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Pharmacological characterization of behavioural responses to SK&F 83959 in relation to 'D_1-like' dopamine receptors not linked to adenylyl cyclase

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1 Behavioural responses to the new benzazepine derivative, SK&F 83959, a compound that both fails to stimulate adenylyl cyclase and inhibits the stimulation of adenylyl cyclase induced by dopamine, were characterized in detail.

2 In rat striatal membrane preparations, radioligand binding studies with [^{3}H]-SCH 23390 and [^{3}H]-spiperone indicated SK&F 83959 had a high affinity and >250 fold selectivity for D_1 over D_2 receptors.

3 Using a rapid time-sampling behavioural check list technique, SK&F 83959 (0.01-1.25 mg kg^{-1}) induced grooming in the manner of all known D_1 receptor agonists, together with some vacuous chewing, which declined at higher doses with the emergence of directed chewing and rearing as an adjunct to prominent sniffing; no stereotyped behaviour was evident.

4 Grooming to SK&F 83959 (0.05 mg kg^{-1}) was blocked by the selective D_1 receptor antagonists, SCH 23390 (0.01-1.0 mg kg^{-1}) and BW 737C (0.04-5.0 mg kg^{-1}) and was attenuated by the selective D_2 receptor antagonist, YM 09151-2 (0.005-0.5 mg kg^{-1}); vacuous chewing to SK&F 83959 was not influenced by either SCH 23390 or BW 737C and was enhanced by YM 09151-2.

5 The paradoxical induction of typical D_1 receptor agonist-induced grooming by SK&F 83959, an agent satisfying criteria for a D_1 receptor antagonist as classically defined, together with its blockade by typical D_1 antagonists, strongly suggests mediation via a 'D_1-like' site that appears to respond similarly to agents independent of whether they exert agonist or antagonist actions at the classical adenylyl cyclase-coupled D_1 receptor. This direct functional evidence for a 'D_1-like' site that is not linked to adenylyl cyclase readily complements neurochemical data suggesting the existence of a cyclase-independent 'D_1-like' receptor that may be coupled to phosphoinositide hydrolysis.

Keywords: Dopamine D_1 receptors; SK&F 83959; SCH 23390; BW 737C; grooming behaviour; 'D_1-like' receptors; adenylyl cyclase

Introduction

Over recent years, molecular biology/gene cloning has revealed dopamine receptors to show broader heterogeneity than was envisaged initially within the classical D_1/D_2 schema; rather, these designations appear to encompass two families of 'D_1-like' [D_{1A} and D_{1B} or D_1 and D_5] and 'D_2-like' [$D_{2L/S}$, D_3 and D_4] receptors (Civelli *et al.*, 1993; Gingrich & Caron, 1993; Sibley *et al.*, 1993). Among these 'D_1-like' receptors, each shows linkage to the stimulation of adenylyl cyclase, in accordance with the original criterion of Kebabian & Calne (1979) for the definition of D_1 receptors. However, there is some evidence to suggest the existence of yet further 'D_1-like' subtypes that may be linked not to adenylyl cyclase but rather to some alternative second messenger/transduction system (Andersen *et al.*, 1990). These notions have their origin in regional and other neurochemical findings (Mailman *et al.*, 1986), which have been complemented indirectly by some subsequent behavioural (Arnt *et al.*, 1988; Murray & Waddington, 1989; Daly & Waddington, 1992) and electrophysiological (Johansen *et al.*, 1991) data suggesting that responsiveness to a range of D_1 receptor agonists appears unrelated to their varying efficacies to stimulate adenylyl cyclase. However, the issue remains controversial.

From a functional perspective, the apparent lack of relationship between behavioural activity and efficacy to stimulate adenylyl cyclase has a number of potential explanations that derive from dependence on studies utilising only a range of partial D_1 receptor *agonist* analogues of the prototypical benzazepine D_1 receptor agonist, SK&F 38393, and more direct evidence is needed. Subsequently, a further benzazepine analogue, SK&F 83959, has been reported not to stimulate adenylyl cyclase and, indeed, to inhibit the stimulation of adenylyl cyclase induced by dopamine, i.e. to show all the defining characteristics of a D_1 receptor *antagonist*, yet this compound appears to exert some paradoxical D_1 receptor agonist-like effects on behaviour (Rogers *et al.*, 1990; Arnt *et al.*, 1992). Recently, we have described preliminary studies which indicated that SK&F 83959 induced grooming, a well-characterized behavioural index of D_1 receptor stimulation, together with vacuous chewing, a more controversial model thereof (Downes & Waddington, 1993). This could appear inconsistent with mediation of grooming via the classical D_1 receptor linked to the stimulation of adenylyl cyclase but the overall profile of responsivity to SK&F 83959 has yet to be defined, particularly using subtype-selective dopamine antagonists. We report here more extensively on behavioural responses to SK&F 83959 and, in particular, we present a detailed pharmacological characterization of these responses using both a benzazepine (SCH 23390) and an isoquinoline (BW 737C)-selective D_1 receptor antagonist in comparison with the selective D_2 receptor antagonist, YM 09151-2, as used recently to characterize behavioural responses to the new full efficacy selective D_1 receptor agonist, A 68930 (Daly & Waddington, 1993). A preliminary account of this study has been presented to the British Pharmacological Society (Deveney & Waddington, 1994).

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Methods

Radioligand binding studies

By use of methods similar to those described by Daly & Waddington, (1994), striata from male Sprague-Dawley rats were homogenized in 30 vol 50 mM Tris-HCl buffer, pH 7.6 at 25°C, and centrifuged at 10000 *g* at 4°C for 5 min. The pellet was twice resuspended, diluted and centrifuged as above. The membrane preparation was finally resuspended at 4–8 mg original wet weight ml^{-1} in Tris-HCl buffer containing (mM): NaCl 120, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, $Na_2S_2O_5$ (as antioxidant) 0.2 and 10 μM pargyline (as monoamine oxidase inhibitor).

The binding of [^{3}H]-SCH 23390 (75 Ci $mmol^{-1}$, Amersham) to D_1 receptors was determined by incubating 0.5 ml membrane suspension (approximately 4 mg ml^{-1}) with 0.0625–2.5 nM ligand in saturation studies or 0.5 nM ligand plus unlabelled drugs in displacement studies at 37°C for 20 min in a total volume of 1 ml; specific binding was defined as that displaced by 100 nM piflutixol (Lundbeck) and typically represented >90% of total binding in displacement studies. Incubations were stopped by filtration through GF/B filters, followed by two 8 ml washes with ice-cold buffer. Radioactivity trapped on the filters was quantified by liquid scintillation spectroscopy after addition of 5 ml Ecoscint A (Medlabs) using a LKB 1214 Rackbeta counter with 45–51% counting efficiency for tritium.

The binding of [^{3}H]-spiperone (24 Ci $mmol^{-1}$, Amersham) to D_2 receptors was determined in membranes prepared as above. Incubations contained 0.5 ml membrane suspension (approximately 8 mg ml^{-1}) with 0.025–1.0 nM ligand in saturation studies or 0.2 nM ligand plus unlabelled drugs in displacement studies in a total volume of 5 ml; specific binding was defined as that displaced by 1 μM domperidone (Janssen) and typically represented >75% of total binding in displacement studies. Incubation and filtration were as described above.

Behavioural studies

Young adult male Sprague-Dawley rats (175–400 g; UCD, 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 (06 h 00 min; 18 h 00 min off) light/dark schedule. On experimental days they were placed individually in clear glass observation cages (36 × 20 × 20 cm) and left undisturbed for a habituation period of 2.5 h.

Behavioural assessments were carried out in a manner similar to that described previously (Daly & Waddington, 1994). Immediately before and at intervals after injection of drug or vehicle, animals were assessed by a rapid time-sampling technique. For this procedure, each rat was observed individually for 5 s periods at 1 min intervals over 15 consecutive min, using a behavioural check list. This allowed the presence or absence of the following individual behaviours (occurring alone or in any combination) to be determined in each 5 s period; stillness (motionless with no behaviour evident); sniffing; locomotion; rearing; grooming (of any form); intense grooming (a characteristic pattern of grooming of the face with the forepaws followed by vigorous grooming of the hind flank with the snout); vacuous chewing (not directed on to any physical material); chewing (directed on to any physical material); the presence of forepaw myoclonus or any other unusual behaviour was also noted. After this 15 min assessment, animals were evaluated on a conventional 0–6 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 behaviour with bursts of licking or gnawing; 6 = continuous licking or gnawing; the classical concept of stereotypy adopted was that of invariant, repetitious, inappropriate and purposeless behaviour, as distinct from non-stereotyped behaviours emitted in a more normally episodic, interpolated and discontinuous manner (Molloy & Waddington, 1987). This cycle of assessment by behavioural check list followed by stereotypy scale was repeated on two further occasions over a total observation period of 1 h. Rats were used on two occasions only, separated by a drug-free interval of at least one week; on each occasion rats were allocated randomly to one of the various treatment groups. All assessments were made by an observer unaware of the treatment given to each animal.

Drugs

The following investigational drugs were used: SK&F 83959 (3-methyl-6-chloro-7,8-dihydroxy-1-[3-methylphenyl]-2,3,4,5-tetrahydro-1H-3-benzazepine; SmithKline Beecham, U.S.A.); SCH 23390 (R-3-methyl-7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; Schering-Plough, U.S.A.); BW 737C (S-6-chloro-1[2,5-dimethoxy-4-propylbenzyl]-7-hydroxy-2-methyl-1,2,3,4-tetrahydroisoquinoline; Wellcome Foundation, U.K.); YM 09151-2 (*cis*-N-[1-benzyl-2-methyl-pyrrolidin-3-yl]-5-chloro-2-methoxy-4-methylaminobenzamide; Yamanouchi, Japan). SK&F 83959, SCH 23390 and BW 737C were dissolved in distilled water; YM 09151-2 was dissolved in a minimum of 0.1 N HCl and made up to volume with distilled water. All drugs were injected subcutaneously into the flank in a volume of 2 ml kg^{-1}, with antagonists or respective vehicles given 30 min prior to challenge with agonist or vehicle.

Data analysis

From application of the behavioural check list, the total 'counts' for each individual behaviour was determined as the number of 5 s observation windows in which a given behaviour was evident, summed over a 1 h period, and expressed as means ± s.e.mean; stereotypy scores were averaged over the 1 h period and expressed similarly. These data were then analysed by analysis of variance (ANOVA) or the Kruskal-Wallis non-parametric ANOVA, followed by Student's *t* test or Mann-Whitney U-test, respectively.

Saturation data from radioligand binding studies were analysed by an iterative direct-fit method (Barlow, 1983) to derive receptor density (B_{max}) and apparent dissociation constant (K_D) values. For displacement studies, data were analysed by an iterative curve-fitting procedure (Barlow, 1983) to derive IC_{50} values; these were converted to K_i values with the Cheng-Prusoff equation: $K_i = IC_{50}/(1 + C/K_D)$ where C is ligand concentration and K_D is the apparent dissociation constant as determined above.

Results

Radioligand binding studies

Saturation studies with [^{3}H]-SCH 23390 indicated a B_{max} of 72.7 ± 5.1 pmol g^{-1} and a K_D of 0.44 ± 0.02 nM ($n = 12$) for D_1 receptors; comparable studies with [^{3}H]-spiperone indicated a B_{max} of 19.7 ± 1.4 pmol g^{-1} and a K_D of 0.05 ± 0.01 nM ($n = 11$) for D_2 receptors.

In displacement studies, SK&F 83959 demonstrated high affinity and >250 fold selectivity for D_1 receptors (Table 1). SCH 23390 was confirmed as showing high affinity and >1000 fold selectivity, with BW 737C showing similarly high affinity and 20 fold selectivity, for the D_1 receptor. YM 09151-2 was confirmed as showing high affinity and >10000 fold selectivity for the D_2 receptor.

Effect of SK&F 83959 on behaviour

As a deliberate consequence of the prolonged habituation period, baseline levels of activity in vehicle-injected animals were low; the majority of 'observation windows' were popu-

Table 1 Displacement of [^{3}H]-SCH 23390 and of [^{3}H]-spiperone from striatal D_1 and D_2 receptors, respectively, by investigational agents

	K_i (nmol l^{-1})		
	[^{3}H]-SCH 23390 (D_1)	*[^{3}H]-spiperone* (D_2)	D_1/D_2
SK&F 83959	5.1 ± 0.9	1471 ± 386	0.003
SCH 23390	0.18 ± 0.03	189 ± 36	0.001
BW 737C	2.9 ± 0.3	58 ± 5	0.05
YM 09151-2	2636 ± 45	0.09 ± 0.01	29290

Values are geometric means ± s.e.mean of at least 3 independent determinations, each performed in duplicate

lated by stillness, between which were interpolated some episodes of sniffing and, occasionally, other behaviours (Figure 1).

Administration of SK&F 83959 (0.01 – 1.25 mg kg^{-1}) resulted in a dose-dependent reduction in, and ultimately the essential abolition of, episodes of stillness (Figure 1). When given at 0.01 – 0.05 mg kg^{-1}, SK&F 83959 induced grooming, to which an important contributory element was intense grooming (see Methods); this response was maximal at 0.05 – 0.25 mg kg^{-1}, with higher doses producing some diminution in grooming and inducing prominent sniffing. Over this same dose-range (0.01 – 0.05 mg kg^{-1}), SK&F 83959 also induced vacuous (but not directed) chewing; this response also became less evident at higher doses, at which there emerged an excess

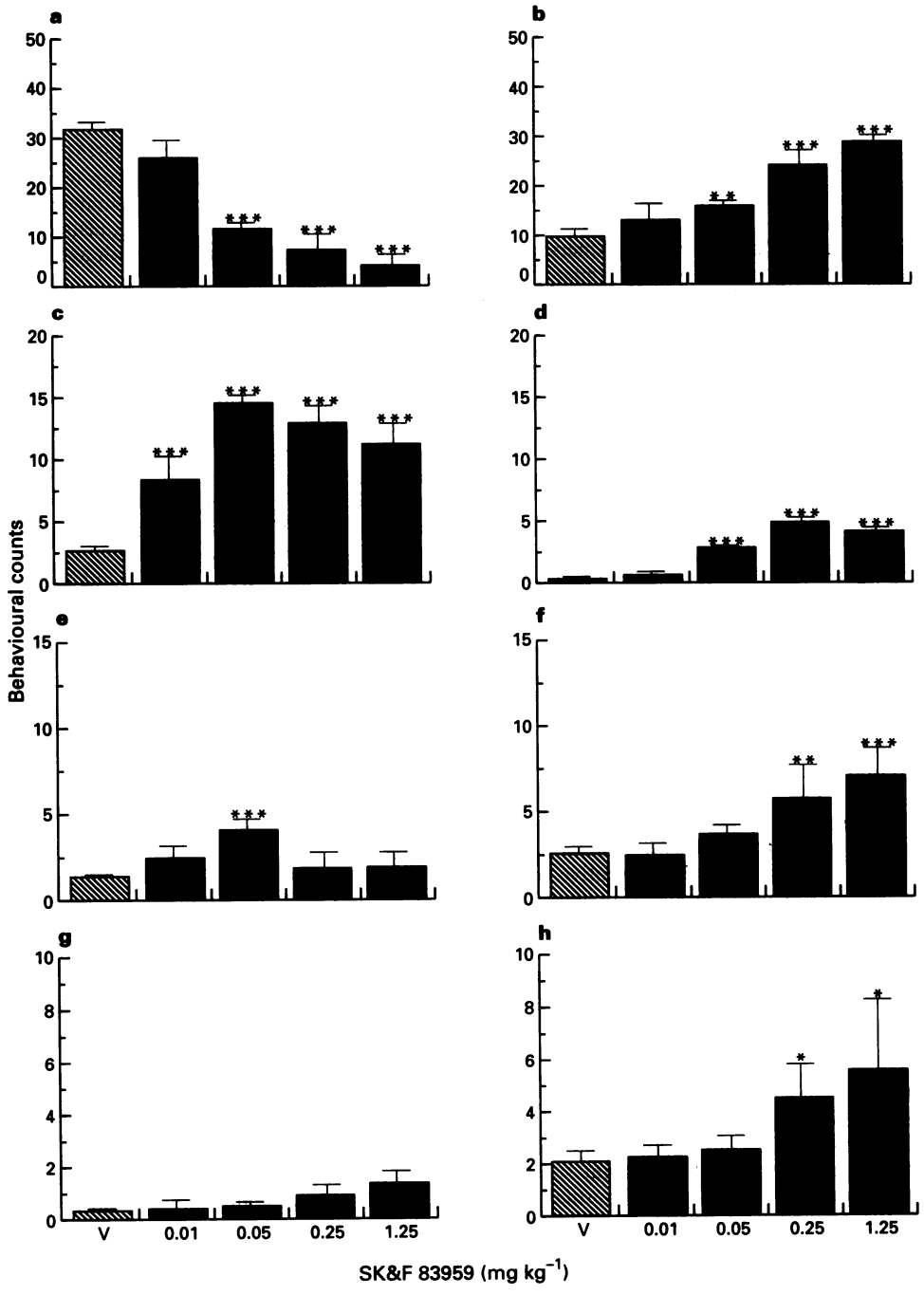

Figure 1 Induction of (a) stillness, (b) sniffing, (c) grooming, (d) intense grooming, (e) vacuous chewing, (f) chewing, (g) locomotion and (h) rearing by 0.01 – 1.25 mg kg^{-1} SK&F 83959 or vehicle (V); data are mean ± s.e.mean of behavioural counts, $n = 8-42$. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ vs vehicle.

of chewing directed onto cage bedding and faecal pellets to accompany prominent sniffing. There was some modest induction of rearing at these higher doses (0.25–1.25 mg kg^{-1}) but no induction of locomotion at any dose of SK&F 83959 administered; nor did it induce licking, gnawing or forepaw myoclonus.

All behaviours induced by SK&F 83959 were episodic, interpolated and discontinuous in nature with mean scores on the 0–6 scale indicating the absence of stereotyped behaviour (vehicle: 0.5±0.1; 0.01 mg kg^{-1}: 0.3±0.1; 0.05 mg kg^{-1}: 1.1±0.2, $P<0.05$; 0.25 mg kg^{-1}: 1.1±0.2, $P<0.05$; 1.25 mg kg^{-1}: 1.5±0.2, $P<0.01$).

Effects of selective D_1 antagonists on SK&F 83959-induced behaviour

The intense grooming response to challenge with 0.05 mg kg^{-1} SK&F 83959 was dose-dependently blocked by pretreatment with 0.01–1.0 mg kg^{-1} SCH 23390 and with 0.04–5.0 mg kg^{-1} BW 737C, such that the higher doses of each antagonist produced essential abolition of this response; the actions of SK&F 83959 on stillness and grooming were blocked similarly. Conversely, pretreatment with these doses of SCH 23390 and BW 737C failed to influence the vacuous chewing response to SK&F 83959 (Figure 2).

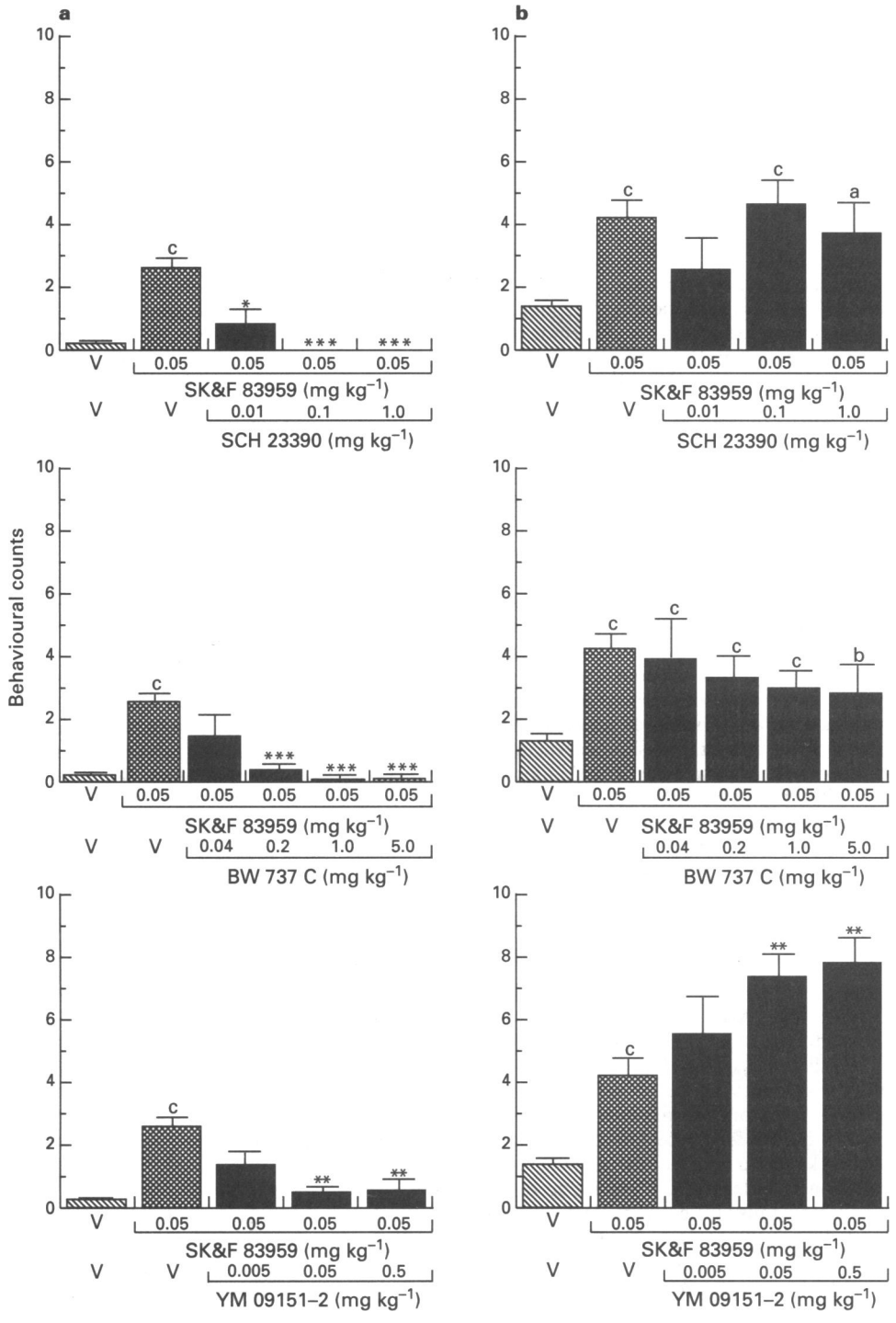

Figure 2 Effects of pretreatment with 0.01–1.0 mg kg^{-1} SCH 23390, 0.04–5.0 mg kg^{-1} BW 737C, 0.005–0.5 mg kg^{-1} YM 09151-2 or vehicle (V) on (column a) intense grooming and (column b) vacuous chewing responses to 0.05 mg kg^{-1} SK&F 83959; data are mean±s.e.mean of behavioural counts, $n=8-42$. $^{c}P<0.001$, $^{b}P<0.01$, $^{a}P<0.05$ vs vehicle; ***$P<0.001$, **$P<0.01$, *$P<0.05$ vs SK&F 83959.

Effects of selective D_2 antagonism on SK&F 83959-induced behaviours

Pretreatment with 0.005–0.5 mg kg^{-1} YM 09151-2 reduced the intense grooming response to challenge with 0.05 mg kg^{-1} SK&F 83959, though less readily than did SCH 23390 or BW 737C; the actions of SK&F 83959 on stillness and grooming were reduced similarly. These doses of YM 09151-2 did not reduce SK&F 83959-induced vacuous chewing; rather, pretreatment with YM 09151-2 resulted in a dose-dependent release of this response to SK&F 83959 (Figure 2).

Effects of selective D_1 and D_2 antagonists given alone

SCH 23390 (0.002–1.0 mg kg^{-1}), BW 737C (0.04–5.0 mg kg^{-1}) or YM 09151-2 (0.001–0.5 mg kg^{-1}) did not induce any form of grooming when given as sole treatment. SCH 23390 increased mean behavioural counts for vacuous chewing (vehicle; 0.4±0.2; 0.002 mg kg^{-1}: 1.6±0.9; 0.01 mg kg^{-1}: 0.9±0.5; 0.1 mg kg^{-1}: 2.5±0.8, $P<0.05$; 1.0 mg kg^{-1}: 4.3±1.2, $P<0.01$; $n=8$), while neither BW 737C nor YM 09151-2 exerted such an effect.

Discussion

At the behavioural level, the primary response to the benzazepine analogue, SK&F 83959, was the induction of grooming, which included episodes of an intense grooming syndrome that is characteristic of all preferential and selective D_1 receptor agonists examined to date (Molloy & Waddington, 1984; Waddington *et al.*, 1995). Such grooming was evident at the very low doses of 10–50 μg kg^{-1} and the tendency towards diminution of this response at higher doses coincided with the emergence of episodes of prominent sniffing and of rearing; this would suggest a response competition/incompatibility effect in relation to grooming/intense grooming and sniffing/rearing. However, in contrast to the prototypical D_1 receptor agonist, SK&F 38393, and a range of related benzazepine agonist analogues that we have studied previously (Murray & Waddington, 1989; Daly & Waddington, 1992), SK&F 83959 *did* induce episodes of vacuous chewing over a dose-range similar to that inducing grooming; this vacuous chewing response also declined at higher doses, to be replaced by chewing directed onto cage bedding and faecal pellets as an adjunct to high dose sniffing and rearing. Perioral dyskinesia/vacuous chewing has proved a more controversial behavioural index of D_1 receptor stimulation (Rosengarten *et al.*, 1983; 1986; Collins *et al.*, 1991; Waddington *et al.*, 1995). While the induction of vacuous chewing by SK&F 83959 distinguished it, in our hands, from the general D_1 receptor partial agonist benzazepine series, this overall profile was similar to that which we have described recently for the new full efficacy isochroman-selective D_1 receptor agonist, A 68930 (Daly & Waddington, 1993).

In vitro, SK&F 83959 shows high (>250 fold) selective affinity for D_1 over D_2 receptors (Arnt *et al.*, 1992) and this finding was readily reproduced here; the affinity of SK&F 83959 for individual 'D_1-like' (D_{1A} and D_{1B}) and 'D_2-like' ($D_{2L/S}$, D_3 and D_4) receptor subtypes has yet to be determined. However, this affinity for D_1 receptors appears unaccompanied by any intrinsic activity, in that SK&F 83959 fails to stimulate dopamine-sensitive adenylyl cyclase and readily inhibits, with nM affinity, the stimulation of adenylyl cyclase induced by dopamine (Arnt *et al.*, 1992); thus, by conventional criteria (Kebabian & Calne, 1979; Civelli *et al.*,1993; Gingrich & Caron, 1993; Sibley *et al.*, 1993), SK&F 83959 shows all the characteristics of a selective D_1 receptor *antagonist*, its neurochemical profile being indistinguishable from that of SCH 23390.

In the face of such fundamental incongruity between clear behavioural evidence for D_1 receptor *agonist*-like effects *in vivo* and neurochemical evidence for D_1 receptor antagonist-like properties *in vitro*, it must be considered whether SK&F 83959 might be metabolized *in vivo* to generate an active D_1 receptor agonist byproduct. However, as outlined by Arnt *et al.* (1992), while di-demethylation of SK&F 83959 would theoretically yield the high efficacy partial D_1 receptor agonist SK&F 81297, only N-demethylation is a potential (but unproven) metabolic reaction and this derivative shows considerably less potency than the parent compound (Weinstock *et al.*, 1985). These considerations, together with the rapid onset (within 5–10 min) of D_1 receptor agonist-like behavioural effects following s.c. challenge (a route that avoids early, first-pass metabolism) at very low doses, indicate strongly that this incongruity is unlikely to be explained in such pharmacokinetic terms. If SK&F 83959 possessed low (but not zero) intrinsic activity at D_1 receptors, i.e. to act as a weak partial agonist, it might be capable of mimicking the actions of SK&F 38393 and related analogues were D_1 receptors to demonstrate a sufficiently large receptor reserve. However, SK&F 83959 has been reported to exert *no* stimulation of dopamine-sensitive adenylyl cyclase (in an assay system sensitive enough to indicate a higher efficacy than has been reported previously e.g. O'Boyle *et al.*, 1989, for the partial selective D_1 receptor agonists, SK&F 38393 and SK&F 75670) and to show agonist-like effects following inactivation of a significant proportion of D_1 receptors with EEDQ (Arnt *et al.*, 1992). These considerations indicate strongly that the incongruity at issue is unlikely to be explained in such pharmacodynamic terms.

The alternative interpretation is that SK&F 83959 exerts its paradoxical effects on typical grooming behaviour directly through stimulating a 'D_1-like' receptor that is (i) not linked to adenylyl cyclase and (ii) thus able to respond similarly to drugs having a common affinity for this 'D_1-like' site independent of whether they exert agonist or antagonist action at the classical, adenylyl cyclase-coupled D_1 receptor. That the selective D_1 receptor antagonists, SCH 23390 and BW 737C (Riddall, 1992) should block the grooming response to SK&F 83959, despite these drugs sharing a common action to inhibit dopamine-sensitive adenylyl cyclase, would indicate further that such grooming has its origins in stimulation of a 'D_1-like' receptor that is not linked to adenylyl cyclase but at which SCH 23390 and BW 737C are antagonists. That the selective D_2 receptor antagonist, YM 09151-2, should attenuate SK&F 83959-induced grooming, as it does also for grooming induced by A 68930 (Daly & Waddington, 1993), would be consistent with the involvement of cooperative/synergistic 'D_1-like': 'D_2-like' interactions in the regulation of this typical behaviour (Murray & Waddington, 1989; Waddington *et al.*, 1994; but see White & Hu, 1993).

In relation to SK&F 83959-induced vacuous chewing, there is evidence that perioral movements can be stimulated also by non-dopamine agents, most notably muscarinic and 5-hydroxytryptamine receptor agonists. However, SK&F 83959 failed to induce *any* elements either of the classical peripheral signs of cholinergic overactivity that follow the stimulation of perioral movements by agents such as pilocarpine (Salamone *et al.*, 1986), or of the typical 5-hydroxytryptaminergic syndrome that follows the stimulation of perioral movements by agents such as the 5-HT_{2C}/5-HT_{2A} (formerly 5-HT_{1C}/5-HT_2) receptor agonist *m*-chlorophenylpiperazine (Stewart *et al.*, 1989; Gong *et al.*, 1992). Importantly, vacuous chewing induced by SK&F 83959 was phenomenologically identical with that induced by the full efficacy D_1 receptor agonist, A 68930, which has negligible affinity for muscarinic, 5-hydroxytryptamine or other non-dopamine receptors (DeNinno *et al.*, 1991); furthermore, pretreatment with the selective D_2 receptor antagonist, YM 09151-2, enhanced vacuous chewing to SK&F 83959, as it does for A 68930 (Daly & Waddington, 1993), in accordance with the involvement of oppositional 'D_1-like': 'D_2-like' interactions in the regulation of this atypical dopaminergic behaviour (Murray & Waddington, 1989; Waddington *et al.*, 1994). SCH 23390 also induced vacuous chewing, as noted previously (Collins *et al.*, 1991; Daly & Waddington, 1993), and can show some low affinity partial agonist activity at 5-HT_{2C} and 5-HT_{2A}

receptors in cloned cell lines. However, this activity is modest and atypical in comparison with 5-HT and SK&F 38393 (Briggs *et al.*, 1991; Woodward *et al.*, 1992), yet we have consistently found SCH 23390 but *not* SK&F 38393 to induce vacuous chewing that is phenomenologically identical to that induced by A 68930, without behavioural evidence of 5-hydroxytryptaminergic activity; furthermore, there is electrophysiological evidence that SCH 23390 can exert paradoxical 'D_1-like' receptor agonist effects on nucleus accumbens neurones (Wachtel & White, 1991). For these reasons it is difficult to equate the present vacuous chewing phenomena with muscarinic or 5-hydroxytryptamine receptor activity. Alternatively, they might be mediated via some other dopamine receptor subtype at which SK&F 83959 (and possibly its fellow benzazepine SCH 23390 but not the isoquinoline BW 737C) can act as an agonist in a manner similar to A 68930, though the involvement of other non-dopamine effects cannot be excluded. Exhaustion of our limited sample of SK&F 83959 precluded further studies.

Irrespective of these considerations in relation to atypical vacuous chewing, the present data on the induction of typical grooming by SK&F 83959 constitute the first direct behavioural evidence for the existence of a 'D_1-like' receptor not linked to dopamine-sensitive adenylyl cyclase. This notion is not readily compatible with the further subtyping (D_{1A} and D_{1B} or D_1 and D_5) that has evolved from molecular biology/gene cloning studies, as each of these entities demonstrates such a cyclase linkage (Civelli *et al.*, 1993; Gingrich & Caron, 1993; Sibley *et al.*, 1993). However, the notion is consistent with earlier (Mailman *et al.*, 1986) and more recent neurochemical studies which have also suggested the existence of 'D_1-like' receptor(s) that are linked not to adenylyl cyclase but perhaps to phosphoinositol metabolism (Mahan *et al.*, 1990; Undie & Friedman, 1990; Schoors *et al.*, 1991; Laitinen, 1993; Giambalvo & Wagner, 1994). In particular, Undie *et al.* (1994) have recently indicated, using a series of benzazepine derivatives, a 'D_1-like' receptor mediating phosphoinositide hydrolysis that is pharmacologically distinct from the classical D_1 receptor coupled to stimulation of cyclic AMP formation.

This convergence of behavioural and neurochemical evidence strengthens the controversial concept of functionally distinct, cyclase-independent 'D_1-like' receptors. The present data indicate additionally that the terms 'agonist' and 'antagonist', as defined in terms of classical D_1-mediated effects on dopamine-sensitive adenylyl cyclase, may be inappropriate for such 'D_1-like' receptor(s) and that SK&F 83959 may be an important tool for elucidating futher their nature and functional role(s).

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