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Research Article

Profile of *in Vitro* Binding Affinities of Neuroleptics at Different Rat Brain Receptors: Cluster Analysis Comparison with Pharmacological and Clinical Profiles

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A series of 21 neuroleptics with different chemical structures (phenothiazines, thioxanthenes, dibenzodiazepines, butyrophenones, benzamides, etc.) was examined for their *in vitro* interactions with 12 neurotransmitter binding sites in the rat brain (alpha- and beta-noradrenergic, dopaminergic, muscarinic, serotoninergic, histaminic, and opioid receptors, calcium channels, and serotonin uptake binding sites). The biochemical profile obtained from the binding data was compared with reported pharmacological and clinical profiles for this class of compounds by cluster analysis. Cluster analysis on binding data classified the compounds in three main subgroups: benzamides, compounds with an affinity mainly for DA2 and 5-HT2 receptors and inactive at muscarinic receptors, and compounds with a high affinity for alpha 1-adrenergic receptors and muscarinic receptors. The main subgroups resulting from cluster analysis of previously published pharmacological and clinical data for neuroleptics contain compounds common to the present study, with some correlations. The results extend previous observations that a complete binding profile corresponds to the pharmacological and clinical profile of this class of compounds.

KEY WORDS: neuroleptics; receptor binding assays; profiles; cluster analysis.

INTRODUCTION

In 1978 Lewi (1) compared the pharmacological profiles of 53 neuroleptic drugs in animals with the clinical physiognomies of 31 neuroleptics (21 were common to both studies) reported by the Liege groups (2). The two profiles were found to be significantly correlated. The incisive, antimanic, and parkinsonian scales of the clinical spectra paralleled catalepsy and the inhibition of apomorphine and amphetamine-induced stereotypes in rats. Similarly, clinical observations of sedative and adrenolytic effects corresponded with inhibition of norepinephrine-induced mortality and possibly also with ptosis in animal studies.

In 1980 (3), the same author reported a further correlation analysis on the basis of a joint description of the neuroleptics in animal pharmacology and in receptor binding assays. Pharmacological profiles of 13 neuroleptics were obtained from the inhibition of apomorphine-, tryptamine-, and norepinephrine-induced symptoms in rats. For the same set of compounds, the affinity for dopamine, serotonin, and norepinephrine binding sites was studied in rat brain tissues. A significant correlation was found by principal-components analysis between pharmacological and receptor binding data.

The present work extends these observations. The *in vitro* effects of 21 neuroleptics were investigated in terms of their interactions with 12 different neurotransmitter binding sites in rat brain membranes. We also compared the *in vitro* affinity profiles of these drugs with the pharmacological and clinical profiles reported by Lewi (1) by means of cluster analysis.

METHODS

General Receptor Binding

Male Sprague Dawley rats weighing 180–230 g (Charles River, Italia) were decapitated and the brains were rapidly removed. Dissected cortical tissue (beta, serotonin-uptake sites, serotonin-1 and -2 and opiate receptors), striatal tissue (dopamine-1 and -2 receptors), or the whole brain minus the cerebellum (alpha-adrenergic, histaminic, and muscarinic receptors, voltage-sensitive calcium channels) was homogenized on ice in buffer (as specified in Table I) using a Polytron homogenizer. The homogenates were centrifuged

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Table I. Binding Assays: Summary of Methods[a]

Details	Receptor				
	Alpha 1	Alpha 2	Beta (1 + 2)	DA1	DA2
Tissue	Brain – cerebell.	Brain – cerebell.	Cortex	Striatum	Striatum
mg tissue/ml	10	10	10	5	5
Buffer (0.05 *M*)	Tris/HCl a	Tris/HCl a	Tris/HCl b	Tris/HCl c	Tris/HCl c
pH (25°C)	7.4	7.4	8.0	7.1	7.4
^{3}H-Ligand	Prazosin	Clonidine	DHA	SCH 23390	Spiperone
Conc. (n*M*)	0.3	1	1	0.4	0.3
Definition of specific binding	Prazosin	l-NE	Propranolol	Flupentixol (−) *cis*	(+)-Butaclamol
Conc. (n*M*)	1,000	100,000	2,000	10,000	1,000
Incubation time (min.)	30	30	10	15	15
Incubation temp.	25	25	37	37	37
K_d of ligand (n*M*)	0.12	1.6	2.5	1.4	0.6

[a] 5-HT, serotonin; QNB, quinuclinidylbenzilate; DA, dopamine. (a) Buffer + 10 μ*M* pargyline + 0.1% ascorbic acid. (b) Buffer + 3 m*M* MgCl2. (c) Buffer + 120 m*M* NaCl + 5 m*M* KCl + 2 m*M* $CaCl_2$ + 1 m*M* $MgCl_2$ + 10 μ*M* pargyline + 0.1% ascorbic acid. (d) Buffer + 50 m*M* NaCl. (e) Buffer + 1 m*M* $MgCl_2$. (f) Buffer + 10 μ*M* pargyline + 0.1% ascorbic acid + 4 m*M* $CaCl_2$. (g) Buffer + 120 m*M* NaCl + 5 m*M* KCl.

(48,000*g*) and resuspended three times in fresh buffer to wash out endogenous ligands. In the nitrendipine binding assay, a 1000*g* centrifugation was run first to precipitate nuclei and gross cell debris which contribute nonspecifically to the binding.

The final pellet was resuspended in an appropriate volume of buffer to obtain the expected tissue concentration in the incubation mixture.

Incubation was generally in 10-ml polyethylene tubes. Tritiated ligand (20 μl) and the compound under evaluation (20 μl) were added before the tissue suspension (1–2 ml). After incubation (see Table I for details), cold buffer was added to each sample, and the contents were rapidly vacuum-filtered through Whatman GF/B glass-fiber filters. The filters were rapidly washed three times with 5 ml of buffer, placed in scintillation vials, and shaken with 10 ml of Filtercount scintillation mixture.

All the drugs were initially tested at a concentration of 3.6 μ*M*. When significant inhibition was observed, a complete competition curve was plotted.

Binding Assays (See Table I for Details)

Alpha 1-adrenergic receptor binding was done according to the method of Greengrass and Bremner (4), alpha 2-adrenergic receptor binding was done according to U'Prichard *et al.* (5), and beta-adrenergic receptor binding (beta 1 + beta 2) was assayed using an adaptation of the methods of Bylund and Snyder (6) and Greenberg and Weiss (7). Dopamine^{-1} (DA1) binding was investigated according to Billard *et al.* (8), DA2 receptor binding according to Matres *et al.* (9), serotonin S1 binding (5-HT1) according to Nelson *et al.* (10), serotonin S2 binding (5-HT2) according to Creese and Snyder (11), and muscarinic (M1 + M2) receptor binding according to Yamamura and Snyder (12). Histamine (H1) and opiate receptor binding was assayed according to Tan Tran *et al.* (13) and Stengaard-Pedersen and Larsson (14), respectively. The binding of [^{3}H]nitrendipine to voltage-sensitive calcium channels was assayed by an adaptation of the methods reported by Bolger *et al.* (15) and Murphy and Snyder (16), and the binding of [^{3}H]imipramine to presynaptic serotonin uptake sites according to Raisman *et al.* (17).

Drugs and Chemicals

All radioactive chemicals were purchased from New England Nuclear: [^{3}H]Prazosin (sp act, 18.8 Ci/mmol), [^{3}H]clonidine (sp act, 66.8 Ci/mmol), [^{3}H]dihydroalprenolol hydrochloride (sp act, 55.4 Ci/mmol), [^{3}H]SCH 23390 (sp act, 77.7 Ci/mmol), [^{3}H]spiperone (sp act, 22.0 Ci/mmol), [^{3}H]5HT (sp act, 24 Ci/mmol), [^{3}H]quinuclinidylbenzylate (sp act, 31.9 Ci/mmol), [^{3}H]pyrilamine (sp act, 24.1 Ci/mmol), [^{3}H]naloxone (sp act, 44.4 Ci/mmol), [^{3}H]nitrendipine (sp act, 77.6 Ci/mmol), and [^{3}H]imipramine (sp act, 44.2 Ci/mmol).

All other compounds and reagents were purchased from standard commercial sources.

The following neuroleptics were examined in the present study: the *phenothiazines* promazine, chlorpromazine, thioridazine, trifluperazine, perphenazine, and fluphenazine; the *thioxanthenes* chlorprothixene, flupentixol (*cis*), and thiothixene; the *dibenzodiazepines* clozapine and clothiapine; the *butyrophenones* haloperidol, trifluperidol, pipamperone, droperidol; and spiroperidol; the *benzamides* metoclopramide, sulpiride, and clebopride; and the *others* pimozide and domperidone.

Analysis of Binding Data

Specific binding was defined as the total binding minus the binding in the presence of the displacing agent (Table I). Binding in the presence of various concentrations of test compound was expressed as a percentage of the specific binding with no drug present. The IC_{50} values were established by simultaneous nonlinear curve fitting according to the logistic equation reported by De Lean *et al.* (18), using the Allfit program running on an IBM AT PC.

Table I. (Continued)

Receptor						
5-HT1	5-HT2	Musc (M1 + M2)	Histamine H1	Opiates	Ca^{2+} channels	5-HT uptake
Cortex	Cortex	Brain − cerebell.	Brain − cerebell.	Cortex	Brain − cerebell.	Cortex
10	10	0.5	33	7	5	33
Tris/HCl	Tris/HCl	Na/K phosphate	Na/K phosphate	Tris/HCl	Tris/HCl	Tris/HCl
f	e			d		g
7.7	7.7	7.4	7.4	7.4	7.7	7.4
5-HT	Spiperone	QNB	Pyrilamine	Naloxone	Nitrendipine	Imipramine
2	1	0.06	1	1	0.4	2
5-HT	Methysergide	Atropine	Pyrilamine	Naloxone	Nifedipine	Amitriptyline
10,000	5,000	5,000	5,000	36,000	1,000	36,000
15	15	60	30	30	60	60
37	37	25	25	25	25	4
2.0	0.4	0.1	1.5	1.8	0.43	6

Data Analysis

The affinities of neuroleptics for the different binding sites considered in this study, expressed as pIC_{50} (−log of IC_{50}, n*M*), were submitted to hierarchical cluster analysis on an IBM PC-AT computer using the SYSTAT (V.2.1) package. The centroid linkage method on the euclidean distance was utilized in cluster analysis. Cluster analysis was also done on the pharmacological and clinical data reported by Lewi (1).

Pharmacological data (ED_{50}, mg/kg) were transformed in pED_{50} (−log ED_{50}, μg/kg) before analysis.

RESULTS

In Vitro Potency of Neuroleptics

The *in vitro* effects of different neuroleptics on rat brain receptors and binding sites are summarized in Table II. Almost all neuroleptics of different structural classes, with the exception of benzamides, showed very marked affinities for alpha 1 binding sites. These data agree with those reported by other laboratories (19–23). Only a few compounds had a weak affinity for alpha 2-adrenergic receptors in the micromolar range, with the exception of clozapine, pipamperone, and perphenazine. Our results agree only partially with those of Leysen (20), who reported that chlorpromazine, thioridazine, fluphenazine, flupenthixol, thiothixene, metoclopramide, and domperidone were all slightly active at this binding site. None of the drugs bound to beta-adrenergic receptors, in agreement with findings of Leysen and Janssen (21) and Chivers (23).

Neuroleptics are thought to exert their therapeutic actions by blocking DA2 receptors in the brain. In fact, all the compounds were highly active in inhibiting [^{3}H]spiperone binding to rat striatum membranes. The affinities reported for DA1 and DA2 binding sites in rat striatum (Table II) are in agreement with those reviewed by other authors (20,21,23–25). Most neuroleptics have a lower affinity for DA1 than DA2 binding sites, with droperidol, pimozide, spiperone, domperidone, clebopride, and sulpiride being the most specific DA2 inhibitors. These findings agree with the finding of Jenner and Marsden that substituted benzamide drugs are selective antagonists of the adenylate cyclase-independent dopamine receptor population (26). All the compounds but the benzamides [with the exception of clebopride (23)] showed a high affinity for 5-HT2 binding sites in rat brain cortex, as also reported by Leysen (20) and Leysen and Janssen (21).

The activity ratios of the *in vitro* IC_{50} values at DA2 over 5-HT2 receptors obtained in the present study correlate significantly ($P = 0.026$) with those reported by Leysen *et al.* (27), with a slope of 1.035. Similarly, all the neuroleptics inhibited [^{3}H]serotonin binding to 5-HT1 receptors but affinities were lower than at 5-HT2 binding sites, confirming the results reported by Wander *et al.* (28) in human brain. The dissociation between the serotonergic affinities was found across all chemical structure and was maximal for clothiapine (dibenzodiazepine) and flupentixol (thioxanthene); promazine, chlorpromazine, and thioridazine (phenothiazines) and thiothixene (thioxanthene) had the lowest degree of serotonergic differentiation.

The appreciable affinity of some neuroleptics for muscarinic cholinergic receptors is well known (20–22,29). Under our experimental conditions chlorpromazine, chlorprotixene, promazine, thioridazine, clothiapine, and clozapine displaced [^{3}H]QNB from its binding sites at nanomolar levels. Similar results have been reported by Leysen and Janssen (21), utilizing [^{3}H]dexetimide as the muscarinic ligand. These authors, however, found flupentixol, pimozide, and pipamperone to be slightly active too. Richelson (22), on the other hand, reported that fluphenazine, perphenazine, haloperidol, and trifluperazine all had a high affinity for muscarinic receptors.

The affinities of tested compounds for the histamine H1 receptors ranged from low (benzamides and domperidone) to medium (butyrophenones) to high (phenothiazines, dibenzodiazepines and thioxanthenes) in agreement with the results of Closse *et al.* (29). Our data, however, differ from those reported by other authors (20,21,23) who found that spiperone, pipamperone, pimozide, metoclopramide, sulpiride, and clebopride had no activity at this binding site. Droperidol, pimozide, and domperidone inhibited [^{3}H]naloxone binding from opiate receptors only at micromolar levels, indicating that very little interaction with these binding sites should be expected at therapeutic doses.

Pimozide displaced [^{3}H]nitrendipine from the 1,4-dihydropyridine binding site at the nanomolar level, while fluphenazine and haloperidol caused significant inhibition

Table II. *In Vitro* Effects of Different Neuroleptic Drugs on Rat Brain Receptors[a]

Drug	Receptor											
	α 1	α 2	β (1 + 2)	DA1	DA2	5-HT1	5-HT2	Muscarinic (M1 + M2)	Histamine H1	Opiates	Ca^{2+} channels	5-HT uptake
Phenothiazines												
Promazine	7.82	5.97	n.a.[b]	5.59	6.55	5.34	5.88	6.08	7.44	n.a.	n.a.	5.92
Chlorpromazine	8.18	n.a.	n.a.	6.81	7.72	5.34	5.88	6.44	8.40	n.a.	n.a.	6.52
Thioridazine	7.47	n.a.	n.a.	6.84	7.57	6.03	6.82	7.11	8.17	n.a.	n.a.	5.61
Trifluperazine	7.00	n.a.	n.a.	7.44	7.80	4.91	6.19	n.a.	7.13	n.a.	n.a.	6.12
Perphenazine	7.46	6.07	n.a.	7.05	9.15	5.49	7.41	n.a.	9.00	n.a.	n.a.	5.65
Fluphenazine	6.90	n.a.	n.a.	7.47	8.62	5.45	7.57	n.a.	8.14	n.a.	4.12	5.48
Thioxanthenes												
Chlorprothixene	8.21	5.87	n.a.	8.02	8.70	6.31	9.23	6.83	8.39	n.a.	n.a.	6.26
Flupenthixol (*cis*)	8.00	n.a.	n.a.	8.26	8.72	5.87	9.21	n.a.	8.03	n.a.	n.a.	5.02
Thiothixene	7.06	n.a.	n.a.	6.74	8.85	5.67	6.53	n.a.	7.17	n.a.	n.a.	5.02
Dibenzodiazepines												
Clothiapine	8.06	n.a.	n.a.	7.21	7.86	5.15	8.80	6.41	8.32	n.a.	n.a.	5.16
Clozapine	7.13	6.72	n.a.	6.07	6.27	5.89	7.98	6.96	8.35	n.a.	n.a.	6.03
Butyrophenones												
Haloperidol	7.30	5.38	n.a.	6.28	7.97	5.55	6.66	n.a.	6.11	n.a.	4.80	4.67
Trifluperidol	7.64	5.28	n.a.	5.99	8.73	5.69	6.76	n.a.	5.84	n.a.	n.a.	5.34
Pipamperone	7.28	6.19	n.a.	5.36	6.42	5.35	8.09	n.a.	5.42	n.a.	n.a.	4.78
Droperidol	7.18	5.72	n.a.	5.40	9.26	6.42	8.57	n.a.	6.32	5.41	n.a.	4.76
Spiperone	7.34	n.a.	n.a.	5.90	9.46	6.00	8.82	n.a.	6.06	n.a.	n.a.	4.89
Benzamides												
Metoclopramide	n.a.	n.a.	n.a.	5.28	6.52	5.62	n.a.	n.a.	4.54	n.a.	n.a.	n.a.
Sulpiride	n.a.	n.a.	n.a.	n.a.	6.60	n.a.	n.a.	n.a.	4.70	n.a.	n.a.	n.a.
Clebopride	n.a.	n.a.	n.a.	4.54	7.78	5.11	6.25	n.a.	4.81	n.a.	n.a.	n.a.
Other												
Domperidone	6.48	n.a.	n.a.	4.96	8.36	5.27	6.20	n.a.	5.14	5.74	n.a.	5.00
Pimozide	7.02	n.a.	n.a.	5.17	9.02	5.59	7.78	n.a.	6.89	6.88	7.42	5.81

[a] Data are the pIC_{50} ($-\log IC_{50}$) M evaluated using nonlinear curve fitting according to the logistic equation. Abbreviations are the same as in Table I, footnote *a*.
[b] Not active or $-\log IC_{50} < 4$.

only at 10–100 μM concentrations. All other compounds tested were inactive at this binding site, in agreement with findings by Quirion *et al.* (30). All the neuroleptics with the exception of benzamides inhibited [^{3}H]imipramine binding at concentrations near the micromolar level.

The correlation matrix (not shown) of the binding data reported in Table II showed that there was no relation among the affinities for the different receptors, with the exception of the activities on serotonin uptake sites that seem to be related to the affinity to alpha 1-adrenergic and DA1 receptors.

Correlation with Pharmacological and Clinical Data

Cluster analysis was used to establish correlations between the biochemical profile of the neuroleptics described and the pharmacological and clinical profile previously reported by Lewi (1).

Figure 1 shows the taxonomic tree produced by hierarchical cluster analysis of the data reported in Table II, excluding binding to beta-adrenergic receptors, for which none of the tested compounds has an affinity. The diagram illustrates the successive fusion of groups and subgroups of compounds until the stage where all the compounds form a single cluster. Three separate clusters can be identified. The first cluster (A), clearly separate from the other two groups of

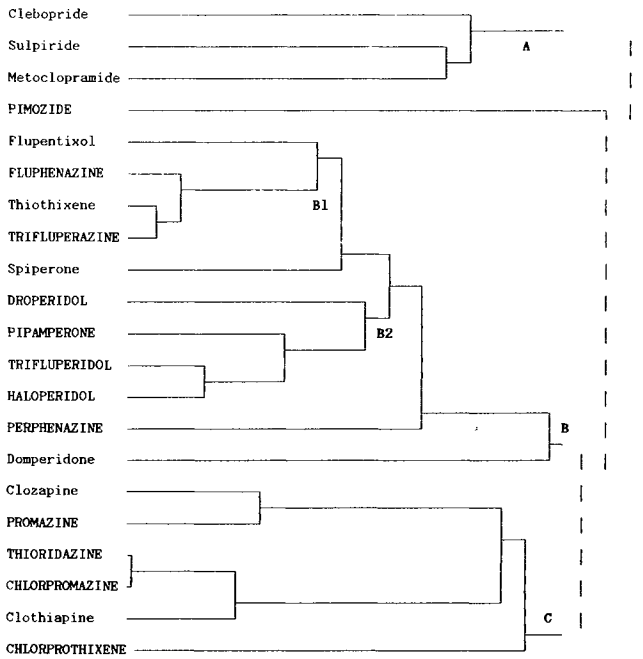

Fig. 1. Taxonomic tree produced by hierarchical cluster analysis on the biochemical data in Table II. Compounds common to Figs. 2 and 3 are printed in all-capital letters.

compounds, includes all the benzamides. Grouping B in Fig. 1 includes two clusters. Cluster B2 contains most of the butyrophenones; subgroup B1 includes spiperone and the two thioxanthenes flupentixol and thiothixene, with the phenothiazines fluphenazine and trifluperazine, whose structure contains the piperazine moiety (31). Perphenezine (a phenothiazine with a piperazine substituent) and domperidone join these two subclusters at a distance. All the compounds in cluster B are characterized by a higher affinity for DA2 and 5-HT2 receptors than for the DA1, alpha 1, and 5-HT1 binding sites and have no activity on muscarinic receptors. Cluster C includes the dibenzodiazepines, chlorprothixene, and three phenothiazines not containing the piperazine moiety in their structure. The compounds in cluster C are characterized by a high affinity for alpha 1-adrenergic receptors and a similar affinity for DA1 and DA2 binding sites. All the compounds included in cluster C have an affinity for muscarinic receptors. Pimozide did not fall into any of these three clusters.

The results of hierarchical cluster analysis on the data related to the clinical profiles of neuroleptics are shown in Fig. 2. Clusters are identified by the same letters as in Fig. 1 and compounds common to the biochemical, pharmacological, and clinical profile are printed in capital letters. The first cluster in the figure (B) includes five of seven compounds grouped in cluster B in Fig. 1, with the exception of pipamperone and droperidol that clustered separately. Cluster C includes four of four compounds common to all three profiles. Pimozide, as in Fig. 1, clustered separately.

Cluster analysis on the pharmacological effects of neuroleptics as reviewed by Lewi (1) is shown in Fig. 3. For simplicity, we analyzed only the most common neuroleptics as reported by Leysen and Niemegeers (31). Cluster C contains chlorprothixene, chlorpromazine, and thioridazine as in Figs. 1 and 2. Pipamperone is also included in this group, as in Fig. 1 but not in Fig. 2, whereas promazine clustered separately, unlike in Figs. 1 and 2. Pimozide, as in the biochemical and clinical dendogram, is in a separate cluster, but the large group B includes the same neuroleptics as in the previous pictures.

DISCUSSION

In the present study, a number of neuroleptics with dif-

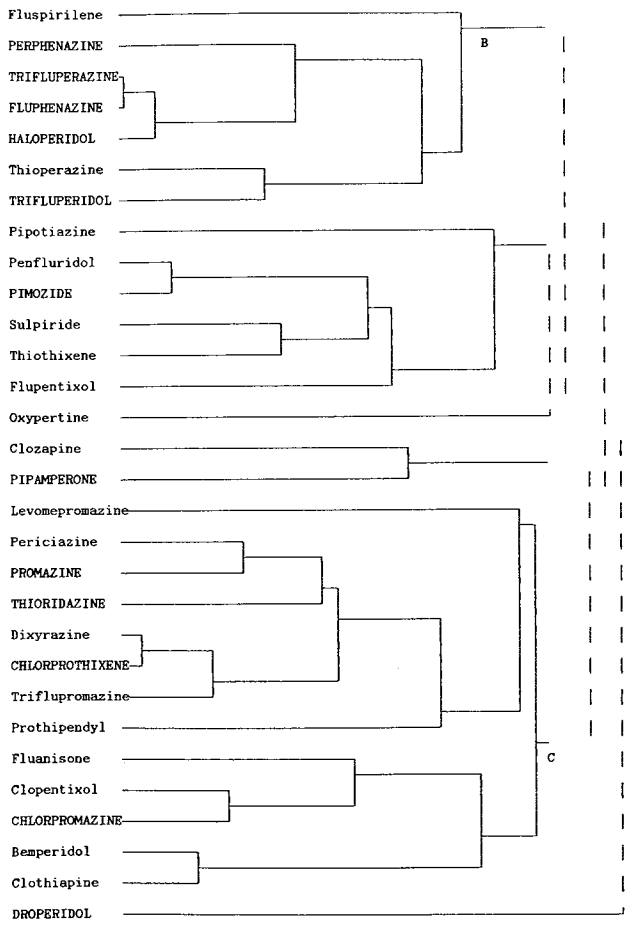

Fig. 2. Taxonomic tree produced by hierarchical cluster analysis on the clinical data reported by Lewi (1). Compounds common to Figs. 1 and 3 are printed in all-capital letters.

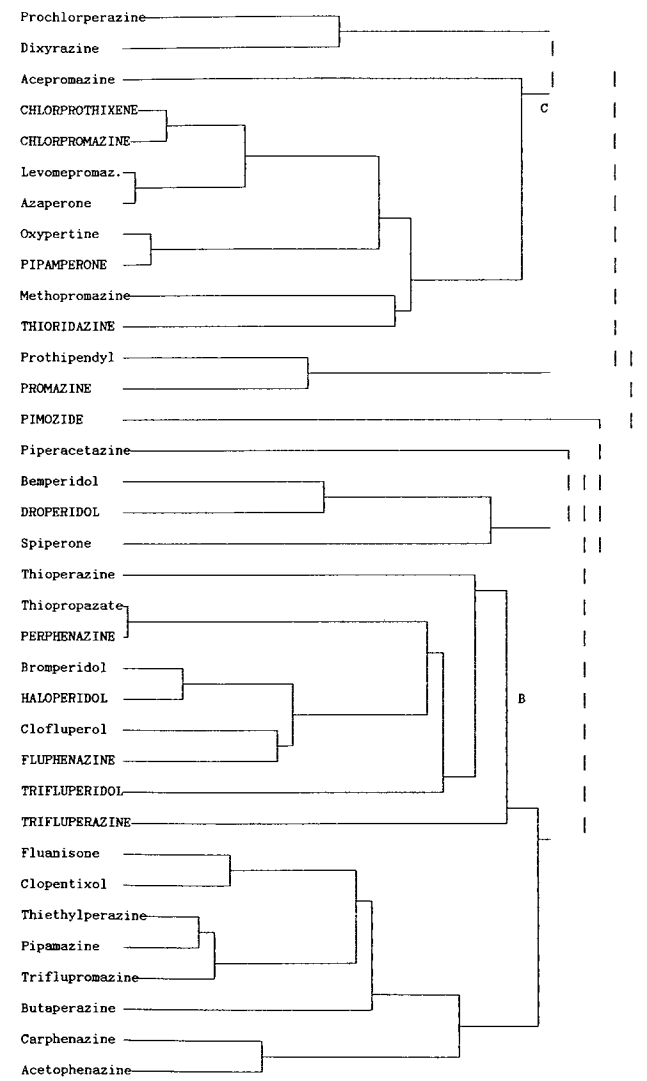

Fig. 3. Taxonomic tree produced by hierarchical cluster analysis on the pharmacological data reported by Lewi (1). Only the most common neuroleptics reviewed by Leysen and Niemeegers (31) are included in the analysis. Compounds common to Figs. 1 and 2 are printed in all-capital letters.

ferent chemical structures (31) were examined for their affinity for the most common neurotransmitter receptors and binding sites in the rat brain. As reported by other authors (20–30), all the compounds examined showed an affinity for alpha 1-adrenergic, DA1 and DA2 dopaminergic, 5-HT1 and 5-HT2 serotonergic, and H1 histaminergic receptors. The 21 neuroleptics studied also showed an affinity for the presynaptic serotonin uptake site labeled by [^{3}H]imipramine. Few compounds were active at alpha 2-adrenergic, muscarinic, and opiate receptors and the 1,4-dihydropyridine binding sites, and none at beta-adrenergic receptors.

At present, most of the functional effects of neuroleptics are ascribed to interactions with DA2 receptors. However, as reported here, compounds such as promazine, chlorpromazine, clozapine, and pipamperone seem to be more active at alpha 1-adrenergic than DA2 dopaminergic receptors. On the other hand, chlorprothixene, flupentixol, clothiapine, clozapine, and pipamperone were more active at 5-HT2 than at DA2 receptors. Drugs with comparable binding affinities for several receptor sites are also expected to have multiple *in vivo* pharmacological effects.

A number of significant correlations have been reported between the *in vitro* binding activities and the *in vivo* potencies of neuroleptics (19,21,27). The correlations indicate that DA2 receptor sites are involved in motor functions, learned and conditioned behavior, emesis, and regulation of dopamine and acetylcholine turnover in animals, as well as in antipsychotic activity and the ability to induce extrapyramidal side effects in humans. Serotonin 5-HT2 receptor blockade is involved in vascular and tracheal contraction, platelet functions, and sleep regulation; further, it may have beneficial effects in dysthymic disorders and on negative symptoms in schizophrenia. Blockade of alpha 1-adrenergic receptor sites is exclusively correlated with antagonism of norepinephrine-induced effects, such as orthostatic hypotension, tachycardia, sedation, and disturbance of sexual functions. Binding to histamine H1 receptors correlates with antagonism of compound 48/80-induced mortality and is related to the known antiallergic effects of neuroleptics (20,21).

These analyses, however, are generally based on a simple linear correlation between *in vitro* and *in vivo* activities and do not take into consideration the complete profile of a compound. Lewi (1) found a significant superimposition of the pharmacological profile of neuroleptics in screening tests in animals (rats and dogs) with the clinical profile obtained by Bobon *et al.* (2) by spectral map and principal components analysis. Lewi (3) also reported that the pharmacological profile of 13 neuroleptics obtained from the inhibition of apomorphine-, tryptamine-, and norepinephrine-induced symptoms in rats correlated with the profile of the affinity for dopamine, serotonin, and norepinephrine binding sites in rat brain tissues.

To test whether a more extended receptor profile of neuroleptics correlates with the pharmacological and clinical data, we compared these profiles by hierarchical cluster analysis. This is a nonparametric method of multivariate statistics, whose purpose is to place objects into groups or clusters, not defined a priori, objects in a given cluster tending to be similar and objects in different clusters dissimilar. The most common cluster method is the hierarchical clustering technique (32), which composes the final classes by hierarchically grouping subclusters; hierarchical clusters are organized so that one cluster may fall entirely within another cluster, but no overlap between clusters is allowed. This analysis of receptor binding data distinguishes three main clusters. Cluster C in Fig. 1 includes clothiapine and clozapine and four drugs positioned in the same zone of the plane of the pharmacological and clinical maps reported by Lewi (1), namely, promazine, thioridazine, chlorpromazine, and chlorprothixene. With the exception of promazine these same drugs also clustered together in the analysis of the clinical profile (Fig. 2) and pharmacological effects (Fig. 3). These results indicate that the clinical observation of sedative and adrenolytic effects corresponding with inhibition of epinephrine-induced mortality and ptosis in animal studies can be predicted by cluster analysis of an extended receptor binding profile of neuroleptics.

The second main group observed in cluster analysis of binding data included the following drugs also common to the pharmacological and clinical profiles: fluphenazine, trifluperazine, droperidol, pipamperone, trifluperidol, haloperidol, and perphenazine. With the exception of droperidol and pipamperone these same compounds clustered together in the analysis of clinical (Fig. 2) and pharmacological (Fig. 3) data. Again, these compounds are positioned in the same zone of the plane in the spectral maps reported by Lewi (1), indicating that the antimanic and extrapyramidal profile of the clinical spectra, the inhibition of apomorphine- and amphetamine-induced stereotypes, and the induction of catalepsy in rats can all be predicted from *in vitro* binding data.

Since no comparable data are available for benzamides (cluster A in Fig. 1), no comparison can be made with pharmacological and clinical spectra.

Recently, El Tayar *et al.* (33) did a cluster analysis on a set of 31 neuroleptics examined for their interactions with DA1 and DA2 receptors and physicochemical parameters such as pK_a and log K_w. The authors claimed that cluster analysis divided the compounds into a number of subgroups according to biological and structural properties. Looking at their dendrogram, however, fluphenazine and haloperidol cluster together with chlorpromazine and thioridazine. Again, spiperone clusters with benzamides, not in agreement with the pharmacological and clinical properties of these drugs.

In conclusion, the results of *in vitro* binding to several neurotransmitter receptors in the central nervous system indicate complex interactions of neuroleptics with these binding sites and lead to a classification of the drugs which is superimposed on that obtained in pharmacological tests and clinical practice. This finding further validates the use of *in vitro* receptor binding as a basic screening test, although additional variables, such as pharmacokinetics and metabolism, also affect the comparison between *in vitro* and *in vivo* test results.

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