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European Journal of Pharmacology 366 (1999) 293–300

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Functional potencies of new antiparkinsonian drugs at recombinant human dopamine D_1, D_2 and D_3 receptors

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Received 3 August 1998; revised 24 November 1998; accepted 27 November 1998

Abstract

We measured the affinities of bromocriptine, pramipexole, pergolide and ropinirole at human recombinant dopamine D_1, D_2 and D_3 receptors in binding and functional tests. All four compounds bound with high affinity at the dopamine D_3 receptor; bromocriptine and pergolide also had high affinity for the dopamine D_2 receptor, while only pergolide had significant, although moderate, affinity for the dopamine D_1 receptor. Only pergolide had high potency and intrinsic activity at the dopamine D_1 receptor for stimulating cyclic AMP accumulation. In addition, the potencies and efficacies of pergolide and bromocriptine, as well as that of dopamine, at the dopamine D_1 receptor were increased in the presence of forskolin, an adenylate cyclase activator. All four compounds were highly potent agonists at dopamine D_2 and D_3 receptors, as measured in a mitogenesis assay. Bromocriptine was ten times more potent and pramipexole and ropinirole ten times less potent at the dopamine D_2 than at the dopamine D_3 receptor, whereas pergolide was equipotent at the two receptors. These results suggest that the activity of recently developed antiparkinsonian drugs at either the dopamine D_1 or the dopamine D_3 and not only the dopamine D_2 receptors should be taken into account in analyses of their mechanisms of action in therapeutics.

Keywords: Bromocriptine; Pergolide; Pramipexole; Ropinirole; Parkinson's disease; Recombinant receptor

1. Introduction

Impaired motor function in Parkinson's disease results from the loss of dopamine-containing neurons in the substantia nigra. Although there is no cure for the disease, the condition is improved by treatment with levodopa, a dopamine precursor, which is still the most widely used treatment of Parkinson's disease (Hornykiewicz, 1966; Wachtel, 1991; Vitti and Ahlskop, 1996). However, fluctuations in drug plasma levels make levodopa difficult to use; in addition, long-term administration of levodopa often results in loss of treatment efficacy and is associated in 60–80% of Parkinson's disease patients with the development of debilitating dyskinesia, including a wide spectrum of abnormal involuntary movements, as well as severe psychological disturbances such as hallucinations (Marsden et al., 1982).

In order to avoid these drawbacks, alternative treatments based on the use of direct agonists have been developed. The dopamine D_2 receptor has long been considered as the sole target for antiparkinsonian drugs. It is highly expressed in the caudate–putamen (Mansour et al., 1990), the brain area where dopamine deficiency is responsible for the motor disturbances in Parkinson's disease, and is responsive to bromocriptine, the prototypical direct agonist developed for the treatment of Parkinson's disease (Calne et al., 1974), whereas this drug showed little affinity at the dopamine D_1 receptor (Urwyler, 1987). Nevertheless, there is considerable evidence indicating functional interactions between dopamine D_1-like and dopamine D_2-like receptors and synergistic interactions have been extensively reported regarding behavioral (Robertson and Robertson, 1986; Clark and White, 1987; Vermeulen et al., 1994; Waddington et al., 1994), electrophysiological (Walters et al., 1987), and biochemical (Paul et al., 1992; LaHoste et al., 1993; Gerfen et al., 1995) responses. Thus, antiparkinsonian drugs combining D_1 and D_2 receptor potencies could be advantageous.

Moreover, additional dopamine receptors should also be considered as targets for antiparkinsonian drugs. The dopamine D_3 receptor is also a target for bromocriptine

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0014-2999/99/$ - see front matter
PII: S0014-2999(98)00896-6

(Sautel et al., 1995), and expression of dopamine D_3 receptor binding and mRNA in the caudate–putamen, although very scarce in rodents (Bouthenet et al., 1991; Lévesque et al., 1992; Diaz et al., 1995), is more abundant in human brain (Hall et al., 1996; Susuki et al., 1998). In addition, the dopamine D_3 receptor is expressed in cortical areas associated with sensorimotor functions in rodent developing brain (Diaz et al., 1997) and in human brain (Susuki et al., 1998). In contrast, the dopamine D_4 and D_5 receptors are expressed in brain regions associated with limbic functions (Tiberi et al., 1991; Meador-Woodruff et al., 1992; Primus et al., 1997), and/or are poorly activated by bromocriptine (Newman-Tancredi et al., 1997).

We recently reported that, in a rat model of Parkinson's disease, the enhanced motor effects of levodopa, observed upon repeated drug administration, result from a dopamine D_1 receptor-mediated induction of dopamine D_3 receptor gene expression in the caudate–putamen (Bordet et al., 1997). Induction of dopamine D_3 receptor expression, likely to occur in the caudate–putamen of Parkinson's disease patients treated with levodopa, may also account for the progressive enhancement of motor recovery. In addition, dopamine D_1 and D_3 receptors frequently colocalise, at least in rat brain, and can act in synergy in vitro (Griffon et al., 1997) and in vivo (Ridray et al., 1998). Thus, levodopa and dopamine agonists active at dopamine D_1 and/or dopamine D_3 receptors in addition to the dopamine D_2 receptor might have superior therapeutic efficacy over selective dopamine D_2 receptor agonists.

Hence, it appears important to evaluate under similar experimental conditions the potency and selectivity of newly developed antiparkinsonian drugs at dopamine receptor subtypes, particularly dopamine D_1, D_2 and D_3 receptors. Among these, pergolide (Langtry and Clissold, 1990; Mizuno et al., 1995; Pezzoli et al., 1995), pramipexole (Hubble et al., 1995; Group, 1997; Guttman, 1997), and ropinirole (Brooks et al., 1995; Adler et al., 1997) were recently introduced in the clinic for the treatment of Parkinson's disease, either as adjuncts to levodopa or as monotherapy. In the present study, we compared these three drugs to bromocriptine, with regard to binding and functional potencies at human recombinant dopamine D_1, D_2 and D_3 receptors.

2. Materials and methods

2.1. Cell lines

Chinese hamster ovary (CHO) cell lines stably expressing the human dopamine D_1, D_2 and D_3 receptors and an NG 108-15 cell line expressing the human dopamine D_3 receptor were obtained by transfecting corresponding cDNAs as described (Lévesque et al., 1992; Sokoloff et al., 1992; Pilon et al., 1994); these cells express 100, 1500, 8500 and 800 fmol of receptors per mg of membrane protein, respectively. Cells were maintained in 10 cm diameter Petri dishes in Dulbecco's modified eagle medium (DMEM, Gibco Life Technologies, Cergy-Pontoise, France), supplemented with 10% foetal calf serum in an atmosphere containing 5% CO_2 and appropriate selecting agents. CHO cells expressing dopamine D_1 and D_2 receptors were maintained in the presence of 40 $\mu g\ ml^{-1}$ phleomycine (Cayla, Toulouse, France), NG 108-15 cells expressing the dopamine D_3 receptor were maintained in the presence of neomycine (800 $\mu g\ ml^{-1}$ of G418, Gibco), and CHO cells expressing the dopamine D_3 receptor were maintained in medium without thymidine and hypoxanthine. Cells were passed at 60% confluence.

2.2. Binding assays

Binding assays were performed with a particulate fraction of transfected CHO cells. Cells were detached from culture dishes in the presence of 0.2% trypsin, harvested by centrifugation and homogenised with a Polytron in 50 mM HEPES–Na^+ containing 1 mM ethylenediaminetetraacetic acid (for dopamine D_1 receptor binding) or 10 mM Tris buffer pH 7.5 containing 5 mM $MgCl_2$ (for dopamine D_2 and D_3 receptor bindings). The membranes were collected by centrifugation at $30{,}000 \times g$ for 15 min and the pellet was resuspended by mild sonication in buffer. The suspension was kept at -80°C until use. Prior to assay, the membranes were diluted in 50 mM HEPES–Na^+ buffer pH 7.5 (for dopamine D_1 receptor binding) or 50 mM Tris buffer pH 7.5 containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 5 mM $MgCl_2$ ('Tris–ions buffer', for dopamine D_2 and D_3 receptor bindings), supplemented with 0.2% bovine serum albumin.

Incubations were run in triplicate in polystyrene test tubes containing radioligand and drug to be tested at 12 increasing concentrations in a volume of 400 µl. Drug dilutions were made in HEPES–Na^+ buffer or Tris–ion buffer and incubations were started by the addition of

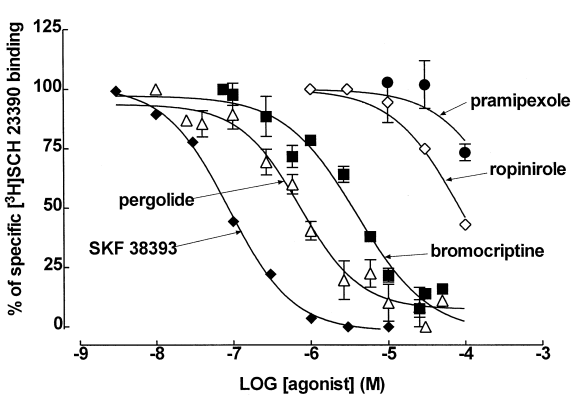

Fig. 1. Inhibition by SKF 38393, pergolide, bromocriptine, ropinirole or pramipexole of [^{3}H]SCH 23390 binding to a particulate fraction of dopamine D_1 receptor-expressing CHO cells. Means ± S.E.M. of values obtained in two to three independent experiments. The curves were fitted by a one-site model.

Table 1
Inhibition constants ($K_i \pm$S.D.) and Hill number ($n_H \pm$S.D.) of various agonists for inhibition of binding to the dopamine D_1 receptor[a]

Agonist	K_i (nM)	n_H
SKF 38393	46 ± 4	1.06 ± 0.08
Pergolide	310 ± 55	1.13 ± 0.09
Bromocriptine	1500 ± 270	0.94 ± 0.08
Pramipexole	$> 50{,}000$	[b]
Ropinirole	$> 50{,}000$	[b]

[a]Inhibition parameters were calculated using a one-site model from competition experiments performed with [^{3}H]SCH 23390 (0.3 nM) as ligand, from data obtained in two different experiments.
[b]Could not be determined.

200 μl membrane suspension (5–10 μg protein), run for 30 min at 30°C and stopped by filtration in a 48 hole-Brandel filtration apparatus. Filters were rinsed 3 times with 3 ml ice-cold 50 mM Tris buffer containing 120 mM NaCl, and the radioactivity was counted by beta or gamma scintigraphy.

D_1 receptor binding was measured using 0.3 nM [^{3}H]SCH 23390, (Amersham, 73 Ci mmol^{-1}), non-specific binding in the presence of 10 μM SKF 38393. D_2 and D_3 receptor binding was measured using 0.1 nM [^{125}I]iodosulpride (Amersham, 2000 Ci mmol^{-1}), non-specific binding in the presence of 3 μM enomapride.

2.3. Cyclic AMP accumulation assay

Cells were plated in a 96-well plate at a density of 20,000 cells well^{-1}. After 24-h culture, the cells were washed twice for 15 min with 200 μl DMEM. The cells were incubated as four replicates for 10 min at 37°C with drugs in the presence of 10 μM isobutyl-methyl-xanthine in 100 μl DMEM. DMEM was then replaced by 50 μl of 0.1 N HCl and the plates were placed on ice. The cells were sonicated for 5 s and the medium was neutralised with a mixture of 2.4 M sodium acetate, pH 6.0 and 2 N NaOH. cAMP levels were measured using the Rianen [^{125}I]cAMP radioimmunoassay kit from Dupont.

2.4. Mitogenesis assay

CHO cells (for D_2 receptor) or NG 108-15 cells (for D_3 receptor) were plated in 96-well plates at a density of 5000 cells well^{-1}. After 24-h culture, the cells were washed twice with culture medium without foetal calf serum and incubated for 16 h with drugs in six replicates (Sautel et al., 1995). Then, [^{3}H]thymidine (1 μCi well^{-1}) was added for 2 h and the cells were harvested by vacuum filtration through Whatman GF/C fibre filters using a Brandel-Beckman automated cell harvester and rinsed 10 times with 200 μl 50 mM potassium phosphate buffer containing 150 mM NaCl. Radioactivity retained on filters was counted by liquid scintigraphy.

2.5. Calculations

IC_{50} or EC_{50} and values were calculated with a computer programme for non-linear regression for a one- or two-site model. Best fits were chosen by an *F*-test on the sum of residual squares and values obtained with the two-site model are reported only when the improvement of the analysis was significant at $P < 0.05$. Curves were drawn with the Prism™ program (GraphPad Software, San Diego, USA). K_i values were then calculated assuming competitive inhibition and taking into account the respective K_d values for the ligand (K_ds = 0.30, 0.51 and 1.31 nM for dopamine D_1, D_2 and D_3 receptors, respectively).

2.6. Drugs

Bromocriptine, pergolide, pramipexole and ropinirole were provided by Athena Neurosciences (San Francisco,

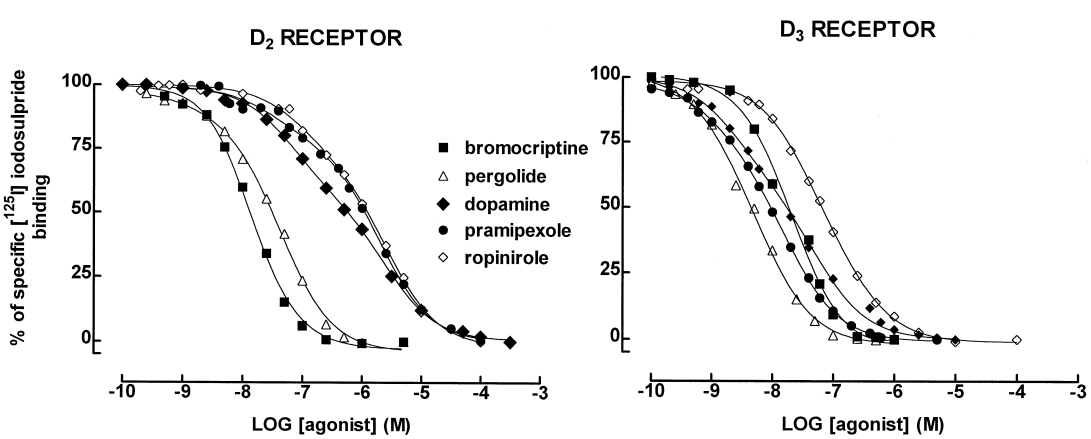

Fig. 2. Inhibition by dopamine, pergolide, bromocriptine, ropinirole or pramipexole of [^{125}I]iodosulpride binding to a particulate fraction of dopamine D_2 receptor- (left) or dopamine D_3 receptor (right)-expressing CHO cells. Means ± S.E.M. of values obtained in two to three independent experiments. The curves were fitted to a two-site model, except that obtained with bromocriptine at the dopamine D_2 receptor, and with bromocriptine and pergolide at the dopamine D_3 receptor.

CA, USA). SFK 38393 ((±)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diaol hydrochloride) was purchased from Research Biochemicals (Natick, MA, USA), dopamine, forskolin and isobutylmethylxanthine from Sigma (St. Louis, MO, USA), and enomapride from Yamanouchi Pharmaceuticals (Tokyo, Japan). [*N*-Methyl-^{3}H]SCH 23390 (*R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine, 73 Ci mmol^{-1}) and [^{125}I]iodosulpride (2000 Ci mmol^{-1}) were obtained from Amersham (Buckinghamshire, UK).

3. Results

3.1. Inhibition of binding to the dopamine D_1 receptor

Displacement of [^{3}H]SCH 23390 (0.3 nM) binding to the dopamine D_1 receptor by bromocriptine, pergolide, pramipexole and ropinirole was first studied in a Tris–HCl buffer containing NaCl, KCl, $MgCl_2$ and $CaCl_2$. SKF 38393 was used as the reference compound. Pergolide and bromocriptine inhibited binding with K_i values (mean ± S.D.) of 180 ± 80 and 3800 ± 1300 nM, respectively, and the competition curves were best fitted if a single binding site was assumed. Pramipexole and ropinirole were less potent, with K_i values higher than 50 μM.

In these experiments, the affinity of SKF 38393 (K_i value = 290 ± 140 nM) was lower than generally reported (Pedersen et al., 1994). We therefore measured the compounds' affinity in a different buffer, made with HEPES–Na^+ and EDTA. In this buffer, competition curves (Fig. 1) also were best fitted by a single-site model and the affinities of SKF 38393 and bromocriptine, but not other compounds, were slightly increased when compared to the values measured in Tris–HCl buffer (Table 1).

Table 2
One-site or two-site analysis of inhibition by dopamine and various agonists of binding at the dopamine D_2 receptor[a]

Agent	One-site model[b]		Two-site model[c]		
	K_i (nM)	n_H	K_H (nM)	R_H (%)	K_L (nM)
Dopamine	590 ± 40	0.57 ± 0.02	56 ± 6	42 ± 2	2300 ± 240
Bromocriptine	12 ± 1	1.14 ± 0.06	[d]	[d]	[d]
Pergolide	33 ± 4	0.77 ± 0.04	0.64 ± 0.4	11 ± 6	38 ± 3
Pramipexole	616 ± 100	0.61 ± 0.03	23 ± 8	21 ± 7	1600 ± 200
Ropinirole	970 ± 100	0.71 ± 0.04	75 ± 29	27 ± 6	2000 ± 380

[a] Inhibition parameters (±S.D.) were calculated for one-site or two-site models from competition experiments performed with [^{125}I]iodosulpride (0.1 nM) as ligand, from data obtained in two different experiments.
[b] K_i and n_H are the inhibition constant and Hill number, respectively.
[c] K_H, R_H and K_L are the inhibition constant at the high-affinity site, the percentage of high-affinity sites and the inhibition constant at the low-affinity site, respectively.
[d] The analysis with the two-site model did not converge.

Table 3
One-site or two-site analysis of inhibition by dopamine and various agonists of binding at the dopamine D_3 receptor[a]

Agent	One-site model[b]		Two-site model[c]		
	K_i (nM)	n_H	K_H (nM)	R_H (%)	K_L (nM)
Dopamine	18 ± 1	0.66 ± 0.02	3.2 ± 0.7	48 ± 6	76 ± 18
Pergolide	4.2 ± 0.3	0.93 ± 0.05	[d]	[d]	[d]
Pramipexole	8.5 ± 0.8	0.76 ± 0.02	0.72 ± 0.14	23 ± 5	15 ± 1
Bromocriptine	12 ± 1	1.14 ± 0.06	[d]	[d]	[d]
Ropinirole	61 ± 3	0.85 ± 0.03	22 ± 8	47 ± 6	140 ± 59

[a] Inhibition parameters (±S.D.) were calculated for one-site or two-site models from competition experiments performed with [^{125}I]iodosulpride (0.1 nM) as ligand. Data obtained in two different experiments.
[b] K_i and n_H are the inhibition constant and Hill number, respectively.
[c] K_H, R_H and K_L are the inhibition constant at the high-affinity site, the percentage of high-affinity sites and the inhibition constant at the low-affinity site, respectively.
[d] The analysis with the two-site model did not converge or was not significantly improved as compared to that with the one-site model.

3.2. Inhibition of binding to dopamine D_2 and D_3 receptors

Competition curves were made in Tris–HCl buffer (Fig. 2), using [^{125}I]iodosulpride (0.1 nM), a highly sensitive and selective ligand for dopamine D_2-like receptors (Martres et al., 1985). Dopamine was used as the reference compound. The competition curves at the dopamine D_2 receptor, obtained with dopamine, pramipexole, pergolide and ropinirole, were biphasic, leading to Hill numbers lower than unity when analysed with the one-site model (Table 2). Best fits were obtained for these compounds with the two-site model, which revealed the presence of a high-affinity site (11–27% of total binding), with affinities 27–68

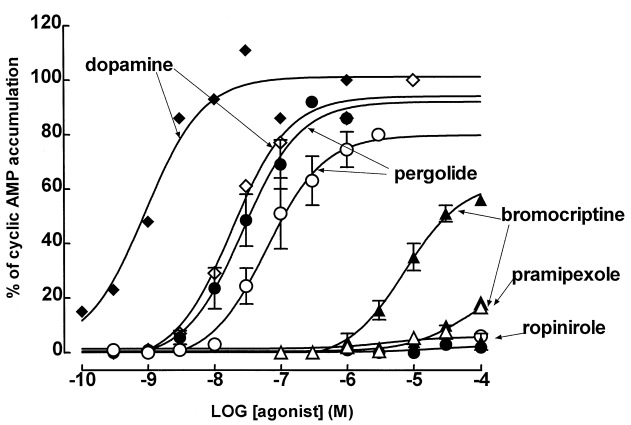

Fig. 3. Cyclic AMP accumulation elicited by dopamine, pergolide, bromocriptine, ropinirole or pramipexole in dopamine D_1 receptor-expressing cells in the absence (open symbols) or presence (filled symbols) of forskolin (1 μM), expressed as percentage of the maximal response induced by dopamine (2 μM). Basal cyclic AMP levels were 0.14 ± 0.01 and 0.40 ± 0.01 pmol well^{-1} in the absence and presence of forskolin, respectively; dopamine-induced cyclic AMP levels were 1.5 ± 0.1 and 4.0 ± 0.7 pmol well^{-1} in the absence and presence of forskolin. Means ± S.E.M. of values obtained in two to three independent experiments.

Table 4
Stimulation by dopamine and various agonists of cyclic AMP accumulation in cells expressing the dopamine D_1 receptor: effects of forskolin

Agent	In the absence of forskolin			In the presence of forskolin (1 μM)		
	EC_{50} value (nM)[a]	R_{max} (%)[b]	R.P.[c]	EC_{50} value (nM)[a]	R_{max} (%)[b]	R.P.[c]
Dopamine	20 ± 3	100 ± 5%	1	1.0 ± 0.4	100 ± 5%	1
Pergolide	61 ± 17	81 ± 5%	0.3	28 ± 8	93 ± 6%	0.04
Bromocriptine	[d]	17% at 10^{-4} M	[d]	7000 ± 1400	62 ± 3%	0.00014
Pramipexole	[d]	4% at 10^{-4} M	[d]	[d]	18% at 10^{-4} M	[d]
Ropinirole	[d]	2% at 10^{-4} M	[d]	[d]	6% at 10^{-4} M	[d]

[a] EC_{50} is the concentration (±S.D.) producing a half-maximal response.
[b] R_{max} is the maximal response (±S.D.) calculated as the percentage of the response produced by dopamine (10 μM). For some compounds, only the maximal response at the maximal concentration tested is given.
[c] R.P. is the relative potency, i.e., the ratio relative to dopamine.
[d] Could not be determined.

times higher than those at the low-affinity site. At the dopamine D_3 receptor, competition curves (Fig. 2) obtained with dopamine and pramipexole and ropinirole could also be resolved into two sites, the high-affinity site representing 23–47% of total binding (Table 3).

Taking into account the best conditions for measurement of binding at the dopamine D_1 receptor and the overall affinity calculated using the one-site model, the following binding selectivities could be established. Bromocriptine had equal affinities at dopamine D_2 and D_3 receptors and a hundred times lower affinity at the dopamine D_1 receptor. Pergolide was more potent at the dopamine D_3 receptor than at the dopamine D_2 or D_1 receptor, at which its affinities were 7.8 and 43 times lower, respectively. Pramipexole was 72 times more potent at the dopamine D_3 than at the dopamine D_2 receptor and almost inactive at the dopamine D_1 receptor. Ropinirole was 16 times more potent at the dopamine D_3 than at the dopamine D_2 receptor and almost inactive at the dopamine D_1 receptor.

3.3. Functional potencies at dopamine D_1 receptor-mediated stimulation of cyclic AMP formation

The accumulation of cAMP in whole cells, measured in the presence of an inhibitor of phosphodiesterase was used as an index of dopamine D_1 receptor activation. Dopamine and pergolide (Fig. 3) stimulated cAMP accumulation in a concentration-dependent manner, while bromocriptine, pramipexole and ropinirole were almost ineffective. When forskolin (1 μM), an activator of adenylate cyclase, was included in the assay, basal cAMP levels were increased by 150% and the responses to all compounds were potentiated. Thus, concentration–response curves for dopamine and pergolide were shifted to the left and bromocriptine became active. Forskolin also potentiated the response to pramipexole, however, to a lesser extent, and had no effects on the response to ropinirole, the drug remaining inactive.

Table 4 shows the EC_{50} values and maximal responses obtained in the absence or presence of forskolin. Potency ratios of the agonists relative to that of dopamine are also given.

3.4. Functional potencies at dopamine D_2 and D_3 receptor-mediated mitogenesis

Stimulation of dopamine D_2 and D_3 receptors activates mitogenesis, as measured by incorporation of [^{3}H]thymidine, through a pertussis toxin-sensitive mechanism involving tyrosine phosphorylation (Pilon et al., 1994; Griffon et

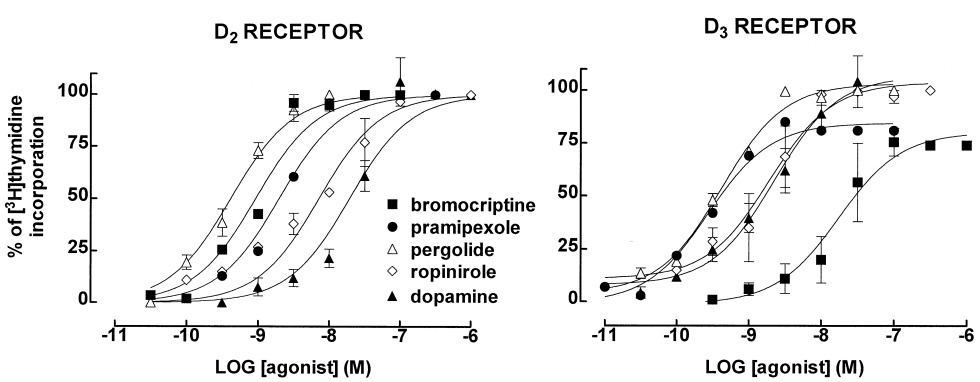

Fig. 4. Mitogenesis elicited by dopamine, pergolide, bromocriptine, ropinirole or pramipexole in dopamine D_2 receptor- (left) or dopamine D_3 receptor-(right) expressing cells. Means (±S.E.M.) of values obtained in three independent experiments, expressed as percentages of the maximal response induced by quinpirole (0.1 μM). Basal and quinpirole-induced [^{3}H]thymidine incorporation was 2350 ± 250 and 5820 ± 460 cpm, respectively, in dopamine D_3 receptor-expressing cells; 2800 ± 200 and 5840 ± 360 cpm, respectively, in dopamine D_2 receptor-expressing cells.

Table 5
Mitogenic responses to dopamine and various agonists in cells expressing dopamine D_2 or D_3 receptors

Agent	D_2 receptor			D_3 receptor		
	EC_{50} (nM)[a]	R_{max} (%)[b]	R.P.[c]	EC_{50} (nM)[a]	R_{max} (%)[b]	R.P.[c]
Dopamine	28±7	104±6	1	1.6±0.4	96±5	1
Pergolide	0.46±0.10	104±2	61	0.46±0.06	103±2	3.5
Bromocriptine	1.1±0.2	106±4	25	9.3±3.1	75±6	0.2
Pramipexole	2.4±0.3	108±3	0.8	0.29±0.05	83±2	5.5
Ropinirole	10±4	100±5	2.8	1.2±0.26	103±5	1.3

[a] EC_{50} is the concentration (±S.D.) producing a half-maximal response.
[b] R_{max} is the maximal response (±S.D.) calculated as the percentage of the response produced by quinpirole (0.1 μM).
[c] R.P. is the relative potency, i.e., the ratio relative to dopamine.

al., 1997). We used this response to evaluate the potency and the intrinsic activity of agonists at dopamine D_2 and D_3 receptors (Fig. 4). Quinpirole was used as the reference agonist.

In dopamine D_2 receptor-expressing cells, all compounds produced maximal responses similar to that produced by quinpirole, a full agonist (Sautel et al., 1995), indicating that they all are full agonists. In dopamine D_3 receptor-expressing cells, pergolide and ropinirole were full agonists, whereas pramipexole and bromocriptine produced responses slightly lower than that to quinpirole. Table 5 gives the EC_{50}, the maximal response and the potency ratio relative to dopamine for each agonist.

4. Discussion

We evaluated the receptor selectivity of four dopamine agonists recently developed for the treatment of Parkinson's disease. For this, we compared their binding affinities and functional potencies at dopamine D_1, D_2 and D_3 receptors, under similar experimental conditions, using recombinant human receptors.

Pergolide was the only compound with significant, although moderate, affinity for the dopamine D_1 receptor, whereas the three other compounds were less potent or completely inactive. All four compounds had high affinity for dopamine D_2 and D_3 receptors. The inhibition of binding to dopamine D_1 and D_3 receptors by pergolide was best described as occurring to a single type of site, indicating that this compound shows similar affinity for the G protein-coupled and uncoupled form of the receptor. Agonists among the ergot derivatives are generally less discriminating for the two affinity states of dopamine D_1-like (Leff et al., 1985) and dopamine D_2-like (Wreggett and De Lean, 1984) receptors. Conversely, pramipexole and ropinirole, which are potent only at dopamine D_2 and D_3 receptors, inhibited binding at these receptors in a biphasic manner, indicating that, like dopamine, they have a much higher affinity for the G protein-coupled form of the receptors. Our results show that the discriminant properties of ligands regarding the two affinity states of the dopamine receptors is not a reliable index of their agonistic activity. For example, pergolide, while not discriminating the two affinity states of the receptors, possesses high potency and intrinsic activity. In addition, the functional potencies of pramipexole and ropinirole at dopamine D_2 and D_3 receptors were found to exceed their binding affinities at the high-affinity site. Furthermore, the binding affinities measured at dopamine D_1 receptors were found to be dependent on buffer composition. It follows that binding selectivity alone, cannot be used to study the receptor profile of the compounds.

Taking into account their respective potency in the functional models used in this study, it appears that pramipexole, ropinirole and, to a lesser extent, bromocriptine show selectivity for dopamine D_2/D_3 over the dopamine D_1 receptor, while pergolide has a much higher potency at the dopamine D_1 receptor than do the other agonists. In addition, pramipexole and ropinirole are ten times more potent at dopamine D_3 than at D_2 receptors, while the reverse applies for bromocriptine. Nevertheless, it should be noted that EC_{50} values, measured in an in vitro assay, are profoundly influenced by receptor expression level and efficacy of coupling to the effector system (Leff, 1995). It is also noteworthy that our transfected cells expressed the dopamine D_1 receptor at a lower level than the other receptor subtypes (see Section 2), which may have led to underestimation of the real potency of the compounds at the dopamine D_1 receptor. Potency ratios, calculated relatively to dopamine and given in Tables 4 and 5, allow a more direct comparison of agonist potencies from one in vitro assay to another. Although this mode of calculation points to the high potency of pergolide and bromocriptine at the dopamine D_2 receptor in vitro, additional factors underlie the level of receptor stimulation by the compounds in vivo.

Among the three receptor subtypes, the dopamine D_1 receptor is the most abundant in the striatum (Mansour et al., 1990), followed by the dopamine D_2 receptor, whereas dopamine D_3 receptor expression is much lower (Lévesque et al., 1992). The distribution of these receptors should also be taken into consideration. Thus, the dopamine D_3 receptor, although less abundant, appears highly concentrated in a limited number of cells, for example, the granule cells of the islands of Calleja, and is present in structures sometimes poorly innervated by dopamine. Ultrastructural immunocytochemical studies showed the dopamine D_1 receptor immunoreactivity to be mostly located at various extrasynaptic sites (Caillé et al., 1996). Dopamine D_1 and D_3 receptors could thus be fully accessible to exogenous direct dopamine agonists, but accessible only to any endogenous dopamine escaping inactivation. In addition, we show here that, unexpectedly, the potency of agonists at the dopamine D_1 receptor was enhanced in the presence of forskolin, indicating that it depends upon the

level of adenylate cyclase stimulation. The potency of agonists at the dopamine D_1 receptor may therefore be increased in neurons stimulated by neurotransmitters acting through receptors positively coupled to adenylate cyclase. It follows that the real potencies of agonists at the dopamine D_1 receptor, and possibly at the dopamine D_3 receptor, might be underestimated when measured in vitro, and it therefore seems likely that pergolide acts through dopamine D_1, D_2 and D_3 receptors in vivo, while other agonists act only at dopamine D_2 and D_3 receptors. In agreement with this, pergolide inhibits [^{3}H]SCH 23390 binding to the dopamine D_1 receptor in vivo (Andersen and Gronvald, 1986) at doses at which it produces cage climbing behaviour in mice (Gianutsos and Palmeri, 1983), a response which requires concomitant stimulations of dopamine D_1-like and D_2-like receptors (Moore and Axton, 1988). Moreover, the locomotor response of acutely reserpinised mice to pergolide is blocked by SCH 23390 (Rubinstein et al., 1988).

The potency of pergolide at both dopamine D_1 and D_2 receptor subtypes could account for its efficacy in the treatment of Parkinson's disease, in view of the synergistic motor effects of dopamine D_1 and D_2 receptors concommittant stimulation (Robertson and Robertson, 1986; Clark and White, 1987; Vermeulen et al., 1994; Waddington et al., 1994). In addition, unlike other compounds, pergolide may also promote the striatal expression of the dopamine D_3 receptor, which a study from this laboratory (Bordet et al., 1997) showed to play a role in motor function. On the other hand, it seems that human caudate–putamen in the normal situation expresses more dopamine D_3 receptor protein (Hall et al., 1996) and mRNA (Susuki et al., 1998) than its rodent counterparts. Thus, the favourable clinical features of pramipexole and ropinirole could derive from their high activity at the dopamine D_3 receptor. In conclusion, the therapeutic efficacy of recently developed antiparkinsonian drugs may be related to their activity at either the dopamine D_1 or D_3 receptor, in addition to the dopamine D_2 receptor.

References

Adler, C.H., Sethi, K.D., Hauser, R.A., Davis, T.L., Hammerstad, J.P., Bertoni, J., Taylor, R.L., Sanchez-Ramos, J., O'Brien, C.F., 1997. Ropinirole for the treatment of early Parkinson's disease. Neurology 49, 393–399.

Andersen, P.H., Gronvald, F.C., 1986. Specific binding of [^{3}H]SCH 23390 to dopamine D_1 receptors in vivo. Life Sci. 38, 1507–1514.

Bordet, R., Ridray, S., Carboni, S., Diaz, J., Sokoloff, P., Schwartz, J.-C., 1997. Induction of dopamine D_3 receptor as a mechanism of behavioral sensitization to levodopa. Proc. Natl. Acad. Sci. USA 94, 3363–3367.

Bouthenet, M.-L., Souil, E., Martres, M.-P., Sokoloff, P., Giros, B., Schwartz, J.-C., 1991. Localization of dopamine D_3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with D_2 receptor mRNA. Brain Res. 564, 203–219.

Brooks, D.J., Torjanski, N., Burn, D.J., 1995. Ropinirole in the symptomatic treatment of Parkinson's disease. J. Neural Transm. 45, 231–238.

Caillé, I., Dumartin, B., Bloch, B., 1996. Ultrastructural localization of D_1 dopamine receptor immunoreactivity in rat striatonigral neurons and its relation with dopaminergic innervation. Brain Res. 730, 17–31.

Calne, D.B., Teychenne, P.F., Leigh, P.N., Bamji, A.N., Greenacre, J.K., 1974. Treatment of parkinsonism with bromocriptine. Lancet 2 (7893), 1355–1356.

Clark, D., White, F.J., 1987. D_1 dopamine receptor.The search for a function: a critical evaluation of the D_1/D_2 dopamine receptor classification and its functional implications. Synapse 1, 347–388.

Diaz, J., Lévesque, D., Lammers, C.H., Griffon, N., Martres, M.-P., Schwartz, J.-C., Sokoloff, P., 1995. Phenotypical characterization of neurons expressing the dopamine D_3 receptor. Neuroscience 65, 731–745.

Diaz, J., Ridray, S., Mignon, V., Griffon, N., Schwartz, J.-C., Sokoloff, P., 1997. Selective expression of dopamine D_3 receptor mRNA in proliferative zones during embryonic development of the rat brain. J. Neurosci. 17, 4282–4292.

Gerfen, C.R., Keefe, K.A., Gauda, E.B., 1995. D_1 and D_2 dopamine receptor function in the striatum: coactivation of D_1- and D_2-dopamine receptors on separate populations of neurons results in potentiated immediate early gene response in D_1-containing neurons. J. Neurosci. 15, 8167–8176.

Gianutsos, G., Palmeri, J.L., 1983. Effects of three dopamine agonists on cage climbing behavior. Psychopharmacology 79, 329–331.

Griffon, N., Pilon, C., Sautel, F., Schwartz, J.-C., Sokoloff, P., 1997. Two intracellular pathways for the dopamine D_3 receptor: opposite and synergistic interactions with cyclic AMP. J. Neurochem. 67, 1–9.

Group, P.S., 1997. Safety and efficacy of pramipexole in early Parkinson's disease. A randomized dose-ranging study. JAMA 278, 125–130.

Guttman, M., 1997. Double-blind comparison of pramipexole and bromocriptine treatment with placebo in advanced Parkinson's disease. International pramipexole–bromocriptine study group. Neurology 49, 1060–1065.

Hall, H., Halldin, C., Dijkstra, D., Wikstrom, H., Wise, L.D., Pugsley, T.A., Sokoloff, P., Pauli, S., Farde, L., Sedvall, G., 1996. Autoradiographic localisation of D_3 dopamine receptors in human brain using the selective receptor agonist (+)-[^{3}H]PD 128907. Psychopharmacology 128, 240–247.

Hornykiewicz, O., 1966. Dopamine (3-hydroxytyramine) and brain function. Pharmacol. Rev. 18, 925–964.

Hubble, J.P., Koller, W.C., Cutler, N.R., Sramek, J.J., Friedman, J., Goetz, C., Ranhosky, A., Korts, D., Elvin, A., 1995. Pramipexole in patients with early Parkinson's disease. Clin. Pharmacol. 18, 338–347.

LaHoste, G.J., Yu, J., Marshall, J.G., 1993. Striatal Fos expression is indicative of dopamine D_1/D_2 synergism and receptor supersensitivity. Proc. Natl. Acad. Sci. USA 90, 7451–7455.

Langtry, H.D., Clissold, S.P., 1990. Pergolide. A review of its pharmacological properties and therapeutic potential in Parkinson's disease. Drugs 39, 491–506.

Leff, P., 1995. The two-state model of receptor activation. Trends Pharmacol. Sci. 16, 89–97.

Leff, S.E., Hamblin, M.W., Creese, I., 1985. Interactions of dopamine agonists with brain D_1 receptors labeled by ^{3}H-antagonists. Evidence for the presence of high and low affinity agonist-binding states. Mol. Pharmacol. 27, 171–183.

Lévesque, D., Diaz, J., Pilon, C., Martres, M.-P., Giros, B., Souil, E., Schott, D., Morgat, J.-L., Schwartz, J.-C., Sokoloff, P., 1992. Identification, characterization and localization of the dopamine D_3 receptor in rat brain using 7-[^{3}H]-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin. Proc. Natl. Acad. Sci. USA 89, 8155–8159.

Mansour, A., Meador-Woodruff, J.H., Bunzow, J.R., Civelli, O., Akil, H., Watson, S.J., 1990. Localization of dopamine D_2 receptor mRNA and D_1 and D_2 receptor binding in the rat brain and pituitary. An in

situ hybridization receptor autoradiographic analysis. J. Neurosci. 10, 2587–2601.

Marsden, C.D., Parkes, J.D., Quinn, N., 1982. Fluctuations in disability in Parkinson's disease: clinical aspects. In: Marsden, C.D., Fahn, S. (Eds.), Movement Disorders. Butterworth Scientific, New York, pp. 96–122.

Martres, M.P., Bouthenet, M.L., Sales, N., Skoloff, P., Schwartz, J.C., 1985. Widespread distribution of brain dopamine receptors evidenced with [^{125}I]iodosulpride, a highly selective ligand. Science 228, 752–755.

Meador-Woodruff, J.H., Mansour, A., Grandy, D.K., Damask, S.P., Civelli, O., Watson, S.J. Jr., 1992. Distribution of D_5 dopamine receptor mRNA in the rat brain. Neurosci. Lett. 145, 209–212.

Mizuno, Y., Kondo, T., Narabayashi, H., 1995. Pergolide in the treatment of Parkinson's disease. Neurology 45, S13–S21.

Moore, N.A., Axton, M.S., 1988. Production of climbing behaviour in mice requires both D_1 and D_2 receptor activation. Psychopharmacology 94, 263–266.

Newman-Tancredi, A., Audinot, V., Chaput, C., Millan, M.J., 1997. [^{35}S]Guanosine-5′-*O*-(3-thio)triphosphate binding as a measure of efficacy at human recombinant dopamine D4.4 receptors: actions of antiparkinsonian and antipsychotic agents. J. Pharmacol. Exp. Ther. 282, 181–191.

Paul, M.L., Graybiel, A.M., David, J.-C., Robertson, H.A., 1992. D_1-like and D_2-like dopamine receptors synergistically activate rotation and c-fos expression in the dopamine-depleted striatum in a rat model of Parkinson's disease. J. Neurosci. 12, 3729.

Pedersen, U.B., Norby, B., Jensen, A.A., Schiodt, M., Hansen, A., Suhr Jenssen, P., Scheideler, M., Thastrup, O., Ansersen, P.H., 1994. Characteristics of stably expressed human D_{1a} and D_{1b} receptors: a typical behavior of the dopamine D_{1b} receptor. Eur. J. Pharmacol. 267, 85–93.

Pezzoli, G., Martignoni, E., Pacchetti, C., Angeleri, V., Lamberti, P., Muratorio, A., Bonuccelli, U., De Mari, M., Foschi, N., Cossutta, E. et al., 1995. A crossover, controlled study comparing pergolide with bromocriptine as an adjunct to levodopa for the treatment of Parkinson's disease. Neurology 45.

Pilon, C., Lévesque, D., Dimitriadou, V., Griffon, N., Martres, M.P., Schwartz, J.-C., Sokoloff, P., 1994. Functional coupling of the human dopamine D_3 receptor in a transfected NG 108-15 neuroblastoma–glioma hybrid cell line. Eur. J. Pharmacol. Mol. Pharmacol. Sect. 268, 129–139.

Primus, R.J., Thurkauf, A., Xu, J., Yevich, E., McInerney, S., Shaw, K., Tallman, J.F., Gallagher, D.W., 1997. II. Localization and characterization of dopamine D_4 binding sites in rat and human brain by use of the novel, D_4 receptor-selective ligand [^{3}H]NGD 94-1. J. Pharmacol. Exp. Ther. 282, 1020–1027.

Ridray, S., Griffon, N., Souil, E., Mignon, V., Carboni, S., Diaz, J., Schwartz, J.-C., Sokoloff, P., 1998. Coexpression of dopamine D_1 and D_3 receptors in rat ventral striatum: opposite and synergistic functional interactions. Eur. J. Neurosci. 10, 1676–1686.

Robertson, G.S., Robertson, H.A., 1986. Synergistic effects of D_1 and D_2 dopamine agonists on turning behaviour in rats. Brain Res. 384, 387–390.

Rubinstein, M., Gershanik, O., Stefano, F.J.E., 1988. Different roles of D_1 and D_2 dopamine receptors involved in locomotor activity of supersensitive mice. Eur. J. Pharmacol. 148, 419–426.

Sautel, F., Griffon, N., Lévesque, D., Pilon, C., Schwartz, J.C., Sokoloff, P., 1995. A functional test identifies dopamine agonists selective for D_3 versus D_2 receptors. NeuroReport 6, 329–332.

Sokoloff, P., Andrieux, M., Besançon, R., Pilon, C., Martres, M.-P., Giros, B., Schwartz, J.-C., 1992. Pharmacology of human D_3 dopamine receptor expressed in a mammalian cell line: comparison with D_2 receptor. Eur. J. Pharmacol. Mol. Pharmacol. Sect. 225, 331–337.

Susuki, M., Hurd, Y.L., Hall, H., Sokoloff, P., Schwartz, J.-C., Sedvall, G., 1998. D_3 dopamine receptor mRNA is widely expressed in the human brain. Brain Res., in press.

Tiberi, M., Jarvie, K.R., Silvia, C., Falardeau, P., Gingrich, J.A., Godinot, N., Bertrand, L., Yang-Feng, T.L., Fremeau, R.T. Jr., Caron, M.G., 1991. Cloning, molecular characterization, and chromosomal assignment of a gene encoding a second D_1 dopamine receptor subtype: differential expression pattern in rat brain compared with the D_{1A} receptor. Proc. Natl. Acad. Sci. USA 88, 7491–7495.

Urwyler, S., 1987. Affinity shifts induced by cations do not reliably predict the agonistic or antagonistic nature of ligands at brain dopamine receptors. J. Neurochem. 49, 1415–1420.

Vermeulen, R.J., Drukarch, B., Sahadat, M.C.R., Goosen, C., Wolters, E.C., Stoof, J.C., 1994. The dopamine D_1 agonist SKF 81297 and the dopamine D_2 agonist LY 171555 act synergistically to stimulate motor behavior of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned Parkinsonian Rhesus monkeys. Mov. Disorders 9, 664–672.

Vitti, R.J., Ahlskop, J.E., 1996. Comparative review of dopamine receptor agonists in Parkinson's disease. CNS Drugs 5, 369–388.

Wachtel, H., 1991. Antiparkinsonian dopamine agonists: a review of the pharmacokinetics and neuropharmacology in animals and humans. J. Neural Transm. (P-D Sect.) 3, 151–201.

Waddington, J.L., Daly, S.A., McCauley, P.G., O'Boyle, K.M., 1994. Levels of functional interaction between D_1-like and D_2-like dopamine receptor systems. In: Niznik, H.B. (Eds.), Dopamine Receptors and Transporters. Pharmacology, Structure, and Function. Marcel Dekker, New York, pp. 511–540.

Walters, J.R., Bergstrom, D.A., Carlson, J.H., Chase, T.N., Braun, A.R., 1987. D_1 dopamine receptor activation of D_2 agonists effects. Science 236, 719–722.

Wreggett, K.A., De Lean, A., 1984. The ternary complex model. Its properties and application to ligand interaction with the D_2-dopamine receptor in the anterior pituitary gland. Mol. Pharmacol. 26, 214–227.