0026-895X/92/050856-08$03.00/0

MOLECULAR PHARMACOLOGY, 42:856-863

Prolonged D_2 Antidopaminergic Activity of Alkylating and Nonalkylating Derivatives of Spiperone in Rat Brain

ROSS J. BALDESSARINI, NORA S. KULA, ALEXANDER CAMPBELL, VENKATESALU BAKTHAVACHALAM, JUN YUAN, and JOHN L. NEUMEYER

Department of Psychiatry and Neuroscience Program, Harvard Medical School, and Mailman Research Center, McLean Division of Massachusetts General Hospital, Belmont, Massachusetts 02178 (R.J.B., N.S.K., A.C.), and Research Biochemicals, Inc., Natick, Massachusetts 01760 (V.B., J.Y., J.L.N.)

Received April 8, 1992; Accepted August 13, 1992

SUMMARY

Alkyl and arylalkyl derivatives of the dopamine (DA) D_2 antagonist spiperone were prepared and characterized chemically and pharmacologically. They included the *N*-methyl, *N*-phenethyl (NPS), and *N*-*p*-aminophenethyl (NAPS) derivatives, as well as the alkylating isothiocyanato (NIPS), bromacetamido, and ethylfumaramido *p*-substituted *N*-phenethylspiperones. These compounds showed high lipophilicity (log *P* up to 6.0 with NIPS), as well as very high *in vitro* D_2 affinity (K_i = 35–280 pM) and D_2 versus D_1 selectivity (540–9000-fold) in radioreceptor assays with corpus striatum of rat brain. Of the alkylating series, NIPS showed the highest D_2 affinity (57 pM) and D_2 versus D_1 selectivity (2040-fold) and so was selected for further evaluation. NPS, NAPS, and NIPS showed little or no affinity for 34 non-DA binding sites defined by radioligand assays for monoamine, amino acid, and peptide neurotransmitters, ion channels, peptide growth factors, and transmission mediators but did show low α_2 and moderate α_1 and 5-hydroxytryptamine (5-HT_2) affinity with rat forebrain tissue *in vitro*; NIPS showed a marked gain in D_2 versus 5-HT_2 selectivity, compared with spiperone (1520- versus 26-fold). Systemic injections of NIPS induced marked decreases in rat striatal D_2 binding sites 24 hr later, with little effect on D_1, 5-HT_2, or α_1 sites; NIPS and NAPS lowered apparent B_{max} values at D_2 receptors with little change in ligand affinity, *ex vivo* as well as *in vitro*. NPS, NAPS, and NIPS all induced dose-dependent lowering of D_2 binding *ex vivo* (ID_{50} = 1–9 μmol/kg, intraperitoneally) and blocked the behavioral effects of the DA agonist apomorphine (0.9 μmol/kg) potently (ID_{50} = 0.3–0.5 μmol/kg) at 24 hr. Recovery from these anti-DA actions required about 1 week after equimolar (15 μmol/kg) and similarly effective doses of NPS and NAPS, as well as NIPS. Thus, highly selective and avidly bound lipophilic D_2 affinity ligands with similarly avid *in vitro* and prolonged *in vivo* anti-DA activities can be derived from *N*-phenethylspiperones with or without an alkylating moiety present. Such affinity ligands may represent useful additions to previously used, generally less selective, D_2 affinity ligands.

Selective alkylating affinity ligands have been developed to study a variety of enzymes (1) as well as receptors of drugs (2) or neurotransmitters (3). Typically, such agents have been developed by attaching an electrophilic moiety to a known selective ligand, while retaining high affinity and selectivity for proposed macromolecular target sites; the electrophilic moiety reacts with a presumed nucleophilic site on a protein of interest (2). Affinity ligands, with or without alkylating moieties, have proven useful in the development of radioreceptor assays, in receptor protein isolation procedures, for histological localization of receptors, and for applications in experimental or clinical brain-scanning technologies (3).

This work was supported by United States Public Health Service (National Institute of Mental Health) Grants MH-34006, MH-47370, and MH-45692 and an award from the Bruce J. Anderson Foundation.

Several alkylating affinity ligands been proposed for DA receptor types in mammalian brain tissue. Among these, the unselective, low affinity, protein-alkylating agent EEDQ has been used repeatedly to study DA receptors as well as other receptors (4–6). We reported previously that phenoxybenzamine, a chloroethyl-mustard with anti-α-adrenergic activity, can also alkylate DA receptors (7) and that the mustard analog of the potent and selective DA agonist (*R*)-(−)-*N*-*n*-propylnorapomorphine, (*R*)-(−)-*N*-(2-chloroethyl)norapomorphine, can bind irreversibly to DA receptors (8), evidently by formation of a reactive aziridinium intermediate (9). A mustard derivative of fluphenazine also has been used to alkylate DA receptors and calmodulin (10). More recently, several D_2-selective affinity probes have been developed, including UV-activated photoaffinity labels (11) and fluorescent-labeled affinity probes (12, 13).

ABBREVIATIONS: DA, dopamine (3,4-dihydroxyphenethylamine); EEDQ, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; SPR, spiperone; NMS, *N*-methylspiperone; NPS, *N*-phenethylspiperone; NAPS, *N*-(*p*-aminophenethyl)spiperone; NIPS, *N*-(*p*-isothiocyanatophenethyl)spiperone; NBPS, *N*-(*p*-bromacetamidophenethyl)spiperone; NEPS, *N*-(*p*-ethylfumaramidophenethyl)spiperone; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NMDA, *N*-methyl-D-aspartate; GABA, γ-aminobutyric acid; 5-HT, 5-hydroxytryptamine; LSD, lysergic acid diethylamide; AMPA, α-amino-3-hydroxyisoxazolepropionic acid.

We have made preliminary reports on the development of a series of putative alkylating agents that appear to be selective for D_2 or D_1 receptor sites in rat brain (14–17). In addition, Xu *et al.* (5) recently reported independently on some pharmacologic characteristics of one compound of this series, the alkylating SPR derivative NIPS. The present report includes detailed information on the synthesis and chemical characteristics of several D_2-selective alkylating and nonalkylating SPR derivatives, as well as extensive neuropharmacologic characterizations of selected examples from this series of compounds, using rat brain tissue for *in vitro*, *ex vivo*, and *in vivo* studies.

Materials and Methods

Chemical preparation of SPR derivatives. *N*-Methylspiperone and most *N*-phenethylspiperones were prepared by the reaction of SPR with methyl iodide and phenethyl bromide, respectively. NAPS was prepared by reaction of SPR with *p*-nitrophenethyl bromide, followed by reduction with stannous chloride. A series of alkylating derivatives of NAPS, in turn, were prepared by reacting its free amino moiety with thiophosgene, ethyl fumaroyl chloride, or bromoacetyl trifluoroacetic anhydride to provide the *p*-isothiocyanate (NIPS), *p*-ethylfumaramido (NEPS), and *p*-bromacetamido (NBPS) derivatives, respectively.

Specific syntheses of experimental compounds (structures in Fig. 1) started with SPR hydrochloride from Research Biochemicals, Inc. (Natick, MA). The preparation of NMS represents the general *N*-alkylating conditions used; the synthesis of its free base was described previously (18). NMS was prepared as follows: a solution of methyl iodide (600 mg, 4.2 mmol) in 10 ml of toluene was added, dropwise, to a mixture of SPR (1.1 g, 2.8 mmol), potassium hydroxide (1.0 g), tetrabutyl ammonium iodide (100 mg), and toluene (100 ml), with stirring at 25° for 8 hr. The mixture was then filtered, toluene was removed *in vacuo*, and the residue was purified by column chromatography on silica gel with chloroform and methanol (99:1, by volume) as the solvent. The recovered product NMS was converted to its HCl salt in a yield of 800 mg (57%). The *N*-phenethyl and *N*-*p*-nitrophenethyl derivatives of SPR were similarly prepared from SPR and a corresponding arylalkyl bromide, with NPS obtained in a yield of 52%.

NAPS was prepared by modifications of previously reported procedures (11, 13), with improved yield and more convenient product isolation. A solution of stannous chloride dihydrate (7.9 g, 35 mmol) in 20 mol of concentrated HCl was added to a solution of *p*-nitrophenethylspiperone (3.2 g, 5.8 mmol) in 40 ml of ethanol and 40 ml of concentrated HCl. This mixture was stirred at 50° for 4 hr, evaporated to half-volume, made alkaline (pH 13), and extracted into ethyl acetate. The extract was then purified by silica gel column chromatography with dichloromethane and methanol (9:1, by volume) as solvent and was converted to the HCl salt of NAPS at a yield of 2.3 g (55% from spiperone).

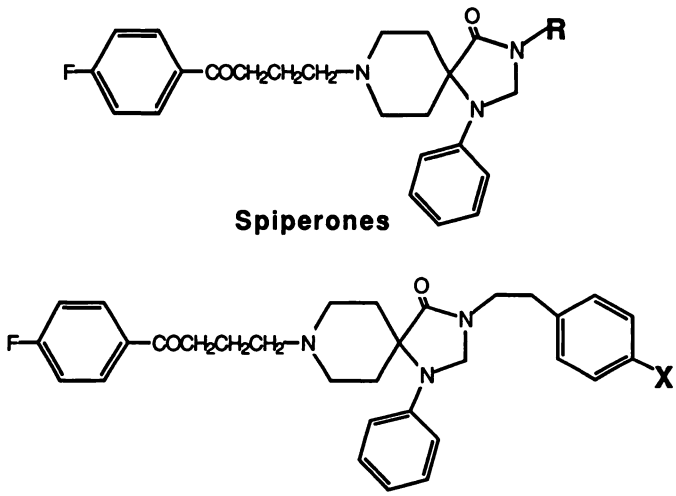

Fig. 1. Structures of SPR and alkylating phenethylspiperone derivatives. Spiperone derivatives include SPR (R = H), NMS (R = CH_3), NPS [R = $(CH_2)_2C_6H_5$], and NAPS [R = $(CH_2)_2C_6H_4NH_2$]. Alkylating phenethylspiperone derivatives include NIPS (X = NCS), NBPS (X = $NHCOCH_2Br$), and NEPS (X = NHCOCH = $CHCO_2CH_2CH_3$).

NIPS was prepared from NAPS by the method of Sharma (19), by adding a solution of thiophosgene (0.7 ml, 9 mmol) to a mixture of NAPS (2.3 g, 4.5 mmol), 50 ml of chloroform, and sodium bicarbonate (3.8 g/50 ml), with constant stirring at 25° for 4 hr. The reaction mixture was then extracted into chloroform, separated on a silica gel column with dichloromethane and methanol (49:1, by volume) as solvent, and converted to the HCl salt of NIPS in a yield of 1 g (37%).

NBPS [*N*-[*p*-(*N*-bromoacetyl)aminophenethyl]spiperone] was prepared by mixing NAPS (80 mg, 156 nmol) with trifluoroacetyl bromoacetic anhydride (5 eq), prepared as described by Ferris and Emmons (20), in 10 ml of dry dichloromethane at 25° under nitrogen with constant stirring. The mixture was washed with aqueous sodium carbonate, dried, and evaporated to yield a residue that was converted to the HCl salt and recrystallized from a mixture of methanol and ether to yield 65 mg (59%) of desired product.

NEPS [*N*-[*p*-(*N*-ethylfumaroyl)aminophenethyl]spiperone] was prepared by acylation of NAPS with fumaric acid monoethyl ester chloride, which was first prepared by refluxing fumaric acid monoethyl ester (Aldrich Chemical Co., Milwaukee, WI) with thionyl chloride until the material was fully dissolved, followed by concentration *in vacuo* with dry toluene to obtain the acid chloride as a brown oil. This reagent (300 mg), dissolved in acetonitrile, was then mixed with a solution of NAPS (90 mg, 175 nmol) in acetonitrile and stirred at 25° for 16 hr. The solvent was removed under reduced pressure, the residue was separated by preparative thin layer chromatography, and the desired product was converted to the HCl salt of NEPS in a yield of 50 mg (40%). The alkylating agents NBPS and NEPS are novel compounds.

Chemical characterization of SPR derivatives. Compounds were characterized by microanalysis of carbon, hydrogen, and nitrogen content by Atlantic Microlab, Inc. (Atlanta, GA), to a criterion of ± 0.4% of predicted quantities. Thin layer chromatography was used to separate novel compounds on F-254 plastic-backed silica gel sheets (E. Merck, Darmstadt, Germany). Purification by medium-pressure column chromatography was performed with flash silica gel (J. T. Baker Chemical Co., Phillipsburg, NJ). Organic extracts were dried with anhydrous magnesium sulfate unless indicated otherwise. Melting points were obtained with a Thomas-Hoover melting point apparatus (Thomas Scientific Co., Philadelphia, PA) and are reported uncorrected. Additional confirmatory physical-chemical testing, not reported here, included obtaining ^1H NMR spectra with an XL-300 (300 MHz) NMR spectrometer (Varian Associates, Sunnyvale, CA) using trimethylsilane as an internal standard, with spectral shifts considered downfield from trimethylsilane. Similarly, mass spectra were obtained by the Department of Chemistry at Northeastern University (Boston, MA) with a model 4021 mass spectrometer (Finnigan MAT, San Jose, CA) under electron impact conditions.

The equivalent of octanol/water partition coefficients (log *P*) were calculated by the method of Hansch and Leo (21), using a published value (22) for SPR (log *P* = 2.67) as a reference standard and computing the effects of added moieties from tables in the reference cited. In addition, chromatographic retention was used as an additional indication of lipophilicity expressed as a chromatographic capacity factor (log *k'*), using the relationship $k' = [t_r - t_v]/t_v$, where t_r is the retention time of the test compound and t_v is the retention time of the reference standard (SPR) for data derived from high pressure liquid chromatography of selected SPR derivatives on a C-18-derivatized silica gel

column with a mobile phase of methanol and aqueous phosphate buffer (50 mM, pH 7.4), in a volume ratio of 3:1 (23). Values obtained by the two methods (log P and log k') were in close agreement ($r = 0.93$), and only the log P values are shown.

Receptor assay methods. All procedures involving animals complied with federal and institutional regulations and were approved by the Institutional Animal Care and Use Committee of McLean Hospital. Binding to D_2 receptor sites was determined in homogenates of corpus striatum tissue of rat brain, prepared from young, adult, male, Sprague-Dawley, albino rats (initially weighing 110–150 g) by homogenization in a hand-held Teflon/glass homogenizer with 200 volumes of Tris hydrochloride buffer (pH 7.4) containing 150 mM sodium chloride, centrifugation at 20,000 × g, and resuspension of the pellet in fresh buffer to provide the equivalent of 5 mg of original tissue/ml and 1 mg (~25 μg of protein) in each assay tube. Tissue was assayed with the selective D_2 antagonist benzamide [^{3}H]YM-09151-2 (DuPont-NEN, Boston, MA) as described previously (6, 24), typically with four to eight tubes for each condition in each experiment and with at least two independent replications. Routinely, the radioligand was incubated at a concentration of 65 pM, slightly above its predetermined apparent K_d (45 pM), or, when saturation isotherm experiments were carried out, at a range of concentrations from 10 to 300 pM, at 30° for 90 min; binding was analyzed by curve-fitting with the LIGAND program of Munson and Rodbard (25) adapted for the Macintosh microcomputer (26). Specific D_2 binding was defined as the difference between binding with and without (+)-butaclamol (0.2 μM; Research Biochemicals) in the incubations; inclusion of additional unlabeled ketanserin and phentolamine (each at 0.2 μM; Research Biochemicals) had no significant effect (≤1% loss) on the specific D_2 binding defined with excess (+)-butaclamol.

In vitro D_2 affinities and D_2 potencies of test SPR analogs were determined with at least six concentrations of test agent, above and below the approximate IC_{50} value. For affinity estimates, test compounds were assayed by direct competition with 65 pM [^{3}H]YM-09151-2, using rat striatal homogenates (15–20 mg/ml) as already described. For estimates of *in vitro* potency, such striatal homogenates were preincubated for 30 min at 30° in a physiological buffer (containing, in mM: NaCl, 124; KCl, 5; KH_2PO_4, 1.4; $MgCl_2$, 1.3; $CaCl_2$, 1.3; $CaCl_2$, 0.8; HEPES, 20; sodium ascorbate, 1.5; glucose, 11; and sucrose, 80; gassed with 95/5 air/CO_2 to pH 7.0) with a range of concentrations of test agent. Unbound test agent was removed by centrifugation (20,000 × g) and then washing (and recentrifuging) five times with excess fresh D_2 radioreceptor assay buffer (4°) before assay of remaining D_2 binding (with 65 pM [^{3}H]YM-09151-2) in the final tissue pellet (with ~2 mg of original tissue/assay). For both *in vitro* affinity and potency experiments, IC_{50} (± standard error) was determined by computer-fitting of the data with the ALLFIT program (27, 28) adapted for the MacIntosh microcomputer[1] and was converted to values of apparent K_i (mean ± standard error) from the relationship of Cheng and Prusoff (29), i.e., $K_i = IC_{50}/(1 + [\text{ligand concentration}]/K_d)$.

For *in vitro* and *ex vivo* demonstrations of radioligand concentration isotherms, single concentrations or doses (typically, approximately half-maximally effective) of test agents were used as a pretreatment, followed by assays with a range of concentrations of [^{3}H]YM-09151-2 (0.01–0.3 nM). Resulting data were evaluated as linearized relationships of specifically bound/free ligand concentrations (B/F) versus specifically bound ligand level (B), such that ligand affinities (apparent K_d) are reflected in the slope and apparent maximum binding (B_{max}) is reflected in the x-intercept.

Ex vivo and *in vivo* potencies of test agents also were determined by D_2 radioreceptor assays of striatal tissue after pretreatment of rats with vehicle (control) or a range of doses of a SPR derivative (50 nmol/kg to 100 μmol/kg, intraperitoneally), given 24 hr earlier to groups of six (replicated at least once; data expressed as percentage of matched controls and pooled). Functional assays of receptor blockade rated inhibition of stereotyped sniffing, licking, and gnawing behaviors in similarly pretreated rats induced over 60 min by a standard dose of (*R*)-(−)-apomorphine·HCl·0.5 H_2O (0.3 mg/kg or 0.9 μmol/kg, intraperitoneally, MacFarlan-Smith, Ltd., Edinburgh, Scotland), as described previously (30). Drug potencies were determined as values of ID_{50} (± standard error) with the ALLFIT program. For time-course experiments, a single equimolar (and similarly effective) dose of 15 μmol/kg, intraperitoneally, of all agents tested was given at times ranging from 1–6 hr to 4–6 days earlier.

D_1 affinity was evaluated in rat striatal homogenates, prepared as already described, using the selective D_1 benzazepine antagonist [^{3}H] SCH-23390 (DuPont-NEN) as described previously (31, 32), at 0.30 nM ($K_d = 0.34$ nM), at 30° for 30 min for determinations of IC_{50} and K_i values. Specific binding (~90%) was defined with (*cis*)-flupenthixol (0.3 μM; donated by Lundbeck Labs., Copenhagen, Denmark) as the blank agent.

Additional affinity characterizations of SPR derivatives were carried out by NovaScreen Division (Nova Pharmaceutical Corp., Baltimore, MD), using a battery of radioligand assay procedures whose literature citations can be obtained from Nova Corp. The SPR derivatives NMS, NPS, NAPS, and NIPS were screened initially at 10 μM; those with submicromolar affinity were characterized with a range of lower concentrations. Screening radioreceptor assays included a confirmatory D_2 assay with another benzamide [(−)-[^{3}H]sulpiride]. We as well as NovaScreen assayed for affinity at 5-HT_2 (serotonin) receptors with [^{3}H]ketanserin (33); this ligand ($K_d = 0.4$ nM) was assayed at 0.5 nM at 37° for 15 min, with 10 μM methysergide (donated by Sandoz Research Institute, Berne, Switzerland) as a blank agent providing 60% specific binding with homogenates of rat forebrain with striatum removed. Similarly, α_1-adrenergic receptors in the same rat forebrain tissue were assayed with [^{3}H]prazosin (34); this ligand ($K_d = 0.2$ nM) was assayed at 0.3 nM at 25° for 45 min, using 2 μM phentolamine (donated by CIBA-Geigy, Ardsley, NY) as blank (78% specific binding). These radioligands also were obtained from DuPont-NEN.

Additional screening by NovaScreen for affinity at monoaminergic receptor sites included radioligand assays for α_2-adrenergic, β-adrenergic, and 5-HT_1 (or other 5-HT) receptors (LSD site). Assays for amino acid binding sites included the glutamate receptors of the quisqualate (AMPA) and NMDA types, kainate site, related phencyclidine and MK-801 binding sites, the glycine-NMDA complex site, and the glycine-strychnine site, as well as GABA receptor types $GABA_A$ and $GABA_B$. Adenosine receptors (types A_1 and A_2) also were screened, as were binding sites for a series of neuropeptides and related growth or tissue-stimulating factors. These included angiotensin II, vasopressin$_1$, bombesin or gastrin-releasing peptide, cholecystokinin (CCK-8 for central nervous system as well as peripheral tissues), neurokinin-1 (substance P), neurokinin-2 (substance K, neurokinin-A), neuropeptide Y, neurotensin, somatostatin, vasoactive intestinal peptide, neuronal growth factor, epidermal growth factor, and atrial natriuretic factor. Sites involved in the mediation of neurotransmitter action that were screened included several cation or anion membrane channels, namely calcium channels of types T-L and N, the calcium-activated potassium channel (apamin site), and the chloride channel, as well as a related benzodiazepine site. Finally, second messenger or receptor-effector molecular elements assayed included stimulatory guanine nucleotide-binding proteins ($G_{s\alpha}$-adenylyl cyclase complex or forskolin site), phosphokinase C site (phorbol ester site), and an inositol trisphosphate site.

Results

Structures of SPR derivatives studied are provided in Fig. 1, with chemical and pharmacologic properties summarized in Table 1. Initial characterization included determinations of the lipophilic properties of selected spiperone derivatives (Table 1). The *N*-phenethyl derivatives had higher calculated log P values than did the precursor SPR or its *N*-methyl derivative (NMS), indicating greater lipophilicity, with somewhat less

[1] M. H. Teicher, personal communication.

TABLE 1

Properties of SPR derivatives

Compound structures are shown in Fig. 1. Molecular weights are formula weights of salts. Octanol/water partion coefficient (log *P*) is a measure of lipophilicity, as described in Materials and Methods. K_i values (mean ± standard error) were determined versus 65 pM [^{3}H]YM-09151-2, 0.3 nM [^{3}H]SCH-23390, 0.5 nM [^{3}H]ketanserin, or 0.2 nM [^{3}H]prazosin with rat forebrain tissues.

Measure	SPR	NMS	NPS	NAPS	NIPS	NEPS	NBPS
Chemical properties							
Molecular formula	$C_{23}H_{26}FN_3O_2 \cdot HCl$	$C_{24}H_{28}FN_3O_2 \cdot HCl$	$C_{24}H_{28}FN_3O_2 \cdot HCl$	$C_{31}H_{35}FN_4O_2 \cdot 3HCl$	$C_{32}H_{33}FN_4O_2S \cdot 2HCl$	$C_{37}H_{41}FN_4O_5 \cdot 2HCl \cdot H_2O$	$C_{33}H_{36}BrFN_4O_3 \cdot 2HCl$
Molecular weight	432	446	563	624	630	732	708
Melting point	252–254°	238–240°	110–112°	205–208°	214–216°	124–126°	156–158°
Octanol/water partition coefficient (log *P*)	2.67	3.33	4.41	5.64	5.97		
Affinities							
D_2 affinity (K_i, nM)	0.17 ± 0.002	0.035 ± 0.004	0.12 ± 0.01	0.13 ± 0.02	0.057 ± 0.007	0.090 ± 0.01	0.28 ± 0.04
D_1 affinity (K_i, nM)	140 ± 17	310 ± 37	28 ± 3	100 ± 12	120 ± 14	46 ± 6	240 ± 30
5-HT_2 affinity (K_i, nM)	0.45 ± 0.08	0.40 ± 0.04	4.54 ± 0.72	1.61 ± 0.11	87.4 ± 16.7		
α_1 affinity (K_i, nM)			2.1 ± 0.4	33 ± 0.4	4.4 ± 0.4		
Selectivity							
D_2 versus D_1 selectivity	8310	8970	4330	810	2040	540	840
D_2 versus 5-HT_2 selectivity	26	12	165	13	1530		
D_2 versus α_1 selectivity			18	290	77		

gain in lipophilicity when a *p*-amino group was added (NAPS); the highest log *P* value was that for NIPS (5.97, indicating 3.30 log units, or 2000 times, greater lipophilicity than that of SPR). SPR derivatives also were evaluated for affinity (K_i values) at D_2 receptor sites in rat striatum (Table 1), which was very high (at the subnanomolar level) for all compounds tested, ranging from 170 pM for SPR itself and 280 pM for NBPS to 35 pM for NMS. In addition, all compounds tested had much lower affinity (540–9000 times) at D_1 sites and, so, very high apparent D_2 versus D_1 selectivity *in vitro*. Of the three derivatives with an alkylating moiety (the bromacetamido, ethylfumaramido, and isothiocyanato derivatives), the last, NIPS, had the highest D_2 affinity (K_i = 57 pM) and D_2 versus D_1 selectivity (2040-fold). Accordingly, it was selected for further pharmacologic characterization and comparison with presumably nonalkylating congeners. Experiments (not shown) with (−)-[^{3}H]sulpiride as a D_2 radioligand supported the conclusion that NMS, NPS, NAPS, and NIPS all have very high D_2 affinity, in the picomolar or low nanomolar range.

Broad screening of the representative SPR derivatives NMS, NPS, NAPS, and NIPS failed to reveal significant interactions with the 37 non-DA receptor and other binding sites (total of 40 assays) defined in Materials and Methods, with only three exceptions. With α_2-adrenergic sites in rat cerebral cortex labeled *in vitro* with [^{3}H]RX-781,094, there were weak interactions with all four test agents, indicating IC_{50} values on the order of 1–10 μM. In contrast, there were significant interactions with similarly assayed α_1 sites labeled with [^{3}H]prazosin, as well as potent interactions with 5-HT_2 sites labeled with [^{3}H]ketanserin (Table 1). The adrenergic affinities (K_i) ranged from a high of 2.1 nM for NMS and 4.4 nM for NIPS to a lower value of 33 nM with NPS, whereas serotonin (5-HT_2) affinities ranged from 40 nM with NMS to 87 nM with NIPS. Thus, for NIPS, the apparent D_2 versus 5-HT_2 selectivity was 1530-fold (0.057 versus 87.4 nM) and D_2 versus α_1 selectivity was 77 (0.057 versus 4.4 nM), not as high as its 2040-fold D_2 versus D_1 selectivity (Table 1).

Given this evidence that NIPS and its congeners had some affinity for 5-HT_2 serotonergic and α_1-adrenergic receptors in rat forebrain tissue (interactions also known to occur with the precursor compound SPR) (35, 36), control experiments on the effects of phenethylspiperone derivatives at these receptor sites were undertaken after systemic *in vivo* administration of NAPS and NIPS at a dose of 15 μmol/kg, intraperitoneally, 24 hr earlier (Table 2). With such pretreatment, reduction of binding of [^{3}H]ketanserin to homogenates of rat forebrain tissue was

TABLE 2

***Ex vivo* receptor-blocking effects of SPR derivatives**

Doses of test agents (15 μmol/kg, intraperitoneally) or vehicle control were given to rats ($n \geq 6$); 24 hr later, brain tissue was recovered and striatum was assayed for receptor levels with the following ligands: D_2, [^{3}H]YM-09151-2 (65 pM); D_1, [^{3}H]SCH-23390 (0.3 nM); α_1-adrenergic, [^{3}H]prazosin (0.2 nM); 5-HT_2, [^{3}H]ketanserin (0.5 nM). Data are mean ± standard error of percentage of loss of binding versus controls.

Receptor type	Loss in striatum	
	NAPS	NIPS
	%	
D_2 dopaminergic	56.7 ± 1.3[a]	66.5 ± 2.1[a]
D_1 dopaminergic	12.6 ± 0.4[a]	4.5 ± 1.9
α_1-Adrenergic	11.9 ± 0.5[a]	2.4 ± 0.3
5-HT_2 serotonergic	29.8 ± 1.0[a]	0.3 ± 0.1

[a] Significant difference from a corresponding control value ($t \geq 2.6$, $p < 0.05$).

insignificant with NIPS (<1%) but moderate with NAPS (30%) and that of [^{3}H]prazosin was small with NIPS (2.4%) and only slightly larger with NAPS (12%). *Ex vivo* decreases in the binding of the D_1 ligand [^{3}H]SCH-23390 were slight with NIPS (4.5%) and moderate with NAPS (13%), whereas striatal D_2 binding (of 65 pM [^{3}H]YM-09151-2) was reduced highly significantly ($p \leq 0.001$ by t test), by 66.5% by NIPS and by 57% by NAPS under the same conditions. Consistent with a receptor-alkylating effect of NIPS, the marked inhibition of striatal D_2 binding obtained under the same conditions involved a selective loss of apparent maximum binding site density/mg of tissue (B_{max}) in a ligand concentration experiment after a moderate dose of this agent (5 μmol/kg, intraperitoneally; Fig. 2A), with minimal change in apparent ligand affinity (K_d), or too little binding to permit reliable determination of affinity at a higher dose (15 μmol/kg; data not shown). Curiously, however, a very similar B_{max}-lowering effect of the nonalkylating congener NAPS also was observed (after a dose of 15 μmol/kg), suggesting that it, too, bound avidly to D_2 sites in cerebral membranes (Fig. 2A). These virtually parallel shifts in Scatchard plots of B/F versus B also suggest that loss of binding detected with a single (approximately K_d concentration of radioligand should be proportional to loss of maximum density (B_{max}).

Selective loss of B_{max} at D_2 sites in striatal tissue similar to that found *ex vivo* after treatment with NIPS or NAPS also was found after treatment of normal striatal tissue *in vitro* with the same agents at a low concentration (3 nM) for 30 min (Fig. 2B). The potency of these agents *in vitro*, determined as IC_{50} (± standard error), was found to be similarly high for both agents (NIPS, 1.2 ± 0.3 nM; NAPS, 1.3 ± 0.2 nM). *In vitro* treatment with SPR had no effect at nanomolar concentrations and, even at concentrations up to 30 μM, led to small and inconsistent losses of D_2 binding. Evidently this difference reflected more efficient removal of SPR than of NAPS or NIPS by the vigorous washing involved in this *in vitro* treatment procedure.

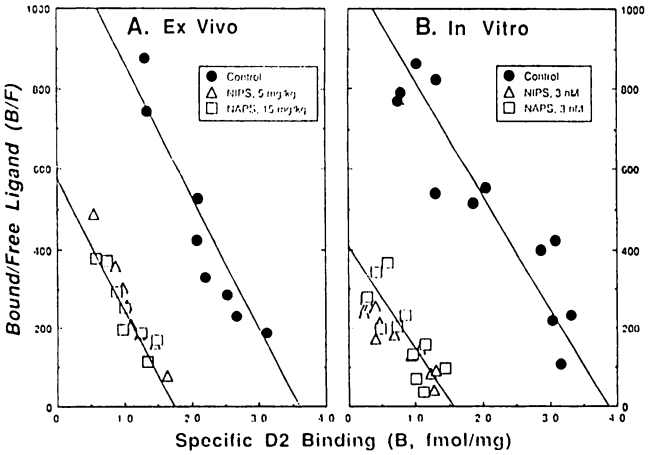

Fig. 2. A, *Ex vivo* anti-D_2 activity of NIPS and NAPS. Striatal tissue was recovered and pooled from four to six rat brains at 24 hr after intraperitoneal injection of NIPS (5 μmol/kg) or NAPS (15 μmol/kg). The experiment was repeated twice, and data from D_2 ligand ([^{3}H]YM-09151-2) concentration (0.01–0.3 nM) assays were pooled. In linearized form (ratio of specifically bound to free ligand concentration, B/F, versus bound ligand, B, in fmol bound/mg of original tissue), the data indicate a marked loss of maximum binding site density (x-intercept, B_{max}) with little change in apparent ligand affinity (indicated by slope). Effects of NIPS and NAPS were indistinguishable, as indicated by a single regression line for all data. Additional treatment with NIPS at 15 μmol/kg led to even more marked loss of specific D_2 binding ($B_{max} \leq 20\%$ of control; data not shown). B, *In vitro* anti-D_2 activity of NIPS and NAPS. Rat striatal tissue homogenate was preincubated with similarly effective concentrations (3 nM) of NIPS or NAPS for 30 min, washed five times, and then incubated with concentrations of [^{3}H]YM-09151-2 (0.01–0.3 nM). Data are pooled from two independent experiments and presented as in A, with similar results.

The potency of representative SPR derivatives (NPS, NAPS, and NIPS) was evaluated further by *ex vivo* assays of striatal D_2 binding 24 hr after a range (0 and 0.05–100 μmol/kg, intraperitoneally) of systemic doses of test agent. In addition, their potency was evaluated as inhibition of behavioral arousal induced by an acute challenge dose of apomorphine under similar conditions. These experiments indicated severalfold greater antiapomorphine (ID_{50} = 0.3–0.5 μmol/kg) than anti-D_2 binding potency (ID_{50} = 1.5–5.6 μmol/kg) for all three compounds (average, 13-fold) under the conditions of testing, particularly with NIPS (ID_{50} = 0.4 versus 5.6 μmol/kg, respectively, or 14-fold potency difference and NAPS; Table 3).

The time course of recovery from the effects of a single dose of a SPR derivative was evaluated as recovery of striatal D_2 binding *ex vivo* at various times over the week after administration of equimolar (15 μmol/kg, intraperitoneally) doses of SPR, NPS, NAPS, or NIPS. Other groups of rats were treated identically and tested behaviorally, once only, during the same time course, with an acute challenge dose of apomorphine (0.9 μmol/kg, intraperitoneally). Results were replicated independently at least once to verify salient differences between compounds and testing models. They indicated that SPR induced nearly unmeasurable apparent *ex vivo* losses of striatal D_2 binding sites, even at 1 day after treatment, whereas its alkylating (NIPS) or nonalkylating (NPS and NAPS) *N*-phenethyl congeners all induced marked early losses (50–70%) followed by similarly slow recovery, with projected full restoration of D_2 receptor binding at about 7–10 days (Fig. 3A). With functional *in vivo* testing, however, apomorphine-induced behavioral arousal was nearly completely inhibited by all agents tested (including SPR) within the first 24 hr (Fig. 3B). Recovery after SPR was rapid and included responses to the DA agonist that tended ($p \leq 0.05$, by one-tailed t test at day 4) to rise above control levels ('supersensitivity') at days 2–4. Recovery after the phenethylspiperones was more gradual and only slightly longer after NIPS than after its nonalkylating congener NAPS.

Loss of D_2 binding *ex vivo* and of responsiveness to apomorphine *in vivo* were similarly well correlated after treatment with the substituted phenethylspiperones, at all doses and times

TABLE 3

***Ex vivo* and *in vivo* potency of SPR derivatives**

Doses of test agents of 0 (vehicle control) or 0.05–100 μmol/kg (intraperitoneally) were given to rats ($n \geq 6$); 24 hr later, rats were challenged with an acute dose of (*R*)-(−)-apomorphine (0.3 mg/kg, intraperitoneally) and scored for stereotyped behaviors, or brain tissue was recovered and striatum was assayed for D_2 receptor levels with [^{3}H]YM-09151-2 (65 pM), as for Tables 1 and 2, or assayed with a range of ligand concentrations to determined B_{max}, as for Fig. 2. Pooled data were analyzed with the ALLFIT program to obtain values of ID_{50} (± standard error).

Agent	ID_{50}		Potency ratio
	Anti-D_2	Anti apomorphine	
	μmol/kg		
NPS	1.5 ± 0.5	0.29 ± 0.04[a]	5.2
NAPS	9.1 ± 2.9	0.47 ± 0.20[a]	19.4
NIPS	5.6 ± 1.2	0.39 ± 0.05[a]	14.4

[a] Differences between the two measures are significant by t test ($t \geq 2.4$, $p < 0.05$).

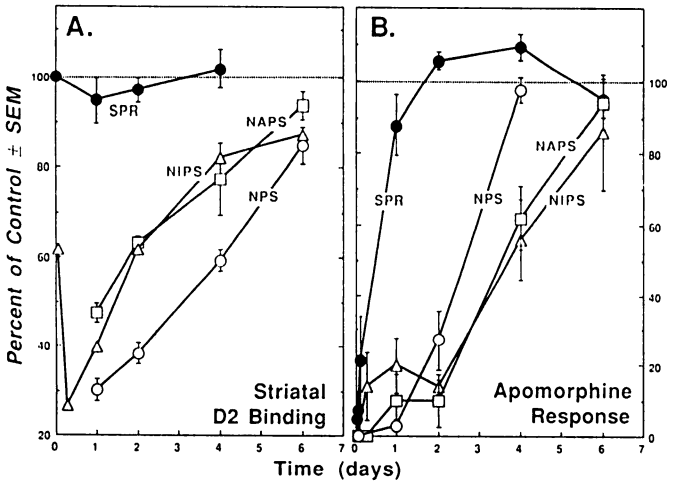

Fig. 3. Time course of *in vivo* recovery from antidopaminergic activity of SPR derivatives. A, Striatal D_2 binding. Rats in groups of four to six were given a single equimolar dose (15 μmol/kg, intraperitoneally) of SPR (●), NPS (○), NAPS (□), or NIPS (△) and monitored for the times indicated, ranging from 1–6 hr to 4–6 days, when groups were sacrificed for assays of D_2 receptor binding with striatal tissue (with 65 pM [^{3}H]YM-09151-2). Data were pooled from at least two replicate experiments and are expressed as percentage of matched vehicle-pretreated control values (mean ± standard error). B, Behavioral response to apomorphine. Groups of four to six rats were treated as for A (with the same agents, doses, and symbols) and tested for stereotyped behavioral responses to (*R*)-(−)-apomorphine (0.3 mg/kg, intraperitoneally) at the times indicated. Data were pooled from at least two separate replicate experiments and are expressed as percentage of matched controls (mean ± standard error). The apparent supersensitivity to apomorphine at 2 and 4 days after SPR is a marginally significant trend ($p \leq 0.05$–0.10, by *t* test).

(linear $r = 0.81$–0.94; all $p \leq 0.0015$) for NIPS, NAPS, or NPS. When all of these data were pooled for all three compounds, the nonparametric rank correlation was highly significant (Spearman $r = 0.83$, $p < 0.001$; not shown) but indicated that a 50% loss of D_2 binding was associated, overall, with a 73.0% loss of apomorphine response.

Discussion

The present experiments indicate that *N*-phenethyl derivatives of SPR (Fig. 1; Table 1), including several containing alkylating *p*-substituents, can retain high D_2 affinity and D_2 versus D_1 selectivity at DA receptors in rat forebrain tissue (Table 1). The isothiocyanate derivative, NIPS, had the highest D_2 affinity and selectivity (Table 1) and so was evaluated further as a potential irreversible D_2 affinity ligand. This compound also had higher D_2 versus 5-HT_2 selectivity *in vitro* (Table 1) and *ex vivo* (Table 2) than did SPR itself or NAPS. A similar gain in D_2 versus 5-HT_2 selectivity over that of SPR was reported recently with a series of analogous *N*-benzyl derivatives of SPR being considered for development as potential positron-emitting neuroradiologic ligands (36). In that report, D_2 and 5-HT_2 affinity (K_i) data were reported (assayed with [^{3}H]SPR and ^{125}I-LSD, respectively) for SPR and its *N*-methyl- and *N*-phenethyl derivatives also evaluated here, with similar results (58 versus our 170 pM D_2 affinity of SPR, 118 versus 35 pM for NMS, and 35 versus 120 pM for NPS; for 5-HT_2 affinity, the values were 450 versus our 450 pM for SPR, 550 versus 400 pM for NMS, and 1.0 versus 4.5 nM for NPS). Moreover, their values for the lipophilicity (log *P*) for NMS and NPS were close to those reported here (3.2 versus our 3.3 for NMS and 5.3 versus 4.4 for NPS; see Table 1). In contrast, D_2 affinity of NIPS with rat striatal tissue, using [^{3}H]SPR as radioligand, was reported by Xu *et al.* (5) to be much lower than reported here (K_i = 10 nM versus our 57 pM; see Table 1), but this agent showed a similar *ex vivo* anti-D_2 potency (~5–10 mg/kg or ~10–20 μmol/kg, subcutaneously versus our 5.6 μmol/kg, intraperitoneally; see Table 3) and duration of action (~8–10 days; see Fig. 3).

In addition to the well known 5-HT_2 affinity of SPR derivatives (35, 36), it now appears that such compounds, including NIPS and other phenylspiperones, also have appreciable affinity at adrenergic receptors, especially the α_1 type, in rat forebrain tissue (Table 1). *Ex vivo*, however, NAPS but not NIPS had a significant impact on α_1 binding, although this was much less than that at D_2 sites (Table 2). NIPS also had a negligible effect at D_1 sites, reflecting its high apparent D_2 versus D_1 selectivity *in vitro*, whereas NAPS had a small but appreciable *ex vivo* effect that is consistent with its 2.5-fold lesser *in vitro* D_2 versus D_1 selectivity, compared with that of NIPS (Tables 1 and 2).

A particularly curious aspect of the present findings is the striking similarity of anti-DA effects of the alkylating agent NIPS and its nonalkylating congeners NPS and NAPS. Thus, whereas SPR seemed to disappear or be washed out of brain tissue during tissue preparation in *ex vivo* studies, NPS and NAPS as well as NIPS induced marked decreases in the binding of the D_2 ligand [^{3}H]YM-09151-2 that survived tissue preparation (Fig. 3A). Moreover, in both *ex vivo* and *in vitro* experiments, the loss in D_2 binding with NAPS as well as NIPS was reflected in a large decrease in B_{max}, with little change in apparent ligand affinity (Fig. 2), suggesting avid and noncompetitive binding of the phenethylspiperones with tenacious D_2 receptor blockade as an apparent result. In contrast, SPR had a potent antiapomorphine effect *in vivo* but yielded little reduction of D_2 binding with homogenized tissue *ex vivo* (Fig. 3A) or after extensive washing following exposure to high concentrations of this agent *in vitro* (data not shown). Furthermore, the time course of recovery of functional responses to *in vivo* challenges with the DA agonist apomorphine, as well as that of D_2 binding *ex vivo*, was at least as prolonged with NPS and NAPS as with NIPS (Fig. 3B). Because the latter time courses (recovery over ~8–10 days) were similar to those found under similar conditions after treatment with the alkylating agent EEDQ (5, 6), it seems likely that the recovery of D_2 receptor availability and function after NIPS may reflect new synthesis of D_2 proteins or, perhaps, the redistribution of preformed "spare" receptors not accessible to the affinity ligand. Evidently, too, the similarly prolonged recovery with nonalkylating, but highly lipophilic and very potently D_2-bound, congeners NPS and NAPS (Table 1) reflects avid binding to D_2 receptors in neuronal membranes. Recovery from such avid ligand–D_2-receptor interactions may also depend on production of new unoccupied D_2 receptors, as is proposed for NIPS. Alternatively, NPS and NAPS may alter the molecular properties of D_2 receptors or their intracellular processing. Because NAPS and NIPS both produced powerful receptor blocking actions *in vitro*, the long lasting effects of NAPS *in vivo* are not likely to reflect a major difference in its metabolic disposition from that of SPR.

The phenethylspiperones (including, NIPS, NAPS, and NPS) blocked functional responses to apomorphine *in vivo*

with higher apparent potency than would be predicted by the smaller, but highly correlated ($r = 0.83$), decreases of striatal D_2 receptor sites *ex vivo* (Table 3). The basis of this difference is not clear. It may reflect technical differences in the two measures, such as the selection of an unrepresentative test dose of apomorphine or tissue sample (striatum) for *ex vivo* experiments; it is also likely that D_2 binding sites in homogenized membrane preparations include receptors not directly related to the behavioral actions of the DA agonist on behavior or on the physiology of intact brain tissue. It was suggested previously, based on studies with the nonspecific alkylating agent EEDQ, that the "receptor reserve" or relative abundance of postsynaptic D_2 receptors (presumably most relevant to the *in vivo* actions of apomorphine) may be limited (37–39) and so these receptors may be more sensitive to avid occupation that may have lesser effects at other sites, such as presynaptic autoreceptors.

In striking contrast to the present observations, we have found much more prolonged anti-DA activity of the analogous D_2 antagonist haloperidol, which can interfere with the behavioral actions of apomorphine in the rat for more than 4 weeks after a single, moderate, systemic dose (40). This time course closely paralleled the slow disappearance of the butyrophenone in rat brain tissue and contrasts with the short duration of action and brain tissue retention of phenothiazine DA antagonists, which, like SPR here (Fig. 3B), can rapidly induce supersensitivity to apomorphine (41). The prolonged blockade of functional DA receptors by haloperidol far exceeds the duration of recovery from alkylating ligands such as NIPS or EEDQ, as well as that of nonalkylating phenethylspiperones (Fig. 3) (4–6). Thus, it may be that haloperidol is not only quite lipophilic and strongly bound to membranes and D_2 receptors but also able to redistribute, either locally in neuronal membranes or cerebral tissue or by systemic recirculation (41), so as to account for its extraordinarily prolonged anti-DA actions. If such redistribution occurs, it may include the ability to interact with newly synthesized D_2 receptors to account for a duration of action that is, paradoxically, much longer than that of the D_2 alkylating agents NIPS and EEDQ. The similarity of the timing of recovery of D_2 binding and function after NPS or NAPS to that after treatment with NIPS or EEDQ, and the dissimilarity of all of these ligands to haloperidol, suggests that the phenylspiperones, like EEDQ, may be bound to D_2 receptors and other membrane sites with high avidity (or presumably covalently in the case of NIPS) and not able to redistribute or bind to newly available or newly synthesized DA receptors. Alternative possibilities include long-lasting molecular alterations of D_2 receptors, or even interference with their synthesis, by haloperidol but not the phenethylspiperones.

This evidence of prolonged retention of nonalkylating lipophilic phenethylspiperones, if (as is likely) it also occurs at non-D_2 sites in DA-poor regions of brain tissue, might make such compounds relatively unfavorable for application in certain D_2-labeling conditions. These may include development of lipophilic SPR derivatives as radioligands for application in positron-emission tomographic or single-photon emission computed tomographic brain-scanning methods, where not only high receptor affinity and selectivity but also a high regional selectivity of bulk distribution and retention are required (36).

In conclusion, the present results support the proposal that phenethylspiperones represent a group of potent and relatively D_2-selective affinity ligands of high avidity, with or without an alkylating moiety present. They may be useful for applications formerly considered with less selective alkylating agents such as EEDQ, including estimates of rates of DA receptor synthesis (5, 6, 16). Finally, such compounds with indicator moieties or radioisotopes incorporated (if they have high affinity and both regional distributional and receptor type selectivity) might be of value for histologic or neuroradiologic applications as well.

Acknowledgments

Dr. Nandkishore Baindur provided helpful discussions of chemical syntheses. Mr. Scott MacLeod determined liquid chromatographic values; Drs. Alexander Tropsha and Raymond G. Booth of the University of North Carolina School of Pharmacy kindly assisted with calculated measures of lipophilicity. Some receptor affinities were screened by NovaScreen Division of Nova Pharmaceutical Corp., with the support of National Institute of Mental Health Contract 278-89-0011. Drug substances were donated by CIBA-Geigy, Lundbeck Laboratories, and Sandoz Research Institute (Berne, Switzerland). Drs. Peter Munson and David Rodbard of the National Institutes of Health Biostatistical and Computing Center (Bethesda, MD) generously provided an electronic copy of their LIGAND program.

References

1. Baker, B. R. *Design of Active-Site Directed Irreversible Enzyme Inhibitors.* J. Wiley and Sons, New York, (1967).
2. Takemori, A. E., and P. S. Portoghese. Development of nonequilibrium opioid receptor probes. *Annu. Rev. Pharmacol. Toxicol.* **25**:193–223 (1985).
3. Newman, A. H. Irreversible ligands for drug-receptor characterization. *Ann. Rep. Med. Chem.* **25**:271–280 (1990).
4. Meller, E., K. Bohmaker, M. Goldstein, and A. J. Friedhoff. Inactivation of D_1 and D_2 dopamine receptors by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline *in vivo*: selective protection by neuroleptics. *J. Pharmacol. Exp. Ther.* **233**:656–662 (1985).
5. Xu, S., Y. Hadata, L. E. Black, I. Creese, and D. R. Sibley. *N*-(*p*-Isothiocyanatophenethyl)spiperone, a selective and irreversible antagonist of D_2 dopamine receptors in brain. *J. Pharmacol. Exp. Ther.* **257**:608–615 (1991).
6. Kula, N. S., T. George, and R. J. Baldessarini. Developmental changes in rate of synthesis of D_1 and D_2 receptors following alkylation with EEDQ. *Dev. Brain Res.* **66**:286–289 (1992).
7. Walton, K. G., P. Liepmann, and R. J. Baldessarini. Inhibition of dopamine-stimulated adenylate cyclase activity by phenoxybenzamine. *Eur. J. Pharmacol.* **52**:231–234 (1978).
8. Baldessarini, R. J., N. S. Kula, G. W. Arana, J. L. Neumeyer, and S.-J. Law. Chloroethylnorapomorphine, a proposed long-acting dopamine antagonist: interactions with dopamine receptors of mammalian forebrain *in vitro*. *Eur. J. Pharmacol.* **67**:105–110 (1980).
9. Cohen, S. A., and J. L. Neumeyer. Aporphines 50. Kinetics of solvolysis of *N*-(2-chloroethyl)norapomorphine, an irreversible dopamine receptor antagonist. *J. Med. Chem.* **26**:1348–1353 (1983).
10. Winkler, J. D., K. Thermos, and B. Weiss. Differential effects of fluphenazine-*N*-mustard, on calmodulin activity and on D_1 and D_2 dopaminergic responses. *Psychopharmacology* **92**:285–291 (1987).
11. Amlaiky, N., and M. G. Caron. Photoaffinity labeling of the D_2 dopamine receptor with novel high affinity radioiodinated probes. *J. Biol. Chem.* **260**:1983–1986 (1985).
12. Barton, A. C., H. C. Kang, M. S. Rinando, F. J. Monsma, R. M. Stewart-Fram, J. A. Macinko, R. F. Haugland, M. A. Ariano, and D. R. Sibley. Multiple fluorescent ligands for dopamine receptors. I. Pharmacological characterization and receptor selectivity. *Brain Res.* **547**:199–207 (1990).
13. Bakthavachalam, V., N. Baindur, B. K. Madras, and J. L. Neumeyer. Fluorescent probes for dopamine receptors: synthesis and characterization of fluorescein and 7-nitro-2-oxa-1,3-diazol-4-yl conjugates of D_1 and D_2 receptor ligands. *J. Med. Chem.* **34**:3235–3241 (1991).
14. Neumeyer, J. L., R. J. Baldessarini, N. S. Kula, V. Bakthavachalam, J. Yuan, N. Baindur, and A. Campbell. Candidate affinity ligands for D_1 and D_2 dopamine receptors. *Soc. Neurosci. Abstr.* **16**:533 (1990).
15. Yuan, J., V. Bakthavachalam, N. S. Kula, A. Campbell, R. J. Baldessarini, and J. L. Neumeyer. Irreversible ligands for dopamine D_2 receptors: synthesis and activity (Abstr. No. 98), in *Proceedings of the American Chemical Society Medicinal Chemistry Division 199th National Meeting* (1990).
16. Baldessarini, R. J., N. S. Kula, T. George, A. Campbell, V. Bakthavachalam, and J. L. Neumeyer. Recovery of central D_1 and D_2 receptors following alkylation in young and adult rat brain. *Soc. Neurosci. Abstr.* **17**:1691 (1991).
17. Neumeyer, J. L., R. J. Baldessarini, N. S. Kula, V. Bakthavachalam, J. Yuan, N. Baindur, and A. Campbell. Selective affinity ligands for D_1 and D_2 receptors, in *Proceedings of the International Brain Research Organization (IBRO) Meeting*, 277 (1991).
18. Burns, H. D., R. F. Dannals, B. Langstrom, H. T. Ravert, S. E. Zemyan, T. Duelfer, D. F. Wong, J. Frost, M. J. Kuhar, and H. N. Wagner. (3-*N*-[^{11}C] Methyl)spiperone, a ligand binding to dopamine receptors: radiochemical

synthesis and biodistribution studies in mice. *J. Nucl. Med.* **25**:1222–1227 (1984).
19. Sharma, S. Thiophosgene in organic synthesis. *Synthesis* 803–820 (1978).
20. Ferris, A. F., and W. D. Emmons. Metathetical reactions of silver salts in solution. I. Synthesis of acyl trifluoroacetates. *J. Am. Chem. Soc.* **75**:232–233 (1953).
21. Hansch, C., and A. Leo. *Substituent Constants for Correlation Analysis in Chemistry and Biology*. John Wiley and Sons, New York (1979).
22. Moerlein, S. M., P. Laufer, and G. Stoecklin. Effect of lipophilicity on the *in vivo* localization of radiolabeled spiperone analogues. *Int. J. Nucl. Med. Biol.* **12**:353–356 (1985).
23. Delgado, J. N., and W. A. Remers, eds. Statistical prediction of pharmacological activity, in *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry*. J. B. Lippincott Co., Philadelphia, 19–25 (1991).
24. Neumeyer, J. L., Y. Gao, N. S. Kula, and R. J. Baldessarini. *R*- and *S*-enantiomers of 11-hydroxy- and 10,11-dihydroxy-*N*-allylnoraporphine: synthesis and affinity for dopamine receptors in rat brain tissue. *J. Med. Chem.* **34**:24–28 (1991).
25. Munson, P. J., and D. Rodbard. LIGAND: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* **107**:220–239 (1980).
26. Munson, P. J., D. Rodbard, and M. S. Hinchcliff. LIGAND: SCAPRE 4.1 and SCAFIT 4.5 software for the MacIntosh computer. National Institutes of Health Biostatistical and Computing Center, Bethesda, MD (1991).
27. DeLean, A., P. J. Munson, and D. Rodbard. Simultaneous analysis of families of sigmoid curves: application to bioassay, radioligand, and physiological dose-response curves. *Am. J. Physiol.* **4**:E97–E102 (1978).
28. Teicher, M. H. Med-65, ALLFIT, GRAFIT (Applesoft). Vanderbilt University Biomedical Computing Technology Information Center, Nashville, TN (1983).
29. Cheng, Y., and W. H. Prusoff. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22**:3099–3108 (1973).
30. Campbell, A., R. J. Baldessarini, Y. Gao, V. J. Ram, and J. L. Neumeyer. *R*(−) and *S*(+) stereoisomers of 11-hydroxy-*N*-*n*-propylnoraporphine: central dopaminergic behavioral activity in the rat. *Neuropharmacology* **29**:527–536 (1990).
31. Billard, W., V. Ruperto, G. Crosby, L. C. Iorio, and A. Barnett. Characterization of the binding of [^{3}H]-SCH 23390: a selective D_1 receptor antagonist ligand in rat striatum. *Life Sci.* **35**:1885–1893 (1984).
32. Faedda, G., N. S. Kula, and R. J. Baldessarini. Pharmacology of binding of ^{3}H-SCH-23390, a ligand selective for D_1 dopamine receptor sites, in rat brain tissue. *Biochem. Pharmacol.* **38**:473–480 (1989).
33. Leysen, J. E., C. J. Niemegeers, J. M. Van-Nueten, and P. M. Laduron. [^{3}H] Ketanserin (R-41,468): a selective tritiated ligand for serotonin$_2$ receptor binding sites: binding properties, brain distribution, and functional role. *Mol. Pharmacol.* **21**:301–314 (1982).
34. Timmermans, B. P. M., W. M. K. Karamat-Ali, H. Y. Kwa, A. M. C. Schoop, F. P. Slothorst-Grisdijk, and P. A. van Zwieten. Identical antagonist selectivity of central and peripheral *alpha*$_1$-adrenoceptors. *Mol. Pharmacol.* **20**:295–301 (1981).
35. Seeman, P. Brain dopamine receptors. *Pharmacol. Rev.* **32**:229–313 (1981).
36. Mach, R. H., J. R. Jackson, R. R. Luedtke, K. J. Ivins, P. B. Molinoff, and R. L. Ehrenkaufer. Effect of *N*-alkylation on the affinities of analogs of spiperone for dopamine D_2 and serotonin 5-HT_2 receptors. *J. Med. Chem.* **35**:423–430 (1992).
37. Meller, E. A., and M. Goldstein. Absence of receptor reserve at striatal dopamine receptors regulating cholinergic neuronal activity. *Eur. J. Pharmacol.* **155**:151–154 (1988).
38. Cox, R. F., and B. I. Waszczak. Differences in dopamine receptor reserve for *N*-*n*-propylnorapomorphine enantiomers: single unit recording studies after partial inactivation of receptors by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline. *Mol. Pharmacol.* **34**:125–131 (1989).
39. Booth, R. G., R. J. Baldessarini, N. S. Kula, Y. Gao, R. Zong, and J. L. Neumeyer. Presynaptic inhibition of dopamine synthesis in rat striatal tissue by enantiomeric mono- and dihydroxyaporphines. *Mol. Pharmacol.* **38**:92–101 (1990).
40. Campbell, A., R. J. Baldessarini, and N. S. Kula. Prolonged antidopamine actions of single doses of butyrophenones in the rat. *Psychopharmacology* **87**:161–166 (1985).
41. Cohen, B. M., T. Tsuneizumi, R. J. Baldessarini, A. Campbell, and S. Babb. Differences between antipsychotic drugs in persistence of brain levels and behavioral effects. *Psychopharmacology* **108**:338–344 (1992).

Send reprint requests to: Dr. R. J. Baldessarini, Mailman Research Center, McLean Hospital, 115 Mill Street, Belmont, MA 02178.