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The Thermodynamics of Agonist and Antagonist Binding to Dopamine D-2 Receptors

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SUMMARY

The ability of dopamine agonists and antagonists to compete with [^{3}H]spiperone binding to rat striatal membrane preparations at 4, 15, 26, and 37° varied markedly with temperature. Dopamine and the dopamine agonist 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide (ADTN) were more potent at lower temperatures. The ability of the dopamine antagonists, haloperidol, *cis*-flupenthixol, *cis*-*N*-(1-benzyl-1-methypyrrolidin-3-yl)-5-chloro-2-methoxy-9-methylaminobenzamide (YM 09151-2), raclopride, and clozapine, and of the agonists apomorphine and pergolide, to compete with [^{3}H]spiperone binding was little altered by temperature. (+)-Butaclamol was more potent at higher temperatures. In contrast, the antagonists sulpiride, metoclopramide, clebopride, sultopride, tiapride, piquindone, and zetidoline were more potent at lower temperatures. The interaction of the agonists dopamine and ADTN was driven by a decrease in enthalpy, allowing an energetically unfavorable decrease in entropy. The binding of the antagonists, haloperidol, *cis*-flupenthixol, YM 09151-2, raclopride, (+)-butaclamol, and clozapine, and also of the agonists, apomorphine and pergolide, was entropy driven. The interaction of the antagonists sulpiride, metoclopramide, clebopride, alizapride, sultopride, tiapride, piquindone, and zetidoline differed from that of other antagonists in being enthalpy driven. The observed entropy changes correlated with the lipophilicity of the displacing drugs and not with their intrinsic activity.

Thermodynamic changes in drug affinity for neurotransmitter receptors can provide information on the type of interaction involved. For example, Weiland *et al.* (1) demonstrated that agonist interaction with turkey erythrocyte β-adrenergic receptors (identified using [^{125}I]iodohydroxybenzylpindolol) was enthalpy driven, whereas the interaction of antagonists was totally entropy driven. Enthalpy is defined as the heat content of a substance per unit mass. Entropy is a measure of unavailable energy, energy existing but unavailable for work, i.e., a measure of the state of order. They proposed that the decrease in both enthalpy and entropy associated with the agonist interaction reflected an agonist-induced conformational change in the receptor. In contrast, the increases in entropy observed on antagonist interaction are thought to represent simple binding events where no information is transferred, akin to the binding of ions and antibodies to proteins (2, 3). Similar thermodynamic changes occur with the interaction of agonist and antagonist drugs with β-adrenergic receptors in rat cerebral cortex, cerebellum, heart, and lung (4). However, not all neurotransmitter systems respond with similar thermodynamic changes on agonist and antagonist interaction. For example, agonist binding to the nicotinic acetylcholine receptor is entropy driven (5). For dopamine receptors, Zahniser and Molinoff (6) reported that both agonist and antagonist interactions with the D-2 receptor identified by [^{3}H]spiperone were entropy driven. However, their data were not in accord with the finding that, for dopamine receptors labeled by the agonist ligand [^{3}H]-*N*-*n*-propylnorapomorphine, agonist binding was enthalpy driven (7). Leysen and Gommeren (8) noted that [^{3}H]spiperone and [^{3}H]apomorphine binding to rat striatal homogenates was little affected by temperature and, hence, presumably entropy driven.

Recently we found that, surprisingly, the selective D-2 antagonist sulpiride was more potent in competing with [^{3}H]spiperone binding at 4° than 37°, whereas the action of haloperidol was little affected by temperature (9). This has led us to examine the thermodynamic changes induced by the interaction of dopamine agonists and of groups of typical and atypical neuroleptic drugs with the rat striatal D-2 receptor identified by [^{3}H]spiperone.

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ABBREVIATIONS: ADTN, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide; YM 09151-2, *cis*-*N*-1-benzyl-1-methoxypyrrolidin-3-yl-5-chloro-2-methoxy-9-methylaminobenzamide; MPS, 3-morpholinopropane sulfonate buffer.

Materials and Methods

[^{3}H]Spiperone (18 or 19 Ci mmol^{-1}) was supplied by Amersham International. The following drugs were employed: ADTN (Wellcome Research Laboratories), (+)-butaclamol hydrochloride (Ayerst Laboratories), clebopride (Almirall), *cis*-flupenthixol hydrochloride (Lundbeck, Copenhagen), haloperidol (Janssen Pharmaceutica, Belgium), (±)-sulpiride, metoclopramide, (−)-sultopride, alizapride, tiapride, (Delagrange, France), YM 09151-2 (Yamanouchi, Japan), raclopride (Astra, Sweden), clozapine (Sandoz, Switzerland), piquindone (Hoffmann-La Roche), zetidoline (Lepetit, Italy), apomorphine hydrochloride (MacFarlan Smith, Edinburgh), and pergolide mesylate (Lilly). Dopamine hydrochloride was supplied by Sigma Chemical Co. All drugs were dissolved in distilled water with minimal use of 1 M HCl; haloperidol was initially dissolved in a minimal quantity of glacial acetic acid.

Preparation of striatal membranes. Female Wistar rats (150 ± 10 g; Bantin & Kingman Ltd.) were stunned and decapitated, the brains were removed and the paired corpora striata were rapidly dissected into 10 volumes of ice-cold 50 mM Tris-HCl buffer at pH 7.4 (at 20°); the final pH measured at 37° was 6.9 and at 4° was 7.8. Paired tissue from 10–20 animals was homogenized using a Polytron homogenizer (setting 5 to 10 sec) and centrifuged at 48,000 × *g* for 10 min in a Sorvall RC5B centrifuge at 4°. The resulting pellet was resuspended in 10 volumes of the same buffer and the process was repeated. The final pellet was resuspended in 270 volumes of original tissue weight and kept on ice before use in the binding assay.

[^{3}H]Spiperone binding to rat striatal membrane preparations. For the determination of [^{3}H]spiperone binding to membrane preparations, aliquots (900 μl) of the final tissue suspension were incubated with 50 μl of a solution of displacing agent or its vehicle and 50 μl of a solution of [^{3}H]spiperone. Sodium chloride at a final concentration of 120 mM was also present. For equilibrium saturation analysis, six ligand concentrations from 0.01–1.0 nM were used. Nonspecific binding was routinely defined by the incorporation of 10^{-5} M (±)-sulpiride. This was justified in control experiments where displacement of [^{3}H]spiperone binding was apparently maximal at 10^{-5} M; Eadie-Hofstee analysis of data suggested a single site for specific binding of [^{3}H]spiperone defined at this concentration of sulpiride at all temperatures. For determinations of the IC_{50} values of drugs to compete with [^{3}H]spiperone (0.1 nM) binding, at least five ascending concentrations of each drug were included in the assay (10^{-10}–10^{-4} M). All samples were examined in triplicate at each ligand or displacing drug concentration. In some experiments samples were incubated with [^{3}H]spiperone (0.1 nM) for varying times (0–90 min) at 4, 15, 26, and 37°. From these experiments equilibrium incubation times of 90, 60, 30, and 10 min were chosen at the respective temperatures. The IC_{50} values of 6,7-ADTN, (±)-sulpiride, (+)-butaclamol, apomorphine, haloperidol, piquindone, and *cis*-flupenthixol were calculated at all temperatures; for the remaining drugs, IC_{50} values were only calculated at 4 and 37°. Reactions were terminated by rapid vacuum filtration through Whatman GF/C filters over Millipore 3025 12 well sampling manifolds at 50 cm Hg vacuum. Filters were washed twice immediately with 5.0 ml of cold buffer. Filters were then placed with 5 ml of Packard ES299 scintillation cocktail and left overnight before counting in a Packard TriCarb 460C scintillation spectrometer at an efficiency of approximately 45%.

Calculation of log k_w. A Siemens S101 chromatograph equipped with an Orlita pump, type DMP-AE 10.4, was used. The detector was a Uvikon 740 LC (Kontron), operating at 254 nm; column length 25 cm, i.d. 4 mm, prepacked with LiChrosorb RP-18, particle size 10 μm (Knauer). A Hewlett-Packard 3390A integrator was used for peak registration and calculation of retention times. *n*-Decylamine (0.2%, v/v) was used as a masking agent to eliminate silanophilic interactions (10–12). The mobile phases were made up volumetrically from combinations of methanol and MPS (0.02 M) and *n*-decylamine (0.2%, v/v) in the range 10–80% methanol. The pH of the mobile phase was adjusted to 7.5 by addition of hydrochloric acid to the aqueous solution of MPS and *n*-decylamine, i.e., the measured pH corresponds to pH in water (without methanol). All solutions were purified by filtration using a Millipore-Q system. Retention times were measured at ambient temperature; the flow rate was 1.5 ml/min and the column dead time (t_o) was determined to be 60 sec using sodium bicarbonate as the non-retained compound. The capacity factor (k) is defined as

$$k = (t_R - t_o)/t_o$$

where t_R is the retention time of the solute. Such capacity factors (log k), determined at various methanol/water ratios, were extrapolated to a 100% water eluent (log k_w values) and corrected for solute ionization (extrapolation to 100% neutral species, log k_w° values) (13, 14) according to the equation log k_w° (true, neutral) = log k_w (apparent) + log $(1 + 10^{pk_a - pH})$.

Data calculation. A computer curve-fitting program (minimization of weighted sum of squares by an iterative process) was used for the calculation of the parameters of equilibrium saturation studies of [^{3}H]spiperone binding by fitting to the equation:

$$b = \frac{B_{max}}{1 + (K_d/F)}$$

where b is the amount bound, K_d the equilibrium dissociation constant, B_{max} the amount bound at infinite ligand concentration (maximum number of specific receptor sites), and F the free (unbound) ligand. K_i values were calculated according to the Cheng-Prussoff equation (15):

$$K_i = \frac{IC_{50}}{1 + (F/K_d)}$$

The IC_{50} of a drug was calculated as the concentration required to displace 50% of specific [^{3}H]spiperone binding (defined by 10^{-5} M (±)-sulpiride) as derived graphically.

The following thermodynamic relationships were employed: $\Delta G^\circ = -RT \ln K_a$, where ΔG° is the Gibbs free energy change in kcal mol^{-1}, R is the gas constant (1.99 cal mol^{-1} − deg), T is the temperature in degrees Kelvin, and K_a is the equilibrium association constant ($1/K_i$). ΔH° (enthalpy change in kcal mol^{-1}) was calculated from the slope of the Van't Hoff plot (ln K_a versus $1/T$). $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$, where ΔS° is the standard entropy change in calories per mol degree.

Results

Time course for association of specific [^{3}H]spiperone binding to membrane preparations of rat striatum. The rate of association of specific [^{3}H]spiperone (0.1 nM) binding to rat striatal membrane preparations increased with increasing temperature (Fig. 1) at 4, 15, 26, and 37°. The following incubation times allowing apparent equilibrium at each temperature were chosen from these data: 10 min at 37°, 30 min at 26°, 60 min at 15°, and 90 min at 4°. The level at which specific [^{3}H]spiperone (0.1 nM) binding reached equilibrium also increased with increasing temperature; at equilibrium, at 37°, specific binding represented 1000–1100 cpm, whereas at 4° this was 600–700 cpm.

The effect of temperature on specific [^{3}H]spiperone binding to striatal membrane preparations. The results of computer curve fitting of equilibrium saturation studies of specific [^{3}H]spiperone binding over a range of concentrations (0.01–1.0 nM; as defined using 10^{-5} M (±)-sulpiride) to the membrane preparation of rat striatum showed no significant change of apparent B_{max} values with temperature. The B_{max} values (pmol/g of tissue ± SE; $n = 3$) were: 4°, 27.4 ± 1.5; 15°, 28.7 ± 1.2; 26°, 30.8 ± 2.2; 37°, 28.0 ± 1.6; $p > 0.05$. The apparent K_d was lower at 4° than at 15, 26, and 37°, but was little changed at each of these three higher temperatures.

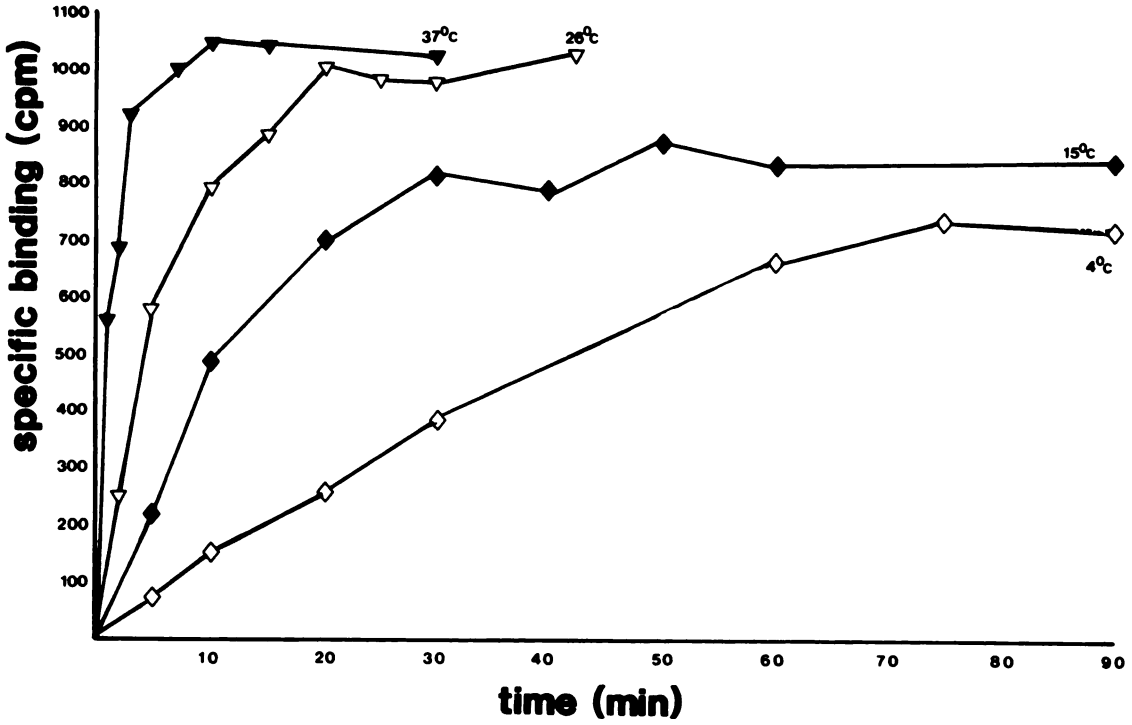

Fig. 1. The association of specific [^{3}H]spiperone [0.1 nM, defined by the incorporation of 10^{-5} M (+)-sulpiride] binding to the membrane preparation of rat striatum at 4 (◇), 15 (◆), 26 (▽), and 37° (▼). Assays were performed in triplicate and terminated by rapid vacuum filtration as described in Materials and Methods.

The K_d values (nM ± SE) were: 4°, 0.37 ± 0.03; 15°, 0.11 ± 0.02; 26°, 0.11 ± 0.02; 37°, 0.13 ± 0.02. The K_d values obtained here were used to calculate K_i values from the IC_{50} values of displacing drugs at the different temperatures. Eadie-Hofstee analysis of this binding data was linear at all temperatures, the correlation coefficient never being less than 0.95.

The effect of temperature on the drug competition for specific [^{3}H]spiperone binding to striatal membrane preparations. These results are presented in Table 1. Dopamine and the agonist drug, ADTN, were greater than 5 times more potent at competing with the specific binding of [^{3}H]spiperone at 4° than 37°, but apomorphine and pergolide were equipotent.

The dopamine receptor antagonists haloperidol, clozapine, and *cis*-flupenthixol showed little temperature dependence in their ability to compete with [^{3}H]spiperone binding. Haloperidol was slightly more potent at lower temperatures and *cis*-flupenthixol was slightly more potent at higher temperatures; (+)-butaclamol was more potent at 37°. The substituted benzamide group of antagonist drugs, sulpiride, sultopride, tiapride, alizapride, metoclopramide, and clebopride, was ≥10 times more potent in competing with [^{3}H]spiperone binding at the lower temperatures as were the non-substituted benzamide dopamine antagonists, piquindone and zetidoline. In contrast, the substituted benzamide drugs, YM 09151-2 and raclopride, showed little temperature dependence for competing with [^{3}H]spiperone binding.

The final buffer pH varied from 7.8 (at 4°) to 6.9 (at 37°). In control experiments performed at 37°, where the final pH was corrected to 7.8 and 6.9, specific [^{3}H]spiperone binding and the IC_{50} values of (±)-sulpiride, 6,7-ADTN, and (+)-butaclamol were not different. There was also no significant difference in the IC_{50} values of these drugs if the incubation time (at 37°) was increased to 90 min (data not shown).

Seven drugs belonging to different chemical classes (ADTN, apomorphine, *cis*-flupenthixol, (+)-butaclamol, haloperidol, (±)-sulpiride, and piquindone) were tested for the ability to displace [^{3}H]spiperone binding at two further temperatures (15° and 26°) (see Table 1). The affinity of ADTN, (±)-sulpiride, and piquindone was negatively related to temperature. The affinity of *cis*-flupenthixol, haloperidol, and apomorphine was little affected by temperature, whereas the affinity of (+)-butaclamol was positively related to temperature. The slope of the IC_{50} curves for ADTN and sulpiride were apparently reduced at lower temperatures, as were those of dopamine. Competition curves of [^{3}H]spiperone binding at 4, 15, 26, and 37° by 15 different concentrations of (±)-sulpiride between 10^{-10} and 10^{-5} M revealed that the Hill number at 37° was 1.01, at 26° it was 0.75, at 15°, 0.61, and at 4°, 0.73 (data not shown). Similar competition curves with ADTN also showed a reduction in Hill number with temperature.

Thermodynamic parameters of drugs competing with [^{3}H]spiperone binding to rat striatal membrane preparations. Fig. 2 (A–G) shows the Van't Hoff plots at 4, 15, 26, and 37° for ADTN, piquindone, (±)-sulpiride, apomorphine, haloperidol, *cis*-flupenthixol, and (+)-butaclamol competition for [^{3}H]spiperone binding. Fig. 2H shows the Van't Hoff plot for [^{3}H]spiperone binding. ΔH° (enthalpy changes) and subsequent ΔS° (entropy changes) calculated from these plots are shown in Table 2. For the remaining drugs the slope of the Van't Hoff plot was taken as a straight line between the two points.

Apparent ΔH° values varied from +13.5 kcal mol^{-1} for (+)-butaclamol to −21.1 kcal mol^{-1} for (±)-sulpiride. ΔS° values

TABLE 1

K_i values of dopamine agonist and antagonist drugs to inhibit binding of [^{3}H]spiperone to membrane preparations of rat striatum

IC_{50} values were calculated as the concentration of drug causing displacement of 50% of specific binding (defined using 10^{-6} M (±)-sulpiride). K_i values are calculated as $K_i = IC_{50}/(1 + F/K_d)$. Results are the mean (±SE) of three separate experiments. IC_{50} values were calculated graphically from each separate experiment.

	K_i (nM)				K_i 4°/K_i 37°
	4°	15°	26°	37°	
Agonists					
1 6,7-ADTN	23 ± 5	65 ± 4	111 ± 4	657 ± 250	0.03
2 Dopamine	379 ± 72			1,999 ± 507	0.19
3 Pergolide	23 ± 1			20 ± 3	1.15
4 Apomorphine	134 ± 25	127 ± 21	129 ± 9	113 ± 43	1.19
Antagonists					
11 (±)-Sulpiride	1.3 ± 0.3	8.3 ± 1.1	21 ± 3	88 ± 1	0.01
12 Piquindone	0.7 ± 0.2	2.0 ± 0.3	7.4 ± 1.0	15 ± 2	0.04
13 Tiapride	5,700 ± 1,290			86,830 ± 8,550	0.07
14 Metoclopramide	8.3 ± 1.0			104 ± 2.1	0.08
15 Clebopride	0.19 ± 0.01			2.2 ± 0.4	0.08
16 Alizapride	7.0 ± 3.8			73 ± 28	0.10
17 (−)-Sultopride	1.1 ± 0.3			11.9 ± 0.2	0.11
18 Zetidoline	1.9 ± 0.2			17.0 ± 1.3	0.11
19 YM 09151-2	0.21 ± 0.02			0.12 ± 0.04	1.75
20 Raclopride	19 ± 7			9.5 ± 1.5	1.99
21 Haloperidol	0.70 ± 0.15	0.92 ± 0.05	1.2 ± 0.1	1.2 ± 0.1	0.59
22 Clozapine	157 ± 47			146 ± 6	1.08
23 *cis*-Flupenthixol	20 ± 6	9.3 ± 1.1	8.5 ± 2.4	9.8 ± 2.8	2.07
24 [^{3}H]Spiperone[a]	0.37 ± 0.03	0.11 ± 0.02	0.11 ± 0.02	0.13 ± 0.01	4.13
25 (+)-Butaclamol	2.0 ± 1	6.0 ± 0.4	3.6 ± 2.2	1.3 ± 0.4	15.27

[a] $K_i = K_d$.

varied from 84 cal mol K^{-1} for (+)-butaclamol to −36 cal mol K^{-1} for (±)-sulpiride. Dopamine and ADTN showed negative enthalpy and entropy changes, whereas the other agonists, apomorphine and pergolide, showed positive enthalpy and entropy changes. The dopamine antagonists haloperidol, *cis*-flupenthixol, (+)-butaclamol, and clozapine, and the ligand, [^{3}H]spiperone, showed small enthalpy changes (−3.0 to −13.5 kcal mol^{-1}) and positive entropy changes (30.7 to 84.7 cal mol^{-1} K^{-1}). The substituted benzamide antagonists, sulpiride, metoclopramide, clebopride, alizapride, sultopride, and tiapride, showed negative enthalpy and entropy changes, as did the novel dopamine antagonists piquindone and zetidoline. The substituted benzamide antagonists YM 09151-2 and raclopride showed positive enthalpy and entropy changes.

The relationship between observed entropy changes for drugs competing with [^{3}H]spiperone binding and the drug lipophilicity. Fig. 3 (A and B) shows plots of the observed entropy change for each drug (from Table 2) against the apparent lipophilicity (log k_w at pH 7.4, Fig. 3A) and the lipophilicity of the neutral species (log k_w^o, Fig. 3B). There appears to be a good correlation such that the more lipophilic the drug, the more positive is the entropy change. Linear regression analysis revealed a correlation coefficient of 0.83 (Fig. 3A) and 0.73 (Fig. 3B).

Correlation matrix of the thermodynamic and physicochemical parameters. The correlation matrix of all thermodynamic and physicochemical parameters determined in the present study is given in Table 3. The relationship between entropy change and lipophilicity is already noted above. In fact, the thermodynamic values are strongly intercorrelated and, and, as a result, lipophilicity also correlates with ΔG^o_{37} and ΔH^o. The complete correlation between ΔS^o_4 and ΔS^o_{37} indicates temperature invariance of the entropy of the binding process.

Discussion

Our results demonstrate the marked temperature dependence of most drugs investigated in competing with [^{3}H]spiperone from its binding to rat striatal preparations. Only a few compounds (pergolide, apomorphine, YM 09151-2, and clozapine) show K_i values that are almost invariant in the temperature range investigated. Use of the Cheng-Prussoff equation (15) to derive K_i values may not be entirely applicable for compounds with low Hill numbers such as dopamine and ADTN. However, it has previously been shown that the K_i values for dopamine remain constant over a wide range of [^{3}H]spiperone concentrations (6). Furthermore, the Hill number for both ADTN and sulpiride competition for [^{3}H]spiperone-binding sites decreased with decreasing temperature. This is unexpected for an antagonist such as sulpiride and necessitates further investigation.

The use of the Van't Hoff isochore is based on the assumption that ΔH is independent of T over the temperature range investigated, i.e., that $\Delta H/\Delta T$ is constant. Fig. 3 presents the Van't Hoff plots from eight compounds which were investigated at four temperatures. Globally, the linearity ranges from excellent [e.g., (±)-sulpiride, piquindone, and (+)-butaclamol] to fair (e.g., [^{3}H]spiperone and *cis*-flupenthixol). Zahniser and Molinoff (6) found that the K_d values of [^{3}H]spiperone varied linearly with temperature. Our study indicates little variation with temperature, in agreement with Leysen and Gommeren (8). Such discrepancies cannot be satisfactorily explained at this stage. From available results, we assume that Van't Hoff plots (ln K_a versus $1/T$) are linear for all compounds and, as a consequence, we determined the K_i values of 11 compounds at two temperatures only (4 and 37°).

Inspection of Table 1 reveals that K_i values either increase or decrease with temperature, excepting those compounds noted above which show little temperature dependence. These variations are indicative of differences in thermodynamic behavior. Indeed, Table 2 reveals major differences in the ΔH^o

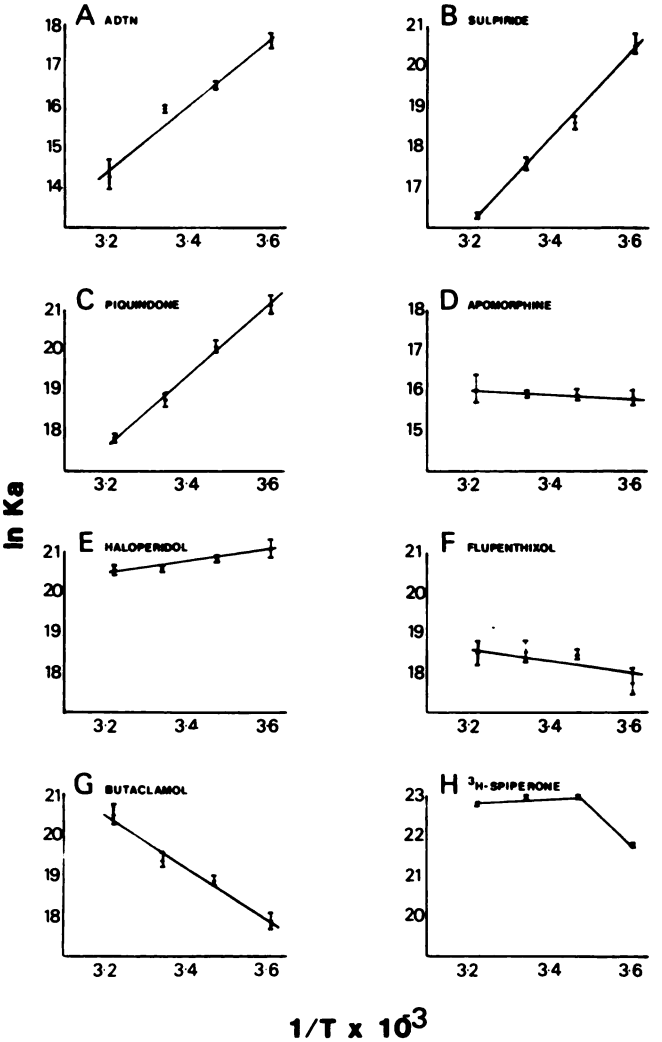

Fig. 2. Van't Hoff plots (ln K_a vs $1/T$) for ADTN (A), (+)-sulpiride (B), piquindone (C), apomorphine (D), haloperidol (E), *cis*-flupenthixol (F), (+)-butaclamol (G), and [^{3}H]spiperone (H). ln K_a (K_a being $1/K_i$) was determined at each temperature as described in Materials and Methods. *Points* represent mean (± standard error) of three separate experiments. ΔH^0 was determined from the slope of these plots.

and ΔS° values of the investigated dopamine receptor compounds. Among the agonists, dopamine and ADTN show exothermic binding which more than compensates for an energetically unfavorable decrease in entropy (enthalpy-driven binding). In contrast, the binding of pergolide and apomorphine is entropy driven. This study reveals that the binding of agonists to the dopamine D-2 receptor is not governed by the same thermodynamic driving forces for all compounds. Structural and physicochemical differences among agonists must account for much variation as discussed later.

Classical neuroleptics in Table 2 display entropy-driven binding, whereas most substituted benzamides as well as piquindone and zetidoline, but not YM 09151-2 and raclopride, exhibit enthalpy-driven binding. Substituted benzamide drugs are recognized as atypical dopamine antagonists because of their very low ability to inhibit or cause dopamine receptor-mediated behaviors (see Ref. 16) when administered peripherally. However, on focal injection these drugs are more potent than expected from 37° binding or pharmacology (17). The observation that YM 09151-2 does not behave as the other benzamide drugs is perhaps not surprising since this compound reveals itself as a typical dopamine antagonist in behavioral tests (18), e.g., induction of catalepsy and inhibition of apomorphine-induced stereotypy. Novel salicylamide analogues such as raclopride seem to differ from other benzamides in their separation between stereotypy and hyperactivity (19). Piquindone (20) and zetidoline (21) are related to the substituted benzamides by the fact that binding to the D-2 receptor is strongly Na^+ dependent (22, 23). The role played by Na^+ in the binding process is still unclear. It may, for example, facilitate binding by provoking or preventing a change in the drug-receptor complex (24). Enthalpy-driven receptor binding has been interpreted by Weiland *et al.* (1) to represent a conformational change in the receptor upon ligand binding. Thus, the net decrease in entropy would be the sum of the increase associated with binding (liberation of water molecules, see below) and the large decrease associated with conformational changes in the receptor-ligand complex. In contrast, entropy-driven binding is consistent with a receptor interaction where no information is transferred (3, 25). Klotz and Urquhart (2) had already postulated that such reactions are driven by an increase in entropy resulting from the displacement of water molecules ordered around the receptor site.

A ligand can bind to a receptor when its overall chemical structure (size, shape, electronic features) meets the necessary requirements. The molecules included in the present study vary considerably in size, from being comparatively small (dopamine), to bulky (butaclamol), to quite long (haloperidol). This would suggest differences in the binding to the receptor. However, Andrews *et al.* (26), when studying group contribution of drugs to receptor interaction, found that a significant portion of the butaclamol molecules does not interact with the dopamine receptor. This implies that only specific parts of the ligand play a role in binding and that size variation among ligands may not be a governing factor.

Lipophilicity is one of the most important factors influencing the absorption, distribution, and receptor binding of drugs. The lipophilicity of the investigated compounds was assessed in the present study by their log k_w (apparent) and log k_w° (neutral species) values. The correlation matrix in Table 3 indicates that both lipophilicity parameters are positively correlated with ΔS°. In other words, the more lipophilic the ligand, the more entropy driven its binding to the receptor. This finding contrasts with the observation of Zahniser and Molinoff (6) that entropy changes due to antagonist binding to the D-2 receptor were not related to lipophilicity. Such a conclusion, however, was based on only two drugs, namely, butaclamol and spiperone.

The relationship between ΔS° at 4° and lipophilicity is shown in Fig. 3. Besides the above-mentioned global relationship involving all ligands investigated, Fig. 3A and, especially, Fig. 3B suggest the existence of two subgroups, namely, the ligands with enthalpy-driven binding and those with entropy-driven binding. In Fig. 3B the linear relationship for the first group ($n = 9$) is 0.81; for the second group ($n = 10$) it is 0.83.

The mechanistic implications, at the receptor level, of changes in thermodynamic parameters as a function of ligand lipophilicity necessitates some discussion. The binding of a ligand to a receptor involves the formation of various electrostatic and hydrophobic bonds (ΔS° and ΔH° decrease) accompanied by the release of previously bound water molecules (ΔH°

TABLE 2

Thermodynamic parameters of the displacement of [^{3}H]spiperone from rat striatal preparations, and lipophilicity and ionization constants of the compounds

		Temperature	ΔG° (kcal·mol^{-1})	ΔH° (kcal·mol^{-1})	ΔS° (cal·K^{-1} mol^{-1})	log k_w[a] (pH 7.5)	log k_w[e]	pK_a[b]
	Agonists							
1	6,7-ADTN	4	−9.7	−16.4	−24.2	−0.42	1.5	9.4
		15	−9.5		−24.0			
		26	−9.5		−23.0			
		37	−8.8		−24.6			
2	Dopamine	4	−8.2	−8.7	−1.8	−0.81	0.68	8.9[c,d]
		37	−8.1		−1.8			
3	Pergolide	4	−9.7	0.7	37.4	3.5	3.6	7.1[e]
		37	−10.9		37.4			
4	Apomorphine	4	−8.7	0.8	34.4	3	2.4	8.9/7.2[c]
		15	−9.1		34.4			
		26	−9.4		34.2			
		37	−9.9		34.4			
	Antagonists							
11	(±)-Sulpiride	4	−11.3	−21.2	−35.7	0.61	2.2	9.1
		15	−10.7		−36.5			
		26	−10.5		−35.6			
		37	−10.0		−36.0			
12	Piquindone	4	−11.6	−17.4	−20.9	1.4	1.9	7.9[f]
		15	−11.5		−20.5			
		26	−11.1		−20.9			
		37	−11.0		−20.7			
13	Tiapride	4	−6.7	−14.2	−27.0	−0.54	1.1	9.1
		37	−5.8		−27.0			
14	Metoclopramide	4	−10.3	−13.1	−10.3	1.1	2.8	9.3
		37	−9.9		−10.3			
15	Clebopride	4	−12.4	−12.8	−1.4	2.9	3.6	8.2
		37	−12.3		−1.4			
16	Alizapride	4	−10.4	−12.2	−6.6	2.5	3.7	8.7/7.4
		37	−10.1		−6.6			
17	(−)-Sultopride	4	−11.4	−12.2	−3.0	0.77	2.7	9.4
		37	−11.3		−3.0			
18	Zetidoline	4	−11.1	−11.5	−1.3	2.1	3.9	9.2
		37	−11.0		−1.3			
19	YM 09151-2	4	−12.3	2.9	54.8	3.5	4.0	7.8
		37	−14.1		54.8			
20	Raclopride	4	−9.8	3.6	48.3	2.4	2.5	6.7[g]
		37	−11.4		48.3		3.1	8.2[h]
21	Haloperidol	4	−11.6	−3.0	31.0	3.1	4.3	8.7[c]
		15	−11.9		30.9			
		26	−12.2		30.7			
		37	−12.7		31.1			
22	Clozapine	4	−8.6	0.4	32.5	3.0	3.3	7.5
		37	−9.7		32.5			
23	*cis*-Flupenthixol	4	−9.8	3.6	48.4	4.4	4.9	7.8[f]
		15	−10.6		49.4			
		26	−11.1		49.2			
		37	−11.4		48.5			
24	[^{3}H]Spiperone	4	−12.0	5.2	61.8	3.3	4.9	9.1[i]
		15	−13.2		63.6		4.2	8.3[c]
		26	−13.7		63.0			
		37	−14.1		62.0			
25	(+)-Butaclamol	4	−9.8	13.5	84.2	5.0	5.0	5.9[j]
		15	−10.9		84.7		5.2	7.2[k]
		26	−11.6		84.0		5.5	7.9[c]
		37	−12.6		84.4			

[a] High performance liquid chromatography lipophilicity measurements were performed at 22 ± 2°.
[b] pK_a values are at 25°.
[c] J. P. Tollenaere, personal communication.
[d] Pomona files (C. Hansch and A. Leo); pK_a dopamine = 8.81, 8.88, 8.93 (27).
[e] Ionization constant in 30% MeOH/70% H_2O, not corrected.
[f] G. L. Olson, personal communication.
[g] T. de Paulis, personal communication.
[h] Estimated value.
[i] Ref. 30.
[j] Ref. 28.
[k] Ref. 29.

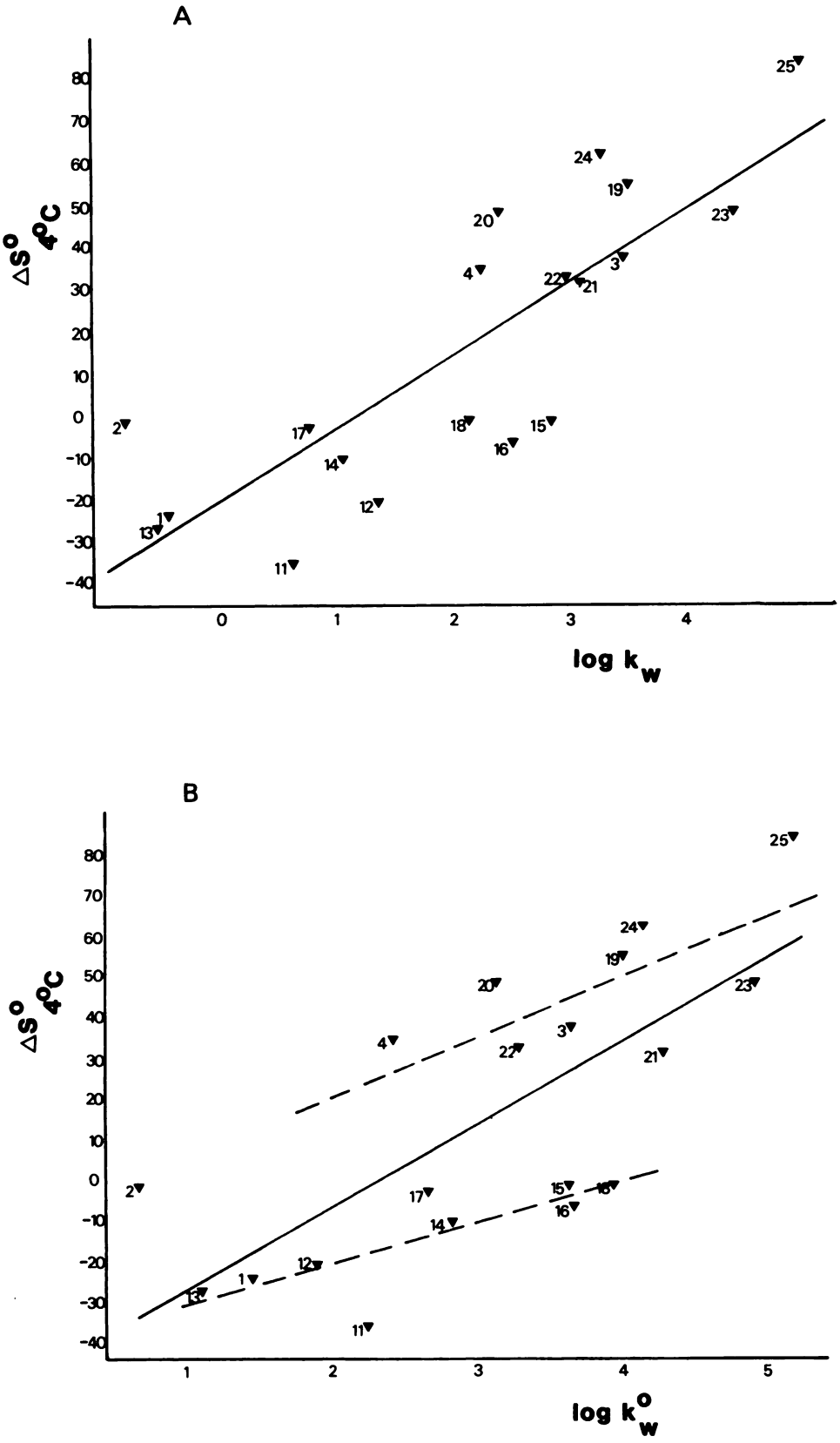

Fig. 3. Correlation between apparent (A) and true or neutral (B) drug lipophilicity and observed entropy change (ΔS^{o} at 4°C). Drugs are numbered according to Tables 1 and 2. Log *k* was determined by reverse phase high performance liquid chromatography as explained. The correlation coefficients are 0.83 (A) and 0.73 (B). B also indicates that separate relationships can be seen for the two subgroups of ligands displaying enthalpy-driven ($n = 9$; $r = 0.81$) and entropy-driven ($n = 10$; $r = 0.83$) binding, respectively.

and ΔS^{o} increase). Conformational changes following the binding process may involve the formation of additional bonds (ΔH^{o} decreases) or the liberation of additional water molecules (ΔS^{o} increases). It is commonly stated (see, for example, Ref. 31) that entropy changes are larger for antagonists than for agonists due to conformational changes induced by the latter. In contrast, we demonstrate that entropy changes are, in the first place, related to ligand lipophilicity which is itself related to size. The positive correlation between lipophilicity and ΔS^{o} observed in the present series of compounds suggests that the

TABLE 3

Correlation matrix of thermodynamic and physicochemical parameters

All values were obtained experimentally (log k_w, pK_a, K_i, pK_i) or calculated (log K_w^o, ΔH, ΔS, ΔG) as described in the text. Subscripts 4 or 37 refer to the temperature at which measurements were made.

log k_w	0.94										
pK_a	−0.46	−0.73									
ΔG_{37}	−0.78	−0.75	0.37								
ΔG_4	−0.46	−0.35	−0.06	0.81							
ΔH	0.66	0.77	−0.73	−0.52	0.07						
ΔS_{37}	0.73	0.83	−0.72	−0.65	−0.09	0.99					
ΔS_4	0.73	0.83	−0.72	−0.65	−0.09	0.99	0.999				
K_{i4}	−0.42	−0.41	0.23	0.64	0.61	−0.20	−0.30	−0.30			
K_{i37}	−0.40	−0.40	0.24	0.62	0.58	−0.21	−0.30	−0.30	0.998		
pK_{i4}	0.48	0.36	0.07	−0.82	−0.998	−0.05	0.11	0.11	−0.62	−0.59	
pK_{i37}	0.78	0.76	−0.37	−0.99	−0.87	0.51	0.64	0.64	−0.65	−0.63	0.82
	log k_w^o	log k_w	pK_a	ΔG_{37}	ΔG_4	ΔH	ΔS_{37}	ΔS_4	K_{i4}	K_{i37}	pK_{i4}

binding of bulky, lipophilic drugs is accompanied by a marked dehydration of receptor and/or ligand, post-binding conformational changes also being involved. However, such conformational changes, if occurring at all, must also be induced by a range of antagonists and cannot be those believed to transfer information following agonist binding. This information must thus occur at a stage energetically distinct from the binding process, and independent from thermodynamic characteristics of the latter.

The present study offers perhaps a clue discriminating atypical neuroleptics from classical neuroleptics. The sodium-dependent and enthalpy-driven binding of the former could imply that they induce a conformational change in the receptor complex.

In conclusion, the results show marked variation in the temperature dependency of drugs competing with [^{3}H]spiperone binding to striatal preparations. Substituted benzamide analogues resemble the agonists ADTN and dopamine in their mode of binding, whereas lipophilic agonists and antagonists share entropy-driven binding. Thus, it is clear that the thermodynamic parameters ΔS^o and ΔH^o do not distinguish between the interaction of agonist and antagonists at the dopamine D-2 receptor. These parameters appear to be related to drug lipophilicity.

References

1. Weiland, G. A., J. P. Minneman, and P. B. Molinoff. Fundamental diffrences between the molecular interactions of agonists and antagonists with the β-adrenergic receptor. *Nature (Lond.)* **281**:114–117 (1979).
2. Klotz, I. M., and J. M. Urquhart. The binding of organic ions by proteins. Effect of temperature. *J. Am. Chem. Soc.* **71**:847–851 (1949).
3. Singer, S. J., and D. H. Campbell. Physical chemical studies of soluble antigen-antibody complexes. V. Thermodynamics of the reaction between ovalbumin and its rabbit antibodies. *J. Am. Chem. Soc.* **77**:4851–4855 (1955).
4. Weiland, G. A., K. P. Minneman, and P. B. Molinoff. Thermodynamics of agonist and antagonist interactions with mammalian β-adrenergic receptors. *Mol. Pharmacol.* **18**:341–347 (1980).
5. Maelicke, A., B. W. Fulpius, R. P. Klett, and E. Reich. Acetylcholine receptor responses to drug binding. *J. Biol. Chem.* **252**:4811–4830 (1977).
6. Zahniser, N. R., and P. B. Molinoff. Thermodynamic differences between agonist and antagonist interactions with binding sites for [^{3}H]spiroperidol in rat striatum. *Mol. Pharmacol.* **23**:303–309 (1983).
7. Niehoff, D. L., J. M. Palacios, and M. J. Kuhar. Dopamine receptors: temperature effects on (^{3}H)spiperone and (^{3}H)*N*-*n*-propyl-norapomorphine binding. *Soc. Neurosci. Abstr.* **6**:252 (1980).
8. Leysen, J. E., and W. Gommeren. Optimal conditions for (^{3}H)apomorphine binding and anomalous equilibrium binding of (^{3}H)apomorphine and (^{3}H)spiperone to rat striatal membranes: involvement of surface phenomena versus multiple binding sites. *J. Neurochem.* **36**:201–219 (1981).
9. Kilpatrick, G. J., P. Jenner, and C. D. Marsden. Properties of rat striatal D-2 dopamine receptors solubilised with the Zwitterionic detergent CHAPS. *J. Pharm. Pharmacol.* **37**:320–328 (1985).
10. Sokalowski, A., and K.-G. Wahlung. Peak tailing and retention behavior of tricyclic antidepressant amines and related hydrophilic ammonium compounds in RP-ion-pair liquid chromatography on alkyl bonded passes. *J. Chromatogr.* **189**:299–316 (1980).
11. Unger, S. H., and G. H. Chiang. Octanol-physiological buffer distribution coefficients of lipophilic amines by reversed-phase HPLC and their correlation with biological activity. *J. Med. Chem.* **24**:262–270 (1981).
12. Fekete, J., P. Del Castilho, and J. C. Kraak. Reversed-phase liquid chromatograph for separation of chlorpromazine, imipramine and some of their metabolites. *J. Chromatogr.* **204**:319–327 (1981).
13. El Tayar, N., H. Van de Waterbeemd, and B. Testa. Lipophilicity measurements of protonated basic compounds by reversed phase high performance liquid chromatography. I. Relationship between capacity factors and the methanol concentration in methanol/water eluents. *J. Chromatogr.* **320**:293–304 (1985).
14. El Tayar, N., H. Van de Waterbeemd, and B. Testa. Lipophilicity measurements of protonated basic compounds by reversed phase high performance liquid chromatography. II. Procedure for the determination of a lipophilic index measured by RP-HPLC. *J. Chromatogr.* **320**:305–312 (1985).
15. Cheng, Y.-C., and W. H. Prussof. Relationship between the inhibition constant and the concentration of inhibitor which causes 50 percent inhibition of an enzymatic reaction. *Biochem. Pharmacol.* **22**:3099–3108 (1973).
16. Jenner, P., and C. D. Marsden. Multiple receptors in brain and pharmacological action of substituted benzamide drugs. *Acta Psychiatr. Scand.* (Benzamine supplement). **69**:109–123 (1984).
17. Costall, B., D. H. Fortune, and R. J. Naylor. Differential activities of some benzamide derivatives on peripheral and intracerebral administration. *J. Pharm. Pharmacol.* **30**:796–798 (1978).
18. Usuda, S., K. Nishikori, O. Noshiro, and H. Maeno. Neuroleptic properties of *cis*-*N*-(1-benzyl-2-methylpyrrolidin-3yl)-5-chloro-2-methoxy-9-methylaminobenzamide (YM 09151-2) with selective anti-dopaminergic activity. *Psychopharmacology* **73**:103–109 (1981).
19. De Paulis, T. Substituted benzamides. The development of dopamine antagonists, in *Proceedings from the VIIIth International Symposium on Medicinal Chemistry* (R. Dahlbom and J. L. C. Nilsson, eds.). Swedish Pharmaceutical Press, 405–425 (1985).
20. Nakajima, T., and K. Iwata. [^{3}H]Ro-22-1319 (piquindone) binds to the D-2 dopaminergic receptor subtype in a sodium-dependent manner. *Mol. Pharmacol.* **26**: 430–438 (1984).
21. Barone, O., A. Assandin, G. Galliani, A. Glasser, and C. Tarzia. Characterization of (^{3}H)zetidoline binding to rat striatal membranes. *J. Pharm. Pharmacol.* **37**:180–187 (1985).
22. Theodorou, A. E., M. D. Hall, P. Jenner, and C. D. Marsden. Cation regulation differentiates specific binding of (^{3}H)sulpiride and (^{3}H)spiperone to rat striatal preparations. *J. Pharm. Pharmacol.* **32**:441–444 (1980).
23. Stefanini, E., A. M. Marchisio, P. Devoto, F. Vernaleone, R. Collu, and P. F. Spano. Sodium-dependent interaction of benzamides with dopamine receptors. *Brain Res.* **198**:229–233 (1980).
24. Woodruff, G. N., and S. B. Freedman. Sulpiride binding in rat striatum—effect of dopamine agonists and sulphydryl group reagents. *Acta Pharm. Suec. Suppl.* **1**:118–129 (1983).
25. Minneman, K. P., G. A. Weiland, and P. B. Molinoff. A comparison of the β-adrenergic receptor of the turkey erythrocyte with β_1 and β_2 receptors. *Mol. Pharmacol.* **17**:1–7 (1980).
26. Andrews, P. R., D. J. Criak, and J. L. Martin. Functional group contributions to drug-receptor interactions. *J. Med. Chem.* **27**:1648–1657 (1984).
27. Pomona Files. Log P and parameter database: a tool for the quantitative prediction of bio-activity. *Pomona College Medicinal Chemistry Project* (C. Hansch, chairman, A. Leo, director), Comptex Scientific Corporation, New York (1983).
28. Philipp, A. H., L. G. Humber, and K. Voith. Mapping the dopamine receptor. 2. Features derived from modifications in the rings A/B region of the neuroleptic butaclamol *J. Med. Chem.* **22**:768–773 (1979).
29. Chrzanowski, F. A., B. A. McGrogan, and B. E. Marynoff. The pKa of butaclamol and the mode of butaclamol binding to central dopamine receptors. *J. Med. Chem.* **28**:399–400 (1985).

30. Tollenaere, J. P., H. Moereels, and M. H. J. Koch. On the conformation of neuroleptic drugs in the three aggregation states and their conformational resemblance to dopamine. *Eur. J. Med.* **12**:199–211 (1977).
31. Closse, A., W. Frick, R. Markstein, R. Maurer, and R. Nordmann. ^{3}H-205-501, a non-catechol dopaminergic agonist, labels selectively and with high affinity dopamine D-2 receptors. *J. Neural Transm.* **62**:231–248 (1985).

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