0022-3565/02/3032-805–814$7.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 303, No. 2
39875/1021571
JPET 303:805–814, 2002
Printed in U.S.A.

Differential Actions of Antiparkinson Agents at Multiple Classes of Monoaminergic Receptor. II. Agonist and Antagonist Properties at Subtypes of Dopamine D_2-Like Receptor and α_1/α_2-Adrenoceptor

ADRIAN NEWMAN-TANCREDI, DIDIER CUSSAC, VALÉRIE AUDINOT, JEAN-PAUL NICOLAS, FRÉDÉRIC DE CEUNINCK, JEAN-A. BOUTIN, and MARK J. MILLAN

Departments of Psychopharmacology (A.N.-T., D.C., F.D., M.J.M.) and Molecular and Cellular Pharmacology (V.A., J.-P.N., J.-A.B.), Institut de Recherches Servier, Paris, France

Received June 12, 2002; accepted July 22, 2002

ABSTRACT

The accompanying multivariate analysis of the binding profiles of antiparkinson agents revealed contrasting patterns of affinities at diverse classes of monoaminergic receptor. Herein, we characterized efficacies at human (h)$D_{2SHORT(S)}$, $hD_{2LONG(L)}$, hD_3, and $hD_{4.4}$ receptors and at $h\alpha_{2A}$-, $h\alpha_{2B}$-, $h\alpha_{2C}$-, and $h\alpha_{1A}$-adrenoceptors (ARs). As determined by guanosine 5′-*O*-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) binding, no ligand displayed "full" efficacy relative to dopamine (100%) at all "D_2-like" sites. However, at hD_{2S} receptors quinpirole, pramipexole, ropinirole, quinerolane, pergolide, and cabergoline were as efficacious as dopamine ($E_{max} \geq$ 100%); TL99, talipexole, and apomorphine were highly efficacious (79–92%); piribedil, lisuride, bromocriptine, and terguride showed intermediate efficacy (40–55%); and roxindole displayed low efficacy (11%). For all drugs, efficacies were lower at hD_{2L} receptors, with terguride and roxindole acting as antagonists. At hD_3 receptors, efficacies ranged from 33% (roxindole) to 94% (TL99), whereas, for hD_4 receptors, highest efficacies (~70%) were seen for quinerolane, quinpirole, and TL99, whereas piribedil and terguride behaved as antagonists and bromocriptine was inactive. Although efficacies at hD_{2S} versus hD_{2L} sites were highly correlated ($r = 0.79$), they correlated only modestly to hD_3/hD_4 sites ($r = 0.44–0.59$). In [^{35}S]GTPγS studies of $h\alpha_{2A}$-ARs, TL99 (108%), pramipexole (52%), talipexole (51%), pergolide (31%), apomorphine (16%), and quinerolane (11%) were agonists and ropinirole and roxindole were inactive, whereas piribedil and other agents were antagonists. Similar findings were obtained at $h\alpha_{2B}$- and $h\alpha_{2C}$-ARs. Using [^{3}H]phosphatidylinositol depletion, roxindole, bromocriptine, lisuride, and terguride displayed potent antagonist properties at $h\alpha_{1A}$-ARs. In conclusion, antiparkinson agents display diverse agonist and antagonist properties at multiple subtypes of D_2-like receptor and α_1/α_2-AR, actions, which likely contribute to their contrasting functional profiles.

Although treatment of Parkinson's disease has long centered on administration of the dopamine precursor L-dihydroxyphenylacetyl acid (L-DOPA), there is increasing interest in the therapeutic use of dopaminergic agonists, both in association with L-DOPA and as monotherapy (Hughes, 1997). Inasmuch as dopaminergic agents currently used as antiparkinson agents interact principally with "D_2-like" receptors, an important question concerns their comparative actions at D_2 receptors (of which functionally distinct short D_{2S} and long D_{2L} isoforms exist), D_3 receptors, and D_4 receptors. D_{2S} versus D_{2L} receptor isoforms differ in both their localization and their functional roles. The D_{2S} isoform is principally responsible for presynaptic control of dopamine release, whereas postsynaptic D_{2S} and D_{2L} receptors in the basal ganglia, via contrasting patterns of interaction with D_1 sites, differentially influence motor function; notably, blockade of D_{2L} sites underlies the extrapyramidal motor effects of dopaminergic antagonists (Wang et al., 2000). As shown in the accompanying article (Millan et al., 2002), therapeutically used antiparkinson agents recognize D_{2S} and D_{2L} isoforms with similar affinity, and many antiparkinson agents also interact with dopamine D_3 receptors. Although the density of striatal D_3 receptors is reduced upon degeneration of nigrostriatal dopaminergic pathways, exposure to L-DOPA may induce their up-regulation, reflecting complex regulatory mechanisms involving dopamine D_1 receptors and brain-derived neurotrophic factor (Quik et al., 2000; Guillin et al., 2001; Joyce, 2001). Nevertheless, the precise nature of functional interrelationships among D_3, D_2, and D_1 receptors, and the implication of D_3 receptors in the therapeutic com-

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.
DOI: 10.1124/jpet.102.039875.

ABBREVIATIONS: L-DOPA, L-dihydroxyphenylacetic acid; AR, adrenoceptor; SNPC, substantia nigra pars compacta; NA, noradrenaline; h, human; CHO, Chinese hamster ovary; DA, dopamine; [^{35}S]GTPγS, guanosine 5′-*O*-(3-[^{35}S]thio)triphosphate; PI, phosphatidylinositol.

pared with dyskinetic effects of antiparkinson agents, remain to be clarified (Joyce, 2001). The majority of antiparkinson agents also show significant affinity for D_4 receptors (Millan et al., 2002), but their engagement does not improve motor function; furthermore, antagonist properties at D_4 receptors may minimize psychiatric side effects and improve cognitive function (Newman-Tancredi et al., 1997; Arnsten et al., 2000).

Several antiparkinson drugs display pronounced affinities for hα_{2A}-, hα_{2B}-, and hα_{2C}-ARs (Millan et al., 2002). This is of note in light of the importance of adrenergic mechanisms in the etiology and treatment of Parkinson's disease (Brefel-Courbon et al., 1998; Bezard et al., 2001). In addition to their postsynaptic localization, α_{2A}-ARs are expressed as inhibitory autoreceptors on adrenergic neurons (Nicholas et al., 1997; Millan et al., 2000a,b). Furthermore, α_{2A}-ARs exert an inhibitory influence upon ascending serotonergic pathways, frontocortical and subcortical dopaminergic pathways (Kable et al., 2000; Millan et al., 2000a,b) as well as corticolimbic cholinergic and glutamatergic pathways (Horn et al., 1982; Tellez et al., 1997; Boehm, 1999). Correspondingly, α_{2A}-ARs fulfill an important role in the control of motor function, mood, and cognition (Arnsten, 1997; Kable et al., 2000; Millan et al., 2000b). Furthermore, α_{2B}-ARs are enriched in the thalamus, a structure interlinked with the basal ganglia and involved in the disruption of motor function in Parkinson's disease, whereas α_{2C}-ARs are concentrated in the striatum itself (Nicholas et al., 1997; Bezard et al., 2001). Gene knockout studies have indicated a modulatory influence of central α_{2C}-ARs, complementary to α_{2A}-ARs, upon motor and cognitive function (Kable et al., 1999; Bjorklund et al., 2000). Although the precise significance of individual α_2-AR subtypes remains unclear, there is evidence that α_2-AR antagonist properties may be useful in the management of Parkinson's disease. First, in rats sustaining unilateral 6-hydroxydopamine lesions of the substantia nigra pars compacta (SNPC), α_2-AR agonists and antagonists, respectively, inhibit and enhance amphetamine-induced rotation (Mavridis et al., 1991). Second, in primates displaying Parkinson's disease-like symptoms after treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, α_2-AR antagonists increase locomotor activity and reduce dyskinesias induced by L-DOPA (Brefel-Courbon et al., 1998; Bezard et al., 2001). Third, after enhancement of adrenergic transmission by blockade of α_{2A}-AR autoreceptors, noradrenaline (NA) may (independently of α_2-ARs) exert neuroprotective actions at dopaminergic neurons in the SNPC (Troadec et al., 2001). Fourth, small-scale clinical studies indicate that the α_2-AR antagonist idazoxan improves motor performance in Parkinson's disease patients receiving L-DOPA (Brefel-Courbon et al., 1998).

Several antiparkinson agents also interact with hα_{1A}-, hα_{1B}-, and hα_{1D}-ARs (Millan et al., 2002). Although the relevance of α_1-ARs to management of Parkinson's disease is less apparent than for their α_2-AR counterparts, they modulate ascending serotoninergic and dopaminergic transmission and play an important role in motor control (Millan et al., 2000a; Spreng et al., 2001; Stone et al., 2001). Indeed, α_1-AR antagonists interfere with the induction of rotation by antiparkinson agents in rats sustaining unilateral lesions of the SNPC (Mavridis et al., 1991). Furthermore, frontocortical α_1-ARs are implicated in the control of cognitive function (Arnsten, 1997). The perturbation of cardiovascular function associated with pronounced activation or blockade of α_1-ARs should also be accentuated (Guimarães and Moura, 2001).

The above-mentioned observations exemplify the importance of characterizing functional actions of antiparkinson agents at subtypes of "hD_2-like" receptor and h$\alpha_{1/2}$-AR. However, studies have been restricted to a few ligands at poorly characterized native sites compared with defined classes of (cloned) human receptor (see *Discussion*). Knowledge of the comparative agonist/antagonist profiles of antiparkinson agents remains, thus, fragmentary. The present study expanded, therefore, the multivariate analyses of binding profiles presented in the preceding article (Millan et al., 2002) in evaluating efficacies of diverse antiparkinson agents at cloned hD_{2S}, hD_{2L}, hD_3, and hD_4 dopamine receptors, and at hα_{2A}-, hα_{2B}- hα_{2C}-, and hα_{1A}-ARs, stably expressed in a common cellular system, Chinese hamster ovary (CHO) cells.

Materials and Methods

Determination of Drug Efficacies at hD_2-Like Receptors and at hα_2-AR Subtypes by [^{35}S]GTPγS Binding. Efficacies at CHO-expressed recombinant hD_{2S}, hD_{2L}, hD_3, and hD_4 (h$D_{4.4}$ isoform) receptors, and at CHO-expressed hα_{2A}-, hα_{2B}-, and hα_{2C}-ARs were determined by measuring the influence of drugs alone and, where appropriate, in interaction with DA or NA upon [^{35}S]GTPγS binding. The protocols used have been described in detail previously (Newman-Tancredi et al., 1997, 1999a,b; Millan et al., 2001). Briefly, the concentration of [^{35}S]GTPγS was 0.1 nM (hD_{2S}, hD_{2L}, and hD_4), 0.2 nM (hα_{2A}-AR, hα_{2B}-AR, and hα_{2C}-AR) or 1.0 nM (hD_3). The pH was 7.4 in each case and the temperature 22°C. Incubation time was 40 min for hD_{2S}, hD_{2L}, and hD_3 sites, 20 min for hD_4 sites, and 60 min for hα_2-AR subtypes. The buffer contained 20 mM HEPES, 100 or 150 mM NaCl, 3 μM GDP, and 3 or 10 mM $MgCl_2$. Membranes were incubated with the antiparkinson agent alone and/or with DA (3 μM-hD_{2S}, 10 μM-hD_{2L}, and 1 μM-hD_4) or NA (10 μM for each subtype) for 15 min before the addition of [^{35}S]GTPγS. Agonist efficacies were expressed as a percentage of the effect observed with maximally effective concentrations of DA (10 μM) or NA (10 μM). Experiments were terminated by rapid filtration through GF/B filters (Whatman, Maidstone, UK) using a 96-well cell harvester (Packard Instrument Company, Inc., Downers Grove, IL), and radioactivity was determined by liquid scintillation counting.

Determination of Drug Efficacies at hα_{1A}-ARs by [^{3}H]Phosphatidylinositol ([^{3}H]PI) Depletion. The efficacies of drugs alone, and in interaction with NA, were determined in CHO-expressed hα_{1A}-ARs as described previously (Millan et al., 2001). Briefly, cells were labeled with 2 μCi/ml of [^{3}H]myoinositol (10–20 Ci/mmol) for 24 h. Cells were washed and then incubated at 37°C for 30 min with the drug alone in Krebs-LiCl buffer: 15.6 mM NaH_2PO_4 pH 7, 120 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM $CaCl_2$, 0.6% (w/v) glucose, 0.04% (w/v) bovine serum albumin, and 10 mM LiCl. In the absence of NA, ~40,000 dpm was typically detected, compared with ~25,000 in the presence of a maximally effective concentration of NA (30 μM). Drug efficacies were expressed as a percentage of the effect observed with a maximally effective concentration of NA (30 μM). For antagonist studies, cells were preincubated for 15 min with drug before the addition of NA (10 μM) and incubation continued for 30 min. Membranes were recovered by rapid filtration through GF/B filters (Whatman) using a 96-well cell harvester (Packard Instrument Company, Inc.), and the [^{3}H]PI content was determined by scintillation counting (Millan et al., 2001).

Data Analyses. [^{35}S]GTPγS and [^{3}H]PI isotherms were analyzed by nonlinear regression using the program PRISM (GraphPad Software, San Diego, CA). K_B values for inhibition of DA- or NA-stimulated [^{35}S]GTPγS binding at hD_2-like or hα_2-ARs, and of NA-induced

[^{3}H]PI depletion at hα_{1A}-ARs, were calculated as described previously (Lazareno and Birdsall, 1993; Newman-Tancredi et al., 1999a,b) according to the equation $K_B = IC_{50}/\{[(2 + (agonist/EC_{50})^{nH})^{nH-1}] - 1\}$, where IC_{50} is the inhibitory concentration$_{50}$ of the antagonist, agonist is DA or NA concentration, EC_{50} is effective concentration$_{50}$ of DA or NA alone, and n_H is Hill coefficient of the DA or NA stimulation isotherm. EC_{50} values for NA at hα_{2A}-, hα_{2B}-, hα_{2C}-, and hα_{1A}-ARs were 354, 316, 302, and 329 nM, respectively. EC_{50} values for DA at hD_{2S}, hD_{2L}, hD_3, and hD_4 receptors were 350, 340, 11, and 100 nM, respectively. Protein concentrations were determined by use of a bichinconic acid kit (Sigma, St. Quentin Fallavier, France). Pearson product-moment correlation coefficients were calculated for pEC_{50} values determined herein compared with pK_i values determined in the accompanying article (Millan et al., 2002).

Drugs. Pramipexole dihydrochloride, piribedil hydrochloride, and ropinirole were synthesized by Servier Institut de Recherches (Paris, France). Lisuride maleate and terguride were donated by Schering (Berlin, Germany). Bromocriptine, (−)-quinpirole HCl, pergolide methanesulfonate, and TL99 (6,7-dihydroxy-*N*,*N*-dimethyl-2-aminotetralin) were purchased from Sigma/RBI (Natick, MA). Apomorphine hydrochloride was purchased from Sigma. Roxindole methanesulfonate was donated by Merck (Darmstadt, Germany) and talipexole by Boehringer Ingelheim GmbH (Ingelheim, Germany). Cabergoline was obtained from Farmitalia Carlo Erba (Rueil-Malmaison, France). Quinelorane dihydrochloride was a gift from Eli Lilly & Co. (Indianapolis, IN).

Results

Drug Actions at hD_{2S} Receptors. At hD_{2S} receptors (B_{max} = 1.4 pmol/mg), at a maximally effective concentration (10 μM), DA enhanced [^{35}S]GTPγS binding by ~2.5-fold; it displayed a pEC_{50} value of 6.5 (Fig. 1; Table 1). Quinpirole, pramipexole, quinelorane, pergolide, and cabergoline behaved as highly efficacious agonists at hD_{2S} receptors in stimulating G protein activation ([^{35}S]GTPγS binding) to a degree at least as marked as that of DA (E_{max} defined as 100%). TL99, talipexole, and apomorphine also showed high efficacies, whereas other ligands displayed less pronounced efficacies, ranging from 40% for terguride to 55% for lisuride. Roxindole showed very low efficacy. Drug potencies for stimulation of [^{35}S]GTPγS binding (pEC_{50} values) at hD_{2S} recep-

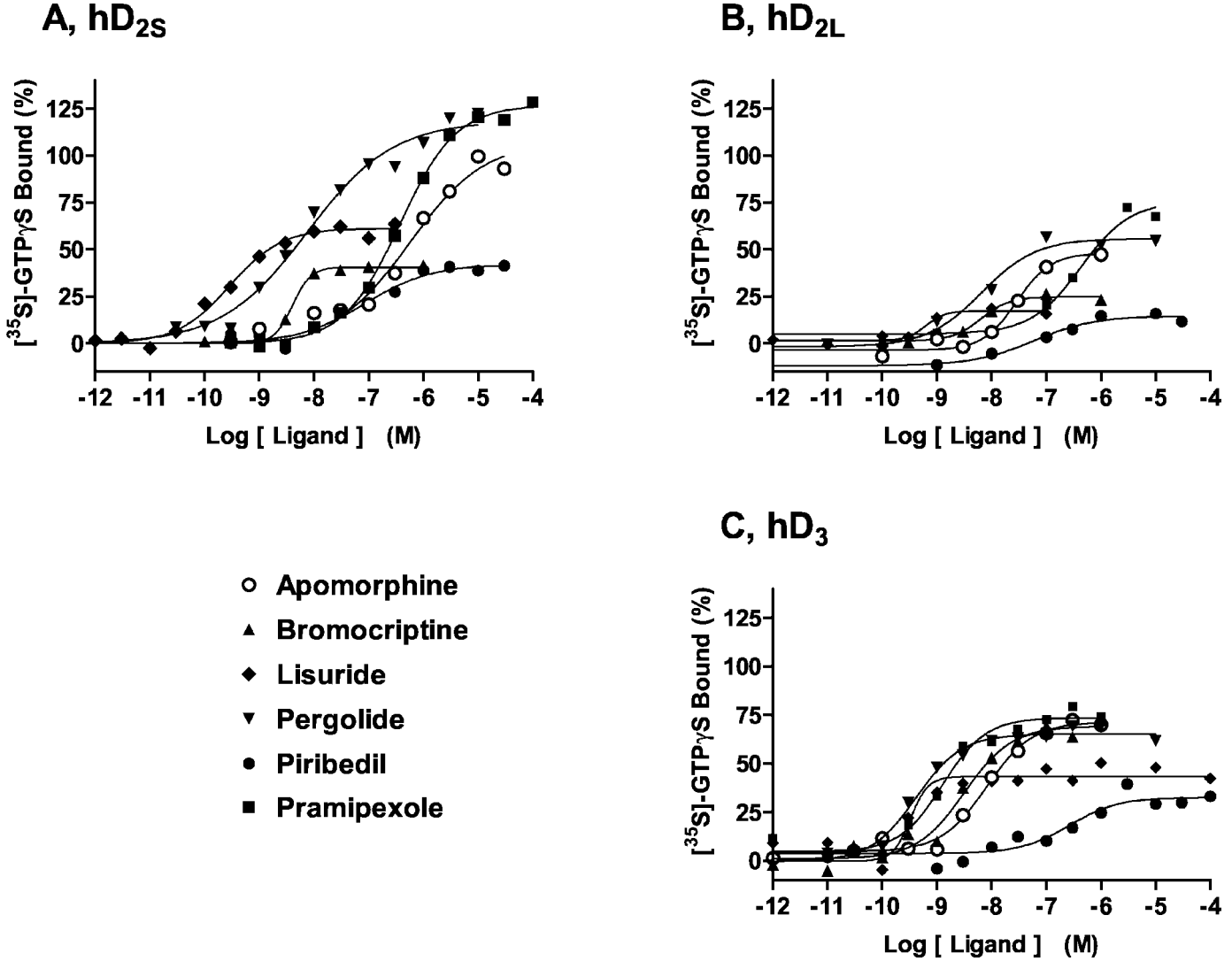

Fig. 1. Influence of antiparkinson agents upon G protein coupling at hD_{2S} (A), hD_{2L} (B), and hD_3 (C) receptors expressed in CHO cells. [^{35}S]GTPγS binding was carried out as described in Table 1. Binding is expressed as a percentage of that observed with a maximally effective concentration (10 μM) of dopamine (defined as 100%). Values shown are from representative experiments performed in triplicate and repeated on at least three occasions.

TABLE 1

Efficacies (E_{max} values) and potencies (pEC_{50} or $\mathbf{pK_b}$ values) of antiparkinson agents at recombinant hD_{2S}, hD_{2L}, hD_3, and hD_4 receptors

Efficacy (E_{max}) and potency (pEC_{50} or $\mathbf{pK_b}$) values at hD_{2S}, hD_{2L}, hD_3, and hD_4 receptors were determined by [^{35}S]GTPγS binding. pEC_{50} values for stimulation are indicated in normal case and $\mathbf{pK_b}$ values for inhibition are indicated in bold. E_{max} values are percentages of the stimulation observed with a maximally efficacious (E_{max} = 100%) concentration of dopamine (see *Materials and Methods*) and are expressed as means ± S.E.M. values of at least three independent determinations performed in triplicate. pEC_{50} values are means of at least three independent determinations: S.E.M.s values (not shown) were less than 0.2 log units. Dopamine exhibited pEC_{50} values of 6.46, 6.49, 7.95, and 7.00 at hD_{2S}, hD_{2L}, hD_3, and hD_4 receptors, respectively. For piribedil, the $\mathbf{pK_b}$ at hD_4 receptors is indicated in the table. It exhibited an agonist pEC_{50} value of 6.4 at these sites. For roxindole, the $\mathbf{pK_b}$ at hD_{2S} receptors is given in the table. It exhibited an agonist pEC_{50} value of 8.11 at these sites.

Ligand	hD_{2S}		hD_{2L}		hD_3		hD_4	
	E_{max}	pEC_{50} or $\mathbf{pK_b}$	E_{max}	pEC_{50} or $\mathbf{pK_b}$	E_{max}	pEC_{50}	E_{max}	pEC_{50} or $\mathbf{pK_b}$
Apomorphine	79 ± 6	7.71	53 ± 3	7.66	82 ± 5.7	7.93	45 ± 3	8.23
Bromocriptine	41 ± 1	8.35	28 ± 2	8.41	68 ± 7.2	8.38	0[a]	< 5[a]
Cabergoline	102 ± 1	9.27	75 ± 5	9.39	86 ± 6.7	9.11	49 ± 1	7.09
Lisuride	55 ± 1	9.54	21 ± 3	9.14	49 ± 9.3	9.24	32 ± 3[a]	8.23[a]
Pergolide	112 ± 3	8.06	52 ± 3	8.07	71 ± 9.4	9.29	56 ± 4[a]	7.53[a]
Piribedil	42 ± 8	6.71	21 ± 2	6.56	34 ± 2.3	6.91	7 ± 3	**5.37**
Pramipexole	130 ± 2	6.37	70 ± 8	6.47	70 ± 2.2	8.65	42 ± 1	6.89
Quinelorane	119 ± 6	6.85	105 ± 8	6.71	79 ± 1.5	9.17	72 ± 2[a]	7.83[a]
Quinpirole	132 ± 8[b]	6.09[b]	74 ± 2	6.23	67 ± 2.1[b]	8.17[b]	69 ± 4[c]	7.21
Ropinirole	108 ± 6	6.18	52 ± 3	6.38	59 ± 4	7.56	74 ± 2[a]	5.54[a]
Roxindole	11 ± 1[c]	**9.05**	0	**9.54**	30 ± 5.4[c]	9.23[c]	35 ± 3[c]	7.69[c]
Talipexole	89 ± 11	6.43	71 ± 5	6.48	88 ± 9.0	7.69	49 ± 4	6.21
Terguride	39 ± 4	9.34	0	**9.62**	36 ± 3.2	9.18	0	**8.31**
TL 99	92 ± 3	7.75	46 ± 4	7.86	94 ± 8.5	8.91	71 ± 2	7.16

[a] Newman-Tancredi et al., 1997.
[b] Newman-Tancredi et al., 1999b.
[c] Newman-Tancredi et al., 1999a

tors correlated well ($r = 0.82$, $P < 0.05$) with their pK_i values determined in competition binding experiments (data not shown; Millan et al., 2002).

Drug Actions at hD_{2L} Receptors. At hD_{2L} receptors (B_{max} = 2.2 pmol/mg), at a maximally effective concentration (10 μM), DA enhanced [^{35}S]GTPγS binding by ~1.9-fold; it displayed a pEC_{50} value of 6.5 (Fig. 1; Table 1). At hD_{2L} receptors, efficacies for all ligands were markedly lower than at hD_{2S} sites. Indeed, all ligands, except quinelorane, behaved as partial agonists. Roxindole and terguride induced no stimulation of [^{35}S]GTPγS binding and displayed antagonist properties. The correlation coefficient for efficacies at hD_{2L} versus hD_{2S} receptors was 0.79 ($P < 0.05$). Drug potencies for stimulation of [^{35}S]GTPγS binding (pEC_{50} values) at hD_{2L} receptors correlated well ($r = 0.93$, $P < 0.05$) with their pK_i values determined in competition binding experiments (data not shown; Millan et al., 2002).

Drug Actions at hD_3 Receptors. At hD_3 receptors (B_{max} = 15.6 pmol/mg), at a maximally effective concentration (10 μM), DA enhanced [^{35}S]GTPγS binding by ~1.6-fold; it displayed a pEC_{50} value of 7.8 (Fig. 1; Table 1). All drugs behaved as agonists at hD_3 receptors, with efficacies varying from 30% (roxindole) and 34% (piribedil) to 88% (talipexole) and 94% (TL99). The lower efficacies of quinelorane and quinpirole at hD_3 versus hD_{2S} and hD_{2L} sites are of note, whereas roxindole and bromocriptine showed higher efficacies at hD_3 than hD_{2S} and hD_{2L} sites. Indeed, there was no consistent pattern of drug efficacies at hD_3 relative to hD_{2S} and hD_{2L} receptors. Accordingly, correlation coefficients for efficacies at hD_3 compared with hD_{2L} and hD_{2S} sites, although significant ($P < 0.05$), were only 0.59 and 0.49, respectively. Drug potencies for stimulation of [^{35}S]GTPγS binding (pEC_{50} values) at hD_3 receptors correlated significantly ($r = 0.62$, $P < 0.05$) with pK_i values determined in competition binding experiments (data not shown; Millan et al., 2002).

Drug Actions at hD_4 Receptors. At hD_4 receptors (B_{max} = 1.4 pmol/mg), at a maximally effective concentration (10 μM), DA enhanced [^{35}S]GTPγS binding by ~2.2-fold; it displayed a pEC_{50} value of 7.0 (Table 1). Although bromocriptine did not interact with hD_4 receptors, agonist efficacies of the other drugs varied widely. Thus, although TL99, quinelorane, and quinpirole showed relatively high efficacies (~70%), pergolide, talipexole, cabergoline, apomorphine, roxindole, pramipexole, and lisuride showed less marked efficacies of 32 to 56%. Piribedil displayed very low efficacy (7%) and antagonized the stimulation by DA of [^{35}S]GTPγS binding. Terguride, which was inactive alone, similarly blocked the action of DA. On the other hand, roxindole was more efficacious at hD_4 than at hD_{2S} and hD_{2L} receptors. Thus, there was no consistent pattern of drug efficacies at hD_4 versus hD_{2S}, hD_{2L}, and hD_3 receptors and correlation coefficients, although significant ($P < 0.05$), were modest: hD_{2S}, $r = 0.57$; hD_{2L}, $r = 0.55$; and hD_3, $r = 0.44$. Drug potencies for stimulation of [^{35}S]GTPγS binding (pEC_{50} values) at hD_4 receptors correlated well ($r = 0.78$, $P < 0.05$) with their pK_i values determined in competition binding experiments (data not shown; Millan et al., 2002).

Drug Actions at $h\alpha_{2A}$-, $h\alpha_{2B}$-, and $h\alpha_{2C}$-ARs. At $h\alpha_{2A}$-, $h\alpha_{2B}$-, and $h\alpha_{2C}$-ARs, a maximally effective concentration of NA (10 μM) increased [^{35}S]GTPγS binding by 7.2-, 6.6-, and 2.7-fold, respectively; pEC_{50} values were 6.2, 6.5, and 6.5, respectively (Figs. 2, 3 and 4; Table 2). Antiparkinson agents differed markedly concerning their functional activities at $h\alpha_{2A}$-, $h\alpha_{2B}$-, and $h\alpha_{2C}$-ARs. TL99 behaved as a high-efficacy agonist at each subtype of $h\alpha_2$-AR, whereas talipexole behaved as a partial agonist at each subtype. On the other hand, pergolide showed pronounced efficacy at $h\alpha_{2B}$-ARs, intermediate efficacy at $h\alpha_{2A}$-ARs, and low efficacy at $h\alpha_{2C}$-ARs. Pramipexole revealed partial agonist properties at $h\alpha_{2A}$-ARs; actions were not evaluated at $h\alpha_{2B}$- and $h\alpha_{2C}$-ARs owing to its low affinities at these sites (Millan et al., 2002). Apomorphine showed low efficacy only at $h\alpha_{2A}$-ARs, whereas high concentrations of quinelorane and quinpirole revealed weak partial agonist actions at $h\alpha_{2A}$- and $h\alpha_{2B}$-ARs, respectively. Agonist pEC_{50} values for stimulation of [^{35}S]GTPγS

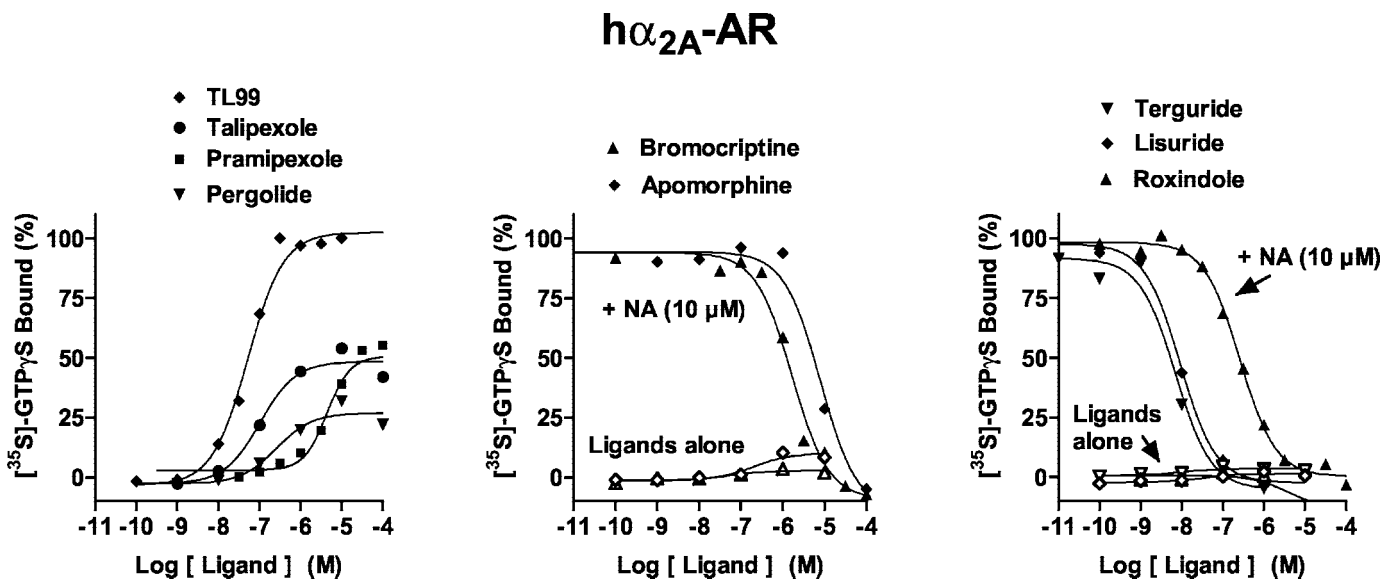

Fig. 2. Influence of antiparkinson agents upon G protein coupling at $h\alpha_{2A}$-adrenoceptors expressed in CHO cells. [^{35}S]GTPγS binding was carried out as described in Table 1. Binding is expressed as a percentage of that observed with a maximally effective concentration (10 μM) of noradrenaline (defined as 100%). Values shown are from representative experiments performed in triplicate and repeated on at least three occasions.

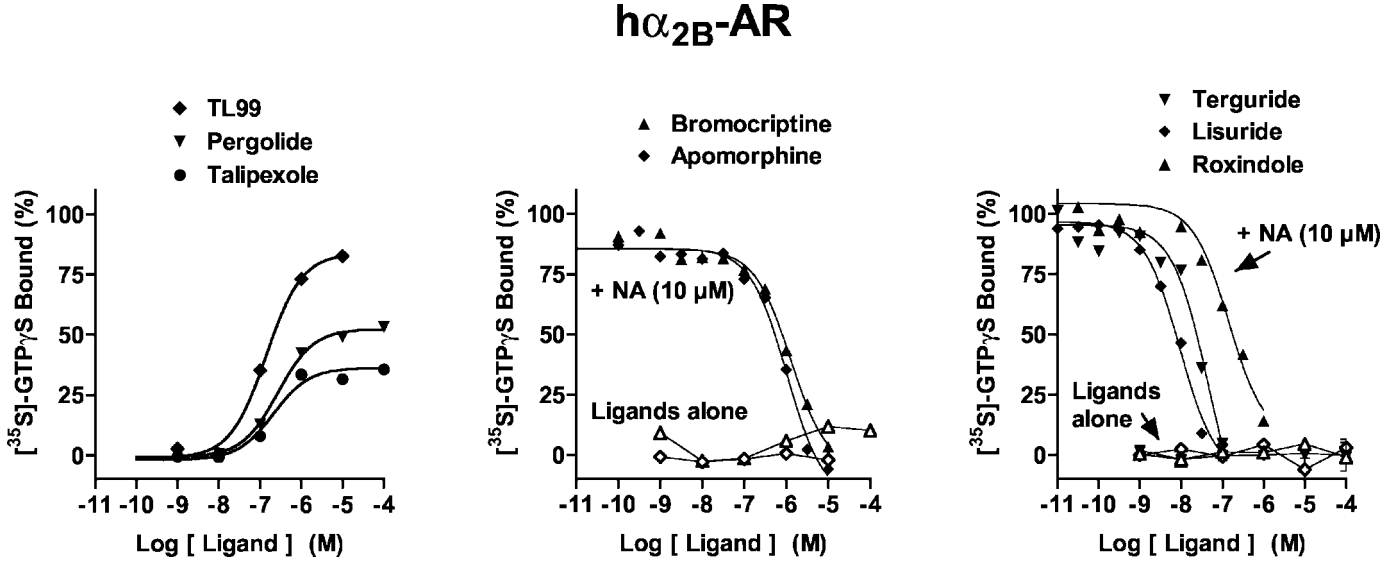

Fig. 3. Influence of antiparkinson agents upon G protein coupling at $h\alpha_{2B}$-adrenoceptors expressed in CHO cells. [^{35}S]GTPγS binding was carried out as described in Table 1. [^{35}S]GTPγS binding is expressed as a percentage of that observed with a maximally effective concentration (10 μM) of noradrenaline (defined as 100%). Values shown are from representative experiments performed in triplicate and repeated on at least three occasions.

binding corresponded well to their respective pK_i values as defined in competition binding assays (Millan et al., 2002). In view of its high affinity and low efficacy at $h\alpha_{2A}$-AR subtypes, apomorphine was further evaluated in interaction with NA and shown to behave as an antagonist. Furthermore, several other drugs also reversed NA-stimulated [^{35}S]GTPγS binding. For drugs behaving as antagonists, pK_B values correlated well ($P < 0.05$) with their respective pK_i values derived from competition binding studies (data not shown; Millan et al., 2002): $h\alpha_{2A}$-ARs, $r = 0.94$; $h\alpha_{2B}$-ARs, $r = 0.86$; and $h\alpha_{2C}$-ARs, $r = 0.87$.

Drug Actions at $h\alpha_{1A}$-ARs. Ligands that exhibited significant binding affinity at $h\alpha_{1A}$-ARs (pK_i values ≥6.0; Millan et al., 2002) were evaluated in a functional test of phospholipase C activation, depletion of membrane-bound [^{3}H]PI (Fig. 5; Table 3). In this procedure, NA itself revealed a pEC_{50} value of 6.51. No compound stimulated phospholipase C activity when tested alone, indicating an absence of agonist properties. In contrast, in order of decreasing potency, roxindole, bromocriptine, lisuride, terguride, cabergoline, and piribedil all reversed the stimulation of [^{3}H]PI hydrolysis induced by noradrenaline (10 μM), demonstrating antagonist properties. pK_B values at $h\alpha_{1A}$-ARs correlated well ($r = 0.96$, $P < 0.05$) with pK_i values obtained from competition binding assays (data not shown; Millan et al., 2002).

Discussion

This comprehensive comparison of the actions of 14 antiparkinson agents at eight classes of cloned "hD_2-like" and $h\alpha_1/h\alpha_2$-AR revealed marked differences in efficacies, obser-

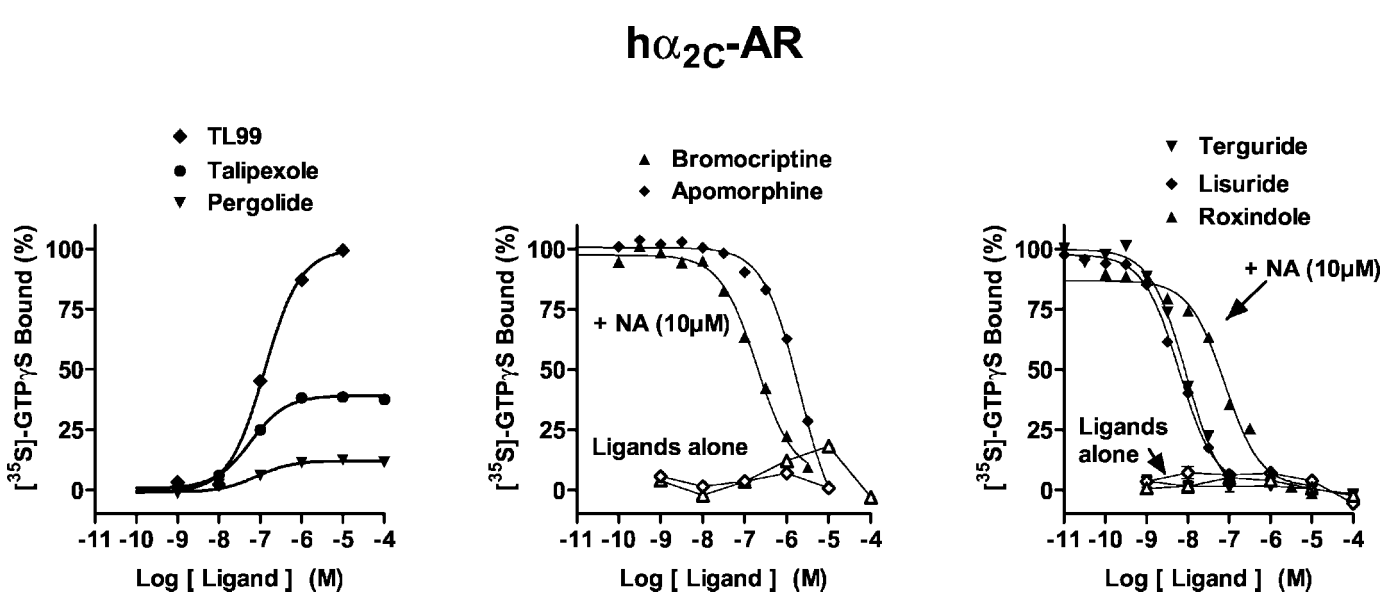

Fig. 4. Influence of antiparkinson agents upon G protein coupling at hα_{2C}-adrenoceptors expressed in CHO cells. [^{35}S]GTPγS binding was carried out as described in Table 1. [^{35}S]GTPγS binding is expressed as a percentage of that observed with a maximally effective concentration (10 μM) of noradrenaline defined as 100%). Values shown are from representative experiments performed in triplicate and repeated on at least three occasions.

TABLE 2

Efficacies (E_{max} values) and potencies (pEC_{50} or **pK_b** values) of antiparkinson agents at recombinant hα_{2A}-, hα_{2B}-, and hα_{2C}-adrenoceptors

Efficacy (E_{max}) and potency (pEC_{50} or **pK_b**) values were determined by [^{35}S]GTPγS binding. pEC_{50} values for stimulation are indicated in normal case, and **pK_b** values for inhibition are indicated in bold. E_{max} values are percentages of the stimulation observed with a maximally efficacious (E_{max} = 100%) concentration of noradrenaline (10 μM) and are expressed as means ± S.E.M. values of at least three independent determinations performed in triplicate. pEC_{50} or **pK_b** values are means of at least three independent determinations: S.E.M. values (not shown) were less than 0.2 log units. The B_{max} at hα_{2A}-, hα_{2B}-, and hα_{2C}-ARs was 1.8, 1.0, and 1.3 pmol/mg, respectively. Noradrenaline exhibited pEC_{50} values of 6.45, 6.50, and 6.52 at hα_{2A}-, hα_{2B}-, and hα_{2C}-ARs, respectively. Apomorphine displayed a **pK_b** of **6.58** at hα_{2A}-ARs, and pergolide displayed a **pK_b** of **6.96** at hα_{2C}-ARs.

Ligand	hα2A		hα2B		hα2C	
	E_{max}	pEC_{50} or **pK_b**	E_{max}	pEC_{50} or **pK_b**	E_{max}	pEC_{50} or **pK_b**
Apomorphine	16 ± 2	6.92	0	**6.84**	0	**6.55**
Bromocriptine	0	**7.42**	0	**6.64**	0	**7.38**
Cabergoline	0	**7.46**	0	**6.30**	0	**6.79**
Lisuride	0	**9.53**	0	**9.03**	0	**8.97**
Pergolide	31 ± 2	6.50	70 ± 10	6.46	16 ± 5	6.16
Piribedil[a]	0	**6.50**	0	<5	0	**6.87**
Pramipexole	52 ± 5	5.45	IA	IA	IA	IA
Quinelorane	11 ± 3	**4.97**	IA	IA	IA	IA
Quinpirole	IA	IA	27 ± 6	5.86	IA	IA
Ropinirole	IA	IA	IA	IA	IA	IA
Roxindole	0	IA	0	**7.80**	0	8.13
Talipexole	51 ± 5	6.90	39 ± 2	6.70	46 ± 1	6.64
Terguride	0	**9.66**	0	**8.95**	0	**9.07**
TL 99	108 ± 3	7.19	79 ± 9	6.91	81 ± 9	7.25

IA, inactive (no agonist or antagonist effect at a concentration of 10 μM).
[a] Piribedil data are from Millan et al., 2001.

vations of significance to their contrasting functional profiles in experimental models and in human.

D_{2S} and D_{2L} Receptors. In the only previous comparison of antiparkinson agonists at hD_{2S} versus hD_{2L} receptors (ropinirole, talipexole, pergolide, lisuride, and bromocriptine), no marked differences in efficacy were apparent (Gardner et al., 1997; Gardner and Strange, 1998). In the present, more extensive work, however, drug efficacies were invariably higher at hD_{2S} sites. The reasons underlying this difference require elucidation, but it may be of pertinence that hD_{2S} and hD_{2L} receptors differentially interact with distinct subtypes of G protein, as implicated in their contrasting patterns of coupling to calcium channels (Wolfe and Morris, 1999). Furthermore, the expression level (2.2 pmol/mg) of hD_{2L} sites herein was higher than that of hD_{2S} sites (1.4 pmol/mg), whereas the inverse was true for Gardner et al. (1997) (1.3 versus 2.7 pmol/mg, respectively). In the light of these comments, it should briefly be pointed out that receptor density can play an important role in determining drug efficacy (Newman-Tancredi et al., 2001). Although receptor density (B_{max}) can easily be determined at pure populations of transfected receptors (see *Results*), equivalent information for defined networks of neurons is not available because B_{max} estimations in native tissue almost inevitably incorporate neurons not expressing the receptor in question. Thus, although the receptor densities of hD_{2S} and hD_{2L} sites here were in the same range as previous studies (Coldwall et al., 1999; Perachon et al., 1999), they cannot be compared with certainty to cerebral populations. Furthermore, it is important to note differences in density between pre- versus postsynaptic populations of D_2 (and D_3) receptors (Seyfried and Boettcher, 1990), as well as the up-regulation of postsyn-

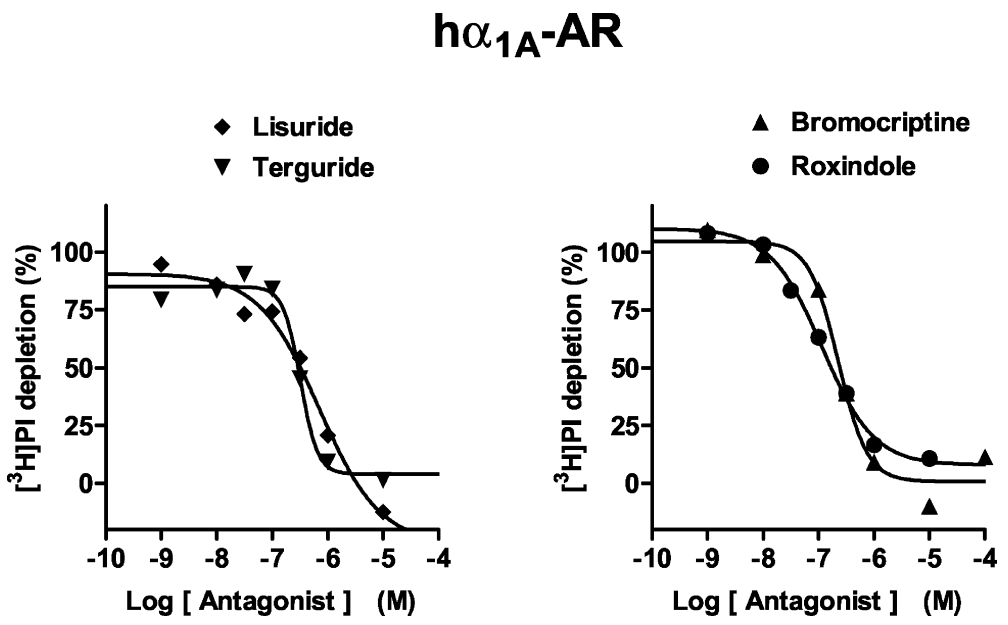

Fig. 5. Influence of antiparkinson agents upon stimulation of phospholipase C activity by noradrenaline at $h\alpha_{1A}$-receptors expressed in CHO cells. [^{3}H]PI depletion studies were carried out as described under *Materials and Methods*. The antagonist actions of drugs were examined against noradrenaline (10 μM). Values shown are from representative experiments performed in triplicate and repeated on at least three occasions.

TABLE 3

Efficacies and potencies ($\mathbf{pK_b}$ values) of antiparkinson agents at recombinant $h\alpha_{1A}$-ARs

Efficacies (E_{max}) are for drugs alone compared with a maximally effective (100%) concentration of NA (30 μM). Potencies ($\mathbf{pK_b}$) at $h\alpha_{1A}$-ARs (B_{max} = 2.5 pmol/mg) were determined by blockade of NA-stimulated [^{3}H]PI depletion. Noradrenaline exhibited a pEC_{50} value of 6.48. Pramipexole, ropinirole, quinelorane, apomorphine, pergolide, talipexole, TL99, and quinpirole, which have low affinities at $h\alpha_{1A}$-ARs (pK_i values of <6.0, see accompanying paper) were not tested. $\mathbf{pK_b}$ values are means of at least three independent determinations: S.E.M. values were less than 0.2 log units. Experiments were carried out in triplicate.

Ligand	$h\alpha_{1A}$-AR	
	E_{max}	$\mathbf{pK_b}$
Bromocriptine	0	**8.24**
Cabergoline	0	**6.16**
Lisuride	0	**8.01**
Piribedil[a]	0	**5.59**
Roxindole	0	**8.37**
Terguride	0	**8.00**

[a] Piribedil data are from Millan et al., 2001.

aptic sites after damage to dopaminergic innervation (Kostrzewa, 1995; Newman-Tancredi et al., 2001), an experimental manipulation that mimics the pathology of Parkinson's disease (see below).

Although the relative degree of D_{2S} versus D_{2L} receptor stimulation required for optimal control of Parkinson's disease remains to be clarified, quinelorane was the only drug to exhibit efficacy equivalent to dopamine at both hD_{2S} and hD_{2L} receptors, in line with its high efficacy at native, rat D_2 receptors (Sánchez and Arnt, 1992; Newman-Tancredi et al., 2001). The relatively high efficacies of quinpirole, ropinirole, pramipexole, and talipexole at hD_{2S} (Terasmaa et al., 2000) and hD_{2L} sites for stimulation of [^{35}S]GTPγS binding coincide with measures of extracellular acidification and mitogenesis (Mierau et al., 1995; Coldwall et al., 1999; Perachon et al., 1999; Alberts et al., 2000; Gilliland and Alper, 2000). The present [^{35}S]GTPγS approach likewise revealed high efficacies at hD_{2S} and hD_{2L} sites of cabergoline and TL99 (Hughes, 1997). Like bromocriptine and lisuride, piribedil displayed intermediate efficacy. This is interesting because piribedil is highly active in rodent and primate models of antiparkinson activity; furthermore, piribedil improves motor and cognitive function in patients both alone and in association with L-DOPA (Rondot and Ziegler, 1992; Maurin et al., 2001; Nagaraja and Jayashree, 2001). Interestingly, terguride failed to activate hD_{2L} receptors, in line with its low efficacy in rodent models of hypothermia, locomotion, and drug discrimination (Arnt and Hyttel, 1990; Sánchez and Arnt, 1992). Although terguride showed weak partial agonist activity in hD_{2L} receptor-expressing SH-SY5Y cells, its efficacy was much lower than that of quinpirole (Gilliland and Alper, 2000). Furthermore, although terguride showed modest antiparkinson activity and attenuated L-DOPA-induced dyskinesia in primates, it was effective in only a small percentage (10–20%) of Parkinson's disease patients in (subsequently discontinued) clinical trials (Filipova et al., 1988; Akai et al., 1993). Furthermore, roxindole, which likewise exhibited low efficacy at hD_{2L} and hD_{2S} receptors (Newman-Tancredi et al., 1999a), failed to reduce L-DOPA-induced dyskinesias in Parkinson's disease patients and has not, as yet, been shown to possess antiparkinson activity in human.

Correspondingly, a certain, minimal "threshold" of efficacy may be necessary for antiparkinson properties. However, "full" agonism at the level of G protein-coupling ([^{35}S]GTPγS binding) is not essential for robust clinical activity in Parkinson's disease because 1) efficacy is "amplified" by intracellular cascades downstream of G proteins (Cussac et al., 2002); 2) postsynaptic striatal D_2 receptors (probably the D_{2L} isoform) are hypersensitive due to loss of dopaminergic input from the SNPC (Kostrzewa, 1995; Geurts et al., 1999; Newman-Tancredi et al., 2001); and 3) submaximal efficacy is sufficient to activate highly sensitive D_{2S} autoreceptors implicated in neuroprotective properties of dopaminergic agonists (Seyfried and Boettcher, 1990). Moreover, antiparkinson agents of intermediate efficacy may preferentially engage nigrostriatal D_2 receptors implicated in the treatment of

Parkinson's disease compared with other populations mediating side effects. Thus, "submaximal" efficacy at the G protein level for drugs such as piribedil or bromocriptine may be advantageous in optimizing the therapeutic index between clinical efficacy and side effects.

hD_3 Receptors. Although hD_3 receptors couple less efficiently to G proteins in CHO cells than their hD_{2S}/hD_{2L} counterparts, DA stimulated [^{35}S]GTPγS binding in the high-expressing cell line used herein (Newman-Tancredi et al., 1999b). The substantial affinities of apomorphine, quinpirole, pramipexole, talipexole, bromocriptine, and pergolide corroborate studies of their actions in models of microphysiometry and mitogenesis (Mierau et al., 1995; Coldwell et al., 1999; Perachon et al., 1999). The high efficacy of TL99 at hD_3 receptors is of note in view of its marked efficacy at hD_{2S}/hD_{2L} and hD_4 sites, whereas the modest efficacies of terguride and roxindole at hD_3 sites mimic their low efficacies at hD_{2L} and (terguride) hD_4 sites. As concerns piribedil, its intermediate efficacy at hD_3 receptors resembles its actions at hD_{2S}/hD_{2L} receptors and is consistent with agonist properties in vivo at D_3 autoreceptors (Millan et al., 1995). As discussed elsewhere (Joyce, 2001), the role of D_3 sites in the expression of beneficial and deleterious actions of antiparkinson agents remains unclear, a question of particular importance because, as shown herein, all antiparkinson agents activated D_3 receptors.

hD_4 Receptors. In line with studies of CHO cells expressing the $hD_{4.2}$ isoform (Gilliland and Alper, 2000) and of cloned, rat D_4 sites (Gazi et al., 2000), quinpirole showed substantial efficacy at hD_4 ($hD_{4.4}$) receptors. This characteristic was shared by quinerolane and TL99. The agonist properties of pergolide, apomorphine, talipexole, and pramipexole at hD_4 sites complement work using other measures of drug efficacy and/or other hD_4 isoforms (Mieurau et al., 1995; Coldwell et al., 1999; Gazi et al., 2000; Gilliland and Alper, 2000). Like pergolide, two other ergolines, cabergoline and lisuride, similarly showed agonist properties at hD_4 sites. In contrast, piribedil displayed low efficacy at hD_4 receptors, whereas bromocriptine was inactive. Because bromocriptine and piribedil are clinically efficacious antiparkinson agents, these data support the argument that activation of D_4 receptors is not necessary for therapeutic efficacy (Newman-Tancredi et al., 1997). Moreover, the essentially D_4 antagonist properties of piribedil may limit psychiatric side effects and contribute to its improvement of cognitive function (Arnsten et al., 2000; Nagaraja and Jayashree, 2001).

hα_2-ARs. Striking differences in drug efficacies were seen at hα_2-AR subtypes. In analogy to piribedil (Millan et al., 2001), lisuride, bromocriptine, and apomorphine displayed antagonist properties, observations amplifying functional studies of isolated organs and hippocampal NA release in rats (McPherson, 1984; Jackisch et al., 1985). In line with their high affinities for hα_2-ARs (Millan et al., 2002), roxindole and two further ergot-related ligands, terguride and cabergoline, also manifested potent α_2-AR antagonist properties. In contrast, in line with in vivo studies (at undefined α_2-AR subtypes) in rodents (Horn et al., 1982; Meltzer et al., 1989; Sánchez and Arnt, 1992), TL99 displayed agonist, and talipexole partial agonist, properties at hα_{2A}-, hα_{2B}-, and hα_{2C}-ARs. Extending observations of partial agonist properties at central α_2-ARs in rodents (Ferrari et al., 1993), pramipexole displayed modest efficacy at hα_{2A}-ARs. Any potential significance of this (low-potency) action in vivo, however, remains to be clarified. On the other hand, in vivo studies in rodents have revealed agonist actions of pergolide at central α_2-ARs (Langtry and Clissold, 1990) and particularly pronounced agonist properties at hα_{2B}-ARs were observed here.

These contrasting actions of antiparkinson agents are of considerable significance in light of evidence that blockade of α_2-ARs improves motor performance, cognitive function, and perhaps mood in Parkinson's disease (Brefel-Courbon et al., 1998). Indeed, experimental and clinical studies with piribedil support the notion that "built-in" α_2-AR antagonist actions may be beneficial in Parkinson's disease (Maurin et al., 2001; Millan et al., 2001; Nagaraja and Jayashree, 2001). In contrast, α_2-AR agonists interfere with the facilitory influence of antiparkinson agents upon motor function (Mavridis et al., 1991; Bezard et al., 2001). Indeed, talipexole-induced stereotypy in rats (which reflects agonist properties at striatal D_2 receptors) is only apparent upon prevention of its α_2-AR agonist properties by coadministration of idazoxan (Meltzer et al., 1989). Similarly, TL99-induced hypomotility has been attributed to its α_2-AR agonist properties (Horn et al., 1982), whereas it only elicits rotation in unilateral SNPC-lesioned rats upon cotreatment with α_2-AR antagonists (Martin et al., 1983).

Nevertheless, future studies should address the significance of α_2-AR subtypes in the clinical actions of antiparkinson drugs. Although α_{2A}-ARs are certainly of key importance (Kable et al., 2000; Millan et al., 2000a), α_{2B}- and α_{2C}-ARs sites should not be neglected. The former are concentrated in the thalamus, a structure intimately involved in the motor deficits of Parkinson's disease, whereas α_{2C}-ARs are enriched in the striatum itself (Nicholas et al., 1997; Bezard et al., 2001). Moreover, gene knockout studies indicate that α_{2C}-ARs contribute to the modulation of monoaminergic transmission, cognitive function, and motor performance (Bjorklund et al., 2000; Kable et al., 2000). Although no antiparkinson agent showed agonist versus antagonist actions at distinct α_2-AR subtypes, the lack of antagonist actions of piribedil at α_{2B}- versus $\alpha_{2A/2C}$-ARs sites, and the preferential agonist actions of pergolide at α_{2B}- versus α_{2A}- and α_{2C}-ARs, may prove instructive in elucidating their relevance to Parkinson's disease and its treatment.

Actions at hα_{1A}-ARs. Roxindole and the ergot derivatives bromocriptine, lisuride, and terguride interact with cloned hα_1-ARs (Millan et al., 2002), although, with the exception of a study of bromocripine at peripheral α_1-ARs (McPherson, 1984), no information on their functional activities is available. Thus, their potent antagonism of NA-induced [^{3}H]PI depletion at cloned hα_{1A}-ARs is of note, whereas cabergoline and piribedil showed weak antagonist properties in line with their low affinities at these sites (Millan et al., 2002). Potent blockade of hα_1-ARs may be unfavorable inasmuch as α_1-AR antagonists interfere with antiparkinson properties in experimental models (Mavridis et al., 1991). Although blockade of α_1-ARs in the cortex and on pars reticulata GABAergic neurons may be involved, generalized sedative/motor-suppressive effects due to blockade of α_{1A}-ARs in motor nuclei of the brainstem and the spinal cord may also be of significance (Stone et al., 2001). Blockade of segmental and peripheral α_{1A}-ARs may also be deleterious in that it exacerbates the perturbation of cardiovascular function elicited via stimula-

tion of spinal dopaminergic receptors (Guimarães and Moura, 2001). On the other hand, inasmuch as frontocortical α_1-ARs inhibit working memory, their blockade might improve cognitive function (Arnsten, 1997), although there is currently no clinical support for this possibility. Further investigations should determine the potential importance for antiparkinson agents of actions at hα_{1B}- and hα_{1D}-AR subtypes (Millan et al., 2002), which likewise modulate motor, cognitive, and cardiovascular function (Guimarães and Moura, 2001; Spreng et al., 2001; Stone et al., 2001).

Conclusions

The present data reveal striking differences among antiparkinson agents concerning efficacies at multiple classes of hD_2-like receptor and ha_1/ha_2-AR. These observations amplify receptor-binding analyses of the accompanying article (Millan et al., 2002) in demonstrating that antiparkinson drugs are heterogeneous rather than a common group of "dopaminergic agonists". In this light, as discussed above, partial agonist and agonist properties at D_{2S}/D_{2L} receptors are favorable in the management of motor symptoms of Parkinson's disease, whereas blockade of α_{2A}-ARs may improve cognitive-attentional function and mood. The present data provide a framework for additional studies of the significance of these and other subtypes of dopaminergic receptor and α_1/α_2-AR in the etiology of Parkinson's disease, and in the beneficial and deleterious properties of antiparkinson agents.

Acknowledgments

We thank M. Soubeyran for secretarial assistance, and Laurence Verrièle, Manuelle Touzard, Christine Chaput, Valérie Pasteau, Laetitia Marini, Nelly Fabry, and Anne Bonnard for technical assistance.

References

Akai T, Yamaguchi M, Mizuta E, and Kuno S (1993) Effects of terguride, a partial D_2 agonist on MPTP-lesioned parkinson cynomolgus monkeys. *Ann Neurol* **33:**507–511.

Alberts GL, Pregenzer JF, and Bin Im W (2000) Advantages of heterologous expression of human D_{2LONG} dopamine receptors in human neuroblastoma SH-SY5Y over human embryonic kidney 293 cells. *Br J Pharmacol* **131:**514–520.

Arnsten AFT (1997) Catecholamine regulation of the prefrontal cortex. *J Psychopharmacol* **11:**151–162.

Arnsten AFT, Murphy B, and Merchant K (2000) The selective dopamine D_4 receptor antagonist, PNU-101387G, prevents stress-induced cognitive deficits in monkeys. *Neuropsychopharmacology* **23:**405–410.

Arnt J and Hyttel J (1990) Dopamine D_2 agonists with high and low efficacies: differentiation by behavioural techniques. *J Neural Transm* [Gen Sect] **80:**33–50.

Bezard E, Brotchie JM, and Gross CE (2001) Pathophysiology of Levo-dopa induced dyskinesia: potential for new therapies. *Nat Rev Neurosci* **2:**577–588.

Bjorklund M, Sirvio J, Riekkinen M, Sallinen J, Scheinin M, and Riekkinen JR (2000) Overexpression of α_{2C}-adrenoceptors impairs water maze navigation. *Neuroscience* **95:**481–487.

Boehm S (1999) Presynaptic α_2-adrenoceptors control excitatory, but not inhibitory, transmission at rat hippocampal synapses. *J Physiol (Lond)* **519:**439–449.

Brefel-Courbon C, Thalamas C, Peyro Saint Paul H, Senard JM, Montastruc JL, and Rascol O (1998) α_2-Adrenoceptor antagonists: a new approach to Parkinson's Disease? *CNS Drugs* **10:**189–207.

Coldwell MC, Boyfield I, Brown T, Hagan JJ, and Middlemiss DN (1999) Comparison of the functional potencies of ropinirole and other dopamine receptor agonists at human $D_{2(long)}$, D_3 and $D_{4.4}$ receptors expressed in Chinese hamster ovary cells. *Br J Pharmacol* **127:**1696–1702.

Cussac D, Amphoux A, Hubert D, Newman-Tancredi A, and Millan MJ (2002) Differential actions of antiparkinson (AP) agents at dopamine D_{2L} receptors revealed by G protein but not MAP-kinase activation. *32th Society for Neuroscience Meeting (USA)*, in press.

Ferrari F, Pelloni F, and Giuliani D (1993) Behavioural evidence that different neurochemical mechanisms underlie stretching-yawning and penile erection induced in male rats by SND 919, a new selective D_2 dopamine receptor agonist. *Psychopharmacology* **113:**172–176.

Filipova M, Filip V, Macek Z, Mullerova S, Markova J, Kas S, Zizkova B, Krivka J, Votavova M, and Krejcova H (1988) Terguride in parkinsonism. A multicenter trial. *Eur Arch Psychiatry Neurol Sci* **237:**298–303.

Gardner B, Hall DA, and Strange PG (1997) Agonist actions at D_2(short) dopamine receptors determined in ligand binding and functional assays. *J Neurochem* **69:**2589–2598.

Gardner B and Strange PG (1998) Agonist actions at D_2(long) dopamine receptors: ligand binding and functional assays. *Br J Pharmacol* **124:**978–984.

Gazi L, Schoeffter P, Nunn C, Croskery K, Hoyer D, and Feuerbach D (2000) Cloning, expression, functional coupling and pharmacological characterization of the rat dopamine D_4 receptor. *Naunyn-Schmiedeberg's Arch Pharmacol* **361:**555–564.

Geurts M, Hermans E, Cumps J, and Maloteaux JM (1999) Dopamine receptor-modulated [^{35}S]GTPγS binding in striatum of 6-hydroxydopamine-lesioned rats. *Brain Res* **841:**135–142.

Gilliland SL and Alper RH (2000) Characterization of dopaminergic compounds at hD_{2SHORT}, h$D_{4.2}$ and h$D_{4.7}$ receptors in agonist-stimulated [^{35}S]GTPγS binding assays. *Naunyn-Schmiedeberg's Arch Pharmacol* **361:**498–504.

Guillin O, Diaz J, Carroll P, Griffon N, Schwartz and Sokoloff P (2001) BDNF controls dopamine D_3 receptor expression and triggers behavioural sensitization. *Nature (Lond)* **411:**86–89.

Guimarães S and Moura D (2001) Vascular adrenoceptors: an update. *Pharmacol Rev* **53:**319–356.

Horn AS, De Vries J, Dijkstra D, and Mulder AH (1982) Is TL99 a selective presynaptic dopamine receptor agonist? *Eur J Pharmacol* **83:**35–45.

Hughes AJ (1997) Drug treatment of Parkinson's disease in the 1990s: achievements and future possibilities. *Drugs* **53:**195–205.

Jackisch R, Moll S, Feuerstein TJ, and Hertting G (1985) Dopaminergic modulation of hippocampal noradrenaline release: evidence for α_2-antagonistic effects of some dopamine receptor agonists and antagonists. *Naunyn-Schmiedeberg's Arch Pharmacol* **330:**105–113.

Joyce JN (2001) Dopamine D_3 receptors as a therapeutic target for antipsychotic and antiparkinsonian drugs. *Pharmacol Ther* **90:**231–259.

Kable JW, Murrin LC, and Bylund DB (2000) *In vivo* gene modification elucidates subtype-specific functions of α_2-adrenergic receptors. *J Pharmacol Exp Ther* **293:**1–7.

Kostrzewa RM (1995) Dopamine receptor supersensitivity. *Neurosci Biobehav Rev* **19:**1–17.

Langtry HD and Clissold SP (1990) Pergolide: a review of its pharmacological properties and therapeutic potential in Parkinson's disease. *Drugs* **39:**491–506.

Lazareno S and Birdsall NJM (1993) Estimation of antagonist K_B from inhibtion curves in functional experiments: alternatives to the Cheng-Prusoff equation. *Trends Pharmacol Sci* **14:**237–239.

Martin GE, Jones JH, and Bendesky RJ (1983) Different actions of TL-99 and 3-PPP in producing contraversive turning in the 6-OHDA-lesioned rat. *Eur J Pharmacol* **92:**275–278.

Maurin B, Baunez C, Bonhomme C, Chezaubernard C, Nieoullon A, and Amalric M (2001) Cognitive deficits in 6-OHDA lesioned rats are improved by chronic treatment with the dopamine agonist, piribedil. *Behav Pharmacol* **12:**S63.

Mavridis M, Colpaert FC, and Millan MJ (1991) Differential modulation of (+)-amphetamine-induced rotation in unilateral substantia nigra-lesioned rats by α_1 as compared to α_2 agonists and antagonists. *Brain Res* **562:**216–224.

McPherson GA (1984) *In vitro* selectivity of lisuride and other ergot derivatives for α_1- and α_2-adrenoceptors. *Eur J Pharmacol* **97:**151–155.

Meltzer LT, Wiley JN, and Heffner TG (1989) The α_2-adrenoceptor antagonists idazoxan and yohimbine can unmask the postsynaptic dopamine agonist effects of B-HT 920. *Eur J Pharmacol* **170:**105–107.

Mierau J, Schneider FJ, Ensinger HA, Chio CL, Lajiness ME, and Huff RM (1995) Pramipexole binding and activation of cloned and expressed dopamine D_2, D_3 and D_4 receptors. *Eur J Pharmacol* **290:**29–36.

Millan MJ, Cussac D, Milligan G, Carr C, Audinot V, Gobert A, Lejeune F, Rivet JM, Brocco M, Duqueyroix D, et al. (2001) The antiparkinsonian agent, piribedil, displays antagonist properties at native rat and cloned human α_2-adrenoceptors: a cellular and functional characterization. *J Pharmacol Exp Ther* **297:**1–12.

Millan MJ, Lejeune F, and Gobert A (2000a) Reciprocal autoreceptor and heteroreceptor control of serotonergic, dopaminergic and noradrenergic transmission in the frontal cortex: relevance to the actions of antidepressant agents. *J Psychopharmacol* **14:**114–138.

Millan MJ, Lejeune F, Gobert A, Brocco M, Auclair A, Bosc C, Rivet JM, Lacoste JM, Cordi A, and Dekeyne A (2000b) S18616, a highly potent spiroimidazoline agonist at α_2-adrenoceptors: II. Influence on monoaminergic transmission, motor function and anxiety in comparison with dexmedetomidine and clonidine. *J Pharmacol Exp Ther* **295:**1206–1222.

Millan MJ, Maiofiss L, Cussac D, Audinot V, Boutin JA, and Newman-Tancredi A (2002) Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned, human receptor subtypes. *J Pharmacol Exp Ther* **303:**791–804.

Millan MJ, Peglion JL, Vian J, Rivet JM, Brocco M, Gobert A, Newman-Tancredi A, Dacquet C, Bervoets K, Girardon S, et al. (1995) Functional correlates of dopamine D_3 receptor activation in the rat in vivo and their modulation by the selective antagonist, (+)-S 14297: I. Activation of postsynaptic D_3 receptors mediates hypothermia, whereas blockade of D_2 receptors elicits prolactin secretion and catalepsy. *J Pharmacol Exp Ther* **275:**885–898.

Nagaraja D and Jayashree S (2001) Randomized study of the dopamine receptor agonist piribedil in the treatment of mild cognitive impairment. *Am J Psychiatry* **158:**1517–1519.

Newman-Tancredi A, Audinot V, Chaput C, Verrièle L, and Millan MJ (1997) [^{35}S]GTPγS binding as a measure of efficacy at human recombinant dopamine $D_{4.4}$ receptors: actions of antiparkinsonian and antipsychotic agents. *J Pharmacol Exp Ther* **282:**181–191.

Newman-Tancredi A, Cussac D, Audinot V, and Millan MJ (1999a) Actions of roxindole at dopamine hD_2 hD_3 hD_4 and serotonin h5-HT_{1A}, h5-HT_{1B} and h5-HT_{1D} receptor subtypes: preferential activation of hD_3 and h5-HT_{1A} receptors revealed by [^{35}S]GTPγS binding. *Naunyn-Schmiedeberg's Arch Pharmacol* **359:**447–453.

Newman-Tancredi A, Cussac D, Audinot V, Pasteau V, Gavaudan S, and Millan MJ (1999b) G-Protein activation by human dopamine D_3 receptors in high-expressing chinese hamster ovary cells: a [^{35}S]GTPγS binding and antibody study. *Mol Pharmacol* **55:**564–574.

Newman-Tancredi A, Cussac D, Brocco M, Rivet JM, Chaput C, Touzard M, Pasteau V, and Millan MJ (2001) Dopamine D_2 receptor-mediated G-protein activation in rat striatum: functional autoradiography and influence of unilateral 6-hydroxydopamine lesions of the substantia nigra. *Brain Res* **920:**41–54.

Nicholas AP, Hökfelt T, and Pieribone VA (1997) The distribution and significance of CNS adrenoceptors examined with *in situ* hybridization. *Trends Pharmacol Sci* **18:**210–211.

Perachon S, Schwartz J-C, and Sokoloff P (1999) Functional potencies of new antiparkinsonian drugs at recombinant human dopamine D_1, D_2 and D_3 receptors. *Eur J Pharmacol* **366:**293–300.

Quik M, Police S, He L, Di Monte DA, and Langston JW (2000) Expression of D(3) receptor messenger RNA and binding sites in monkey striatum and substantia nigra after nigrostriatal degeneration: effect of levodopa treatment. *Neuroscience* **98:**263–273.

Rondot P and Ziegler M (1992) Activity and acceptability of trivastal in Parkinson's disease: a multicenter study. *J Neurol* **239:**528–534.

Sánchez C and Arnt J (1992) Effects on body temperature in mice differentiate between dopamine D_2 receptor agonists with high and low efficacies. *Eur J Pharmacol* **211:**9–14.

Seyfried CA and Boettcher H (1990) Central D_2-autoreceptor agonists, with special reference to indolylbutylamines. *Drugs Future* **15:**819–832.

Spreng M, Cotecchia S, and Schenk F (2001) A behavioral study of α-$_{1B}$ adrenergic receptor knockout mice. Increased reaction to novelty and selectively reduced learning capacities. *Neurobiol Learning Memory* **75:**214–229.

Stone EA, Lin Y, Itteera A, and Quartermain D (2001) Pharmacological evidence for the role of central α_{1B}-adrenoceptors in the motor activity and spontaneous movement of mice. *Neuropharmacology* **40:**254–261.

Tellez S, Colpaert F, and Marien M (1997) Acetylcholine release in the rat prefrontal cortex *in vivo*: modulation by α_2-adrenoceptor agonists and antagonists. *J Neurochem* **68:**778–785.

Terasmaa A, Finnman U-B, Owman C, Ferré S, Fuxe K, and Rinken A (2000) Modulation of [^{35}S]GTPγS binding to Chinese hamster ovary cell membranes by $D_{2(short)}$ dopamine receptors. *Neuroscience Lett* **280:**135–138.

Troadec JD, Marien M, Darios F, Hartmann A, Ruberg M, Colpaert F, and Michel PP (2001) Noradrenaline provides long-term protection to dopaminergic neurons by reducing oxidative stress. *J Neurochem* **79:**200–210.

Wang Y, Xu R, Sasaoka T, Tonegawa S, Kung MP, and Sankoorikal EB (2000) Dopamine D_2 long receptor-deficient mice display alterations in striatum-dependent functions. *J Neurosci* **20:**8305–8314.

Wolfe SE and Morris SJ (1999) Dopamine D_2 receptor isoforms expressed in AtT20 cells differentially couple to G-proteins to acutely inhibit high voltage-activated calcium channels. *J Neurochem* **73:**2375–2382.

Address correspondence to: Dr. Mark J. Millan, Institut de Recherches Servier, Centre de Recherches de Croissy, 125 chemin de Ronde, 78290 Croissy/Seine, Paris, France. E-mail: mark.millan@fr.netgrs.com