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COMMENTARY

SOLUBILIZATION OF BRAIN MUSCARINIC, DOPAMINERGIC AND SEROTONERGIC RECEPTORS: A CRITICAL ANALYSIS

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In vitro binding studies using labelled ligands have considerably facilitated a better understanding of neurotransmitter receptors in the brain and provided a basis for the elucidation of the mechanisms of drug action. However, in spite of an almost explosive increase in the number of studies in this field, we have very little idea about the cascade of molecular events which begins with the formation of the ligand–receptor complex and terminates in a physiological response. Why do certain substances act as agonists and others as antagonists? This represents one of the most fundamental unanswered problems in the field of brain neurotransmitter receptors, but is to some extent solved for the nicotinic receptor from Torpedo [1]. To achieve this goal, two prerequisite steps are needed: first the conversion of the receptors into a solubilized form and then their purification.

The purpose of this commentary is to discuss the solubilization of the muscarinic acetylcholine, dopamine and serotonin receptors. The majority of difficulties encountered during their solubilization were more or less common to all three.

Before considering each receptor site in particular, we will describe some general principles and more especially the criteria that must be fulfilled when assessing whether the receptors have been effectively obtained in a soluble form while retaining their original high affinity properties.

Principles for the solubilization of membrane-linked proteins

Maddy and Dunn [2] define the solubilization as a conversion of the complex membrane system into a relatively simpler state which can only be characterized by using methods that cannot be applied to the intact membrane. All the receptors involved in brain neurotransmission are membrane-linked proteins; their role is to bring information from outside the neuronal cell into its interior. The interaction of signal-molecules with a macromolecular complex constitutes the basis of the neurotransmission concept.

After fractionation by differential centrifugation, muscarinic, dopaminergic and serotonergic receptors were mainly enriched in the microsomal (P) fraction; their distribution pattern resembles that of 5′ nucleotidase, a marker enzyme of plasma membranes [3–5]. Subfractionation of digitonin-treated membranes in isopycnic centrifugation provided evidence that the synaptic membranes bearing dopamine and muscarinic receptors behave as plasma membranes, thus having a higher content of cholesterol than other intracellular membranes [5].

What is the topography of receptors in the molecular organization of membranes? There is now a consensus [cf. 2, 6] that membrane proteins are attached to membranes in two different ways: first, proteins can be embedded within the lipid bilayer of the membranes (Fig. 1); they are, thus, called integral or intrinsic or even endo-membrane proteins. They are firmly bound to membranes and may have functionally important interactions with the membrane [6]. One of the greatest differences between membrane enzymes and "soluble" or cytoplasmic enzymes is just the interrelationships of the former with lipids [7]. The second type of proteins are located at the surface of the membranes; they are called peripheral or extrinsic or even exo-membrane proteins; they are weakly bound by electrostatic forces or by intermediate divalent cations. They do not interact with the membrane lipids [6] and are expected to retain their original conformation in aqueous solutions [2].

Although the distinction between intrinsic and extrinsic membrane proteins remains conceptually valuable, the experimental criteria leading to such classification must be applied with caution [2, 6]. It is widely accepted that most of the membrane proteins (70–80%) are easily recognized as intrinsic [6]; they include membrane-associated enzymes, antigenic and transport proteins as well as neurotransmitter and certain hormone receptors [6, 8]. As a rule, these transmembrane proteins are amphipathic, their hydrophobic ends being embedded within the non-polar interior of the lipid bilayer and their hydrophilic ends protruding from the membrane [6]. These external regions of intrinsic proteins are particularly important from a functional point of view because they contain the binding or recognition sites of neurotransmitters, hormones or substrates [8]. Such transmembrane topography was recently reported for the nicotinic receptor from Torpedo [9].

How to solubilize or extract extrinsic and intrinsic proteins?

The present consensus is that the different sorts of proteins require different solubilization pro-

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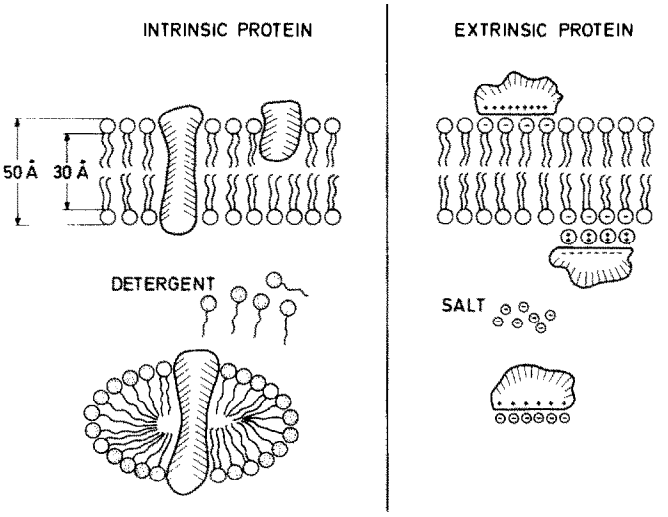

Fig. 1. Schematic membrane topography of intrinsic (integral) or extrinsic (peripheral) proteins and their solubilization by mild detergents or high salt concentrations.

cedures [2, 10–12]; the extrinsic proteins are readily solubilized by relatively mild or gentle methods involving chelating agents and changes in ionic strength and pH. The fact that these loosely bound proteins can be extracted by relatively high salt concentration (2 M NaCl or KCl) may be regarded as evidence for the occurrence of electrostatic binding [2].

When the salt concentration exceeds 3 M, it is not clear whether the solubilizing effect may be attributed to a diminution of electrostatic forces or to a chaotropic effect [2]. More tightly bound to the membrane, the intrinsic proteins cannot be released by the former manipulations; their extraction requires the use of detergents, organic solvents or chaotropic agents [2, 10–12]. In fact, detergents (synthetic, bile salts and saponins) may be considered as the most useful solubilizing agents presently available [10].

Why are detergents the tool of choice for effective solubilization of biologically active proteins?

Detergent molecules are a special class of lipids; they contain polar and apolar groups. The solubilizing effects of detergents stem from their amphiphilic character which enables them to interact with both hydrophilic and lipophilic regions in an essentially disruptive fashion but not denaturating [2]. In contrast, all organic solvents used for lipid extraction and certain ionic detergents cause some degree of protein denaturation [7]. At low concentrations in water, detergents exist mostly as monomers; at higher concentrations members of them aggregate to form, more or less, spherical micelles with polar groups at the surface and the hydrocarbon chain forming a core (cf. Fig. 1) [13]. At low detergent–membrane ratios the detergent monomers bind to lipid or proteins in the original membrane [10].

Appropriate detergents can fully substitute for membrane phospholipid (phospholipid simulation) and can offer, in micelles, the same kind of local environment to the protein as a phospholipid bilayer (cf. Fig. 1) [2, 8]. The most useful property of micelles is their ability to 'dissolve' hydrophobic materials in their interior [13]. However, some proteins require more specific phospholipid molecules in their environment to exhibit activity (reconstitution experiment) [11].

Solubilization criteria of specific receptor sites

Two types of criteria must be considered; first, the criteria that allow assessment of the identity of binding sites measured *in vitro* with labelled ligands; one has to ascertain that such binding sites really correspond to the receptor sites responsible for the physiological effects of a given neurotransmitter. These criteria of specificity are practically identical to those which are applied to binding on membrane preparations; they have been discussed in detail elsewhere [4, 14].

The second group of criteria concerns the solubilization criteria themselves which allow to ascertain that membrane-bound receptors are truly converted into a molecularly dispersed system. Finally, in the last part of this paragraph, certain factors for optimalizing receptor solubilization will be considered.

Let us examine briefly the first group, which is summarized in Table 1. High affinity, saturability, reversibility and stereospecificity are certainly required, and even necessary, conditions for specific binding but they are far from being decisive. When these criteria are fulfilled one cannot be certain that the binding site is relevant physiologically. More crucial are the criteria which concern the regional distribution in the brain, the drug displacement and, last but not least, the correlations with pharmacological or clinical effects. Regional distribution is particularly evident for dopamine and serotonin receptors which are specifically located in a few brain regions. In this regard, it is often useful to perform a solubilization from a brain region devoid of receptors; this solubilized material may be considered as a tissue blank. However, a regional distribution is not always a good indication; for instance, the spirodecanone sites are markedly enriched in the frontal

Table 1. Solubilization of specific brain receptors

A. Criteria of receptor specificity
1. High affinity
2. Saturability
3. Reversibility
4. Stereospecificity
5. Regional distribution
6. Drug displacement (agonists and antagonists belonging to different chemical and pharmacological class)
7. Correlation between drug affinity in solubilized and membrane preparations
8. Correlation between drug affinity *in vitro* and pharmacological potency in *in vivo*

B. Operational criteria to assess receptor solubilization
1. Lack of sedimentation in low density media (100,000 g × 60 min)
2. No retention on small pore size filters (millipore 0.22 μ)
3. Higher retention on gel filtration than membranes
4. Lower sedimentation coefficient than membranes
5. Disappearance of lamellar membrane structure
6. Decrease of thermostability

C. Conditions to optimalize receptor solubilization
1. Different animal species
2. Choice of a brain region
3. Subcellular fraction enriched in receptors
4. Selection of mild detergents
5. Medium composition
6. Choice of appropriate binding assays
7. Labelling of receptors before solubilization (prelabelling conditions)

cortex but cannot be considered as receptor sites [14–17].

Drug displacement is one of the most, if not the most, important criteria. It consists of determining the affinity of various drugs (agonists and antagonists) for the receptor. However, to be valid, one needs to test drugs belonging to different chemical and pharmacological classes. Why is it necessary to select compounds from different chemical series? To rule out the possibility that the binding sites are only recognition sites for compounds having the same structural moiety but devoid of any physiological identity, as has often been found with numerous labelled ligands. Therefore, we believe that 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 with a large number of compounds. When this has been achieved one can try to correlate the drug affinity measured *in vitro* with the drug potency obtained *in vivo* from pharmacological tests. One cannot stress enough upon the fact that all the criteria listed in Table 1A must be fulfilled to be sure that the *in vitro* binding reveals a specific and physiologically active receptor. In the case of solubilized receptors, an additional criterion is that the binding sites must display the same high affinity properties in the soluble state, as in membrane preparations.

Now the question arises; how is it possible to assess that a receptor site has been really solubilized? Here, the criteria are mostly operational; they are essentially based on the use of different biochemical and even morphological techniques. These solubilization criteria are listed in Table 1B; two points need further comments. It is generally believed that the lack of sedimentation at 100,000 g for 60 min represents an important, if not decisive, criterion for solubilization; this is true if the medium that contains the receptor sites, has a density lower than all the membrane structures. Indeed when high salt concentrations are used as solubilizing agents, the density of the medium becomes so high as to prevent certain membrane elements from being spun down; in these conditions, membrane-like structures may remain in solution or simply above the solution. In our opinion, the criterion of sedimentation in sucrose gradients represents one of the most severe tests; it allows one to clearly differentiate the membrane and soluble state, the former being recovered in the bottom of the tube and the latter above in the first fractions.

Before examining the three brain receptors in detail, we would like to mention certain factors which can be useful to optimalize the solubilization conditions (Table 1C). As was the case for the dopamine receptors, switching to another animal species may help to solve certain problems encountered during the solubilization process. Brain regions and subcellular fraction enriched in receptors will be selected as well as an appropriate detergent at a given concentration. Sometimes the composition of the medium may be determining; the presence of sucrose can affect the state of association and binding of detergent on membrane proteins [10, 18]. Another point is the technique to separate the ligand–macromolecular complex from the free ligand; the use of gel-filtration though ideal in many cases is sometimes of too long duration and can lead to a dissociation of the ligand–receptor complex. Finally, an important problem concerns the relatively low yield in receptors (5–20%). The reason for this is unclear, but one of the most plausible hypotheses is that during the solubilization process, endogenous inhibitors perhaps basic proteins, are also solubilized and then can inhibit the binding. Two other possibilities are, the inhibition by the detergent itself or the dissociation of the receptor into inactive subunits. In favor of the first hypothesis, is the fact that the binding on solubilized receptors is only linear in a relatively narrow range of protein concentration; at higher concentration, even the binding may decrease. The fact that yields can sometimes exceed 60% when the receptors are first labelled on membranes and then solubilized does not allow any distinction to be made between the above possibilities.

Solubilization of muscarinic receptors

Initial attempts to obtain muscarinic receptors in a soluble form were not successful; membranes were first labelled with high concentrations of radioactive ligands and then extracted with organic solvents [19, 20] or with detergents [21]; however, these results suffered from a lack of characterization of specific solubilized receptors; moreover they have not been confirmed. More successful were the preliminary results of Beld and Ariens [22] who used the plant glycoside digitonin as solubilizing agent. Unlike Triton X-100, this detergent did not inhibit atropine binding. Although two different ligands [^{3}H]atropine and [^{3}H]dexetimide were used, the physical characteristics and the receptor specificity were not reported.

Thereafter, all the reports may be divided into two groups according to whether high salt concentrations or mild detergents were used as solubilizing agents. That both procedures would provide similar results is rather unlikely, since it would imply that the muscarinic receptor is, at one and the same time, an intrinsic and extrinsic protein. Indeed, two groups claimed to have obtained muscarinic receptors in solution by means of high salt concentration (2 N NaCl or NaI) [23–26] but this was not confirmed in many other laboratories [27–34]. The reason for this has been recently elucidated [33, 34]; in fact, the first solubilization criterion (lack of sedimentation, cf. Table 1) had been misinterpreted since the density of the medium was too high (NaCl 2 N $\rho = 1.08$) to allow a complete sedimentation of all the membranes. The median density of muscarinic receptors bearing membranes equals 1.12 with a distribution pattern of about 10–20% of membranes equilibrating between 1.08 and 1.09. Consequently, it is obvious that the high speed centrifugation had selected membranes of low density which were taken as 'solubilized' material. A strict application of the solubilization criteria enabled us to demonstrate that muscarinic receptors solubilized with 2 N NaCl behave in sedimentation gradient and through gel-filtration as membranes, but not as digitonin-solubilized receptors [34]. The failure of the salt treatment to solubilize muscarinic receptors rules out the possibility that this macromolecule is an extrinsic protein.

The second group of workers used mild detergents such as 1% digitonin [28–31, 33, 34] but also Lubrol PX [35] lysolecithin [36; Gorissen, unpublished results), SP_{15}, deoxycholate, tauro-cholate [31, 32] and a mixture of digitonin–cholate [37]. Muscarinic receptors were solubilized from brain of rat [28, 33–35], cat, dog, calf [34], ox [29–32], pig [36] and from porcine heart [37].

Various ligands (quinuclidinyl benzilate [^{3}H]QNB [29, 30, 35–37], propylbenzilylcholine mustard [^{3}H]PBCM [31, 38] and [^{3}H]dexetimide [22, 28, 33, 34]) were successfully used. Among these ligands, [^{3}H]dexetimide is the only one which can be compared directly to its labelled inactive enantiomer [^{3}H]levetimide. A pronounced stereospecific effect (about 4000 times) was observed between both enantiomers; moreover the use of [^{3}H]levetimide allowed to precisely evaluate the non-specific binding [4, 28, 39]. Although a large number of binding assays including gel-filtration [28, 34–36], polyethyleneglycol precipitation [37], adsorption on DEAE filters [29, 34, 37], ammonium sulphate precipitation [29, 34] and equilibrium dialysis [29, 30, 37] were successfully used, the charcoal adsorption method appears to be the most convenient procedure by giving fast and quite reproducible results with a high yield in specific binding sites [34].

Solubilized muscarinic receptors apparently display similar physical characteristics within various animal species: indeed, a sedimentation coefficient of 9S was found in digitonin extracts from rat [28] and bovine brain [29]. Interestingly, when muscarinic receptors were solubilized with 0.1% Lubrol PX, a value of 3S was obtained which allows to estimate a molecular weight of 86,000 [35]. This is in good agreement with the value of 83,000 obtained by SDS–polyacrylamide gel electrophoresis [38]. Until now, the heterogeneity of the agonist binding sites is not reflected in a corresponding heterogeneity in molecular weight [40].

As a rule, the receptor specificity criteria were fulfilled: drug displacement [28–30, 35], correlation between the drug affinity in detergent solubilized and membrane preparations [4, 28–30, 34, 36] and correlation with the drug potency in pharmacological tests [34, 41].

Interestingly, the solubilization yield in muscarinic receptors seems to depend mostly upon whether the receptor sites have been labelled before or after the solubilization procedure; using [^{3}H]QNB as ligand, the extraction yield varied between 27 and 43% according to the animal species [29, 37] in post-labelling conditions, but it exceeded 60% in prelabelling conditions [31].

From the foregoing results, one may conclude that only mild detergents are able to solubilize muscarinic receptors; therefore, they must be considered as intrinsic proteins.

Solubilization of dopamine receptors

The first attempts to obtain dopamine receptors in solution were not very encouraging; [^{3}H]spiperone binding sites solubilized from rat striatum by means of 1% digitonin had lost the high affinity properties (except for spiperone) normally found on membrane preparations [42]. Similar results were obtained from calf striatum [43]. The choice of another animal species was quite decisive; in choosing the dog, we were able, for the first time, to solubilize high-affinity dopamine receptors with digitonin [44, 45]. We now know why the dog was a better choice than the rat; in fact, the specific binding sites solubilized from rat striatum but not from dog striatum were heavily masked by a large proportion of non-specific but displaceable [^{3}H]spiperone binding sites (spirodecanone sites) [46]. Successful solubilizations were, then, achieved by other groups in canine [47, 48], bovine [49], rat [50] and human brains [47].

Digitonin is currently used, but other mild detergents like Lubrol PX [49], CHAPS [50] and lysolecithin [5, 49] have also been employed. Recently a curious mixture of sodium cholate and ammonium sulphate was described [51] but the solubilization criteria were not fulfilled. More surprising was the procedure used by Clement-Cormier and Kendrick [52] which was further discussed in a recent commentary [53]. This work is based on the use of saturating concentrations of KCl which is equal to 4.2 M at 25° (density, $\rho = 1.78$). Their standard procedure was performed at 0° with 50% KCl (w/w or g salt/g original wet weight of tissue); this corresponds approximately to 3.4 M KCl. Note that KCl concentrations higher than 50% (reaching even 500%) were also used [52]. One must be suspicious about the use of such high salt concentration, since it should imply that dopamine receptors could be an extrinsic protein. In fact we are, again, faced with the same problem encountered throughout the solubilization of muscarinic receptors; the too high density of the medium falsely allows the sedimentation criterion to be validated. Although the authors

claimed to have submitted their 'solubilized' material to different tests (except the sedimentation gradient) in order to verify the soluble state of the [^{3}H]spiperone binding sites, they did not present any results to prove this. In fact numerous points argue against effective solubilization; first, even after centrifugation at 100,000 *g* for 2 hr, membrane fragments are not sedimented since the density of the medium exceeds that of the membranes ($\rho = 1.121$); secondly, the observation of microlamellar-like structures by electromicroscopy [52], the complete retention of KCl-'solubilized' binding sites on GF/B filters [53] and the relative thermostability of these sites. Finally, in our laboratory, H. Gorissen (unpublished results) was only able to demonstrate the presence of membrane binding sites in KCl extracts; when the density of the medium was lowered after 'solubilization', no binding sites were detected in the supernatant. It is, thus, unlikely that the dopamine receptor may be considered as an extrinsic protein.

Different binding assay procedures were proposed for detergent-solubilized dopamine receptors: gel-filtration [44–48, 52], polyethyleneglycol [48, 50] or ammonium sulphate precipitation [46], equilibrium dialysis [46] and adsorption on BSA-coated charcoal [46, 49, 52, 53]. The latter method seems to be one of the most sensitive techniques and is especially appropriate for digitonin-solubilized dopamine receptors from rat striatum [46]. Indeed, the concomitant use of this technique and of the spirodecanone site blocker (R 5260, Ref. 46) allowed to greatly diminish the proportion of non-specific binding and to reveal the existence of solubilized dopamine receptors [46] instead of spirodecanone binding sites [42].

A large series of dopamine antagonists belonging to different chemical classes and of agonists were tested in solubilized extract and a good correlation was obtained between the drug affinity in soluble and membrane preparations [4, 44, 45, 47]. The macromolecular complex solubilized by digitonin was much more sensitive to thermal inactivation than the membrane-bound receptors and had a sedimentation coefficient of 9S [45]; this corresponds to a molecular weight of 200,000, but this estimation is probably an overestimate.

Now the question arises as to whether the dopamine receptor in soluble form is the same as the receptor responsible for the physiological effects of dopamine? Moreover, what is the significance of such a dopamine receptor site seen against the multiple dopamine sites concept? The solubilized dopamine sites labelled with [^{3}H]spiperone fulfilled the receptor specificity criteria: high affinity and saturability [44, 47, 48, 50], reversibility [44], stereospecificity [44–48, 50], regional distribution [44, 45, 47] as well as drug displacement and correlation between soluble and membrane preparations [4, 44, 45, 47].

Why does the membrane or soluble site correspond to the dopamine D_2 receptor site [45]?

It is beyond doubt that the [^{3}H]spiperone binding site from dopaminergic brain regions is the physiologically active dopamine receptor; indeed the binding on D_2-sites in membrane and solubilized preparations perfectly correlates with the antagonism of apomorphine-induced emesis [4, 14, 44, 54]. Moreover, as reported elsewhere [4, 14, 54], there are more than 17 pharmacological, behavioural and biochemical parameters related to the effects of dopamine agonists and antagonists which also correlates with the binding on the D_2-receptor. In contrast, such a correlation was not found for the D_1, D_3 and D_4-subtypes [14, 54]; the dopamine-sensitive adenylate cyclase, defined as the D_1-site [55] is an enzyme [14, 54] and no evidence permits it to be considered as a receptor [56]; the D_3-subtype [55] is quite unrelated to any dopaminergic activity [14] and probably is a recognition site for catechol derivatives [14] including norepinephrine [55]. Recently, two different groups claimed to have obtained solubilized [^{3}H]dopamine binding sites; they were identified as a D_2-site by the one [57] but as a D_3-site by the other [58]. This needs some comments. The occurrence of the D_3-subtype is based on its high affinity (nanomolar range) for dopamine, norepinephrine and other catechol derivatives and low affinity (micromolar range) for neuroleptics or dopamine antagonists. This single criterion of specificity is quite insufficient to merit calling it a receptor or even a dopaminergic binding site. Indeed, dopaminergic means activity related to dopamine; a fact which implicates *ipso facto* the existence of a receptor. As a matter of fact, the D_3-site is not recognized by dopamine antagonists and therefore cannot be considered as a physiologically relevant receptor. That this binding site has now been solubilized [57, 58] is certainly of minor importance and remains an enigma; it is quite unlikely that [^{3}H]dopamine could remain firmly bound to a physiologically active macromolecular complex during the long-lasting gel-filtration procedure [58]; this is also against the basis of the neurotransmission concept; the interaction of an agonist with its receptor is a very short-lasting event which should not permit the detection of a dopamine–receptor complex after such a long-lasting filtration procedure. There are many reasons to believe that the D_3-site is purely artefactual.

Solubilization of serotonergic receptors

Serotonin (5-HT) receptors have been identified *in vitro* with various ligands such as [^{3}H]LSD, [^{3}H]spiperone, [^{3}H]ketanserin and [^{3}H]-5-HT. However, they do not all label the same site [59, 60]. In the frontal cortex, LSD, spiperone and ketanserin were found to label a site [61, 62] which was designated as the serotonin-S_2 receptor [63]. In contrast, 5-HT selectively labels on S_1-site [59, 63–65] whose physiological relevance remains unclear [60, 63]. This site more likely represents a recognition site for indol-like compounds since most of the serotonin antagonists are either weakly active or inactive [59, 60, 63]. In contrast, the S_2-site is certainly a physiological receptor. Indeed, the relative potency of drugs in preventing the bilateral clonic seizures induced by tryptamine [60, 61] or the head twitches induced by 5-hydroxytryptophan [63] and the 5-HT-induced vasoconstriction of rat caudal arteries [60, 62] parallels their affinity for the S_2-site.

Serotonin (S_2) receptors were solubilized for the first time by using the mild detergent, lysolecithin

[66]. They were extracted from a microsomal (P) membrane fraction from rat frontal cortex which contains the higher proportion in S_2-receptors [62]. The first results were not entirely convincing since the specific [^{3}H]spiperone binding represented only 10% of the total binding. As was the case for the solubilization of dopamine receptors from rat striatum [46], we were also faced with the occurrence of a very high non-specific binding. When R 5573, an analgesic compound having a spirodecanone moiety, was added to the incubation medium the proportion of specific binding increased to 60% [66]. Afterwards, we found that the non-specific binding of [^{3}H]spiperone to the spirodecanone sites could be also abolished by performing the incubation at 30° during 20 min instead of 18 hr at 0° [5, 67]. In such conditions, the solubilized macromolecular complex labelled with [^{3}H]spiperone retained the high-affinity characteristics of the serotonin (S_2) receptor in the original membrane [5, 66]; the correlation coefficient between the drug potencies in both soluble and membrane preparations was equal to 0.976 [5]. [^{3}H]spiperone binding to the solubilized receptor sites was stereoselective, saturable and reversible [5]. No specific binding sites could be extracted from the cerebellum; in contrast, few binding sites endowed with the characteristics of serotonin receptors were detected in lysolecithin extracts from rat striatum but only when a tetralin-derivative was added to the incubation medium in order to prevent the [^{3}H]spiperone binding on solubilized dopamine receptors [5]. Interestingly, when microsomal membranes from rat striatum were treated with digitonin, only dopamine receptors were measurable [5]. Therefore, the latter detergent seems inappropriate for serotonin receptors. Finally, the serotonin (S_2) receptors solubilized by lysolecithin from rat frontal cortex fulfilled all the solubilization criteria and displayed a low sedimentation coefficient in sucrose density gradients [5].

More recently, [^{3}H]ketanserin was found to be a ligand of choice for solubilized serotonin (S_2) receptors [67]. The main advantage of this new ligand is its relatively low non-specific binding; all the difficulties encountered throughout the solubilization with [^{3}H]spiperone as ligand were not present here (no labelling on dopamine receptors or on other displaceable sites like recognition sites for compounds, structurally related to the ligand, and slow dissociation rate). [^{3}H]Ketanserin is certainly a more appropriate ligand for this kind of work; its use allowed the successful solubilization of serotonin S_2-receptors from rat, dog and human brains [67, Schotte, unpublished results]. Although the solubilization yield, under post-labelling conditions, was rather low (about 5%), when prelabelling conditions were followed, this yield was brought to 40% [67]. Here again, the reasons for this low solubilization effectiveness remains unclear; further experimental data are needed to confirm whether, or not, endogenous inhibitors of the binding occur.

Conclusion

The three neurotransmitter receptors, muscarinic, dopaminergic and serotonergic could be solubilized by means of mild detergents; this suggests that these receptor sites are intrinsic proteins. Numerous criteria must be fulfilled to assess not only that the binding sites were effectively solubilized but that they correspond to a physiologically active receptor specific for a given neurotransmitter. The solubilization is not a goal in itself; but it is a prerequisite step to the purification of the receptor and the attempt to unlock the molecular events behind the binding site.

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