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STEREOSPECIFICITY IN BINDING STUDIES

A USEFUL CRITERION THOUGH INSUFFICIENT TO PROVE THE PRESENCE OF RECEPTORS

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Abstract—In binding studies, stereospecificity is not a property restricted to receptor sites; indeed stereospecific binding has also been observed for acceptor sites. Therefore it does not represent a decisive criterion to make a binding site, a receptor site. However, in some well established cases, it can be useful especially when the difference between the active and inactive enantiomer exceeds 1000-fold as is the case for dexetimide and levetimide on muscarinic receptors.

Stereospecific effect is also detectable with acceptor sites, e.g. spirodecanone sites, levocabastine displaceable neurotensin and, presumably, many other ones. Since the membrane is chiral (L-aminoacid) one should expect that non-specific displaceable binding would also display stereospecificity. In this regard, as most of the Scatchard plots reported throughout the literature are curvilinear, even if a straight line is drawn, one may assume that this is due to the presence of acceptor sites that are labelled by the ligand in addition to receptor sites. One cannot exclude the repetition of another "levocabastine story" with other neuropeptides.

Hence, as the biochemical criteria like high affinity, saturability, reversibility and stereospecificity cannot differentiate a receptor from an acceptor (see Table 1), the most important and decisive criteria remain: (1) the drug displacement with compounds belonging to different pharmacological classes but mostly to different chemical classes, and (2) the functional correlates between the binding affinity and the potency in pharmacological or physiological tests *in vitro* or *in vivo*. When these points are fulfilled a binding site may be called a receptor site.

Binding studies are becoming more and more legion; today most of the pharmacological and neurological laboratories are currently using *in vitro* binding assays. Of course, the introduction of this technique has been quite a decisive step for the identification of brain receptors and more recently for the molecular dissection of them. Binding studies have also contributed to the localization of brain receptors and even to their visualization in human brain (PET scanning), to the exploration of the cellular and dynamic mechanisms in which receptors are involved and also to a better understanding of the mechanism of action of numerous drugs. Today binding is becoming quite useful in discovering new drugs. However, besides numerous advantages, binding also has serious drawbacks and limitations; it has been, and still remains today, the source of numerous controversies, in particular in the multiplication of receptor subtypes. Perhaps one of its major drawbacks is that it is too easy to perform, while the interpretation of the results remains extremely difficult. Sometimes people believe that some of my statements on the binding are too negative while my aim is only to emphasize the possible pitfalls of them and the real difficulties encountered through the interpretation of binding results. What I was trying to say is that binding studies have to be integrated into a multidisciplinary approach because the biochemical criteria are not sufficient to make sure that the binding sites measured *in vitro* really represent physiological receptors. Functional correlates are thus an absolute requirement (cf. Table 1).

Table 1. Binding specificity criteria for receptor and acceptor sites

	Criteria	Receptor	Acceptor
1.	High affinity	+	+
2.	Reversibility	+	+
3.	Saturability	+	+
4.	Stereospecificity	+	+
5.	Region or tissue specificity	+	+
6.	Drug displacement with compounds belonging to		
	(a) the same chemical class	+	+
	(b) different chemical classes	+	−
7.	Functional correlates between binding affinity and pharmacological potency	+	−

One of the biochemical criteria is the stereospecificity which was the topic of the third Biochemical Pharmacology Symposium.

The purpose of the present paper is to examine to what extent, when a drug possesses active and inactive enantiomers, this may help in assessing receptor specificity. In this regard, various examples of binding stereospecificity not only on receptor sites but also on acceptor sites will be considered and discussed.

MUSCARINIC RECEPTORS

Among all the specificity criteria required to make a binding site a receptor site, stereospecificity seems to be essential. We will see, however, that it is not decisive, because stereospecificity also exists for acceptor sites or binding sites unrelated to physiological receptors. Only in some well established cases, it may be considered as determining to ascertain the binding specificity; the muscarinic receptor is such an example.

Some years ago, we were looking at the binding of the two enantiomers; the dextro form, [^{3}H] dexetimide was found to bind to muscarinic receptors with high affinity and with a very low dissociation rate whereas the non-specific binding was extremely low [1]. In this binding the affinity of dexetimide (IC_{50}; 3 nM) was more than 2000 times that of levetimide, the inactive enantiomer. Similar experiments were carried out with [^{3}H] levetimide as ligand. Interestingly, the binding with [^{3}H] levetimide did not reveal such a stereospecific displacement but both enantiomers revealed the same very flattened inhibition curve (Fig. 1). In fact, the shape of a displacement curve is already determining; when a displacement occurs over a range of concentrations which exceeds 2 to 3 log, one may be suspicious about the nature of the binding which is not compatible with the presence of physiological receptors. In the case of [^{3}H] levetimide, it was definitively unrelated to muscarinic receptors and even to any

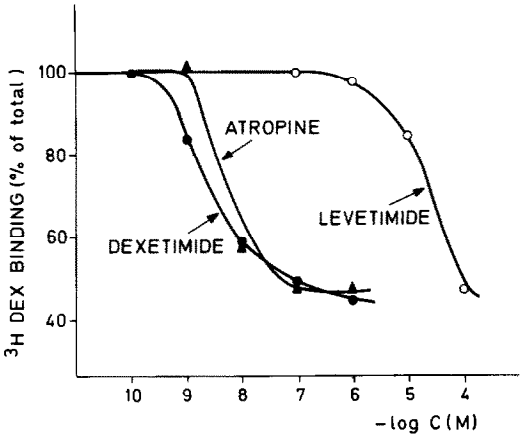

Fig. 2. Inhibition of [^{3}H] dexetimide binding in a total homogenate of dog splenic nerves by atropine, dexetimide and levetimide.

receptor sites [1]. Subsequently when muscarinic receptors were measured using [^{3}H] dexetimide, [^{3}H] QNB or [^{3}H] methylscopolamine as ligand, the use of dexetimide and levetimide as displacers was quite decisive. For instance, the first evidence for the existence of axoplasmic transport of neuroreceptors was found in ligated dog splenic nerves, and the muscarinic receptors were labelled *in vitro* with [^{3}H] dexetimide [2]. Three displacement curves were necessary to prove the muscarinic nature of [^{3}H] dexetimide binding (Fig. 2): dexetimide (IC_{50}; 2 nM), atropine (6 nM) and levetimide (28 μM). In fact, three criteria were so fulfilled: high affinity (dexetimide), stereospecificity (more than 10,000 between both enantiomers) and displacement with a drug, atropine, belonging to another chemical class as the ligand but having the same pharmacological property (cf. Table 1). More recently, a 10,000-fold stereospecificity was found with dexetimide and levetimide in 108CC15 neuroblastoma cells (mem-

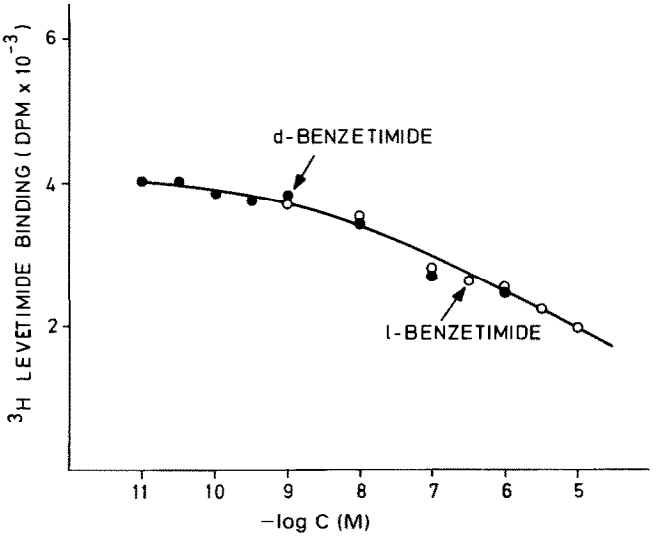

Fig. 1. Dose–response inhibition of D-benzetimide (dexetimide) and L-benzetimide (levetimide) in the [^{3}H] levetimide assay using a total homogenate from rat striatum.

branes and intact cells) using [^{3}H] dexetimide, [^{3}H] QNB and [^{3}H] methylscopolamine [3]. Interestingly, the use of [^{3}H] dexetimide and [^{3}H] QNB in intact cells allowed to detect a trapping phenomenon within the cells, presumably, in the lysosomes. Displacement with dexetimide gave rise to a biphasic curve, the second part of the curve being parallel to that obtained with levetimide.

Consequently, the stereospecificity observed with anticholinergic ligands may be considered as a decisive specificity criterion if the IC_{50} value for the active enantiomer, dexetimide, ranges from 1 to 3 nM, and 5 to 10 μM for the inactive enantiomer, levetimide. In addition to this, if the binding reveals atropine active between 5 and 10 nM, one can be sure that the ligand binds on muscarinic receptors (Fig. 2). It was certainly not the case in recent binding studies performed on lymphocytes [4–6]; the two low IC_{50} values obtained with atropine and other anti-muscarinic agents definitely exclude the possibility that these binding sites represent muscarinic receptors; they are simply acceptor sites.

DOPAMINE RECEPTORS AND SPIRODECANONE ACCEPTORS

In the course of the solubilization of dopamine receptors, we were faced, for the first time, with the problems raised by acceptor sites. Indeed when [^{3}H] spiperone binding sites were solubilized from rat striata, they did not reveal the high affinity properties of dopaminergic receptors normally found in membrane preparations [7]. Only spiperone and two drugs having the same chemical moiety (spirodecanone) displayed high affinity properties (Fig. 3), while benperidol, a butyrophenone as spiperone that lacks the spirodecanone moiety, was 3800 times less potent in soluble preparations from rat striatum than in membrane preparations. Similarly, haloperidol, (+) butaclamol and flupenthixol were much less active than in membrane preparations (Fig. 3). Nevertheless (+) butaclamol was more potent than (−) butaclamol, the inactive enantiomer. In fact at that time, we had solubilized spirodecanone sites which are acceptor sites or recognition sites for molecules having a spirodecanone moiety. The lack of these spirodecanone sites in dog striatum enabled us to solubilize dopamine receptors from dog striatum that retained the high affinity properties of the membranes (Fig. 3). Interestingly, both receptor and acceptor sites revealed stereospecificity towards (+) and (−) butaclamol. However, the stereospecificity was much pronounced for the solubilized dopamine receptor (300-fold) than for the acceptor site (~10-fold). As was the case for the muscarinic receptors, stereospecificity becomes more important if the active enantiomer displays the expected high affinity.

There are only a few ligands which are suitable for labelling brain receptors in *in vivo* conditions; spiperone was found to fulfil the conditions to label *in vivo* dopamine but also serotonin receptors [8, 9]. Figure 4 shows clearly the drugs acting on serotonin (frontal cortex) and on dopamine receptors (striatum): ketanserin, pipamperone and methysergide mostly prevented the labelling of [^{3}H] spiperone in the frontal cortex. Interestingly (+) butaclamol like spiperone was competitive in both regions whereas (−) butaclamol was totally inactive at the doses which produced a maximal prevention with (+) butaclamol. This demonstrates that stereospecificity may also be achieved *in vivo* on two different brain receptors using one ligand.

NEUROTENSIN ACCEPTOR

A crucial point, perhaps the most important in binding studies, concerns the meaning of displaceable binding. As already pointed out in previous papers [cf. Refs 10 and 11], displaceable binding corresponds often to the sum of specific and non-

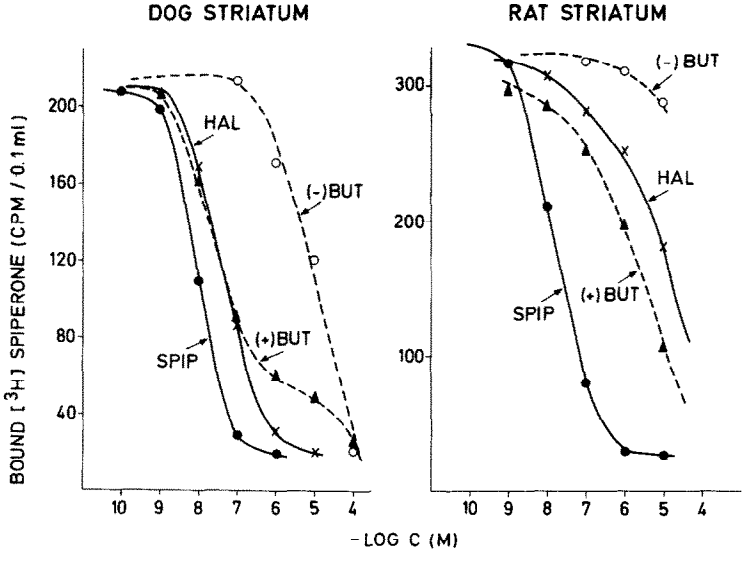

Fig. 3. Dose–response inhibition of spiperone (Spip), haloperidol (Hal) and (+)- and (−)-butaclamol (But) in the [^{3}H] spiperone binding using solubilized extracts from dog and rat striatum.

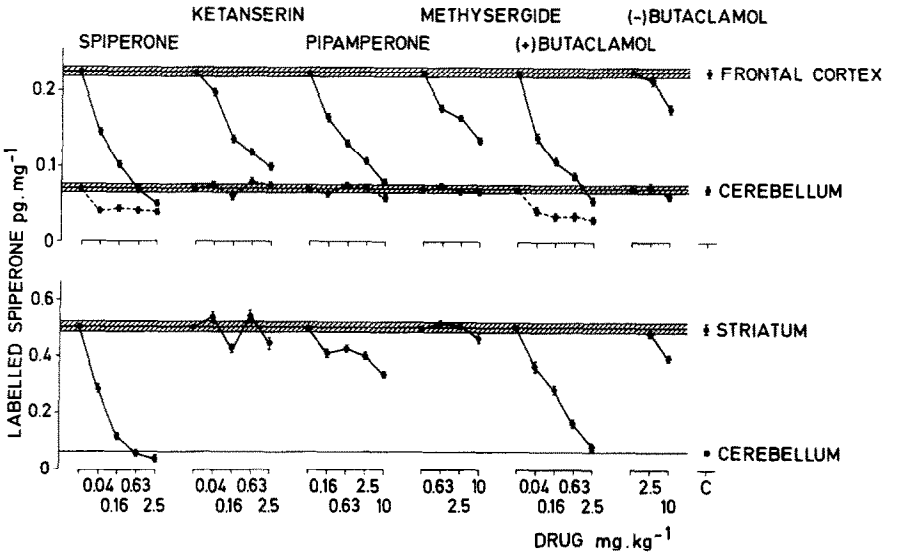

Fig. 4. Prevention of *in vivo* [^{3}H] spiperone binding in the frontal cortex (serotonin S_2) and in the striatum (dopamine D_2) by various drugs given s.c. to rats 1 hr before [^{3}H] spiperone (5 $\mu g \cdot kg^{-1}$ i.v.). The level in the cerebellum is taken as "blank". Means of six determinations ± SEM.

specific binding. Specific binding is defined, here, as binding on true receptors and non-specific binding on acceptors or recognition sites without physiological response. It is noteworthy that the term non-specific displaceable does not preclude that the binding may occur on a specific protein which, however, is not endowed with receptor properties according to the Langley's definition [cf. Refs 10 and 11].

A good example of non-specific displaceable binding was recently reported for [^{3}H] neurotensin binding in rat brain [12]. In this case, levocabastine, a potent H_1 antagonist was found to displace, even stereospecifically, 60% of the [^{3}H] neurotensin binding from an acceptor site in rat brain, thus unrelated to a physiological receptor. When these sites were occluded by levocabastine, it was possible to reveal, after displacement with unlabelled neurotensin, the neurotensin receptor which corresponded to about 30% of the total displaceable sites. Interestingly this levocabastine displaceable site was not present in guinea-pig, dog or human brain.

Interestingly, marked differences in affinity on the acceptor site were observed for five different isomers of levocabastine. Changes of configuration led to a more than 400-fold difference in the IC_{50} values. However, the most remarkable thing was that the order of potency for the neurotensin acceptor was exactly parallel to that for the histamine H_1 receptor. Therefore this represents an example where the stereospecificity was similar for a receptor and an acceptor site suggesting that both entities share perhaps in common some identical binding subunits or peptide fragments. Nevertheless it is noteworthy that all the other antihistamines H_1 were totally inactive on the neurotensin site displaceable by levocabastine.

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