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INTERACTIONS OF AGONISTS WITH D-2 DOPAMINE RECEPTORS: EVIDENCE FOR A SINGLE RECEPTOR POPULATION EXISTING IN MULTIPLE AGONIST AFFINITY-STATES IN RAT STRIATAL MEMBRANES

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Abstract—Several lines of evidence previously indicated that [^{3}H]spiroperidol (SPIRO) or [^{3}H]domperidone (DOMP) might label heterogenous populations of striatal dopamine receptors in radioligand binding studies. We have examined this possibility in rat striatum using computerized non-linear curve fitting and a method to block the unwanted [^{3}H]SPIRO binding to S-2 serotonergic receptors. In the absence of a serotonergic antagonist, [^{3}H]SPIRO saturation data produce curved Scatchard plots which are best computer fit by assuming the presence of two sites of different [^{3}H]SPIRO affinities. In the presence of appropriate concentrations of ketanserin to block S-2 serotonergic binding, Scatchard plots are linear, with data modeling best to a single population of homogenous binding sites. The D-2 dopamine receptor B_{max} and [^{3}H]SPIRO K_D determined in this fashion are indistinguishable from that obtained for the higher affinity binding site by computer analysis of data obtained in the absence of ketanserin. [^{3}H]DOMP produced indistinguishable values for D-2 receptor B_{max} as well. Competitions by (−)sulpiride, metoclopramide, and DOMP for [^{3}H]SPIRO binding sites in the presence of ketanserin are steep (Hill slope approximately 1), demonstrating that the previously observed heterogeneity of these sites is due entirely to serotonergic [^{3}H]SPIRO binding. In contrast, agonist/^{3}H-antagonist competition curves in the presence of ketanserin are best computer fit by assuming two independent receptor sites of high (R_H) and low (R_L) agonist affinity. With the addition of 5′-guanylylimidodiphosphate [Gpp(NH)p] computer analyses of agonist/^{3}H-antagonist competition curves show an increased ratio R_L/R_H concomitant with apparent decreases in agonist affinities for both sites. Under some conditions, some agonist/^{3}H-antagonist competition curves are best fit by a single site model in which agonist affinity is indistinguishable from the agonist's affinity at R_L determined in the absence of Gpp(NH)p.

These data are consistent with the presence of a single dopaminergic ^{3}H-butyrophenone binding site, representing the D-2 receptor, which exists in two interconverting states differing in agonist affinity.

Numerous studies have demonstrated that [^{3}H]spiroperidol, [^{3}H]domperidone, and [^{3}H]haloperidol label similar sites in mammalian striatal membranes, and that the majority of this binding is to sites displaying the characteristics expected of a D-2 dopamine receptor [1, 2]. It is now clear, however, that the ligand generally found most useful because of its high affinity, [^{3}H]SPIRO, labels multiple sites from which it is displaceable by the antagonists commonly used to determine "specific" dopaminergic binding. Some of this binding is to serotonergic S-2 receptors [3–7] but controversy remains as to whether the remaining sites represent single or multiple subtypes of dopamine receptors [8, 9]. Agonist competition for sites labeled by all these antagonist ligands yields shallow curves with Hill slopes <1 [2, 8, 10–14] suggesting that even [^{3}H]DOMP, perhaps the most D-2 selective ligand available [15] labels a heterogenous population of binding sites [7, 14]. We have re-evaluated the binding of [^{3}H]SPIRO and [^{3}H]DOMP to rat striatal membranes, devising and validating a method to quantitatively eliminate the serotonergic component of [^{3}H]SPIRO binding. Using this method and computer assisted curve fitting, we have demonstrated that when this serotonergic artifact is eliminated, these two ligands do label a homogenous population of D-2 receptors. The previously observed heterogeneity of agonist affinities at these sites may be explained by the existence of two interconverting states of a single striatal D-2 receptor, as has been demonstrated for the anterior pituitary by D-2 receptor [16].

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5′-Guanylylimidodiphosphate, Gpp(NH)p; spiroperidol, SPIRO; domperidone, DOMP.

Computer fitted parameter abbreviations: R_H, R_L, receptor concentrations for high and low affinity components respectively; K_H, K_L, dissociation/inhibition constants for high and low affinity components respectively; K_G, dissociation/inhibition constant for single (low affinity) binding component observed for agonists in the presence of guanine nucleotides.

METHODS

Albino rats 175–250 g (Charles River Laboratories) were decapitated and their striata removed, chilled, pooled, weighed, and homogenized (Ultra-Turrax, setting 7, 10 sec) in 100 vol of ice-cold Tris–HCl, pH 7.7 at 25° (Tris), and centrifuged at 0–4° 50,000 *g* for 10 min. This initial pellet was then resus-

pended in 50 vol Tris containing 2 mM $MgSO_4$, incubated for 15 min at 37°, cooled with an equal volume of 0° Tris, briefly chilled on ice, and centrifuged as before. After suspension of the tissue pellet in fresh 0° Tris and given a final centrifugation, the tissue was suspended in assay buffer containing (to yield the listed final incubation concentration allowing for dilution with ligand and drugs): 20 mM morpholinopropane sulfonic acid (MOPS), 1 mM ascorbic acid, 10 mM pargyline, 1 mM EDTA and 19 mM Tris base produce a final assay pH of 7.2 at 22°. Assay buffer also included 4 mM $MgSO_4$ except when deleted as noted. Incubation was initiated by adding tissue to triplicate tubes containing ligand, ions and drugs, made up in 1 mM ascorbic acid. Nonspecific binding was defined as that remaining in the presence of 100 nM (+)butaclamol for [^{3}H]SPIRO and [^{3}H]-DOMP, and DOMP at 20 times [^{3}H]dopamine concentration for D-2 specific binding of this ligand. Final tissue concentration was 0.4 mg/ml and final assay volume was 5 ml. Incubation proceeded in temperature controlled water baths until terminated after 180 min by rapid filtration under reduced pressure through Whatman GF/C filters. These were then rapidly washed with 20 ml of ice-cold Tris. The entire filtration and wash procedure required 10–15 sec. Radioactivity trapped on the filters was assessed using liquid scintillation spectroscopy at efficiencies of approximately 45%.

Some experiments were performed under the conditions used by Sibley *et al.* [16] as noted. For this, tissues was prepared as above, omitting the second suspension with incubation and its succeeding centrifugation. The final tissue pellet was suspended in ice cold assay buffer containing 120 mM NaCl, 2 mM $CaCl_2$, 5 mM KCl, 1 mM $MgCl_2$, 0.1% ascorbic acid, and 10 μM pargyline made up in 50 mm Tris–HCl pH 7.7 (25°). Incubation was started by addition of 1.7 ml tissue suspension to 0.3 ml of ligand and other drugs made up in 0.1% ascorbic acid. Incubation proceeded for 15 min at 37° before filtration as above.

Preliminary experiments revealed that the observed K_D for the two antagonist ligands employed varied as a funtion of tissue concentration, with higher K_Ds observed at higher tissue concentrations. This presumably reflected artifactual effects of the depletion of labeled ligand to specific and nonspecific sites observed with high tissue concentration [17]. Further reduction of tissue concentration below 0.4 mg/ml did not alter observed K_Ds.

Specific binding of [^{3}H]SPIRO and [^{3}H]DOMP at the lowest concentrations used in saturation studies required 3 h to reach a steady state when performed in the absence of NaCl. It is important to note that we found, similar to a previous study [18], that in the presence of 100 mM NaCl, equilibrium was reached more rapidly. Thus, unless equilibrium is reached under all conditions of assay, it may be spuriously concluded that [^{3}H]SPIRO labels more sites in the presence of sodium ion than in its absence, a conclusion not supported by our data at equilibrium (see Results). Binding of the other ligand was also at equilibrium after 3 hr incubation, and all maintained this steady state for at least 3 hr thereafter.

Other preliminary experiments similar to those described in Leff *et al.* [19] established that the concentration of ascorbic acid used did not reduce specific binding of any ligand, and that the concentration of drugs used to determine non-specific binding, the filter thickness, and the wash procedure was optimal.

Ligands used were [^{3}H]SPIRO (30–35 Ci/mmol), [^{3}H]DOMP (65 Ci/mmol) and [^{3}H]dopamine (44–48 Ci/mmol), from New England Nuclear. Dopamine, pargyline, apomorphine, guanosine-5′-triphosphate (Tris salt, Type VI), and guanylylimidodiphosphate (sodium salt) were obtained from Sigma. Sources of other drugs were: (+)butaclamol, Ayerst Laboratories; (±)2-amino-dihydroaminotetralin (ADTN), Burroughs Wellcome: *n-N*-propylnorapomorphine, Sterling-Winthrop; (-)sulpiride, Ravizza; DOMP, ketanserin, SPIRO, Janssen Pharmaceutica; cinnanserin, Nutritional Biochemicals. Other reagents were obtained from commercial sources in the highest available grade.

Data analysis was performed similar to the method described by De Lean *et al.* [20]. Competition and saturation data was analyzed using the computer program "LIGAND" of Munson and Rodbard [21] as adapted by De Lean *et al.* [22] and employed in our laboratory [16]. Data was analyzed according to a general model for the interaction of multiple independent sites based on the law of mass action [23]. This analysis provided an estimate of the affinity constant for each radioligand or competitor at one, two, or more sites, along with the concentration of each of the several binding sites. The statistical goodness of fit was assigned for each derived curve by the calculation of the weighted residual variance. A two site fit was accepted only if significantly better than a one site fit using a partial F-test, $P < 0.05$. Saturation experiments are presented as Scatchard plots for clarity. The assumption of three or more binding sites did not improve the fit for data described in this paper. The affinity of ^{3}H-ligands as determined in saturation experiments are used in the determination of K_i values in competition studies simultaneous fits. The affinities of [^{3}H]SPIRO for D-2 and S-2 sites used in analysis of competition studies were those determined by computer analysis of total (+)butaclamol displaceable [^{3}H]SPIRO binding (Table 1). Data analysis was performed using a program version in BASIC for a VAXII. Values given are arithmetic means ± standard error of the mean, unless stated otherwise. For some experiments, curves were analysed simultaneously. Competition curves were first analyzed constraining only the affinities of the labeled ligand constant. Subsequently residual variances were compared between these fits and fits in which cold ligand K parameters or R parameters were shared across curves. Thus, for example, if sharing K parameters for both curves for any site did not significantly (F-test, $P < 0.05$) worsen the fits, it was accepted that K values for each site could be the same in both curves.

RESULTS

Use of ketanserin (R41468) to eliminate [^{3}H]*SPIRO binding to S-2 serotonergic sites in dopamine receptor binding assays.* As previously reported by Fields *et al.* [24] and List and Seeman [6] in the absence of

any serotonergic antagonist to mask ligand binding to S-2 receptors, (+)butaclamol displaceable [^{3}H]SPIRO binding displayed curved Scatchard plots, indicating the presence of two or more populations of [^{3}H]SPIRO binding sites with differing [^{3}H]SPIRO affinities (Fig. 1). Computer assisted analysis revealed that in the presence of 4 mM $MgSO_4$, the [^{3}H]SPIRO binding was best modeled to two sites with $K_D = 0.018 \pm 0.002$ nM and $B_{max} = 22.1 \pm 1.9$ pmoles/g tissue, and $K_D = 0.7 \pm 0.3$ nM and $B_{max} = 21 \pm 6$ pmoles/g tissue (N = 3). Similar results were obtained in the presence of both 4 mM $MgSO_4$ and 100 mM NaCl (Table 1).

Several reports [4–6, 8] have buttressed the original observation of Leysen *et al.* [3] that "specific" [^{3}H]SPIRO binding defined using (+)butaclamol as blank includes binding to both dopaminergic (D-2) and serotonergic (S-2) sites. List and Seeman [6] and Withy *et al.* [5] demonstrated the effectiveness of including low concentrations of serotonergic antagonists R43448 and mianserin respectively to prevent [^{3}H]SPIRO binding to S-2 sites without affecting D-2 binding. We sought to validate the use of another reportedly more specific S-2 antagonist, ketanserin (R41468) [25], as a serotonergic "mask" for use in dopamine receptor binding studies.

Ketanserin/[^{3}H]SPIRO competition curves in the presence of 4 mM $MgSO_4$ and 100 mM NaCl were biphasic when the [^{3}H]SPIRO concentration exceeded about 0.1 nM (Fig. 2). Computer assisted modeling yielded K_i values for the high and low affinity sites of 0.33 nM (putative S-2 receptors) and 740 nM (putative D-2 receptors) respectively, or a selectivity of approximately 2200-fold. Similar results but with lower selectivity were obtained when assays were performed in the presence of 4 mM $MgSO_4$ without 100 mM NaCl. Another serotonergic S-2

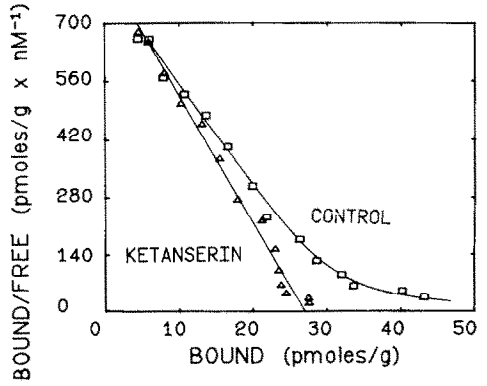

Fig. 1. Saturation of [^{3}H]SPIRO binding of rat striatal membranes. In the absence of serotonergic antagonists, Scatchard plots were nonlinear, consistent with the presence of multiple (+)butaclamol displaceable [^{3}H]SPIRO binding sites. These curves were resolved by computer analysis to two binding sites of roughly equal B_{max} but different K_Ds as shown in Table 1. Addition of the serotonergic antagonist ketanserin in a concentration 200 times that of radioligand eliminated the low [^{3}H]SPIRO affinity phase of the curve. The curves shown here were obtained in the presence of 4 mM $MgSO_4$, using 0.01–1.2 nM [^{3}H]SPIRO and 100 nM (+)butaclamol to define non-specific binding.

Table 1. Parameters of [^{3}H]spiroperidol binding to D-2 dopaminergic and S-2 serotonergic binding sites in rat caudate membranes obtained by two different methods

Ions added to assay	Total (+)butaclamol displaceable binding (all curves best fit to two sites)				(+)Butaclamol displaceable binding, presence of ketanserin (all curves best fit to one site)		Ketanserin displaceable binding			
	D-2		S-2		D-2		S-2			
	K_D	B_{max}	K_D	B_{max}	K_D	B_{max}	K_D	B_{max}		
4 mM Mg^{2+}	0.018 nM ± 0.002 N = 3	22.1 pmoles/g ± 1.9	0.73 nM ± 0.29 N = 3	20.9 pmoles/g ± 6.0	0.021 nM ± 0.002 N = 3	24.0 pmoles/g ± 2.5	0.82 ± 0.52 N = 3	16.0 pmoles/g ± 7.8		
4 mM Mg^{2+} + 100 mM Na^+	0.016 ± 0.002 N = 2	23.6 ± 3.8	0.39 ± 0.25 N = 2	10.3 ± 2.4	0.016 ± 0.001 N = 3	24.8 ± 2.7	0.57 ± 0.01 N = 2	9.6 ± 3.5 N = 2		
None					0.0091 nM ± 0.0011 N = 2	24.7 pmoles/g ± 5.0	0.83 nM N = 1	28.6 pmoles/g		
4 mM Mg^{2+} + 300 μM GTP					0.015 ± 0.001 N = 2	24.7 ± 4.8				

Use of the S-2 serotonergic antagonist ketanserin to displace S-2 specific [^{3}H]spiroperidol binding (column 4) and to mask S-2 sites in the determination of D-2 specific [^{3}H]spiroperidol binding (column 3) gave K_D and B_{max} values indistinguishable from those obtained by the use of the computer program "LIGAND" of Munson, Rodbard and De Lean in the analysis of total (+)butaclamol displaceable [^{3}H]spiroperidol binding (column 1 and 2). Addition of 100 mM NaCl to the standard assay buffer, which contained 4 mM $MgSO_4$, EDTA 1 mM, MOPS 20 mM, Tris 19 mM, and ascorbic acid 1 mM produced a slight reduction in D-2 K_D which reached significance using the ketanserin mask method ($P < 0.05$, Student's t-test) but not by computer fitting. Assays routinely used 0.007–1.2 nM [^{3}H]spiroperidol with 100 μM (+)butaclamol to determine specific binding. Ketanserin concentrations were 200 times that of [^{3}H]spiroperidol at each concentration point. Values are mean ± standard error of the mean for N independent determinations.

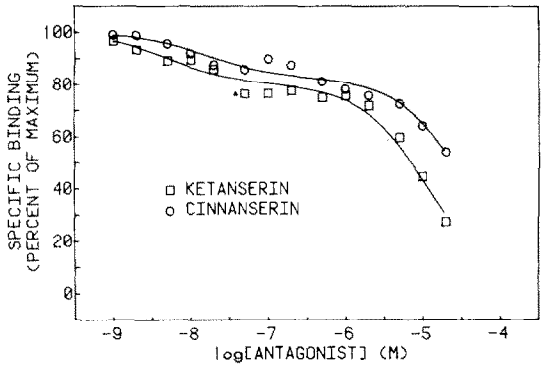

Fig. 2. Competition by ketanserin (R41468) and cinnanserin for [^{3}H]SPIRO binding in rat striatal membranes. Competition by both serotonergic antagonists produced biphasic curves with high (S-2 serotonergic) and low (D-2 dopaminergic) serotonin antagonist affinity components. The points represent actual data points while curves are computer generated best fits. The [^{3}H]SPIRO concentration here was 0.75 nM, and 100 nM (+)butaclamol was used to determine nonspecific binding.

antagonist, cinnanserin, produced similar competition curves, with respective affinities for the two sites of 0.99 and 2300 nM. That both compounds were competing with high affinity for the same subset of non-dopaminergic [^{3}H]SPIRO binding sites was supported by the observation that approximately equal fractions of [^{3}H]SPIRO binding were displaced with high affinity by both of these compounds, and that the high affinity components of the competitions by the S-2 antagonists were not additive. Separate experiments demonstrated similar results using both rat and bovine caudate under the conditions employed by Creese *et al.* [11]. Competition by the proposed D-2 selective blank 10 μM (±)ADTN [26] was additive to the high affinity component of the competition by these serotonergic antagonists, further confirming that ketanserin was relatively selective for the non-dopaminergic component of [^{3}H]SPIRO binding. Over the range of [^{3}H]SPIRO concentrations employed in saturation studies (0.007–1.2 nM), a concentration of ketanserin 200 times the [^{3}H]SPIRO concentration was selective for blocking the presumed S-2 specific [^{3}H]SPIRO binding in the presence or absence of 100 mM NaCl. Under these conditions, Scatchard plots became linear and modeled best to a single site (Fig. 1). The K_D and B_{max} values for D-2 specific [^{3}H]SPIRO binding determined in this fashion closely matched respective values determined for the higher affinity site by computer analysis of total (+)butaclamol displaceable binding (Table 1). Similarly, Scatchard plots of ketanserin displaceable (S-2 specific) [^{3}H]SPIRO binding were linear (not shown) and these data modeled to a single site. The parameters of this site were nearly identical to those of the low affinity [^{3}H]SPIRO site from the analysis of total (+)butaclamol displaceable binding (Table 1). Thus, the higher affinity component of [^{3}H]SPIRO binding is to a dopaminergic site. Similar conclusions using other compounds have been reached by Peroutka and Snyder [4], List and Seeman [6] and Withy *et al.* [5]. Addition of ketanserin at 200 times the [^{3}H]SPIRO concentration thus provided a very selective method for the elimination of S-2 binding in the study of dopaminergic [^{3}H]SPIRO binding, and was employed in all further studies below.

Inclusion of 100 mM NaCl to the assay significantly reduced the [^{3}H]SPIRO D-2 K_D from 0.021 to 0.016 nM as determined in the presence of ketanserin (Table 1). A similar slight reduction in this parameter was seen in computer analysis of total (+)butaclamol displaceable binding although using this method the difference did not reach statistical significance. NaCl addition did not alter significantly any other parameter of [^{3}H]SPIRO D-2 or S-2 specific binding (Table 1), nor did the addition of 300 μM GTP or the deletion of $MgSO_4$.

[^{3}H]*Domperidone saturation studies: labeling of equal numbers of non-serotonergic sites by* [^{3}H]*DOMP and by* [^{3}H]*SPIRO.* Several studies have now demonstrated the similar pharmacological profiles of the specific binding of [^{3}H]SPIRO and the binding of the butyrophenone-like ligand [^{3}H]DOMP, and some evidence indicates that [^{3}H]DOMP is completely D-2 specific [15]. After elimination of the major S-2 serotonergic [^{3}H]SPIRO binding artifact, it would then be expected that [^{3}H]SPIRO and [^{3}H]DOMP would specifically label equal numbers of (+)butaclamol displaceable sites representing the D-2 receptor. This was confirmed in saturation studies of [^{3}H]DOMP, which in the presence of $MgSO_4$ yielded a $B_{max} = 21.4 \pm 2.1$ pmoles/g tissue (N = 8) which was not significantly different from that obtained for [^{3}H]SPIRO (Fig. 3). The [^{3}H]DOMP K_D under these conditions was 0.25 ± 0.11 nM. As was seen with [^{3}H]SPIRO, inclusion of 100 mM NaCl in addition to 4 mM $MgSO_4$ did not alter either [^{3}H]DOMP K_D (0.25 ± 0.01 nM) or B_{max} (26 ± 6.5 pmoles/g, N = 2).

Antagonist competition for [^{3}H]*SPIRO binding.* In the presence of ketanserin to mask S-2 specific [^{3}H]SPIRO binding, antagonist competition for [^{3}H]SPIRO binding displayed a typical D-2 pharmacologic profile [1]. Curves were monophasic and steep ($n_H = 1$) and thus were best computer modeled to one site (Fig. 4). This was true not only of "typical" dopamine antagonists such as *cis*-flupentixol, and

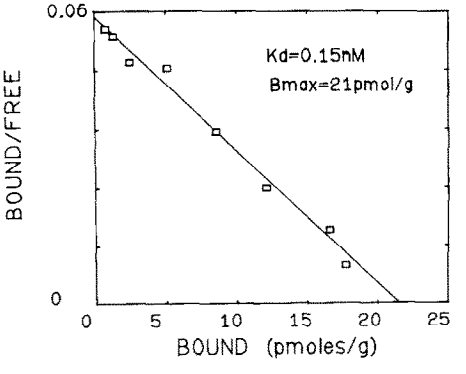

Fig. 3. Scatchard plot of [^{3}H]DOMP saturation. The data were computer fit best to one site. [^{3}H]DOMP concentration ranged from 0.006 to 0.90 nM. One hundred nanomolar (+)butaclamol was used to determine nonspecific binding.

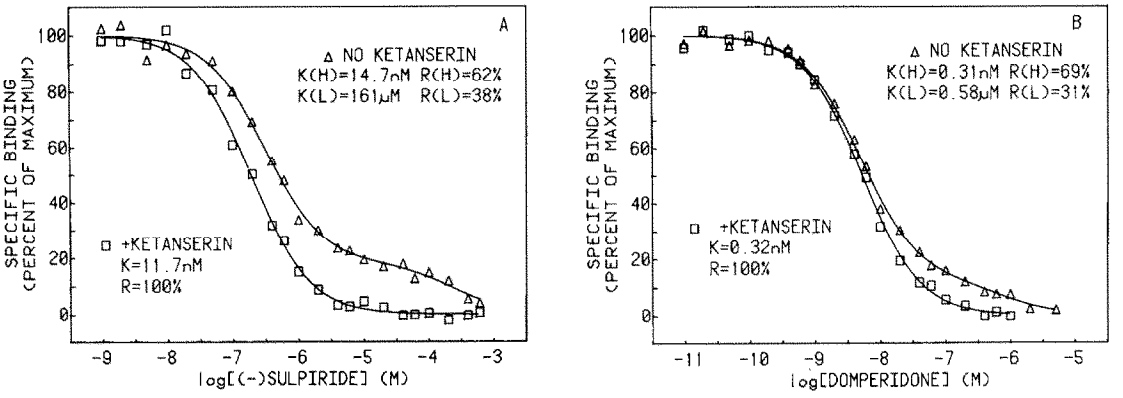

Fig. 4. Antagonist competition for [^{3}H]SPIRO binding in the presence and absence of ketanserin. Representative (−)sulpiride (A) and DOMP (B) competition curves fit best to a two-site model in the absence of ketanserin, but both competition curves are resolved by a one-site model when 50 nM ketanserin is included in the assay. Assays were conducted at 37° in the presence of 100 mM NaCl. In the absence of ketanserin, the dissociation constants for [^{3}H]SPIRO were constrained to 20 and 800 pM for the high and low affinity sites, respectively, and the inhibition constants for the competitors are denoted K_H and K_L for these two sites. In the presence of ketanserin the dissociation constant for [^{3}H]SPIRO was constrained to 20 pM. [^{3}H]SPIRO concentrations used in these experiments were 0.29–0.44 nM.

(+)butaclamol, but also for such "atypical" antagonists as DOMP, (−) sulpiride and metoclopramide. This is noteworthy as previous reports [27, 28] and our own observations (Fig. 4) demonstrated Hill slopes <1 for these atypical agents in competition for [^{3}H]SPIRO sites in the absence of serotonin blocking agents. This provided additional support for the homogeneity of (+)butaclamol displaceable [^{3}H]SPIRO binding in the presence of appropriate concentrations of ketanserin, and the identity of these sites as D-2 receptors. Domperidone affinity (K_i = 0.26 ± 0.11 nM, N = 4, absence of NaCl) at [^{3}H]SPIRO sites labeled in the presence of ketanserin under certain conditions (MOPS–Tris–EDTA–Mg buffer, 22°) in Fig. 3 was not significantly different from [^{3}H]DOMP affinity observed in saturation studies, confirming that [^{3}H]DOMP and [^{3}H]SPIRO (with ketanserin) label identical populations of sites.

A concentration of 100 mM NaCl has a negligible effect on the affinities of *cis*-flupentixol, (+)butaclamol, and DOMP for [^{3}H]SPIRO labeled D-2 sites (Fig. 5). This contrasted with the profound influence of NaCl on the affinity of the substituted benzamides, (-)sulpiride and metoclopramide. As was reported by Stefanini *et al.* [29], inclusion of sodium greatly increased potency of sulpiride at [^{3}H]SPIRO binding sites, by 400-fold from 360 ± 110 nM (N = 3) to 9 ± 3 nM (N = 2). We obtained similar results with the related substituted benzamide, metoclopramide. Competition curves in the presence or absence of 100 mM NaCl were both best fit by assuming a single population of sites for metoclopramide, with an 8-fold shift in affinity induced by NaCl, from 1100 to 130 nM (Fig. 5). Addition of 300 μM GTP had no effect on (−)sulpiride affinity (370 nM) in the presence of 4 mM magnesium ion.

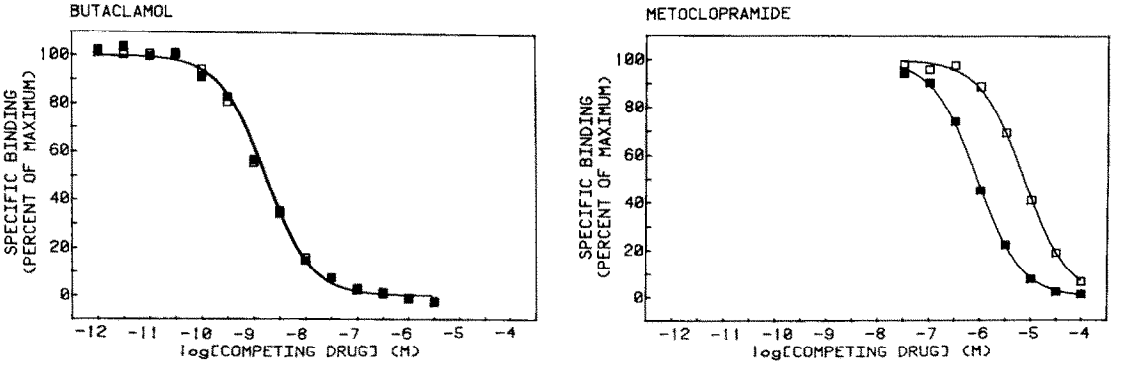

Fig. 5. Antagonist competition for [^{3}H]SPIRO binding in the presence of ketanserin. Addition of 100 nM NaCl had no effect on the affinity of butaclamol for D-2 sites labeled by [^{3}H]SPIRO. NaCl produced an approximate 8-fold increase in affinity of the substituted benzamide antagonist metoclopramide. All curves were best fit to a single site, confirming the homogeneity of sites labeled under these conditions. [^{3}H]SPIRO concentration used in these experiments was 0.11–0.18 nM, with 100 nM (+)butaclamol used to determine nonspecific binding ■, + 100 mM NaCl; □, no NaCl addition.

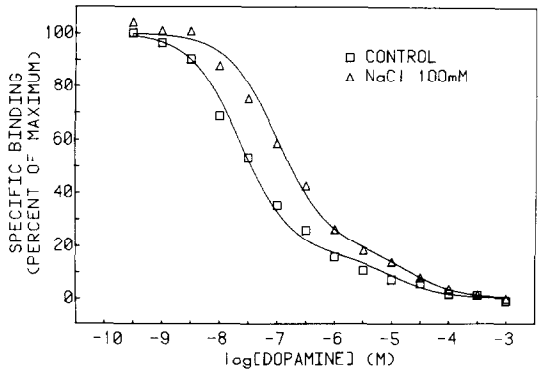

Fig. 6. Dopamine/[^{3}H]SPIRO competition: effect of NaCl. Unlike antagonist/[^{3}H]SPIRO competition curves, dopamine/[^{3}H]SPIRO curves were biphasic and were computer modeled best to two sites. The addition of 100 mM NaCl produced a rightward shift in the curve while maintaining heterogeneity of agonist affinity for labeled sites. The parameters for the two fitted sites in this experiment were: control, K_H = 3.0 nM, K_L = 573 nM, R_H = 82%, R_L = 18%; with NaCl, K_H = 13.2 nM, K_L = 2522 nM, R_H = 81%, R_L = 19%. The [^{3}H]SPIRO concentration used was 0.14 nM; nonspecific binding was determined in the presence of 100 nM (+)butaclamol. Ketanserin was included as described above to prevent labeling of S-2 serotonergic sites.

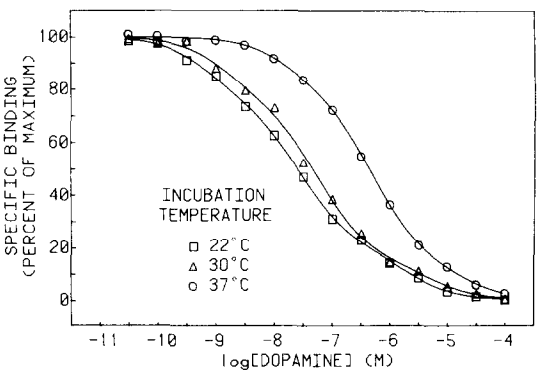

Fig. 7. Dopamine/[^{3}H]SPIRO competition: effect of incubation temperature. Elevation of incubation temperature to 30° had little effect on dopamine/[^{3}H]SPIRO curves in comparison with those obtained at the standard 22°. Elevation to 37° produced a marked rightward shift. The [^{3}H]SPIRO concentration here was 0.072 nM, with ketanserin included as above to block S-2 serotonergic binding. 100 nM (+)butaclamol was used as blank.

Agonist competition at D-2 sites labeled by ^{3}H-DOMP and ^{3}H-SPIRO. In contrast to the apparent site/state homogeneity of D-2 receptors observed in antagonist/^{3}H-antagonist competition experiments, the competition of agonists for ^{3}H-antagonist ligands was complex, modeling best to two sites (Fig. 6). Similar results were recently reported for agonist/^{3}H-antagonist competition at the anterior pituitary D-2 receptor [16]. Similar to what is observed at the frog erythrocyte beta adrenergic [22] and mammalian α_2-adrenergic receptor [30, 31], the D-2 receptor of bovine anterior pituitary exists in two convertible states with equal antagonist affinities but with differing agonist affinities. In rat striatal membranes at 22°, the high affinity phase of dopamine/[^{3}H]SPIRO competition had an affinity, denoted K_H, of 3.7 ± 0.7 nM (N = 5) and the low affinity sites possessed an affinity, denoted K_L, of 470 ± 160 nM. Similarly, dopamine/[^{3}H]DOMP competition curves modeled to two sites with K_H = 3.7 ± 1.8 nM (N = 4) and K_L = 250 ± 160 nM. The high affinity state, denoted R_H, accounted for 73 ± 4 and 81 ± 6% of [^{3}H]SPIRO and [^{3}H]DOMP binding, respectively. When combined with the B_{max} values for these ligands these yield approximate absolute values for [^{3}H]SPIRO R_H of 17.5 pmoles/g and for [^{3}H]DOMP R_H of 17.3 pmoles/g tissue.

We recently demonstrated [32] that under these conditions, selected to maximize high affinity specific agonist binding, [^{3}H]dopamine labeled with high affinity a portion of D-2 receptors in a magnesium ion dependent fashion. Under these conditions, using a 20-fold ratio of DOMP to [^{3}H]dopamine to define D-2 specific binding [32] saturation experiments demonstrated a [^{3}H]dopamine K_D of 3.7 ± 0.6 nM and B_{max} of 20 ± 2 pmoles/g tissue (N = 2). These values do not differ significantly from the K_H and R_H values obtained in dopamine/^{3}H-antagonist competition studies, providing additional evidence that under low temperature, sodium-free conditions [^{3}H]dopamine labels a high affinity state of the same D-2 receptor labeled by [^{3}H]SPIRO and [^{3}H]DOMP.

Agonist/[^{3}H]antagonist competition: effect of sodium ions and temperature. Previous studies demonstrated that high affinity D-2 specific [^{3}H]dopamine binding to magnesium preincubated tissue was greatly reduced with the addition of 10–100 mM NaCl or other sodium salts [32]. Because, as shown in Table 2, among the monovalent cations examined, sodium was, with lithium, the most potent ion in

Table 2. Inhibition by monovalent cations of D-2 specific [^{3}H]dopamine binding

	Salt added (100 nM)					Tris–HCl
	LiCl	NaCl	KCl	RbCl	CsCl	(pH 7.2/25°)
Percent of control D-2 specific binding	42.5 ± 5.2	55.6 ± 6.5	79.5 ± 9.1	98.6 ± 1.4	88.9 ± 11.1	90.0 ± 5.3
N	3	3	3	2	2	3

Values are percent specific binding relative to control containing no added monovalent metal cations. ± standard error of the mean for N independent determinations. All salts were added for a final cation concentration of 100 mM. All conditions included 4 mM $MgSO_4$. D-2 specific [^{3}H]dopamine binding was determined using 1 nM [^{3}H]dopamine and either spiroperidol or 20 nM domperidone as blank. Assays for these experiments only were performed as described [32], with 1 ml incubation vol and a 60 min incubation time. Tris–HCl used was titrated so as not to alter incubation pH.

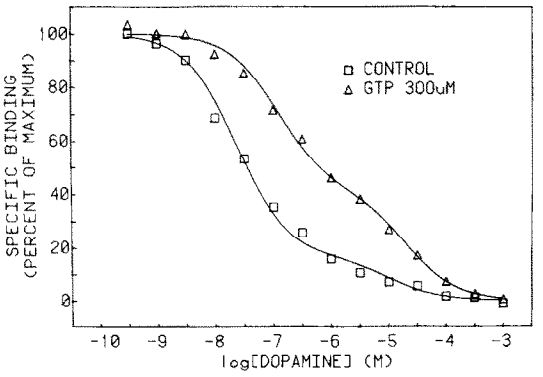

Fig. 8. Dopamine/[^{3}H]SPIRO competition: effect of guanine nucleotides at 22° in the absence of sodium. Addition of 300 μM GTP increased K_H, K_L, and R_L in dopamine competition at sites labeled by [^{3}H]SPIRO. Similar results were obtained with agonists other than dopamine or with 100 μM Gpp(NH)p instead of GTP. [^{3}H]SPIRO binding was performed in the presence of ketanserin as described above. The concentration of [^{3}H]SPIRO was 0.14 nM. (+) Butaclamol, 100 nM, was used as blank.

inhibiting D-2 specific [^{3}H]dopamine binding, we examined the effect of NaCl on dopamine/[^{3}H] SPIRO competition.

As would be expected, addition of 100 mM NaCl also produced a rightward shift in dopamine/[^{3}H] DOMP and [^{3}H]SPIRO competition curves (Fig. 6) by effecting a decrease in affinity of dopamine for both affinity states. Their respective proportions were not significantly changed. Values in the presence of 100 mM NaCl were $K_H = 40 \pm 24$ nM, $K_L = 2290 \pm 400$ nM, $R_H = 77 \pm 4\%$ and $R_L = 23 \pm 4\%$.

Most experiments reported here were performed with binding at 22°, which was previously found to be much more favorable for high affinity D-2 specific [^{3}H]dopamine binding than higher temperatures (unpublished observations). Conducting binding at 30° produced a small rightward shift in dopamine/[^{3}H]SPIRO competition curves, while at 37° this effect was much more marked (Fig. 7). Increasing temperature had an effect similar to that seen with addition of sodium—an increase in both K_H and K_L to 40 ± 28 nM and 2500 ± 1800 nM respectively with no change in proportion R_H (71 ± 10%, N = 2).

Agonist/[^{3}H]antagonist competition: effect of guanine nucleotides. The effect of GTP and its hydrolysis resistant analog, Gpp(NH)p, in reducing agonist affinity at striatal D-2 receptors is well known [12, 18]. Sibley *et al.* [16] recently demonstrated that similar effects at anterior pituitary D-2 receptors are accomplished by a conversion of the R_H state to the R_L state. The agonist/^{3}H-antagonist competition curves obtained at anterior pituitary D-2 receptors are similar to the two-site curves obtained above. In the presence of maximally effective concentrations of guanine nucleotides, curves become homogenous with respect to agonist affinity, and displacement curves model best to a single site with affinity, K_G, identical to the K_L observed in the absence of guanine nucleotide. We found that in our current system developed to maximize high affinity [^{3}H]dopamine binding, addition of 300 μM GTP or 100 μM Gpp(NH)p, concentrations which maximally inhibit D-2 specific [^{3}H]dopamine binding [32], had a different effect (Fig. 8). With the addition of 300 μM GTP, dopamine/[^{3}H]SPIRO competition curves were still best computer fit to two sites. Unlike the complete disappearance of R_H observed in anterior pituitary, only an incomplete decline in R_H, to 58 ± 1% (N = 3), was observed. Furthermore, both K_H and K_L values were increased, to 31 ± 9 and 5400 ± 2300 nM. Similar results were observed when [^{3}H] DOMP was used as ligand, or when Gpp(NH)p was used as nucleotide.

Further competition experiments were performed under conditions used by Sibley *et al.* [16]. While occasional experiments under these conditions, which include 96 mM NaCl and other ions (see Methods) in an incubation at 37°. shows that guanine nucleotide additions alter these agonist/^{3}H-antagon-

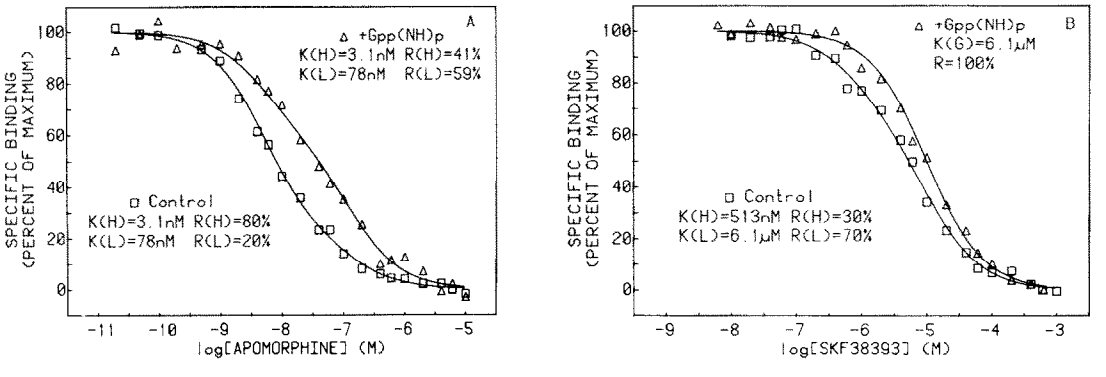

Fig. 9. Agonist/[^{3}H]DOMP competition: effect of guanine nucleotides at 37° in the presence of sodium. (A) Apomorphine competes for [^{3}H]DOMP biphasically in both the presence and absence of 100 μM Gpp(NH)p. The rightshift of the curve by Gpp(NH)p was modeled as a decrease in $\%R_H$. Curves were computer analyzed constraining K values to be shared for each component. The residual variances were not significantly different (F-test) from those arising when the curves were analyzed without this constraint. (B) SKF 38393/[^{3}H]DOMP competition is biphasic in the control but monophasic in the presence of Gpp(NH)p. $\%R_H$ is only 30% in control and K_G is modeled equal to K_L by simultaneous analysis of the two curves. Residual variances were not significantly different when K_L–K_G were shared or analyzed unconstrained.

Table 3. Computer fitted parameter estimates for affinities and relative site densities for high and low affinity interaction with D-2 receptor specific [^{3}H]spiroperidol and [^{3}H]domperidone binding

Ligand	Agonist	Constraints K_{cold} parameters	Control K_H (nM)	Control K_L (μM)	Control $\%R_H$	+Gpp(NH)p K_H (nM)	+Gpp(NH)p K_L (μM)	+Gpp(NH)p $\%R_H$	N
[^{3}H]SPIRO +ketanserin	Dopamine	unshared	56.8 ± 5.4	1.56± 0.23	69.8± 4.0	76.2 ± 9.4	2.56 ± 0.22	46.8 ± 1.9	6
		shared	68.4 ± 6.4	2.31 ± 0.19	76.4 ± 3.5	68.4 ± 6.4	2.31 ± 0.19	44.1 ± 1.5	
	Apomorphine	unshared	3.40 ± 0.51	61.0 ± 17.8	59.2 ± 7.7	4.21 ± 1.24	74.2 ± 14.7	34.2 ± 7.3	4
		shared	4.42 ± 0.35	68.9 ± 9.4	68.8 ± 1.3	4.42 ± 0.35	68.9 ± 9.4	34.8 ± 5.9	
[^{3}H]DOMP	Dopamine	unshared	41.4 ± 4.3	0.79 ± 0.08	68.2 ± 5.1	141 ± 23	4.80 ± 0.47	55.4 ± 2.8	5
		shared	80.0 ± 8.3	3.75 ± 0.26	86.3 ± 0.3	80.0 ± 8.3	3.75 ± 0.26	46.6 ± 4.0	
	Apomorphine	unshared	1.5 ± 0.4	33.8 ± 6.1	57.8 ± 7.1	5.3 ± 1.7	115 ± 29	42.0 ± 6.2	6
		shared	2.96 ± 1.06	72.8 ± 5.7	73.8 ± 2.4	2.96 ± 1.06	72.8 ± 5.7	32.7 ± 4.3	

Experiments were conducted under conditions described in Fig. 9. Computer analyses were conducted constraining K_D values for [^{3}H]spiroperidol and [^{3}H]domperidone to 20 and 330 pM respectively for all sites. "Unshared" fits allowed K_H, K_L, R_H and R_L values for each curve absence (control) or presence of 100 μM Gpp(NH)p to be derived from the best two-site fit. For shared K parameter fits, K_H and K_L parameters from paired control and +Gpp(NH)p curves were shared while R_H and R_L values were unconstrained for both curves. Such constraints did not significantly worsen the fits in 14 of 21 comparisons. Values shown are means ± S.E.M. Ligand concentrations were 0.30–0.44 nM and 0.20–0.43 nM for [^{3}H]spiroperidol and [^{3}H]domperidone respectively. $\%R_H$ represents the percent of total sites having high affinity for agonists. K_{cold} represents affinity parameter estimated (K_H and K_L) for the cold ligands (agonists)

ist competitions such that assuming a two-site model no longer fit significantly better than a one-site model, more detailed experiments indicate that agonist/^{3}H-antagonist competitions using the full D-2 agonists, dopamine and apomorphine, are still best fit by a two site model in both the presence and absence of guanine nucleotides. These findings (Fig. 9, Table 3) are similar to those shown in Fig. 8. Table 3 indicates that computer fitted apparent inhibition constants for agonists at both sites are increased (affinity decreased) by the addition of guanine nucleotides. In addition, the percentage of receptors existing in a state of high affinity for agonists ($\%R_H$) is decreased by guanine nucleotide addition. When competition data from experiments conducted in the presence and absence of guanine nucleotides were *simultaneously* computer analyzed, an equivocal result was obtained. In a slight majority of cases (14 of 21 total comparisons), constraining control agonist affinity constants K_H and K_L to share values with their respective constants from competitions containing guanine nucleotides did not significantly worsen the fits. However, in half the cases constraining fitted R_H and R_L values to be shared across curves [i.e. R_H (control) = R_H (guanine nucleotide); R_L (control) = R_L (guanine nucleotide)] also did not significantly worsen the fits. Nevertheless, Table 3 shows fitted parameter values for both unconstrained curves and curves where K_H and K_L values are shared across curves.

Similar to the agonists dopamine and apomorphine, competition experiments using a novel agonist SKF 38393 (Fig. 9B) and SKF 82526 (not shown) demonstrate biphasic curves in the absence of guanine nucleotides. However, unlike dopamine and apomorphine, the high affinity phase of competition for the SKF drugs comprises only about 30% of the total displacement curve. Furthermore, the addition of guanine nucleotides elicits monophasic competition curves whereby the affinity constants of the SKF drugs in the presence of guanine nucleotides (K_G) are not significantly different from their K_L values determined in the absence of guanine nucleotides (Fig. 9B).

DISCUSSION

Several arguments have previously been made to suggest that [^{3}H]SPIRO labels more than one subtype of dopamine receptor in mammalian striatum. Support for this contention includes non-linear Scatchard plots of [^{3}H]SPIRO saturations, complex curves with Hill slopes <1 for the competition of agonists and atypical neuroleptics for [^{3}H]SPIRO binding sites, and differences between the binding characteristics of [^{3}H]SPIRO and the atypical dopamine antagonist ligand, [^{3}H]sulpiride (for review, see [1]). In this paper we have addressed these difficulties and have presented evidence for the contrary view—that [^{3}H]SPIRO and [^{3}H]DOMP both label a single D-2 type dopamine receptor. We have described the use of serotonin antagonist ketanserin in the quantitative discrimination of S-2 serotonergic and D-2 dopaminergic [^{3}H]SPIRO binding. We have further presented evidence that this single D-2 receptor exists in two agonist-affinity states whose intercon-

version is mediated by GTP, as was recently demonstrated for the bovine anterior pituitary receptor [16].

It is now well documented that SPIRO or (+) butaclamol displaceable [^{3}H]SPIRO binding to mammalian striatal tissue is complex, labeling multiple sites as revealed by non-linear Scatchard plots [6, 28, 33–35] or by competition with atypical dopamine antagonists such as sulpiride, which yields complex curves (Hill slopes <1) [6, 7, 8, 28, 34]. This may be explained by the high affinity of [^{3}H]SPIRO for serotonergic S-2 receptors, allowing these, in addition to D-2 receptors, to be labeled by this ligand [3, 4]. The use of the dopamine agonist ADTN in place of (+)butaclamol as a D-2 selective blank [26] or of the serotonin antagonists mianserin [5, 8] or quinazolinedione R43448 [6] to mask serotonergic binding have been advocated as methods to direct [^{3}H]SPIRO specific binding exclusively to dopaminergic sites. Because of its high S-2/D-2 selectivity [25], we evaluated ketanserin (R41468) for suitability as an S-2 mask, and found that the use of ketanserin to define D-2 and S-2 specific [^{3}H]SPIRO binding yields values for K_D and B_{max} of these receptors indistinguishable from those obtained by evaluation of total (+)butaclamol displaceable binding by computer fitting. Ketanserin therefore represents a tool to quantitatively discriminate D-2 and S-2 type [^{3}H] SPIRO binding in *in vitro* assays. In the presence of ketanaserin, Scatchard plots of [^{3}H]SPIRO were linear. Using this technique, addition of sodium ion produced a very slight increase in the affinity of D-2 specific [^{3}H]SPIRO binding, although this was not apparent using computer modelling techniques. Addition of sodium or GTP, or the deletion of magnesium ion did not significantly alter other parameters of either D-2 or S-2 binding.

Previous studies [5, 6] also established that in the presence of a serotonergic blocker, [^{3}H]SPIRO saturations became linear, consistent with binding site homogeneity. This does not rule out, however, the possibility that binding to sites that are homogenous with respect to [^{3}H]SPIRO affinity might in fact be pharmacologically distinguishable as multiple sites using other compounds. It was thus important to demonstrate that the shallow competition curves observed for atypical antagonists at [^{3}H]SPIRO sites could be explained exclusively on the basis of S-2 serotonergic [^{3}H]SPIRO binding for which these drugs also had differential affinities. In the absence of ketanserin, (−)sulpiride and DOMP inhibited about 67% of [^{3}H]SPIRO binding with high (nanomolar) affinity and about 33% of the binding with much lower affinity (Fig. 4) suggesting that this lower affinity portion of the competition curve represents interaction at an S-2 site. Contrary results have been reported regarding this question [9] and by Huff and Molinoff [7] who failed to block S-2 sites. Data from competition experiments such as shown in Fig. 4 do not conform with the D-2 site A–site B hypothesis of Huff and Molinoff [7]. While these authors report that site A, comprising 20–30% of [^{3}H]SPIRO binding sites, has higher affinity for DOMP and lower affinity for sulpiride than does site B (70–80% of [^{3}H]SPIRO binding), we find that the minority site found under the conditions described for Fig. 4 (37°, 100 mM NCl) has lower affinity for both of these neuroleptics. The computed K_i values (DOMP 475 nM; (−)sulpiride 200 μM) were more consistent with an interaction at a serotonergic site. Furthermore we demonstrated that after elimination of S-2 binding, competition for [^{3}H]SPIRO sites by (−) sulpiride, metoclopramide, and DOMP were indeed homogenous, with competition curves being best fit to a single site. These results thus confirm the findings of Withy *et al.* [8], who examined binding to bovine caudate membranes in the presence of physiologic concentrations of sodium. We have now extended these results to rat striatal tissue, finding no evidence for a (possibly species specific) butaclamol displaceable "spirodecanone binding site" reported to interfere with [^{3}H]SPIRO D-2 assays by Gorissen *et al.* [36]. Furthermore, we demonstrate that the presence or absence of sodium ion, which exerts profound effects on both the binding of [^{3}H]sulpiride [37] and the competition of sulpiride for high affinity [^{3}H] SPIRO sites [29] has no effect on the conclusion reached—that [^{3}H]SPIRO, in the presence of ketanserin, labels a single non-serotonergic population of D-2 receptor sites.

[^{3}H]DOMP is a butyrophenone-like ligand thought to be completely specific for D-2 receptors [15]. It was therefore important to demonstrate that this ligand labeled sites equal in number to those labeled by [^{3}H]SPIRO in the presence of ketanserin. The B_{max} values for these two ligands were not significantly different, although the K_D of [^{3}H]DOMP was approximately 10 times that of [^{3}H]SPIRO. The affinity of [^{3}H]DOMP determined in saturation studies was in excellent agreement with the K_i of DOMP at D-2 specific [^{3}H]SPIRO sites, again confirming the identity of the sites labeled by these two ligands. We have also demonstrated that in addition to D-1 sites, [^{3}H]flupenixol also labels approximately 23 pmoles/g of D-2 sites under these conditions, with a K_D indistinguishable from the *cis*-flupentixol/[^{3}H] SPIRO K_i (0.1 nM), further strengthening this point (data not shown). It should be noted that exact correspondence of B_{max} values in the comparison of two such ligands may be impossible to obtain, as up to 10% error is expected in the determination of the ligand specific activity upon which the calculation of B_{max} is based (Nancy Wilbar, New England Nuclear Corp., personal communication). [^{3}H]SPIRO, when used with an appropriate S-2 blocking agent such as ketanserin, remains the D-2 ligand of choice for most applications, because its specific binding comprises a greater fraction of total binding: approximately 85 vs 65% for [^{3}H]SPIRO and [^{3}H]DOMP at their respective K_Ds.

The characteristics of binding of yet another antagonist radioligand, [^{3}H]sulpiride, has led some authors [37–39] to suggest that this ligand labels sites, at least in part, distinct from those labeled by [^{3}H]SPIRO. We found, however, that in the presence or absence of sodium ion, (−)sulpiride/[^{3}H]SPIRO competition curves modeled best to a single site. In the presence of sodium ion, the sulpiride K_i was as low as 9 nM, a value closely comparable to that observed for the K_D in our own preliminary saturation studies with [^{3}H]sulpiride (unpublished observations), as well as those reported by others [37, 40, 41] in the presence of sodium. The possibility that [^{3}H]sulpiride labels

additional sites is not excluded, but these data strongly suggest that [^{3}H]sulpiride must label with uniform affinity all D-2 sites labeled by [^{3}H]SPIRO. Freedman *et al.* [39] have demonstrated antagonist potencies and guanine nucleotide effects at [^{3}H]sulpiride binding sites similar to those observed at [^{3}H] SPIRO binding sites, lending additional support to our suggestion that these two ligands, in addition to [^{3}H]DOMP, label identical populations of D-2 receptors.

Sulpiride affinity at [^{3}H]SPIRO labeled D-2 sites was dependent upon sodium ion concentration, as was also reported by Stefanini *et al.* [29], with addition of 100 mM NaCl producing an approximate 40-fold increase in affinity. A similar increase of 8-fold was observed for metoclopramide. Sodium ion had little effect on the K_i of (+)butaclamol, *cis*-flupentixol, and DOMP, and little effect on the affinity of the radioligands [^{3}H]SPIRO and [^{3}H]DOMP. These results suggest that sodium may interact with the substituted benzamide molecules rather than the receptor itself. On the other hand, the interaction of a portion of the substituted benzamide molecule with an accessory site on the receptor may be sodium dependent. This effect does not then imply the existance of separate sodium dependent and independent dopamine antagonist binding sites as suggested by Theodorou *et al.* [37, 38].

Unlike the antagonist/^{3}H-antagonist curves described above which modeled best to a single class of sites, agonist/^{3}H-antagonist curves displayed a shallow slope ($n_H < 1$) and were computer fit to two receptor sites (R_H and R_L) with respective high (K_H) and low (K_L) agonist affinities. Sokoloff *et al.* [14] and Seeman [2] suggest on the basis of similar data that this is evidence that both [^{3}H]SPIRO and [^{3}H] DOMP label two distinct dopamine receptors distinguishable on the basis of different agonist affinities, which they refer to as D-2 and D-4 receptors. Recently, however, Sibley *et al.* [16] conclusively demonstrated that for the D-2 dopamine receptor labeled by [^{3}H]SPIRO in bovine anterior pituitary, such complex agonist/^{3}H-antagonist binding site interactions do not reflect the existence of multiple receptors, but of two interconvertible receptor states. GTP and the stable GTP analog guanylylimidodiphosphate [Gpp(NH)p] appear to convert the high agonist affinity (R_H) state of the receptor into the low agonist affinity (R_L) state. Evidence from striatal tissue suggests that magnesium or other divalent metal cations may promote the conversion of the R_L to the R_H state [32].

These data are consistent with a general "ternary complex model" as first applied to the frog erythrocyte beta adrenergic receptor, wherein adenylate cyclase stimulation by agonist is dependent upon the prior formation of a ternary complex of ligand, receptor, and a separate membrane-bound guanine nucleotide binding factor known as the "G-protein" [42]. A wealth of data demonstrates that the D-2 receptor does not stimulate adenylate cyclase [43] and in fact is probably linked in an inhibitory fashion to adenylate cyclase in striatum [44]. Ample evidence also exists that a G-protein is involved in the action of some receptors negatively as well as those positively coupled to adenylate cyclase [45]. Agonist and G-protein reciprocally increase the affinity of the other for their separate binding sites on the beta receptor [45]. This high affinity complex represents the receptor in the R_H state, and may be dissociated by GTP or Gpp(NH)p binding to G, causing a conversion to the R_L state. Antagonist affinity is not altered by and does not promote the attachment of G to the receptor.

Our data concerning the rat striatal D-2 binding site is in partial accord with this model. However, addition of guanine nucleotides does not abolish the heterogeneity of full-agonist's affinity for D-2 sites, and Hill coefficients remain less than one [46]. Competition curves in the presence of GTP are computer modeled best to a two site model with agonist affinities (K_H and K_L) similar to but slightly lower than those determined with no added GTP. In contrast, the novel agonists SKF 38393 and SKF 82526 behave comparably to agonists at the D-2 receptor in the bovine anterior pituitary as noted here (Fig. 9B) and earlier by Sibley *et al.* [47]. Such behaviour exhibited by the SKF drugs may be related to their activity as partial agonists [47], although the intrinsic activity of these two novel compounds at D-2 receptors has yet to be carefully evaluated. Nevertheless, SKF 38393 has been demonstrated to exert agonist activity on rat neurointermediate D-2 receptors [48]. If, however, one considers these data according to a two-step, three component ternary complex model the proportion of the high affinity agonist binding state should be dependent upon the ratio of receptor (R) and G-protein in the membrane and the dissociation constant for their association [49]. Consequently, full agonists at the D-2 receptor in the brain may still show some high affinity binding in the presence of maximally effective concentrations of guanine nucleotides if the affinity of the G-protein for the receptor is only moderately decreased in the presence of guanine nucleotides. In the case of the SKF drugs (partial agonists?) R-G-protein affinity may be weaker than for full agonists in both the presence and absence of guanine nucleotides. Thus, the percentage of R_H (A-R-G) is less (30%) than seen for full agonists (60–70%) in both control curves and in the presence of guanine nucleotides (0 vs 30–50%). While our data are not conclusively linked to such a model, the model offers a logical alternative to invoking multiple D-2 receptor subtypes. Previously, utilizing kainate lesion of the striatum to isolate D-2 receptors on cortical striate terminals [50], we found that dopamine displacement of [^{3}H]SPIRO binding was no longer GTP sensitive although the curves were still shallow. This suggested the presence of both GTP sensitive and insensitive [^{3}H]SPIRO binding sites in the striatum. We are presently reevaluating these findings utilizing computer assisted curve analysis to determine the veracity of these findings in lesioned tissue.

We recently described conditions under which D-2 specific high affinity [^{3}H]dopamine binding could be obtained [32]. Previous studies employing incubation buffers including sodium ion [51] or incubation at 37° [52] failed to demonstrate D-2 type [^{3}H]dopamine binding [32]. We previously determined that sodium ion at 10–100 mM and elevated temperature decreased high affinity D-2 specific [^{3}H]dopamine binding.

We have now demonstrated that high affinity competition at the R_H state of the D-2 receptor labeled by [^{3}H]SPIRO displays similar sensitivities. Addition of 100 mM NaCl increases both K_H and K_L without affecting the relative proportion of R_H and R_L. Increasing temperature from 22° used in our standard assays to 37° has a similar effect. Saturation studies of high affinity [^{3}H]dopamine binding displaceable by low concentrations of DOMP demonstrate a nearly identical affinity and capacity to the K_H and R_H determined under identical conditions in dopamine/^{3}H-antagonist competition experiments. These experiments confirm our previous studies which demonstrated that such high affinity [^{3}H]dopamine binding is to a high affinity state of the D-2 receptor.

Our studies demonstrate that interconversion of the R_H and R_L state is not identical under all assay conditions or for all agonists. When performed under conditions that maximize high affinity agonist binding, dopamine/^{3}H-antagonists reveal a very high proportion of R_H that is partially resistant to the effects of GTP. The contribution of the different variables contributing to this effect is under current study.

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