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[^{3}H]Ro 22-1319 (Piquindone) Binds to the D_2 Dopaminergic Receptor Subtype in a Sodium-Dependent Manner

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SUMMARY

Ro 22-1319, a novel pyrroloisoquinoline compound, is a potent antipsychotic agent with low potential for extrapyramidal effects and tardive dyskinesia. In this study, the specific binding of [^{3}H]Ro 22-1319 to the rat striatal homogenates has been examined. The binding of [^{3}H]Ro 22-1319 was found to be critically dependent on the presence of sodium in the incubation medium. There appeared to be a single saturable binding component for [^{3}H]Ro 22-1319 with a high affinity. The binding sites showed a stereochemical specificity for (−)Ro 22-1319, (+)butaclamol, and (α)flupenthixol. Ro 22-1319 and three D_2 antagonistic antipsychotics (sulpiride, metoclopramide, and molindone) exerted a more potent inhibition of [^{3}H]Ro 22-1319 binding than of [^{3}H]spiroperidol binding, whereas other classical antipsychotics were almost equipotent at both binding sites. The requirement for sodium to detect Ro 22-1319 binding was also verified by the use of [^{3}H] spiroperidol binding. The competition curves of Ro 22-1319, sulpiride, metoclopramide, and molindone for [^{3}H]spiroperidol binding were shifted to the right by the omission of sodium in the incubation medium, whereas spiroperidol, chlorpromazine, and domperidone were equiactive under both conditions. These results suggest that Ro 22-1319 has a sulpiride-like property and binds to a D_2 dopaminergic receptor subtype in a sodium-dependent manner. Nineteen pyrroloisoquinoline derivatives were also tested for their inhibitory effects on [^{3}H]Ro 22-1319 and [^{3}H] spiroperidol binding. An interesting finding was that small changes in chemical structure modulated the potency at D_2 dopaminergic receptor subtypes. Thus, the compounds having a nonlipophilic functional group on the basic nitrogen (Ro 22-1319, Ro 22-3822, etc.) showed a stronger potency at [^{3}H]Ro 22-1319 receptors whereas the compounds having a lipophilic group (Ro 22-6600, etc.) were nonselective antagonists at both [^{3}H]Ro 22-1319- and [^{3}H]spiroperidol-binding sites. However, all pyrroloisoquinoline derivatives including Ro 22-6600 showed a sodium dependency for [^{3}H]spiroperidol-binding sites, indicating that the functional moiety which displays a sodium dependency may be the pyrroloisoquinoline moiety itself.

INTRODUCTION

Ro 22-1319 (2,6-dimethyl-3-ethyl-4,4*a*,5,6,7,8,8*a*,9-octahydro-4*a*,8*a*-*trans*-1*H*-pyrrolo[2,3-*g*]isoquinolin-4-one, piquindone, Fig. 1) is a new antipsychotic pyrroloisoquinoline derivative which was designed on the basis of a hypothetical model of the interaction of antipsychotic drugs with the dopamine receptor (1, 2). Ro 22-1319 was reported to be at least five times more potent than chlorpromazine and approximately half as potent as haloperidol in two discrete avoidance procedures in rats (lever press and pole climb) and in continuous avoidance in rats and squirrel monkeys, with low potential for extrapyramidal effects and tardive dyskinesia (3). In biochemical tests, Ro 22-1319 exerted no inhibition of dopamine-stimulated adenylate cyclase activity and a weak inhibition of [^{3}H]spiroperidol binding (2). These data suggest the possibility that Ro 22-1319 elicits its antipsychotic effect through receptors different from those identified by classical antipsychotics. To substantiate this hypothesis, we have examined the binding characteristics of [^{3}H]Ro 22-1319 to rat striatal homogenates, and compared them with properties of [^{3}H]spiroperidol binding. The conclusion drawn from the present work is that Ro 22-1319 has a sulpiride-like property and binds to a D_2 dopaminergic receptor subtype in a sodium-dependent manner. The chemical structure required for a selective D_2 antagonist is also discussed.

MATERIALS AND METHODS

Tissue preparations. For [^{3}H]Ro 22-1319-binding studies, the striatal homogenates were prepared from male Wistar rats (weighing 180–250 g). Unless otherwise stated, the striatum was homogenized in 19 volumes of 50 mM Tris-maleate buffer (pH 7.5) containing 0.1 mM EGTA[1]

[1] The abbreviation used is: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

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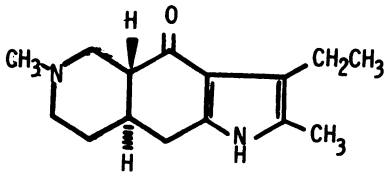


FIG. 1. *Structure of Ro 22-1319*

using a glass-Teflon Potter homogenizer, and stored in small aliquots at −20° until use. For [^{3}H]spiroperidol-binding studies, the striatal P_2 fraction was prepared from bovine brain. The P_2 fraction (crude mitochondrial fraction) was prepared according to the method of Gray and Whittaker (4) and was suspended in 50 mM Tris-HCl (pH 7.5) or 50 mM Tris-maleate buffer (pH 7.5) containing 0.1 mM EGTA.

[^{3}H]Ro 22-1319 binding assay. Unless otherwise stated, 5 nM [^{3}H] Ro 22-1319 was incubated under constant shaking for 15 min at 37° with the striatal homogenates (1.5 mg protein) in 50 mM Tris-maleate buffer (pH 7.5) containing 0.1 mM EGTA and compounds to be tested in a total volume of 1 ml. Following incubation, the samples were rapidly filtered through Whatman GF/B glass fiber filters under reduced pressure. The filters were washed three times with 5 ml of ice-cold buffer, and were subsequently counted by liquid scintillation spectrometry. The binding of [^{3}H]Ro 22-1319 to the striatal homogenates in the presence of 10 μM unlabeled Ro 22-1319 was referred to as the nonspecific binding of [^{3}H]Ro 22-1319. Other competitors reduced binding to the same amount, and thus competition with 10 μM Ro 22-1319 appears to be a valid definition for specific binding. Under the standard assay conditions in the presence of 5 nM [^{3}H]Ro 22-1319 at 37°, the specifically bound radioactivity was about 70% of the total bound radioactivity. The [^{3}H]Ro 22-1319 (13 Ci/mmol) was shown to be at least 95% radiochemically pure throughout this study by TLC.

[^{3}H]Spiroperidol binding assays. Unless otherwise stated, the bovine striatal P_2 fraction (1.5 mg protein) was incubated for 15 min at 37° with 0.25 nM [^{3}H]spiroperidol and 50 mM Tris-maleate buffer, pH 7.5, containing 0.1 mM EGTA in a total volume of 10 ml. In experiments designed to examine the sodium dependency, 50 mM Tris-HCl buffer (pH 7.5) was used with or without 120 mM NaCl. The binding of [^{3}H] spiroperidol to bovine striatal P_2 fractions in the presence of 1 μM (+)butaclamol was defined as the nonspecific binding of [^{3}H]spiroperidol.

Protein content was determined by the method of Lowry *et al.* (5) using bovine serum albumin as standard.

We obtained comparable IC_{50} values for antipsychotics competing for [^{3}H]spiroperidol binding to bovine and rat striatal preparations (Fig. 2). Consequently, we feel it is valid to compare [^{3}H]spiroperidol binding in bovine striatal preparations to [^{3}H]Ro 22-1319 binding obtained in rat striatal membranes. All data shown for [^{3}H]spiroperidol were obtained with bovine striatal P_2 fractions.

Drugs and chemicals. Tritium-labeled Ro 22-1319 (*N*-methyl[^{3}H], 13 Ci/mmol) was donated by Dr. G. Olson (F. Hoffmann-La Roche, Nutley, NJ). 1-Phenyl-4-[^{3}H]spiroperidol (25.64 Ci/mmol) was purchased from New England Nuclear. All antipsychotics were donated by F. Hoffmann-La Roche (Basle, Switzerland and Nutley). All other chemicals and materials were purchased from local commercial sources.

RESULTS

Establishment of experimental conditions for [^{3}H]Ro 22-1319 binding. To ensure proper kinetic conditions and to optimize and maximize the specific [^{3}H]Ro 22-1319 binding, the effects of varying the protein and electrolyte composition of the medium were examined. The specific [^{3}H]Ro 22-1319 binding at 37° was linearly related to tissue concentration up to 2.5 mg protein per assay, when the ligand concentration was set at 5 nM. The binding of [^{3}H]Ro 22-1319 was found to be critically dependent on the presence of sodium in the incubation medium. The

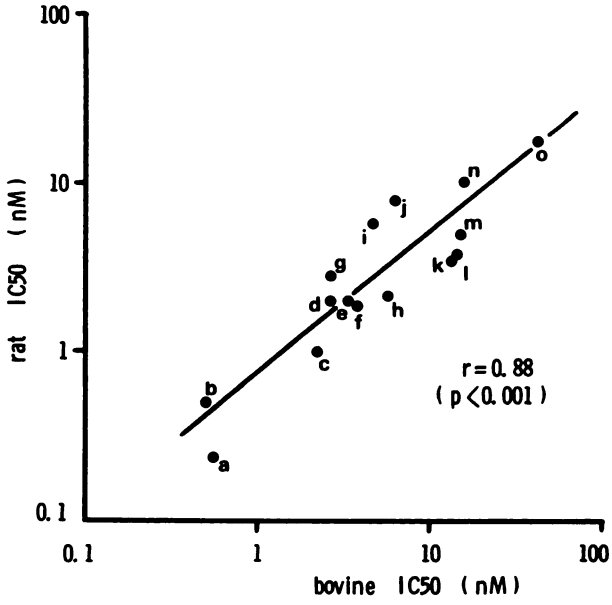


FIG. 2. *Correlation of antipsychotic drug affinity for [^{3}H]spiroperidol binding to bovine and rat striatal preparations*

The P_2 fraction of bovine or rat striatum was incubated with 0.25 nM [^{3}H]spiroperidol and 50 mM Tris-maleate buffer (pH 7.5) containing 0.1 mM EGTA for 15 min at 37°. The concentration of each compound that inhibited specific binding 50% (IC_{50}) was determined by log-probit analysis of at least five different concentrations. Points shown are the means from at least three separate experiments. *a*: spiroperidol; *b*: octoclothepine; *c*: pimozide; *d*: fluphenazine; *e*: trifluperidol; *f*: triflupromazine; *g*: (+)butaclamol; *h*: flupenthixol; *i*: chlorprothixene; *j*: penfluridol; *k*: prochlorperazine; *l*: haloperidol; *m*: thiethylperazine; *n*: chlorpromazine; *o*: thioridazine.

TABLE 1

Effects of different monovalent cations on [^{3}H]Ro 22-1319 binding

The binding assay was performed on whole homogenates of rat striatum. The tissue was incubated with 5 nM [^{3}H]Ro 22-1319 and 50 mM Tris-HCl buffer (pH 7.5) for 120 min at 0° in the presence and absence of 10 μM unlabeled Ro 22-1319. Each value represents the mean ± standard error of the mean of three separate experiments; each was performed in triplicate.

Salt	Concentration	[^{3}H]Ro 22-1319 binding	
	mM	*fmol/mg protein*	*% control*
None		56.2 ± 10.0	100
NaCl	30	149.9 ± 8.1	267
	150	146.1 ± 23.1	260
KCl	30	66.8 ± 1.7	119
	150	83.9 ± 11.8	149
LiCl	30	94.4 ± 14.2	168
	150	94.8 ± 11.5	169
Choline Cl	30	57.8 ± 11.8	105
	150	27.4 ± 4.1	49

specificity of NaCl on [^{3}H]Ro 22-1319 binding was investigated by comparative studies with the chlorides of different monovalent cations (Table 1). LiCl was stimulatory, although less so than NaCl, and KCl was weakly stimulatory. Choline Cl had an inhibitory effect at high concentrations. These results show that the stimulatory activity of NaCl was not attributable to an increase in the ionic strength of the medium or to Cl^-. Figure 3 shows the concentration-response relationships for the

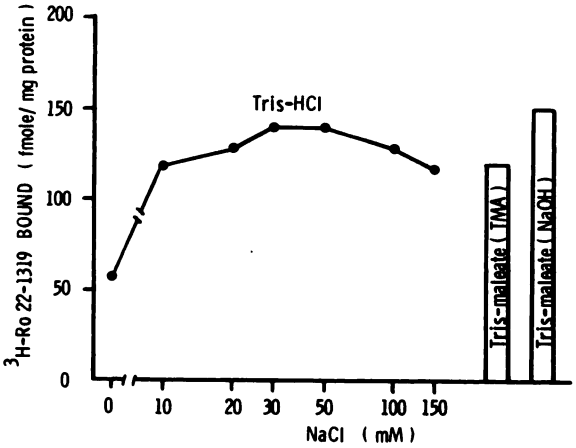


FIG. 3. *Effects of NaCl on [^{3}H]Ro 22-1319 binding to rat striatal homogenates*

Rat striatal homogenates were incubated with 5 nM [^{3}H]Ro 22-1319 and 50 mM Tris-HCl buffer (pH 7.5) for 120 min at 0°. Tris-maleate (TMA) and Tris-maleate (NaOH) denote 50 mM Tris-maleate buffer in which pH was adjusted by using tetramethylammonium and NaOH, respectively. Tris-maleate (TMA) contained 60 mM NaCl and Tris-maleate (NaOH) contained 60 mM Na^+; Points shown are the means from two separate experiments.

effect of NaCl. Incubation of the rat striatal homogenates with 10–150 mM NaCl resulted in an up to 3-fold stimulation of [^{3}H]Ro 22-1319 binding. The maximum binding of [^{3}H]Ro 22-1319 was observed at a sodium concentration of around 50 mM when 50 mM Tris-HCl buffer (pH 7.5) was used. The amount of specifically bound [^{3}H]Ro 22-1319 at 5 nM was somewhat higher in 50 mM Tris-maleate buffer (pH 7.5) containing 0.1 mM EGTA than in 50 mM Tris-HCl buffer (pH 7.5) containing 10–150 mM NaCl. Tris-maleate buffer contains about 60 mM Na^+ due to the pH adjustment by NaOH. The amount of specifically bound [^{3}H]Ro 22-1319 was slightly lower in Tris-maleate buffer by using tetramethylammonium to adjust the pH (containing 60 mM NaCl). Accordingly, throughout this investigation, Tris-maleate buffer (NaOH) was used.

Time course of [^{3}H]Ro 22-1319 binding to the rat striatal homogenates. As shown in Fig. 4a, binding of 5 nM [^{3}H]Ro 22-1319 increased rapidly and reached a plateau at 10 min, and steady state levels of binding remained stable for at least until 60 min when assayed at either 37 or 25°. At 0°, the binding proceeded more slowly and reached steady state at 30 min. The steady state levels of the binding at 37 or 25° were approximately half the values at 0°. The data at 0° presented in Fig. 4a were replotted in the Fig. 4b and a second order association rate constant k_{+1} = 0.00236 nM^{-1} min^{-1} (n = 3) was calculated. As shown in Fig. 4c, we examined the rate of dissociation using 10 μM unlabeled Ro 22-1319 to compete for the rebinding of specifically bound [^{3}H]Ro 22-1319. A first order dissociation rate constant k_{-1} of 0.0057 min^{-1} (n = 3) was obtained. The ratio k_{-1}/k_{+1} (2.4 nM) of the rate constants at 0° provides an estimate of the dissociation constant (K_D) for the interaction of [^{3}H]Ro 22-1319 with the striatal binding sites. At 37°, the rate of dissociation was rapid with k_{-1} value of about 1.0 min^{-1}. The K_D value at 37° could not be calculated using

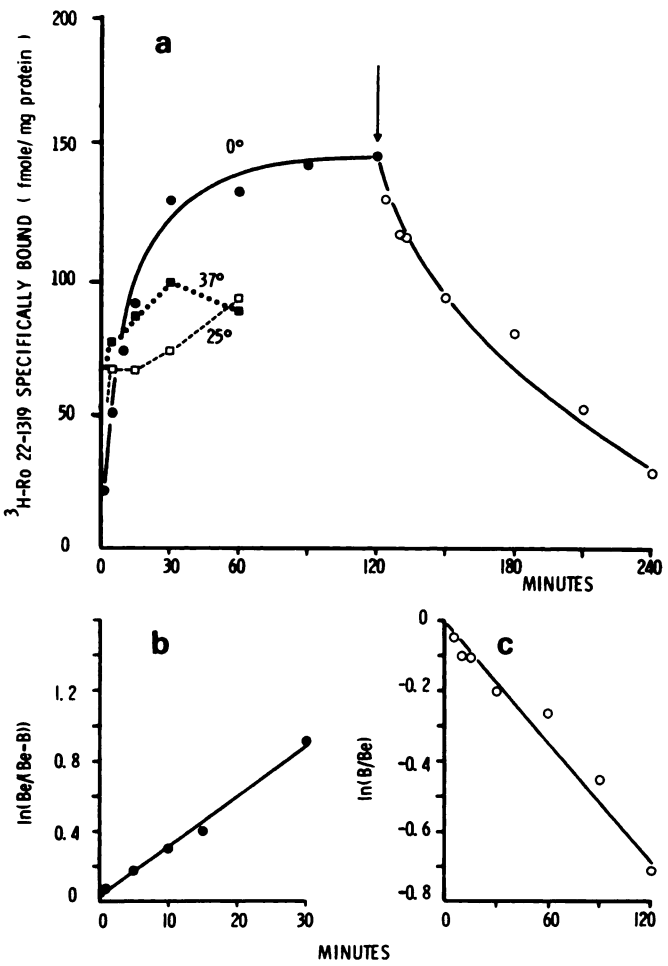


FIG. 4. *Kinetics of specific [^{3}H]Ro 22-1319 binding to rat striatal homgenates*

a, time course of specific [^{3}H]Ro 22-1319 binding at 37, 25, and 0° at a ligand concentration of 5 nM. Rat striatal homogenates (1.5 mg protein) were incubated with 50 mM Tris-maleate buffer (pH 7.5) containing 0.1 mM EGTA in the presence and absence of 10 μM unlabeled Ro 22-1319 in a total volume of 1 ml. At the arrow, Ro 22-1319 (10 μM) was added to a parallel set of tubes and both total binding and dissociation of the [^{3}H]Ro 22-1319:receptor complex were monitored for an additional 2 hr at 0°. b, pseudo-first order kinetic plot of initial [^{3}H]Ro 22-1319 binding at 0°, in which B is the amount of [^{3}H]Ro 22-1319 specifically bound at each time and Be is the amount of [^{3}H]Ro 22-1319 specifically bound at equilibrium. The slope of this line is k_{ob}, equal to the observed rate constant for the pseudo-first order reaction. The second order association rate constant, k_{+1} is calculated from $k_{+1} = (k_{ob} - k_{-1})/([^3H]Ro\ 22\text{-}1319)$ in which k_{-1} is the first order rate constant for dissociation and ([^{3}H]Ro 22-1319) is the concentration of [^{3}H]Ro 22-1319 used in the experiment (5 nM). c, rate of dissociation for [^{3}H]Ro 22-1319 specifically bound. Points shown are the means from three separate experiments.

rate constants because of the rapid rate of association and dissociation at this temperature.

Steady state studies of [^{3}H]Ro 22-1319 binding. As shown in Fig. 5, the specific binding of [^{3}H]Ro 22-1319 increased with increasing concentrations of free [^{3}H]Ro 22-1319 and was saturable. Scatchard analysis of three separate experiments revealed a single class of binding sites with an apparent dissociation constant (K_D) of 3.1 ± 0.3 nM (mean ± standard error) at 0° for the 120-min

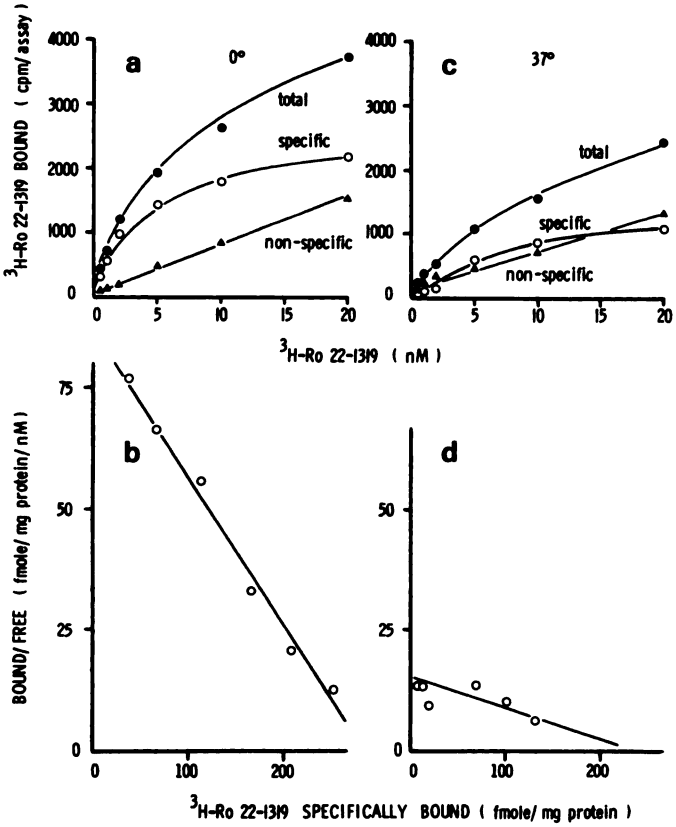


FIG. 5. *Saturation of specific [³H]Ro 22-1319 binding to rat striatal homogenates*

Rat striatal homogenates (1.5 mg protein) were incubated with increasing concentrations of [³H]Ro 22-1319 (0.5–20 nM) at 0°(a) and 37°(c), and binding was determined as described under Materials and Methods. Specific [³H]Ro 22-1319 binding (O) represents the difference between total (●) and nonspecific (▲) binding ([³H]Ro 22-1319 bound in the presence of 10 μM unlabeled Ro 22-1319). Scatchard analysis of specific [³H]Ro 22-1319 binding at 0°(b) and 37°(d). Each point represents the mean from a typical single experiment, each performed in triplicate.

incubation and of 17.6 ± 0.7 nM at 37° for the 15-min incubation. The maximal number of binding sites (B_{max}) was the same under both conditions (260 ± 21.3 and 287 ± 31.3 fmol/mg protein, respectively), and the respective Hill coefficients were 1.00 ± 0.02 and 1.04 ± 0.08.

Regional distribution of [³H]Ro 22-1319 and [³H]spiroperidol binding sites in rat brain. The result of regional distribution for [³H]Ro 22-1319 binding sites is summarized in Table 2. For these studies, the radioligand was present at 5 nM and incubations were for 120 min at 0°. The highest level of binding was observed in the striatum, followed by the olfactory tubercle and olfactory bulb. The binding capacity in the striatum was 260 fmol/mg for [³H]Ro 22-1319 and 1050 fmol/mg protein for [³H] spiroperidol. The distribution profile of [³H]Ro 22-1319-binding sites was similar to that of [³H]spiroperidol-binding sites, although [³H]spiroperidol bound to the cortical regions more intensely than [³H]Ro 22-1319.

Influence of various antipsychotics and other agents on specific [³H]Ro 22-1319 binding at 37°. Although the optimal temperature for determination of [³H]Ro 22-1319 binding was 0°, the competition experiments were

TABLE 2

Regional distribution of [³H]Ro 22-1319 and [³H]spiroperidol binding in rat brain

The binding assay was performed on whole homogenates of various regions of rat brain. For [³H]Ro 22-1319 binding, the tissue was incubated with 5 nM [³H]Ro 22-1319 and 50 mM Tris-maleate buffer (pH 7.5) containing 0.1 mM EGTA for 120 min at 0° in the presence and absence of 10 μM unlabeled Ro 22-1319. For [³H]spiroperidol binding, the tissue was incubated with 0.25 nM [³H]spiroperidol and 50 mM Tris-maleate buffer (pH 7.5) containing 0.1 mM EGTA for 15 min at 37° in the presence and absence of 1 μM (+)butaclamol. Each value represents the mean ± standard error of the mean of three separate experiments, each performed in triplicate.

Brain region	Specifically bound	
	[³H]Ro 22-1319	[³H]Spiroperidol
	fmol/mg protein	
Olfactory bulb	37 ± 5	37 ± 8
Olfactory tubercle	39 ± 7	78 ± 15
Septum	26 ± 5	36 ± 2
Hippocampus	13 ± 4	16 ± 6
Striatum	136 ± 15	199 ± 29
Hypothalamus	24 ± 5	24 ± 4
Midbrain	20 ± 4	26 ± 6
Pons-medulla	20 ± 2	13 ± 4
Cerebellum	9 ± 1	11 ± 1
Frontal cortex	21 ± 6	72 ± 10
Motor cortex	18 ± 4	46 ± 2
Sensory cortex	29 ± 5	56 ± 6
Visual cortex	20 ± 1	28 ± 7
Auditory cortex	14 ± 2	28 ± 2

conducted at 37°, because antipsychotics are known to bind poorly at 0°. Indeed, the amounts of [³H]spiroperidol and [³H]haloperidol binding to the rat and bovine striatum at 0° were far lower than those at 37° (data not shown). [³H]Ro 22-1319 binding sites showed a stereochemical specificity for (−)Ro 22-1319, (+)butaclamol, and (α)flupenthixol (Fig. 6); the pharmacologically potent (−)Ro 22-1319, (+)butaclamol, and (α)flupenthixol, had a 1700-, 150-, and 37-fold higher potency than their respective, pharmacologically weak enantiomers. As shown in Table 3, all antipsychotics tested inhibited the [³H]Ro 22-1319 binding with nanomolar range with Hill coefficients of about 1.0 (data not shown). Ro 22-1319 and three D_2 antagonistic antipsychotics (sulpiride, metoclopramide, and molindone) exerted a more potent inhibition of [³H]Ro 22-1319 binding than of [³H]spiroperidol binding, whereas all the other antipsychotics tested had a strong potency at [³H]spiroperidol binding sites. Indeed, these four compounds displayed ratios of IC_{50} for [³H]spiroperidol/[³H]Ro 22-1319 greater than 10, while others had ratios less than 2 except domperidone (Table 3). Dopamine, (+)-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydro-naphthalene, and apomorphine weakly inhibited [³H]Ro 22-1319 binding with respective Hill coefficients of 0.50, 0.64, and 0.65, whereas serotonin, propranolol, phentolamine, mianserine, and levallorphan demonstrated low potencies at [³H]Ro 22-1319-binding sites (Table 4).

Competition for [³H]spiroperidol binding in the presence and absence of 120 mM NaCl. Inhibitory effects of

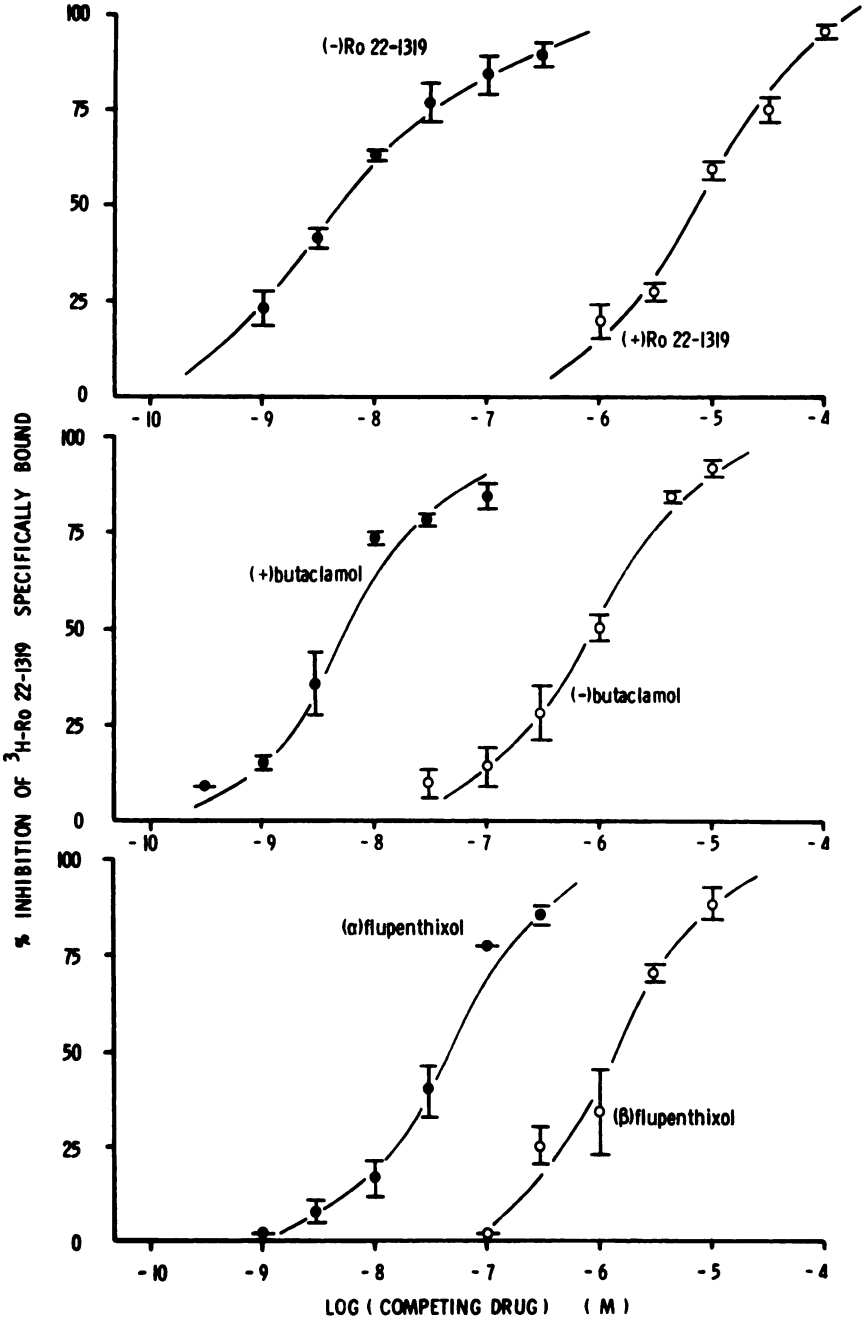


FIG. 6. *Stereochemical specificity of [^{3}H]Ro 22-1319 binding*
The binding assay was performed at 37° as described under Materials and Methods. Each point represents the mean + standard error of three to four separate experiments.

Ro 22-1319 and some antipsychotics on [^{3}H]spiroperidol binding to bovine striatal P_2 fraction were tested in the presence and absence of 120 mM NaCl. Figure 7 gives the competition binding curves of the compounds under both conditions. The competition curves of Ro 22-1319, sulpiride, molindone, and metoclopramide for [^{3}H]spiroperidol binding were shifted to the right when sodium was omitted from the incubation medium, whereas spiroperidol chlorpromazine and domperidone were equiactive in both the absence and presence of sodium.

Competition of pyrroloisoquinoline derivatives for [^{3}H] Ro 22-1319 and [^{3}H]spiroperidol binding at 37°. Nineteen pyrroloisoquinoline derivatives all competed for [^{3}H]Ro 22-1319 binding in the nanomolar range (Table 5). Among them, the compounds having nonlipophilic moieties on the basic nitrogen (Ro 22-1319, Ro 22-3822, Ro 22-4645, Ro 22-3439, etc.) were stronger competitors at [^{3}H]Ro 22-1319-binding sites than at [^{3}H]spiroperidol-binding sites (these compounds had ratios of greater than 5). In contrast, an introduction of a lipophilic functional group to the molecule seems to create nonselective competitors (Ro 22-6600, Ro 22-4353, Ro 22-5092, Ro 22-5226, etc.; these compounds had ratios of less than 1). However, the sodium dependency of the binding of these compounds in competing for [^{3}H]spiroperidol binding sites was also observed (Table 5).

TABLE 3

Comparison between potencies of antipyschotics at [^{3}H]Ro 22-1319- and [^{3}H]spiroperidol-binding sites

The competition for specific [^{3}H]Ro 22-1319 binding to rat striatal homogenates and of [^{3}H]spiroperidol binding to bovine striatal P_2 fraction by various antipsychotics was assayed using the standard procedures under Materials and Methods. The ligand concentration was 5 nM for [^{3}H]Ro 22-1319 and 0.25 nM for [^{3}H]spiroperidol. Incubation was performed for 15 min at 37°. The concentration of each compound that inhibited specific binding 50% (IC_{50}) was determined by log-probit analysis of at least five different concentrations. Each value represents the mean ± standard error of the mean of at least three separate experiments, each performed in triplicate.

Compounds	IC_{50} [^{3}H]Ro 22-1319 (a)	IC_{50} [^{3}H]Spiroperidol (b)	Ratio (b)/(a)
	nM		
Spiroperidol	0.62 ± 0.20	0.31 ± 0.04	0.50
Domperidone	1.8 ± 0.48	13 ± 1.8	7.2
Octoclothepin	3.4 ± 0.41	0.82 ± 0.10	0.24
Perphenazine	4.7 ± 0.27	6.2 ± 0.94	1.3
Pimozide	6.1 ± 0.55	2.3 ± 0.57	0.38
Fluphenazine	6.9 ± 2.2	2.8 ± 0.57	0.41
Chlorprothixene	9.4 ± 2.8	5.0 ± 0.84	0.53
Ro 22-1319	11 ± 2.0	110 ± 8.8	10
Chlorpromazine	21 ± 0.58	14 ± 8.8	0.67
Metoclopramide	29 ± 6.0	420 ± 50	14
Flupenthixol	36 ± 10	5.9 ± 0.66	0.16
Molindone	40 ± 5.7	740 ± 33	19
Thioridazine	61 ± 9.8	42 ± 15	0.69
Sulpiride	64 ± 15	2300 ± 500	36
Clozapine	360 ± 110	120 ± 6.2	0.33

TABLE 4

Effect of dopamine, serotonin, and several compounds on [^{3}H]Ro 22-1319 and [^{3}H]spiroperidol binding

The binding assay was performed as described in Table 3. The concentration of each compound that inhibited specific binding 50% (IC_{50}) was determined by log-probit analysis of at least five different concentrations. Each value represents the mean ± standard error of the mean of at least three separate experiments, each performed in triplicate. The Hill coefficient was calculated from the logit-log plot.

Compounds	[^{3}H]Ro 22-1319 IC_{50}	[^{3}H]Ro 22-1319 Hill coefficient	[^{3}H]Spiroperidol IC_{50}	[^{3}H]Spiroperidol Hill coefficient
	nM		*nM*	
Dopamine	2,300 ± 390	0.50	70,000 ± 8,400	0.69
ADTN[a]	1,400 ± 290	0.64	11,000 ± 3,600	0.65
Apomorphine	94 ± 18	0.65	380 ± 54	0.67
Serotonin	31,000 ± 5,400	0.96	13,000 ± 2,100	0.75
Mianserine	4,300 ± 620	0.76	1,500 ± 300	0.63
Propranolol	8,600 ± 1,400	1.0	18,000 ± 2,000	0.68
Phentolamine	2,000 ± 560	0.83	6,500 ± 500	0.77
Levallophan	2,600 ± 200	0.80	6,200 ± 210	0.71

[a] ADTN, (+)-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene.

DISCUSSION

It is well known that antipsychotics interact with high potency in several receptor systems (6). The clinical efficacy of these agents appears to correlate well with their ability to interact with dopamine receptor sites in the striatum as labeled by either [^{3}H]spiroperidol or [^{3}H]haloperidol. However, this correlation does not appear to hold for some atypical antipsychotics (7, 8). Because of a heterogeneous population of dopamine receptors in the brain, the question arises whether antipsychotics interact in a similar manner with all dopamine receptors or whether there is a selective interaction of the antipsychotics with one subpopulation of the sites.

The present study demonstrates that Ro 22-1319 has a sulpiride-like property and binds to the D_2 dopaminergic receptor subtype in a sodium-dependent manner. This conclusion is justified by the following results. 1) [^{3}H]Ro 22-1319 binding is saturable and shows a high affinity in the presence of sodium. 2) Binding of [^{3}H]Ro 22-1319 is stereoselective with respect to the optical isomers of several derivatives, the biologically active isomer being more potent in each case. 3) Among the antipsychotics tested, D_2 antagonistic antipsychotics (sulpiride, metoclopramide, and molindone) exhibit more potent inhibition of [^{3}H]Ro 22-1319 binding than of [^{3}H]spiroperidol binding. 4) The distribution of binding sites is consistent with the distribution of dopamine content in the brain, as binding is greatest in the striatum. 5) Like sulpiride, metoclopramide, and molindone, the competition curve of Ro 22-1319 for [^{3}H]spiroperidol binding is shifted to the right when sodium is omitted from the incubation medium.

It has been reported that the presence of sodium in the incubation mixture is necessary for the dopamine antagonists sulpiride, sultopride, metoclopramide, and molindone to effectively compete for [^{3}H]spiroperidol-binding sites in the rat striatum (8–11). For reasons discussed below, [^{3}H]Ro 22-1319 appears to be the most valuable ligand for labeling the D_2 dopaminergic subtype. Thus, in the case of [^{3}H]sulpiride and [^{3}H]sultopride bindings, there is a positive correlation between the IC_{50} values of antipsychotics for [^{3}H]sulpiride/[^{3}H]sultopride binding and those for [^{3}H]spiroperidol/[^{3}H]haloperidol binding (10, 12). In contrast, Ro 22-1319, sulpiride, metoclopramide, and molindone are the stronger competitors for [^{3}H]Ro 22-1319 binding than for [^{3}H]spiroperidol binding, and indeed there is no correlation of the IC_{50} values of various antipsychotics between the two bindings ($r = 0.09$). Of the two enantiomers of Ro 22-1319, (−)Ro 22-1319 is 1700 times more active in competing for [^{3}H]Ro 22-1319 binding than is (+)Ro 22-1319, whereas (−)sulpiride/(−)sultopride is only 10 to 68 times more active in competing for [^{3}H]sulpiride/[^{3}H]sultopride binding than is (+)sulpiride/(+)sultopride (10, 12–14). In addition, the serotonin antagonist mianserine is a weak competitor of [^{3}H]Ro 22-1319 binding. Moreover, flupenthixol, a more selective D_1 antagonist (15–17), is a weak competitor of [^{3}H]Ro 22-1319 binding whereas this compound is a potent competitor of [^{3}H]sulpiride binding (12, 14). These results, including a lack of inhibitory effect of Ro 22-1319 on dopamine-stimulated adenylate cyclase activity, indicate that [^{3}H]Ro 22-1319 binds to the D_2 dopaminergic receptor subtype (sodium-dependent D_2 receptor) and not to D_1 or serotonin receptors. Domperidone, a selective D_2 antagonist (18), was more active in [^{3}H]Ro 22-1319 binding than in [^{3}H]spiroperidol binding. However, the binding site of domperidone seems to differ from that of [^{3}H]Ro 22-1319 because

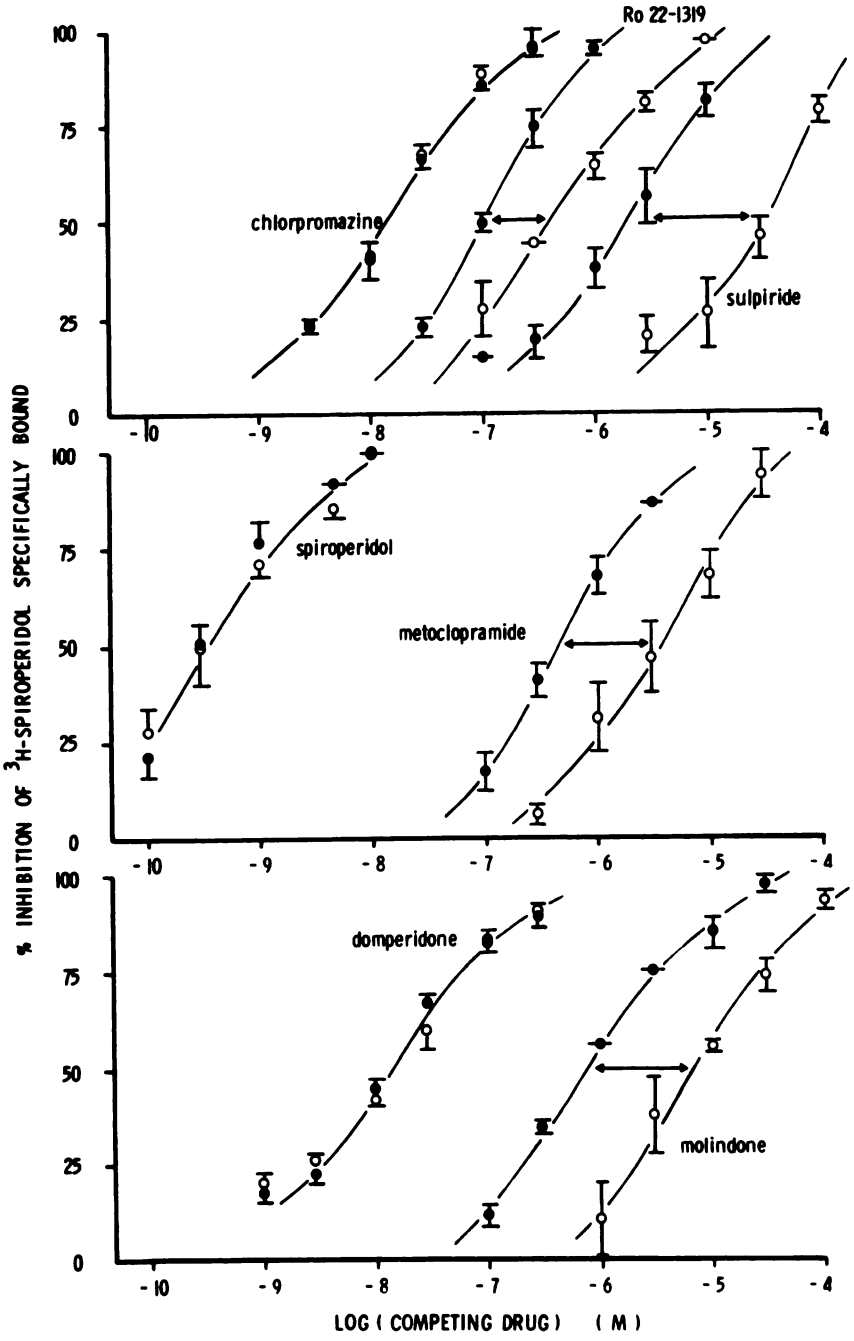


FIG. 7. *The displacement of [^{3}H]spiroperidol binding to bovine striatal P_2 fraction in the presence and absence of 120 mM NaCl*

Bovine striatal P_2 fraction (1.5 mg protein) was incubated with 0.25 nM [^{3}H]spiroperidol and 50 mM Tris-HCl buffer (pH 7.5) for 15 min at 37° in the presence (●) and absence (○) of 120 mM NaCl. Each point represents the mean ± standard error of three to five separate experiments.

domperidone shows no sodium dependency for [^{3}H]spiroperidol-binding sites.

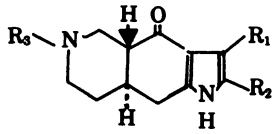
The B_{max} values in the rat striatum were 260 fmol/mg protein for [^{3}H]Ro 22-1319 and 1050 fmol/mg protein for [^{3}H]spiroperidol. The factors which may contribute to this discrepancy are not readily apparent. First, [^{3}H] spiroperidol has high affinity for serotonergic sites. However, because of the low density of these sites in the striatum, this does not appear to be a valid explanation. Second, saturation experiments with [^{3}H]spiroperidol clearly indicate that at least two binding sites of [^{3}H] spiroperidol are present in the striatum, where low affinity components represented more than 50% of the total components (7). Therefore, this apparent heterogeneity may contribute to this discrepancy. In addition, it should be noted that the overall IC_{50} of dopamine agonists for the binding of [^{3}H]spiroperidol are higher than for the binding of [^{3}H]Ro 22-1319, suggesting that [^{3}H]Ro 22-1319 may bind only to a select small population of [^{3}H] spiroperidol-binding sites. Further characterization of the interaction of [^{3}H]Ro 22-1319 will have to be performed in order to resolve this point.

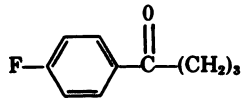
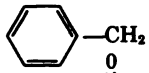
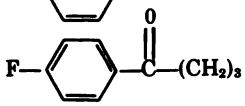
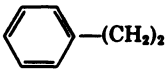
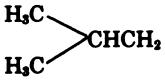
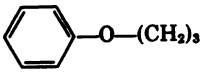
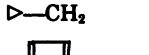
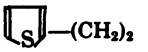
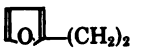
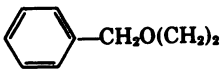
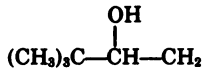
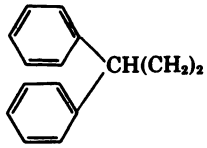
It is interesting to note that small changes in the chemical structure of the pyrroloisoquinoline series mod-

TABLE 5

Comparison between affinities of pyrroloisoquinoline derivatives for [^{3}H]Ro 22-1319- and [^{3}H]spiroperidol-binding sites and their sodium dependency for [^{3}H]spiroperidol-binding sites

Competition experiments were performed and analyzed as described in Table 3 and Fig. 7. Each value represents the mean ± standard error of the mean of at least three separate experiments, each performed in triplicate.



Compounds	Chemical structure			[^{3}H]Ro 22-1319	[^{3}H]Spiroperidol		Ratio	
	R_1	R_2	R_3		(+)NaCl (b)	(−)NaCl (c)	(b)/(a)	(c)/(b)
				IC_{50} (*nM*)(a)	IC_{50} (*nM*)			
Ro 22-4272	CH_2CH_3	CH_3		1.4 ± 0.05	1.6 ± 0.24	6.6 ± 0.76	1.1	4.1
Ro 22-3891	CH_2CH_3	CH_3		1.7 ± 0.12	5.4 ± 0.47	35 ± 5.5	3.2	6.5
Ro 22-6600	CH_3	CH_3		2.4 ± 0.66	2.2 ± 0.28	12 ± 0.33	0.92	5.5
Ro 22-4353	CH_2CH_3	CH_3		2.4 ± 0.65	2.3 ± 0.15	18 ± 2.4	0.96	7.8
Ro 22-5430	CH_2CH_3	CH_3		2.8 ± 0.52	26 ± 3.1	190 ± 26	9.3	7.3
				3.7 ± 1.1	1.3 ± 0.06	11 ± 1.2	0.35	8.5
Ro 22-4066	CH_2CH_3	CH_3		3.7 ± 1.2	19 ± 1.9	93 ± 9.5	5.1	4.9
Ro 22-7610	CH_2CH_3	CH_3		3.8 ± 0.95	5.6 ± 0.54	23 ± 1.6	1.5	4.1
Ro 22-4354	CH_2CH_3	CH_3	$CH_3CH_2O(CH_2)_2$	4.0 ± 0.70	30 ± 4.2	200 ± 32	7.5	6.7
Ro 22-7993	CH_2CH_3	CH_3		4.1 ± 0.67	9.7 ± 1.5	29 ± 2.6	2.4	3.0
Ro 22-4924	CH_2CH_3	CH_3	$CH_3(CH_2)_2$	4.4 ± 0.53	45 ± 4.6	160 ± 15	10	3.6
Ro 22-6559	CH_2CH_3	CH_3		5.4 ± 0.81	6.3 ± 0.94	42 ± 2.5	1.2	6.7
Ro 22-4645	CH_2CH_3	CH_3		6.2 ± 1.3	85 ± 5.7	410 ± 30	14	4.8
Ro 22-1319	CH_2CH_3	CH_3	CH_3	11 ± 2.0	110 ± 8.8	540 ± 43	10	4.9
Ro 22-4065	CH_2CH_3	CH_3	$H_2C{=}CHCH_2$	12 ± 2.3	80 ± 15	270 ± 21	6.7	3.4
Ro 22-3924	CH_2CH_3	CH_3	$HO{-}CH_2CH_2$	19 ± 3.6	140 ± 17	510 ± 76	7.4	3.6
Ro 22-4521	$-(CH_2)_5-$		CH_3	30 ± 4.6	440 ± 31	620 ± 48	15	1.4
Ro 22-3822	CH_3	CH_3	CH_3	31 ± 7.5	350 ± 40	1700 ± 130	11	4.9
Ro 22-3439	CH_2CH_3	CH_3	H	45 ± 7.4	350 ± 22	1500 ± 340	7.8	4.3
Ro 22-5226	CH_2CH_3	CH_3		78 ± 11	7.2 ± 1.1	28 ± 3.9	0.092	3.9

ulate the binding affinity to the D_2 dopaminergic receptor subtype. The compounds having a nonlipophilic functional group on their basic nitrogen (Ro 22-1319, Ro 22-3822, etc.) possessed a stronger potency at [^{3}H]Ro 22-1319 receptors whereas the compounds having a lipophilic moiety at the basic nitrogen became nonselective antagonists of both [^{3}H]Ro 22-1319 and [^{3}H]spiroperidol receptors. Introduction of a lipophilic group to the basic nitrogen seems to enhance the potencies of the pyrroloisoquinoline series not only at [^{3}H]spiroperidol-binding

sites but also at D_1 receptors, since Ro 22-6600 and Ro 22-4353 inhibit dopamine-stimulated adenylate cyclase of rat striatum (19). However, the most impressive finding is that all pyrroloisoquinoline series show a sodium dependency for interacting with [^{3}H]spiroperidol-binding sites, indicating that the functional moiety showing the sodium dependency may be a pyrroloisoquinoline moiety itself.

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REFERENCES

1. Olson, G. L., H.-C. Cheung, C. D. Morgan, J. F. Blount, L. Tadaro, L. Berger, A. B. Davidson, and D. Boff. Dopamine receptor model and its application in the design of a new class of rigid pyrrolo[2,3-g]isoquinoline antipsychotics. *J. Med. Chem.* **24**:1026–1034 (1981).
2. Olson, G. L., H.-C. Cheung, E. Chiang, and L. Berger. Conformationally defined pyrroloisoquinoline antipsychotics. Implications for the model of interaction of antipsychotic drugs with the dopamine receptor, in *Dopamine Receptors* (J. W. Kebabian and C. Kaiser, eds.), 251–273 (1983).
3. Davidson, A. B., E. Boff, D. A. MacNeil, J. Wenger, and L. Cook. Pharmacological effects of Ro 22-1319: a new antipsychotic agent. *Psychopharmacology* **79**:32–39 (1983).
4. Gray, E. G. and V. P. Whittaker. The isolation of nerve endings from brain: an electron microscopic study of cell fragments derived by homogenization and centrifugation. *J. Anat. (Lond.)* **96**:79–87 (1962).
5. 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).
6. Peroutka, S. J., and S. H. Snyder. Relationship of neuroleptic drug effects at brain dopamine, serotonin, α-adrenergic and histamine receptors to clinical potency. *Am. J. Psychiat.* **137**:1518–1522 (1980).
7. Nakajima, T., E. Egawa, and I. Kuruma. A comparison of ^{3}H-haloperidol and ^{3}H-spiroperidol bindings to dopaminergic receptors. *Jpn. J. Pharmacol.* **229**:suppl. 56P (1979).
8. Stefanini, E., A. M. Marchisio, P. Devoto, F. Vernaleone, R. Collu, and P. F. Spano. Sodium-dependent interaction of benzamides with dopamine receptors. *Brain Res.* **198**:229–233 (1980).
9. Clements-Jewery, S., and P. A. Robson. Are striatal [^{3}H]spiperone radioreceptor assays indicative of multiple dopamine receptor interactions? *Neuropharmacology* **20**:1295–1298 (1981).
10. Mizuchi, A., N. Kitagawa, S. Saruta, and Y. Miyachi. Characteristics of [^{3}H] sultopride binding to rat brain. *Eur. J. Pharmacol.* **84**:51–59 (1982).
11. Makman, M. H., B. Dvorkin, and P. N. Klein. Sodium ion modulates D_2 receptor characteristics of dopamine agonist and antagonist binding sites in striatum and retina. *Proc. Natl. Acad. Sci. U.S.A.* **79**:4212–4216 (1982).
12. Freedman, S. B., J. A. Poat, and G. N. Woodruff. [^{3}H]Sulpiride, a ligand for neuroleptic receptors. *Neuropharmacology* **20**:1323–1326 (1981).
13. Theodorou, A., P. Crockett, P. Jenner, and C. D. Marsden. Specific binding of [^{3}H]sulpiride to rat striatal preparations. *J. Pharm. Pharmacol.* **31**:424–426 (1979).
14. Woodruff, G. N., and S. B. Freedman. Binding of [^{3}H]sulpiride to purified rat striatal synaptic membranes. *Neuroscience* **6**:407–410 (1981).
15. Hyttel, J. Effects of neuroleptics on ^{3}H-haloperidol and ^{3}H-cis(*Z*)-flupenthixol binding and on adenylate cyclase activity in vitro. *Life Sci.* **23**:551–556 (1978).
16. Kebabian, J. W., and D. B. Calne. Multiple receptors for dopamine. *Nature (Lond.)* **277**:93–96 (1979).
17. Hyttel, J. Further evidence that [^{3}H]-cis(*Z*)-flupenthixol binds to the adenylate cyclase associated dopamine receptor (D-1) in rat corpus striatum. *Psychopharmacology* **67**:107–109 (1980).
18. Lazareno, S., and S. R. Nahorski. Selective labelling of dopamine (D_2) receptors in rat striatum by [^{3}H]domperidone but not by [^{3}H]spiperone. *Eur. J. Pharmacol.* **81**:273–285 (1982).
19. Nakajima, T., I. Kuruma, K. Nakamura, G. Olson, H. Cheung, E. Chiang, L. Berger, G. Bautz, R. A. O'Brien, C. Burghardt, T. Mowles, E. Gamzu, A. B. Davidson, and E. Boff. Interaction of pyrroloisoquinoline antipsychotics with a D-2 dopamine receptor subtype—evaluation by ^{3}H-Ro 22-1319 binding. *Soc. Neurosci. Abstr.* **9**:431 (1983).

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