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Locomotor inhibition, yawning and vacuous chewing induced by a novel dopamine D_2 post-synaptic receptor agonist

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Abstract

The *N*-*n*-propyl analog of dihydrexidine ((±)-*trans*-10,11-dihydroxy-5,6,6*a*,7,8,12*b*-hexahydrobenzo[*a*]phenanthridine) is a dopamine receptor agonist with high affinity for dopamine D_2 and D_3 receptors ($K_{0.5} = 26$ and 5 nM, respectively). Members of the hexahydrobenzo[*a*]phenanthridine structural class are atypical because they display high intrinsic activity at post-synaptic dopamine D_2 receptors, but low intrinsic activity at dopamine D_2 autoreceptors. The present study examined the effects of (±)-*N*-*n*-propyl-dihydrexidine on unconditioned behaviors in rats. The most striking results observed were large, dose-dependent decreases in locomotor activity (e.g., locomotor inhibition), and increases in vacuous chewing; yawning was also increased at the highest dose of (±)-*N*-*n*-propyl-dihydrexidine. The locomotor inhibition and yawning induced by (±)-*N*-*n*-propyl-dihydrexidine were blocked by pre-treatment with (−)-remoxipride (*S*(−)-3-bromo-*N*-((1-ethyl-2-pyrrolidinyl)-methyl)-2,6-dimethoxybenzamide), a dopamine D_2 receptor antagonist, but not by the dopamine D_1 receptor antagonist (+)-SCH23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine). Vacuous chewing was decreased by both (−)-remoxipride and (+)-SCH23390. These data support the hypothesis that a subpopulation of post-synaptic dopamine D_2 receptors has a critical role in decreases in locomotor activity and induction of vacuous chewing and yawning.

Keywords: Dihydrexidine; Dopamine D_2 receptor; Locomotor activity; Sedation; Vacuous chewing; Yawning

1. Introduction

The development of novel ligands with high selectivity for dopamine receptor isoforms has been important for understanding brain dopaminergic function in normal and disease states (e.g., Parkinson's disease and schizophrenia). Dopamine receptors comprise a subset of the superfamily of G-protein coupled receptors (Dohlman et al., 1987). Currently there are two known pharmacologically similar families of dopamine receptors, usually categorized as D_1 and D_2 (Garau et al., 1978; Kebabian and Calne, 1979). At least five genes code for unique dopamine receptors, some having splice variants (Gingrich and Caron, 1993). Each molecular subtype has a unique regional distribution in the brain, the functional significance of which is, at present, poorly understood. The five genes can be divided into two families that are often referred to as D_1-like and D_2-like. D_1-like dopamine receptors (the D_{1A} and D_{1B} or D_5) have intron-less genes, are expressed as proteins having a relatively short third intracellular loop and a relatively long carboxy tail, and show high affinity for phenyl-tetrahydrobenzazepines such as SCH23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benza-

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zepine) and SKF38393 (*R*(+)-1-phenyl-7,8-dihydroxy-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine). D_2-like dopamine receptors (D_{2short} and D_{2long} splice variants, D_3, and D_4) are genes having multiple introns, are expressed as proteins having a long third intracellular loop and a short carboxy tail, and show high affinity for butyrophenones (e.g., spiperone) and benzamides (e.g.,(−)-sulpiride). Currently available ligands have limited ability to discriminate between molecular isoforms within each family. Throughout this paper, our references to dopamine D_1 and D_2 receptors will be to each family, although if specific information is available about a particular receptor isoform, (e.g., D_2 vs. D_3), this will be stated explicitly.

Dopamine D_2 receptors are expressed both as autoreceptors on dopamine neurons and terminals, and as post-synaptic receptors on target cells. Systemically administered dopamine receptor agonists acting at dopamine D_2 receptors are known to have a biphasic dose-response effect on unconditioned behaviors in rodents: low doses inhibit spontaneous locomotor activity, whereas high doses increase locomotor activity and elicit oral stereotypies (Di Chiara et al., 1976; Strömbom, 1976). This biphasic dose-response effect has been attributed to the ability of low agonist doses to stimulate selectively dopamine D_2 autoreceptors on the dopamine neuron, thereby decreasing synaptic concentrations of dopamine via down-regulation of neural firing rate, dopamine synthesis, and dopamine release (Strömbom, 1976; Walters and Roth, 1976; Westfall et al., 1976; Skirboll et al., 1979; Costall et al., 1981). Consistent with an autoreceptor hypothesis of decreased locomotor activity, several atypical dopamine receptor agonists displaying functional selectivity for dopamine D_2 autoreceptors are observed to dose-dependently inhibit locomotor activity in rodents, even at high doses (e.g., Hjorth et al., 1981; Pugsley et al., 1992; Nisoli et al., 1993).

Yet not all reports are consistent with this autoreceptor hypothesis (e.g., Ståhle and Ungerstedt, 1987; Ståhle, 1992 review). The most recent challenge to this notion offers the alternative hypothesis that a post-synaptic subpopulation of dopamine D_2 receptors (possibly the D_3 molecular isoform of the receptor) can mediate decreases in spontaneous locomotor activity. This is based on the evidence that purported antagonists with selectivity for the dopamine D_3 (i.e., at least in vitro) vs. the D_2 receptor increase locomotor activity without affecting dopamine release or utilization (Waters et al., 1993; Svensson et al., 1994).

Several years ago, we reported on dihydrexidine ((±)-*trans*-10,11-dihydroxy-5,6,6*a*,7,8,12*b*-hexahydrobenzo[*a*]-phenanthridine), the parent compound of a novel class of dopamine receptor agonists (Lovenberg et al., 1989). It is now known that both the D_1 and D_2 affinity and functional potency reside in the (+)-enantiomer (Knoerzer et al., 1994). Although originally designed as a ligand for the dopamine D_1-like receptor, it was found that dihydrexidine and several of its analogs also were high affinity ligands for dopamine D_2-like receptors as well. Of particular interest was the fact that dihydrexidine and other structural analogs could functionally activate post-synaptic dopamine D_2 receptors in striatum and pituitary, yet displayed little or no activity at release- or synthesis-modulating terminal dopamine autoreceptors (Mottola et al., 1992; unpublished observations). The unique post-synaptically selective pharmacology of hexahydrobenzo[*a*]phenanthridine ligands is supported further by a lack of agonist effects at impulse-regulating D_2 autoreceptors located on the somatodendritic membranes of dopamine neurons. These differential functional effects occur despite equivalent binding affinity for pre- and post-synaptic receptor sites (unpublished observations).

The present work used the *N*-*n*-propyl analog of (±)-dihydrexidine to assess the effects of selective activation of post-synaptic dopamine D_2 receptors on unconditioned behaviors in rats. Binding studies had shown that (±)-*N*-*n*-propyl-dihydrexidine has 10-fold selectivity for native dopamine D_2 receptors vs. D_1 receptors in rat striatum ($K_{0.5}$ approx. 26 nM vs. 325 nM, respectively, Mottola et al., 1992), and also has high affinity for the cloned dopamine D_3 receptor ($K_{0.5}$ approx. 5 nM, Watts et al., 1993). The purpose of the present study was to determine whether the unique functional selectivity of (±)-*N*-*n*-propyl-dihydrexidine for post-synaptic vs. pre-synaptic dopamine D_2 receptors would induce a linear dose-dependent increase in spontaneous locomotor activity and oral stereotypy as predicted by the autoreceptor hypothesis of dopamine receptor agonist effects on unconditioned behaviors.

To our surprise, we observed just the opposite effect: systemic administration of (±)-*N*-*n*-propyl-dihydrexidine in unhabituated, drug-naive rats induced a linear dose-dependent decrease in spontaneous locomotor activity, and did not induce oral stereotypy typically seen after administration of high doses of dopamine receptor agonists. In addition, we observed significant dose-dependent increases in yawning and vacuous chewing, behaviors usually observed after activation of dopamine D_2 autoreceptors and D_1 receptors, respectively. These data support the hypothesis that a sub-population of post-synaptic dopamine D_2 receptors (possibly the D_3 receptor) mediate suppression of spontaneous locomotor activity. The activation of these receptors also appears to be important in potentiating yawning and vacuous chewing behaviors.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Charles River, 200–300 g) were housed 4–6 per cage under standard conditions (lights on, 06:00–18:00 h; 22–23°C; humidity 30–70%), with food and water available ad libitum. All rats were drug-

naive prior to testing and were used in only one experimental session.

2.2. Drugs

(±)-*N*-*n*-Propyl-dihydrexidine was synthesized according to previously published methods (Brewster et al., 1990). It was dissolved in 0.1% ascorbic acid vehicle, and administered over a dose range (expressed as the free base) of 0.062–4.0 mg/kg s.c. (0.0002–0.0129 mmol/kg). The reference compound (−)-apomorphine (Research Biochemicals International, Natick, MA, USA) was dissolved in 0.1% ascorbic acid vehicle, and administered at a free base dose of 0.025 mg/kg and 1.0 mg/kg, s.c. For the antagonist studies, the α_2-adrenoceptor antagonist (±)-idazoxan (Research Biochemicals International) was dissolved in ultrapure water and administered at a dose of 0.5 mg/kg s.c. (+)-SCH23390 (Research Biochemicals International) and (−)-remoxipride (*S*(−)-3-bromo-*N*-((1-ethyl-2-pyrrolidinyl)-methyl)-2,6-dimethoxybenzamide; obtained from Astra Pharmaceuticals, Sodertalje, Sweden)) were dissolved in 0.1% tartaric acid vehicle and administered at doses of 0.01–0.03 mg/kg and 3–10 mg/kg s.c., respectively.

2.3. Behavior observation methodology

A computer-supported modified frequency analysis for the observation of discrete behavioral topographies was used (Lewis et al., 1985). Brief descriptions of the various behaviors recorded are presented in Table 1. This method is amenable to quantifying unexpected behaviors, although none were noted in these experiments.

In some cases, subtypes of the target behaviors were recorded. For example, distinct grooming elements (face, flank, anogenital, tail) and types of inactivity (characterized by body posture and eyes open vs. closed) were recorded. The description of vacuous chewing deserves special note. While vacuous chewing was often observed concomitantly with facial tremor, facial tremor alone was never scored as vacuous chewing. No attempt was made to quantify tongue protrusions accompanying vacuous chewing.

Animals were tested between 10:00–16:00 h in a dimly illuminated room. A trained observer blind to drug condition recorded behavioral activity immediately post-injection for a 1-h duration. Observers achieved reliability κ coefficient scores > 0.80 (Cohen, 1960) prior to this study on all behaviors scored. Four rats were observed per test session, and were not habituated to the test cages. The test cages consisted of $23 \times 23 \times 43$ cm clear polycarbonate cages turned upside-down over a wire-mesh floor placed over wood chip bedding.

A time sampling strategy was employed. An individual animal was observed for four successive 15-s periods. During each 15-s period, the presence of any of the target behavior categories was recorded. At the end of the 1-min trial, observation shifted to the next animal. Thus, each animal was observed for 1 min every 4 min, for a total of 15 trials (60 periods of 15 s) in a 1-h observation session.

Although this methodology does not allow for the measurement of absolute counts of individual behaviors (e.g., number of yawns), two separate categories were used to distinguish between continuous (occurring throughout a 15-s scoring interval, indicative of high intensity or frequency) or intermittent (occurring once or sporadically throughout a 15-s scoring interval, indicative of lower intensity or frequency) behaviors. A target behavior occurring in an individual 15-s interval could be counted as either continuous or intermittent, but never both. This judgement was made for oral behaviors (sniffing, gnawing, licking, chewing on objects) and grooming.

It is important to note that, with the observation system employed, the absolute frequency of any recorded behavior depends on the total number of observation periods. In the present case, the maximum score for a behavior that occurred during each observation period would be 60. To facilitate interpretation of the data, the absolute frequency for each behavior category was transformed to a relative frequency by dividing by the number of observation periods. This transformation accounts for the influence of the number of sampling periods on the initial frequency scores.

Table 1
Description of behavioral categories recorded during observation session

Behavioral topography	Description
Locomotion	Directed movement of four limbs
Rearing	Balancing on hind limbs not associated with grooming
Nose poking	Directing snout through holes in floor grid
Grooming	Includes normal-appearing licking and biting of any body surface as well as scratching
Inactivity	Movement of less than four limbs *and* no directed head movement throughout the scoring interval
Vacuous chewing	Large amplitude repetitive up/down jaw movements resembling chewing that is not directed to an object
Yawning	Protracted gaping of jaws so that incisors are visible
Sniffing	Head-directed movement of snout with movement of vibrissae
Licking	Protrusion of tongue to contact cage or floor
Gnawing	Chewing on floor grids
Chewing on object	Chewing directed to feces or bedding

For those behaviors that were scored for continuous/discontinuous, separate relative frequencies were calculated for the continuous and intermittent categories (e.g., continuous or intermittent grooming). In some cases (e.g., grooming) counts were combined to provide a measure of the number of intervals with any occurrences of the target behavior (independent of intensity).

2.4. (−)-Apomorphine dose effects on behavior as a standard reference

Rats ($n = 16$) were injected with 0 ($n = 4$), 0.025 or 1 mg/kg ($n = 6$ each) (−)-apomorphine. Behavioral observation began immediately and continued for a 1 h post-injection interval. These data with (−)-apomorphine are presented for comparison with (±)-*N*-*n*-propyl-dihydrexidine effects on unconditioned behaviors.

2.5. (±)-N-n-Propyl-dihydrexidine dose effects on behavior

Rats ($n = 64$) were injected with 0, 0.062, 0.12, 0.25, 0.5, 1.0, 2.0, or 4.0 mg/kg (±)-*N*-*n*-propyl-dihydrexidine ($n = 8$ per dose), and observed for a 1-h post-injection interval that began immediately following dosing.

2.6. (±)-N-n-Propyl-dihydrexidine-induced behavior: effects of selective dopamine receptor blockade

A separate experiment was conducted to determine the contribution of dopamine D_1 or D_2 receptors to the behaviors induced by (±)-*N*-*n*-propyl-dihydrexidine. Rats ($n = 60$) were pretreated by injection with either vehicle, 0.01 or 0.03 mg/kg (+)-SCH23390, or with 3 or 10 mg/kg (−)-remoxipride. Thirty minutes following this pretreatment, rats received a second injection of either vehicle or 2 mg/kg (±)-*N*-*n*-propyl-dihydrexidine. The vehicle/vehicle and vehicle/2 mg/kg (±)-*N*-*n*-propyl-dihydrexidine treatment control groups consisted of 12 subjects each. All other treatment groups consisted of 6 subjects each.

2.7. (±)-N-n-Propyl-dihydrexidine-induced behavior: effects of α_2-adrenoceptor blockade

In a separate study, rats were injected with 0.5 ($n = 4$ each) mg/kg (±)-idazoxan 5 min prior to injection of 0 or 2 mg/kg (±)-*N*-*n*-propyl-dihydrexidine to determine the contribution of α_2-adrenoceptor effects on behavior (cf., Johansen et al., 1988). The data obtained with idazoxan were compared qualitatively to those from treatment control groups (administration of vehicle alone or 2 mg/kg (±)-*N*-*n*-propyl-dihydrexidine alone) included in the earlier dose-response study. These across-experiment comparisons, although not ideal, were necessary because limitations on the amount of (±)-*N*-*n*-propyl-dihydrexidine available did not allow reassessment of the control levels in the α_2-adrenoceptor antagonist study.

2.8. Statistical analysis of data

SYSTAT for Windows (version 6.01; Systat, Evanston, IL, USA) was used to perform univariate analysis of variance (ANOVA) assessment of group differences for each dependent variable. Post-hoc tests compared drug-treatment scores with control scores by Dunnett's two-sided test. In some cases, there was significant heterogeneity of variance among groups. To maintain ANOVA robustness, degrees of freedom for the omnibus test were reduced according to recommendations by Brown and Forsythe (1951), and any subsequent post-hoc tests employed separate rather than pooled variance estimates and reduced degrees of freedom (Welch, 1938). In these cases, Bonferroni adjustment was applied to maintain alpha at the nominal 0.05 level.

3. Results

3.1. Choice of behavioral categories for data presentation

All of the data presented for oral stereotypies (gnawing, sniffing, licking and chewing on object) are derived from tallies of the high intensity, repetitive category of each of these behaviors (i.e., calculated from the number of 15 s intervals where the observer judged the behavior to occur continuously). This decision was based on the greater sensitivity of the continuous (rather than the intermittent)

Table 2
Effects of (−)-apomorphine (APO) on unconditioned behavior in rats

Behavior	Vehicle	APO	
		0.025 mg/kg	1.0 mg/kg
Locomotion	70 ± 11	31 ± 6 [a]	41 ± 11
Rearing	32 ± 3	9 ± 3 [a]	0 [a]
Nose poking	30 ± 10	6 ± 2 [a]	2 ± 2 [a]
Grooming [c]	25 ± 8	16 ± 3	0 [a]
Inactivity	0	38 ± 7 [a]	0
Vacuous chewing	0	16 ± 3 [a]	0
Yawning	0	1 ± 1	1 ± 1
Oral stereotypy			
Sniffing [b]	71 ± 13	32 ± 2 [a]	100 ± 0 [a]
Licking [b]	0	0	14 ± 9
Gnawing [b]	1 ± 1	0	59 ± 12 [a]
Chewing object [b]	2 ± 1	7 ± 2	3 ± 2

Unhabituated rats were injected s.c. with either vehicle ($n = 4$), 0.025 mg/kg ($n = 6$) or 1.0 mg/kg (−)-apomorphine ($n = 6$) and scored by treatment-blind observer. Values are mean percent frequencies ± S.E.M. for 0–20 min post-injection.

[a] $P < 0.05$ vs. vehicle condition by Dunnet's or Bonferroni multiple comparison procedure following significant univariate ANOVA.

[b] High-intensity, repetitive behavior (scored as continuous).

[c] All forms of grooming combined.

category of these behaviors to the treatments administered. The inactivity data reflect the relative frequency of sustained (scored as continuous) periods of this behavior, as required by the definition presented in Table 1. Data for grooming represent occurrences of any form of grooming, as analysis of subtypes did not prove illuminating in the present studies. Intermittent and continuous categories for each of the other behavior topographies (vacuous chewing, yawning, nosepoking, locomotion and rearing) were combined so that the data presented reflect the relative frequencies of any occurrence of each of these target behaviors. This combined analysis occurred because the relative frequencies of continuous forms of these behaviors were too low to be reliable in many treatment conditions.

3.2. Behavioral profile observed following a low or a high dose of (−)-apomorphine

Table 2 presents the data obtained following administration of either vehicle or a low or high dose of the standard mixed dopamine receptor agonist (−)-apomorphine to drug-naive, unhabituated rats. These data are presented as a benchmark to establish the suitability of the scoring system, and to provide a comparison to the effects of the novel compound (±)-*N*-*n*-propyl-dihydrexidine. Although observation was continued for 1 h following dosing, data from the initial 20-min observation period only are shown, as habituation to the novel environment occurred quickly in vehicle-treated subjects, and differences among the various treatments could be seen most clearly at the early time points prior to habituation.

Rats administered vehicle were active continuously throughout the initial 20-min observation period (i.e., inactivity = 0%). Locomotion and repetitive sniffing were the two most frequent behaviors recorded. Rearing, grooming and nose poking occurred with moderate frequency. Licking, gnawing, vacuous chewing and chewing on objects were absent or rare.

The behavioral profiles observed with the two doses of (−)-apomorphine differed markedly from one another and from the vehicle condition. At the lower dose of (−)-apomorphine (0.025 mg/kg), there was a depression in a

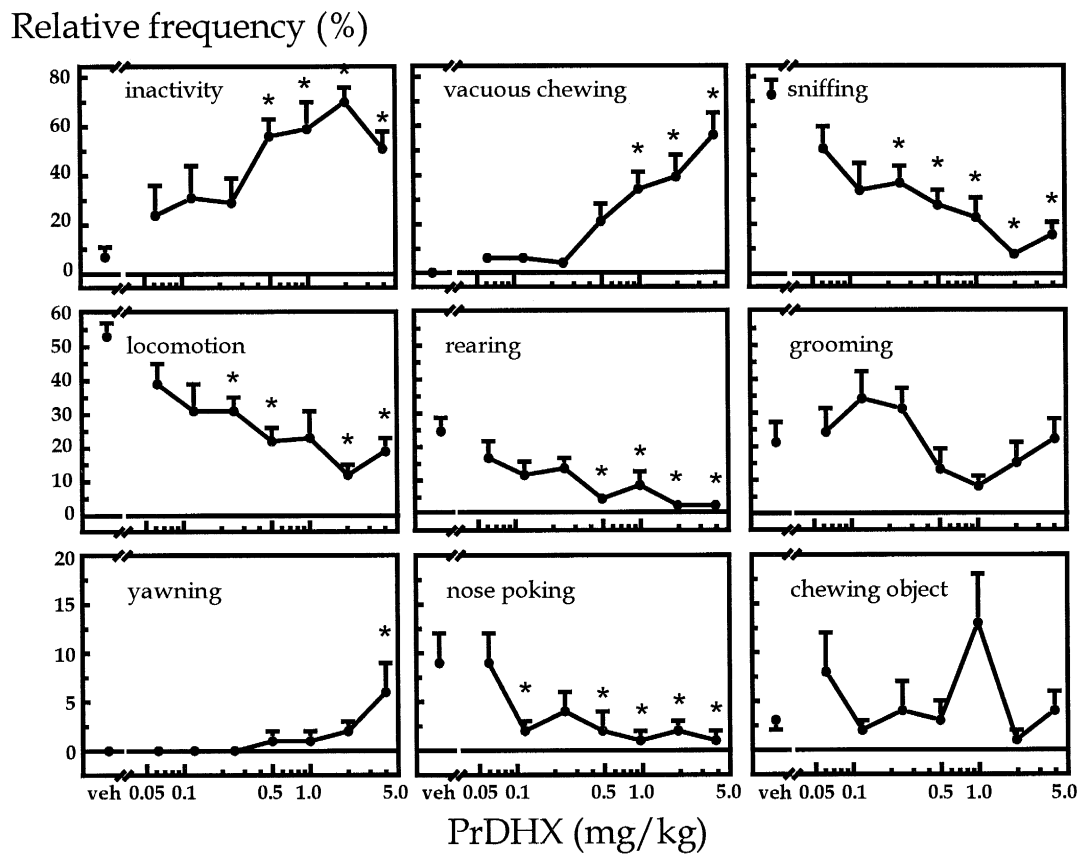

Fig. 1. Behavioral profile induced by administration of the novel dopamine D_2 receptor agonist (±)-*N*-*n*-propyl-dihydrexidine (PrDHX). Unhabituated, drug-naive male rats ($n = 6$ for each condition) were administered vehicle or (±)-*N*-*n*-propyl-dihydrexidine (0.062–4.0 mg/kg s.c.) and behaviors recorded by a treatment-blind observer during the 20-min period immediately following drug administration. Values are mean relative frequencies and their standard errors. Note the change in ordinate scale for the top, middle and lower panels to accommodate differing maximal frequencies of the behaviors depicted. Sniffing data presented are the relative frequencies of high intensity, repetitive (continuous) form of this behavior. Grooming data reflect relative frequency of any subtype of grooming. * $P < 0.05$ vs. vehicle condition by Dunnett's multiple comparison procedure applied following significant univariate ANOVA.

number of behavioral elements compared to the vehicle condition. Rearing, locomotion, repetitive sniffing and nose poking were reduced significantly. There was both a large increase in inactivity and a moderate increase in vacuous chewing. In contrast, rats administered the higher dose of (−)-apomorphine showed no periods of inactivity or vacuous chewing, and there were large increases in repetitive sniffing and gnawing. Repetitive licking was increased moderately, although this increase failed to achieve significance. There were also significant reductions in several behaviors at the high dose of (−)-apomorphine, including rearing, grooming and nose poking, when compared to the vehicle condition.

3.3. Dose–response effects of (±)-N-n-propyl-dihydrexidine on unconditioned behavior

Fig. 1 illustrates the data obtained in a separate experiment after unhabituated, drug-naive rats were injected with test doses of (±)-*N*-*n*-propyl-dihydrexidine ranging from 0.062–4.0 mg/kg s.c. or vehicle. Rats that received (±)-*N*-*n*-propyl-dihydrexidine expressed significant dose-dependent increases in the frequency of inactivity. Inactivity was not found to be associated with any characteristic body posture or eyelid tone (eyes open, closed and ptosis were equally observed; data not shown). The inactivity caused by (±)-*N*-*n*-propyl-dihydrexidine was comparable visually, as well as in frequency, to inactivity induced by the low dose of (−)-apomorphine (Table 2). The increase in inactivity observed with (±)-*N*-*n*-propyl-dihydrexidine was accompanied by significant dose-dependent decreases in sniffing, locomotion, rearing and nosepoking. There was a dramatic increase in vacuous chewing at the higher doses of (±)-*N*-*n*-propyl-dihydrexidine. In addition, at the highest dose of (±)-*N*-*n*-propyl-dihydrexidine tested, a significant increase in the frequency of yawning was observed. Chewing on objects and grooming were not affected systematically by (±)-*N*-*n*-propyl-dihydrexidine. Licking and gnawing were rare in all treatment conditions (% less than 5; data not shown). When taken together, the behavioral profile induced over the entire dose range of (±)-*N*-*n*-propyl-dihydrexidine corresponds closely to that seen with the low dose of (−)-apomorphine.

3.4. Effects of dopamine receptor antagonists on behaviors induced by (±)-N-n-propyl-dihydrexidine

In order to assess the relative contribution of dopamine D_1 and D_2 receptor activation with respect to the behavioral effects of (±)-*N*-*n*-propyl-dihydrexidine, a separate group of animals were administered either the D_1 selective antagonist, (+)-SCH23390, or the D_2 selective antagonist,

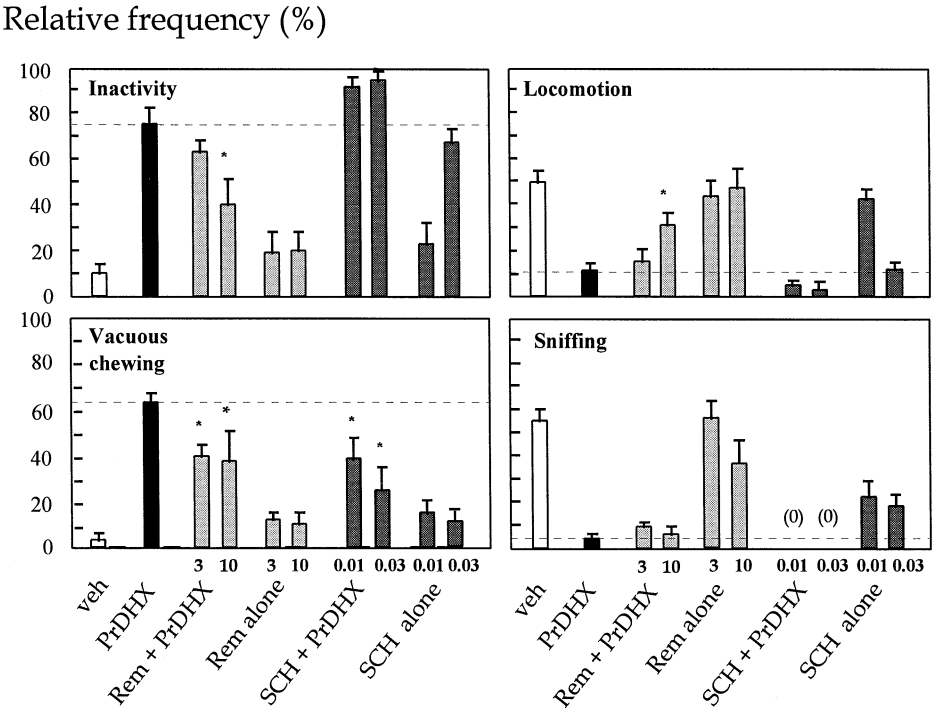

Fig. 2. Effects of dopamine D_1 and D_2 receptor antagonists on behaviors induced by (±)-*N*-*n*-propyl-dihydrexidine (PrDHX). Unhabituated drug-naive male rats were pretreated s.c. with either (−)-remoxipride (Rem; dopamine D_2 receptor antagonist) or (+)-SCH23390 (SCH; dopamine D_1 receptor antagonist) 30 min prior to receiving a 2 mg/kg s.c. dose of *N*-*n*-propyl-dihydrexidine. Behaviors were recorded by a treatment-blind observer during the 20-min period following (±)-*N*-*n*-propyl-dihydrexidine dosing. Values are mean relative frequencies ± S.E.M. The numbers below the *x*-axis refer to the antagonist drug dose in mg/kg. Results of statistical comparisons between (±)-*N*-*n*-propyl-dihydrexidine alone and combined with dopamine D_1 or D_2 receptor antagonists are indicated. Sniffing data presented are the relative frequencies of the high intensity, repetitive (continuous) form of this behavior.
* $P < 0.05$ vs. PrDHX alone by Dunnett's or Bonferroni multiple comparison procedure.

Table 3
Effects of the α_2-adrenoceptor antagonist (±)-idazoxan on behaviors induced by (±)-*N*-*n*-propyl-dihydrexidine

Behavior	Idazoxan alone	Idazoxan + PrDHX	PrDHX alone (from Fig. 1)	Vehicle (from Fig. 1)
Locomotion	81 ± 4	35 ± 9	12 ± 3	53 ± 4
Rearing	34 ± 5	5 ± 2	2 ± 1	24 ± 4
Nose poking	15 ± 5	11 ± 5	2 ± 1	8 ± 3
Grooming [b]	22 ± 7	18 ± 7	15 ± 6	21 ± 6
Inactivity	0	46 ± 11	70 ± 6	7 ± 4
Vacuous chewing	2 ± 3	41 ± 12	39 ± 9	0
Yawning	0	1 ± 1	2 ± 1	0
Oral stereotypy				
Sniffing [a]	84 ± 6	21 ± 6	8 ± 3	72 ± 6
Licking [a]	0	0	0	0
Gnawing [a]	0	0	1 ± 1	2 ± 1
Chewing object [a]	1 ± 1	1 ± 1	1 ± 1	3 ± 1

Unhabituated drug-naïve, male rats were injected s.c. with 0.5 mg/kg (±)-idazoxan followed in 5 min by vehicle ($n = 4$) or (±)-*N*-*n*-propyl-dihydrexidine (PrDHX; 2.0 mg/kg; $n = 4$) and behaviors were recorded by a treatment-blind observer. For comparison purposes, data from the previous dose–response study (Fig. 1) are included in the table (rats treated with 2.0 mg/kg (±)-*N*-*n*-propyl-dihydrexidine alone or vehicle). Values are mean percent frequencies ± S.E.M. for 0–20 min post-injection.
[a] High-intensity, repetitive behavior (scored as continuous).
[b] All forms of grooming combined.

(−)-remoxipride, prior to receiving (±)-*N*-*n*-propyl-dihydrexidine. Fig. 2 presents results obtained for inactivity, locomotion, vacuous chewing and repetitive sniffing. These behaviors were selected for illustration because they represent ones that were affected most clearly when (±)-*N*-*n*-propyl-dihydrexidine was administered alone in the previous dose-response study (see Fig. 1). (−)-Remoxipride was able to attenuate significantly the inactivity, vacuous chewing, and locomotor inhibition induced by 2 mg/kg (±)-*N*-*n*-propyl-dihydrexidine. (+)-SCH23390 was without effect on (±)-*N*-*n*-propyl-dihydrexidine-induced inactivity or locomotor inhibition, but did attenuate vacuous chewing behavior (Fig. 2). Note that the higher dose of 0.03 mg/kg (+)-SCH23390, while not affecting the inactivity produced by (±)-*N*-*n*-propyl-dihydrexidine, was by itself 'sedating'. Neither the dopamine D_1, nor the D_2 receptor, antagonist blocked the reduction in repetitive sniffing induced by (±)-*N*-*n*-propyl-dihydrexidine. The modest amount of yawning induced by 2.0 mg/kg (±)-*N*-*n*-propyl-dihydrexidine, 3.8 ± 1.8% (S.E.M.), was abolished by the lowest dose of remoxipride (mean of 0.0%; $P < 0.05$) but was not affected significantly by the higher dose of remoxipride or by either dose of the D_1 antagonist (data not shown).

3.5. Effects of α_2-adrenoceptor blockade on behaviors induced by (±)-*N*-*n*-propyl-dihydrexidine

It is known that (±)-*N*-*n*-propyl-dihydrexidine has some, albeit weak, α_2-adrenoceptor activity in vitro (i.e., $K_{0.5}$ approx. 200 nM; Mottola et al., 1992). To verify that the effects of (±)-*N*-*n*-propyl-dihydrexidine were not influenced by adrenoceptors, a separate group of animals were administered either the α_2-adrenoceptor-selective antagonist (±)-idazoxan alone, or (±)-idazoxan, prior to receiving 2 mg/kg (±)-*N*-*n*-propyl-dihydrexidine. As shown in Table 3, the behavioral profile induced by (±)-idazoxan plus (±)-*N*-*n*-propyl-dihydrexidine was similar qualitatively to that observed with (±)-*N*-*n*-propyl-dihydrexidine alone in the prior dose-response study (data taken from Fig. 1). That is, rats administered (±)-*N*-*n*-propyl-dihydrexidine combined with (±)-idazoxan showed large reductions in locomotion, rearing and repetitive sniffing, and large increases in inactivity and vacuous chewing. It should be noted that quantitative comparisons between the effects of (±)-*N*-*n*-propyl-dihydrexidine alone, and (±)-*N*-*n*-propyl-dihydrexidine plus (±)-idazoxan, were not possible because the treatments were not included within the same study due to limitations on the amount of (±)-*N*-*n*-propyl-dihydrexidine available.

4. Discussion

The present findings provide a comprehensive description of the behavioral effects of the novel dopamine receptor agonist (±)-*N*-*n*-propyl-dihydrexidine. Administration of this compound induced marked increases in inactivity and vacuous chewing and decreases in a number of other specific behavioral elements, including locomotion, rearing and repetitive sniffing. Increased yawning was seen at the highest dose of (±)-*N*-*n*-propyl-dihydrexidine. Oral stereotypies (licking and gnawing) were notably absent. The use of dopamine receptor subtype-selective antagonists confirmed the dopaminergic basis for the prominent behavioral effects of this novel agonist. The increased inactivity induced by (±)-*N*-*n*-propyl-dihydrexidine was mediated by dopamine D_2 receptors, while both dopamine D_1 and

D_2 receptors contributed to the observed increases in vacuous chewing. Taken together, the behavioral effects observed with (±)-*N*-*n*-propyl-dihydrexidine resembled those obtained with a low dose of the traditional dopamine D_2 receptor agonist (−)-apomorphine.

The behavioral profile observed with (±)-*N*-*n*-propyl-dihydrexidine is of special relevance for evaluating traditional concepts concerning the neurobiological substrate for dopamine receptor-mediated behaviors. The modification of spontaneous locomotion and oral behaviors in rats by dopamine D_2 receptor agonists is typically biphasic, as is the case for (−)-apomorphine. Electrophysiological data indicating that dopamine D_2 autoreceptors are functionally more sensitive to low doses of dopamine agonists than are post-synaptic dopamine D_2 receptors initiated an autoreceptor hypothesis to explain the biphasic dose effects of dopamine receptor agonists on unconditioned behavior (Strömbom, 1976; Walters and Roth, 1976; Westfall et al., 1976; Skirboll et al., 1979; Costall et al., 1981). This hypothesis has been supported further by data indicating that agonists with functional selectivity for dopamine D_2 autoreceptors induce monotonic decreases in locomotor activity (Hjorth et al., 1981; Pugsley et al., 1992; Nisoli et al., 1993; however, note that some data on 'autoreceptor-selective' agonists have been determined later to be the result of multiple pharmacological effects, e.g., Johansen et al., 1988; Ferrari and Giuliani, 1993).

When viewed against this background, the behavioral effects obtained with *N*-*n*-propyl-dihydrexidine would be considered as indicative of actions exclusively at dopamine D_2 autoreceptors. Yet this conclusion is at odds with other data indicating that hexahydrobenzo[*a*]phenanthridine ligands (e.g., (±)-*N*-*n*-propyl-dihydrexidine) are dopamine receptor agonists that have little functional activity at dopamine D_2 autoreceptors while having potent functional activity at post-synaptic dopamine D_2 receptors. For example, administration of either (±)-dihydrexidine or its *N*-*n*-propyl analog reduces serum prolactin levels to an extent equivalent to that of the full agonist (−)-quinpirole, actions that are mediated by dopamine D_2 heteroreceptors located on pituitary lactotrophs. Likewise, members of the hexahydrobenzo[*a*]phenanthridine class produce full inhibition of the enzyme adenylate cyclase in models that reflect dopamine D_2 postsynaptic receptor function primarily (e.g., cAMP efflux in superfused striatal slices). Conversely, these same compounds display little or no efficacy at dopamine D_2 receptors controlling dopamine release or cell firing in substantia nigra. For example, *N*-*n*-propyl dihydrexidine has minimal effects on dopamine release as measured using in vivo voltammetry in striatal slices. Finally, it is of special interest that the differing functional profiles of this novel class of agonists at dopamine D_2 pre- vs. post-synaptic receptors occur despite equal binding affinity to each receptor population (unpublished observations).

Given the evidence that (±)-*N*-*n*-propyl-dihydrexidine does not activate any of the autoreceptor-linked processes leading to decreased dopaminergic transmission, the dose effects of this agonist on unconditioned behavior are not consistent with an autoreceptor hypothesis. Decreased locomotor activity was shown to be dopamine D_2 receptor-mediated, therefore we conclude that decreases in spontaneous locomotor activity can be induced by post-synaptic dopamine D_2 receptor activation. This is important since drug effects on spontaneous locomotor activity are routinely used as a pharmacological screen for determining autoreceptor vs. post-synaptic dopamine D_2 receptor activation; our data advocate extreme caution in using locomotor activity as a measure of functional selectivity for novel agonists.

There have been some data in the literature to suggest that post-synaptic dopamine D_3 receptors might have functional significance in decreasing spontaneous locomotor activity in rats. The evidence for this is limited for two reasons: (1) there are no highly selective dopamine D_3 receptor ligands, yet there is a relatively larger expression of dopamine D_2 receptors in the relevant brain regions, thus complicating interpretation, particularly in vivo (Landwehrmeyer et al., 1993); and (2) the same inavailability of a highly selective dopamine D_3 ligand has prevented learning about the biochemical endpoints that dopamine D_3 receptor activation mediates in vivo.

Nevertheless, some classes of dopamine agonists with high affinity for the cloned dopamine D_3 receptor have been shown to decrease spontaneous locomotor activity over a larger dose range than other agonist classes (e.g., (±)-7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (7-OHDPAT); Daly and Waddington, 1993a; Svensson et al., 1994). The decreased locomotor activity caused by (±)-7-OHDPAT occurs over a dose range at which terminal dopamine release is not affected (Svensson et al., 1994), suggesting post-synaptic mechanisms. Yet other structural classes having equivalent high dopamine D_3 receptor affinity show discretely biphasic effects on locomotor activity ((−)-quinpirole; Johansson et al., 1987)). A novel ligand (U99194A), determined to be a dopamine D_3 receptor antagonist based on its affinity in vitro and the steep slope of its binding curve, has been shown to increase locomotor activity in rats without altering terminal dopamine release, consistent with the hypothesis that post-synaptic dopamine D_3 receptors have functional significance in decreasing locomotor activity (Waters et al., 1993). The high affinity of (±)-*N*-*n*-propyl-dihydrexidine for the D_3 receptor (Watts et al., 1993), and its functional selectivity for post-synaptic vs. pre-synaptic dopamine D_2 receptors, are consistent with a hypothesis that post-synaptic dopamine D_3 receptors functionally mediate decreases in spontaneous locomotor activity.

Yawning behavior also has been hypothesized to be mediated by selective stimulation of dopamine D_2 autoreceptors (Gower et al., 1984; Yamada et al., 1986). Our data indicate that high doses of (±)-*N*-*n*-propyl-dihy-

drexidine, functionally activating post-synaptic dopamine D_2 receptors, significantly increase the frequency of yawning observed in rats. This supports other evidence that yawning is not exclusively associated with the activation of dopamine D_2 autoreceptors (e.g., Morelli et al., 1986; Ståhle, 1992). (±)-7-OHDPAT has been shown to increase yawning frequency within the same dose range as it decreases terminal dopamine release (Damsma et al., 1993). Taken together with our data, this suggests that both autoreceptors and post-synaptic dopamine D_2/D_3 receptors have functional significance in mediating yawning.

An unexpected result was the potent dose-dependent increase in vacuous chewing observed with (±)-*N*-*n*-propyl-dihydrexidine. This became a high-intensity behavior (i.e., nearly continuous throughout observation time) at doses equal to or higher than 2 mg/kg. Vacuous chewing is not in the category of oral stereotypies typically observed in the high dose range of dopamine D_2 receptor agonists (e.g., see Table 2 for high-dose (−)-apomorphine oral stereotypies quantified). Moreover, vacuous chewing is typically associated with acute administration of dopamine D_1 receptor agonists (Rosengarten et al., 1983, 1993; Daly and Waddington, 1993b) or the chronic administration of dopamine D_2 receptor antagonists (for review, see Waddington, 1990). Interestingly, our antagonist studies for (±)-*N*-*n*-propyl-dihydrexidine effects on vacuous chewing behavior provide evidence that both dopamine D_1 and D_2 receptors contribute to the potentiation of this behavior. This is in distinct contrast to most of the literature on acutely induced, dopamine D_1 receptor-mediated vacuous chewing, in which an antagonistic functional interaction between dopamine D_1 and D_2 receptors is observed (Rosengarten et al., 1983, 1986, 1993; Johansson et al., 1987; Molloy and Waddington, 1988; Daly and Waddington, 1993b). Currently available pharmacological ligands have limited selectivity for distinguishing between molecular isoforms of the dopamine D_1 (D_1 and D_5) and D_2 (D_2, D_3 and D_4) receptor families. With the introduction of new ligands that are highly selective for molecular subtypes of the receptor, conflicting issues of dopamine D_1–D_2 receptor functional interaction may be clarified by a discovery that certain sub-populations of the dopamine receptor families have antagonistic, while others have cooperative/synergistic, functional interactions.

In summary, these findings demonstrate that locomotor inhibition and yawning, often considered as markers of dopamine D_2 presynaptic receptor activation, can occur following administration of a novel dopamine receptor agonist that does not produce the neurochemical changes (e.g., changes in dopamine release) that have been linked to activation of dopamine D_2 presynaptic receptors. Although the mechanism for the unusual profile of this compound has not been elucidated, a role for postsynaptic dopamine D_2-like receptor subpopulations (D_3 receptors) is hypothesized. These findings lend weight to recent challenges (for review, see Ståhle, 1992) to established ideas about the behavioral consequences of dopamine D_2 presynaptic receptor function. In a similar vein, the unexpected emergence of vacuous chewing observed with *N*-*n*-propyl-dihydrexidine should prompt efforts to reformulate current concepts concerning the role of the dopamine D_1 and D_2 receptor subtypes in the generation of this behavior.

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