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# Inhibition of dopamine neuron firing by pramipexole, a dopamine $D_3$ receptor-preferring agonist: comparison to other dopamine receptor agonists

Montford F. Piercey *, William E. Hoffmann, Martin W. Smith, Deborah K. Hyslop

*CNS Research Pharmacia & Upjohn, Inc., Kalamazoo, MI 49001, USA*

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**Abstract**

Pramipexole, an amino-benzathiazole [(*S*)-4,5,6,7-tetrahydro-*N*-6-propyl-2,6-benzothiazolediamine dihydrochloride monohydrate] direct-acting dopamine receptor agonist effective in treating Parkinson's disease, bound selectively and with high affinity to dopamine D2-like receptors, with highest affinity at dopamine $D_3$ receptors. Ergot dopamine receptor agonists (bromocriptine, lisuride, pergolide) bound to both dopamine and non-dopamine receptors. Although all agonists depressed dopamine neuron firing, only pramipexole and quinpirole completely silenced firing when administered in slowly-accumulating doses. High-dose pergolide, but not other ergots, completely suppressed firing when given by a prompt bolus i.v. injection, suggesting efficacy limitations may have involved receptor desensitization for pergolide, but not for bromocriptine and lisuride. We conclude that pramipexole differs from ergot dopamine receptor agonists currently used in the treatment of Parkinson's disease by virtue of its selectivity for dopamine receptors, its preferential affinity for the dopamine $D_3$ receptor subtype, and its greater efficacy for stimulating dopamine receptors, as indicated in these electrophysiology assays.

*Keywords:* $D_3$ receptor; Dopamine receptor agonist; Dopamine neuronal firing; Pramipexole

## 1. Introduction

Parkinson's disease, which is caused by a progressive degeneration of dopamine-producing cells in the substantia nigra, has been managed with L-dopa (L-3,4-dihydroxyphenylalanine) therapy since the 1960s. Such treatment has improved prognosis, quality of life, and survival, but is associated with drug-induced adverse effects and diminishing benefit during long-term use (Yahr, 1993). To minimize such complications, a number of strategies have been proposed. These include the replacement or augmentation of L-dopa treatment with dopamine receptor agonists which act directly on the postsynaptic dopamine receptors of the striatum (Goetz, 1990; Mierau and Schingnitz, 1992).

Currently, the most widely used dopamine receptor agonist is bromocriptine [2-bromo-12′-hydroxy-2′-(1-methylethyl)-5′-(2-methylpropyl)ergotamin-3′-6′-18-trione], an ergot alkaloid with agonist activity at postsynaptic D2 [1] receptors. Bromocriptine is typically somewhat less effective than L-dopa alone in reducing neurologic disability in early Parkinson's disease, and its usefulness is limited by dose-related adverse effects (Olanow, 1988). Bromocriptine is, however, associated with a much lower incidence of drug-induced dyskinesias and motor fluctuations than is L-dopa (Olanow, 1988). Other dopamine receptor agonists currently used to treat Parkinson's disease include pergolide (D-6-*n*-propyl-8β-methylmercaptomethylergoline] and lisuride [3-(9,10-didehydro-6-methyl-ergolin-8α-yl)-1,1-diethylurea], (Goetz, 1990; Langtry and Clissold, 1990); like bromocriptine, these agonists are members of the ergot class and are also less efficacious than L-dopa.

* Corresponding author. CNS Research 7251-209-419, Pharmacia & Upjohn, Inc., 301 Henrietta Street, Kalamazoo, MI 49001, USA. Tel.: (616) 833-7847; fax: (616) 833-2525.

[1] Because of the confusion of the similarities in the names of the D2 receptor subfamily and the actual $D_2$ receptor subtype, the distinction will be made throughout this document by the use of subscripts for the subtypes (i.e. $D_2$ subtypes), in conformity with current nomenclature practices (Watson and Girdlestone, 1995), and the lack of subscripts for the subfamilies (i.e. D1 subfamily and D2 subfamily).


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Pramipexole [(*S*)-4,5,6,7-tetrahydro-*N*-6-propyl-2,6-benzothiazolediamine dihydrochloride monohydrate], which is being evaluated clinically as an anti-parkinsonian agent, is a synthetic amino-benzathiazole derivative previously shown to have agonist activity at the presynaptic and postsynaptic dopamine receptors belonging to the D2 subfamily, and to be effective in rodent and primate Parkinson's disease models (Mierau and Schingnitz, 1992). In human subjects as well, pramipexole has been demonstrated to have a dopamine D2 agonist profile (Mierau and Schingnitz, 1992; Schilling et al., 1992) and to be efficacious in treating Parkinson's disease (Hubble et al., 1995).

In recent years, it has become clear that many dopamine receptor agonists have complex activity profiles (Clark et al., 1985; Piercey et al., 1987; Svensson et al., 1991; Lahti et al., 1992). Dopamine D2 receptor agonists vary greatly in their intrinsic efficacies (Clark et al., 1985; Piercey et al., 1987; Lahti et al., 1992), their behavioral effects (Svensson et al., 1991), their binding affinities for adrenoceptor and 5-HT receptors (Svensson et al., 1991), and their activities in limbic or striatal brain regions (Hjorth et al., 1983). In addition, the selectivity of compounds for receptor subtypes in the dopamine D2 receptor subfamily must be considered (Sokoloff et al., 1990; Chio et al., 1994a). An unanswered question is whether alterations in these various properties could yield a more effective dopamine receptor agonist treatment for Parkinson's disease.

Classic dopamine receptor agonists and antagonists have characteristic effects on the firing rates of dopamine neurons. Direct-acting dopamine receptor agonists typically reduce firing rates through their stimulation of dopamine autoreceptors, while dopamine receptor antagonists reverse dopamine receptor agonist-induced inhibition of firing rates (Aghajanian and Bunney, 1977). Dopamine receptor antagonists also reverse depression of firing rates in dopamine neurons caused by amphetamine, an indirect dopamine receptor agonist that stimulates a negative feedback control loop (Bunney et al., 1973). Haloperidol, a classic neuroleptic agent, is considered to be one of the most potent of the dopamine receptor antagonists (Bunney et al., 1973). This agent reverses amphetamine-induced depression of dopamine neuron firing rates (Bunney et al., 1973; Piercey et al., 1987).

The case is somewhat more complex for partial dopamine receptor agonists, such as terguride (Piercey et al., 1987; Svensson et al., 1991) or preclamol (Clark et al., 1985), terguride suppressed spontaneous dopamine neuron firing rates by 53% (Piercey et al., 1987), while preclamol depressed firing rates by 80% (Clark et al., 1985). Terguride also partially reversed amphetamine-induced depression of dopamine neuron firing (Piercey et al., 1987).

Thus, to characterize the activity of a dopamine receptor agonist, it is important to specifically evaluate the receptor populations with which the agonist might interact, as well as obtain some estimate of intrinsic activity. In the current study, we evaluated some of the binding and electrophysiological properties of pramipexole, a dopamine D2 receptor subfamily agonist with highest affinity for the dopamine $D_3$ receptor subtype (Svensson et al., 1994; Mierau et al., 1995), and compare these properties to those of other dopamine receptor agonists.

## 2. Materials and methods

### 2.1. Binding assays

The binding profiles of pramipexole and six other dopamine receptor agonists were evaluated in competition binding experiments using 11 half-log dilutions of drugs run in duplicate tubes (Piercey et al., 1994). The starting concentrations ranged from 1 to 10 μM, depending on the results of pilot studies using drug at the 1 μM concentration. The radioligands, all tritiated, were: prazosin ($\alpha_1$-adrenoceptor sites, 76 Ci/mmol, 1.2 nM), clonidine ($\alpha_2$-adrenoceptor sites, 60 Ci/mmol, 3.8 nM), dihydroalprenolol (β-adrenoreceptors, 52 Ci/mmol, 1.9 nM), oxotremorine-M (muscarinic receptors, 87 Ci/mmol, 0.4 nM), SCH 23390 ($D_1$ receptors, 71 Ci/mmol, 0.3 nM), U-86170 ($D_2$ receptors, 62 Ci/mmol, 1–2 nM, Lahti et al., 1991), *R*(+)-7-OH-DPAT ((+)-7-hydroxy-di-*n*-propylaminotetralin for $D_3$ receptors, 139 Ci/mmol, 0.9 nM), spiperone ($D_4$ receptors, 97 Ci/mmol, 0.7 nM), 8-OH-DPAT (8-hydroxy-di-*n*-propylaminotetralin for 5-$HT_{1A}$ receptors, 85 Ci/mmol, 1.2 nM), and ketanserin (5-$HT_2$ receptors, 62 Ci/mmol, 0.8 nM). Non-specific binding (5% to 20% of total) was defined with 3 μM of the following cold compounds (listed in the same order as the radioligands above): phentolamine, clonidine, alprenolol, atropine, SCH 23390, haloperidol ($D_2$, $D_3$ and $D_4$), lisuride, and spiperone. The sources of binding receptors were as follows: homogenized rat cortex ($\alpha_1$-adrenoceptors, $\alpha_2$-adrenoceptors, β-adrenoceptors, muscarinic receptors and 5-$HT_2$ receptors), chinese hamster ovary (CHO) cell membranes prepared from cells expressing the rat dopamine $D_1$ receptor (Sunahara et al., 1990) CHO cell membranes prepared from cells expressing the rat dopamine $D_{21}$ receptor (Chio et al., 1990), CHO cell membranes from cells expressing the rat dopamine $D_3$ receptor (Chio et al., 1994a), HEK 293 cell membranes from cells expressing the human $D_{4.2}$ receptor (Chio et al., 1994b), and CHO cell membranes prapared from cells expressing the human 5-$HT_{1A}$ receptor (Fargin et al., 1988). Buffers used were 50 mM Tris, 5 mM $MgCl_2$, pH 7.4 ($\alpha_1$, $\alpha_2$, β, muscarinic, 5-$HT_{1A}$ and 5-$HT_2$ assays), 50 mM Tris, 120 mM NaCl, 5 mM $CaCl_2$, 5 mM KCl, 1 mM $MgCl_2$, pH 7.4 ($D_1$ assay), 20 mM Hepes, 10 mM $MgSO_4$, pH 7.4 ($D_2$ assay), and 20 mM Hepes, 10 mM $MgSO_4$, 150 mM NaCl, 1 mM EDTA, pH 7.4 ($D_3$ and $D_4$ assays). Incubation of the 0.9 ml binding reactions was for 1 h at room temperature. Reactions were stopped by vacuum filtration using ice cold 50 mM Tris, 5

mM $MgCl_2$, pH 7.4. Filter paper was Skatron no. 11734 previously soaked for 5 min in 0.05% polyethylenimine. $IC_{50}$ values were estimated by fitting the data to a one-site model by non-linear least squares minimization using GraphPad Prism. $K_i$ values were calculated according to Cheng and Prusoff (1973). These are expressed in Table 1 with 95% confidence intervals for the weighted average of the mean ($n = 1–5$) constructed using individual standard deviations (Finney, 1978). In some cases the drug failed to produce 50% inhibition at the 1 μM concentration in the pilot study. Here dose-response studies were not performed, and the $IC_{50}$ is expressed as greater than 1000 nM.

### 2.2. Electrophysiology

Standard microelectrode recording techniques, which are described in more detail elsewhere (Piercey et al., 1987), were used for single neuron recording in chloral hydrate-anesthetized (400 mg/kg) male Charles River Sprague-Dawley rats, weighing 250–300 g. The femoral vein and artery were cannulated for administration of drug and monitoring of blood pressure, respectively. Body temperature was maintained at 37°C using a Delta phase isothermal pad (Braintree Scientific, Braintree, MA, USA). Stereotaxic coordinates were used for electrode placement in the substantia nigra pars compacta and ventral tegmental area for dopaminergic neurons. Dopaminergic neurons were identified according to the criteria of Bunney et al. (1973). Electrode locations were verified by histological localization of iontophoresed pontamine sky blue dye spots. Drug solutions were prepared in distilled water with equimolar citric acid added as needed. Drugs (quinpirole, Research Biochemicals; lisuride, Schering AG; bromocriptine, Sandoz; pergolide, Eli Lilly; pramipexole, Boehringer-Ingelheim) were administered intravenously in volumes of 0.1 ml or less. For dose-response studies based on cumulative dosing schedules, injections were timed to allow a maximal response to occur, but to accumulate drug sufficiently quickly so that cumulative doses approximated what could be expected with single bolus injections. Thus, the timing of the intervals between injections was determined by how long it took for a maximal response to a drug injection to occur (typically 1–2 min). Drug effects were measured as changes in neuronal firing rates monitored by an integrated rate meter. $ED_{50}$s are doses required to depress firing rates by 50% of maximal response and are calculated by point interpolation on the dose-response curve (log-linear coordinates) for each cell.

## 3. Results

### 3.1. Binding assays

The results of the binding assays are shown in Table 1. Pramipexole bound with high affinity to the D2 subfamily receptors. Its highest affinity was for the dopamine $D_3$ receptor subtype, although its affinity for dopamine $D_3$ receptors was less than one order of magnitude different from that for the dopamine $D_2$ receptor subtype. Pramipexole bound with moderate affinity for $\alpha_2$-adrenoceptors, but with very low affinity for $\alpha_1$- and β-adrenoceptors, acetylcholine receptors, dopamine $D_1$ and 5-HT receptors. Quinpirole, a selective dopamine D2 subfamily receptor agonist, bound to $D_2$, $D_3$ and $D_4$ sites with

Table 1
Binding affinities of $D_2$ receptor agonists for adrenoceptors, acetylcholine, dopamine and 5-HT receptors

| Receptor | $K_i$ (nM) with 95% confidence intervals | | | | | | |
|---|---|---|---|---|---|---|---|
| | Pramipexole | (−)Quinpirole | Bromocriptine | Pergolide | Lisuride | (+)-7-OH-DPAT | Ropinirole |
| $\alpha_1$-Adreno- | > 1 000 [a] | > 1 000 [a] | 18 (17 – 19) | 888 (660 – 1195) | 55 (47 – 64) | > 1 000 [a] | > 1 000 [a] |
| $\alpha_2$-Adreno- | 188 (160 – 220) | > 1 000 [a] | 26 (23 – 29) | 47 (41 – 54) | 2 (1.9 – 2.0) | > 1 000 [a] | > 1 000 [a] |
| β-Adreno- | > 1 000 [a] | > 1 000 [a] | > 1 000 [a] | > 1 000 [a] | N.D. [b] | N.D. | N.D. |
| Acetylcholine | > 1 000 [a] | > 1 000 [a] | > 1 000 [a] | > 1 000 [a] | > 1 000 [a] | N.D. | > 1 000 [a] |
| Dopamine $D_1$ | > 1 000 [a] | > 1 000 [a] | 3 418 (2 444 – 4 779) | 1 163 (819 – 1652) | 62 (55 – 70) | > 7 000 | > 1 000 [a] |
| DDopamine $D_2$ | 6.9 (6.5 – 7.4) | 3.3 (3.0 – 3.6) | 10 (9.5 – 10.5) | 4.0 (3.4 – 4.7) | 0.3 (0.3 – 0.4) | 1.0 (0.9 – 1.1) | 7.2 (7.1 – 7.3) |
| Dopamine $D_3$ | 0.9 (0.8 – 1.0) | 5.0 (4.6 – 5.4) | 87 (75 – 101) | 4.0 (3.7 – 4.3) | 1.7 (1.5 – 2.0) | 0.2 (0.17 – 0.23) | 19 (17 – 22) |
| Dopamine $D_4$ | 15 (14 – 17) | 18 (16 – 20) | 373 (309 – 450) | 6.2 (6.0 – 6.4) | 3.2 (3.1 – 3.4) | 135 (112 – 163) | > 1 000 [a] |
| 5-$HT_{1A}$ | > 1 000 [a] | > 1 000 [a] | 24 (21 – 28) | 5.7 (5.6 – 5.9) | 0.5 (0.47 – 0.53) | 47 (40 – 55) | > 1 000 [a] |
| 5-$HT_2$ | > 1 000 [a] | > 1 000 [a] | 119 (93 – 153) | 26 (23 – 30) | 5.1 (5.0 – 5.1) | N.D. | > 1 000 [a] |

[a] $IC_{50}$ estimated from competition with 1000 nM drug. [b] Not done.

roughly the same affinities. (+)-7-OH-DPAT, which has been reported to have very low affinity for dopamine $D_2$ receptors when competing with antagonist radioligands (Sokoloff et al., 1990; Levesque et al., 1992; Gonzalez and Sibley, 1995), demonstrated much less difference in affinities for dopamine $D_3$ and $D_2$ receptors when using agonist radioligands in both the dopamine $D_2$ and $D_3$ binding assays (see also Gonzalez and Sibley, 1995).

The ergot dopamine receptor agonists (bromocriptine, lisuride, pergolide) were found to have either equivalent affinities for dopamine $D_2$ and $D_3$ receptors, or to have modest selectivity for the dopamine $D_2$ receptor subtype. In addition, these compounds were found to have moderate to high affinities for α-adrenoceptor, $D_1$, $D_4$ or 5-HT receptors.

Ropinirole is an experimental compound that is effective in animal models of Parkinson's disease (Eden et al., 1991). Although this compound binds with very low affinity to dopamine $D_2$ receptors when competing with antagonist ligands (Bowen et al., 1993), we found it to have slightly higher affinity for dopamine $D_2$ than for dopamine $D_3$ sites when tested at the high affinity agonist conformations of these receptors. Ropinirole was found to have very low affinity for all receptors evaluated, except dopamine $D_2$ and $D_3$ sites.

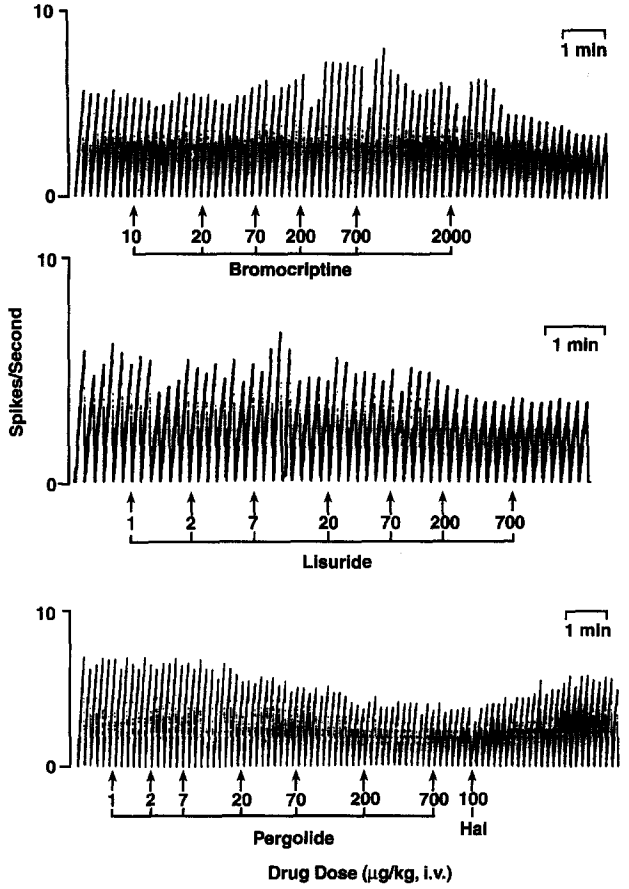


Fig. 1. Effects of dopamine receptor agonists on firing rates of single dopamine neurons in substantia nigra pars compacta, as plotted by an integrating ratemeter on a chart recorder. Ordinates give firing rates, whereas abscissae give times. The arrows indicate the times of administration of bromocriptine (top trace), lisuride (middle trace), and pergolide (bottom trace). Hal = haloperidol. Numbers associated with each arrow are the non-cumulative doses in μg/kg i.v.

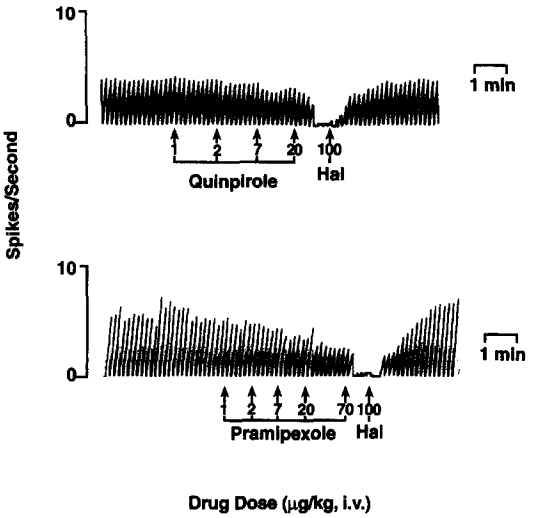


Fig. 2. Effects of dopamine receptor agonists on firing rates of single dopamine neurons in substantia nigra pars compacta, as plotted by an integrating ratemeter on a chart recorder. Ordinates give firing rates, whereas abscissae give times. The arrows indicate the times of administration of quinpirole (top trace) and pramipexole (bottom trace). Hal = haloperidol. Numbers associated with each arrow are the non-cumulative doses in μg/kg i.v.

### 3.2. Electrophysiology studies

All of the dopamine receptor agonists depressed substantia nigra pars compacta firing, albeit with large differences in potencies and efficacies (Fig. 1 and Fig. 2). These differences are not likely due to variations in the cell populations studied since firing rates (2–6/s) of the substantia nigra pars compacta neurons tested with the various dopamine receptor agonists did not differ ($P > 0.05$, ANOVA).

The mean effective doses for 50% inhibition ($ED_{50}$) of cell firing rates in the substantia nigra pars compacta are shown in Table 2. Bromocriptine weakly depressed substantia nigra pars compacta firing rates in three animals (Fig. 1) and, in one of these, the maximal inhibition was less than 50%. In two other subjects, either no effect or an enhanced firing rate was observed with doses as high as 3000 μg/kg. Like bromocriptine, pergolide depressed firing rates in only half the cells tested, but pergolide decreased firing rates of all substantia nigra pars compacta neurons tested (Fig. 1). Although more potent than bromocriptine ($ED_{50} = 1804$ μg/kg, $n = 2$ of 5 cells), both pergolide ($ED_{50} = 18 \pm 5$ μg/kg, $n = 6$) and lisuride ($ED_{50} = 74 \pm 29$ μg/kg, $n = 3$ of 6 cells) had only weak inhibitory effects on cell firing rates, and neither compound completely inhibited firing when given in slowly

Table 2
$ED_{50}$ (μg/kg ± S.E.M.) for inhibition of substantia nigra pars compacta neuronal firing by dopamine receptor agonists

| $D_2$ receptor agonist | $ED_{50}$ (μg/kg ± S.E.M.) | Number of cells |
|---|---|---|
| Pramipexole | 51.0 ± 17 | 11 |
| Quinpirole | 6.3 ± 1.6 | 8 |
| Bromocriptine | 1 804 | 2 [a] |
| Pergolide | 18 ± 5 | 6 |
| Lisuride | 74 ± 29 | 3 [a] |

[a] Bromocriptine and lisuride were each tested in 3 additional cells in cumulative doses up to 3000 μg/kg without sufficiently depressing firing for accurate measurements of $ED_{50}$s.

accumulating doses (Fig. 1). Quinpirole and pramipexole consistently depressed substantia nigra pars compacta cell firing rates in a dose-dependent manner; at higher doses, cell firing was completely silenced by a haloperidol-sensitive mechanism (Fig. 2).

Dose-response curves for depression of substantia nigra pars compacta dopamine neuron populations for pramipexole, bromocriptine, pergolide, lisuride, and quinpirole are illustrated in Fig. 3. Bromocriptine, pergolide, and lisuride failed to completely silence substantia nigra pars compacta neuronal firing, even at the highest doses. Mean inhibitory efficacies were as follows: bromocriptine, 56 ± 14%; pergolide, 72 ± 10%; and lisuride, 19 ± 6%. In contrast, both quinpirole and pramipexole were more potent (Table 2) and had efficacies of 100%. Since the dose-response curve for bromocriptine did not demonstrate a ceiling, it is possible that higher doses might have silenced dopamine neuron firing. However, the extremely high bromocriptine doses used are far outside the range where dopamine receptor agonists typically depress dopamine neuron firing. And, for one cell, the bromocriptine dose was increased to 10 mg/kg without increasing the depression.

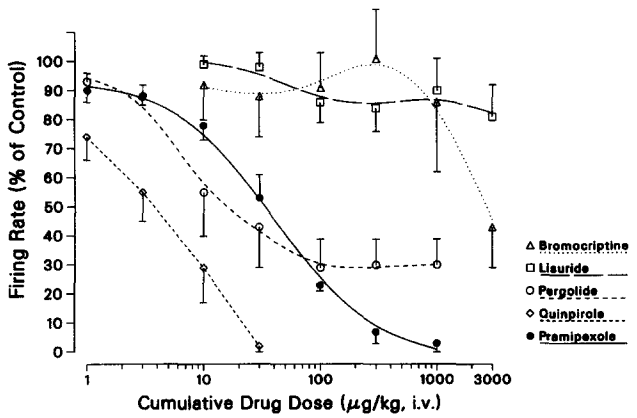


Fig. 3. Dose-response curves comparing bromocriptine (triangles, $n = 5$), lisuride (squares, $n = 6$), pergolide (open circles, $n = 6$), quinpirole (diamonds, $n = 8$), and pramipexole (filled circles, $n = 11$) on substantia nigra pars compacta dopamine neuronal firing rates. Results are expressed as percentages of control firing rate, indicated on the ordinate by the mean values ± S.E.M. The abscissa gives the cumulative dose in μg/kg i.v.

Table 3
Effect of injection schedule on maximal effects of ergot agonists on dopamine neuron firing rates

| Drug | Percentage depression | |
|---|---|---|
| | Cumulative dosing [a] | Bolus dosing [b] |
| Bromocriptine, 3 mg/kg | 56 ± 14 (5) | 53 ± 14 (5) |
| Lisuride, 1 mg/kg | 19 ± 6 (6) | 22 ± 14 (4) |
| Pergolide, 1 mg/kg | 72 ± 10 (6) | 100 ± 0 (4) [c] |

[a] Percentage depression in firing rates following accumulation of drug in amounts indicated in leftwardmost column; dosing schedules accumulated doses by increasing half-log intervals. See Methods and Fig. 3 for precise schedules. The numbers of substantia nigra pars compacta dopamine neurons evaluated are given in parentheses. [b] Percentage depression in firing rates following single bolus injections of drugs as indicated by the leftwardmost column. The numbers of substantia nigra pars compacta dopamine neurons evaluated are given in parentheses. [c] $P < 0.05$ compared to results with cumulative dosing schedule, Mann-Whitney test.

For lisuride and bromocriptine, the firing rate depressions obtained in the cumulative injection experiments were unaltered when high doses were given by a single

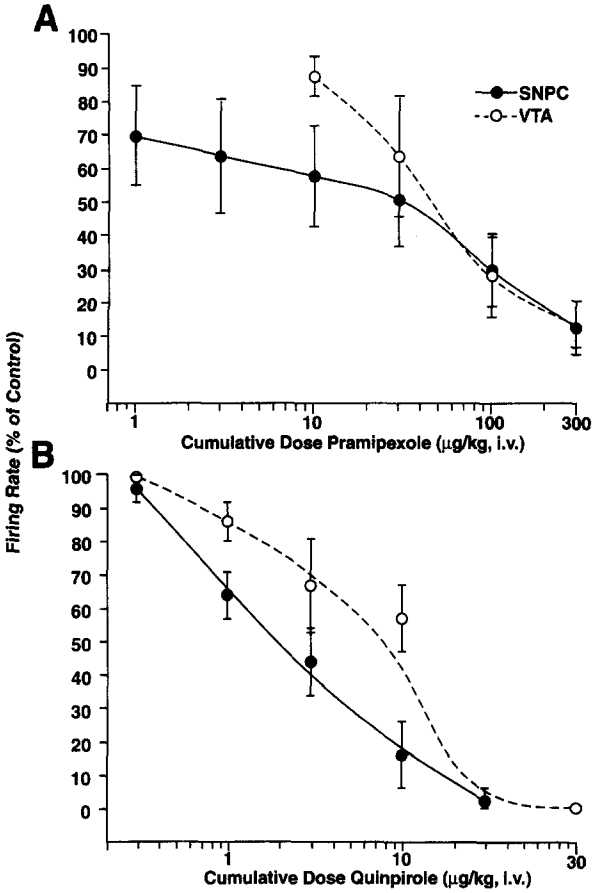


Fig. 4. Comparison of pramipexole's (A) and quinpirole's (B) effects on rat substantia nigra pars compacta (solid lines, $n = 11$ for pramipexole, $n = 8$ for quinpirole) and ventral tegmental area (dotted lines, $n = 6$ for pramipexole and $n = 6$ for quinpirole) dopamine neuron firing rates. Results are shown as percentages of control firing rate, indicated on the ordinates by means ± S.E.M. Abscissae give cumulative doses in μg/kg i.v.

bolus injection (Table 3). Interestingly, amphetamine (1 mg/kg) which in control animals depresses dopamine cell firing by 69 ± 8% (Piercey et al., 1996), was unable to depress firing at all following these bolus injections of lisuride and bromocriptine. Pergolide given in a 1 mg/kg bolus injection was able to totally silence all dopamine neurons tested, even though it did not do so when administered in a cumulative dosing schedule (Table 3).

Similar to its effects in substantia nigra pars compacta, pramipexole inhibited dopaminergic neuronal firing in the ventral tegmental area in a dose-dependent manner (Fig. 4A). Although the threshold for inhibition was lower in the substantia nigra pars compacta than in the ventral tegmental area, no statistically significant differences were found in $ED_{50}$s (substantia nigra pars compacta, 51 ± 17 μg/kg, $n = 11$; ventral tegmental area, 82 ± 40 μg/kg, $n = 6$) or in effects at the two sites; findings were similar for quinpirole, with no significant differences between $ED_{50}$s (substantia nigra pars compacta, 6.3 ± 1.6, $n = 8$; ventral tegmental area, 8.5 ± 2.3 μg/kg, $n = 6$; Fig. 4B).

## 4. Discussion

As the number of identified dopamine receptors grows, it becomes increasingly important to pinpoint the binding affinities of dopamine receptor agonists for specific dopamine receptor subtypes as well as for non-dopamine receptors. Pramipexole, a compound previously identified pharmacologically as a dopamine receptor agonist effective in treating Parkinson's disease (Mierau and Schingnitz, 1992; Schilling et al., 1992; Hubble et al., 1995), binds in a highly selective manner to members of the dopamine D2 receptor family, with an approximate sevenfold higher affinity for the dopamine $D_3$ receptor subtype over dopamine $D_2$ sites (also see Mierau et al., 1995). Its affinity for dopamine $D_4$ receptors has been measured directly by saturation binding analysis to be 5 nM (Mierau et al., 1995). The several-fold discrepancy between this value and the 15 nM reported in the present study is most likely attributable to our use of an antagonist radioligand to measure dopamine $D_4$ affinities. Pramipexole apparently binds with very low affinity to dopamine $D_1$ receptors. Although the use of an antagonist radioligand may lead to an underestimate for dopamine $D_1$ receptor affinities, our result is consistent with pramipexole's lack of dopamine $D_1$ pharmacological effects (Mierau and Schingnitz, 1992). Pramipexole was found to bind with moderate affinity to the $\alpha_2$-adrenoceptor, but with very low affinity for $\alpha_1$- and β-adrenoceptor, acetylcholine receptor, dopamine $D_1$ and 5-HT receptors.

The aminotetralin, (+)-7-OH-DPAT, has been used as a $D_3$-preferring dopamine receptor agonist for evaluation of $D_3$ functional activities and neuroanatomical distribution (Levesque et al., 1992; Svensson et al., 1994). When (+)-7-OH-DPAT's affinities for dopamine receptors are estimated by competition with antagonist ligands, it appears to be a very selective dopamine $D_3$ agonist (Sokoloff et al., 1990). However, it has been appreciated that when affinities are expressed in terms of the high affinity binding to the agonist receptor conformations, (+)-7-OH-DPAT has preferential, rather than selective, affinity for the dopamine $D_3$ receptor (Chio et al., 1994a; Freedman et al., 1994; Gonzalez and Sibley, 1995). Similarly, quinpirole (Sokoloff et al., 1990) and ropinirole (Bowen et al., 1993) were reported to be selective dopamine $D_3$ agonists when using antagonist binding at dopamine $D_2$ receptors when, in fact, these compounds show no preference for binding to the dopamine $D_3$ receptors when evaluations are limited to agonist states of the dopamine $D_2$ and $D_3$ receptor subtypes.

By increasing receptor-G protein association, agonists induce a dopamine receptor conformation that retains a high affinity for agonists whereas, through receptor-G protein dissociation, antagonists induce a conformation with a low affinity for agonists; like antagonists, excess GTP can induce the low affinity state via receptor-G protein dissociation (Dolphin, 1987). Since our binding measurements for dopamine $D_2$ and $D_3$ receptors were obtained by competition with radiolabeled agonists ([$^3$H]U-86170E for dopamine $D_2$ receptors and [$^3$H](+)-7-OH-DPAT for dopamine $D_3$ receptors), the data we have presented are for agonist binding to the high affinity (agonist) states of these receptors. Associated with the weak ability of GTP to alter agonist affinities for binding to the cloned dopamine $D_3$ receptor, affinities for high affinity (agonist) and low affinity (antagonist) states of this receptor are fairly similar (Sokoloff et al., 1990; Chio et al., 1994a), and it is usually reasonable to detect high affinity states by two-site analysis even when using antagonist ligands like spiperone (Chio et al., 1994a). However, it is very difficult to detect binding to the high affinity states when agonists compete with radiolabeled antagonist ligands for dopamine $D_2$ receptors (Gonzalez and Sibley, 1995). Thus, when comparing binding affinities for the high affinity agonist conformations, the relative preference we found with pramipexole for the dopamine $D_3$ as opposed to the dopamine $D_2$ receptor is comparable to what we observed with (+)-7-OH-DPAT (see also Gonzalez and Sibley, 1995). It is important to note that it is the high affinity state that is associated with biological activity for agonists. Functional assays suggest that pramipexole has slightly higher dopamine $D_3$ preference compared to (+)-7-OH-DPAT (Svensson et al., 1994). Although (+)-7-OH-DPAT binds to σ receptors (Wallace and Booze, 1995a), pramipexole does not (Wallace and Booze, 1995b). Thus, although neither compound can be claimed as a specific $D_3$ ligand, pramipexole appears to be slightly superior to (+)-7-OH-DPAT as a dopamine $D_3$-preferring ligand.

The binding profile of pramipexole may be contrasted

with those of the ergot dopamine receptor agonists used to treat Parkinson's disease. These compounds (Table 1) were found to have either equivalent affinities for dopamine $D_2$ and $D_3$ agonist sites, or to have modest selectivity for the dopamine $D_2$ subtype. In addition, these compounds were found to have moderate to high affinities for α-adrenoceptors, dopamine $D_1$, $D_4$ or 5-HT receptors. Ropinirole resembles pramipexole in that it is a non-ergot dopamine receptor agonist which is found to have very low affinity for all receptors except those for dopamine $D_2$ and $D_3$ sites. However, ropinirole, if anything, bound with slightly greater affinity for dopamine $D_2$ than for dopamine $D_3$ receptors. Thus, pramipexole may be distinguished from other clinically effective anti-parkinsonian dopamine receptor agonists by its selectivity for dopamine D2 subfamily receptors and its preferential affinity for the dopamine $D_3$ receptor subtype.

It should be noted that antagonist radioligands were used in the $\alpha_1$-adrenoceptor, β-adrenoceptor, dopamine $D_1$, dopamine $D_4$, and serotonin 5-$HT_2$ receptor binding assays. Because these radioligands presumably occupy both G-protein-coupled and uncoupled receptors at the concentrations used, the affinities reported in Table 1 for these assays may be underestimated. Nonetheless, these data clearly demonstrate differences in binding profiles among the various dopamine receptor agonists evaluated, and previous studies have also demonstrated that the ergots, bromocriptine, lisuride, and pergolide, bind to non-dopaminergic receptors in vitro and exhibit non-dopaminergic neurochemical effects in vivo (Wachtel, 1991). Additionally, pramipexole's in vivo pharmacological profile is similar to that for other dopaminergic agonists selective for the dopamine D2 receptor subfamily; in particular, non-dopaminergic effects are not observed (Mierau and Schingnitz, 1992).

Like quinpirole, a full agonist for dopamine D2 subfamily receptors, pramipexole, completely suppressed firing of single neurons in the substantia nigra pars compacta and ventral tegmental area of rats anesthetized with chloral hydrate. Although the $ED_{50}$s for pramipexole and quinpirole were slightly higher for the substantia nigra pars compacta than for the ventral tegmental area neurons, the differences were not statistically significant. (+)-7-OH-DPAT which, like pramipexole, binds with higher affinity to dopamine $D_3$ than dopamine $D_2$ receptors, has also been reported to completely suppress dopamine neuron firing in both the substantia nigra pars compacta and ventral tegmental area, and with similar potencies in both areas (Liu et al., 1994).

Depression of dopamine neuron firing occurs via activation of somatodendritic autoreceptors (Aghajanian and Bunney, 1977). Although pramipexole is a dopamine $D_3$-preferring agonist, we cannot use the data presented in this study to distinguish whether the depression of dopamine neuronal firing is due to dopamine $D_2$ or $D_3$ receptor stimulation, or both. Based on the inability of a dopamine $D_3$ antagonist, U-99194A, to increase dopamine release, it has been suggested that dopamine $D_3$ receptors may not function as autoreceptors at dopamine nerve terminals (Waters et al., 1993).

Although depression of dopamine neurons is an autoreceptor function, it can be used as one measure to evaluate efficacy at dopamine D2 subfamily receptors. By this measure, pramipexole and quinpirole are both full dopamine receptor agonists. In vitro functional assays in cell lines expressing cloned receptors confirm our observation that pramipexole is a full dopamine receptor agonist (Svensson et al., 1994).

Although bromocriptine, pergolide, and lisuride all depressed dopamine neuron firing rates, these compounds failed to completely suppress substantia nigra pars compacta neuron firing when given in slowly accumulating doses. These data indicate that, in contrast to quinpirole and pramipexole which are full D2 agonists, the dopamine receptor agonists currently used for treatment of Parkinson's disease are partial dopamine D2 agonists. This conclusion is especially convincing when one considers the large numbers of spare somatodendritic autoreceptors (Meller et al., 1986).

Other investigators also report that the ergot dopamine receptor agonists lisuride (Rogawski and Aghajanian, 1979; Gessa, 1988) and bromocriptine (Bannon et al., 1980; Scarnati et al., 1980; Yarbrough et al., 1984) only partially depress dopamine neuron firing. Consistent with partial activity, Bannon et al. (1980) report that not all cells they evaluated with bromocriptine were completely depressed. Nonetheless, the majority of cells were; the mean dose required for complete cessation in sensitive cells was 6 mg/kg. This dose is extremely high compared to those typically required for dopamine receptor agonist-induced depression of substantia nigra pars compacta firing rates, and is higher than the highest bromocriptine dose (3 mg/kg) we typically tested (but lower than the 10 mg/kg dose we tested on one cell without silencing it). Bannon et al. (1980) report extreme difficulties in reversing bromocriptine's effects on substantia nigra pars compacta neuron firing rates with the dopamine receptor antagonist haloperidol, requiring an average of 1.1 mg/kg haloperidol to only partially reverse effects when administered within 5 min of the bromocriptine injection. This is more than 10 times the haloperidol doses typically used to completely reverse the depression of dopamine neurons caused by other dopamine receptor agonists (see, for example, Fig. 2). Haloperidol had absolutely no effect when administered more than 20 min after bromocriptine. Bannon et al. (1980) suggested that bromocriptine irreversibly binds to dopamine receptors. However, their data could also be interpreted to indicate that the bromocriptine effects involved non-dopaminergic mechanisms. Clearly, our binding data and those of others (Wachtel, 1991) demonstrate that bromocriptine interacts with a number of non-dopaminergic receptors.

The inability to increase efficacy with bolus injections indicates that the weak effects of bromocriptine and lisuride probably do not result from dopamine receptor desensitization. It is possible that these low efficacies result from low intrinsic activities, since high doses of these ergots blocked amphetamine's effects similar to actions observed with other dopamine receptor partial agonists (Piercey et al., 1987; Svensson et al., 1991). However, given the ability of these compounds to interact with adrenoceptors and 5-HT receptors, we cannot rule out non-dopaminergic influences.

Carlson et al. (1987) found that pergolide completely suppressed substantia nigra pars compacta neuronal firing rates. Since their methodology appears similar to ours, the reasons that our results differ are not clear. However, pergolide was the most efficacious of the ergot agonists that we evaluated, maximally depressing dopamine neuron firing by 72%, on average, when dosed cumulatively. Moreover, the $ED_{50}$ we report for pergolide (18 μg/kg) is similar to the potency reported by Carlson et al. (1987) (10 μg/kg). Since we did find total suppression of firing with bolus pergolide, the weak efficacy of pergolide could be due to rapid receptor desensitization occurring during the cumulative pergolide dosing schedule we used. But, this does not explain why Carlson et al. (1987) got complete suppression, since they used cumulative dosing schedules.

Dopamine receptor agonist treatment of Parkinson's disease is mediated via postsynaptic dopamine receptors rather than via somatodendritic autoreceptors. However, it is probable that, like the autoreceptors, the postsynaptic receptors stimulated by anti-parkinsonian dopamine receptor agonists belong to the dopamine D2 receptor subfamily. Bromocriptine, pergolide, and lisuride all appear to bind with higher affinities for receptors in the dopamine D2 receptor subfamily than for the dopamine $D_1$ receptor. Of these three agonists, only pergolide demonstrates even partial D1-like agonist effects on striatal dopamine-sensitive cAMP stimulation, and all three compounds act as dopamine D2-like rather than dopamine D1-like agonists when measuring effects on striatal acetylcholine (Wachtel, 1991). Thus, it seems likely that the anti-parkinsonian properties of these compounds are dependent on their agonist effects at dopamine D2 subfamily receptors, even though their intrinsic activity or efficacies at these sites appear low.

Behavioral experiments confirm low potency and efficacy for bromocriptine as a postsynaptic dopamine receptor agonist (Yarbrough et al., 1984). In electrophysiology experiments, pergolide is as effective as apomorphine in exciting globus pallidus neurons via presumed dopaminergic postsynaptic receptors (Carlson et al., 1987). However, pergolide only demonstrates partial agonist activity when stimulating presynaptic dopamine receptors responsible for decreasing tyrosine hydroxylase activity in vitro (Rabey et al., 1981) or dopamine synthesis in vivo (unpublished data cited in Rabey et al., 1981). Similarly, pergolide, lisuride and bromocriptine (V.H. Sethy, unpublished) are partial agonists at postsynaptic dopaminergic receptors responsible for increases in striatal acetylcholine in vivo.

Since Parkinson's disease is associated with loss of dopamine neurons, one might expect that a dopamine receptor agonist interacting directly with postsynaptic dopamine receptors would provide a more effective treatment than L-dopa, which has some dependency on existing dopamine neurons for dopamine synthesis and release. In the face of this theoretical premise, the low efficacy associated with dopamine receptor agonists is problematic. Low intrinsic activities for bromocriptine and lisuride, and rapid receptor desensitization for pergolide provides some explanation for their poor anti-parkinsonian efficacy when compared to L-dopa. Alternatively, the efficacies of the currently used dopamine receptor agonists could be dose-limited by side effects. The limiting side effect observed with the dopamine receptor agonists is more likely to be hallucinations rather than dyskinesias, whereas the opposite is true for L-dopa (Goetz, 1990; Montastruc et al., 1993). Although hallucinations could be due to excessive stimulation of dopamine receptors, hallucinations are also a property of 5-HT receptor agonists (Heym and Jacobs, 1987). Interestingly, all three of the dopamine receptor agonists used to treat Parkinson's disease bind to 5-HT receptors and adrenoceptors with affinities rivaling those for binding to receptors in the dopamine D2 receptor subfamily (Table 1).

Pramipexole is a new dopamine receptor agonist currently being evaluated for the treatment of Parkinson's disease. Pramipexole differs from the dopamine receptor agonists currently being used to treat Parkinson's disease in that, within the dopamine D2 receptor subfamily, it is a full agonist that binds with only very low affinity to adrenoceptors and 5-HT receptors. Thus, this compound can provide a test of the hypotheses that limits on dopamine receptor agonist efficacy in Parkinson's disease result either from low intrinsic activity or interactions with non-dopamine receptors.

Pramipexole also differs from other dopamine receptor agonists by having a higher affinity for dopamine $D_3$ receptors than for dopamine $D_2$ and $D_4$ receptors. Although mRNA studies suggest that the dopamine $D_3$ receptor is highest in mesolimbic pathways, dopamine $D_3$ receptor mRNA is also found in the dorsal striatum (Bouthenet et al., 1991). Some antibody studies indicate that dopamine $D_3$ receptor protein is low in caudate (Boundy et al., 1993), while others suggest it is quite high (Ariano and Sibley, 1994). Work in our laboratory suggests that pramipexole stimulates caudate neurons via a possible dopamine $D_3$ receptor mechanism (Hyslop et al., 1993). Thus, it is possible that pramipexole's dopamine $D_3$ receptor preference could contribute to its efficacy in treating Parkinson's disease. One additional consequence of pramipexole's high affinity for dopamine $D_3$ receptors, based on the possible preferential distribution of this receptor subtype in mesolimbic reward pathways, is antidepres-

sant activity through activation of motivational pathways (Willner et al., 1994). Such activity could have consequences for treatment of Parkinson's disease, where depression occurs concomitantly with motor dysfunction (Cummings, 1992).

Thus, the receptor binding profile and intrinsic activity of pramipexole make this an interesting compound to evaluate for treatment of Parkinson's disease. Although more extensive studies will be needed to address these questions fully, some data supporting clinical efficacy for pramipexole are already available (Hubble et al., 1995).

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