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Binding of [^{125}I]-*N*-(*p*-Aminophenethyl)spiroperidol to the D-2 Dopamine Receptor in the Neurointermediate Lobe of the Rat Pituitary Gland: A Thermodynamic Study

TAKASHI AGUI, NOURDINE AMLAIKY, MARC G. CARON, and JOHN W. KEBABIAN[1]

Experimental Therapeutics Branch, NINCDS, Bethesda, Maryland 20892 (T.A., J.W.B.) and Department of Physiology, Duke University Medical School, Durham, North Carolina 27710 (N.A., M.G.C.)

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SUMMARY

The novel iodinated ligand [^{125}I]-*N*-(*p*-aminophenethyl)spiroperidol ([^{125}I]NAPS) was used to identify the D-2 dopamine receptor in the intermediate lobe of the rat pituitary gland. The binding of [^{125}I]NAPS was of high affinity and saturable, given that the dissociation constant and the maximal binding were 34.7 ± 4.8 pM and 21.1 ± 2.5 fmol/mg of protein, respectively. The ability of dopaminergic agonists and antagonists to compete with [^{125}I]NAPS varied markedly with incubation temperature. The marked decrease of the molar potency associated with increasing incubation temperature in the competitive displacement curve suggested that the binding of five agonists, dopamine, (−)-apomorphine, (−)-*n*-propylnorapomorphine, N-0434, and LY-171555, to the D-2 dopamine receptor was enthalpy-driven, with a negative change in entropy. In contrast, the binding of three antagonists, fluphenazine, (+)-butaclamol, and domperidone, was entropy-driven, with positive change in entropy, suggesting less temperature-sensitive change in the molar potency. Several molecules gave unanticipated results; the molar potency of two dopamine agonists, bromocriptine and lisuride, was much less temperature-sensitive than the other agonists used in this study. The thermodynamic parameters for the atypical agonists indicated entropy-driven binding. Conversely, the molar potency of (+)-apomorphine, a dopamine receptor antagonist, was markedly affected by incubation temperature, indicating enthalpy-driven binding. Another antagonist, YM-09151-2, was affected by the inclusion of sodium chloride in the assay system: in the absence of sodium chloride, the drug was relatively weak and displayed enthalpy-driven binding; in the presence of sodium chloride, its molar potency was increased and its binding manner turned into entropy-driven.

Dopamine receptors can be divided into two general categories designated as the D-1 and D-2 dopamine receptors (1). The D-2 receptor occurs in both the central nervous system and many peripheral tissues. The D-2 receptor occurring in the IL of the rat pituitary gland is a useful experimental system because the concentration of D-2 receptors in the IL is extremely high (2–4). The binding properties of this receptor have been studied (2, 5–7); however, the small size of the rat IL limits its experimental accessibility in direct binding assays. In addition, the IL D-2 receptor is amenable to indirect assays. The dopaminergic inhibition of adenylate cyclase activity in homogenates of IL tissue provides a convenient *in vitro* correlate to receptor occupancy (3, 5). The available biochemical evidence suggests that the IL D-2 receptor is linked to adenylate cyclase by an inhibitory guanyl nucleotide-regulatory protein (N_i) (8). The organization of the IL D-2 receptor, N_i, and adenylate cyclase and the involvement of this complex in the dopaminergic regulation of hormone release from the IL has been recently reviewed (9).

Binding assays provide insight into the interaction between ligands and receptors. In the case of the D-2 receptor (among others), binding assays were valuable in identifying the existence of the high and low affinity states of the receptor for agonists and the participation of guanyl nucleotides in the interconversion of these two states (10–13). Binding assays also facilitate the investigation of the thermodynamics of the interactions of ligands with receptors (14). In the case of the β-adrenoceptor, the thermodynamics of the binding reaction permit a discrimination between agonists and antagonists. The binding of β-adrenergic antagonists is relatively insensitive to temperature; their binding is envisioned to be almost completely entropy-driven. The binding of β-adrenergic agonists is

[1] Present address: Abbott Laboratories, Abbott Park, IL 60064.

ABBREVIATIONS: IL, intermediate lobe; N-0434, 2-(*N*-propyl-*N*-2-phenylethyl-amino)-5-hydroxytetralin; SKF 38393, 7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; YM-09151-2, *N*-[(2*RS*,3*RS*)-1-benzyl-2-methyl-3-pyrrolidinyl]-5-chloro-2-methoxy-4-methylaminobenzamide; SCH 23390, *R*(1)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol; APO, apomorphine; NPA, *N*-*n*-propylnorapomorphine; SKF 103108A, 8-hydroxy-7-iodo-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine, hydrochloride; Gpp(NH)p, 5′-guanylyl imidodiphosphate; [^{125}I]NAPS, [^{125}I]-*N*-(*p*-aminophenethyl)spiroperidol; e.u., entropy unit (cal·mol^{-1}·°K^{-1}); NIL, neurointermediate lobe; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid.

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affected by the incubation temperature; the binding is envisioned to be enthalpy-driven with marked (and thermodynamically unfavorable) decreases in entropy.

The present experiments utilize the iodinated D-2 receptor antagonist [^{125}I]NAPS to characterize the properties of the D-2 receptor in the rat IL (15). The high specific activity of [^{125}I]NAPS (approximately 2200 Ci/mmol) allows binding assays to be performed with small quantities of tissue. The temperature dependency of the binding reaction is also investigated with a view towards differentiating between agonists and antagonists.

Experimental Procedures

Materials. Experimental reagents were obtained from the following sources: ATP, cAMP, GTP, Gpp(NH)p, EGTA, theophylline, Triton X-100, and bromocriptine mesylate, Sigma Chemical Co. (St. Louis, MO); (+)- and (−)-APO, (−)-NPA, and (+)-butaclamol, Research Biochemicals Inc. (Wayland, MA); fluphenazine and SCH 23390, Schering Corp. (Kenilworth, NJ); SKF 38393 and SKF 103108A, Smith Kline & French Laboratories (Philadelphia, PA); dopamine, Calbiochem-Behring Corp. (La Jolla, CA); LY-171555, Lilly (Indianapolis, IN); domperidone, Janssen (New Brunswick, NJ); YM-09151-2, Yamanouchi Pharmaceutical Co. Ltd. (Tokyo, Japan); glass filter no. 30, 7 mm, Schleicher & Schuell (Keen, NH); and [^{3}H]cAMP (specific activity 30–50 Ci/mmol), New England Nuclear Corp. (Boston, MA). N-0434 was a kind gift of Dr. Alen Horn (University of Groningen, Groningen, The Netherlands). NAPS was radioiodinated by a chloramine-T procedure as reported previously (15) and was stored in absolute ethanol.

Tissue. Sprague-Dawley rats (either male or female as indicated) were decapitated, the pituitary gland was removed, and the NIL was separated from the anterior lobe. NILs obtained from rats killed at the National Institutes of Health were used immediately; NILs from rats obtained from a commercial vendor (Hazelton Laboratories, Vienna, VI) were frozen on dry ice and kept at −80° until used. NIL tissue was homogenized in a solution containing 2 mM Tris-HCl (pH 7.4) and 2 mM EGTA. Routinely, each NIL was homogenized in 0.09 ml of this buffer.

Radioiodinated ligand-binding assay. Two assay systems were used to characterize the binding of [^{125}I]NAPS to the NIL. The first assay system was designed to reproduce (as closely as possible) the conditions used to assay adenylate cyclase activity. This assay system (system I) contained (in a final volume of 200 μl) 80 mM Tris-HCl (pH 7.4), 1 mM $MgSO_4$, 0.8 mM EGTA, 10 mM theophylline, 0.25 mM ATP, 50 pM [^{125}I]NAPS (except as shown in Fig. 1), and drugs as indicated. The second assay system was used to obtain maximal changes in the binding parameters. This assay system (system II) contained (in a final volume of 200 μl) 80 mM Tris-HCl (pH 7.4), 100 mM $MgSO_4$, 0.8 mM EGTA, 50 pM [^{125}I]NAPS, and drugs as indicated. Agonists were dissolved in a 2% (w/v) aqueous solution of sodium ascorbate (pH 6.5); all dilutions of stock solutions were made with this solution. Agonists were added to either assay system in a volume of 10 μl to achieve the indicated final concentration. Antagonists were dissolved and diluted in water; antagonists were added to either assay system in a volume of 10 μl to achieve the indicated final concentration. Nonspecific binding of the radiolabeled ligand was estimated by including 10 μM fluphenazine in the otherwise complete assay system.

Either binding assay was initiated by adding a 10-μl aliquot of [^{125}I]NAPS (to achieve a final concentration of 50 pM, except where indicated otherwise) and a 30-μl aliquot of tissue homogenate. The experimental incubation was conducted for 2 hr at 25°. Binding of [^{125}I]NAPS reached equilibrium within 2 hr under these conditions. In some experiments, the temperature of the binding assay was changed. Changing the temperature of the incubation medium introduced a slight change in the pH of the assay system: at 25° the pH was 7.4; at 22° the pH was 7.5, whereas at 37° the pH was 7.2. To assess whether these slight temperature-induced changes in pH affected the interaction of ligands with the receptor, we determined the molar potency of (−)-APO at 25°, at pH 7.4 and 7.2. There was no difference in the displacement curves for (−)-APO obtained at the two pH levels. Similarly, [^{125}I]NAPS displayed the same affinity toward the specific binding site in the NIL at 25° and 37°. The incubation was terminated by filtering the experimental assay system through a glass fiber filter and washing the filter four times with 200 μl of a washing buffer containing 80 mM Tris-HCl (pH 7.4), 1 mM $MgSO_4$, 0.8 mM EGTA, 0.01% (v/v) Triton X-100, and 0.1% (w/v) bovine serum albumin. The amount of radioactivity retained on the filter was estimated with a gamma counter.

The decreased potency of agonists at the higher incubation temperature which we report under Results was not due to an accelerated breakdown of either the radiolabeled NAPS or the nonradioactive ligand at the higher temperature. If the assay system to test the molar potency of (−)-APO (complete except for the presence of tissue) was first incubated at 37° for 2 hr and then ligand binding to tissue homogenate was tested at 25°, the molar potency of (−)-APO was the same as when the assay system was incubated only at 25° (i.e., without the 2-hr incubation at 37°). Conversely, if the tissue-free assay system was first incubated at 25° for 2 hr, the molar potency of an agonist, e.g., (−)-APO, was diminished when the complete assay system was incubated at 37° rather than 25°.

Determination of adenylate cyclase activity. Adenylate cyclase activity was assayed as described by Frey *et al.* (5). The NIL was dissected from male Sprague-Dawley rats and IL tissue was separated from posterior lobe, collected by centrifugation (200 × *g*, for 5 min), and homogenized in a solution containing 2 mM Tris-HCl (pH 7.4) and 2 mM EGTA. The standard assay system (final volume 60 μl) contained 80 mM Tris-HCl (pH 7.4), 1 mM $MgSO_4$, 0.8 mM EGTA, 10 mM theophylline, 0.25 mM ATP, 0.01 mM GTP, and drugs as indicated, and tissue homogenate (containing 2–10 μg of protein). The assay of enzyme activity was initiated by addition of the tissue homogenate to the otherwise complete assay system and was conducted for 10 min at 30°. The reaction was terminated by placing the tubes in boiling water for 3 min. The amount of cAMP was determined with the protein-binding assay of Brown *et al.* (16). In the adenylate cyclase assay, the intrinsic activity of a compound at the D-2 receptor was determined relative to (−)-APO. In each assay, the inhibitory effect of 30 μM (−)-APO upon forskolin (10 μM)-activated enzyme activity was determined. In addition, the maximal inhibitory effect of the other tested drug was determined and expressed as a percentage of the inhibitory effect of (−)-APO. Previous studies had determined that 30 μM (−)-APO produced the maximal inhibitory effect (5).

Computer modeling. Analysis of binding data to determine the dissociation constant of nonradioactive drugs was performed with the curve-fitting program LIGAND (17). For each tested compound, data were fit to both one-site and two-site models in order to determine which model gave a better fit.

Thermodynamic analysis. The association constant (K_a) obtained from the computer program LIGAND was used for calculating thermodynamic parameters. The standard state-free energy change (ΔG°) of binding was calculated from the equation:

$$\Delta G^\circ = -2.3\ R\ T \log K_a \qquad (1)$$

where R is the gas constant (1.99 cal mol^{-1} deg^{-1}), T is the temperature in degrees Kelvin, and K_a is the equilibrium association constant. Routinely, drugs were tested at 25° and 37°, and ΔH° was calculated from the difference between association constants at 25° and 37°, with the following equation:

$$\Delta H^\circ = -2.3\ R \frac{(T_1 \cdot T_2)}{T_2 - T_1} [\log K_{a_{25}} - \log K_{a_{37}}] \qquad (2)$$

where T_1 and T_2 are the temperatures in degrees Kelvin at 25° and 37°,

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respectively. Change in entropy ($\Delta S°$) was calculated from this equation:

$$\Delta S° = (\Delta H° - \Delta G°)/T \quad (3)$$

Protein determination. Protein concentration was routinely determined by the method of Lowry *et al.* (18), with bovine serum albumin as the standard.

Results

[125I]NAPS identifies the D-2 receptor in the rat IL. Homogenates of the rat NIL avidly bound [125I]NAPS in experiments using standard assay system I (Fig. 1A, total). Substantially less radiolabeled ligand was bound in the presence of 10 μM fluphenazine (Figure 1A, nonspecific). The specific binding of [125I]NAPS, the difference between total and nonspecific binding of the ligand, was of high affinity and saturable (Fig. 1B). A Scatchard plot (Fig. 1B, *inset*) showed that the specific binding of [125I]NAPS to the NIL homogenate had a dissociation constant of 34.7 ± 4.8 pM (mean ± SE, $n = 4$), and that the binding capacity of the NIL homogenate was 21.1 ± 2.5 fmol/mg of protein (mean ± SE, $n = 4$).

The specific binding site identified with [125I]NAPS in the NIL displayed pharmacological properties similar to those of the D-2 dopamine receptor known to occur in this pituitary structure (Fig. 2, Table 1). YM-09151-2, a potent and selective antagonist of the D-2 dopamine receptor, was at least 1100 times more potent (on a molar basis) than either SCH 23390 or SKF 103018A, antagonists of the D-1 dopamine receptor. Likewise, N-0434, a potent D-2 agonist, was 300 times more potent than SKF 38393, a D-1 receptor agonist, in competing with [125I]NAPS for occupancy of the specific binding site. In

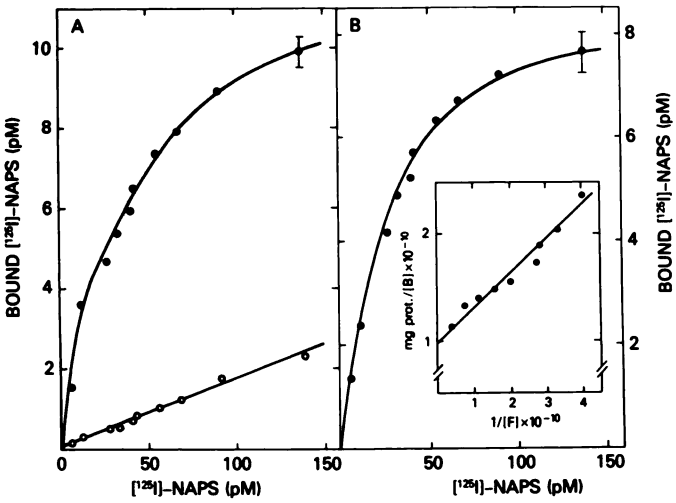

Fig. 1. A. Binding of [125I]NAPS to rat NIL. Binding of [125I]NAPS to a homogenate of male rat NIL was determined using system I (as described under Experimental Procedures), and the indicated concentrations of [125I]NAPS at each concentration were determined in three replicate samples in either the absence (i.e., total binding, ●) or the presence (i.e., nonspecific binding, ○) of 10 μM fluphenazine. Data are expressed as the concentration of [125I]NAPS bound as a function of the concentration of [125I]NAPS added to the assay system. This experiment is representative of data obtained in four independent experiments. Each point represents the mean ± standard error. B. Specific binding of [125I]NAPS to a homogenate of rat NIL. Using data in A, the difference between total and nonspecific binding was calculated and is presented as a function of the concentration of [125I]NAPS in the assay system. *Inset:* A Scatchard plot of the data in this panel in which [*B*] is the concentration of bound ligand and [*F*] is the concentration of free ligand in the assay system.

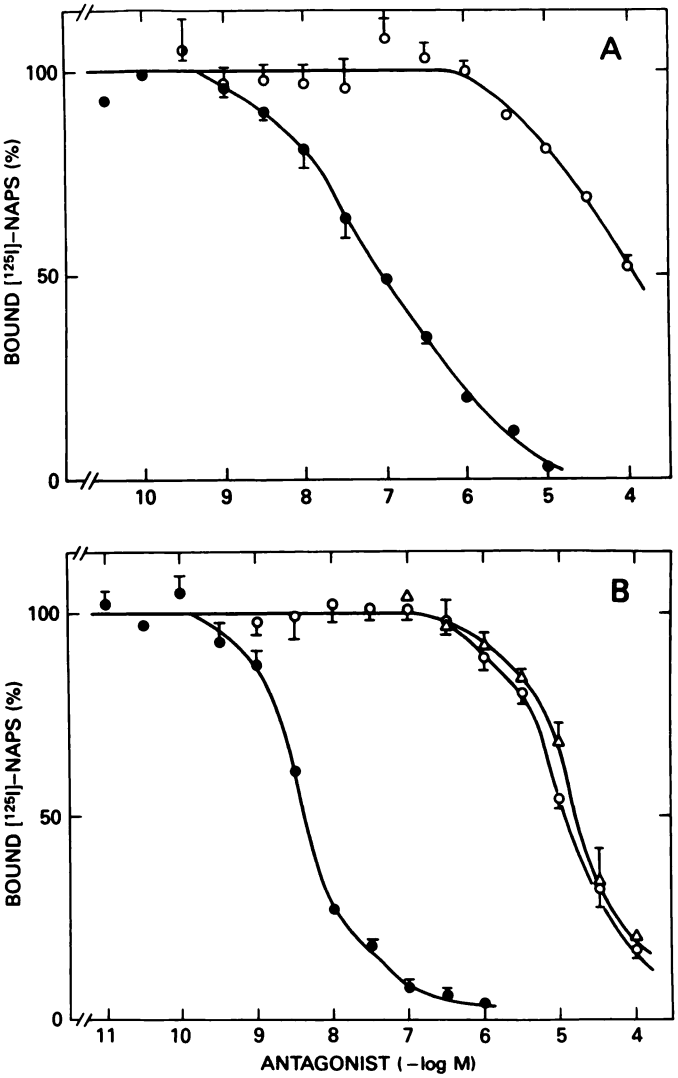

Fig. 2. Competition between [125I]NAPS and drugs for occupancy of specific binding sites in the rat NIL. A. Agonists. Binding of [125I]NAPS to a homogenate of female rat NIL was determined using system I (as described under Experimental Procedures) in the presence of the indicated concentrations of either N-0434, a D-2 agonist (●) or SKF 38393A, a D-1 agonist (○). Data represent the mean ± standard error of triplicate samples obtained in a single assay. B. Antagonists. Using the procedures described in A, binding of [125I]NAPS was determined in the presence of the indicated concentrations of YM-09151-2, a D-2 antagonist (●), SCH 23390, a D-1 antagonist (○), or SK&F 103108A, a D-1 antagonist (△). Data represent the mean ± standard error of triplicate samples obtained in a single assay.

TABLE 1

An affinity of drugs for the NIL D-2 receptor

Additional experiments similar to those in Fig. 2 were performed and the affinities of the drugs for the specific binding site were determined. Data represent the mean ± standard error for values obtained from three independent experiments performed with assay system I.

Compound	Dissociation Constant	
	K_H	K_L
	nM	
N-0434	13.7 ± 1.72	780 ± 388
SKF 38393	4,140 ± 1.930	77,900 ± 2,690
YM-09151-2	1.48 ± 0.126	
SCH 23390	1,640 ± 260	
SKF 103108A	2,610 ± 1,090	

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accordance with previous publications, the antagonist displacement data could be best fit by a monophasic curve with a single affinity constant, whereas agonist displacement data could be best fit by a biphasic curve with two affinity constants (Table 1).

Thermodynamics of binding. Temperature affected the interaction between agonists and the IL D-2 receptor. When the assay temperature was increased from 25° to 37°, the molar potency of (−)-APO was reduced (compare *open* and *solid circles*, Fig. 3). Either displacement curve was shallow and could be best fit by a two-site binding model. Binding of (−)-APO was tested at temperatures between 22° and 37° and the association constants from the high and low affinity states were calculated. Changing the incubation temperature from 25° to 37° did not cause a statistically significant difference in the dissociation constant of the [^{125}I]NAPS for the specific binding site. Fig. 3 (*inset*) is a van't Hoff plot of the log K_a versus the inverse of absolute temperature: the negative slope of the line provides an estimate of the enthalpy change associated with the binding of (−)-APO to the IL D-2 receptor. For both the high and low affinity state of the D-2 receptor, the binding of (−)-APO was associated with a large negative enthalpy change (Table 2).

The temperature dependency of the binding reaction for other agonists was tested (Table 2). Four of these agonists (dopamine, NPA, N-0434, and LY-171555) yielded results similar to those obtained with (−)-APO. The binding curves were shallow and were best fit by the two-state model. For each ligand, the dissociation constant for the two affinity states of the receptor differed by a factor ranging between 30 and 145. Inclusion of Gpp(NH)p (100 μM) in the assay system caused a substantial decrease in the affinity of the ligand for the receptor. Increasing the incubation temperature decreased the molar potency of the ligand in the competition assay. Consequently, the change in enthalpy of the binding reaction to either the high or the low affinity state of the receptor was a negative value. The change in entropy associated with the binding of the ligands to the D-2 receptor was calculated from the enthalpy changes. With one exception, these values for both the high and low affinity states of the D-2 receptor were negative values. The one exception was the small positive value obtained for the interaction between N-0434 and the low affinity state of the receptor.

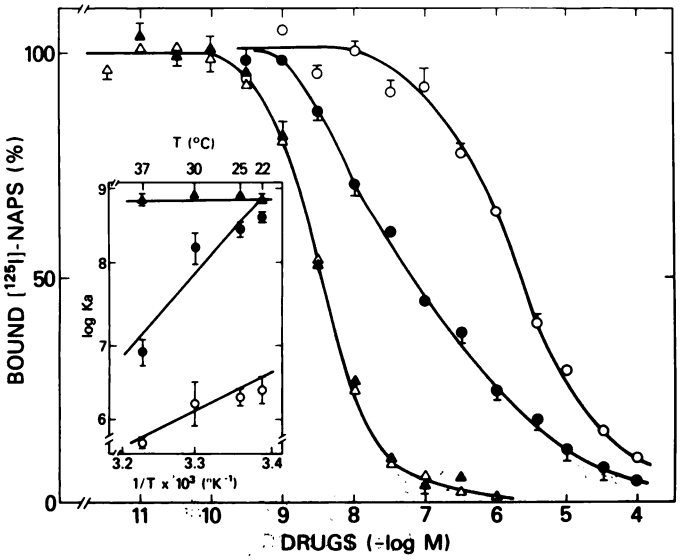

Fig. 3. Temperature dependency of drug binding to rat NIL D-2 receptors. Binding of [^{125}I]NAPS to a homogenate of female rat NIL was determined using system II (as described under Experimental Procedures) in the presence of the indicated concentrations of either fluphenazine (▲, △) or (−)-APO (●, ○) at incubation temperatures of either 25° (▲, ●) or 37° (△, ○). Data represent the mean ± standard error of observations obtained in three independent experiments. *Inset*: Similar experiments were conducted with the incubation temperature ranging between 22° and 37°. The association constant of the drugs for the specific binding site was determined, and data are presented as a van't Hoff plot. Fluphenazine fit a one-site model, whereas, (−)-APO fit a two-site model. Three independent experiments were performed at each incubation temperature. ▲, data for fluphenazine; ●, data for the (−)-APO and the high affinity state of the D-2 receptor; ○, (−)-APO and the low affinity state of the D-2 receptor. Data represent the mean ± standard error of observations obtained in three independent experiments.

Antagonists. The molar potency of antagonists in the [^{125}I]NAPS binding assay (system II) was not markedly altered by temperature. Comparison of the data in Fig. 3 for fluphenazine with the data for (−)-APO exemplifies the difference between the effect of temperature upon antagonist and agonist binding. The van't Hoff plot of the data for fluphenazine (Fig. 3, *inset*) suggests that there is only a small enthalpy change associated with its binding to the D-2 receptor. Two other antagonists, (+)-butaclamol and domperidone, gave results similar to those obtained with fluphenazine (Table 2). YM-09151-2, a substituted benzamide, gave slightly different results (Fig. 5). As had been shown previously (19), binding of YM-09151-2 to the D-2 receptor was affected by the presence of sodium ions in the assay system (Fig. 5). In the absence of sodium, YM-09151-2 was relatively weak in the binding assay and had a large temperature dependency. In the presence of 120 mM NaCl, the molar potency of the drug in the binding assay was increased and the temperature dependency was diminished. The inclusion of 120 mM NaCl did not alter the pH of the assay system. With the exception of YM-09151-2 in the absence of NaCl, binding of these D-2 antagonists to the receptor was associated with a positive increase in entropy.

Previous studies have established that (+)-APO is an antagonist of the D-2 receptor (20, 21). However, the data obtained

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TABLE 2

Intrinsic activity and binding parameters of agonists and antagonists for D-2 dopamine receptor

Compound	Physiological assay: intrinsic activity (Adenylate cyclase)	Binding assay							
		K_L/K_H (at 25°)[a]	K_H (at 25°)	Guanine nucleotide Shift[b]	ΔS			ΔH	
					K_H	(at 25°)	K_L	K_H	K_L
		%			$cal \cdot mol^{-1} \cdot °K^{-1}$			$kcal \cdot mol^{-1}$	
R-(−)-APO	1.0	142 ± 55.6	66.0 ± 1.04	54.2 ± 7.74	−141 ± 24.8		−43.3 ± 18.9	−53.5 ± 7.39	−21.5 ± 5.63
with Gpp(NH)p		–[c]	–[c]		−29.5 ± 5.10			−16.9 ± 1.52	
R-(−)-NPA	1.24 ± 0.07	30.3 ± 13.9	58.6 ± 9.45	28.4 ± 10.0	−19.8 ± 21.7		−32.2 ± 19.6	−18.7 ± 6.48	−20.4 ± 5.84
Dopamine	1.03 ± 0.15	145 ± 35.4	63.6 ± 3.50	122 ± 24.9	−66.8 ± 28.1		−31.7 ± 26.0	−30.3 ± 8.38	−16.9 ± 7.74
LY-171555	1.03 ± 0.13	84.8 ± 33.0	66.4 ± 5.26	54.2 ± 21.2	−134 ± 16.8		−99.3 ± 22.8	−50.3 ± 5.00	−37.3 ± 6.79
N-0434	0.77 ± 0.16	136 ± 75.7	72.7 ± 1.58	108 ± 3.9	−37.3 ± 22.7		+15.9 ± 37.6	−23.2 ± 6.76	−4.58 ± 11.2
Lisuride	0.73 ± 0.15	9.01 ± 4.10	79.7 ± 5.45	1.09 ± 0.17	+88.3 ± 19.1		+60.4 ± 5.74	+14.1 ± 5.70	+7.04 ± 1.70
Bromocriptine	0.64 ± 0.13	–[c]	–[c]	1.58 ± 0.003		+54.0 ± 0.10		+4.58 ± 0.02	
Fluphenazine	0	–[c]	–[c]	1.71 ± 0.25		+35.0 ± 9.46		−1.76 ± 2.82	
(+)-Butaclamol	0	–[c]	–[c]	0.65 ± 0.06		+42.6 ± 1.06		+0.282 ± 0.30	
Domperidone	0	–[c]	–[c]	0.59 ± 0.08		+32.5 ± 14.8		−2.11 ± 4.40	
YM-09151-2	0	–[c]	–[c]	0.88 ± 0.09		−20.5 ± 9.40		−18.0 ± 2.80	
with 120 mM NaCl		–[c]	–[c]	1.65 ± 0.40		+13.4 ± 4.73		−9.50 ± 1.41	
S-(+)-APO	0	16.7 ± 3.12	55.9 ± 13.0	10.9 ± 1.9	−24.3 ± 64.8		−74.8 ± 13.7	−16.9±19.3	−30.3 ± 4.08

[a] K_H and K_L are the high and low affinity states of the D-2 receptor.
[b] IC_{50} in the presence of 100 μM Gpp(NH)p/IC_{50} in the absence of Gpp(NH)p.
[c] –, one affinity state model was better fitting in the computer analysis.

for (+)-APO differed from that obtained for the other antagonists. The competition curve for (+)-APO was shallow (Fig. 4); the binding data were best fit by a two-site model (Table 2). The molar potency of (+)-APO in the binding assay was diminished by increasing the incubation temperature (Fig. 4A). Inclusion of Gpp(NH)p in the binding assay decreased the molar potency of the molecule; binding data obtained in the presence of Gpp(NH)p could be best fit by a one-site model of the D-2 receptor (Fig. 4B). The calculated changes in both enthalpy and entropy associated with the binding of (+)-APO to the D-2 receptor were relatively large negative values, more typical of those obtained for conventional agonists (Table 2).

Discussion

The homogeneous structure of the IL, its high concentration of D-2 dopamine receptors, and its innervation by dopaminergic neurons that synapse upon the parenchymal cells make it an attractive structure in which to study receptors for dopamine. The present results show that [125I]NAPS identifies the D-2 dopamine receptor in the NIL of the rat pituitary gland. This conclusion is in accord with the recent use of the azido congener of [125I]NAPS as a photoaffinity probe of the D-2 receptor in the rat NIL (6). The high specific activity of [125I]NAPS allows binding experiments to be performed with small quantities of NIL tissue; this technical advance may facilitate the use of the NIL as a model of a postsynaptic D-2 dopamine receptor. The quantity of specific binding sites identified with [125I]NAPS is greater than the quantity of binding sites identified with [3H] spiroperidol. In this report, we calculate the maximal binding capacity for ligand to be 21 pmol/g of NIL tissue. This value is approximately twice the value of 9.4 pmol/g of NIL tissue reported earlier from this laboratory by Frey *et al.* (5) from experiments performed with [3H]spiroperidol. Either of the values obtained in this laboratory is substantially higher than the value of 3.8 pmol of binding sites/g of NIL tissue reported by Stefanini *et al.* (22). The reason(s) for the differences in binding capacity of the NIL of the rat pituitary gland remain(s) to be determined. However, in each report of ligand binding to the rat NIL, sufficient data were presented to support the conclusion that the binding site was a D-2 dopamine receptor.

The temperature dependency of the binding of ligands to the NIL D-2 receptor gave some insight into the pharmacology of the D-2 receptor. The binding of "conventional" antagonists (exemplified by fluphenazine) to the D-2 receptor was characterized by a net increase in entropy. In contrast, the binding of "conventional" agonists [exemplified by (−)-APO] was enthalpy-driven with a net decrease in entropy. In accord with previous results (10–12, 23), the competition curves for conventional agonists fit a two-site model, whereas antagonist curves fit a one-site model; the guanyl nucleotide-induced shifts in agonist binding curves have been previously reported by others. When considered together, the phenomena reported in this communication for the "conventional" agonists and antagonists are reminiscent of the situation at the β-adrenergic receptor of turkey erythrocytes described by Molinoff and his colleagues (14). However, our results differ from those reported by Zahniser and Molinoff (24), who studied the temperature dependency of the binding of agonists and antagonists to striatal D-2 dopamine receptor in experiments using [3H]spiroperidol. In these studies, binding of either agonists or antagonists to the striatal dopamine receptor was associated with an increase in entropy: the increases in entropy associated with antagonist binding were greater than the increases associated with agonist binding. The discrepancy between the results which we obtained from the NIL and those obtained from the striatum may have arisen from technical differences between the tissues used (striatum versus NIL), the ligands used (spiroperidol versus NAPS), or some other variation in the assay conditions. The results obtained in this study differ substantially from those in a recent study using tritiated spiroperidol to study the thermodynamics of agonist and antagonist binding to the D-2 receptor in the striatum (25). This earlier study reported that the molar potency of (−)-APO in the binding assay was unaffected by incubation temperature, whereas the potency of dopamine was affected by the incubation temperature. Conversely, some substituted benzamide antagonists were reported to be extremely sensitive. However, in accord with the present results, other antagonists [including (+)-butaclamol and YM-09151-2] were relatively insensitive to the incubation temper-

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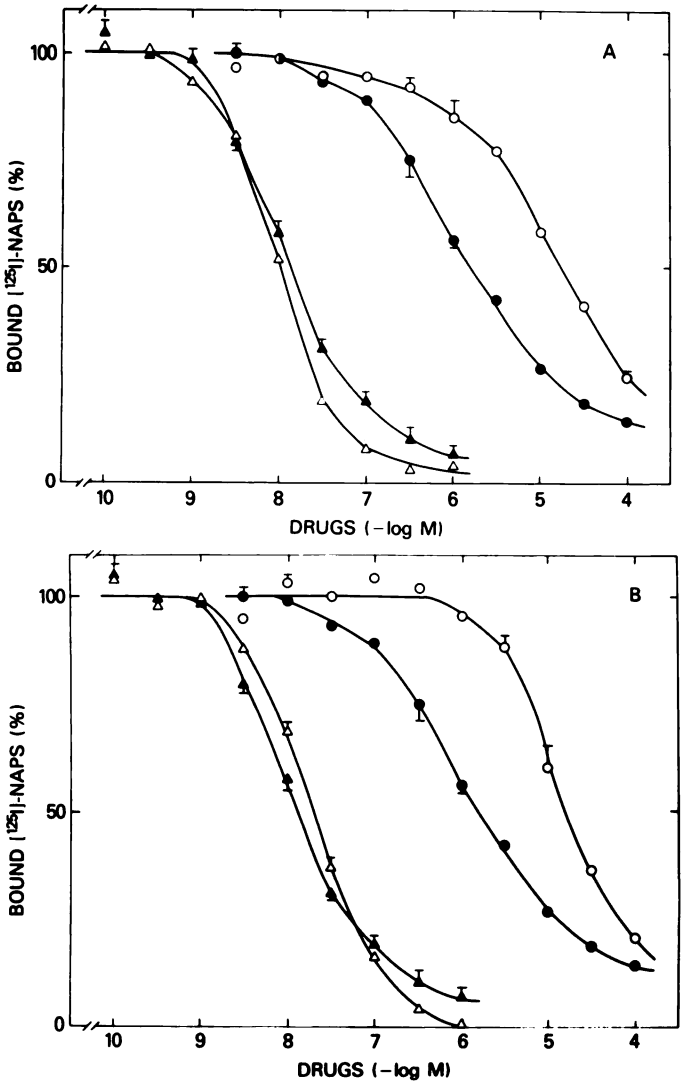

Fig. 4. Binding of atypical agonists and antagonists to the rat NIL D-2 receptor. A. Binding of [^{125}I]NAPS to a homogenate of female rat NIL was determined using system II (as described under Experimental Procedures) in the presence of the indicated concentrations of either bromocriptine (▲, △) or (+)-APO (●, ○) at either 25° (▲, ●) or 37° (△, ○). B. Using procedures described in A, binding of either bromocriptine (▲, △) or (+)-APO (●, ○) was determined in the absence (△, ○) or presence (▲, ●) of 100 μM Gpp(NH)p. Incubation temperature was 25°. Data in A and B represent the mean ± standard error of results obtained in three replicate experiments.

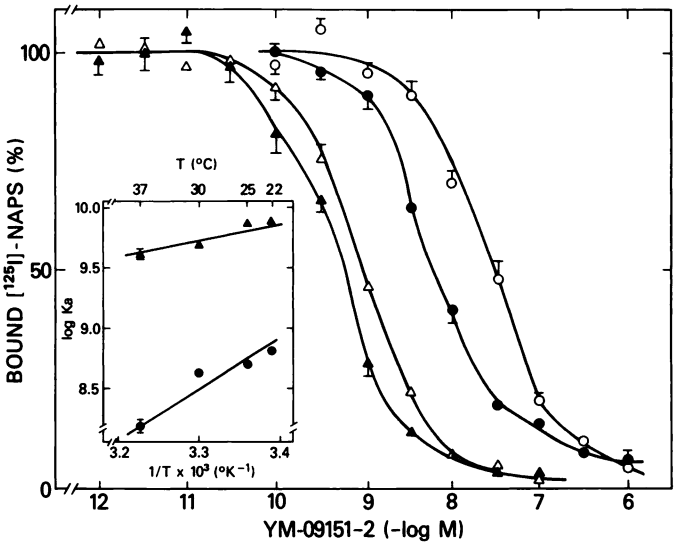

Fig. 5. Temperature dependency of the binding of YM-09151-2 to rat NIL D-2 receptors: the effect of sodium chloride. Binding of [^{125}I]NAPS to a homogenate of female rat NIL was determined using system II (as described under Experimental Procedures) in the presence of the indicated concentrations of YM-09151-2 at either 25° (●, ▲) or 37° (○, △) in the absence (●, ○) or presence (▲, △) of 120 mM NaCl. Data represent the mean ± standard error of data obtained in three independent experiments. *Inset*: Similar experiments (▲, with 120 mM NaCl; ● no added NaCl) were conducted with the incubation temperature ranging between 22° and 37°. The association constant of the drug for the specific binding site was determined, and data are presented as a van't Hoff plot. Three independent experiments were performed at each incubation temperature. Data represent the mean ± standard error of results obtained in three replicate experiments.

ature. The reasons for the discrepancies between our study and the earlier report (25) are not immediately evident.

Several of the compounds studied in this report gave unanticipated results. The first of these compounds was (+)-APO. Although (+)-APO is well known as a dopamine receptor antagonist, its properties in the binding assay were characteristic of an agonist (20, 21). The shallow displacement curve and marked shifts induced by temperature or guanyl nucleotide are comparable to the results obtained with "conventional" agonists. In the future, it will be of interest to identify how the inversion of carbon 6a in apomorphine from the *R*-(−) to the *S*-(+) configuration removes efficacy from the molecule in functional assays while preserving agonist-like properties in the binding assay. A second anomalous compound was bromocriptine. Previously, bromocriptine was identified as possessing antagonist-like properties in the binding assays, although this compound is a well known D-2 agonist (2, 5, 11). The present results show that in the thermodynamic determinations, bromocriptine also resembles an antagonist. Lisuride, another D-2 agonist, more closely resembled an antagonist than an agonist in the thermodynamic assays. It remains to be determined whether the hypothesis of Sibley and Creese (26), that the side chain of the ergot nucleus contributes to the "antagonist-like" properties of lisuride and bromocriptine, is accurate. The third anomalous compound was YM-09151-2. The ability of sodium chloride to affect the binding of YM-09151-2 is well known (19); however, the mechanism(s) underlying this phenomenon is(are) unknown. In the absence of sodium, binding of YM-09151-2 to the D-2 receptor displays "agonist-like" thermodynamic properties (i.e., negative values for enthalpy and entropy changes) but "antagonist-like" properties in the competition assays (i.e., one binding site and no guanyl nucleotide shift). In the presence of sodium, binding becomes more "antagonist-like" (i.e., the entropy change becomes a positive value). In the future, it will be of interest to determine whether the effect of sodium upon interaction between benzamide and the D-2 receptor is an intrinsic property of the receptor or is mediated by some other biological entity. Recent advances in the technology of the solubilization and the reconstitution of the D-2 receptor may permit experimental examination of this question (27). The existence of these anomalous compounds makes it difficult to correlate intrinsic activity with the thermodynamic parameters of the binding reaction as was possible for the β-adrenoceptor (14). Furthermore, these apparently "anomalous" compounds highlight the limits of our present understanding of the

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properties of the D-2 receptor. It seems reasonable to anticipate that attempts to gain insight into the basis for these unanticipated results may be a productive avenue for future investigations.

References

1. Kebabian, J. W., and D. B. Calne. Multiple receptors for dopamine. *Nature* (*Lond.*) **277**:93–96 (1979).
2. Sibley, D. R., and I. Creese. Dopamine receptor binidng in bovine intermediate lobe pituitary membranes. *Endocrinology* **107**:1405–1409 (1980).
3. Cote, T. E., C. W. Grewe, K. Tsuruta, J. C. Stoof, R. L. Eskay, and J. W. Kebabian. D-2 dopamine receptor-mediated inhibition of adenylate cyclase activity in the intermediate lobe of the rat pituitary gland requires guanosine 5′-triphosphate. *Endocrinology* **110**:812–819 (1982).
4. Lightman, S. L., M. Ninkovic, and S. P. Hunt. Localization of [^{3}H]spiperone binding sites in the intermediate lobe of the rat pituitary gland. *Neurosci. Lett.* **32**:99–102 (1982).
5. Frey, E. A., T. E. Cote, C. W. Grewe, and J. W. Kebabian. [^{3}H]Spiroperidol identifies a D-2 dopamine receptor inhibiting adenylate cyclase activity in the intermediate lobe of the rat pituitary gland. *Endocrinology* **110**:1897–1904 (1982).
6. Amlaiky, N., and M. G. Caron. Identification of the D-2-dopamine receptor binding subunit in several mammalian tissues and species by photoaffinity labeling. *J. Neurochem.* **47**:196–204 (1986).
7. DeSouza, E. B. Serotonin and dopamine receptors in the rat pituitary gland: autoradiographic identification, characterization, and localization. *Endocrinology* **119**:1534–1542 (1986).
8. Rodbell, M. The role of hormone receptors and GTP-regulatory proteins in membrane transduction. *Nature* (*Lond.*) **284**:17–22 (1980).
9. Cote, T. E., E. A. Frey, R. D. Sekura, and J. W. Kebabian. Dual regulation of adenylate cyclase activity and hormone release in the intermediate lobe of the rat pituitary gland: evidence for the involvement of membrane components of the stimulatory β_2-adrenergic system and of the inhibitory D-2 dopaminergic system. *Adv. Cyclic Nucleotide Res.* **19**:151–167 (1985).
10. De Lean, A., B. F. Kilpatrick, and M. G. Caron. Dopamine receptor of the porcine anterior pituitary gland. Evidence for two affinity states discriminated by both agonists and antagonists. *Mol. Pharmacol.* **22**:290–297 (1982).
11. Sibley, D. R., A. De Lean, and I. Creese. Anterior pituitary dopamine receptors. Demonstration of interconvertible high and low affinity states of the D-2 dopamine receptor. *J. Biol. Chem.* **257**:6351–6361 (1982).
12. Wreggett, K. A., and A. De Lean. The ternary complex model. Its properties and application to ligand interactions with D-2-dopamine receptor of anterior pituitary gland. *Mol. Pharmacol.* **26**:214–227 (1984).
13. De Lean, A., J. M. Stadel, and R. J. Lefkowitz. A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled β-adrenergic receptor. *J. Biol. Chem.* **255**:7108–7117 (1980).
14. Weiland, G. A., K. P. Minneman, and P. B. Molinoff. Fundamental difference between the molecular interactions of agonists and antagonists with the β-adrenergic receptor. *Nature* (*Lond.*) **281**:114–117 (1979).
15. Amlaiky, N., B. F. Kilpatrick, and M. G. Caron. A novel radioiodinated high affinity ligand for the D-2-dopamine receptor. *FEBS Lett.* **176**:436–440 (1984).
16. Brown, B. L., J. D. M. Albano, R. P. Ekins, and A. M. Sgherzi. A simple and sensitive saturation assay method for the measurement of adenosine 3′:5′-cyclic monophosphate. *Biochem. J.* **121**:561–562 (1971).
17. Munson, P. J., and D. Rodbard. LIGAND: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* **107**:220–239 (1980).
18. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
19. Niznik, H. B., D. E. Grigoriadis, I. Pri-Bar, O. Buchman, and P. Seeman. Dopamine D-2 receptors selectively labeled by a benzamide neuroleptic: [^{3}H]-YM-09151-2. *Naunyn-Schneideberg's Arch. Pharmacol.* **329**:333–343 (1985).
20. Lehmann, J., R. V. Smith, and S. Z. Langer. Stereoisomers of apomorphine differ in affinity and intrinsic activity at presynaptic dopamine receptors modulating [^{3}H]acetylcholine release in slices of cat caudate. *Eur. J. Pharmacol.* **88**:81–88 (1983).
21. Goldman, M. E., and J. W. Kebabian. Aporphine enantiomers. Interactions with D-1 and D-2 dopamine receptors. *Mol. Pharmacol.* **25**:18–23 (1984).
22. Stefanini, E., P. Devoto, A. M. Marchisio, F. Vernaleone, and R. Collu. [^{3}H] Spiroperidol binding to a putative dopaminergic receptor in rat pituitary gland. *Life Sci.* **26**:583–587 (1980).
23. George, S. F., M. Watanabe, T. Di Paolo, P. Falardeau, F. Labrie, and P. Seeman. The functional state of the dopamine receptor in the anterior pituitary is in the high affinity form. *Endocrinology* **117**:690–697 (1985).
24. 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).
25. Kilpatrick, G. J., N. El Tayar, H. Van De Waterbeemd, P. Jenner, B. Testa, and C. D. Marsden. The thermodynamics of agonist and antagonist binding to dopamine D-2 receptors. *Mol. Pharmacol.* **30**:226–234 (1986).
26. Sibley, D. R., and I. Creese. Interactions of ergot alkaloids with anterior pituitary D-2 dopamine receptors. *Mol. Pharmacol.* **23**:585–593 (1983).
27. Senogles, S. E., N. Amlaiky, A. L. Johnson, and M. G. Caron. Affinity chromatography of the anterior pituitary D-2-dopamine receptor. *Biochemistry* **25**:749–753 (1986).

Send reprint requests to: Dr. John W. Kebabian, 47U, AP10, Abbott Laboratories, Abbott Park, IL 60064.

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