also observed in rat brain [29] and during the ontogenetic development which markedly differed for both [30]. In our subcellular fractionation studies, the 5HT uptake was mainly recovered in the mitochondrial (M and L) fractions whereas $^3$H-imipramine binding was mainly enriched in the nuclear fraction [18] indicating thus the occurrence of two different entities. Subsequent studies [31, 32], although they did not exactly confirm our results, did not reveal an identical subcellular profile for both entities. In fact, these studies further strengthen the idea of a lack of specificity for the $^3$H-imipramine binding. First, a large proportion of $^3$H-imipramine sites was found in the microsomal fractions, which surprisingly disappeared even more than in the mitochondrial fractions after chemical denervation with 5,7-dihydroxytryptamine [31]. Moreover, after chemical denervation, $^3$H-imipramine binding was reduced by 36% whereas more than 90% of the serotonergic neurones disappeared. The second study in which no marker enzymes were used to characterize the subcellular fractions (for this reason it may not be called a subcellular fractionation) and in which even the myelin and mitochondrial fractions were localized at a wrong place also revealed a different distribution profile for $^3$H-imipramine binding and 5HT uptake. Therefore $^3$H-imipramine is certainly not a specific ligand to label the 5HT uptake sites but binds to recognition sites for tricyclic drugs. These high affinity sites were even detected in the liver where their subcellular localization was purely mitochondrial (unpublished results). Owing to such a lack of specificity, it was not surprising to learn that imipramine may interact with the ionic channel of the nicotinic receptor in *Torpedo* [33] and that $^3$H-imipramine binding sites were found in *Escherichia coli* [34].

*High affinity agonists for non specific binding.* Sometimes an error may be profitable for other scientists. Unfortunately this was not the case in the binding studies; the same mistake, i.e. to try to identify receptor sites with labelled agonists has been repeated for practically all the neurotransmitters. First it was clearly established that binding assays using labelled catecholamines [35] were not a suitable approach to identify $\beta$-adrenergic receptors [36, 37]. Despite this, labelled catecholamines were again taken to try to label $\alpha$-adrenergic receptors [38]. Thereafter, $^3$H-dopamine [39], $^3$H-serotonin [40], $^3$H-histamine [41] and $^3$H-nicotine [42] were also reported to bind to "receptor" sites, generally non accessible to antagonists. The common idea was that because the binding is saturable, reversible and mostly exhibits high affinity properties, these sites might have an important role though different from that of the receptor sites labelled with antagonists which only display low affinity for agonists. However, the major problem is that none of these agonists act in physiological conditions at such low concentrations (nanomolar); all of them need at least micromolar concentrations not only *in vivo* including cerebral injections but also in binding assay when labelled antagonists are used as ligand. In fact, the agonists bind with high affinity to non specific sites; until now all these so-called agonist sites are only recognition sites for catechol, indol or imidazol like structures; there is a complete lack of correlation with any physiological or pharmacological activity. It is now obvious that the dopamine $D_3$ and serotonin $S_1$ sites are certainly not involved in neurotransmission [1, 43].

These agonist sites have evidently generated a lot of speculations about the occurrence of multiple "receptor" subtypes [39, 44, 45]. More surprising—or perhaps not surprising at all—was the modulation of conformational states of the $S_1$ site by antidepressant drugs possibly through an allosteric process and its stabilization by GTP [46]. Last but not least these $S_1$ sites were even generated *de novo* in test tubes [47]; indeed after solubilization the recovery of $S_1$ sites reached the extraordinary value of 217%! That GTP was found to regulate most of these agonist sites remains somewhat strange; this should mean that GTP can also interact with non specific binding sites, a fact which is, however, compatible with the detergent properties of the nucleotide [48].

*Displaceable does not mean specific.* The classical equation, displacement = specific and non displaceable = non specific is certainly not true; agonist bindings are good examples of displaceable non specific binding. Such an oversimplification has generated not only a lot of fancy but is largely responsible for the explosive increase of "receptor" subtypes. This problem has been discussed in detail elsewhere [49].

The question arises how such a non specific binding may indirectly influence specific binding on receptor sites. When $^3$H-tamoxifen was found to label the estrogen receptor and distinct anti-estrogen binding sites, the authors pertinently concluded that these binding sites may not be directly involved in mediation of the classically recognized estrogen antagonism of anti-estrogens; however, they might influence the action of anti-estrogens *in vivo*, possibly in a passive manner by altering the distribution of these drugs and, hence their biological potency [50]. Presumably, the same may be true also *in vitro* in the binding assay itself. Figure 3 shows the inhibition curves of astemizole in the $^3$H-pyrilamine assay in

two different conditions; surprisingly when the incubation volume was brought from 1.1 to 10 ml, the affinity of astemizole increased from 40 to 4 nM. There is no theoretical basis to explain such an observation. However $^3$H-astemizole is known to bind with high affinity to non specific sites and even to the glass-ware [51]; therefore one can assume that relatively more drugs are available in larger incubation volume in such a way that non specific sites would be more readily "saturated". One has to remember that when a drug is tested *in vitro* for its ability to inhibit a specific ligand binding, one assumes (sometimes erroneously) that the affinity of this drug for non specific binding sites is low enough as to not influence the specific binding on the receptor site. For astemizole the opposite was probably true. Let us recall this important point that the affinity of the cold drugs for non specific sites in binding studies remains nearly always an unknown parameter which can sometimes influence binding on receptor sites.

*Membrane heterogeneity and multiple receptor localization.* Brain fractionation studies performed according to an analytical approach reveal that the subcellular distribution of receptors is bimodal [cf. 52]. The highest number of receptors is generally recovered in the microsomal fractions whereas a lesser amount is associated with nerve endings. Obviously the neurotransmitter receptors possess a multiple postsynaptic localization [52, 53].

It is quite important that one be aware of the heterogeneity of membrane structures bearing receptor sites for interpreting binding studies on membrane preparations. Indeed, since the biological material used is so heterogeneous, one may expect that the binding of labelled or displacer drugs (agonist or antagonist) will not necessarily occur in the same way in all the different membrane structures. Figure 3 illustrates schematically the possible subcellular localization of receptor sites: external membrane of nerve endings, within the nerve endings and in small vesicles as demonstrated by axonal flow experiments [54]. It is likely that the accessibility of the different drugs will not necessarily be the same in all the intracellular membrane structures. Drugs like agonists could be taken up into vesicles or into nerve endings in a way that their concentration in the vicinity of certain receptor sites will be higher than in the incubation medium. Another possibility could be that a given drug prevents the penetration of labelled ligand towards certain receptor sites. In this regard, hydrophilic or lipophilic substances will not behave similarly namely the agonists which are known to be much less hydrophobic. Therefore all these possibilities must be taken into consideration for interpreting intriguing results namely flattened, biphasic or multiphasic displacement curves. In my opinion, the heterogeneity of receptor bearing membranes can provide a more plausible explanation to binding studies for which hypothetic subtypes of muscarinic receptors (super high; high and low affinity sites or $M_1$ and $M_2$) were proposed [55, 56]. Supporting this view is the fact that changes of ion concentrations are already sufficient to modify binding properties [57] and that, after solubilization, such anomalous displacement curves are not found.

*Ligand trapping in intact cells.* Binding studies on intact cells require even more caution than those on membrane preparations. Here, $^3$H-ligand can also be taken up by a trapping phenomenon (permeation) into the lysosomes. Numerous bases are known to enter into the lysosomes by permeation [58]. Recently such a trapping phenomenon was observed on lymphocytes when performing *in vitro* $^3$H-spiperone [59] and $^3$H-dihydroalprenolol binding [60], but also on fibroblasts, hepatocytes and neuroblastoma cells [59]. As a rule, chloroquine, a well-known lysosomotropic drug is one of the most potent drugs to displace the entrapped $^3$H-ligand. As pointed out in detail elsewhere [59], this trapping phenomenon must be regarded as a serious drawback in binding studies when carried out on intact cells.

*Acknowledgement*—I thank D. Ashton for his help in preparing the manuscript. This work was partially supported by a grant from I.W.O.N.L.

## REFERENCES

1. P. Laduron, in *Advances in Dopamine Research* (Eds. M. Kohsaka *et al.*), Vol. 37, p. 245. Pergamon Press, Oxford (1982).
2. Z. M. Bacq, *Trends Pharmac. Sci.* **1**, 141 (1980).
3. J. N. Langley, *Proc. R. Soc. B* **78**, 170 (1906).
4. J. P. Changeux, *Harvey Lect.* **75**, 85 (1981).
5. P. M. Laduron and B. Ilien, *Biochem. Pharmac.* **31**, 2145 (1982).
6. P. Cuatrecasas, *Biochem. Pharmac.* **23**, 2353 (1974).
7. W. P. Burkard, *Eur. J. Pharmac.* **50**, 449 (1978).
8. D. A. Kendall, J. W. Ferkany and S. J. Enna, *Life Sci.* **26**, 1293 (1980).
9. I. R. Smith, M. T. Cleverley, C. R. Ganellin and K. M. Metters, *Agents Actions* **10**, 422 (1980).
10. D. R. Bristow, J. R. Hare, J. R. Hearn and L. E. Martin, *Br. J. Pharmac.* **72**, 547P (1981).
11. T. J. Rising, D. B. Norris, S. E. Warrender and T. P. Wood, *Life Sci.* **27**, 199 (1980).
12. N. Subramanian and T. A. Slotkin, *Molec. Pharmac.* **20**, 240 (1981).
13. G. A. Gajtkowski, D. B. Norris, T. J. Rising and T. P. Wood, *Nature* **304**, 65 (1983).
14. F. C. Colpaert, F. M. Lenaerts, C. J. E. Niemegeers and P. A. J. Janssen, *Archs. int. Pharmacodyn. Thér.* **125**, 40 (1975).
15. C. H. Lee and S. H. Snyder, *Proc. natn. Acad. Sci. U.S.A.* **78**, 5250 (1981).
16. S. Z. Langer, R. Raisman and M. Briley, *Eur. J. Pharmac.* **72**, 423 (1981).
17. M. Rehavi, P. Skolnick, B. Hulikan and S. M. Paul, *Eur. J. Pharmac.* **70**, 597 (1981).
18. P. M. Laduron, M. Robyns and A. Schotte, *Eur. J. Pharmac.* **78**, 491 (1982).
19. D. A. Kendall, D. P. Taylor and S. J. Enna, *Molec. Pharmac.* **23**, 594 (1983).
20. B. H. Bulis, M. A. Gordon and I. B. Wilson, *Biochim. biophys. Acta* **643**, 398 (1980).
21. S. J. Zalcman, L. M. Neckers, O. Kaayalp and R. J. Wyatt, *Life Sci.* **29**, 69 (1981).
22. J. M. Bidart, C. Moninger and C. Bohuon, *Res. Commun. Chem. Path. Pharmac.* **39**, 169 (1983).
23. J. E. Leysen, C. J. E. Niemegeers, J. M. Van Nueten and P. M. Laduron, *Molec. Pharmac.* **21**, 301 (1982).
24. L. Maître, P. Moser, P. A. Baumann and P. C. Waldmeier, in *Biogenic Amines and Affective Disorders* (Eds. Svensson and Carlsson), *Acta physiol. scand.* **280**, suppl. 61, 97 (1980).
25. R. Raisman, M. Briley and S. Z. Langer, *Nature* **281**, 148 (1979).

26. R. Raisman, M. S. Briley and S. Z. Langer, *Eur. J. Pharmac.* **61**, 373 (1980).
27. S. M. Paul, M. Rehavi, P. Skolnick and F. K. Goodwin, *Life Sci.* **26**, 953 (1980).
28. L. Ahtee, M. Briley, R. Raisman, D. Lebuc and S. Z. Langer, *Life Sci.* **29**, 2323 (1981).
29. R. C. Arora, C. Tong, H. L. Jackman, D. Stoof and H. Y. Meltzer, *Life Sci.* **33**, 437 (1983).
30. I. Mocchetti, N. Brunello and G. Racagni, *Eur. J. Pharmac.* **83**, 151 (1982).
31. M. Sette, M. Ruberg, R. Raisman, B. Scatton, B. Zivkovic, Y. Agid and S. Z. Langer, *Eur. J. Pharmac.* in press (1983).
32. M. Rehavi, P. Skolnick and S. M. Paul, *Eur. J. Pharmac.* **87**, 335 (1983).
33. M. E. Eldefrawi, J. E. Warnick, G. G. Schofield, E. X. Abbuquerque and A. T. Eldefrawi, *Biochem. Pharmac.* **30**, 1391 (1981).
34. J. Molnar, K. Csiszár and G. Tóth, *Res. Commun. Chem. Path. Pharmac.* **39**, 127 (1983).
35. R. J. Lefkowitz and E. Haber, *Proc. natn. Acad. Sci. U.S.A.* **68**, 1773 (1971).
36. P. Cuatrecasas, G. P. E. Tell, V. Sica, I. Parikh and K. J. Chang, *Nature* **247**, 92 (1974).
37. R. J. Lefkowitz, L. E. Limbird, C. Mukherjee and M. C. Caron, *Biochim. biophys. Acta* **457**, 1 (1976).
38. D. C. U'Prichard and S. H. Snyder, *Life Sci.* **20**, 527 (1977).
39. P. Seeman, *Pharmac. Rec.* **32**, 229 (1980).
40. G. Fillion, M. P. Fillion, C. Spirakis, J.-M. Bakers and J. Jacob, *Life Sci.* **18**, 65 (1976).
41. G. Barbin, J. M. Palacios, E. Rodergas, J. C. Schwartz and M. Garbarg, *Molec. Pharmac.* **18**, 1 (1980).
42. C. Romano and A. Goldstein, *Science* **210**, 647 (1980).
43. J. E. Leysen and J. P. Tollenaere, in *Annual Report in Medicinal Chemistry* (Ed. J. McDermed), Vol. 17, p. 1. Academic Press, New York (1982).
44. S. H. Snyder and R. R. Goodman, *J. Neurochem.* **35**, 5 (1980).
45. D. L. Nelson, N. W. Pedigo and H. I. Yamamura, in *Psychopharmacology and Biochemistry of Neurotransmitter Receptors* (Eds. H. I. Yamamura, R. W. Olsen and E. Usdin), p. 325. Elsevier North-Holland, Amsterdam (1980).
46. G. Fillion and M. P. Fillion, *Nature* **292**, 349 (1981).
47. S. R. Vanderberg, R. L. Allgren, R. D. Todd and R. D. Ciaranello, *Proc. natn. Acad. Sci. U.S.A.* **80**, 3508 (1983).
48. D. Godelaine, H. Beaufay, M. Wibo and A. M. Ravoet, *J. Cell. Biol.* **97**, 340 (1983).
49. P. Laduron, *Trends Pharmac. Sci.* **4**, 333 (1983).
50. K. Sudo, F. J. Monsma and B. S. Katzennellenbogen, *Endocrinology* **112**, 425 (1983).
51. P. M. Laduron, P. F. M. Janssen, W. Gommeren and J. E. Leysen, *Molec. Pharmac.* **21**, 294 (1982).
52. P. M. Laduron, P. F. M. Janssen and B. Ilien, *J. Neurochem.* **41**, 84 (1983).
53. P. Laduron, in *Apomorphine and Other Dopaminomimetics. Vol. I. Basic Pharmacology* (Eds. G. L. Gessa and G. U. Corsini), p. 95. Raven Press, New York (1981).
54. P. M. Laduron, *Biochem. Pharmac.* **33**, 897 (1984).
55. N. J. M. Birdsall and E. C. Hulme, *Trends Pharmac. Sci.* **4**, (1983).
56. M. Watson, H. I. Yamamura and W. R. Roeske, *Life Sci.* **32**, 3001 (1983).
57. J. E. Leysen and W. Gommeren, *J. Neurochem.* **36**, 201 (1981).
58. C. de Duve, T. de Barsy, B. Poole, A. Trouet, P. Tulkens and F. Van Hoof, *Biochem. Pharmac.* **23**, 2495 (1974).
59. J. M. Maloteaux, A. Gossuin, C. Waterkeyn and P. M. Laduron, *Biochem. Pharmac.* in press (1983).
60. H. Meurs, W. Van den Bogaard, H. F. Kauffman and P. L. B. Bruynzeel, *Eur. J. Pharmac.* **85**, 185 (1982).