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Antidepressant-Like Effect of $D_{2/3}$ Receptor-, but not D_4 Receptor-Activation in the Rat Forced Swim Test

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Dopamine plays a role in the pathophysiology of depression and therapeutic effects of antidepressants but the contribution of individual D_2-like receptor subtypes (D_2, D_3, D_4) to depression is not known. We present evidence that activation of D_2/D_3, but not D_4 receptors, can affect the outcome in the rat forced swim test (FST). Nomifensine, a dopamine uptake inhibitor (7, 14, and 28 μmol/kg); quinpirole, a D_2-like receptor and agonist (0.4, 1.0, and 2.0 μmol/kg); PD 12,8907, a preferential D_3 receptor agonist (0.17, 0.35, and 0.7 μmol/kg); PD 168077 (0.1, 0.3, and 1.0 μmol/kg) and CP 226269 (0.3, 1.0, and 3.0 μmol/kg), both selective D_4 receptor agonists, were administered s.c. 24, 5, and 0.5/1 h before testing. Nomifensine, quinpirole at all doses and PD 128907 at the highest dose decreased immobility time in FST. PD 168077 and CP 226269 had no effect on the model. To further clarify what type of dopamine receptors were involved in the anti-immobility effect of quinpirole, we tested different antagonists. Haloperidol, a D_2-like receptor antagonist (0.27 μmol/kg), completely blocked the effect of quinpirole; A-437203 (LU-201640), a selective D_3 receptor antagonist (17.46 μmol/kg), showed a nonsignificant trend to attenuate the effect of the low dose of quinpirole, and L-745,870, a selective D_4 receptor antagonist (1.15 μmol/kg), had no effect. The pharmacological selectivity of the compounds tested suggests that the antidepressant-like effects of quinpirole are most likely mediated mainly by D_2 and to a lesser extent by D_3 but not D_4 receptors.
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INTRODUCTION

Depression has been associated with the dysfunction of neurotransmitter systems, mainly norepinephrine and serotonin. Dopamine is also proposed to play an important role in the pathophysiology of depression as well as in the mechanism of action of antidepressant drugs (Willner, 1983a, b; Borsini *et al*, 1985a, b; Pulvirenti and Samanin, 1986; Fibiger, 1995; Charney, 1998; Naranjo *et al*, 2001; Klimek *et al*, 2002; Pania and Gessab, 2002). Mesolimbic dopamine pathways are involved in the control of motivation and reward-related behaviors (Koob, 1996; Robbins and Everitt, 1996; Schultz, 1997; Berridge and Robinson, 1998) and hypofunction of the dopamine system is implicated in the loss of motivation and/or anhedonia described as core symptoms in human depressive states (Nelson and Charney, 1981). Administration of dopamine receptor antagonists, or drugs that reduce dopamine levels such as reserpine, induces dysphoria and many symptoms resembling those of endogenous depression (Wise *et al*, 1978; Willner, 1983a). Conversely, dopamine receptor agonists, as well as drugs that increase dopamine function, have antidepressant-like profiles in animal models of depression (Muscat *et al*, 1992) and have been reported to have efficacy in the treatment of human depression (Forrest *et al*, 1977; Bouras and Bridges, 1982; Lopez-Ibor Alino *et al*, 1982; Kinney, 1985). Moreover, long-term administration of antidepressant drugs or repeated administration of electroconvulsive shock (ECS) increases behavioral response to dopaminergic agonists and dopaminergic neurotransmission in the limbic system (Spyraki and Fibiger, 1981; Willner and Montgomery, 1981; Maj *et al*, 1984a, b, 1998b; Serra *et al*, 1992; Ainsworth *et al*, 1998a).

Dopamine receptors can be classified into two families: the D_1-like receptors (D_1 and D_5) and the D_2-like receptors (D_2, D_3, and D_4 receptor subtypes). Previous studies have reported the role of dopamine in the mechanism of action of antidepressants, focusing on behavioral responses to dopamine agonists after chronic antidepressant treatment or on how selective dopamine receptor antagonists can

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affect the ability of antidepressants to elicit their behavioral response (Willner and Montgomery, 1981; Maj *et al*, 1984b, 1989; Serra *et al*, 1990; Gambarana *et al*, 1995; D'Aquila *et al*, 2000a). For example, dopamine agonists have shown efficacy in models of behavioral despair and chronic mild stress (Porsolt *et al*, 1979; Duterte-Boucher *et al*, 1988; Muscat *et al*, 1992). Most of the studies have assigned a critical role to D_2-like receptors, as compared to D_1-like receptors (Spyraki and Fibiger, 1981; Borsini *et al*, 1988; Maj *et al*, 1989, 1996a; Nunes Junior *et al*, 1994; Ainsworth *et al*, 1998b; Rogoz and Dziedzicka-Wasylewska, 1999); however, little is known about the involvement of the individual dopamine receptor subtypes within the D_2-like family in preclinical models of depression such as the forced swim test (FST). The involvement of the D_3 receptor in antidepressant activity has been proposed previously based on the preclinical and clinical effects of compounds that activate the D_3 receptor as well as a change in the D_3 receptor functioning following chronic antidepressant treatments (Sokoloff *et al*, 1992; Levant *et al*, 1993; Willner *et al*, 1994; Maj *et al*, 1997, 1998a; Corrigan *et al*, 2000). Interestingly, although the D_4 receptor has been proposed as a potential target for schizophrenia treatment, its relevance in other psychiatric disorders such as depression has yet to be investigated.

The rat FST is one of the most widely used animal models for assessing antidepressant-like activity (Porsolt *et al*, 1978; Porsolt *et al*, 1979). This model is based on the principle that an animal exposed to an inescapable stressor will show altered behavior in response to subsequent stressors. Rats are placed in cylinders filled with water. After initial vigorous attempts to struggle and escape, they develop an immobile posture, which has been called behavioral despair and has been hypothesized to reflect lowered mood or hopelessness. Upon subsequent exposure, rats become immobile faster and for a longer period of time than naive animals, suggestive of a 'depressive-like state'. FST is sensitive to a broad variety of antidepressant treatments, and they significantly reduce the immobility period (Porsolt *et al*, 1979). Furthermore, Detke *et al* (1995) has proposed that the rats can display various behavioral patterns in this model, differentially related to different classes of antidepressant drugs.

The goal of this study was to investigate the involvement of each of the D_2-like receptors subtypes (D_2, D_3, and D_4) in an animal model of depression considered to have good predictive value for detecting antidepressant activity, FST, using selective dopamine receptor agonists and/or antagonists. We compared the effects of nomifensine, a dopamine uptake inhibitor (Meiergerd and Schenk, 1994); quinpirole, a D_2-like receptor agonist ($K_i = 38.4$, 4.5, and 91.0 nM for D_2, D_3, and D_4 receptors, respectively) (Moreland *et al*, 2004); PD 128907, a preferential D_3 receptor agonist ($K_i = 15.1$, 1.1, and 1040.0 nM for D_2, D_3, and D_4 receptors, respectively) (Moreland *et al*, 2004); PD 168077 ($K_i =$ 1050.0, 2540.0, and 11.9 nM for D_2, D_3, and D_4 receptors, respectively) and CP 226269 ($K_i = 120.0$, 521.0, and 0.8 nM for D_2, D_3, and D_4 receptors, respectively), both selective D_4 receptor agonists (Moreland *et al*, 2004). To further clarify the role of specific dopamine receptors in the anti-immobility effect of quinpirole, we assessed the ability of haloperidol, a nonselective D_2-like receptor antagonist ($K_i = 0.3$, 3.6 and 1.9 nM for D_2, D_3, and D_4 receptors, respectively) (Moreland *et al*, 2004); A-437203 (LU-201640), a D_3 receptor antagonist ($K_i = 71.0$, 1.6, and 6220.0 nM for D_2, D_3, and D_4 receptors, respectively) (Unger *et al*, 2002; Chaperon *et al*, 2003); and L-745,870, a highly selective D_4 receptor antagonist ($K_i = > 10\,000$, $> 10\,000$ and 0.4 nM for D_2, D_3, and D_4 receptors, respectively) (Moreland *et al*, 2004; Patel *et al*, 1997) to attenuate the quinpirole-induced antidepressant-like response. Results support a major role for dopamine D_2 receptors, a moderate role for D_3 receptors, and no involvement of D_4 dopamine receptors in antidepressant-like effects in the rat FST.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (Charles River Laboratories, Inc.) weighing 250–350 g were used for these experiments. Animals were allowed to habituate to our facilities at least 1 week prior the start of the experiments. Rats were group housed (five per cage) and maintained on a 12 : 12-h light–dark schedule (lights on 0600, lights off 1800), in a temperature- and humidity-controlled environment ($22 \pm 1^{\circ}$C, 60–70% humidity). Animals had free access to food and water. All studies were approved by the Abbott Laboratories Institutional Animal Care and Use Committee, according to the guidelines of the Association for the Assessment and Accreditation of Laboratory Animals Care.

Drugs

(−)-Quinpirole hydrochloride (Sigma, St Louis, MO), PD 168077, and CP 226269 (both synthesized at Abbott Laboratories) were dissolved in 0.1% ascorbic acid in saline solution (0.9%). Nomifensine maleate salt (Sigma, St Louis, MO) was dissolved in 10% DMSO and 90% hydroxypropyl β-cyclodextrin. Haloperidol (Sigma, St Louis, MO) was dissolved in acetic acid and water, and pH was adjusted to 5.0–5.5 with NaOH. A-437203 (LU-201640), a D_3 receptor antagonist synthesized at Abbott Laboratories, was dissolved in NaOH and sterile water and pH was adjusted to 10.5 with 1 N HCl. Imipramine hydrochloride (Sigma, St Louis, MO), L-745,870 trihydrochloride, and PD 128907 (both from Tocris, Ellisville, MO) were dissolved in sterile water.

Forced Swim Test

The procedure was based on the behavioral test described by Porsolt *et al* (1978). A single experiment consisted of a preswim and a test swim. Naive rats were individually placed inside vertical cylinders (height: 40 cm, diameter: 30 cm) containing 25 cm of water at 23–25°C for 15 min. Following this preswim, animals were removed and allowed to dry in a heated enclosure before returning to their home cages. After 24 h, the test swim occurred in which the rats were replaced in the cylinder for 5 min and the total duration of immobility and escape behaviors was measured. Test swims were videotaped and subsequently assessed for the following behaviors: immobility (the animal remains floating passively in the water without struggling and shows

the minimal movements necessary to keep its head above water), climbing (very vigorous, active movements with animal's forepaws breaking the water surface usually against the walls of the water container), and swimming (described as active movements more than those necessary to keep the head of the rat above the water, and mainly distinguished as propelling the rat around the cylinder). Total time spent engaged in each activity was recorded. Both swimming sessions were performed during the afternoon between 1200 and 1600 h. Animals were used only once in a single experiment including preswim and test swim.

Locomotor Activity

Animals were habituated to the testing room under dim lighting conditions for 1.5 h before drug administration and testing. Locomotor activity was measured by individually placing naive rats into automated activity chambers (Versamax from Accuscan, Columbus, OH), measuring 42 cm × 30 cm × 42 cm (length × height × depth) immediately following drug application. Activity was detected by infrared photo beam sensors placed at 4.5 cm and 15.5 cm above the cage floor. Data were expressed as total distance moved during the 90-min testing period. Locomotor activity experiments were performed between 1000 and 1600 h.

Experimental Procedure

Forced swim test. For most of the experiments, a reference compound, imipramine 95 μmol/kg (30 mg/kg) was tested. Imipramine was administered i.p. 24, 5, and 1 h before the test swim. In all the experiments, the first administration of the compound/s took place 15 min after the 15-min preswim test. Nomifensine (7, 14, and 28 μmol/kg = 2.5, 5, and 10 mg/kg) was administered i.p. 24, 5, and 1 h before the test swim. Quinpirole (0.4, 1.0, and 2.0 μmol/kg = 0.1, 0.25, and 0.5 mg/kg), PD 128907 (0.17, 0.35, and 0.7 μmol/kg = 0.05, 0.1, and 0.2 mg/kg), PD 168077 (0.1, 0.3, and 1.0 μmol/kg = 0.045, 0.135, and 0.45 mg/kg), and CP 226269 (0.3, 1.0, and 3.0 μmol/kg = 0.13, 0.43, and 1.28 mg/kg) were administered s.c. 24, 5, and 0.5 h before the test swim. Haloperidol (0.27, 1.33, and 2.66 μmol/kg = 0.1, 0.5, and 1.0 mg/kg i.p.), A-437203 (LU-201640) (0.52, 1.75, 5.24, and 17.46 μmol/kg = 0.3, 1.0, 3.0, and 10.0 mg/kg i.p.), and L-745,870 (0.23, 1.15, 2.3, and 5.7 μmol/kg = 0.1, 0.5, 1.0, and 2.5 mg/kg i.p.) were tested initially alone in order to determine effective dose ranges. In those experiments, haloperidol, A-437203, and L-745,870 were administered i.p. 24, 5, and 0.5 h before the test swim. In the subsequent antagonism experiments, haloperidol (0.27 μmol/kg), A-437203 (17.46 μmol/kg) or L-745,870 (1.15 μmol/kg) were injected i.p. 15 min prior to each quinpirole injection (0.4 and 1.0 μmol/kg s.c.).

Locomotor activity. To differentiate the anti-immobility effect of quinpirole from general locomotor activation, locomotor activity was evaluated in rats treated acutely with quinpirole 0.4, 1.0, and 2.0 μmol/kg, as well as following a subchronic quinpirole treatment according to the same protocol treatment used for rat FST (three injections, 0.4 and 1.0 μmol/kg).

Statistical Analyses

Experimental design for FST experiments were between-subjects, where each animal was observed for three different behaviors: immobility, climbing, and swimming. The number of rats in each experimental group was $n = 10$, except for quinpirole ($n = 8$–10), PD 168077 ($n = 8$–9), haloperidol ($n = 9$), and the combined treatment haloperidol + quinpirole experiment ($n = 15$). Data for the FST experiments testing dopamine agonists or the dopamine uptake inhibitor were analyzed using a one-way ANOVA for treatment groups including in the analysis the data for the reference compound imipramine. When the ANOVA revealed a significant effect of treatment, *post hoc* analysis for individual group comparisons were followed (Fisher's protected least significant difference test). Those experiments using an antagonist to block the effect of quinpirole were analyzed using a two-way ANOVA where the main factors under consideration were antagonist (vehicle or antagonist) and quinpirole (different doses tested). When a statistically significant antagonist × quinpirole interaction was revealed, individual group comparisons followed the overall ANOVA (Fisher's protected least significant difference test). When no significant antagonist × quinpirole interaction was achieved but significant main effects were observed, the main effect of the independent variables were analyzed using a *post hoc* Fisher's protected least significant difference test, for the collapsed data over the levels of all other factors in the design.

For the locomotor activity study, data for distance moved were analyzed using a one-way ANOVA for treatment, followed by a *post hoc* analysis (Fisher's protected least significant difference test).

Data are expressed as the mean ± SEM. For all tests, significance was defined as $p < 0.05$.

RESULTS

Effect of Nomifensine, a Selective Dopamine Uptake Inhibitor, on Rat FST

ANOVA revealed a significant treatment effect for immobility time ($F_{4,45} = 35.403$, $p < 0.001$), climbing time ($F_{4,45} = 28.735$, $p < 0.001$), and swimming time ($F_{4,45} = 9.711$, $p < 0.001$). *Post hoc* mean contrast indicated a significant decrease in immobility time and a significant increase in climbing behavior for the three doses of nomifensine and for the reference compound imipramine compared to vehicle control group ($p < 0.001$; Figure 1). *Post hoc* analysis for swimming time showed that nomifensine 7 and 14 μmol/kg increased swimming time ($p < 0.05$ and $p < 0.001$, respectively), while the higher dose of nomifensine (28 μmol/kg) interestingly did not (Figure 1). It is important to mention that nomifensine did not increase locomotor activity at the dose of 7 μmol/kg or lower. However, nomifensine at 14 and 28 μmol/kg induced a significant increase in locomotor activity (data not shown).

Effect of Quinpirole, a D_2-Like Receptor Agonist, on Rat FST

A one-way ANOVA indicated a significant treatment effect for immobility time ($F_{4,43} = 24.986$, $p < 0.001$), and a significant treatment effect for climbing behavior

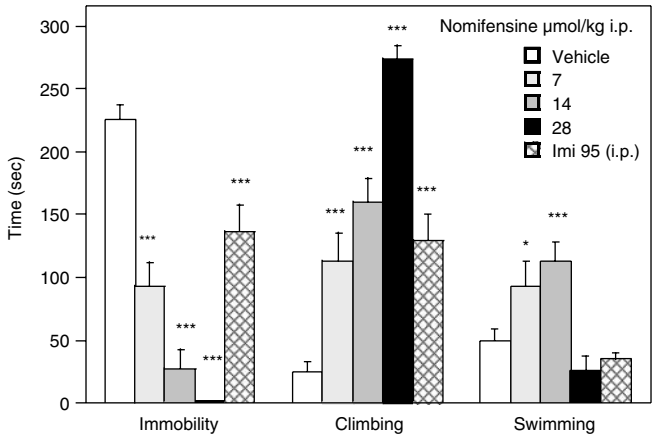

Figure 1 Effect of nomifensine, a selective dopamine uptake inhibitor on rat FST. Graph represents mean ± SEM for immobility, climbing, and swimming time during the 5-min test swim. Nomifensine (7, 14, and 28 µmol/kg i.p.) was administered 24, 5, and 1 h before testing ($n = 10$/group). $^*p < 0.05$ and $^{***}p < 0.001$ compared to vehicle-treated rats (Fisher *post hoc* test). Imi = imipramine.

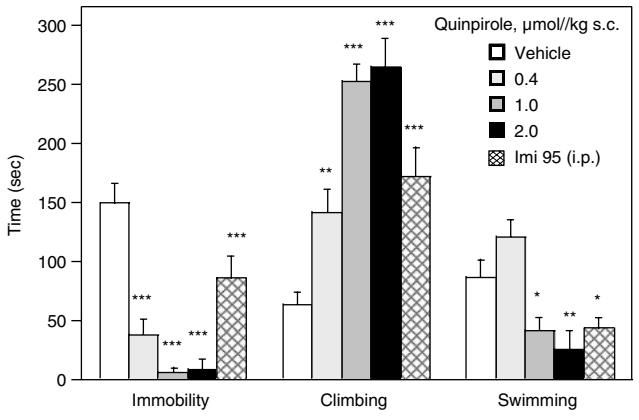

Figure 2 Effect of quinpirole, a D_2-like agonist, on rat FST. Graph represents mean ± SEM for immobility, climbing, and swimming time during the 5-min test swim. Quinpirole (0.4, 1.0, and 2.0 µmol/kg s.c.) was administered 24, 5, and 0.5 h before testing ($n = 8$–10/group). $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to vehicle-treated rats (Fisher *post hoc* test).

($F_{4,43} = 22.567$, $p < 0.001$; Figure 2). *Post hoc* mean comparisons revealed a significant decrease in immobility time at all doses of quinpirole and for the reference compound imipramine compared to the vehicle group ($p < 0.001$), and a significant increase in climbing behavior compared to control rats ($p < 0.01$ for quinpirole 0.4 µmol/kg and $p < 0.001$ for quinpirole 1.0 and 2.0 µmol/kg and imipramine). ANOVA also indicated a significant treatment effect for swimming time ($F_{4,43} = 9.171$, $p < 0.001$), while mean comparisons showed a significant decrease in swimming time for quinpirole 1.0 µmol/kg ($p < 0.05$), 2.0 µmol/kg ($p < 0.01$), and for imipramine ($p < 0.05$).

Effect of PD 128907, a Preferential D_3 Receptor Agonist, on Rat FST

ANOVA revealed a significant treatment effect for immobility time ($F_{4,45} = 7.692$, $p < 0.001$), a significant treatment effect in climbing ($F_{4,45} = 8.928$, $p < 0.001$), but no significant effect in swimming ($F_{4,45} = 2.339$, $p = 0.07$; Figure 3a). *Post hoc* mean comparisons revealed a significant decrease in immobility time and a significant increase in climbing for the highest dose of PD 128907 (0.7 µmol/kg, $p < 0.001$), as well as a significant effect of imipramine compared to vehicle group in both parameters ($p < 0.01$ for immobility and $p < 0.001$ for climbing). PD 128907 was tested in locomotor activity assay at the doses of 0.0425, 0.17, 0.7, 2.8, and 5.6 µmol/kg (0.0125, 0.05, 0.2, 0.8, and 1.6 mg/kg). All the doses of PD 128907 up to 2.8 µmol/kg (0.8 mg/kg) significantly reduced locomotor activity. PD 128907 5.6 µmol/kg (1.6 mg/kg) produced a nonsignificant trend towards increasing locomotor activity (data not shown), allowing separation between the antidepressant-like effects in FST from changes in spontaneous activity.

Effect of PD 168077 and CP 226269, Two Selective D_4 Receptor Agonists, on Rat FST

A one-way ANOVA for PD 168077 data revealed a significant treatment effect for immobility time ($F_{4,37} = 3.829$, $p < 0.05$), a significant treatment effect in climbing ($F_{4,37} = 16.146$, $p < 0.001$), but no significant effect in swimming (Figure 3b). *Post hoc* mean comparisons revealed a significant decrease in immobility time and a significant increase in climbing only for the reference compound imipramine compared to vehicle group ($p < 0.001$). PD 168077 failed to induce a significant effect in the FST at any of the doses tested.

A one-way ANOVA for CP 226269 data showed a significant treatment effect for immobility time ($F_{4,45} = 4.727$, $p < 0.01$), a significant treatment effect in climbing ($F_{4,45} = 19.543$, $p < 0.001$), and a significant effect in swimming ($F_{4,45} = 5.104$, $p < 0.01$, Figure 3c). *Post hoc* mean comparisons revealed only a significant decrease in immobility and swimming time ($p < 0.001$ and $p < 0.05$, respectively), and a significant increase in climbing ($p < 0.001$) for the reference compound imipramine compared to vehicle group. CP 226269 did not elicit any significant change in the FST at any of the doses tested (Figure 3c).

Effect of Haloperidol, a D_2-Like Receptor Antagonist, on Rat FST

No significant treatment effect was observed in any of the behaviors analyzed in the model at any of the tested doses of haloperidol ($F_{3,31} = 1.98$, $p = 0.137$ for immobility, $F_{3,31} = 1.059$, $p = 0.381$ for climbing, and $F_{3,31} = 0.969$, $p = 0.42$ for swimming; Table 1), although the highest dose of haloperidol (2.66 µmol/kg) showed a trend towards an increase in immobility time (Table 1). Considering that haloperidol can suppress spontaneous locomotor activity at doses higher than 0.27 µmol/kg (internal data), the dose of haloperidol 0.27 µmol/kg (0.1 mg/kg) was selected for subsequent studies.

Effect of Haloperidol on the Effect of Quinpirole on Rat FST

Figure 4 shows the effects of the pretreatment with a D_2-like receptor antagonist, haloperidol (0.27 µmol/kg), on the effects induced by quinpirole in rat FST. Two-way ANOVA

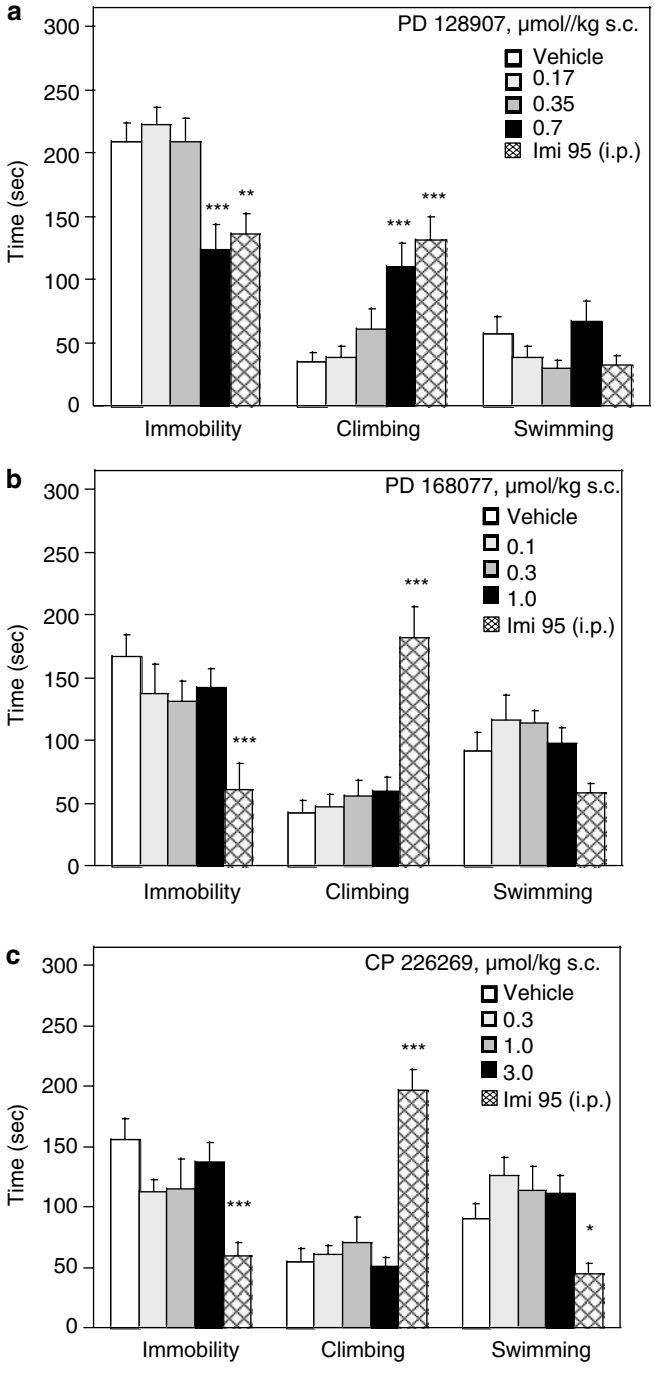

Figure 3 (a) Effect of PD 128907, a preferential D_3 receptor agonist, on rat FST. PD 128907 (0.17, 0.35, and 0.7 µmol/kg s.c.) was administered 24, 5, and 0.5 h before testing ($n = 10$/group). (b) Effect of PD 168077, a selective D_4 agonist, on rat FST. PD 168077 (0.1, 0.3, and 1.0 µmol/kg s.c.) was administered 24, 5, and 0.5 h before testing ($n = 8$–9/group). (c) Effect of CP 226269, a selective D_4 agonist, on rat FST. CP 226269 (0.3, 1.0, and 3.0 µmol/kg s.c.) was administered 24, 5, and 0.5 h before testing ($n = 10$/group). Graph represents mean ± SEM for immobility, climbing, and swimming time during the 5-min test swim. $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to vehicle-treated rats (Fisher *post hoc* test).

for immobility time indicated a significant antagonist treatment effect ($F_{1,84} = 129.19$, $p < 0.001$), a significant quinpirole effect ($F_{2,84} = 17.50$, $p < 0.001$), and a significant antagonist × quinpirole interaction ($F_{2,84} = 18.99$, $p < 0.001$). *Post hoc* analysis showed a significant reduction in immobility time of vehicle/quinpirole (0.4 and 1.0 µmol/kg) compared to vehicle/vehicle group ($p < 0.001$). Haloperidol/vehicle-treated animals did not statistically differ from the control group (vehicle/vehicle) in immobility time; however, haloperidol pretreatment antagonized the effect on immobility induced by the two doses of quinpirole ($p < 0.001$; Figure 4). Analysis of climbing behavior showed a significant antagonist treatment effect ($F_{1,84} = 105.64$, $p < 0.001$), a significant quinpirole effect ($F_{2,84} = 39.82$, $p < 0.001$), and a significant antagonist × quinpirole interaction ($F_{2,84} = 35.77$, $p < 0.001$). *Post hoc* analysis revealed that the vehicle/quinpirole group (0.4 and 1.0 µmol/kg) showed a significant increase in climbing behavior compared to the vehicle/vehicle group ($p < 0.01$ and $p < 0.001$, respectively) and these effects were blocked by pretreatment with haloperidol ($p < 0.001$). The haloperidol/vehicle group (0.27 µmol/kg) did not show any statistically significant difference compared to the vehicle/vehicle control group in climbing behavior. Analysis of swimming behavior indicated a significant antagonist treatment effect ($F_{1,84} = 17.16$, $p < 0.001$), a significant quinpirole effect ($F_{2,84} = 10.48$, $p < 0.001$), and a significant antagonist × quinpirole interaction ($F_{2,84} = 9.68$, $p < 0.001$). *Post hoc* analysis revealed that the vehicle/quinpirole group (0.4 µmol/kg dose only) showed a significant increase in swimming behavior compared to the vehicle/vehicle group ($p < 0.001$) and the effects observed at this lower dose were blocked by pretreatment with haloperidol ($p < 0.001$).

Table 1 Effect of Selective Dopamine Antagonists on Rat FST

Drug (µmol/kg)	Immobility (s)	Climbing (s)	Swimming (s)
Haloperidol (n = 9)			
0	177.33 ± 15.82	64.33 ± 12.08	58.33 ± 6.28
0.27	**190.13 ± 10.52**	**58.125 ± 8.27**	**51.75 ± 8.37**
1.33	177.89 ± 19.44	70.89 ± 10.69	51.22 ± 17.17
2.66	228.11 ± 17.04	40.22 ± 16.09	31.67 ± 9.28
A-437203 (n = 10)			
0	207.6 ± 13.70	49.70 ± 10.98	42.70 ± 9.54
0.52	234.30 ± 12.13	42.70 ± 10.36	23.00 ± 3.57
1.75	235.33 ± 17.36	35.44 ± 8.20	31.50 ± 9.78
5.24	189.50 ± 17.49	53.50 ± 12.12	57.00 ± 15.48
17.46	**200.80 ± 20.57**	**44.00 ± 7.52**	**55.20 ± 15.07**
L-745,870 (n = 10)			
0	153.56 ± 20.63	65 ± 15.50	81.44 ± 8.67
0.23	179.3 ± 9.29	55.1 ± 10.70	65.6 ± 8.01
1.15	**164.78 ± 17.74**	**54.11 ± 8.11**	**81.11 ± 19.46**
2.3	204.2 ± 23.95	34.1 ± 8.27	76.3 ± 19.84
5.7	210.2 ± 14.41	38.2 ± 6.66	51.6 ± 10.25

Doses highlighted are those selected for further studies.

Effect of A-437203, a Selective D_3 Receptor Antagonist, on Rat FST

A-437203, a selective D_3 receptor antagonist, was initially tested alone in rat FST and results are expressed in Table 1.

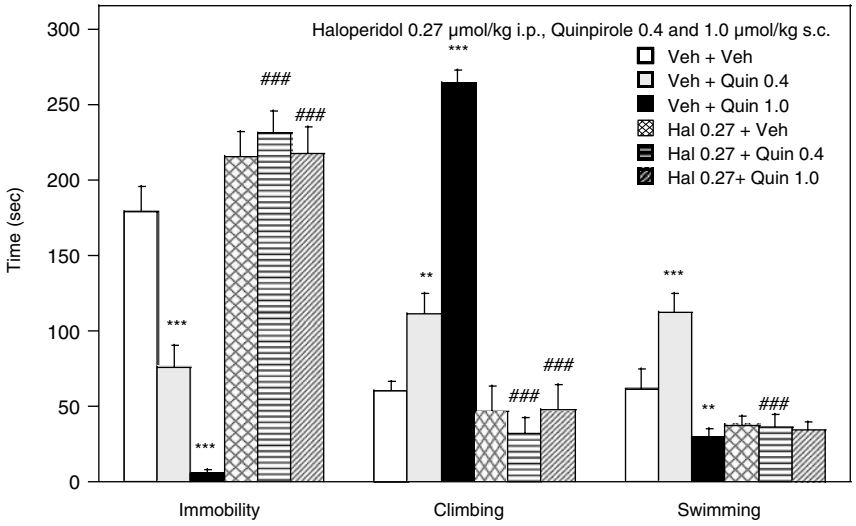

Figure 4 Effect of haloperidol on the effect of quinpirole on rat FST. Graph represents mean ± SEM for immobility, climbing, and swimming time during the 5-min test swim. Haloperidol (0.27 μmol/kg i.p.) was administered 15 min before each quinpirole injection. Quinpirole (0.4 and 1.0 μmol/kg s.c.) was injected 24, 5, and 0.5 h before testing ($n = 15$/group). $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to vehicle/vehicle-treated rats; $^{###}p < 0.001$ compared to vehicle/quinpirole-treated rats (Fisher *post hoc* test).

Doses of A-437203 evaluated were 0.52, 1.75, 5.24, and 17.46 μmol/kg i.p. Doses were chosen based on the selectivity of A-437203 for D_3 *vs* D_2 dopamine receptors (Chaperon *et al*, 2003) and reports indicating that the effects of A-437203 at doses of 17.46 μmol/kg (10 mg/kg) or lower are clearly mediated by D_3 but not D_2 receptors, since higher doses of the compound such as 174.6 μmol/kg (100 mg/kg) are necessary to bind and block D_2 receptor from the irreversible inactivation induced by the alkylating agent EEDG (Drescher *et al*, 2002; Ramirez *et al*, 2003). ANOVA revealed no significant difference between the treatments for any of the behaviors analyzed ($F_{4,45} = 1.12$, $p = 0.359$ for immobility, $F_{4,45} = 0.188$, $p = 0.943$ for climbing, and $F_{4,45} = 1.634$, $p = 0.182$ for swimming). Based on these results, the dose of 17.46 μmol/kg i.p. of A-437203 was selected for further experiments.

Effect of A-437203 on the Effect of Quinpirole on Rat FST

As observed in previous experiments, administration of quinpirole induced a dose-dependent reduction in immobility time and an increase in climbing time. The D_3 receptor antagonist A-437203 did not have an effect alone in the model; however, it showed a trend to attenuate the anti-immobility effect at the lower dose of 0.4 μmol/kg but not at the higher dose of 1.0 μmol/kg of quinpirole.

The effect of administration of the D_3 receptor antagonist A-437203 on the effect of quinpirole in rat FST is shown in Figure 5. Two-way ANOVA for immobility time indicated a significant quinpirole effect ($F_{2,54} = 59.4318$, $p < 0.001$), a significant antagonist treatment (A-437203) effect ($F_{1,54} = 9.8622$, $p < 0.01$), but a nonsignificant antagonist × quinpirole interaction ($F_{2,54} = 2.4319$, $p = 0.0974$). While there was no significant interaction, it appears that A-437203 showed a nonsignificant trend to attenuate the quinpirole-induced antidepressant-like activity at the dose of quinpirole 0.4 μmol/kg dose (Figure 5). Two-way ANOVA

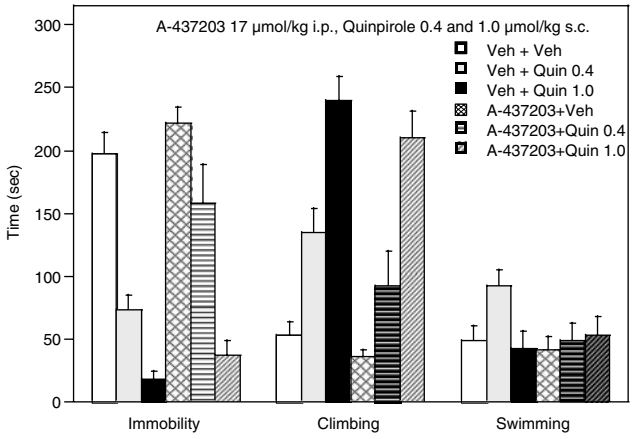

Figure 5 Effect of A-437203 on the effect of quinpirole on rat FST. Graph represents mean ± SEM for immobility, climbing, and swimming time during the 5-min test swim. A-437203 (17.46 μmol/kg i.p.) was administered 15 min before each quinpirole injection. Quinpirole (0.4, and 1.0 μmol/kg s.c.) was injected 24, 5, and 0.5 h before testing ($n =$ 10/group). No significant antagonist × quinpirole interaction was observed. Quinpirole induced a decrease in immobility and increase in climbing time that was revealed through a significant main effect of quinpirole and the collapsed data of each quinpirole dose (0.4 and 1.0 μmol/kg) as compared to vehicle ($p < 0.001$).

for climbing revealed a significant quinpirole effect ($F_{2,54} = 48.3166$, $p < 0.001$), an almost significant antagonist treatment (A-437203) effect ($F_{1,54} = 3.8044$, $p = 0.0563$), and a nonsignificant antagonist × quinpirole interaction ($F_{2,54} = 0.2455$, $p = 0.7831$). The effect of quinpirole decreasing immobility and increasing climbing time in the model was revealed through the significant main effect of quinpirole and the collapsed data of each quinpirole dose (0.4 and 1.0 μmol/kg), as compared to vehicle ($p < 0.001$). Statistical analysis for swimming indicated no significant effect for quinpirole, the antagonist or for the interaction.

Effect of L-745,870, a Selective D_4 Antagonist, on Rat FST

Doses of L-745,870 were selected according to previous work reporting the behavioral profile of this compound (Bristow *et al*, 1997; Cao and Rodgers, 1997; Patel *et al*, 1997). ANOVA indicated no significant effect for any of the doses of L-745,870 in any of the parameters tested ($F_{4,43} = 1.867$, $p = 0.134$ for immobility time, $F_{4,43} = 1.593$, $p = 0.193$ for climbing time, and $F_{4,43} = 0.805$, $p = 0.529$ for swimming time). There was a trend towards an increase in immobility at the higher doses of L-745,870 (2.3 and 5.7 μmol/kg) as can be observed in Table 1. The dose of 1.15 μmol/kg of L-745,870 was therefore selected for the subsequent blockade study of quinpirole effects.

Effect of L-745,870 on the Effect of Quinpirole on Rat FST

Figure 6 shows the effects of pretreatment with the D_4 receptor antagonist, L-745,870, on quinpirole-induced effects in rat FST. Two-way ANOVA for immobility time indicated a significant quinpirole effect ($F_{2,54} = 61.261$, $p < 0.001$), a nonsignificant antagonist treatment effect ($F_{1,54} = 0.115$, $p = 0.736$), and a nonsignificant antagonist × quinpirole interaction ($F_{2,54} = 0.7275$, $p = 0.488$). Statistical analysis for climbing time also revealed a similar result. Two-way ANOVA for climbing time showed a significant quinpirole effect ($F_{2,54} = 85.589$, $p < 0.001$), but a nonsignificant antagonist treatment effect ($F_{1,54} = 2.5619$, $p = 0.1153$), and a nonsignificant antagonist × quinpirole interaction ($F_{2,54} = 0.6278$, $p = 0.538$). The effect of quinpirole decreasing immobility and increasing climbing time in the model was revealed through the significant main effect of quinpirole and the collapsed data of each quinpirole dose (0.4 and 1.0 μmol/kg) as compared to vehicle ($p < 0.001$).

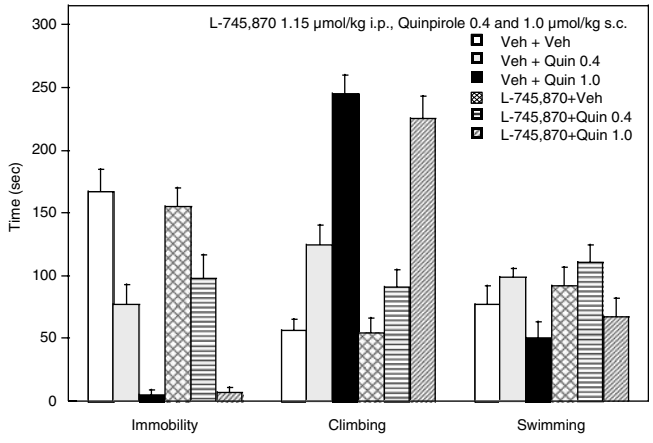

Figure 6 Effect of L-745,870 on the effect of quinpirole on rat FST. Graph represents mean ± SEM for immobility, climbing, and swimming time during the 5-min test swim. L-745,870 (1.15 μmol/kg i.p.) was administered 15 min before each quinpirole injection. Quinpirole (0.4 and 1.0 μmol/kg s.c.) was injected 24, 5, and 0.5 h before testing ($n = 10$/group). No significant antagonist × quinpirole interaction was observed. Quinpirole induced a decrease in immobility and increase in climbing time that was revealed through a significant main effect of quinpirole and the collapsed data of each quinpirole dose (0.4 and 1.0 μmol/kg) as compared to vehicle ($p < 0.001$).

Two-way ANOVA for swimming time showed a significant quinpirole effect ($F_{2,54} = 5.995$, $p < 0.01$), but a nonsignificant antagonist treatment effect ($F_{1,54} = 1.837$, $p = 0.181$), and a nonsignificant antagonist × quinpirole interaction ($F_{2,54} = 0.017$, $p = 0.983$). Thus, quinpirole induced a decrease in immobility time and an increase in climbing time at both tested doses (0.4 and 1.0 μmol/kg). Administration of the D_4 antagonist L-745,870 alone (1.15 μmol/kg) did not induce any change either in immobility, climbing, or swimming time compared to vehicle/vehicle control rats. Moreover, pretreatment with L-745,870 did not antagonize the effects of any of the doses of quinpirole in any of the parameters recorded.

Effect of Quinpirole on Locomotor Activity

Acute administration of quinpirole induced a biphasic effect in the locomotor activity assay, consisting of a significant reduction in total distance moved at the lowest dose of quinpirole (0.4 μmol/kg), but a significant increase in locomotion at higher doses (1.0 and 2.0 μmol/kg) during 90 min of testing (data not shown). Chronic administration of dopamine agonists, such as quinpirole, can result in behavioral sensitization to the activity response to the drug (Szechtman *et al*, 1993; Koeltzow *et al*, 2003; Lomanowska *et al*, 2004). Considering that subchronic treatment with quinpirole, similar to that used in FST (three injections in a 24-h period), might also be associated with induction of behavioral sensitization, rats were tested for spontaneous activity after three injections of quinpirole (0.4 and 1.0 μmol/kg = 0.1 and 0.25 mg/kg) according to the same protocol treatment used for rat FST. Figure 7 shows the effect of subchronic quinpirole administration on spontaneous locomotor activity in rats and the time course of the activity effects induced by quinpirole. ANOVA for total distance moved indicated a significant treatment effect ($F_{2,27} = 5.6058$, $p < 0.01$). Subchronic treatment with quinpirole 0.4 μmol/kg did not increase locomotor activity compared to vehicle-treated rats, but quinpirole 1.0 μmol/kg induced a significant increase in activity ($p < 0.01$). Activity data from quinpirole permit the differentiation of the anti-immobility effect observed in FST from general activation.

DISCUSSION

The present study supports the hypothesis that activation of D_2-like receptors is associated with an antidepressant-like profile in rat FST. This detailed study assessed the involvement of specific dopamine D_2-like receptors, and is the first to show that D_2 and to a lesser extent D_3, but not D_4 receptors, are implicated in the modulation of depressive-like behaviors in a behavioral despair test, the FST. Nomifensine, a selective dopamine reuptake inhibitor that increases dopamine concentration in the synapses, and quinpirole, a dopamine D_2-like receptor agonist, were associated with a significant decrease in immobility time and a significant increase in climbing behavior (Figures 1 and 2). The administration of a D_3 preferential agonist, PD 128907, also induced a modest but significant decrease in immobility time and increase in climbing time at the

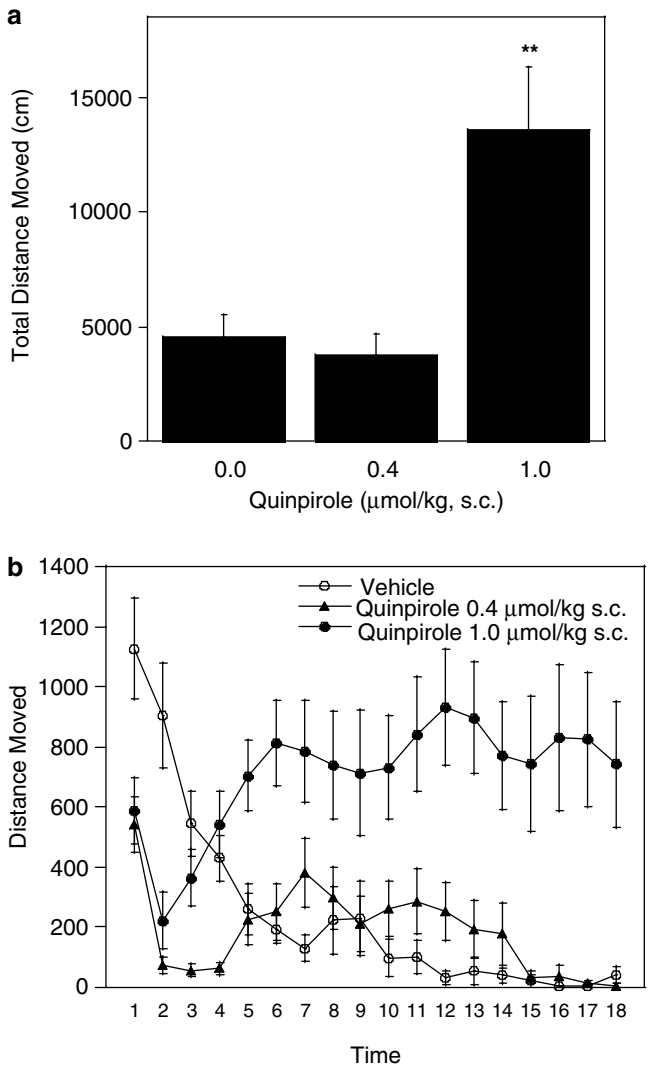

Figure 7 Effect of quinpirole a D_2-like agonist on locomotor activity. Graph represents mean ± SEM of total distance moved during 90 min (a) and time course of activity in 5-min intervals (b) following quinpirole administration ($n = 10$/group). Locomotor activity was recorded after three quinpirole injections, following the same administration schedule used for rat FST (0.4 and 1.0 μmol/kg s.c. administered 25, 5, and 0 h before the test). $^{**}p < 0.01$ compared to vehicle-treated rats.

highest dose tested. In contrast, the administration of selective D_4 agonists, such as PD 168077 and CP 226269, did not induce significant behavioral changes in rat FST compared to vehicle control rats (Figure 3). However, since the availability of agonists showing true selectivity among receptor subtypes in the D_2-like receptor family is limited, we sought to further test the involvement of different receptors by taking the complementary approach, that is, assessing the ability of D_2, D_3, and D_4 selective antagonists to attenuate quinpirole-induced changes in the FST. The $D_{2/3/4}$ receptor antagonist haloperidol, completely antagonized the effect of all doses of quinpirole in FST; however, the selective D_3 receptor antagonist A-437203, only seemed to attenuate the effect of quinpirole at the low dose but not at the higher dose (Figures 4 and 5). In contrast, the selective D_4 receptor antagonist L-745,870 did not attenuate any of the effects of quinpirole in rat FST (Figure 6). Thus, consistent with the agonist studies, results indicated there was blockade of quinpirole-induced antidepressant-like effects by a D_2-like receptor antagonist, a trend towards a partial attenuation by a D_3 receptor antagonist, but no effect of a D_4 receptor antagonist. These findings suggest that an enhancement of dopamine function, and more specifically activation of dopamine D_2 and, to a lesser extent, D_3 receptors with dopamine agonists, may have relevance for the therapeutic treatment of depression.

The D_3 receptor has been implicated in multiple neuropsychiatric disorders including depression and schizophrenia. The literature reports antidepressant effects for dopamine D_2/D_3 receptor agonists such as bromocriptine, piribedil, pramipexole, and roxindole (Post *et al*, 1978; Bouras and Bridges, 1982; Willner, 1983a; Grunder *et al*, 1993; Willner *et al*, 1994; Maj *et al*, 1996b; Corrigan *et al*, 2000). Pramipexole, a D_3 receptor preferring agonist, has been reported to reverse the suppression of sucrose intake in animals chronically exposed to mild unpredictable stress, and showed efficacy in FST (Willner *et al*, 1994; Maj *et al*, 1997). Pramipexole has also demonstrated similar efficacy to fluoxetine for the treatment of major depressive disorder in a clinical trial (Corrigan *et al*, 2000). Within our study, the effects of the D_3 preferential compound were modest but consistent; however, there remains the possibility that the effects of the D_3 preferential compound were due to affinity for the D_2 receptor. PD 128907 is a preferential D_3 agonist, which shows preference for D_3 *vs* D_2 receptors in binding and functional studies using recombinant hD_3 and hD_2 receptors; however, the selectivity for D_3 *vs* D_2 is modest (14-fold). Thus, the finding that PD 128907 only induces a modest antidepressant-like effect at the highest dose tested may suggest that this dose is beginning to stimulate D_2 receptors which are in turn mediating the antidepressant effect. However, the dose of PD 128907 that showed antidepressant-like activity in our study (0.7 μmol/kg = 0.2 mg/kg; Figure 3) has been previously reported to be selective for stimulating D_3 receptors suggesting that this modest effect may reflect a modest ability of D_3 receptor activation to induce an antidepressant effect in FST (Bristow *et al*, 1996; Chaperon *et al*, 2003). Potential involvement of D_3 receptors might also be indicated by the data obtained in the antagonism study. A-437203 is an antagonist with high affinity for D_3 receptors and relatively high selectivity compared to other dopamine receptor subtypes (44-fold selective for D_3 *vs* D_2). Since quinpirole, a D_2-like receptor agonist, shows some degree of selectivity for the D_3 receptors (8.5-fold more selective for D_3 over D_2 receptors) (Levant *et al*, 1993), the finding that A-437203 tended to attenuate the lower but not the higher dose of quinpirole may suggest that the antagonist was capable of antagonizing the more D_3-mediated effects of quinpirole, but unable to compete with the effect of the higher dose of quinpirole that fully stimulated D_2 receptors (Figure 5). Thus, although D_3 receptors do not appear to play as robust of a role as D_2 receptors, they appear to contribute to this effect. However, further investigation is necessary to conclude whether this effect is specifically related with D_3 receptors or due to nonspecific stimulation of dopamine receptors (ie D_2 receptors).

Similar to D_3 receptors, D_4 receptors have been extensively investigated as a target for the treatment of schizophrenia. Interestingly, distribution of D_4 receptors in brain areas associated with regulation of emotions and cognition such as prefrontal cortex and mesolimbic areas (Matsumoto *et al*, 1995; Ariano *et al*, 1997; Defagot and Antonelli, 1997; Defagot *et al*, 1997; Tarazi *et al*, 1997; Oak *et al*, 2000) also make them an intriguing candidate to play a role in depressive-like behaviors. However, using selective D_4 agonists and antagonists, we found no indication of the involvement of D_4 receptors in the FST. The marked selectivity of the D_4 agonists (PD 168077, 100-fold selective *vs* D_2 receptors and 200-fold selective *vs* D_3 receptors; and CP 226269, 150-fold selective *vs* D_2 receptors and 650-fold selective *vs* D_3 receptors) (Moreland *et al*, 2004), and their lack of efficacy in the rat FST model, as well as for the D_4 antagonist L-745,870 (10000-fold selective for D_4 receptors *vs* D_2 and D_3) (Moreland *et al*, 2004), and the lack of efficacy blocking the effect of quinpirole in FST suggest that D_4 receptors are clearly not involved in the mediation of antidepressant-like activity.

Since dopamine agonists like quinpirole can induce hyperactivity acutely as well as induce sensitization following repeated administration (Szechtman *et al*, 1993; Lomanowska *et al*, 2004), it could be proposed that the anti-immobility effects seen in the present study were merely the reflection of nonspecific locomotor activation. In order to address this, quinpirole was tested in a locomotor activity assay after a subchronic administration schedule identical to the administration regime used for rat FST. While there was a significant increase in locomotor activity levels following subchronic treatment with 1.0 μmol/kg of quinpirole, there was no change in locomotor activity with 0.4 μmol/kg. Thus, the effect of quinpirole in rat FST can be differentiated from general locomotor stimulation since antidepressant-like effects were clearly seen at doses not associated with hyperactivity (0.4 μmol/kg = 0.1 mg/kg; Figure 2). In addition, potential increases in motor activity induced by stereotyped behavior do not seem likely since stereotypy usually occurs at higher doses than hyperlocomotion (Kurashima *et al*, 1995) and animals showed clear escape-oriented behaviors in the model (ie increase in climbing behavior). The effect observed in FST with PD 128907, as well as with low doses of nomifensine, can also be differentiated from general activation effect (data not shown).

Dopamine is believed to play a critical role in the neurobiology of depression and in the mechanism of action of antidepressant drugs, a hypothesis based on clinical and preclinical observations (Willner, 1983b; Fibiger, 1995; Charney, 1998; D'Aquila *et al*, 2000b). As stated above, compounds acting as agonists on dopamine D_2/D_3 receptors, such as bromocriptine, piribedil, pramipexole, and roxindole (Post *et al*, 1978; Bouras and Bridges, 1982; Willner, 1983a; Borsini *et al*, 1988; Muscat *et al*, 1992; Grunder *et al*, 1993; Willner *et al*, 1994; Maj *et al*, 1996b; Corrigan *et al*, 2000), and compounds that enhance dopamine levels inhibiting selective dopamine reuptake like amineptine, nomifensine, and bupropion (Kinney, 1985; Dalery *et al*, 1997), have shown antidepressant-like activity in both preclinical models and clinical settings. Interestingly, it has been proposed that an increase in dopamine activity induced by chronic antidepressant treatments is one of the mechanisms of action underlying the efficacy of these compounds, since their effects are antagonized by low doses of D_2/D_3 antagonists (Borsini *et al*, 1984; Borsini *et al*, 1985a, b; Pulvirenti and Samanin, 1986; Sampson *et al*, 1991; Serra *et al*, 1992; D'Aquila *et al*, 2000a). Moreover, antidepressant treatments are associated with increased dopaminergic function in the mesolimbic system and increased behavioral response to dopamine agonists (Spyraki and Fibiger, 1981; Maj *et al*, 1984a, b; Cervo and Samanin, 1987; De Montis *et al*, 1990; Ainsworth *et al*, 1998a; D'Aquila *et al*, 2000b, 2003), which takes place after chronic treatment (2–3 weeks), a period of time that correlates with that necessary for the onset of antidepressant activity in the clinic.

Dopaminergic deficits are associated with several psychiatric symptoms in man, such as depression (Kapur and Mann, 1992; Fibiger, 1995; Pania and Gessab, 2002). A deficiency of mesolimbic dopaminergic pathway plays a critical role for the etiology of symptoms of depression like anhedonia and decreased motivation (Willner, 1983b; Schultz, 1997; Berridge and Robinson, 1998; Naranjo *et al*, 2001). Mesolimbic dopamine projections are a crucial component in the neural circuitry of reward and/or incentive motivation, both dysfunctional in major depression disorder (Fibiger, 1995; Naranjo *et al*, 2001). Dopaminergic abnormalities such as reduced dopamine transporter and upregulation of D_2/D_3 receptors have been found in amygdaloid nuclei in postmortem brain of major depressed patients (Klimek *et al*, 2002) as well as reduced concentration of homovalinic acid, a dopamine metabolite, in cerebrospinal fluid (Willner, 1983b) and plasma of depressed patients (Lambert *et al*, 2000). Furthermore, administration of dopamine antagonists as well as drugs that produce depletion of catecholamines (ie reserpine) can elicit symptoms in healthy volunteers that resemble those described in depression such as anhedonia, and lack of volition and energy (Wise *et al*, 1978; Willner, 1983a), suggesting that reduction in dopamine transmission is an important neurochemical substrate associated with depression. Studies with schizophrenic patients treated with antipsychotics indicate that they may experience common side effects such as neuroleptic dysphoria (Weiden *et al*, 1989; Voruganti and Awad, 2004), negative affective states and depressive symptoms (Harrow *et al*, 1994). Moreover, D_2 receptor blockade associated with typical antipsychotic treatment directly correlates with and contributes to the emergence of severe depressive symptoms in schizophrenic patients (Bressan *et al*, 2002; de Haan *et al*, 2004). Increased immobility in rat FST is associated with a depressive-like state in the FST. In the present study, we did not see that kind of effect by dopamine antagonists at the doses tested in the animal model (Table 1). However, it is well known that antipsychotics induce hypolocomotion at high doses, and despite the sedative effects of these compounds this behavior could be representative of depressive-like states (Porsolt *et al*, 1978).

In summary, dopamine receptor agonists, especially dopamine D_2/D_3 receptor agonists, could be potentially effective for the treatment of depression (Forrest *et al*, 1977; Bouras and Bridges, 1982; Lopez-Ibor Alino *et al*, 1982; Kinney, 1985; Corrigan *et al*, 2000). However, given the

implication of the D_2 receptor in reward and addiction, one would have to clearly separate antidepressant activity from the risk for potential abuse (Caine *et al*, 1997; Haddad, 1999; Ellinwood *et al*, 2002; Rouge-Pont *et al*, 2002; Farvolden *et al*, 2003). Furthermore, activation of dopamine function and/or supersensitivity of dopaminergic neurotransmission may play an important role in the induction of mania episodes in patients with bipolar disorders (D'Aquila *et al*, 2003), another obstacle for the use of D_2 receptor agonists for the treatment of depression (Massat *et al*, 2002; Yatham, 2002; Serretti *et al*, 2004). Mood changes observed in bipolar patients from mania to depression are critical events in the course of the disorder and might depend upon parallel changes in mesolimbic dopamine system sensitivity. Finally, the understanding of the neurobiological basis of depression, including the importance of the dopamine system, will be important in the development of new treatments for depression with better efficacy and tolerability, faster onset of action and fewer side effects.

ACKNOWLEDGEMENTS

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