Dopamine Receptor Binding Predicts Clinical and Pharmacological Potencies of Antischizophrenic Drugs

Abstract. *Tritiated haloperidol and tritiated dopamine label postsynaptic dopamine receptors in mammalian brain. Clinical potencies of butyrophenones, phenothiazines, and related drugs correlate closely with their ability to inhibit tritiated haloperidol binding. These binding methods provide a simple in vitro means for evaluating new drugs as potential antischizophrenic agents.*

Neuroleptic phenothiazine and butyrophenone drugs are thought to alleviate schizophrenic symptoms and induce parkinsonism-like extrapyramidal side effects by blocking dopamine receptors in the brain (*1*). While molecular modeling indicates how phenothiazines can assume the preferred conformation of dopamine, the conformation of butyrophenones at their receptor sites is unclear (*2*). Nevertheless, in behavioral tests both phenothiazines and butyrophenones block dopamine-mediated behaviors induced by amphetamine and apomorphine in proportion to their clinical potency (*3*).

A dopamine-sensitive adenylate cyclase, localized to areas of the brain rich in dopamine terminals, appears to be associated with the postsynaptic dopamine receptor and might therefore predict potencies of dopamine antagonists (*4*). While there are some correlations between the pharmacological potencies of phenothiazines and their inhibition of the dopamine-sensitive adenylate cyclase, major discrepancies exist for butyrophenones (*5*). Although butyrophenone neuroleptics such as spiroperidol and pimozide are weaker than chlorpromazine in inhibiting the dopamine-sensitive cyclase, in vivo behavioral and clinical data show them to be considerably more potent than chlorpromazine (*3*). These discrepancies can be construed as challenges to hypotheses that antischizophrenic drugs produce their therapeutic effects by blocking postsynaptic dopamine receptors; indeed it has been suggested instead that the drugs might act by inhibiting dopamine release (*6*).

Recently, dopamine receptor binding has been demonstrated in brain membranes by labeling the receptor both with the agonist [^{3}H]dopamine and with the antagonist [^{3}H]haloperidol (*7*). The regional distribution of receptor binding and the relative potencies of catecholamines and a variety of drugs ensure that binding of these ligands is associated with postsynaptic dopamine receptors, while the failure of destruction of dopamine nerve terminals to decrease binding indicates that presynaptic receptors are not involved. [^{3}H]Dopamine and [^{3}H]haloperidol appear to label distinct agonist and antagonist states of the receptor, respectively (*8*). Thus dopamine and other agonists have a much greater affinity for dopamine than haloperidol

Table 1. Antischizophrenic drugs: comparison of affinities for [^{3}H]haloperidol and [^{3}H]dopamine binding sites with in vivo pharmacological potencies. Drugs are listed in order of affinity for [^{3}H]haloperidol binding sites of calf striatal membranes. For each drug competition of binding of both ligands was measured at two to four concentrations of drug, and 50 percent inhibitory concentrations, IC_{50}, were derived by log probit analysis. These values were converted to apparent K_i's according to the equation $K_i = IC_{50}/(1 + C/K_D)$, where C is the concentration of radioactive ligand and K_D is its dissociation constant. Each value is the mean ± standard error of the mean for three to ten determinations (N is given in parentheses). In vivo animal data and clinical potencies were calculated from published results (*12, 13*); ID_{50} and PD_{50} are 50 percent inhibitory dose and 50 percent protective dose, respectively.

Drug	Inhibition of [^{3}H]haloperidol binding, K_i (nM)	Inhibition of apomorphine stereotypy in rat, ID_{50} (μmole/kg)	Inhibition of apomorphine-induced emesis in dog, PD_{50} (μmole/kg)	Inhibition of amphetamine stereotypy in rat, ID_{50} (μmole/kg)	Average clinical daily dose (μmole/kg)	Inhibition of [^{3}H]dopamine binding, K_i (nM)	Rank order, inhibition of [^{3}H]dopamine binding
Spiroperidol	0.25 ± 0.02 (4)	0.177	0.0006	0.051	0.058	1400 ± 190 (3)	16
Benperidol	0.33 ± 0.02 (4)	0.118	0.0012	0.071	0.060	4100 ± 540 (4)	21
Clofluperol	0.50 ± 0.03 (4)	0.198		0.117	0.077	360 ± 20 (3)	4
(+)-Butaclamol	0.55 ± 0.09 (8)		0.095		2.14	70 ± 10 (10)	1
Fluspirilene	0.60 ± 0.13 (4)				0.066	1400 ± 220 (4)	15
Pimozide	0.80 ± 0.07 (4)	0.370	0.024	0.242	0.108	4100 ± 1140 (4)	22
Trifluperidol	0.95 ± 0.19 (3)	0.067	0.016	0.056	0.096	740 ± 20 (3)	10
Droperidol	1.0 ± 0.10 (4)	0.185	0.003	0.095		880 ± 80 (3)	12
α-Flupenthixol	1.1 ± 0.22 (4)	0.867		0.650	0.099	180 ± 30 (8)	3
Fluphenazine	1.2 ± 0.12 (6)	0.255	0.012	0.196	0.168	180 ± 30 (5)	2
Bromoperidol	1.4 ± 0.15 (4)	0.324	0.038	0.126	0.153	600 ± 90 (3)	7
cis-Thiothixene	1.4 ± 0.11 (4)	1.42		0.803	0.393	540 ± 140 (6)	6
Haloperidol	1.5 ± 0.14 (9)	0.532	0.050	0.101	0.152	650 ± 90 (4)	8
Moperone	1.9 ± 0.26 (4)	0.638	0.050	0.059	0.802	1200 ± 160 (4)	14
Triflupromazine	2.1 ± 0.12 (4)	4.62	0.50	0.746	4.59	530 ± 80 (5)	5
Trifluoperazine	2.1 ± 0.34 (4)	1.14	0.08	0.520	0.297	740 ± 80 (5)	9
Fluanisone	3.8 ± 0.80 (4)	6.17	0.40	0.757	3.44	800 ± 180 (4)	11
Penfluridol	5.6 ± 1.40 (7)				0.466	1600 ± 310 (4)	17
Azaperone	10.0 ± 0.6 (4)	27.4		9.16		1700 ± 290 (4)	18
Chlorpromazine	10.3 ± 0.2 (5)	18.3	2.0	3.09	12.0	900 ± 200 (7)	13
Thioridazine	14.0 ± 0.2 (5)				12.6	1780 ± 332 (4)	19
Pipamperone	31.3 ± 5.2 (4)	635	3.5	11.1	11.1	4900 ± 500 (4)	23
Promazine	71.5 ± 3.2 (4)	> 250	60	99.6	33.3	7100 ± 1640 (8)	24
Clozapine	100 ± 6 (6)				24.6	1890 ± 340 (5)	20
Promethazine	238 ± 32 (4)					12000 ± 3600 (7)	25
Correlation with [^{3}H]haloperidol binding		$r = .94$ $P < .001$	$r = .93$ $P < .001$	$r = .92$ $P < .001$	$r = .87$ $P < .001$	$r = .58$ $P < .01$	
Correlation with [^{3}H]dopamine binding		$r = .46$ $P < .05$	$r = .22$ $P > .05$	$r = .41$ $P > .05$	$r = .27$ $P > .05$		

Table 2. Regional comparison of competition for [^{3}H]haloperidol binding by antischizophrenic drugs. Experiments were performed as described in Table 1. Fresh calf brain was dissected into regions and assayed, using the same drug dilutions. Except for clozapine and thioridazine, striatal data are from other experiments than those in Table 1. Each value is the mean ± standard error of the mean (N is given in parentheses).

Drug	K_i (nM)		
	Corpus striatum	Olfactory tubercle	Nucleus accumbens
Dopamine	550 ± 100 (6)	700 ± 40 (2)	475 ± 75 (2)
Haloperidol	1.4 ± 0.1 (5)	0.8 ± 0.3 (3)	0.8 ± 0.1 (2)
Pimozide	0.6 ± 0.2 (2)	0.6 ± 0.04 (2)	0.6 ± 0.1 (2)
Fluphenazine	0.6 ± 0.1 (6)	0.5 ± 0.05 (2)	0.8 ± 0.2 (2)
Thioridazine	14 ± 1.3 (5)	14 ± 2 (3)	16 (1)
Clozapine	100 ± 6 (6)	64 ± 24 (2)	80 ± 0 (2)

sites, while the reverse holds true for dopamine antagonists. We now report that the relative affinities of an extensive series of butyrophenones, phenothiazines, and other dopamine antagonists in competing for [^{3}H]haloperidol binding to the dopamine receptor predict the pharmacological activities of these drugs in animal behavioral tests and their clinical potencies in psychiatric patients.

Methods were essentially as described previously (*7*). Homogenates (Brinkmann Polytron) of fresh calf caudate in cold tris(hydroxymethyl)aminomethane (tris) buffer were washed twice by centrifugation. The final resuspension (in cold 50 mM tris buffer containing 0.1 percent ascorbic acid, 10 μM pargyline, and ions as follows: 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, giving a final pH of 7.1 at 37°C) was warmed to 37°C for 5 minutes and returned to ice. Each tube received 2 ml of caudate suspension (20 mg, wet weight) and contained 5 nM [^{3}H]dopamine ([ethyl-1-^{3}H]dopamine, 8.4 c/mmole, New England Nuclear) or 2 nM [^{3}H]haloperidol (0.86 c/mmole, custom tritiated by New England Nuclear and purified by a thin-layer chromatography) and various concentrations of nonradioactive drugs. Triplicate tubes were incubated at 37°C for 10 minutes and rapidly filtered under vacuum through Whatman GF/B filters with two 5-ml rinses of cold buffer. The filters were counted by liquid scintillation spectrometry.

Saturable or specific binding of [^{3}H]dopamine was measured as the excess over blanks taken in the presence of 1 μM dopamine or 10 μM (+)-butaclamol. Blank tubes for [^{3}H]haloperidol binding contained 100 μM dopamine or 0.1 μM (+)-butaclamol. By these criteria half of the total [^{3}H]dopamine binding and about 40 percent of the [^{3}H]haloperidol binding were specific. Binding of [^{3}H]dopamine has a dissociation constant, K_D, of about 20 nM, while that of [^{3}H]haloperidol binding is about 2 nM.

In general, butyrophenones and related drugs are the most potent class of neuroleptics in treating schizophrenia and in antagonizing dopamine-mediated behaviors in animals. The most potent of the drugs examined in this study, spiroperidol, substantially protects dogs against apomorphine-induced emesis and rats against amphetamine-induced stereotyped behavior at doses under 0.05 μmole/kg, a dose level similar to that used therapeutically in humans (Table 1). Spiroperidol is also the most potent inhibitor of [^{3}H]haloperidol binding with a value of the inhibition constant, K_i, indicating 50 percent receptor occupation, of 0.25 nM. It thus possesses a 5-fold higher affinity for [^{3}H]haloperidol binding than fluphenazine, a potent phenothiazine, a 40-fold greater affinity than chlorpromazine, and a 125-fold to 950-fold greater affinity than the weak neuroleptics pipamperone, promazine, and promethazine (Table 1). There is an excellent correlation between the molar pharmacological potencies of the butyrophenones, phenothiazines, and related compounds in animals and man and their affinities for [^{3}H]haloperidol binding sites. On a log-log graph (not shown) of the data in Table 1, the affinities of these 25 drugs for [^{3}H]haloperidol binding sites display a correlation coefficient (r) of .94 ($P < .001$) with pharmacological potencies in antagonizing apomorphine stereotypy and an r of .92 ($P < .001$) in antagonizing amphetamine stereotypy in rats; an r of .93 ($P < .001$) with potencies in antagonizing apomorphine-induced emesis in dogs and an r of .87 ($P < .001$) with clinical potencies in man. These impressive correlations indicate that affinity for [^{3}H]haloperidol binding to dopamine receptors is a powerful predictor of clinical activity. The correlations are all the more impressive because binding studies were conducted in vitro and animal behavioral and human studies conducted in vivo.

Dopamine receptor blockade in the corpus striatum is presumed to be responsible for extrapyramidal parkinsonism-like side effects of neuroleptic drugs, while antischizophrenic actions may involve dopamine receptors in other brain regions as well (*1*). Some neuroleptics, such as thioridazine and clozapine, elicit a much lower incidence of extrapyramidal effects than most agents, which could arise from differential influences on dopamine receptors in various areas. This is unlikely, because our regional studies indicate that clozapine, thioridazine, other neuroleptics, and dopamine have about the same affinity for [^{3}H]haloperidol binding sites in the corpus striatum as in two meso-limbic areas of the brain, the olfactory tubercle and nucleus accumbens (Table 2). Neuroleptics also affect the dopamine-sensitive adenylate cyclase to a similar extent in these three areas (*4*). The relative affinities of clozapine and thioridazine for [^{3}H]haloperidol binding correspond reasonably well with their clinical potencies (Table 1). The anticholinergic properties of these drugs may well account for their low incidence of extrapyramidal effects (*1*).

The great potency of butyrophenones and phenothiazines in competing for [^{3}H]haloperidol binding to the dopamine receptor contrasts with their relatively low potency in competing for the binding of [^{3}H]dopamine. The absolute potencies of all drugs correlate significantly for the two types of binding ($r = .58$, $P < .01$), although there are discrepancies in rank order for some drugs. Competition of the drugs for [^{3}H]dopamine binding correlates much less well than for [^{3}H]-haloperidol binding with the behavioral activities of these agents in rat (for apomorphine antagonism $r = .46$, $P < .05$, and for amphetamine antagonism $r = .41$, $P > .05$) and dog (for apomorphine antagonism $r = .22$, $P > .05$) and with their clinical potencies ($r = .27$, $P > .05$).

We have attributed the fact that phenothiazines and butyrophenones are much less potent in competing for [^{3}H]-dopamine than for [^{3}H]haloperidol binding to the selective labeling by [^{3}H]dopamine of the agonist state of the dopamine receptor and the labeling by [^{3}H]haloperidol of the antagonist state (*7*). One might expect that the relative affinities of drugs for [^{3}H]dopamine and [^{3}H]haloperidol sites would indicate the extent to which the drugs are pure agonists, pure antagonists, or mixed agonist-

antagonists. This is confirmed by data showing that D-lysergic acid diethylamide, a mixed agonist-antagonist of the dopamine-sensitive adenylate cyclase (*9*), has similar affinities for both [^{3}H]dopamine and [^{3}H]haloperidol binding sites (*10*). Conceivably the different relative affinities of antischizophrenic drugs for [^{3}H]dopamine and [^{3}H]haloperidol binding sites indicate that these drugs vary in how they affect the dopamine receptor. For instance, some may be more "pure" antagonists than others.

The data reported here demonstrate an extremely close correlation between the clinical and pharmacological potencies of butyrophenones and phenothiazines and their affinities in competing for the binding of [^{3}H]haloperidol to dopamine postsynaptic receptors. This result argues that these drugs do act by blocking postsynaptic dopamine receptors. Reasons for discrepancies between results with the dopamine-sensitive adenylate cyclase and the in vivo and binding data are unclear but may be related to variable degrees of coupling of dopamine receptor sites with the adenylate cyclase (*11*).

Labeling of postsynaptic dopamine receptors by [^{3}H]haloperidol provides a simple, sensitive, and specific means for screening phenothiazines, butyrophenones, and related agents as potential antischizophrenic drugs.

IAN CREESE
DAVID R. BURT
SOLOMON H. SNYDER
Department of Pharmacology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

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Neuronal Substrate of Classical Conditioning in the Hippocampus

Abstract. *Neuronal activity in dorsal hippocampus was recorded in rabbits during classical conditioning of nictitating membrane response, with tone as conditioned stimulus and corneal air puff as unconditioned stimulus. Unit activity in hippocampus rapidly forms a temporal neuronal "model" of the behavioral response early in training. This hippocampal response does not develop in control animals given unpaired stimuli.*

The hippocampus has been implicated in learning by many investigators (*1*). Recent studies of hippocampal neurons in the intact, behaving animal have demonstrated clear changes in unit activity during learning (*2*). However, the role of hippocampus in learning remains obscure. We have recently adopted classical conditioning of the nictitating membrane response of the rabbit (*3*) as a model system in which to study neuronal substrates of learning (*4*). The parametric effects of stimulus and training variables and the properties of the response are well established in this system (*5*). Here we report results of an initial study of hippocampal activity during nictitating membrane conditioning.

Animals were anesthetized with halothane, and insulated stainless steel microelectrodes with approximately 5- to 7-μm tip diameters and 40- to 50-μm exposed shafts were permanently implanted (one per animal) in the dorsal hippocampus. Electrodes were localized both with stereotaxic coordinates and physiological recordings during implantation. After 1 week of recovery, animals in the conditioning group were given standard training (*6*): 13 blocks of trials per day, with eight CS-UCS (*7*) paired trials and one CS-alone (1-khz, 85-db, 350-msec tone) test trial per block (117 trials total per day); the intertrial interval was a random sequence of 50, 60, or 70 seconds. The UCS was a 100-msec air puff to the cornea, onset 250 msec after CS onset (CS and UCS overlap). Animals were given one, sometimes two, days of conditioning and then extinguished with at least 13 blocks of CS-alone trials, nine trials per block. Control animals received 13 blocks of unpaired CS and UCS presentations per day, with eight CS-alone trials and eight UCS presentations per day, for 16 unpaired trials per block (204 trials total per day). The sequence was random with a 20-, 30-, or 40-second intertrial interval. To nearly equalize the number of stimulus presentations, the number of unpaired trials was approximately double that of paired trials. All animals were held in a restraining apparatus throughout training. Data from 18 conditioning and 11 control animals are reported here; only acquisition results are given.

Neural activity was recorded on AM-FM tapes and band-pass filtered at 500 to 5000 hertz. Although individual neuron waveforms could be examined if desired, the present analysis was limited to discharges of relatively small groups of units ("multiple unit" discharges) as defined by a pulse-height discriminator set to pass only larger unit spikes. The level of the discriminator was set to maintain a spontaneous mean count of approximately 2 to 6 counts per second. Records were used only where the signal-to-noise