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COMMENTARY

ANTI-SCHIZOPHRENIC DRUGS—MEMBRANE RECEPTOR SITES OF ACTION

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Neuroleptic sites-of-action strategy

The anti-psychotic drugs, introduced in 1952 [21], effectively reduce many of the symptoms of schizophrenia and to a large extent prevent relapse and rehospitalization of schizophrenic patients [31]. These drugs thus provide a powerful research tool and strategy for determining the abnormal site or sites in the brain in schizophrenia.

Such a pharmacological strategy [72] requires a thorough search to locate all of the many possible sites of neuroleptic receptors in normal brain before a subsequent search can be made for an abnormal subset of neuroleptic receptors in tissues from schizophrenic or psychotic patients. This neuroleptic receptor approach differs in practice from the psychosis-exacerbation strategy [25, 40, 50, 1, 101, 64] and from the biochemical approach to schizophrenia [36, 54–56, 46, 64].

The purpose of this Commentary is primarily to compare the pharmacological properties of the different reported sites of neuroleptic action and to consider critically which of these might be of particular significance in the clinical action of neuroleptic drugs.

Neuroleptic sites: criteria for specific and non-specific sites

Many different sites of action have been suggested for the neuroleptic drugs. These sites may be classified as either specific or non-specific according to the following criteria.

Stereoselective criterion of neuroleptic action. The neuroleptic drugs are very fat-soluble [73, 24, 69] and surface-active [74, 72, 60]. The drugs are, therefore, very soluble in membranes [81]. Hence, it is not surprising that the neuroleptics interfere with many membrane-associated events [73, 84] because the neuroleptic concentration within the membrane phase attains extremely high values. In fact, the drug molarity (or molality) within the membrane phase itself can go as high as 20 mM (that is, 20 m-moles drug/kg or liter of membrane phase) when, for example, the aqueous phase contains 10^{-6} M or 10^{-5} M chlorpromazine [73]. Such enormous concentrations within the membrane phase produce a wide variety of non-specific membrane-disturbing actions. These include expansion of membrane proteins, membrane fluidization, alterations of *trans*-membrane fluxes, and inhibition of excitability (anaesthetic action) [73].

These non-specific membrane actions depend primarily on the membrane solubility of the drug, and there is very little difference between stereoisomers in producing such effects. For example, opiate enantiomers (dextrorphan and levorphanol) are equally active in their local anaesthetic action when applied in the 10^{-6} to 10^{-4} M concentration range [79]. In the nM concentration range, however, the *levo-* form of the opiate is generally 100–1000 times more active than the *dextro-* form on opiate receptor systems.

Because the neuroleptics are so very membrane-soluble, any neuroleptic action on, or binding to, biological membranes is not a sufficient criterion for identifying that action or binding as being associated with a "specific" neuroleptic site. It requires the stereoselective action of (+)-butaclamol [7, 97] in order to identify that site as truly specific for the neuroleptic. For example, in the 0.1 to 1 μM zone the neuroleptics are non-specifically membrane-anaesthetic [91], while in the nM region they are stereoselective [87, 88, 8]. Furthermore, since neuroleptic solubility in octanol (or in the membrane) generally goes up in accordance with clinical potency, it is essential to use the neuroleptic enantiomers to define neuroleptic specificity because the enantiomers have identical partition coefficients (Table 1). *Cis-trans* isomers, however, may not have identical partition coefficients. Thus, *cis-* and *trans*-flupenthixol are not ideal drugs for establishing true neuroleptic specificity, since their membrane concentrations may not be identical. Differences in action between *cis-* and *trans*-neuroleptics are a necessary but not a sufficient criterion for identifying a specific neuroleptic site. The meager solubility data available on *cis-* and *trans*-isomers of neuroleptics, however, do suggest that their partition coefficients may be similar (Table 1).

Nanomolar concentration criterion for specific neuroleptic action. Of the many neuroleptic sites proposed, only those affected by nanomolar concentrations (1–100 nM) ought to be further considered as being truly specific and clinically significant. Therapeutic concentrations of the neuroleptics in plasma water are between 0.1 and 50 nM (Table 2). Aqueous concentrations exceeding 100 nM are generally toxic.

In a typical biochemical pharmacology experiment with neuroleptics (*in vitro*), considerable amounts of the neuroleptic are adsorbed to the tissue and the glassware [72]. This absorption amounts to 20–90 per cent for chlorpromazine [76], 29–50 per cent for haloperidol [87, 49], 70 per cent for pimozide [49], and 86 per cent for clopimozide [49]. Even with [^{3}H]apomorphine, the amount removed from the aqueous medium can be high (~45 per cent; Ref. 89).

Table 1. Partition coefficients (P)

	Octanol/water*	Membrane/buffer [84]†
Chlorpromazine sulfoxide	1,900 [50A]	
Promethazine	22,400 ‡	19
Promazine	35,500 [50A]	30
Imipramine	41,700 [50A]	295
Chlorpromazine	191,000 [50A]	1,700
Haloperidol	555,000 ‡	200
Fluphenazine	912,000 ‡	
Prochlorperazine	1,350,000 ‡	
Trifluperidol	1,700,000 ‡	
Pimozide	2,000,000 [49]	
Clopimozide	12,600,000 [49]	
Penfluridol	40,000,000 [49]	
(+)-Butaclamol	Log P = 2.52 §	
(−)-Butaclamol	Log P = 2.56 §	
cis-Flupenthixol	R_{Mo} = 0.90 ‖	
trans-Flupenthixol	R_{Mo} = 0.90 ‖	

* Coefficient for the non-ionized form of the drug.
† Numbers in brackets refer to reference numbers.
‡ Calculated from Ref. 50A.
§ At pH 7.45; using pK of 8.15, P can be derived (see Ref. 60); data of L. G. Humber, Ayerst Res. Labs., Montreal.
‖ R_{Mo} = Log $P \cdot r$, where P is the partition coefficient in a thin-layer chromatographic system and r is the constant; see Ref. 61.

These enormous adsorptions must be considered before applying the Cheng–Prusoff equation [11] for calculating the neuroleptic K_i on a particular system. No study has systematically attempted to do this. Existing K_i values [18, 8] for neuroleptics are difficult to interpret because no allowance has been made for adsorption [49]. Until this problem is resolved, it would be better if authors reported the actual inhibitory concentrations observed experimentally (IC_{50} values).

Types of specific sites for neuroleptics

Using the criteria of neuroleptic specificity outlined in the previous sections, it is possible to consider the sensitivities *in vitro* of various pre-synaptic and post-synaptic sites to neuroleptics, and to comment critically whether the sites might be pharmacologically vulnerable *in vivo*.

Pre-synaptic nerve impulses. Neuroleptics can block conduction of nerve impulses [73, 66, 26, 30, 81–83, 91]. In general, however, this

Table 2. Neuroleptic concentrations in serum water*

Neuroleptic dose range (mg/day)	Previous days on drug	No. of patients	Average concn in plasma		Per cent bound to plasma proteins	Average concn in plasma water (nmoles/l.)	IC_{50}‡ (nM)
			Peak† (ng/ml)	Pre-dose (ng/ml)			
Haloperidol							
9–15 [102]§	Over 14	14	2.4		76.2 ± 1.2‖	1.5	1.2
7–14 [23]	Chronic		4.5			2.8	
Chlorpromazine							
400–900 [67]	7–42	13	246	216	94.5 [19]	39–45	29
400 [15]	24	18	210	114		21–39	
400 [15]	53	18	526	186		34–96	
300 [71]	8	8	100	58		11–18	
300–600 [52]	9.6 yr	36	50			9	
600 [32]	30–90	13	55			10	
200–800 [90]	42	5	70			13	
Thioridazine							
300–600 [2]	Over 8	56		700	94.4 [3]	10	12
30–600 [53]	Over 14	46	700	400		8.2	

* Determined by gas chromatography.
† Usually 3 hr after oral dose.
‡ Neuroleptic concentration which inhibits the specific binding of [^{3}H]haloperidol by 50 per cent (see Ref. 87).
§ Numbers in brackets refer to reference numbers.
‖ T. Inaba and P. Seeman (1976, unpublished observations); measured at 3 ng/ml; method of Ref. 37.

local anaesthetic-like action of the neuroleptics occurs in the sub-micromolar concentration range (Table 3). According to the nanomolar concentration criterion, therefore, the impulse-blocking actions occur at concentrations which are about ten times higher than those found clinically in the serum water (Table 2). It is known that small diameter axones are considerably more sensitive to anaesthetic blockade (by neuroleptics) than are large ones [91] (A. Staiman and P. Seeman, manuscript to be published). It is possible, therefore, that very small pre-synaptic fibers (0.1 μm wide) may be blocked, but this would probably still occur at rather higher serum drug levels.

The stereoselective anaesthetic action of butaclamol has not yet been studied. If it should turn out that the butaclamol enantiomers are equiactive, one may then conclude that the impulse-blocking sites of action are not vulnerable *in vivo*, since only (+)-butaclamol is effective *in vivo* [97].

Pre-synaptic coupling-blockade by neuroleptics. Neuroleptics can inhibit the entry of Ca^{2+} into excitable cells [65, 82, 48]. Thus, the drugs are every effective in blocking the coupling between the pre-synaptic impulse and the entry of Ca^{2+} (Table 3) [77].

Although there is a highly stereoselective action of (+)-butaclamol on the pre-synaptic coupling site, the concentrations of the neuroleptics range from 50 to 1000 mM; these are about ten times higher than those found in serum water *in vivo* (Table 2).

Unlike all the other pre-synaptic actions of neuroleptics, the potency for inhibiting pre-synaptic coupling correlates very well with the clinical potency of the neuroleptic (Fig. 1) [77]. However, despite meeting this clinical potency criterion, and despite meeting the stereoselectivity criterion, the neuroleptic concentrations required for coupling-blockade are still too high to be realized clinically. An equally important and rather demanding criterion is that neuroleptic potencies *in vitro* and *in vivo* should correlate 1:1, but the regression coefficient for these data (Fig. 1) does not meet this ideal.

Pre-synaptic membrane fluidization by neuroleptics (enhanced release of transmitter). At rather high concentrations (in the μM range), the neuroleptics fluidize all pre-synaptic membranes, including vesicle membranes [73], thus promoting membrane–membrane fusion. This leads to an enhanced spontaneous release of neurotransmitter (Table 3). Although the stereoselectivity of this process has not yet been studied, it is unlikely that it can be of therapeutic significance because of the extremely high concentrations required (compare with Table 2). This transmitter-releasing action, however, may be of importance in toxic neuroleptic states such as tardive dyskinesia [84].

Pre-synaptic uptake of transmitters. The uptake processes for dopamine, norepinephrine and serotonin are all rather insensitive to neuroleptics (Table 3), requiring supra-micromolar concentrations for signifi-

Table 3. Possible pre-synaptic sites for neuroleptics

	Neuroleptic IC_{50} values (nM)							
	Block of nerve impulses	Block of stimulated release of dopamine	Enhancement of dopamine release	Block of dopamine uptake	Block of noradrenalin uptake	Block of 5-HT uptake	Reversal of apomorphine-inhibited tyrosine hydroxylase	Block of autoreceptors
Promazine	7,000 [81]*	6,100 [77]		16,000 [39]	160 [29]	12,000 [29]		
Chlorpromazine	400 [81]	700 [77]	3,500 [75]†	10,000 [75]	500 [33]	3,800 [28]	> 1,000 [5]	
		900 [39]	2,600 [39]	10,600 [27]	180 [29]	5,100 [29]	500 [39]	
		100 [62]		1,000 [62]				
				10,000 [39]				
Thioridazine	170 [81]	800 [77]		5,800 [27]			Nil [12]	
Trifluoperazine		160 [77]		9,000 [39]			300 [29]	
				10,000 [27]	9,000 [33]			
Clozapine		440 [77]					Nil [12]	
Prochlorperazine		315 [77]			3,700 [29]	16,000 [29]		
Fluphenazine	40 [81]	54 [77]		11,000 [27, 39]			500 [12]	1,000 [100]
		> 50 [100]					20 [5]	
Haloperidol	100 [81]	95 [77]	100 [75]	4,400 [27]			20 [5]	
		1900 [39]	4,000 [39]	1,300 [62]			16 [39]	
		100 [62]		300 [75]			110 [13]	
							500 [12]	
Pimozide		51 [77]	100 [75]	1,100 [27]			100 [5]	
		100 [62]		400 [62]			55 [39]	
(+)-Butaclamol		150 [77]					200 [39]	
							10 [5]	
(−)-Butaclamol		> 100,000 [77]					1,000 [39]	
							Nil [5]	
α-Flupenthixol		59 [77]		3,500 [39]				
β-Flupenthixol		13,000 [77]		5,800 [39]				

* Numbers in brackets refer to reference numbers.

† The IC values for Ref. 75 refer to the threshold concentrations of neuroleptics.

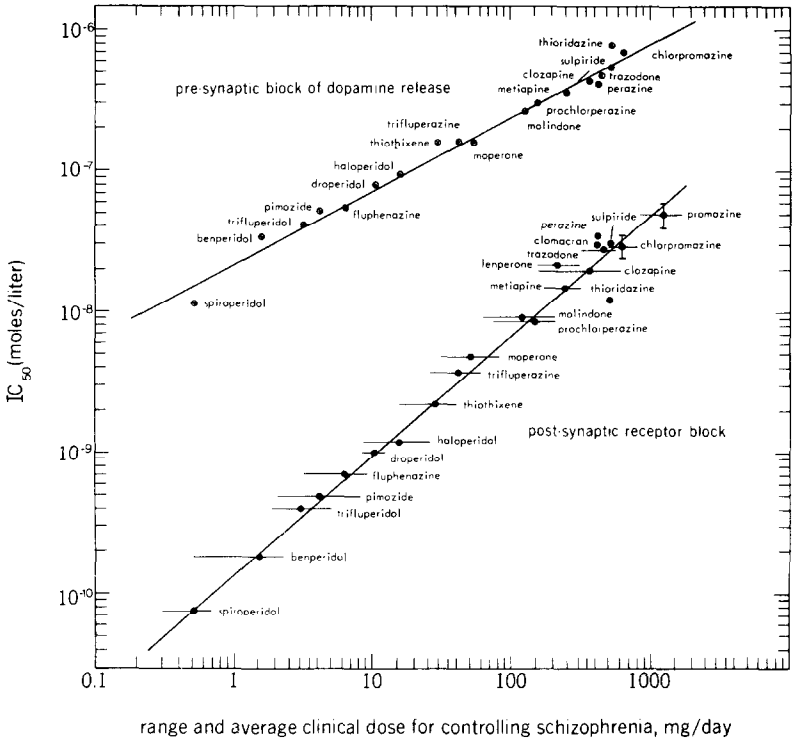

Fig. 1. Correlations between the dopamine synapse-blocking actions of neuroleptics and their clinical potencies. Although the pre-synaptic blocking concentrations [77, 78] (of stimulated release of [^{3}H]dopamine from rat striatal slices) correlate very well, the values are too high (50–1000 nM) to be observed clinically in the patient's serum water. The post-synaptic inhibiting concentrations of the neuroleptics (on butaclamol-specific [^{3}H]haloperidol binding to either rat or calf caudate homogenate [80, 85–88]) exhibit a better correlation and are effective at concentrations seen clinically (Table 2).

cant inhibition. Although no studies have yet been reported on the stereoselective actions of butaclamol, there is little difference between the two geometric isomers, alpha- and beta-flupenthixol (on dopamine uptake). Thus, pre-synaptic uptake or re-uptake is not likely to be a vulnerable site of neuroleptic action *in vivo*.

Pre-synaptic enzymes as sites for neuroleptics. The only pre-synaptic enzyme which has received serious consideration as a possible specific site of neuroleptic action is tyrosine hydroxylase. This enzyme is inhibited by apomorphine, and this inhibition is in turn antagonized by many neuroleptics (Table 3). Strangely, clozapine and thioridazine do not exert such antagonism [12]. None of the three criteria (listed in the section entitled "Neuroleptic Sites: Criteria for Specific and Non-specific Sites") are fulfilled in this particular site of neuroleptic action: there is relatively poor stereoselectivity *in vitro* by butaclamol [39], the neuroleptic concentrations are high, and the correlation with clinical potency is poor.

Pre-synaptic receptors (autoreceptors) as sites for neuroleptics. It is known that there are pre-synaptic membrane receptors for neurotransmitters (autoreceptors) [10, 45] which are important in regulating tyrosine hydroxylase and dopamine synthesis [68, 98, 59].

There is very little information, however, on the sensitivity *in vitro* of such pre-synaptic receptors to neuroleptics. Westfall *et al.* [100] found that 1000 nM fluphenazine antagonized the dopamine-inhibited synthesis of new dopamine, a process presumably mediated by pre-synaptic dopamine receptors. It is difficult to know how sensitive these pre-synaptic receptors are to fluphenazine since only one fluphenazine concentration had been tested. It is possible that the neuroleptic inhibition of pre-synaptic coupling is somehow associated with such pre-synaptic receptors, since apomorphine can antagonize the neuroleptic inhibition of the [^{3}H]dopamine release from electrically cally stimulated striatal slices (T. Lee and P. Seeman, unpublished observations), a finding also observed *in vitro* by Perkins and Westfall [62]. If both these two sets of results are truly indicative of the pre-synaptic receptor sensitivity to neuroleptics, then these autoreceptors cannot be considered as being particularly vulnerable to neuroleptics *in vivo* because of the high neuroleptic concentrations needed.

Post-synaptic adenylate cyclases as neuroleptic sites. Considerable data have now accumulated on the neuroleptic inhibition of the catecholamine-sensitive adenylate cyclases (see Fig. 2 and Table 4). For the phenothiazines and butaclamol there is a crude but definite correlation between their inhibitory potencies and their clinical antipsychotic potencies. For the butyrophenones, however, there seems to be no such correlation. Iversen *et al.* [39, 57] have found a correlation within the butyrophenones between data *in vitro* and animal data. It is possible, as suggested by Iversen, that there are two sets of neuroleptic receptors, one for the phenothiazines and another for the butyrophenones; the specific binding data for

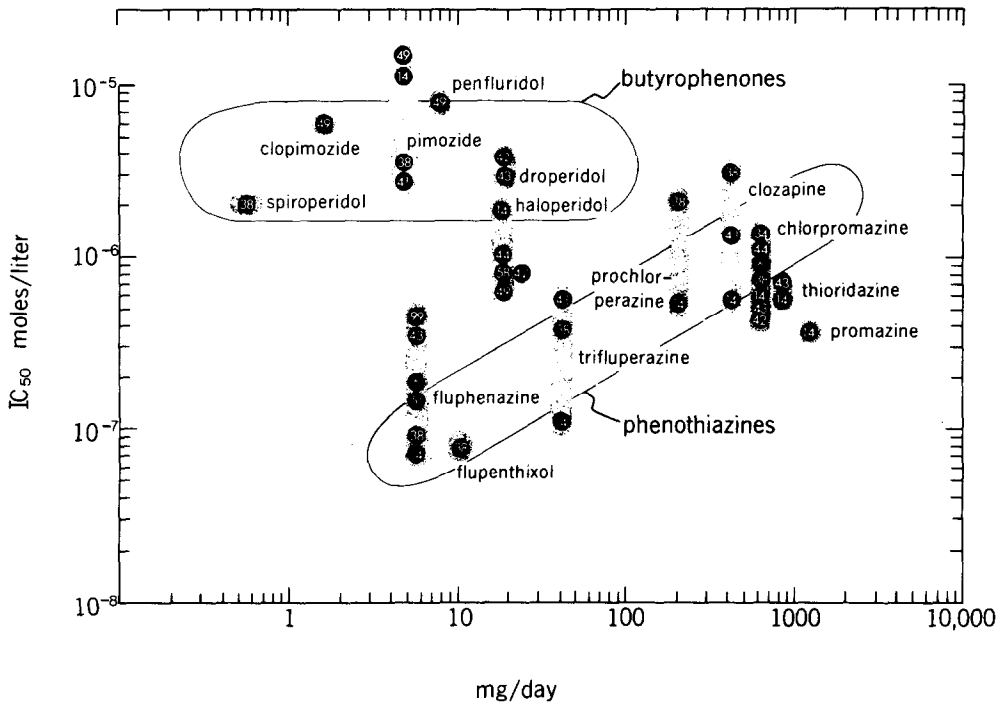

Fig. 2. Correlation between clinical potency and inhibitory dosage of phenothiazines and butyrophenone neuroleptics. The inhibitory potencies of the phenothiazines (against dopamine-sensitive adenylate cyclase in striatal or caudate homogenates) generally correlate with the clinical potencies; there is no such correlation for the butyrophenone neuroleptics. Numbers refer to references.

[^{3}H]haloperidol, however, suggest that only one set of neuroleptic receptors need be proposed (see next section).

The dopamine-sensitive adenylate cyclase enzyme does not quite fulfill the necessary criteria for being of pharmacological significance *in vivo*, although it is stereoselectively blocked by (+)-butaclamol [63], and although there is a crude correlation with clinical potency within the phenothiazines. The correlation within the phenothiazine family (Fig. 2) and the distinctly different correlation within the butyrophenone family [39] can be almost entirely explained by the increasing partition coefficients of each set of congeners (Table 1). Furthermore, the inhibiting concentrations of neuroleptics on this enzyme are extremely high, all of them being in the μM range. While it is true that the so-called K_i values are in the nanomolar range, the Cheng–Prusoff relation between the neuroleptic IC_{50} values and the K_i values does not take into account the fact that the C_{total} and C_{free} values for the neuroleptics are far from identical; furthermore, the K_i value is not a concentration that can be freely applied to the situation *in vivo*, since one does not know the dopamine concentration in the synaptic cleft during the discharge of dopaminergic vesicles (the concentration, in fact, may be in the μM to mM range for short periods). A final difficulty with dopamine-sensitive adenylate cyclase as a candidate for being a meaningful neuroleptic receptor is that sulpiride, a new antipsychotic drug, and metoclopramide do not inhibit this enzyme [70].

Post-synaptic receptors for dopamine/apomorphine and neuroleptics. A number of specific receptor sites have now been investigated in order to determine whether the properties of ligand binding might establish the site as being "specific" for neuroleptic drugs (see Table 4). Of these, the one most sensitive to neuroleptic drugs is that for [^{3}H]haloperidol itself (Fig. 1).

The properties of [^{3}H]haloperidol binding in the striatum fulfill all the criteria required of a specific neuroleptic receptor; namely, the binding is stereoselectively blocked by (+)-butaclamol, the neuroleptics compete for binding in a 1:1 relation with their clinical potency, and the concentrations *in vitro* are of the same order as those found in the serum water of patients who are using these drugs. Earlier reports of neuroleptic binding [95, 47] did not reveal results which fit these criteria.

The neuroleptic IC_{50} values for inhibition of specific haloperidol binding have been reported from both this laboratory [80, 85–88] and Snyder's laboratory [92, 94, 16–18, 93, 8]. Although there is good agreement between the two sets of data from these laboratories, there are two or three neuroleptics which seem to give different results. For example, Creese *et al.* [18] find that chlorpromazine is ten times stronger than clozapine in blocking the binding of [^{3}H]haloperidol, while Seeman *et al.* [87] find that clozapine is about twice as potent as chlorpromazine; clinically, the anti-psychotic dosage of clozapine is about half that of chlorpromazine. A possible explanation for this apparent discrepancy is that "specific" binding of haloperidol is defined differently in the two laboratories; Burt *et al.* [8, 9] define it as the difference in [^{3}H]haloperidol binding in the absence and presence of 100 nM (+)-butaclamol, while Seeman *et al.* [87] define it as that difference in the pres-

Table 4. Possible post-synaptic sites for neuroleptics

	Neuroleptic or butaclamol IC_{50} values (nM) (+)-Isomer	(−)-Isomer	Ref.
Dopamine receptors	70	30,000	*; 86
	100	16,000	22; †
Chlorpromazine	2,000		87
	900		8
Haloperidol	300		87
	920		8
Haloperidol receptors	1	300	‡, 86
	1	1,300	22; §
Apomorphine receptors	80	20,000	‖; 89
Alpha-Adrenergic receptors	80	8,000	¶
Beta-Adrenergic receptors	1,000	1,000	**
	No effect	No effect	22; ††
Serotonin receptors	~ 30	> 1,000	‡‡
	1,000	8,000	§§
D-LSD-receptors	50	7,000	22
GABA receptors	No effect	No effect	‖‖
	No effect	No effect	22
Dopamine (DA)-sensitive adenylate cyclase	180	No effect	58
Noradrenalin (NA)-sensitive adenylate cyclase			
Butaclamol	~ 1,000	~ 50 μM	35
Chlorpromazine	10,000		35
Haloperidol	10,000		35
Pimozide	75		4

* Rat striatum; 1 nM [^{3}H]DA; using 1 μM (+)-butaclamol for non-specific binding.

† [^{3}H]DA (5 nM); using 1 μM DA or 10 μM (+)-butaclamol for non-specific binding; calf striatum.

‖ [^{3}H]Apomorphine (1.5 nM); using 100 nM (+)-butaclamol for non-specific binding; calf striatum.

‡ [^{3}H]Haloperidol (2.14); using 100 nM (+)-butaclamol for non-specific binding; rat striatum.

§ [^{3}H]Haloperidol (2 nM); using 100 nM (+)-butaclamol or 100 μM DA for non-specific binding; calf striatum.

¶ Calf striatum; 0.8 to 1 nM [^{3}H]dihydroergocryptine; using 1 μM phentolamine for non-specific binding (P. Seeman, unpublished observations).

** [^{3}H]Dihydroalprenolol (0.7 nM); using 100 nM propranolol for non-specific binding (P. Seeman, unpublished observations).

†† [^{3}H]Dihydroalprenolol (1 nM); using 1 μM (−)-alprenolol for non-specific binding; rat cortex.

‡‡ [^{3}H]Serotonin (4.6 nM); using excess 5-HT for non-specific binding; calf striatum; (P. Whitaker and P. Seeman, unpublished observations).

§§ Calf striatum; 7 nM [^{3}H]5-HT; using 10 μM 5-HT for non-specific binding.

‖‖ [^{3}H]GABA (13 nM); (P. Seeman, unpublished observations), calf striatum.

ence of 100 nM (−)-butaclamol and (+)-butaclamol. Apparently, 100 nM (−)-butaclamol has no effect in the results of Burt *et al.*, but does in the results of Seeman *et al.* A further difference may reside in the preincubation procedure.

Of all the neurotransmitter ligands studied, the dopaminergic ones (dopamine and apomorphine) are those most stereoselectively blocked by (+)-butaclamol (Table 4). This is strong evidence that the neuroleptic sites and the dopamine sites are closely associated, if not identical, as first proposed by Van Rossum [96]. The situation is not so simple, however, since (+)-butaclamol does not have an absolute stereospecificity; this neuroleptic has considerable stereoselective action on both the alpha-adrenergic and serotonin receptors (Table 4) [20, 22].

Conclusions

Although the neuroleptic drugs may provide a strategy for locating the "schizophreno-genic" sites in human brain, there are a number of pitfalls. The drugs are highly membrane-soluble and thus non-specifically bind to many sites, producing many types of membrane perturbations. Stereoselective action by the (+)-butaclamol neuroleptic does occur in the nM region, and of all sites studied, the post-synaptic binding sites for haloperidol and for dopamine (apomorphine) are the ones most stereoselectively blocked. These assays for neuroleptic receptors and dopamine receptors are now ready to be applied to the study of diseased human brain tissues (Parkinson's disease, schizophrenia, Huntington's disease, tardive dyskinesia, and others). It should be emphasized, however,

that merely measuring the number of receptor sites and their dissociation constant (K_D) will not be sufficient to characterize the diseased neurotransmitter pathway, since synaptic "transmissivity" or synaptic sensitivity is a function of both the neurotransmitter turnover (synthesis and release) as well as the number and K_D of the transmitter receptors. A complicating factor is that prolonged exposure of agonist drugs to receptors induces tachyphylaxis, particularly with beta-adrenergic receptors. If this receptor desensitization is a general phenomenon for many types of receptors, including the dopamine/neuroleptic receptors, it will be necessary to develop methods to ensure that the receptors are all re-sensitized before their measurement in disease states.

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