Characterization of Unconditioned Behavioral Effects of Dopamine D₃/D₂ Receptor Agonists

BETH GETER-DOUGLASS, JONATHAN L. KATZ, KEN ALLING, JANE B. ACRI and JEFFREY M. WITKIN

Drug Development Group, Preclinical Pharmacology Laboratory, NIDA Addiction Research Center, National Institutes of Health, Baltimore, Maryland

Accepted for publication June 12, 1997

ABSTRACT

A series of experiments examined the ability of dopamine D₁/D₂ receptor agonists [(+)-(4aR,10bR)-3,4,4a,10b-tetrahydro-4-propyl-2H,5H-[1]benzopyrano-[4,3-b]-1,4-oxazin-9-ol hydrochloride (PD 128,907), (+)-(7)-hydroxy-dipropylaminotetralin hydrobromide (7-OH-DPAT), quinpirole and bromocriptine] to produce a variety of dopaminergically mediated behaviors. The effects of these drugs with selectivity for D₃/D₂ receptors over D₁ receptors were compared with those produced by the selective D₁ agonists [(±)-Phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride (SKF 82958), (+)-(6)-Chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide (SKF 82958)], a nonselective dopaminergic agonist (apomorphine), and an indirect dopamine agonist (cocaine). The D₃/D₂ agonists decreased locomotor activity, had no effect on gnawing and only inconsistently induced climbing in mice. Further, these agonists dose-dependently produced scratching in squirrel monkeys. In contrast, the D₁ agonists, SKF 82958 and SKF 38393, did not produce scratching in squirrel monkeys. Whereas the full D₁ agonist, SKF 82958, produced increases in locomotor activity and in climbing and gnawing, the partial D₁ agonist, SKF 38393, did not increase the frequencies of these behaviors. The nonselective dopamine agonist, apomorphine, produced decreases in locomotor activity and increases in climbing and gnawing in mice. Apomorphine dose-dependently produced scratching in squirrel monkeys. The indirect dopamine agonist, cocaine, produced increases in locomotor activity and climbing, but had no effect on climbing or gnawing in mice and did not produce scratching in squirrel monkeys. These findings suggest that D₃/D₂ agonists can be distinguished on various behavioral measures from the nonselective agonist, apomorphine (gnawing), D₁ agonists (scratching) and the indirect agonist, cocaine (locomotor activity and scratching). Behaviors once attributed to stimulation of D₂ (locomotor activity and scratching) or D₃/D₂ (climbing and gnawing) receptors may also involve dopamine D₃ receptors.

The dopaminergic system is involved in a variety of functions including motor control, sexual functions, cardiovascular homeostasis, endocrine regulation and cognition (for review see Jackson and Westlind-Danielsson, 1994). Disruption of this system is associated with neurological disorders such as schizophrenia, Parkinson’s disease and Huntington’s chorea. In addition, central dopaminergic pathways have been implicated in the reinforcing and subjective effects of drugs of abuse such as cocaine (Johanson and Fischman, 1989; Kuhar et al., 1991; Witkin, 1994). A major focus of research involving pharmacological treatment for all of these disorders has been the development of drugs that target specific subtypes of the various dopamine receptors (Seeman and Van Tol, 1994). Some success in this area has been attained, including the use of dopamine D₁/D₂ agonists (e.g., bromocriptine) in treating Parkinson’s disease and D₂/D₃ antagonists in treating psychosis (for review see Seeman and Van Tol, 1994; Seeman, 1995).

At present, at least five dopamine receptor subtypes have been identified and have been designated as D₁-D₅ (Kebabian and Calne, 1979; Sibley and Monsma, 1992). D₃, D₁ and D₅ dopamine receptor subtypes share molecular characteristics with either D₁ or D₂ receptors and based on these simi-
larities have been grouped into two subfamilies: “D1-like” which include D1 and D2 subtypes and “D2-like” which include D2, D3 and D4 subtypes (Sibley and Monsma, 1992). Until recently, however, dopamine receptors were only divided into two subtypes: D1 receptors that activate adenyl cyclase and D2 receptors that inhibit or have no effect on the enzyme (Keubabian and Calne, 1979). After the development of ligands selective for the D1 and D2 receptor subtypes, experimental evidence revealed that selective stimulation of each subtype produced different types of unconditioned behavioral effects (for review see Clark and White, 1987). For example, D1 agonists produce intense grooming and abnormal perioral movements in rats in the absence of stereotyped behaviors (Rosengarten et al., 1983; Molloy and Waddington, 1985; Murray and Waddington, 1989). In squirrel monkeys, full D1 agonists produce increases in the frequencies of stationary postures and head movements (Rosenzweig-Lipson et al., 1994). Conversely, D2 agonists produce stereotypies such as sniffing, rearing and head movements in rats (Molloy and Waddington, 1985; Arnt et al., 1987) and scratching in squirrel monkeys (Rosenzweig-Lipson et al., 1994; Pellón et al., 1995). Given that D1 and D2 agonists have been previously demonstrated to produce distinct behavioral effects, the question of whether the newly identified subtypes (D3, D4 or D5) share functions with other subtypes in their respective subfamilies is of interest.

At present, the availability of ligands that selectively target each of the dopamine receptor subtypes is limited. However, drugs have been identified that bind with varying degrees of selectivity for the various receptor subtypes. For example, agonists have been shown to bind to both D2 and D1 receptors, including PD 128,907, (-)-7-OH-DPAT and quinpirole. As for their selectivity at D3 and D4 receptors, these compounds display a slightly higher affinity for the high-affinity D3 receptor than for the high-affinity D4 receptor (Chio et al., 1994; Burris et al., 1995; Malmberg and Mohell, 1995; Pugsley et al., 1995). Under different assay conditions that did not take into account high- vs. low-affinity dopamine binding sites, the D3-like agonist, bromocriptine, has been shown to have similar affinity for D3 and D4 receptors (Sokoloff et al., 1992; Freedman et al., 1994). Although quinpirole has been shown to have similar affinities for D3 and D4 receptors (Seeman and Van Tol, 1994), bromocriptine, 7-OH-DPAT and PD 128,907 have a greater selectivity for D3 vs. D4 receptor subtypes, with a rank order of potency of D3>D2>D4 (Seeman and Van Tol, 1994; Pugsley et al., 1995). PD 128,907, 7-OH-DPAT bromocriptine, quinpirole are referred to throughout the manuscript as D3/D2 agonists. Our series of experiments was designed to examine the behavioral effects produced by these D3/D2 agonists. These effects were compared to those produced by dopamine agonists that have been used in the past to define dopamine-mediated behaviors, including the D1 agonists SKF 38393 and SKF 82958, the nonselective dopamine agonist, apomorphine, and the indirect (uptake blocker) dopamine agonist, cocaine.

The ability of the dopaminergic agonists to produce climbing, immobility and gnawing was examined in mice. Climbing and gnawing previously have been suggested to require stimulation of both D1 and D2 receptor subtypes given that apomorphine or D3/D2 agonist combinations have been demonstrated to readily produce these behaviors in rodents (Arnt et al., 1987; Moore and Axton, 1988; Vasse et al., 1988; Murray and Waddington, 1989). Conversely, neither D3 or D2 agonists (Arnt et al., 1987; Arnt et al., 1988; Moore and Axton, 1988; Vasse et al., 1988; Murray and Waddington, 1989; Daly and Waddington, 1992; Tirelli and Witkin, 1995) nor cocaine (Tirelli and Witkin, 1994a) have been shown to readily produce climbing or gnawing when administered alone.

Reductions in locomotor activity and/or increases in immobility previously have been found in rodents after the administration of D2 agonists (Martin and Bendesky, 1984; Martin et al., 1984; Eilam and Szechtmann, 1989; Puglisi-Allegre et al., 1990; Jackson and Westlind-Daniellsen, 1994) and apomorphine (Tirelli and Witkin, 1994a). Conversely, increases in locomotor activity and/or decreases in immobility have been associated with D1 agonists (Murray and Waddington, 1989; Daly and Waddington, 1992, 1993; Tirelli and Terry, 1993; Deveney and Waddington, 1995) or cocaine (Johanson and Fishman, 1989; Kelley and Lang, 1989; Pierce and Berc, 1990; French and Witkin, 1993; Witkin, 1993) administration. Given the broad spectrum of effects that dopamine agonists have on locomotor activity and immobility, these behaviors were also measured in mice.

Scratching in squirrel monkeys was also examined after administration of these dopaminergic compounds because it has been suggested as an in vivo model of D2 receptor stimulation (Rosenzweig-Lipson and Bergman, 1993; Rosenzweig-Lipson et al., 1994; Pellón et al., 1995). Whereas apomorphine has been shown to produce scratching in squirrel monkeys (Pellón et al., 1995), neither D1 agonists nor cocaine has been shown to produce this effect in monkeys (Rosenzweig-Lipson et al., 1994; Pellón et al., 1995).

PD 128,907, 7-OH-DPAT, quinpirole and bromocriptine generally produced behavioral effects that could be distinguished from those produced by apomorphine, cocaine and the D1 agonists. These findings suggest that D2 and/or D3 receptor stimulation may be involved in producing behaviors that were once considered to be mediated exclusively by stimulation of D2 receptors (decreased locomotor activity and scratching) or D1/D2 receptors (climbing and gnawing).

Methods

Behavioral Observations

Subjects. Male Swiss-Webster mice (Taconic Farms, Germantown, NY) were housed in groups of five in a temperature controlled vivarium (22–25°C) with a 12-hr light/dark cycle. Food (Zeigler Small Animal Feed, St. Louis, MO) and water were available ad libitum. Behaviorally naive mice were studied only once (n = 6/dose).

Apparatus and procedure. As previously described by Tirelli and Witkin (1994a), behavioral observation cages were constructed of wire screen (1 cm mesh, 15 × 15 × 26 cm high). During testing, the bottomless cages were placed on a smooth surface. Testing sessions always involved six cages with one mouse per cage. After drug treatments (i.p.), mice were immediately placed into a cage for a 65-min session. During 5-min observation periods beginning at 10, 35 and 60 min postinjection, a subject was observed every 30 sec for the presence or absence of climbing, immobility, nose poking, switching sides and gnawing as described below. For each behavior, each subject could receive a total of 10 points during each observation period and, therefore, 30 points over the entire session. Climbing was recorded when the wire of the test chamber was grasped with all four paws for at least 15 sec (Tirelli and Witkin, 1994a). Climbing could simultaneously occur with immobility, nose poking, switching

Downloaded from jpet.aspetjournals.org on July 10, 2017
sides and gnawing. Immobility was scored when no movement occurred anywhere within the cage for at least 15 sec; immobility could simultaneously occur with climbing, but could not occur simultaneously with any other of the behaviors being scored. Nose poking was recorded when the nose was projected through the wire-mesh in the absence of gnawing. Switching sides was recorded after movement from one wall of the screen to another (including the ceiling and floor). Gnawing was recorded when the incisors were placed over the wire-mesh and was often accompanied by the typical jaw movements of biting (Tirelli and Witkin, 1994a). A single experimenter scored each experimental session.

**Locomotor Activity**

**Subjects.** Male Swiss-Webster mice (Taconic Farms, Germantown, NY) were housed and fed as above. Eight mice were tested at each dose of dopamine agonist.

**Apparatus and procedure.** Immediately after an i.p. injection of a dopamine agonist, subjects were individually placed into a 40-cm³ activity monitor equipped with photoelectric detectors placed 2.5 cm apart along the perimeter that were capable of sensing ambulatory activity at a height of 2.5 cm above the floor (Omnitech Electronics, Columbus, OH). Horizontal locomotor activity was recorded in 30-min intervals for a total of 180 min.

**Scratching**

**Subjects.** Four adult male squirrel monkeys (Saimiri sciureus) with mean body weights of 860 to 1,050 g were housed individually in a temperature and humidity-controlled room with a 12-hr light/dark cycle. Cages permitted two-way visual and olfactory (not tactile) access to other squirrel monkeys and to the observer. Monkeys had unrestricted access to food (Lab Diet, High Protein Monkey Diet, St. Louis, MO and Golden Squares, BioServ, Frenchtown, NJ, supplemented with fresh fruit) and water. They had been studied previously in experiments involving acute administration of drugs or cocaine self-administration, but had not participated in an experiment for 5 mo before the start of this study.

**Procedure.** Within a cumulative dosing procedure, monkeys (n = 4) received four successive injections (i.m. in thigh) of incremental doses of a dopamine agonist separated by an interval of 15 min. Immediately after each injection, they were observed in their home cages during three 1-min periods. Observations occurred at 5, 9 and 13 min after an injection. During each observation period, scratching was recorded for each monkey if it occurred on at least one occasion for at least 2 sec. Each monkey could receive three positive scores at each dose tested. Across subjects, there was a maximum of 12 positive scores possible at each dose. A single experimenter scored each experimental session.

**Drugs**

PD 128,907 (Research Biochemicals Inc., RBI, Natick, MA), 7-OH- DPAT (RBI), trans-1-4aR-4,4a,5,6,7,8a,9-Octahydro-5-propyl-1H-pyrazolo[3,4-g]quinoline HCl (quinpirole HCl (LY 171555); RBI), SKF 38393 (RBI), SKF 82958 (RBI), R(-)-10,11-Dihydroxyaporphine HCl (apomorphine; Sigma Chemical Co., St. Louis, MO) and 2B-carbethoxy-3b-benzoyloxytropane HCl (cocaine HCl; Sigma Chemical Company, St. Louis, MO) were dissolved in distilled water. Bromocriptine mesylate (Sigma) was dissolved in distilled water and minimal HCl. If necessary, mild heat and sonication were used for dissolution. For mouse experiments, injections were given i.p. in volumes of .01 ml/g body weight. For squirrel monkey experiments, injections were given i.m. and no more than 1.5 ml was injected into each animal during each testing session. Vehicle injections were of a volume similar to that of the drugs in each experiment.

**Data Analysis**

For observational experiments in mice, the data taken at each of the three time intervals (10, 35 and 65 min) did not consistently vary at each interval; hence, the data were collapsed over the entire session. The total number of occurrences of each behavior made during the session were averaged across all six subjects at each dose of dopamine agonist. Percentages were obtained by dividing the average by the maximum possible score that each subject could receive during the session (30); that result was multiplied by 100. These data were analyzed using Fischer's exact probability test by comparison to vehicle control values. For locomotor activity data, individual contrasts at each 30-min interval for each dose were compared to vehicle control values using Dunnett's test (two-tailed) after significant ANOVA. Means (± S.E.M) were also calculated across two 90-min intervals (0–90 min; 90–180 min) at each dose. Individual contrasts at each dose during these 90-min periods were compared against control values using Dunnett's test (two-tailed). The percentage of observations with scratching was obtained by dividing the total number of observations that contained at least one instance of scratching by the overall maximum number of observations in all four monkeys (12). ED₅₀ values with 95% CI were obtained according to the methods described by Litchfield and Wilcoxon (1949) with specific comparisons between treatments and vehicle control values using Fischer's exact probability test. For each analysis, error probabilities of more than .05 were considered to be nonsignificant.

**Results**

**Behavioral observations.** Vehicle-treated control mice displayed climbing (35% ± 0.83) and few occurrences of immobility and gnawing (0 and 9% ± 2, respectively) during the 65-min observation period (figs. 1–3). The nonselective dopamine agonist apomorphine (3–30 mg/kg) and the dopamine uptake inhibitor cocaine (0.3–30 mg/kg) increased climbing at all doses studied (fig. 1). Whereas apomorphine increased immobility at the lowest dose tested (3 mg/kg), cocaine had no effect on immobility. Gnawing was increased in a dose-dependent manner by apomorphine but was not affected by cocaine.

As illustrated in figure 2, climbing decreased after the highest dose tested of the partial D₂ agonist SKF 38393 (100 mg/kg). Climbing was increased at two doses of the full D₁ agonist SKF 82958 (3 and 30 mg/kg); this increase was not dose-dependent. Neither compound significantly altered immobility. Gnawing was not affected after SKF 38393 (10–100 mg/kg) but was increased by SKF 82958 at the highest dose tested (100 mg/kg).

PD 128,907 (0.3 and 3 mg/kg), 7-OH-DPAT (30 mg/kg) and quinpirole (3 mg/kg) increased the frequency of climbing at select doses (fig. 3); this increase was not dose dependent. 7-OH-DPAT also decreased climbing at the highest dose tested (100 mg/kg). Bromocriptine decreased climbing at one dose (10 mg/kg). Each agonist increased periods of immobility across most doses tested. Gnawing was not affected by these compounds at any dose.

Vehicle-treated control mice displayed nose poking (40%) and switching sides (25%) during the observation period. All of the dopamine agonists that were tested significantly decreased nose-poking and switching-sides during the session within the wire-mesh box; each compound decreasing the occurrence of these behaviors less than 10% at the highest doses tested (data not shown).

**Locomotor activity.** During the first 30 min of the locomotor activity session, vehicle-treated mice displayed moderate levels of activity (4000–8000 counts) that decreased over the remainder of the 180-min session (figs. 4 and 5, filled
circles). Locomotor activity after apomorphine (fig. 4; 3–30 mg/kg), SKF 38393 (fig. 4; 100 mg/kg), PD 128,907 (fig 5; 0.3–30 mg/kg), 7-OH-DPAT (fig 5; 1–30 mg/kg), quinpirole (fig 5; 0.01–100 mg/kg) and bromocriptine (fig. 5; 3 and 30 mg/kg) was decreased relative to control subjects during the first 30 min. These decreases in activity were not evident when data were averaged across the first 90 min of the session (figs. 4 and 5, insets). During the remainder of the session, activity after these compounds was similar to control.

Fig. 1. Effects of the nonselective dopamine agonist, apomorphine, and the dopamine uptake inhibitor, cocaine, on climbing, immobility and gnawing in mice (n = 6 per dose) within a wire-mesh box for 65 min. Data presented are the percentage of observations in which these behaviors were observed. Dashed lines represent data for vehicle-treated control subjects (± S.E.M.). Statistical comparisons with control values were determined using Fisher’s Exact Probability test (*P < .05).

Fig. 2. Effects of the partial D₁ agonist, SKF 38393, and the full D₁ agonist, SKF 82958, on climbing, immobility and gnawing. Other details as in figure 1.
levels at most of the 30-min time intervals. At higher doses of these compounds (with the exception of bromocriptine) activity levels increased during at least one of the latter 30-min time intervals. Increases in activity were evident during the latter half of the session following apomorphine (fig. 4, inset; 10 and 30 mg/kg), SKF 38393 (fig. 4, inset; 30 and 100 mg/kg), 7-OH-DPAT (fig. 5, inset; 10 mg/kg) and quinpirole (fig. 5, inset; 30 and 100 mg/kg).

In contrast, cocaine (fig. 4) increased activity (10 and 30 mg/kg) during the first half of the session, producing counts of more than 18,000 during the first 30 min (30 mg/kg) that gradually decreased to control levels (less than 2,000 counts) by the end of the session. This increase was evident during the first half of the session at 30 mg/kg (fig. 4, inset). Locomotor activity was similar to controls at each dose during the latter half of the session. SKF 82958 (fig. 4) increased activity (7,000–10,000 counts). This increase was evident at each dose during the first half of the session (fig. 4, inset; 1–100 mg/kg). Although activity never reached levels comparable to that of cocaine, increases lasted throughout the 180-min session (10–100 mg/kg).

Scratching. Cumulative doses of apomorphine (ED$_{50}$ = 0.10 μmol/kg; 95% CI = 0.03–0.23 μmol/kg), PD 128,907 (ED$_{50}$ = 0.10 μmol/kg; 95% CI = 0.07–0.24 μmol/kg), 7-OH-DPAT (ED$_{50}$ = 0.02 μmol/kg; 95% CI = 0.003–0.09 μmol/kg) and quinpirole (ED$_{50}$ = .12 μmol/kg; 95% CI = 0.03–0.39 μmol/kg) dose-dependently increased scratching in squirrel monkeys (fig. 6). The maximum frequency of scratching episodes after each of these compounds was approximately 80%. Neither SKF 38393, SKF 82958 nor cocaine significantly increased scratching. Within this cumulative dosing procedure, bromocriptine also did not produce scratching. However, bromocriptine did produce scratching when a single dose (0.3 mg/kg) was given at the onset of testing and scratching was recorded at 15-min intervals for a total of 90 min. At this dose, scratching episodes reached their highest levels (75%) during the 60- to 75-min interval.

**Discussion**

Our series of experiments examined the behavioral effects of several dopamine agonists that bind to D$_3$, D$_2$ and minimally to D4 receptors (PD 128,907, 7-OH-DPAT, quinpirole and bromocriptine). As summarized in table 1, during behavioral observations in mice, the D$_3$/D$_2$ agonists produced immobility that resulted in a decrease in locomotor activity over a wide dose range, had no effect on gnawing, and only inconsistently induced climbing. In squirrel monkeys, these D$_3$/D$_2$ agonists dose-dependently produced scratching. Although there was some overlap between the behaviors produced by the D$_3$/D$_2$ agonists and the other types of dopamine agonists tested, behavioral effects could be differentiated. For exam-
ple, whereas the nonselective agonist, apomorphine, increased gnawing, the D3/D2 agonists had no effect on gnawing across a wide range of doses that readily produced other behavioral effects. The D3/D2 agonists also produced a different spectrum of effects (immobility, locomotor activity decreases and scratching) from those produced by the indirect dopamine agonist, cocaine and the D1 agonists, SKF 38393 and SKF 82958 (scratching). Together these findings demonstrate that D3/D2 agonists produce behavioral effects that can be differentiated from those produced by nonselective dopaminergic agonists, indirect dopamine agonists and selective D1 agonists.

Many of the compounds (e.g., quinpirole) used to define D2-mediated behaviors in the past have now been shown also to bind to D3, D2 and D1 receptors. That these agonists, as well as the newly identified D3/D2 agonists, PD 128,907 and 7-OH-DPAT, quinpirole and bromocriptine decreased locomotor activity over a wide dose range within the first 30 min of the test session suggests that D3 receptors also may be involved. At present, it is not clear if D3 receptor involvement in this, or any other behavior, is due to stimulation of presynaptic D3 autoreceptors (Meller et al., 1993; Starr and Starr, 1995) or postsynaptic D3 receptors (Waters et al., 1993; Svensson et al., 1994).

Scratching in squirrel monkeys also has been characterized previously as a D2-mediated behavior (Rosenzweig-Lipson et al., 1994; Pellón et al., 1995). For example, Pellón et al. (1995) recently concluded that scratching in squirrel monkeys is D2-mediated given that several dopamine agonists that had been demonstrated previously to bind to D2 receptors (quinpirole, (-)-NPA, RU 24213, pergolide, quinelorane and piribedil) dose-dependently increased scratching in these subjects. However, quinelorane (Sokoloff et al., 1992) and pergolide (Sokoloff et al., 1990) have also been shown to bind to D3 receptors. Given that these compounds (Pellón et al., 1995), as well as PD 128,907, 7-OH-DPAT, quinpirole, bromocriptine and apomorphine, produced scratching suggests that scratching may involve D2 and/or D3 receptors. Interestingly the nonselective dopamine agonist, apomorphine,

Fig. 4. Effects of increasing doses of apomorphine, cocaine, SKF 38393 and SKF 82958 on horizontal locomotor activity in mice (n = 8 per data point). Injections (i.p.) were given immediately before testing and data were recorded at 30-min intervals for a total of 180 min. Activity at each 30-min interval and the mean (± S.E.M) of this activity over the first and second 90-min periods is also shown (inset). Individual contrasts at each 30-min interval were compared against saline control values (closed circles) using Dunnett’s test (two-tailed; *P < .05). Individual contrasts at each dose during the 90-min periods were compared against saline control values (0 mg/kg) using Dunnett’s test (two-tailed; *P < .05).
produced scratching when the indirect dopamine agonist cocaine did not. Differences in the behavioral effects produced by apomorphine versus cocaine have been reported previously (Tirelli and Witkin, 1994a, b; Pellón et al., 1995) and elsewhere in the present report on measures of immobility, gnawing (fig. 1) and locomotor activity (fig. 4). Stereotyped climbing and gnawing have been previously discussed in terms of the simultaneous stimulation of D1 and D2 receptors given that apomorphine or D1/D2 agonist combinations have been demonstrated to readily produce these behaviors (Arnt et al., 1987; Moore and Axton, 1988; Vasse et al., 1988; Murray and Waddington, 1989). In our experiment, climbing was increased after apomorphine and cocaine at each dose tested (fig. 1). Climbing was also increased at select doses of SKF 82958 (fig. 2), PD 128,907, 7-OH-DPAT and quinpirole (fig. 3); these increases, however, were not dose dependent. That climbing was observed after the D1 and D3/D2 agonists, suggests that the amount of stimulation re-

![Fig. 5. Effects of increasing doses of PD 128.907, 7-OH-DPAT, quinpirole and bromocriptine on horizontal locomotor activity in mice (n = 8 per data point). Other details as in figure 4.](image)

![Fig. 6. Effects of cumulative doses of dopamine agonists on scratching in squirrel monkeys (n = 4). Four successive injections were given incrementally and separated by an interval of 15 min. Data presented are the percentage of observations in which scratching was observed. The unconnected, inverted triangle above 0.3 mg/kg represents the percentage of scratching observations after this single dose of bromocriptine recorded during the 60- to 75-min interval of a 90-min session. Control data are presented to the left as unconnected points above “V.” Statistical comparisons with control values were determined using Fisher’s exact probability test (*P < .05).](image)

**TABLE 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Locomotion First 30 Min</th>
<th>Immobility</th>
<th>Gnawing</th>
<th>Scratching</th>
<th>Climbing</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD 128,907</td>
<td>–</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7-OH-DPAT</td>
<td>–</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Quinpirole</td>
<td>–</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Bromocriptine</td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Cocaine</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>SKF 38393</td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>SKF 82958</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
</tbody>
</table>

Significant increases (+), decreases (−) or no change (0) in behavior from control values after dopamine agonists.
quired at multiple dopamine receptor sites to produce this behavior may be less than that required to produce gnawing. Gnawing was dose-dependently produced by apomorphine and the highest dose tested of SKF 82958 (100 mg/kg; fig. 2), a full D1 agonist (O’Boyle et al., 1989; Anderson and Jansen, 1990). Interestingly, SKF 38393, a partial D1 agonist that binds with greater selectivity for D1 vs. D2 receptors than SKF 82958 (O’Boyle et al., 1989; Anderson and Jansen, 1990), did not produce gnawing at any dose tested (1–100 mg/kg). Overall, these findings support past research indicating that the production of climbing and gnawing require the simultaneous stimulation of multiple dopamine receptor sites. At this point, the extent that D2 receptors may be contributing to the production of these behaviors is unclear.

A great deal of interest has been taken in D3 receptors given their abundance in mesolimbic dopaminergic regions (Sokoloff et al., 1990), an area that has been associated with emotionality, the efficacy of antipsychotic drugs and psychomotor stimulant abuse. For this reason, it has been suggested that D3 receptors may be involved in schizophrenia or cocaine abuse, and may serve as targets for drug development in these areas. Recent reports have found relationships between the D2/D3 agonists PD 128,907 and 7-OH-DPAT and the behavioral effects produced by cocaine (Acri et al., 1995; Spealman, 1996). For example, Acri et al. (1995) reported that PD 128,907 and 7-OH-DPAT fully substituted for the discriminative stimulus effects of cocaine in rats suggesting that D3 agonists may serve as a substitution-type for cocaine abusers. However, as reported by Acri et al. (1995) there were also differences in the behavioral effects produced by cocaine and these D2/D3 agonists. The response rate reductions observed with PD 128,907 and 7-OH-DPAT (Acri et al., 1995) were unlike those after cocaine and other compounds that fully substitute for cocaine (Spealman et al., 1991; Baker et al., 1993; Witkin, 1994). Differences in overall activity levels produced by D2/D3 agonists vs. cocaine were also found in our study (fig. 1 vs. 3 and fig. 4 vs. 5). These side effects may mitigate against the therapeutic use of the present drugs. None-the-less, 7-OH-DPAT and quinpirole have been reported to reduce cocaine self-administration in rats at doses that alone were not reinforcing (Caine and Koob, 1993; 1995) further suggesting that D3 receptor agonists may have potential as treatments for cocaine abuse.

The therapeutic potential of D2/D3 receptor ligands may extend beyond that of the treatment of cocaine abuse. As demonstrated by Rodriguez de Fonseca et al. (1995), 7-OH-DPAT can modulate the acquisition and expression of morphine-induced place-preference in rats. Together these findings suggest a potential use of D3 agonists in the treatment of drug abuse, and possibly, other psychiatric and neurological disorders that have been associated with disruptions in dopamine neurotransmission. However, the development of additional D3 ligands of better selectivity (Sokoloff et al., 1990; Rivet et al., 1994; Wright et al., 1995) is essential to exploring this hypothesis further.

The results of our study have shown that D2/D3 agonists produce behavioral effects that can be distinguished from nonselective dopamine receptor agonists, from D1 agonists and from dopamine uptake inhibitors. Furthermore, behaviors once attributed to D2 receptors alone (decreased locomotor activity and scratching) or attributed to the simultaneous stimulation of D1 and D2 receptors (climbing and gnawing) may also involve D3 receptors. This study complements the work of others who have suggested that D3 receptors may play a role in the control of certain behaviors, including yawning (Longoni et al., 1987; Danysz et al., 1993; Bristow et al., 1996), chewing (Daly and Waddington, 1993), sniffing (McElroy et al., 1993), hypothermia (Ahlenius and Salmi, 1994; Millan et al., 1994) and reductions in locomotor activity (Daly and Waddington, 1993; Svensson et al., 1994; Pugsley et al., 1995; Starr and Starr, 1995; Bristow et al., 1996; Depoortere et al., 1996).

Although these findings suggest that D3 receptors play a role in the control of behavior, the identification of agonists and antagonists that more selectively target D3 receptors and other subpopulations of dopamine receptors (e.g., D2a, D2b, D3) is essential to obtaining a more precise understanding of the role these subtypes are playing in drug abuse and other neuropsychiatric disorders, as well as in nonpharmacological states.

Acknowledgments

The authors thank Drs. Nancy Ator, Doreen Grech, Elise Weerts and Troy Zarcone for their helpful comments on an earlier version of this manuscript. We also thank Julie Haak for her expert technical assistance.

References


MURRAY, A. M. AND WADINGTON, J. L.: The induction of grooming and vacuous
behavioural responses to SKF 83859 in relation to “D1-like” dopamine
receptors not linked to adenylyl cyclase. Br. J. Pharmacol. 116: 2120–2126,
1997.

ELAM, D. AND SIECHTMAN, H.: Biphasic effects of the D2 agonist quinpirole on

FREEDMAN, S. B., PATEL, S., MARWOOD, R., EMMS, F., SEABROOK, G. R., KNOWLES,
M. E., MCALLISTER, G.: Expression and pharmacological characterization
of the human D2 dopamine receptor. J. Pharm. Exp. Ther. 268: 417–426,
1994.

FRIEND, D. AND WITKIN, J. M.: Effects of the dopamine release inhibitor, CGS
10746B, on the locomotor stimulant and discriminative stimulus effects
of cocaine and methamphetamine. Pharmaco Behavior. 48: 989–993,
1993.

JACKSON, D. M. AND WESTLAND-DANIELSSON, A.: Dopamine receptors: Molecular
biology, biochemistry and behavioural aspects. Pharmacol. Ther. 64: 291–369,
1994.

JOHANSON, C.-E. AND FISCHMAN, M. W.: The pharmacology of cocaine related to


KELLEY, A. E. AND LANG, C. G.: Neurochemical and functional characterization of

KUHARI, M. J., RITZ, M. C. AND BOAM, J. W.: The dopamine hypothesis of the

LITCHFIELD, J. T. AND WILCOXON, F.: A simplified method of evaluating dose-

LONGONI, R., SPINA, L. AND DI CHIARA, G.: Permissive role of D-1 receptor
stimulation by endogenous dopamine for the expression of postynaptic

MALMBERG, A. AND MORELL, N.: Characterization of [3H]quipirosine binding to
human dopamine D1A and D2 receptors: Effects of ions and guanine nucleotides.

MARTIN, G. E. AND BENDREK, R. J.: Mouse locomotor activity: an in vivo test for
dopamine autoreceptor activation. J. Pharmacol. Exp. Ther. 229: 706–711,
1984.

direct-acting dopamine agonist, (+)-(4-propyl-9-hydroxyxanthone ((+)-

A. L.: In vivo agonist properties of 7-OH-ADPAT, a dopamine D1

MELLER, E., BÖRMADERE, K., GÖLZSTEIN, M. AND BASHAM, D. A.: Evidence that
striatal synthesis-inhibiting autoreceptors are dopamine D2 receptors. Eur.

MELLER, E., BOHMAKER, K., GÖLDSTEIN, M. AND BASHAM, D. A.: Evidence that
the selective dopamine D3 agonist (+)-4-propyl-9-hydroxyxanthone by

direct-acting dopamine agonist, (+)-(4-propyl-9-hydroxyxanthone ((+)-

A. L.: In vivo agonist properties of 7-OH-ADPAT, a dopamine D1

MELLER, E., BÖRMADERE, K., GÖLZSTEIN, M. AND BASHAM, D. A.: Evidence that
striatal synthesis-inhibiting autoreceptors are dopamine D2 receptors. Eur.

MILLAN, M. J., AUDINOTTI, V. A., RIVET, J.-M., GIBERT, A., VAN, J., PROST, J.-F.,
SPEEDING, M. AND PEGGIO, J.-L. S. 14297, a novel selective ligand at cloned

MOSS, N. A. AND ASTON, M. S.: Production of climbing behaviour in mice
requires both D1 and D2 dopamine receptor activation. Psychopharmacology 94:

MURRAY, A. M. AND WADINGTON, J. L.: The induction of grooming and vacuous
chewing by a series of selective D1 dopamine receptor agonists: Two direc-


PERRON, J.-P., BOREY, J.-F., VONZON, A., POULIVETEY, C., BOYER, G., AND
PRÉVÉOL, C.: Expression of the dopamine D2 receptor in the rat striatum using

PUGLISETTI AND CALENTI, S., CARLETI, L. AND CABEL, S. E. L.: 171555-induced
catalepsy and defensive behavior in four strains of mice suggest the involvement
of different dopaminergic pathways in the CNS. Pharmacol. Biochem. Behav.

PUGLISETTI AND CALENTI, S., CARLETI, L. AND CABEL, S. E. L.: 171555-induced
catalepsy and defensive behavior in four strains of mice suggest the involvement
of different dopaminergic pathways in the CNS. Pharmacol. Biochem. Behav.

PUGLISETTI AND CALENTI, S., CARLETI, L. AND CABEL, S. E. L.: 171555-induced
catalepsy and defensive behavior in four strains of mice suggest the involvement
of different dopaminergic pathways in the CNS. Pharmacol. Biochem. Behav.

PUGLISETTI AND CALENTI, S., CARLETI, L. AND CABEL, S. E. L.: 171555-induced
catalepsy and defensive behavior in four strains of mice suggest the involvement
of different dopaminergic pathways in the CNS. Pharmacol. Biochem. Behav.