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Short communication

Dopamine D_2 receptors play a role in the (−)-apomorphine-like discriminative stimulus effects of (+)-PD 128907

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Abstract

The discriminative stimulus effects of the dopamine D_3 receptor-preferring agonist, *S*(+)-(4*aR*,10*bR*)-3,4,4*a*,10*b*-tetrahydro-4-propyl-2*H*,5*H*-[1]benzopyrano-[4,3-*b*]-1,4-oxazin-9-ol ((+)-PD 128907), were examined in rats trained to discriminate (−)-apomorphine (0.16 m/kg) from saline in a two-lever, fixed-ratio 10 drug-discrimination paradigm. Both (−)-apomorphine and (+)-PD 128907 produced dose-related (−)-apomorphine-lever selection, with full substitution observed at 0.16 mg/kg, i.p. (ca. 0.5 μmol/kg). Drug-appropriate responding produced by either (−)-apomorphine or (+)-PD 128907 was antagonized by the putative dopamine D_3 receptor antagonists, (1*S*,2*R*)-*cis*-5-methoxy-1-methyl-2-(*n*-propylamino)tetralin ((+)-AJ76) and *cis*-(+)-5-methoxy-1-methyl-2-(di-*n*-propylamino)tetralin ((+)-UH 232), as well as by the dopamine D_2 receptor antagonist haloperidol. Because haloperidol was approximately 30–150-times more potent than (+)-AJ76 or (+)-UH-232 in blocking the effects of either receptor agonist, the results indicate that dopamine D_2 receptors play a role in the discriminative stimulus effects of (+)-PD 128907.

Keywords: Haloperidol; (+)-AJ76; (+)-UH-232; (+)-PD 128907; (−)-Apomorphine; Discriminative stimulus effect; Dopamine D_3 receptor

1. Introduction

The dopamine D_3 receptor-preferring agonists 7-hydroxy-dipropylaminotetralin ((±)-7-OH-DPAT) and *S*(+)-(4*aR*,10*bR*)-3,4,4*a*,10*b*-tetrahydro-4-propyl-2*H*,5*H*-[1]benzopyrano-[4,3-*b*]-1,4-oxazin-9-ol ((+)-PD 128907) reportedly produce hypolocomotion at low doses and hyperlocomotion at higher doses, with the former effect attributed to the stimulation of a postsynaptic dopamine D_3 receptor that inhibits locomotion (Pugsley et al., 1995; Svensson et al., 1994; Waters et al., 1993). These compounds were once held to be highly selective dopamine D_3 receptor ligands, with reported D_3:D_2 receptor selectivity ratios as high as, in the case of (+)-PD 128907, 1000-fold (DeMattos et al., 1993). However, because this high separation was based on binding affinities for low-affinity receptor agonist sites, i.e., conditions which may not represent receptor affinity states in vivo, their actual selectivity may vary considerably such that their behavioral effects may not be easily differentiated (Starr and Starr, 1995). Indeed, when affinities at high-affinity dopamine D_2 and D_3 sites are compared, (+)-PD 128907 exhibits considerably lower selectivity, i.e., approximately 14-fold higher affinity for dopamine D_3 sites (Pugsley et al., 1995). Similarly, when in vitro functional effects are compared, the reported selectivity ratios for (+)-PD 128907 vary between approximately 6 (Pugsley et al., 1995) and 54 (Sautel et al., 1995a). Thus, it is conceivable that some behavioral effects of this putative dopamine D_3 receptor agonist may be mediated by dopamine D_2 receptors.

The purpose of this study was to characterize in vivo behavioral effects of the novel dopamine D_3 receptor-preferring agonist (+)-PD 128907 in rats trained to discriminate the dopamine D_2-like receptor agonist (−)-apomorphine from saline. Prior pharmacological characterizations of this discrimination indicate that it is mediated by central dopamine D_2 receptors (Colpaert et al., 1976; Tang and Franklin, 1987), and, as such would allow the demonstration of dopamine D_2-mediated behavioral effects of putative selective dopamine D_3 receptor agonists. However, reports that (−)-apomorphine exhibits dopamine D_3 receptor affinity (Sautel et al., 1995a; Sokoloff et al.,

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1990) suggest the possibility that dopamine D_3 receptors may also play a role. In this study, the ability of the dopamine D_2 receptor-preferring antagonist, haloperidol, to block the effects of (−)-apomorphine and (+)-PD 128907 was compared to that of the putative dopamine D_3 receptor-preferring antagonists, (1*S*,2*R*)-*cis*-5-methoxy-1-methyl-2-(*n*-propylamino)tetralin ((+)-AJ76) and *cis*-(+)-5-methoxy-1-methyl-2-(di-*n*-propylamino)tetralin ((+)-UH 232). Although the involvement of dopamine D_3 receptors in the discriminative stimulus effects of 7-OH-DPAT and (+)-PD 128907 has been implicated on the basis of their high in vitro selectivity (Acri et al., 1995; McElroy, 1994), the results of the present study suggest the possibility that, in vivo, dopamine D_2 receptors may also play an important role.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Ico: OFA SD (I.O.P.S. Caw) Iffa Credo, France), weighing between 240 and 260 g at the beginning of the studies were used. Animals were housed in air-conditioned rooms (temperature: 21 ± 1°C; hygrometric degree: 55 ± 5%) with lighting on from 7:00 to 19:00 h. Filtered (0.22 μm) water was freely available, but access to standard laboratory food (A04, 4AR, Epinay sur Orge, France) was limited to 10 g per day, except during weekends when food was freely available between 17:00 h Friday and 14:00 h Sunday. Animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication No. 85-23, revised 1985) and the experimental protocol (No. 009) was carried out in accordance with French law and the local ethical committee guidelines for animal research.

2.2. Apparatus

Experiments were conducted in standard operant conditioning chambers (model E10-10, Coulbourn Instruments, Lehigh Valley, PA, USA) housed in light- and sound-attenuating enclosures that were ventilated by a fan, which also produced a masking noise. Each chamber contained a houselight that was mounted above a food pellet receptacle located between two levers, which were situated 2.5 cm above the grid floor. Food pellets (45 mg dustless pellets, Bioserv, Frenchtown, NJ, USA) were delivered by a pellet dispenser (model E14-12, Coulbourn Instruments). Scheduling of reinforcement contingencies, reinforcement delivery and data recording were controlled by a SKED-11 system (State Systems, Kalamazoo, MI, USA) implemented on a PDP-11 computer (Digital Equipment Corporation, Maynard, MA, USA).

2.3. Procedure

Rats were trained to discriminate (−)-apomorphine (0.16 mg/kg, i.p.) from saline in a two-lever, food-reinforced fixed-ratio 10 drug-discrimination paradigm using methods identical to those described recently (Koek et al., 1995). Briefly, (−)-apomorphine was administered 15 min prior to sessions during which responding on one of two levers, depending upon either saline or drug administration, was reinforced. Discrimination training was continued until less than three responses were made on the injection-inappropriate lever before the first food presentation, during ten consecutive sessions.

Test sessions were conducted on Wednesdays and/or Fridays, while training continued on intervening days. During test sessions, the lever on which 10 responses accumulated first was defined as the selected lever. After lever selection, the animal received the first food pellet, and subsequent reinforcement was made contingent upon pressing the selected lever. A test session ended after 15 min. Testing was postponed to the next scheduled test day if, on either of the two most recent training days, the number of injection-inappropriate responses before the first food presentation exceeded 15. Also, test data were discarded and the test condition later retested if the test session was followed by a training session of which the number of injection-inappropriate responses before the first food presentation exceeded 15.

2.4. Analysis of data

Test sessions generated data on the following two variables: (1) the selected manipulandum, i.e., saline lever or drug lever, representing the measure of discriminative responding, and (2) the response rate, i.e., the total number of responses made on either lever during the 15 min session expressed as a percentage of the response rate during the most recently preceding saline training session. Selection data were used to calculate the percentage of animals at each treatment condition selecting the drug lever. Drug effects on this variable (i.e., slope and potencies) were analyzed by means of the Litchfield and Wilcoxon procedure (Tallarida and Murray, 1987), implemented using the research programming language, RS/1 (Bolt, Beranek and Newman, Cambridge, MA, USA), to estimate ED_{50} values and 95% confidence limits. When less than two intermediate effects were observed, 0 and/or 100% effects were transformed by means of Berkson's adjustment (Hubert, 1984) to permit the use of the Litchfield and Wilcoxon procedure. The order of treatment with individual drugs and doses was unsystematic.

2.5. Drugs

(+)-AJ76 HCl and (+)-UH-232 maleate were obtained from Tocris Cookson (Bristol, UK), and haloperidol and

(−)-apomorphine HBr from Research Biochemicals International (Natick, MA, USA). (+)-PD 128907 HCl was initially obtained from Research Biochemical International, and later synthesized by J. Maurel (Centre de Recherche Pierre Fabre). All drugs were dissolved in distilled water and injected i.p. or s.c. in a volume of 10 ml/kg, with doses expressed as the weight of the free base.

3. Results

3.1. Substitution for (−)-apomorphine

As shown in Fig. 1, (−)-apomorphine and (+)-PD 128907 produced dose-related substitution for the training dose of (−)-apomorphine over nearly identical dose ranges (0.01–0.16 mg/kg, i.p.) and the highest dose (0.16 mg/kg) of both agonists engendered 100% drug-lever selection. The estimated ED_{50} values were nearly identical for (−)-apomorphine (0.068 mg/kg; 95% confidence limits: 0.042–0.11) and for (+)-PD 128907 (ED_{50} 0.055 mg/kg; 95% confidence limits: 0.021–0.15).

Significant decreases in rates of responding were observed after administration of the training dose of (−)-apomorphine (35 ± 10% of control; mean ± S.E.M.; $P < 0.05$ vs. control) whereas the highest dose of (+)-PD 128907 (0.16 mg/kg) did not significantly alter response rate (73 ± 18% of control).

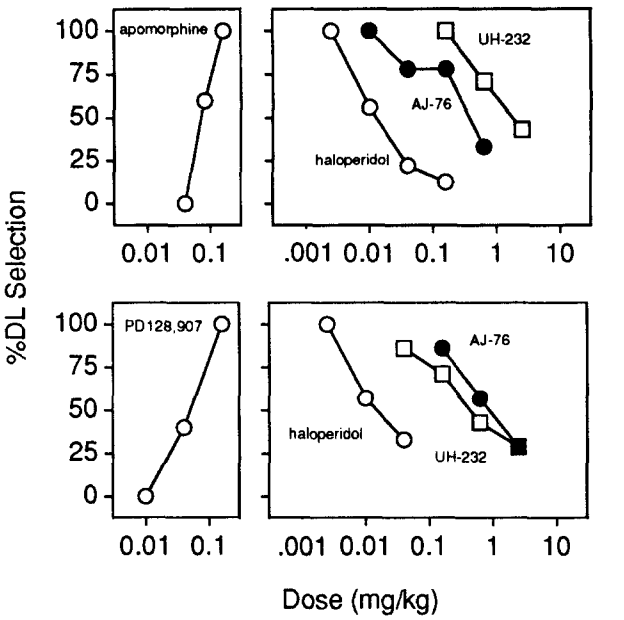

Fig. 1. Effects of haloperidol, (+)-AJ76 and (+)-UH-232 on drug-lever selection produced by (−)-apomorphine (upper panels) and (+)-PD 128907 (bottom panels) in rats ($n = 7–9$/group) trained to discriminate (−)-apomorphine (0.16 mg/kg) from saline. Left panels: Animals were treated with (−)-apomorphine (0.04–0.16 mg/kg) or (+)-PD 128907 (0.01–0.16 mg/kg) i.p., 15 min before test sessions. Right panels: Animals were treated with haloperidol (○), (+)-AJ76 (●), or (+)-UH-232 (□) s.c. 60 min before the session in combination with 0.16 mg/kg (−)-apomorphine or (+)-PD 128907, administered 15 min before the session.

3.2. Antagonism of (−)-apomorphine-lever selection

Haloperidol (Fig. 1, open circles) dose-dependently decreased drug-lever selection engendered by (−)-apomorphine and (+)-PD 128907. Similarly, the putative dopamine D_3 receptor-selective antagonists (+)-AJ76 and (+)-UH-232 dose dependently blocked the discriminative stimulus effects of both (−)-apomorphine and (+)-PD 128907 along dose-response functions that, according to the Litchfield and Wilcoxon procedure, differed significantly in potency, but not slope, from that produced by haloperidol. According to the estimated ED_{50} values shown in Table 1, (+)-AJ76 and (+)-UH-232 were, respectively, 36- and 134-times less potent than haloperidol in blocking the discriminative stimulus effects of (−)-apomorphine. Similarly, (+)-AJ76 and (+)-UH-232 were both more than 30-times less potent than haloperidol in blocking drug-lever selection produced by (+)-PD 128907.

Pretreatment with the dopamine antagonists, haloperidol, (+)-AJ76 or (+)-UH-232, did not significantly reverse the rate-decreasing effects of (−)-apomorphine; conversely, haloperidol decreased (−)-apomorphine-lever selection only at doses that appeared to have additional, rate-decreasing effects. Effects of the antagonists on response rate precluded testing higher doses.

Table 1
Antagonism by dopamine D_2-like receptor antagonists of the ability of (−)-apomorphine and (+)-PD 128907 to engender drug-lever selection in rats trained to discriminate (−)-apomorphine from saline

Antagonist	Affinity (nmol) [a]		Agonist					
	D_2	D_3	(−)-Apomorphine			(+)-PD 128907		
			ED_{50} (mg/kg)	95% C.L. (mg/kg)	ED_{50} (μmol/kg)	ED_{50} (mg/kg)	95% C.L. (mg/kg)	ED_{50} (μmol/kg)
Haloperidol	0.45	9.8	0.018	0.0064–0.050	0.047	0.018	0.0062–0.054	0.049
(+)-AJ76	270	91	0.39	0.16–0.91	1.7	0.87	0.16–4.6	3.7
vs. haloperidol	**600**	**9**			**36**			**76**
(+)-UH232	40	9.2	1.7	0.64–4.7	6.3	0.50	0.11–2.2	1.8
vs. haloperidol	**89**	**1**			**134**			**37**

[a] Data from Sokoloff et al. (1990).

4. Discussion

An important finding of this study is that the dopamine D_2 receptor-selective antagonist, haloperidol, potently and dose-dependently blocked the ability of the dopamine D_2-like receptor agonist, (+)-PD 128907, to substitute for the discriminative stimulus effects of (−)-apomorphine. In contrast, the putative dopamine D_3 receptor-selective antagonists, (+)-AJ76 or (+)-UH-232 (Sokoloff et al., 1990), were approximately 30–130 times less potent than haloperidol in blocking the effects of either the training drug, (−)-apomorphine, or (+)-PD 128907. Such large differences in potency between haloperidol and the putative dopamine D_3 receptor-selective antagonists more closely approximate their reported dopamine D_2 receptor affinities relative to that of haloperidol (89 and 600, (+)-AJ76 and (+)-UH232, respectively, Table 1) than their relative dopamine D_3 affinities (1- to 10-fold), therefore indicating that the ability of (+)-PD 128907 to produce drug-lever selection in (−)-apomorphine-trained rats may be attributed to its dopamine D_2 receptor agonist properties.

Taken in conjunction with findings of several previous pharmacological characterizations of the discriminative stimulus effects of (−)-apomorphine (Colpaert et al., 1976; Tang and Franklin, 1987), the present results are in agreement with the idea that dopamine D_2 receptors play an important role. Therefore, it is not unexpected that dopamine D_2-like receptor agonists may mimic the discriminative stimulus effects of (−)-apomorphine via dopamine D_2 receptors. Importantly, however, in this study (+)-PD 128907 produced (−)-apomorphine-lever selection at doses lower than 1 μmol/kg (ED_{50} = 0.25 μmol/kg), indicating that relatively low doses of a putative dopamine D_3 receptor agonist are capable of producing behavioral effects via interactions at dopamine D_2 receptors.

It should be noted that the present findings do not exclude the possibility that other behavioral effects of relatively low doses of dopamine D_2-like receptor agonists (e.g., hypolocomotion) are mediated by dopamine D_3 receptors. Furthermore, it is conceivable that D_3-mediated discriminative stimulus effects may be demonstrable under conditions where (+)-PD 128907 serves as the training drug. Nevertheless, the findings of this study indicate that low doses of dopamine D_3 receptor-preferring agonists are capable of exerting effects via dopamine D_2 receptors and therefore emphasize the need for caution when attributing effects to dopamine D_3 receptor involvement solely on the basis of in vitro receptor selectivity. Clearly, obtaining evidence of dopamine D_3 receptor involvement in behavioral actions of dopamine D_2-like receptor agonists will be facilitated when newer dopamine D_3 receptor-selective antagonists, such as nafadotride and S14297, which are reportedly as much as 30-fold selective (Newman-Tancredi et al., 1995; Sautel et al., 1995b), become available.

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