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Topographically Based Search for an "Ethogram" Among a Series of Novel D_4 Dopamine Receptor Agonists and Antagonists

Jeremiah J. Clifford, Ph.D., and John L. Waddington, Ph.D., D.Sc.

The effects of three selective D_4 antagonists [CP-293,019, L-745,870, and Ro 61-6270] and two putative selective D_4 agonists [CP-226,269 and PD 168077] were compared with those of the generic D_2-like [$D_{2L/S}$,D_3, D_4] antagonist haloperidol to identify any characteristic "ethogram," in terms of individual topographies of behavior within the natural rodent repertoire, as evaluated using ethologically based approaches. Among the D_4 antagonists, neither L-745,870 (0.0016–1.0 mg/kg) nor Ro 61-6270 (0.2–25.0 mg/kg) influenced any behavior; whereas, CP-293,019 (0.2–25.0 mg/kg) induced episodes of nonstereotyped sniffing, sifting, and vacuous chewing; there were no consistent effects on responsivity to the D_2-like agonist RU 24213. Among the putative D_4 agonists, CP-226,269 (0.2–25.0 mg/kg) failed to influence any behavior; whereas, PD 168077 (0.2–25.0 mg/kg) induced nonstereotyped shuffling locomotion with uncoordinated movements, jerking, and yawning, which were insensitive to antagonism by CP-293,019, L-745,870, or haloperidol. These findings fail to indicate any "ethogram" for selective manipulation of D_4 receptor function at the level of the interaction between motoric and psychological processes in sculpting behavioral topography over habituation of exploration through to quiescence and focus attention on social, cognitive, or other levels of examination.

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Although identification of D_1 and D_2 dopamine (DA) receptor subtypes evolved from classical functional/pharmacological considerations, which included otherwise anomalous DAergic behavioral effects, members of the broader D_1-like [D_{1A} /D_1, D_{1B}/D_5] and D_2-like [$D_{2L/S}$, D_3, D_4] families of receptors have been identified through molecular biology and characterized primarily in terms of their neuroanatomical localization and cellular neurobiology (Missale et al. 1998; Neve and Neve 1997; Waddington et al. 1995, 1998). In particular, any behavioral role for the D_4 receptor (Van Tol et al. 1991) remains poorly understood, primarily because of a paucity of agonists and antagonists showing meaningful selectivity for this site (Tarazi and Baldessarini 1999). It is on this background that interest in the D_4 receptor as a target for antipsychotic therapy evolved indirectly from: (1) its extrastriatal, primarily corticolimbic localization; (2) the discovery that clozapine, an efficacious antipsychotic drug with a very low propensity to induce extrapyramidal side effects, evidenced some modest preference for D_4 over D_2 receptors; and (3) controversial evidence that D_4 receptor density was elevated in schizophrenia (Seeman et al. 1997; Tarazi and Baldessarini 1999); strikingly, this interest evolved in the absence of any substantive body of preclinical evidence for D_4 receptor involvement in behavioral models of antipsychotic activity.

From the Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, Dublin, Ireland.

Address correspondence to: John L. Waddington, Ph.D., D.Sc., Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, St. Stephen's Green, Dublin 2, Ireland.

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Subsequently, a series of selective D_4 antagonists has been identified. Among these, CP-293,019 (Mansbach et al. 1998; Sanner et al. 1998;), L-745,870 (Bristow et al. 1997; Patel et al. 1997), NGD 94-1 (Tallman 1998), PNU-101387 (Merchant et al. 1996), Ro 61-6270 (Hartman et al. 1996), and S 18126 (Millan et al. 1998) have received, to date, the more extensive preclinical evaluation, and there is some consensus that they show little or no activity in traditional models of either antipsychotic activity (e.g., DA agonist-induced responsivity and inhibition of conditioned avoidance responding) or of extrapyramidal side effect liability (e.g., induction of catalepsy); there are only limited and, thus far, contradictory data as to whether they seem to be inactive (Bristow et al. 1997), partially active (Tallman 1998), or active (Mansbach et al. 1998) in such newer models as restoration of DA agonist-induced disruption of prepulse inhibition.

Although little or no effect of selective D_4 antagonists on spontaneous behavior has been noted, this has almost invariably involved assessment in terms of photobeam interruptions, which fail to resolve other than the most elementary components of otherwise composited behavior. Regarding selective D_3 antagonists, we have demonstrated recently (Clifford and Waddington 1998) that evaluation of behavioral topography in rodents using an ethologically based approach (Colgan 1978) can identify drug profiles ("ethograms") that can clearly distinguish between agents seemingly of the same pharmacological class. Furthermore, we recently described (Clifford et al. 1998) how this approach (Gerlai and Clayton 1999) can reveal interactions at the level of individual behaviors between receptor manipulation and such psychological processes as habituation, which sculpts behavioral topography, in a manner that cannot be accessed in detail by photobeam approaches.

Given that studies to date on any behavioral role for D_4 receptors have emerged primarily in the context of antipsychotic potential, even so fundamental a question as the extent to which they might play any role in regulating behavior has received little attention. Therefore, we examined three selective D_4 antagonists (CP-293,019, L-745,870, and Ro 61-6270; Table 1), as compared to the reference D_2-like [$D_{2L/S}$, D_3, D_4] antagonist haloperidol, to identify any associated "ethogram" and have studied in the same manner any effects on behavioral responsivity to the reference D_2-like [$D_{2L/S}$, D_3, D_4] agonist RU 24213 (Euvrard et al. 1980; Waddington et al. 1995; Waddington and O'Boyle 1989;). Very recently, two putative selective D_4 agonists, CP-226,269 and PD 168077 (Glase et al. 1997; Zorn et al. 1997; Table 1) have been described, but their psychopharmacological profiles remain essentially unexplored; we have studied these agents similarly, to probe for any "ethogram" complementary to that for selective D_4 antagonists.

Table 1. D_4 Agonists and Antagonists and Their Selectivities Within the D_2-line [D_2, D_3, D_4] Family of Receptors

	Affinity [Ki, nM]			
	D_2	D_3	D_4	D_2/D_4
Antagonists				
CP-293,019[a]	>3310	>2000	3.4	>1000
L-745,870[b]	960	2300	0.4	>2200
Ro 61-6270[c]	>5000	>5000	5.0	>1000
Agonists				
CP-226,269[d]	>600	NA	6.0	>100
PD 168077[e]	3740	2810	9.0	>415

NA, not available.
[a] Sanner et al. (1998).
[b] Patel et al. (1997).
[c] Hartman et al. (1996).
[d] Zorn et al. (1997).
[e] Glase et al. (1997).

METHODS

Behavioral Studies

Young adult male Sprague–Dawley rats (180–350 g; Beaumont Hospital, Dublin) were housed in groups of five per cage with food and water available ad libitum, and were maintained at 21 ± 1°C on a 12/12 h (0900 on; 2100 off) light/dark regimin. On experimental days, they were placed individually in clear glass observation cages (36 × 20 × 20 cm) and either received drug or vehicle immediately (nonhabituated condition; exploring–habituating to a novel environment;) or were left undisturbed for a habituation period of 3 h (habituated condition) before assessment.

Behavioral assessments were carried out in a manner similar to that described previously (Clifford et al. 1998; Clifford and Waddington 1998; Deveney and Waddington 1996, 1997). Following injection of drug or vehicle, animals were assessed using a rapid time-sampling behavioral checklist technique. For this procedure, each rat was observed individually for 5-s periods at 1-min intervals over 15 consecutive minutes, using an extended, ethologically based behavioral checklist. This made possible the determination of the presence or absence of the following individual behaviors (occurring alone or in any combination) in each 5-s period: stillness (motionless, with no behavior evident); sniffing (flaring of nostrils with movements of vibrissae); locomotion (coordinated movement of all four legs resulting in change of location); rearing (of any form); rearing free (front paws raised off floor with motion upward or outward away from any surface); rearing to wall (front paws raised off floor with motion upward or outward toward cage wall); rearing seated (front paws raised off floor from a seated position); sifting (characteristic pattern of coordinated movements of the forepaws through bedding material on cage floor); grooming (of

any form); intense grooming (characteristic pattern of grooming of the snout and then face with the forepaws, followed by vigorous grooming of the hind flank or anogenital region with the snout); vacuous chewing (not directed onto any physical material); chewing (directed onto any physical material without consumption); eating (chewing with consumption); and licking. After this 15-min assessment using the behavioral checklist, animals were evaluated using a conventional 0- to 6-point stereotypy scale: 0 = asleep or inactive; 1 = episodes of normal activities; 2 = discontinuous activity with bursts of prominent sniffing or rearing; 3 = continuous stereotyped activity such as sniffing or rearing along a fixed path; 4 = stereotyped sniffing or rearing fixated in one location; 5 = stereotyped behavior with bursts of licking or gnawing; 6 = continuous licking or gnawing. This cycle of assessment by behavioral checklist followed by stereotypy scale was repeated on two further occasions over a total observation period of 1 h; some studies were continued into additional periods thereafter. For studies in the nonhabituated condition, rats were used on a single occasion only; otherwise, they were used on two occasions only with exposure only to a single drug, separated by a drug-free interval of at least 1 week, with random allocation to one of the various dosages in each instance. All assessments were made by a single observer unaware of the treatment given to each animal. These studies were approved by the Research Committee of the Royal College of Surgeons in Ireland and were conducted under license from the Department of Health in accordance with Irish legislation and EU regulations for the care and use of experimental animals.

Drugs

The following selective D_4 antagonists were used: CP-293,019 [(7R,9aS)-7-(4-fluorophenoxy)methyl-2-(5-fluoropyrimidin-2-yl)-2,3,4 ,6, 7,8, 9, 9a-octahydro-1H-pyridol](1,2-a)pyrazine; Pfizer, USA]; L-745,870 [3-[4-(4-chlorophenyl)piperazin-1-yl]methyl-1H-pyrrolo[2,3-b]pyridine; Merck Sharpe & Dohme, UK]; Ro 61-6270 [2-amino-benzoic acid-1-benzyl-piperidin-4-yl-ester; Roche, Switzerland]. The following putative selective D_4 agonists were used: CP-226, 269 [5-fluoro-2-(4-pyridin-2-yl-piperazin-1-methyl)-1H-indole; Pfizer, USA]; PD 168077 {N-[methyl-4-(2-cyanophenyl)piperazinyl-3-methylbenzamide] Parke-Davis, USA].

CP-293,019 was dissolved in 40% cyclodextrin (RBI, USA) and injected in a volume of 6 ml/kg; L-745,870 was dissolved in a minimum of 0.1N hydrochloric acid, made up to volume with distilled water and injected in a volume of 2 ml/kg; Ro 61-6270 was dissolved in distilled water and injected in a volume of 2 ml/kg; CP-226,269 was dissolved in 40% dimethylsulphoxide and injected in a volume of 2 ml/kg; PD 168077 was dissolved using ultrasonication in a minimum of glacial acetic acid made up to volume with 40% cyclodextrin and injected in a volume of 4 ml/kg. RU 24213 (Hoechst-Marion-Roussel, France) was dissolved in distilled water and injected in a volume of 2 ml/kg; haloperidol (RBI, USA) was dissolved in a minimum of glacial acetic acid, made up to volume with distilled water and injected in a volume of 2 ml/kg. All drugs or their vehicles were injected subcutaneously into the flank, with antagonists or their vehicles given 30 min before agonists in combination experiments.

Data Analysis

From application of the behavioral checklist, the total "counts" for each individual behavior were determined as the number of 5-s observation windows in which a given behavior was evident, summed over a 1-h period, and expressed as means ± SEM; stereotypy scores were averaged over the 1-h period and expressed similarly. These data were then analyzed using analysis of variance (ANOVA) or the Kruskal–Wallis nonparametric ANOVA, followed by Student's *t*-test or Mann–Whitney U-test.

RESULTS

D_4 Antagonist Effects on Behavior Over Nonhabituated (Exploratory) Condition

Haloperidol (0.004–0.5 mg/kg) dose dependently reduced sniffing ($p < .01$), locomotion ($p < .05$), rearing ($p < .001$), grooming ($p < .01$), and chewing ($p < .01$), with no significant effect on any other topography of behavior over a 1-h period; no additional effects were evident when observations were continued over a second, consecutive 1-h period. Neither CP-293,019 (0.2–25.0 mg/kg), L-745,870 (0.008–1.0 mg/kg) nor Ro 61-6270 (0.2–25.0 mg/kg) significantly influenced any topography of behavior over a 1-h period; no significant effects were evident when observations were continued over a second, consecutive 1-h period.

D_4 Antagonist Effects on Behavior Over Habituated Condition

Haloperidol (0.0008–0.1 mg/kg) dose dependently reduced intense grooming ($p < .01$) but failed to influence significantly any other topography of behavior over a 1-hr period; no additional effects were evident when observations were continued over a second, consecutive 1-h period. CP-293,019 (0.2–25.0 mg/kg) dose-dependently induced sniffing ($p < .001$) and vacuous chewing ($p < .01$), with some induction of sifting ($p < .05$), whereas grooming ($p < .001$) and episodes of stillness ($p < .001$) were reduced over the 1-h period; increases

in stereotypy score were confined to the range of 0–1, indicating that behavioral stimulation was occurring episodically in a nonstereotyped manner; no additional effects were evident when observations were continued over a second, consecutive 1-h period. Neither L-745,870 (0.0016–1.0 mg/kg) nor Ro 61-6270 (0.2–25.0 mg/kg) influenced significantly any topography of behavior over the 1-h period; no significant effects were evident when observations were continued over a second, consecutive 1-h period.

D_4 Antagonist Effects on D_2-Like Agonist-Induced Behavior Over Habituated Condition

RU 24213 (0.1–12.5 mg/kg) dose-dependently induced sniffing ($p < .001$), locomotion ($p < .001$), sifting ($p < .001$), and chewing ($p < .001$) with some yawning ($p < .05$); increases in stereotypy score ($p < .001$) in the range of 2–3 indicated threshold levels in terms of sniffing and locomotion, in the absence of compulsive licking or gnawing. An intermediate dose of 2.5 mg/kg RU 24213 was selected for D_4 antagonist studies to allow detection of either attenuation or potentiation of responsivity. Following challenge with RU 24213 (2.5 mg/kg), sniffing ($p < .001$), locomotion ($p < .001$), rearing ($p < .001$) and chewing ($p < .01$) were dose-dependently blocked by pretreatment with haloperidol (0.02–0.5 mg/kg). CP-293,019 (0.2–25.0 mg/kg) failed to influence significantly any aspect of response topography; L-745,870 (0.04–1.0 mg/kg) antagonized only rearing ($p < .05$), although with an inverse dose dependency, and potentiated sifting ($p < .01$); Ro 61-6270 (0.2–25.0 mg/kg) antagonized only sniffing ($p < .01$), and potentiated episodes of stillness ($p < .01$); the baseline level of grooming ($p < .05$) was reduced by this drug combination. There were no significant effects on any other topography of responsivity to RU 24213 or on stereotypy scores.

D_4 Agonist Effects on Behavior Over Habituated Condition

CP-226,269 (0.2–25.0 mg/kg) failed to influence significantly any topography of behavior over the 1-h period; no significant effects were evident when observations were continued over a second, consecutive 1-h period. PD 168077 (0.2–25.0 mg/kg) dose-dependently induced locomotion ($p < .01$), which took an unusual and characteristic "shuffling" form with uncoordinated movements together with yawning, and episodes of myoclonic jerking; grooming ($p < .01$), and rearing ($p < .05$) were reduced. Increases in stereotypy score were confined to the range of 0–1, indicating that behavioral stimulation was occurring episodically in a nonstereotyped manner. No additional effects were seen when observations were continued over a second, consecutive 1-h period.

D_4 Antagonist Effects on D_4 Agonist-Induced Behavior Over Habituated Condition

Following challenge with PD 168077 (25.0 mg/kg), haloperidol (0.004–0.1 mg/kg) antagonized only sniffing, whereas the baseline level of grooming was reduced by this drug combination; there were no significant effects of haloperidol on any other topography of responsivity to PD 168077. CP-293,019 (0.2–25.0 mg/kg) and L-745,870 (0.04–1.0 mg/kg) failed to influence significantly any topography of responsivity to PD 168077 (Figure 1).

DISCUSSION

CP-293,019, L-745,870, and Ro 61-6270 are novel D_4 antagonists showing >1000-fold selectivity over other members of the D_2-like receptor family and over their D_1-like counterparts; furthermore, they show >300-fold selectivity over numerous non-DAergic receptors, other than a >50-fold selectivity of CP-293,019 over 5-HT_{1A} and 5-HT_{2A} receptors (Sanner et al. 1998; Patel et al. 1997; Hartman et al. 1996). No characteristic "ethogram" for these D_4 antagonists was apparent, using dosage ranges shown to exert biological activity in the brain at alternative levels of examination (Hartman et al. 1996; Holland et al. 1996; Patel et al. 1997). Although CP-293,019 induced episodes of nonstereotyped sniffing, vacuous chewing, and sifting, with attenuation of grooming, no such profile was apparent for L-745,870 or Ro 61-6270; hence, these effects of CP-293,019 are unlikely to have a basis in D_4 antagonism. Similarly, these selective D_4 antagonists as a class failed to evidence any characteristic "ethogram" at the level of responsivity to D_2-like [$D_{2L/S}$, D_3, D_4] receptor stimulation.

CP-226,269 and PD 168077 are the first agents to be identified as putative selective D_4 agonists. They show >100-fold selectivity over other members of the D_2-like receptor family and over their D_1-like counterparts; in addition, CP-226,269 shows an 80-fold selectivity over α_2 and >100-fold selectivity over several other non-DAergic receptors, whereas PD168077 shows a 20-fold selectivity over α_1, and α_2, a 45-fold selectivity over 5-HT_{1A}, and a 460-fold selectivity over 5-HT_{2A} receptors; each agent evidences intrinsic activity at the D_4 receptor in terms of quinpirole-like inhibition of forskolin-stimulated cAMP accumulation (Zorn et al. 1997) or stimulation of [^{3}H]thymidine uptake (Glase et al. 1997) in CHO cells expressing the human D_4 receptor. However, to our knowledge, their psychopharmacological effects have yet to be studied. No "ethogram" for CP-226,269 was apparent over a wide dose range. Conversely, PD 168077 induced nonstereotyped episodes of a "shuffling" form of locomotion with uncoordinated movements, together with yawning and episodes of myoclonic jerking, in the course of which grooming and

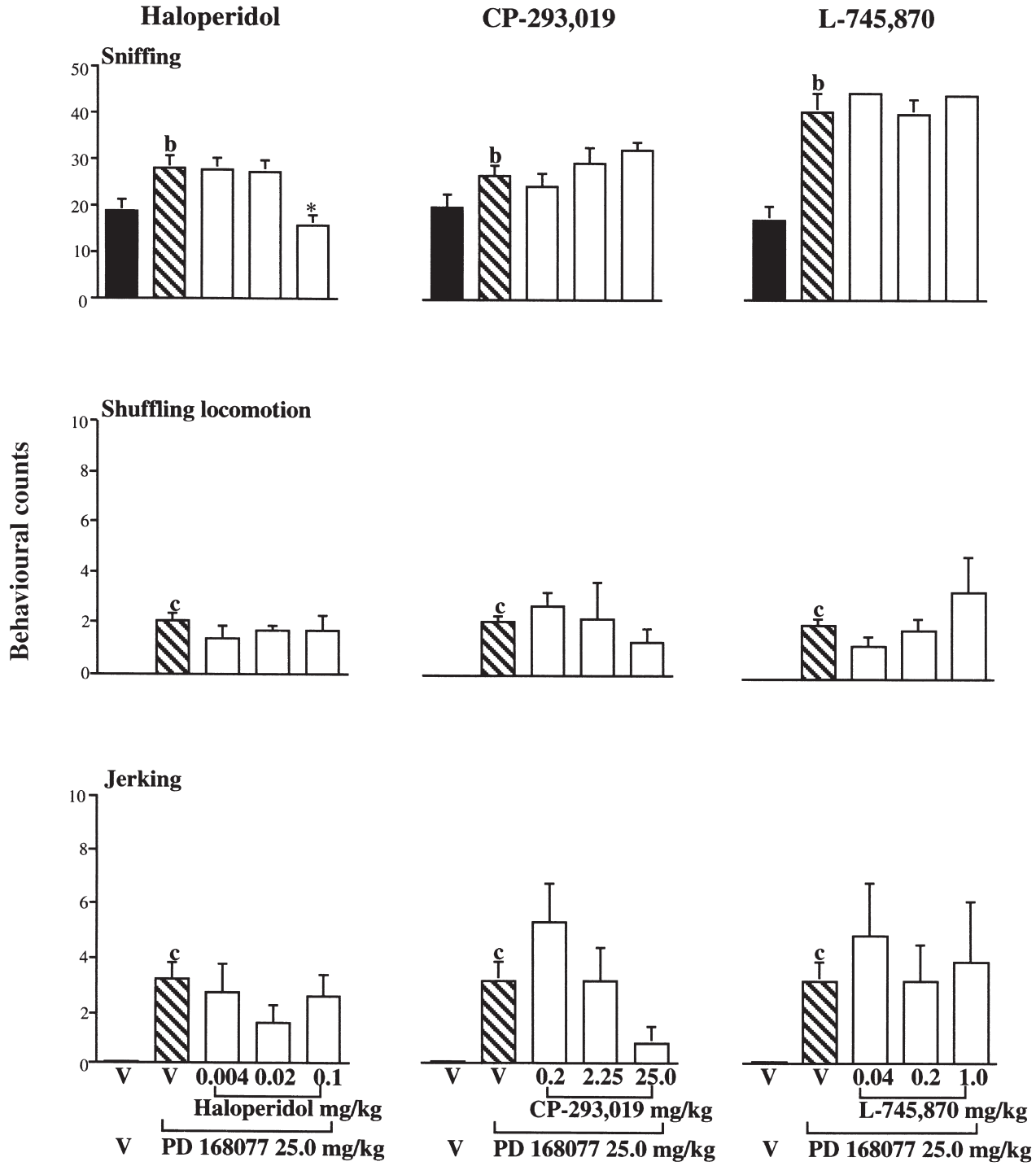

Figure 1. Topographical responsivity to 25.0 mg/kg PD 168077 following pretreatment with 0.004–0.1 mg/kg haloperidol, 0.2–25.0 mg/kg CP-293,019, 0.04–1.0 mg/kg L-745,870 or vehicle over an initial 1-h period. Data are mean counts for each behavior indicated ± SEM of n = 8–32 animals per group. $^{a}p < .05$, $^{b}p < .01$, $^{c}p < .001$ vs. vehicle (V); $^{*}p < .05$, $^{**}p < .01$ $^{***}p < .001$ vs. PD 168077.

rearing were reduced. However, these responses to PD 168077 were insensitive to D_4 antagonism, either by CP-293,019 or by L-745,870; furthermore, they were insensitive to haloperidol. Therefore, it seems that these effects of PD 168077 are not just unrelated to D_4 receptor activation; rather, they seem to have a non-DAergic basis, the nature of which remains to be specified. This conclusion indicates caution in the use of PD 168077 to probe the functional role of D_4 receptors.

In the rat, D_4 receptors are located primarily in corticolimbic areas, particularly in frontal cortex, thalamus, and hypothalamus, with low levels in the striatum/nucleus accumbens (Jaber et al. 1996; Tarazi and Baldessarini 1999). It is, therefore, important to establish the extent to which the low level of D_4 receptors in the striatum/nucleus accumbens (or, indeed, the higher levels elsewhere) might influence the topography of spontaneous behavior under diverse conditions or of D_2-like agonist-induced behavior; it seems that on the basis of studies using selective D_4 agonists and antagonists, they have little role in this regard. Although a recent study in mice with targeted gene deletion ("knockout") of the D_4 receptor has indicated modest reductions in horizontal and vertical movements relative to wild types in terms of photobeam interuptions and heightened responsivity to methamphetamine (Rubinstein et al. 1997), no study with any D_4 antagonist has suggested a comparable profile.

In relation to any antipsychotic potential of selective D_4 antagonists, recent studies in mice with targeted gene deletion of individual members of the D_2-like receptor family indicate amphetamine-induced disruption of prepulse inhibition to be essentially a D_2 rather than a D_3 or D_4 receptor-mediated effect (Ralph et al. 1999). Selective D_4 antagonists appear not to influence phencyclidine-induced stereotyped behavior or social isolation (Sams-Dodd 1998), although they may reverse phencyclidine-induced cognitive deficits (Jentsch et al. 1999). The clozapine cue in drug discrimination responding does not seem to generalize to a selective D_4 antagonist (Goudie et al. 1998). In the only controlled clinical trial of a selective D_4 antagonist in schizophrenia to date, L-745,870 failed to evidence either antipsychotic activity or extrapyramidal effects (Kramer et al. 1997); the D_4/5-HT_{2A} antagonist fananserin (Heuillet et al. 1996) has also been shown recently to evidence such a lack of therapeutic efficacy, although there seemed to be some worsening of akathisia (Truffinet et al. 1999). Although the present lack of psychopharmacological signature for selective D_4 antagonists is complementary to their apparent inactivity both in models of antipsychotic activity and in the clinic, it remains to be clarified whether any agent with antipsychotic activity could be ethologically "silent." Furthermore, the present findings in no way preclude any functional role(s) for the D_4 receptor in social, cognitive, or other processes, which require further study.

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