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Thermodynamic Differences between Agonist and Antagonist Interactions with Binding Sites for [^{3}H]Spiroperidol in Rat Striatum

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SUMMARY

The characteristics of the binding of the dopamine receptor antagonist [^{3}H]spiroperidol to rat striatal membranes were examined at six different incubation temperatures ranging from 1° to 37°. Although the number of receptors labeled at each temperature was identical, the affinity of the receptor for [^{3}H]spiroperidol decreased 10-fold as the incubation temperature was lowered from 37° to 1°. The binding of [^{3}H]spiroperidol was entropy-driven ($\Delta S°$ = +80 cal/mole-deg), endothermic ($\Delta H°$ = +10 kcal/mole), and exergonic ($\Delta G°$ = −13 kcal/mole). Qualitatively similar results were found for (+)-butaclamol, another dopamine receptor antagonist. The binding of the agonists dopamine and (±)-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene to sites labeled by [^{3}H]spiroperidol in the striatum also appeared to be entropy-driven ($\Delta S°$ = +35 cal/mole-deg). In contrast to the results obtained in studies with antagonists, however, the affinity of the receptor for agonists was independent of the incubation temperature between 8° and 37°. Competition curves for the inhibition of [^{3}H]spiroperidol binding by agonists became increasingly complex as the incubation temperature was lowered. The addition of GTP reduced the affinity of the receptor for agonists at all temperatures but did not simplify interpretation of these complex curves. At 1° there was a decrease in the affinity of the receptor for dopamine, and the effect of GTP was abolished.

INTRODUCTION

Structurally similar agonists and antagonists are thought to occupy similar binding sites on a particular receptor. However, only agonist occupation is transduced into a physiological response. This observation has led to the suggestion that the interactions of agonists and antagonists with a receptor must be fundamentally different. Determination of the thermodynamic parameters associated with the binding of agonists and antagonists to *beta*-adrenergic receptors has provided some insight into the molecular events which accompany receptor activation (1, 2). The binding of antagonists to *beta*-adrenergic receptors is entropy-driven; in contrast, the binding of agonists is associated with decreases in both enthalpy and entropy (1, 2). The difference in the energetics of binding of agonists and antagonists to *beta*-adrenergic receptors suggests that agonists can produce a conformational change in, and thus activation of, the receptor. It is impossible, however, to generalize from one hormone or neurotransmitter system to another with respect to specific thermodynamic changes associated with ligand-receptor interactions (3).

The molecular mechanisms underlying the interactions of agonists and antagonists with dopamine receptors are not well understood. In the present studies the thermodynamics of such interactions have been investigated. [^{3}H]Spiroperidol, a butyrophenone antagonist which has been shown to bind with high affinity to striatal dopamine receptors (4, 5), was used in these studies. The binding of [^{3}H]spiroperidol and the inhibition of [^{3}H]spiroperidol binding by dopamine receptor agonists and antagonists were determined over a range of incubation temperatures from 1° to 37°. The results of these studies demonstrate striking differences between the thermodynamic parameters associated with ligand-receptor interactions in the *beta*-adrenergic system and in the dopamine system.

EXPERIMENTAL PROCEDURES

Materials. The sources of the drugs used were as follows: 1-[*phenyl*-4-^{3}H]spiroperidol from New England Nuclear Corporation (Boston, Mass.); dopamine from Sigma Chemical Company (St. Louis, Mo.); (±)-ADTN[2] from Burroughs Wellcome Company (Research Triangle Park, N. C.); (+)-butaclamol from Ayerst Laboratories (Montreal, Canada); GTP from Boehringer-Mannheim

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[2] The abbreviations used are: ADTN, amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; BSA, bovine serum albumin; NPA, *N-n*-propylnorapomorphine.

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(Indianapolis, Ind.). All other chemicals used were reagent-grade. Incubation temperatures were maintained using Thermomix circulators (Model 1441, Braun) which have a temperature range of −30° to +150° ± 0.01°.

Binding assays. The number and characteristics of striatal dopamine receptors were determined using the antagonist [^{3}H]spiroperidol (6). Striata from male Sprague-Dawley rats (180–200 g) were homogenized in 300 volumes of 20 mM Hepes buffer (pH 7.5, adjusted with tetramethylammonium hydroxide at 25°) for 10 sec at speed 4.5 with a Brinkmann Polytron. Membrane pellets, prepared by centrifugation at 20,000 × *g* for 10 min at 4°, were washed by resuspension and centrifugation. Radioligand and drugs were dissolved in 2.8 mM ascorbic acid containing BSA (10 μg/ml). Under the present experimental conditions the final concentration of ascorbic acid (0.28 mM) did not affect the K_d values or the density of binding sites for [^{3}H]spiroperidol (Table 1). The concentrations of [^{3}H]spiroperidol (26 Ci/mmole) used for Scatchard analysis ranged from 0.05 to 0.75 nM; 0.6 nM was used for concentration-inhibition curves. Nonspecific binding of [^{3}H]spiroperidol was defined in the presence of 3 μM (+)-butaclamol at 37°, 30°, and 25°, in the presence of 10 μM (+)-butaclamol at 17° and 8° and in the presence of 30 μM (+)-butaclamol at 1°. The concentration of (+)-butaclamol was increased at lower temperatures to compensate for a decrease in the affinity of the receptor for (+)-butaclamol (see Results). Specific binding of [^{3}H]spiroperidol constituted 65–85% of total binding.

Assays were initiated by addition of 900-μl aliquots of membranes resuspended in 20 mM Hepes buffer (0.10–0.15 mg of protein) to tubes containing 50 μl of [^{3}H] spiroperidol and 50 μl of ascorbate-BSA or appropriate drug. When GTP was included in the assays, 850-μl aliquots of tissue were added to 150 μl of ligand and drugs. Samples were incubated for 20 min at 37°, 30 min at 30°, 45 min at 25°, 60 min at 17°, 90 min at 8°, and 120 min at 1° (see Results). Equilibrium was reached using these incubation times even in the presence of a competing drug. Reactions were terminated by the addition of 10 ml of ice-cold 10 mM Tris buffer (pH 7.5), and samples were immediately filtered through glass-fiber filters (Schleicher and Schuell, No. 30). Each filter was washed with an additional 10 ml of cold Tris buffer. Radioactivity was determined by liquid scintillation spectrometry in 3 ml of a Triton X-100/toluene-based fluor. The counting efficiency for tritium was approximately 32%.

Scatchard analysis. Saturation curves were constructed using six to nine final concentrations of [^{3}H] spiroperidol ranging from 0.05 nM to 0.75 nM. Definition of specific binding of [^{3}H]spiroperidol and times of incubation depended on the temperatures of incubation as explained above. The saturation cuves for specifically bound radioligand were transformed by the method of Scatchard (7) to determine the density of binding sites (B_{max}) and equilibrium dissociation constants (K_d values). Scatchard plots were linear, and the straight lines which best fit the data points were determined by linear regression. Correlation coefficients calculated for these lines ranged from −0.777 to −0.998 but were generally greater than −0.875.

Competition curves. Seventeen concentrations of each drug (0.1 nM–1 mM) were examined for their ability to inhibit the total binding of 0.6 nM [^{3}H]spiroperidol. Total binding was measured in the presence of vehicle. The IC_{50} values and Hill coefficients for the drugs were obtained from the concentration-inhibition curves using the method of Hill (8). K_d values were calculated from the IC_{50} values using the Cheng and Prusoff correction (ref. 9; see also ref. 10).

Thermodynamic parameters. Thermodynamic parameters were determined according to the procedure outlined by Weiland *et al.* (2). The following classical thermodynamic relationships were employed: (a) $\Delta G° = -RT \ln K_a$, where $\Delta G°$ is the Gibbs free energy change in kilocalories per mole, R is the gas constant (1.99 cal/mole-deg), T is the temperature in degrees Kelvin, and K_a is the equilibrium association constant ($1/K_d$). (b) $\Delta H°$ was obtained from the slope of the van't Hoff plot ($-\Delta H°/R$), where $\Delta H°$ is the enthalpy change in kilocalories per mole. (c) $\Delta G° = \Delta H° - T\Delta S°$, where $\Delta S°$ is the standard entropy change in calories per mole-degree.

Protein determinations. Protein concentrations were determined according the the dyebinding method of Bradford (11), using BSA as a standard.

TABLE 1

Effect of ascorbic acid on the binding of [^{3}H]spiroperidol to rat striatal membranes

Homogenates of striatal membranes in 20 mM Hepes buffer (pH 7.5) were incubated at 37° with concentrations of [^{3}H]spiroperidol ranging from 0.05 nM to 0.75 nM and ascorbic acid ranging from 0 to 0.56 mM. The details of the assay are given under Experimental Procedures. The affinity (K_d value) and number of binding sites (B_{max}) for [^{3}H]spiroperidol were determined by Scatchard analysis (7). The values shown are the means ± standard error of the mean for three independent determinations.

Final concentration of ascorbic acid	[^{3}H]Spiroperidol	
	K_d value	B_{max}
mM	*pM*	*fmoles/mg protein*
0	36 ± 5.8	580 ± 64
0.14	32 ± 3.6	540 ± 51
0.28	35 ± 2.7	570 ± 54
0.56	41 ± 3.7	590 ± 53

RESULTS

Time course. The time course of association of [^{3}H] spiroperidol to rat striatal membranes was examined as a function of incubation temperature (Fig. 1). This experiment was carried out using a concentration of [^{3}H] spiroperidol (150 pM) in the lower range of concentrations used for Scatchard analysis. From these results the following times of incubation were chosen for measuring equilibrium binding: 20 min at 37°, 30 min at 30°, 45 min at 25°, 60 min at 17°, 90 min at 8°, and 120 min at 1°.

Definition of specific binding. The affinity of striatal dopamine receptors for antagonists decreased as the temperature of incubation was lowered. Since both [^{3}H]spiroperidol (the radioligand used) and (+)-butaclamol (the compound used to define specific binding) are antagonists, the concentration of (+)-butaclamol used to define specific [^{3}H]spiroperidol binding had to be evaluated at each incubation temperature. The inhibition of [^{3}H]spi-

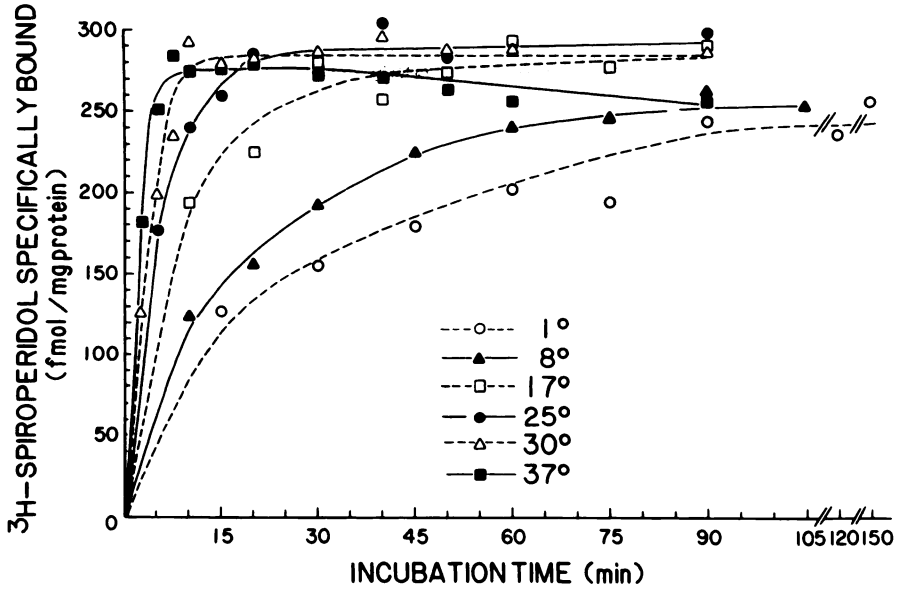

FIG. 1. *Time course of [^{3}H]spiroperidol binding to rat striatal membranes as a function of temperature*

Homogenates of washed striatal membranes were incubated in 20 mM Hepes buffer (pH 7.5) with a low concentration of [^{3}H]spiroperidol (0.15 nM) for the times indicated. Assays were performed as explained under Experimental Procedures. The values shown are means of three independent determinations.

roperidol binding by (+)-butaclamol was examined at each of the six temperatures used (Fig. 2). As the temperature was lowered, the affinity of the receptor decreased to a greater extent for (+)-butaclamol than for [^{3}H]spiroperidol (see Fig. 4). From these results it was evident that at lower temperatures the plateau in the dose-response curve occurred at higher concentrations of (+)-butaclamol. Thus, at temperatures from 37° to 25°, specific binding of [^{3}H]spiroperidol was defined as the difference in total binding in the absence and presence of 3 μM (+)-butaclamol. At 17° and 8° it was defined using 10 μM (+)-butaclamol, and at 1° it was defined using 30 μM (+)-butaclamol.

Calculation of K_d values for inhibition of [^{3}H]spiroperidol binding. Hill coefficients for the inhibition of [^{3}H]spiroperidol binding by either competing agonists or antagonists, in most cases, are significantly less than 1 (6). Since these interactions do not appear to follow simple mass-action principles, it was necessary to validate the use of the Cheng and Prusoff correction (9) to convert IC_{50} values to K_d values (2, 12, 13).

Dose-response curves for dopamine were generated at

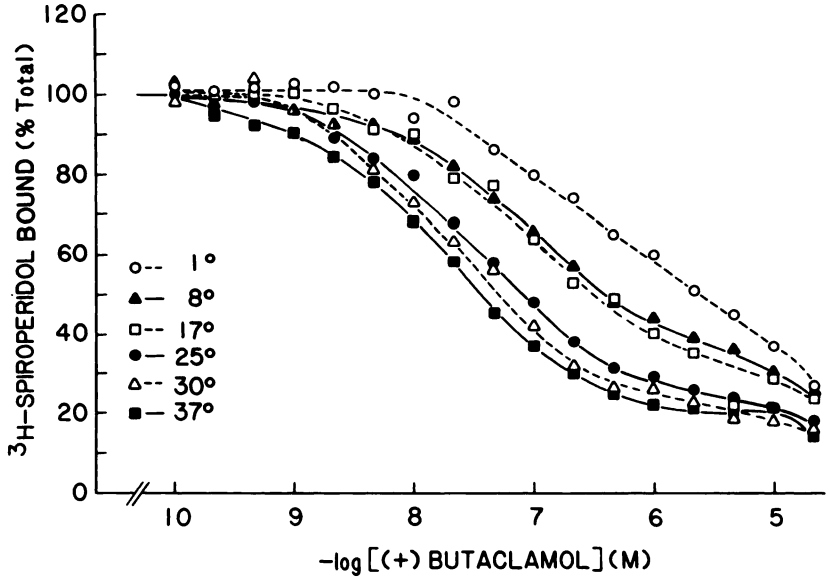

FIG. 2. *Inhibition of [^{3}H]spiroperidol binding to homogenates of washed rat striatal membranes by (+)-butaclamol at different temperatures*

Details of the assay procedure are given under Experimental Procedures. The values shown are expressed as a percentage of the total [^{3}H] spiroperidol binding measured in the absence of (+)-butaclamol and are means of six independent determinations. The lines connecting the data points were drawn by eye.

different concentrations of [^{3}H]spiroperidol. While IC_{50} values for dopamine increased with increasing concentrations of [^{3}H]spiroperidol, the K_d values for dopamine calculated by the Cheng and Prusoff equation (9) were independent of the concentration of [^{3}H]spiroperidol employed (Fig. 3). Thus, it appeared valid to use this method of correction. It should also be noted that the extrapolated value for the IC_{50}, when the concentration of [^{3}H] spiroperidol approaches zero, is equal to the K_d value (Fig. 3). The value calculated when the Hill coefficient is significantly less than 1.0 is a $K_{0.5}$ value rather than a true K_d value. However, it still reflects the concentration of drug that saturates one-half of the binding sites (see ref. 2). Since the Hill coefficients for both antagonists (Fig. 2) and agonists (Fig. 3) were less than 1, it is possible that [^{3}H]spiroperidol was binding to more than a single population of sites. Thus, the K_d values may represent a composite value for two populations of [^{3}H]spiroperidol binding sites. When these same data were replotted as Scatchard plots of [^{3}H]spiroperidol binding in the presence of increasing concentrations of dopamine, the results again suggested that the interaction between [^{3}H] spiroperidol and dopamine was competitive. That is, the K_d values for [^{3}H]spiroperidol increased with increasing concentrations of dopamine whereas the B_{max} values were unaffected.

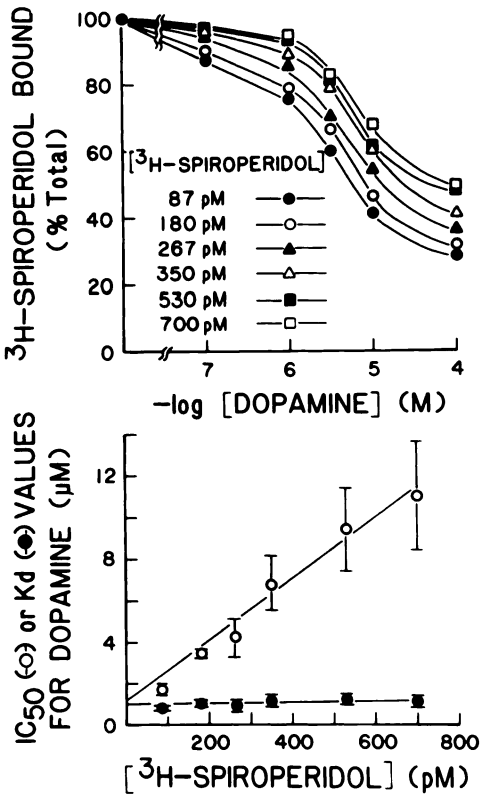

FIG. 3. *Determination of the affinity of striatal dopamine receptors for dopamine measured in the presence of varying concentrations of [^{3}H]spiroperidol*

Top, Dose-response curves for the inhibition of [^{3}H]spiroperidol binding by dopamine were generated at 37° in the presence of the indicated concentrations of [^{3}H]spiroperidol. Assays were performed as described under Experimental Procedures. Bottom, IC_{50} values for dopamine were determined by the method of Hill (8) from the dose-response curves shown above. The IC_{50} values were transformed into K_d values using the Cheng and Prusoff correction (9). The K_d value (53 pM), used for [^{3}H]spiroperidol in this calculation, was that determined by Scatchard analysis (7) at 37° in the absence of dopamine. The values shown are means ± standard error of the mean for three independent determinations.

Thermodynamic analysis of antagonist binding. As the incubation temperature was lowered from 37° to 1°, there was a progressive 10-fold decrease in the affinity of the receptor for [^{3}H]spiroperidol (Table 2). In contrast, the number of receptors labeled by [^{3}H]spiroperidol was unaffected by changing the temperature (Table 2). Scatchard plots were linear at all temperatures, suggesting that only a single class of high-affinity sites was labeled by [^{3}H]spiroperidol.

Data from the (+)-butaclamol dose-response curves (Fig. 2) and the [^{3}H]spiroperidol Scatchard analysis (Table 2) were used to construct van't Hoff plots (Fig. 4). These plots were linear over the range of temperatures examined. The thermodynamic parameters for these two antagonists were then calculated. Although the slope of the plot for (+)-butaclamol was greater than that for [^{3}H] spiroperidol, there was good qualitative agreement between the parameters calculated for the two antagonists (Table 3). The large positive entropy change compensated for the thermodynamically unfavorable positive enthalpy change. The binding reactions revealed a net loss in free energy.

Thermodynamic analysis of agonist binding. Analysis of inhibition of [^{3}H]spiroperidol binding by the agonists dopamine and (±)-ADTN was carried out over the same range of incubation temperatures. In contrast to the results of studies with antagonists, the affinity of the receptor for dopamine was independent of incubation temperature from 37° to 8°. At 1°, however, there was a 5-fold decrease in the apparent affinity of the receptor for dopamine (Table 4). Similar results were found for (±)-ADTN (Table 4). Thus the binding of agonists was entropy-driven, and no change in enthalpy was observed at temperatures from 37° to 8° (Fig. 4; Table 3). At temperatures below 25°, the dose-response curves for dopamine became obviously biphasic (Fig. 5); the same phenomenon was observed for (±)-ADTN. To test

TABLE 2

Effect of temperature on the binding of [^{3}H]spiroperidol to rat striatal membranes

Homogenates of washed striatal membranes were incubated at various temperatures in 20 mM Hepes buffer (pH 7.5) with concentrations of [^{3}H]spiroperidol ranging from 0.05 nM to 0.75 nM. The details of the assay are given under Experimental Procedures. The affinity (K_d value) of the receptor and the number of binding sites (B_{max}) for [^{3}H]spiroperidol were determined by Scatchard analysis (7). The values shown are the means ± standard error of the mean for 6–12 independent determinations.

Temperature	K_d value	B_{max}
	pM	*fmoles/mg protein*
37°	53 ± 5.3	570 ± 26
30	82 ± 5.3	600 ± 18
25	120 ± 12	600 ± 24
17	200 ± 15	560 ± 18
8	270 ± 26	570 ± 33
1	510 ± 57	650 ± 82

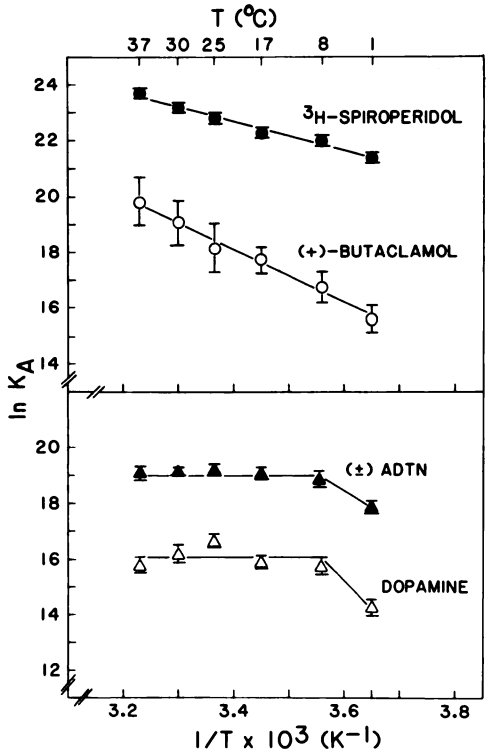

FIG. 4. *Van't Hoff plots for dopamine receptor antagonists and agonists*

The K_a values for [^{3}H]spiroperidol were derived from the K_d values obtained from Scatchard analysis (Table 2). The K_a values for (+)-butaclamol, dopamine, and (±)-ADTN were derived from the K_d values calculated using the Cheng and Prusoff correction (9) on the IC_{50} values obtained from displacement curves and Hill analysis (8). The data shown are means ± standard error of the mean for 4–12 independent determinations.

whether the addition of GTP would affect one component of these complex agonist inhibition curves, dose-response curves to dopamine were carried out at various temperatures in the presence of 300 μM GTP. In the presence of GTP, the affinity of the receptor for dopamine decreased by 5- to 10-fold at all temperatures with the exception of 1° (Fig. 5). The GTP shift in agonist affinity was abolished at 1°. Even in the presence of GTP, however, the Hill coefficients were less than unity (results not shown).

DISCUSSION

The results of the present study demonstrate that the binding of two antagonists to putative dopamine receptors on rat striatal membranes is entropy-driven. It should be noted that the two antagonists examined, spiroperidol and (+)-butaclamol, are structurally dissimilar. Furthermore, the thermodynamic parameters were derived both from direct studies, using Scatchard analysis in the case of [^{3}H]spiroperidol, and from indirect studies using displacement curves in the case of (+)-butaclamol. However, it is impossible to know whether these findings are generally applicable and whether the energetics of the binding of other dopamine receptor antagonists would be qualitatively similar to those described here.

The conclusion that antagonist binding to striatal dopamine receptors is entropy-driven agrees with that published previously by Niehoff and co-workers (15). The magnitude of the increase in entropy (80–100 cal/mole-deg) associated with the binding of the two antagonists studied here is relatively large. However, it is less than that reported for the binding of both α-neurotoxin and low molecular weight agonists to the nicotinic cholinergic receptor [$\Delta S°$ = 99–129 entropy units/mole of ligand (16)]. The adsorption of another neuroleptic, chlorpromazine, to membranes has been shown to be entropy-driven (17). A number of other protein binding reactions, especially those in which no information is transferred, have also been shown to be entropy-driven (1, 2, 18, 19, 20). In these cases the observed increase in entropy is thought to result from the displacement of ordered water molecules from around the radioligand and the receptor. Changes in thermodynamic parameters of the magnitude seen in the present study are consistent with there being substantial modifications in the environment of the receptor promoted by the binding of the ligand to the receptor. The energy for the binding of both spiroperidol and (+)-butaclamol is probably derived primarily from hydrophobic interactions, since both compounds are relatively lipophilic. However, the value of $\Delta S°$ does not

TABLE 3

Thermodynamic parameters calculated from van't Hoff plots for antagonists and agonists of striatal dopamine receptors

The thermodynamic parameters were calculated using the equations shown under Experimental Procedures. The values for $\Delta G°$ and $\Delta S°$ were calculated at each temperature from 37°–1° for the antagonists and from 37°–8° for the agonists. The mean values ± standard error of the mean are shown for 4–12 independent determinations. $\Delta H°$ was derived from the van't Hoff plots shown in Fig. 4.

	$\Delta G°$	$\Delta H°$	$\Delta S°$
	kcal/mole	*kcal/mole*	*cal/mole-deg*
Antagonists			
[^{3}H]Spiroperidol	−13 ± 0.12	+10 ± 0.40	+80 ± 0.07
(+)-Butaclamol	−11 ± 0.29	+20 ± 1.6	+100 ± 0.38
Agonists			
Dopamine	−9.5 ± 0.11	0	+32 ± 0.28
(±)-ADTN	−11 ± 0.14	0	+38 ± 0.21

TABLE 4

Affinity of striatal dopamine receptors for agonists as a function of temperature

Rat striatal membranes were incubated with 0.6 nM [^{3}H]spiroperidol and various concentrations of dopamine or (±)-ADTN as described under Experimental Procedures. K_d values were calculated from IC_{50} values as described by Cheng and Prusoff (9). The values shown are means ± standard error of the mean for three to six independent determinations. Statistically significant differences were identified using Dunnett's multiple comparison test (14).

Incubation temperature	K_d value for dopamine	K_d value for (±)-ADTN
	nM	*nM*
37°	170 ± 38	2.9 ± 0.68
30	120 ± 35	4.7 ± 0.02
25	63 ± 19	4.6 ± 0.33
17	140 ± 31	5.4 ± 0.91
8	170 ± 62	6.5 ± 1.80
1	830 ± 260[a]	17.0 ± 3.3[a]

[a] $p < 0.001$ compared with the K_d value determined at 37°.

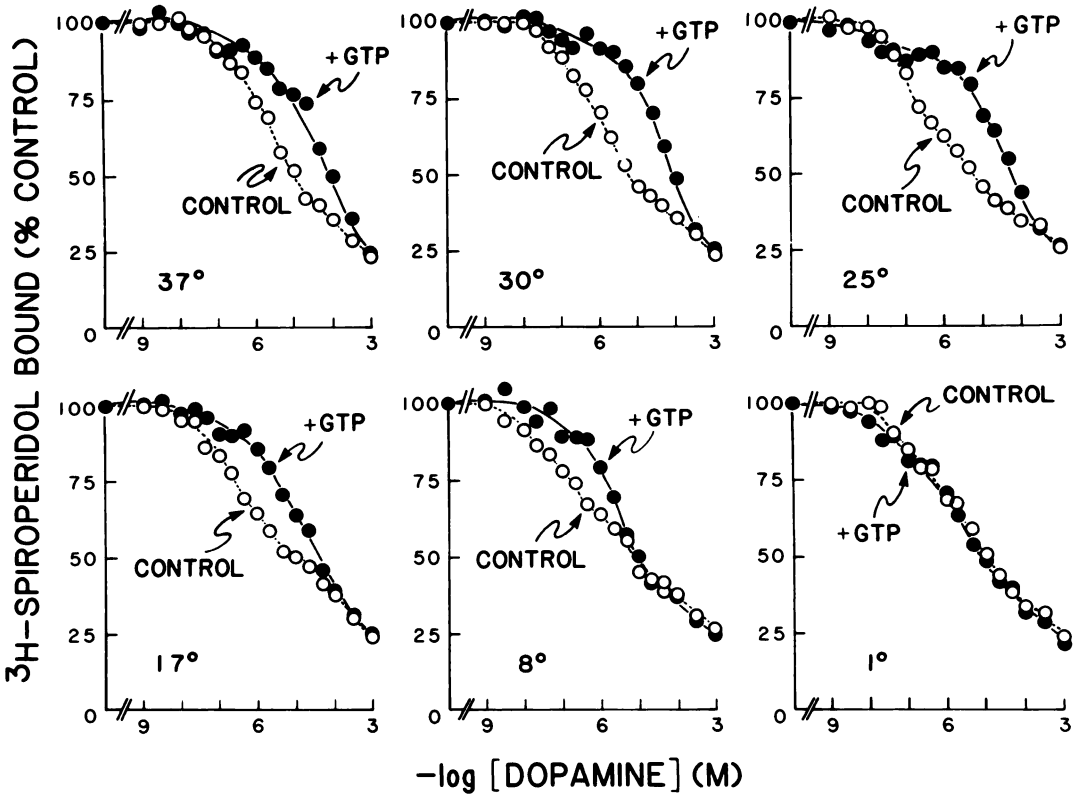

FIG. 5. *Effect of GTP on the inhibition of [^{3}H]spiroperidol binding by dopamine*

Rat striatal membranes were incubated with 0.6 nM [^{3}H]spiroperidol and various concentrations of dopamine in the absence (○) and presence (●) of 300 μM GTP at temperatures from 37° to 1° as described under Experimental Procedures. The values shown are means of three to five independent determinations performed in triplicate.

appear to correlate directly with the lipophilicity of the compound, since spiroperidol is significantly less polar than (+)-butaclamol (21, 22).

The binding of the agonists dopamine and (±)-ADTN also appears to be entropy-driven. Similar results have been reported for the binding of agonists to nicotinic cholinergic receptors (16). The magnitude of the increase in entropy associated with the binding of agonists was only one-half that associated with the binding of antagonists (Table 3). Agonists may produce a smaller net increase in entropy than seen in studies with antagonists because agonists induce a conformational change in the receptor as well as displace water from around the receptor. Unlike antagonists, the affinity of the receptor for agonists was not dependent on the incubation temperature, and, therefore, there was no change in enthalpy associated with agonist binding. In contrast to this result, Niehoff *et al.* (15) reported that the binding of the agonist [^{3}H]NPA to rat striatal membranes was temperature-dependent and enthalpy-driven. This discrepancy may be explained by the suggestion that [^{3}H]spiroperidol and [^{3}H]NPA do not label identical populations of receptor sites or that the findings reported here for dopamine and (±)-ADTN cannot be generalized to other dopamine receptor agonists.

Beta-adrenergic receptors and at least some dopamine receptors are thought to be positively coupled to adenylate cyclase. It has been suggested, however, that high-affinity binding sites for [^{3}H]spiroperidol are not related to the dopamine receptor involved in stimulation of adenylate cyclase activity (23, 24). In this respect, it is interesting to compare the energetics of the interactions of agonists with *beta*-adrenergic and dopamine receptors. The binding of agonists to *beta*-adrenergic receptors is enthalpy-driven (1, 2), while in the present study agonist binding to dopamine receptors was found to be entropy-driven (Table 3). These differences may indicate that the sites labeled by [^{3}H]spiroperidol are either not coupled to adenylate cyclase or are not coupled in the same manner to this enzyme.

Another difference was noted between the binding of agonists to *beta*-adrenergic receptors and to dopamine receptors. In the presence of GTP the affinities of both *beta*-adrenergic receptors (2) and dopamine receptors (6) for agonists are reduced. In the *beta*-adrenergic receptor system, the addition of GTP also changes the characteristics of agonist binding from those of a complex interaction to those of a simple bimolecular interaction (2). Hill coefficients observed in studies of the inhibition of [^{3}H]spiroperidol binding by agonists are consistently less than unity. In the present studies biphasic dose-response curves for dopamine and (±)-ADTN were observed in the absence of GTP at temperatures below 25° (Fig. 5). In the presence of GTP the affinity of the receptor for the agonists was decreased at all temperatures examined above 1°. However, the addition of GTP did not change the characteristics of agonist binding to those associated with a simple bimolecular interaction.

Several practical considerations for the binding of [^{3}H] spiroperidol became apparent from the results of this

study. (a) When an antagonist radioligand such as [^{3}H] spiroperidol and/or a competing ligand such as (+)-butaclamol is used, the definition of specific binding must be evaluated at every incubation temperature to be employed. The affinity of the receptor for antagonists is markedly decreased as the incubation temperature is lowered. The magnitude of this decrease in affinity may also depend on the composition of the buffer-salt mixture used during the incubation. Leysen and Gommeren (22) reported that the K_d value for [^{3}H]spiroperidol was relatively constant between 37° and 25° but that it increased 8-fold between 25° and 0°. Our results using a different buffer system revealed a progressive 10-fold increase in the K_d value for [^{3}H]spiroperidol between 37° and 1°. (b) The time to reach equilibrium must be determined at each temperature used. A spurious increase in the K_d value or a decrease in the B_{max} may result if equilibrium binding is not achieved. Other investigators (22) in addition to ourselves (Table 2) have found that the density of sites labeled by [^{3}H]spiroperidol at incubation temperatures between 37° and 0° is constant; however, another group (15) has reported a decrease in the density of binding sites when assays were carried out at 0°. (c) At temperatures greater than 45°, the binding of [^{3}H]spiroperidol or the binding sites themselves may be labile. Following a 10-min incubation at 44°, the B_{max} for [^{3}H]spiroperidol was identical with that determined at 37°; however, after a 10-min incubation at 50°, there was a 10% decrease in the number of receptors measured.[3] A similar finding has been reported by Lew and Goldstein (25). (d) In this system dopamine and [^{3}H] spiroperidol appear to interact in a purely competitive manner (Fig. 3). This result is in contrast to that reported by Sibley and Creese (26), who reported that NPA appears to interact noncompetitively with [^{3}H]spiroperidol in the bovine anterior pituitary. The decreased number of binding sites labeled by [^{3}H]spiroperidol when NPA is present has been attributed to receptor heterogeneity, however, rather than to a true noncompetitive interaction.

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[3] N. R. Zahniser and P. B. Molinoff, unpublished observations.

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