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Short communication

The dopamine D_4 receptor antagonist L-745,870: effects in rats discriminating cocaine from saline

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Abstract

The contribution of dopamine D_4 receptors to the discriminative stimulus effects of cocaine was evaluated by testing the selective dopamine D_4 receptor antagonist, L-745,870 (3-([4-(4-chlororphenyl) piperazin-1-yl] methyl)-1*H*-pyrrolo[2,3-b] pyridine), alone and in combination with cocaine, in rats trained to discriminate cocaine (10 mg/kg) from saline. The antagonist (1–10 mg/kg) failed to engender cocaine-appropriate responding when injected alone, and failed to modify the cocaine dose-response curve when injected as a pre-treatment; however, it reduced response rates dose-dependently. Conversely, the dopamine 'D_1-like' receptor antagonist, SCH 39166 ((−)-*trans*-6,7,7a,8,9,13b-hexahydro-3-chloro-2hydroxy-*N*-methyl-5*H*-benzo[d]naptho-{2,1-b}azepine), produced surmountable antagonism. Results suggest that dopamine D_4 receptors play a negligible role in cocaine's discriminative stimulus effects, and further support a critical involvement of dopamine D_1-like receptors. © 1998 Elsevier Science B.V.

Keywords: Cocaine; L-745,870; SCH 39166; Drug discrimination

1. Introduction

The relative contributions of different dopamine receptor subtypes to the discriminative stimulus effects of cocaine have been studied extensively over recent years. Important roles have been attributed to each of the two main sub-families of receptor, the so-called dopamine 'D_1-like' and 'D_2-like' receptors (Sibley and Monsma, 1992). For example, dopamine D_1-like and D_2-like receptor agonists substitute, at least partially, for the discriminative stimulus effects of cocaine in rats; similarly, dopamine D_1-like and D_2-like receptor antagonists have been shown to attenuate cocaine's discriminative stimulus effects (e.g., Barrett and Appel, 1989). Although discrepant results have been obtained using primates (e.g., Kleven et al., 1990), both types of agonist have been shown to substitute nearfully in squirrel monkeys (Spealman et al., 1991). Most studies have not indicated a preferential involvement of one class of receptor over the other, but dopamine D_1-like receptors may be more important at low training doses of cocaine in rats (Terry et al., 1994).

Despite these findings, little is known concerning the roles of specific dopamine receptor subtypes within each of the two receptor sub-families (i.e., the dopamine D_1, D_5, and D_2, D_3, D_4 receptors, respectively). Acri et al. (1995) demonstrated full substitution of (relatively) selective dopamine D_3 receptor agonists for the discriminative stimulus effects of cocaine, but further examination of specific dopamine receptor subtypes has been hampered by the limited availability of selective drugs. In the present study, we examine for the first time the involvement of the dopamine D_4 receptor (Van Tol et al., 1991) in the discriminative stimulus effects of cocaine, using the dopamine D_4 receptor antagonist L-745,870 ((3-([4-(4-chlororphenyl) piperazin-1-yl] methyl)-1*H*-pyrrolo[2,3-b] pyridine); Kulagowski et al., 1996). The drug is highly selective for the dopamine D_4 receptor, having >2000-fold and >5000-fold selectivities over dopamine D_2 and D_3 receptors respectively, and $>20\,000$-fold selectivity over dopamine D_1-like receptors. The drug penetrates the brain rapidly, maintains selectivity in vivo, and has no reported intrinsic activity (Patel et al., 1996). Since the experiment found no attenuation of cocaine's discriminative stimulus effects by L-745,870, a comparison was made with the effects of a dopamine D_1-like receptor antagonist, SCH 39166 ((−)-*trans*-6,7,7a,8,9,13b-hexahydro-3-chloro-2hydroxy-*N*-methyl-5*H*-benzo[d] naptho-{2,1-b}azepine); Chipkin et al.,

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1988), to confirm that effective antagonism was demonstrable in the present procedure.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats (Charles River, Margate, UK) weighing 320–400 g, housed with free access to water under a 12 h light/dark cycle (lights on 0700). All testing was between 0900 and 1000. Rats were fed 15 g standard diet daily, 30 min after testing.

2.2. Apparatus

Two-lever operant chambers (Coulbourn and Med-Associates, all controlled by Med-PC software), housed within fan-ventilated, light- and sound-attenuating shells. Ambient illumination was by a lamp set centrally above the two levers (17 cm apart). Reinforced presses activated an audible click, and dispensed one 45 mg pellet (Noyes Precision) into a central tray.

2.3. Procedure

Rats were trained initially to press both levers, separately under a fixed-ratio 1 (FR1) schedule of food reinforcement. They were then trained to discriminate i.p. injections of cocaine (10 mg/kg) from i.p. injections of saline. After cocaine, responses on only one lever were reinforced; after saline, responses on the other lever were reinforced. The assignment of cocaine- and saline-appropriate levers was counterbalanced across rats. Rats were placed in the chambers directly after injection, and a 5-min time-out period was initiated, during which lamps were off and responding was not reinforced. Lamps were then illuminated, and responses on the appropriate lever were reinforced; each reinforcement was followed by a 20 s time-out. The FR value was increased to FR20 over several sessions, and responses on the inappropriate lever reset the FR requirement on the appropriate lever. Sessions ended after 20 food presentations or 20 min, whichever occurred first. As the FR value reached 20, training sessions with cocaine and saline were scheduled in a double-alternating sequence. The criteria for successful completion of a session were: at least 85% injection-appropriate responding overall, and during the first FR of the session. Substitution tests began after six consecutive successful training sessions at FR20: tests were identical to training sessions, except that 20 consecutive responses on either lever were reinforced. Thereafter, tests were conducted when a given rat met criterion on both preceding saline and cocaine training sessions. Cocaine was injected immediately before chamber entry; injections of the antagonists (random order within drug) were 30 min beforehand.

2.4. Data analysis

Data from any rat that pressed fewer than 20 times were not included in the calculation of mean cocaine-appropriate responding on that test, and if less than three rats met the requirement, no mean value was derived on that test. Mean response rates were calculated for all rats on each test. Standard analysis of variance and regression were used for difference testing and to calculate ED_{50} values, their 95% confidence limits (CL), and relative potency estimates (the dose of standard drug (mg/kg) equal to 1 mg/kg of comparison drug; a significant difference was assumed if the 95% CL did not include 1.0).

2.5. Drugs

(−)-Cocaine HCl (Sigma, Poole, UK) and SCH 39166 HCl (Schering, Bloomfield, NJ, USA) were dissolved in 0.9% saline; L-745,870 HCl (Tocris Cookson, Bristol, UK) was dissolved in distilled water. All drugs were injected i.p. at 1 ml/kg.

3. Results

In two generalization tests (one for each antagonist tested), cocaine produced dose-dependent increases in cocaine lever responses (see Table 1 for ED_{50} values). At 1, 3 and 10 mg/kg, the dopamine D_4 receptor antagonist L-745,870 produced no reliable cocaine-appropriate responding in the absence of cocaine, and was without effect when administered as a pretreatment to a series of cocaine doses (Fig. 1, upper panel). The ED_{50} values for the discriminative stimulus effects of cocaine were similar whether preceded by 0, 1 or 3 mg/kg of L-745,870 (Table 1). Although ED_{50} values could not be calculated for the combination with 10 mg/kg of L-745,870, the graph

Table 1
ED_{50} values (mg/kg) and relative potencies for the discriminative stimulus effects of cocaine alone and in combination with a dopamine D_4 (L-745,870) or D_1-like (SCH 39166) receptor antagonist ($N = 5$ for L-745,870; $N = 7$ for SCH 39166)

Drug	ED_{50} (95% CL)	Potency relative to cocaine
Dopamine D_4 receptor antagonist		
Cocaine alone	1.89 (1.52–3.61)	[1.0]
+1.0 mg/kg L-745,870	1.67 (1.20–2.32)	1.07 (0.79–1.46)
+3.0 mg/kg L-745,870	2.29 (1.63–3.23)	0.84 (0.59–1.19)
+10.0 mg/kg L-745,870	[a]	[a]
Dopamine D_1-like receptor antagonist		
Cocaine alone	1.31 (0.51–3.39)	[1.0]
+0.03 mg/kg SCH 39166	5.01 (2.99–8.39)	0.46 (0.16–0.97)
+0.3 mg/kg SCH 39166	8.47 (3.71–19.33)	0.29 (0.10–0.58)

[a] Insufficient data to calculate ED_{50} values: only two dose combinations yielded acceptable response rates.

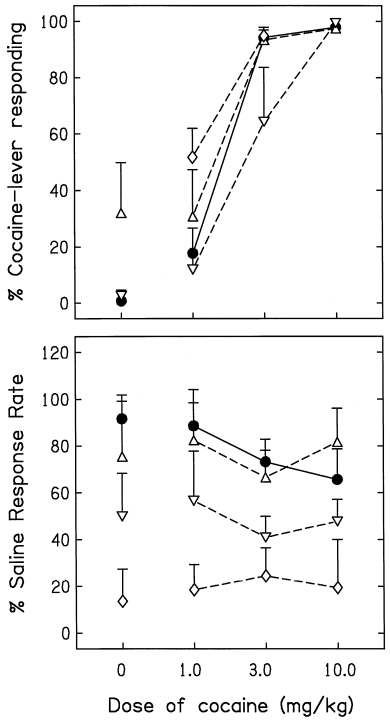

Fig. 1. Upper panel: effects of L-745,870 at 0 (vehicle, ●), 1 (Δ), 3 (∇) and 10 (◇) mg/kg alone and in combination with 1, 3 and 10 mg/kg cocaine on the discriminative stimulus effects of cocaine. L-745,870 was administered i.p. 30 min before each dose of cocaine (or vehicle). Lower panel: rates of responding expressed with respect to saline response rates. Each point represents the mean of at least 3 out of 5 rats tested at each dose.

shows no indication of blockade at either 1 or 3 mg/kg cocaine. Despite the lack of activity in terms of lever-choice behaviour, the antagonist produced a significant dose-related decrease in response rates ($F(3,12) = 18.6$, $P < 0.001$), but no interaction with cocaine dose ($F(9,36) < 1$) (Fig. 1, lower panel).

Pre-treatment with the dopamine D_1-like receptor antagonist SCH 39166 (0.03 and 0.3 mg/kg) produced a significant rightward shift in the dose-response function for cocaine's effects, indicating antagonism. The ED_{50} value for the discriminative stimulus effects of cocaine was significantly increased when preceded by injection with 0.3 mg/kg SCH 39166 (Table 1). The dopamine D_1-like receptor antagonist also produced a dose-related decrease in response rates ($F(2,12) = 26.1$, $P < 0.001$).

4. Discussion

The dopamine D_4 receptor antagonist L-745,870 failed to alter the discriminative stimulus effects of cocaine. Confirmation that the dose-range of the antagonist extended to behaviourally-active doses was provided by the drug's dose-dependent effect on response rate. The lack of any interaction with cocaine dose on response rates also suggests independence of mechanism in the regulation of this aspect of behaviour. The effect on response rate is one of few behavioural effects identified for drugs that selectively block dopamine D_4 receptors: spontaneous and amphetamine-induced locomotor activity have been reported to be resistant to attenuation by the dopamine D_4 receptor antagonist U-101387 (Merchant et al., 1996), and L-745,870 was found to be ineffective in behavioural tests of antipsychotic activity (Bristow et al., 1996). In terms of cocaine's discriminative stimulus effects, Spealman (1996) found no relationship between the substitution potencies of various dopamine receptor agonists and their intrinsic efficacies or binding affinities at dopamine D_4 receptors; instead, drug action at dopamine D_3 receptors was most clearly relevant.

Dopamine D_4 receptor distribution among brain regions still requires clarification: there is agreement that such receptors are expressed in midbrain nuclei, amygdala and frontal cortex (e.g., Van Tol et al., 1991), but there are conflicting reports as to whether they are expressed in nucleus accumbens and basal ganglia (e.g., Defagot and Antonelli, 1997). The brain substrates of cocaine's discriminative stimulus effects have not been widely studied, but intra-accumbens infusions of cocaine, and dopamine receptor antagonists, support a critical role for this region (Callahan et al., 1997). Limited expression of dopamine D_4 receptors in nucleus accumbens (if confirmed) might therefore explain the ineffectiveness of L-745,870 here. However, recent studies have shown that the discriminative stimulus effects of cocaine are also dependent upon at least two brain regions that have consistently shown dopamine D_4 receptor expression: the amygdala and frontal cortex (Callahan et al., 1997); dopamine D_1-like receptor antagonists injected into these regions block cocaine's discriminative stimulus effects. The present findings therefore suggest that dopamine D_4 receptors in amygdala and frontal cortex are probably not important to cocaine's discriminative stimulus effects. On the other hand, the results further emphasize the importance of dopamine D_1-like receptors to the discriminative stimulus effects of cocaine. Previous studies with rats have usually tested antagonists only against the training dose of cocaine (e.g., Barrett and Appel, 1989; Callahan et al., 1997). Our results, testing the full cocaine dose-response curve, suggest that antagonism of dopamine D_1-like receptors is sufficient to attenuate cocaine's discriminative stimulus effects; similar procedures using monkeys have yielded similar results (e.g., Kleven et al., 1990; Spealman et al., 1991).

In summary, the study is the first to investigate dopamine D_4 receptor involvement in the discriminative stimulus effects of cocaine. No evidence for involvement was found, but results confirmed the effectiveness of dopamine D_1-like receptor antagonists at blocking cocaine's discriminative stimulus effects. Experiments using other selective dopamine D_4 receptor antagonists and agonists (when available) should be conducted to confirm the outcome.

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