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Psychopharmacological Distinction Between Novel Full-Efficacy "D_1-like" Dopamine Receptor Agonists

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DEVENEY, A. M. AND J. L. WADDINGTON. *Psychopharmacological distinction between novel full-efficacy "D_1-like" dopamine receptor agonists*. PHARMACOL BIOCHEM BEHAV **58**(2) 551–558, 1997.—The search for full-efficacy agonists selective for the "D_1-like" family of dopamine receptor subtypes has recently generated two novel series of compounds: the isochromans, typified by A 68930, and the phenanthridines, typified by dihydrexidine. This study was undertaken to compare systematically the effects of these two agents on the spectrum of unconditioned motor behaviour (i.e., construction of their drug ethograms) in the intact adult rat and to determine the sensitivity of these responses to selective antagonists of "D_1-like" (SCH 23390) vs. "D_2-like" (YM 09151-2) receptors. A 68930 (0.0625–4.0 mg/kg) readily induced grooming, including intense grooming, the most widely accepted behavioural model of "D_1-like" receptor stimulation; it also induced vacuous chewing, a more controversial model thereof, and sniffing. Conversely, dihydrexidine (0.25–16.0 mg/kg) induced grooming, but little intense grooming was evident; it failed to induce vacuous chewing but did induce sniffing. Grooming and sniffing responses to A 68930 were readily blocked by SCH 23390 (0.01–1.0 mg/kg) but were only attenuated or spared by YM 09151-2 (0.005–0.5 mg/kg). Conversely, the grooming and sniffing responses to dihydrexidine were readily blocked both by SCH 23390 and by YM 09151-2. A 68930 and dihydrexidine do not show identical psychopharmacological profiles; they appear to differ in the specificity of their effects on "D_1-like" vs. "D_2-like" function and may interact differentially with putative subtypes of "D_1-like" receptors that are indicated behaviourally. © 1997 Elsevier Science Inc.

"D_1-like" dopamine receptors Full-efficacy "D_1-like" agonists Behaviour A 68930 Dihydrexidine Ethogram

BENZAZEPINE D_1 dopamine (DA) receptor agonists derived from the prototypical compound SK&F 38393 (32,43,45) have proven useful in probing the functional role(s) of what is now recognised to be a broader family of molecular biologically defined brain "D_1-like" (D_{1A}, D_{1B}, D_{1C}, D_{1D}) receptors (4,8,13,33,34,41). On the basis of functional considerations, there appear to exist additional "D_1-like" subtypes with which these agents may interact (41). However, the benzazepines are, in the main, partial agonists with various degrees of intrinsic activity to stimulate adenylyl cyclase, the defining characteristic of a D_1 agonist (16), and some show only modest selectivity and/or limited penetration into the brain following peripheral administration. Furthermore, those members of this series with putative full efficacy [SK&F 82958, SK&F 83189] appear to stimulate adenylyl cyclase in an anomalous manner and/or have low in vivo potency (23,26,27,41,43).

These limitations, in juxtaposition with preliminary evidence for therapeutic potential in Parkinson's disease (40), have stimulated a search for potent, full-efficacy D_1 agonists of high selectivity, and over recent years two such series of compounds have been offered. Among the isochromans, A 68930 is a very selective, full-efficacy "D_1-like" agonist of high in vivo potency (9); it has been reported to readily induce grooming, the most widely accepted rodent behavioural model of "D_1-like" receptor stimulation, together with vacuous chewing, a more controversial model thereof, and at higher doses it stimulates sniffing and rearing but not locomotion (6,41). Conversely, among the phenanthridines, dihy-

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drexidine is a full-efficacy "D_1-like" agonist of more limited selectivity vis-á-vis "D_2-like" ($D_{2L/S}$, D_3, D_4) receptors and lower in vivo potency (23); it has also been reported to induce grooming, though no vacuous chewing response has been noted, and it stimulates sniffing and locomotion but not rearing (7). The psychopharmacological profiles of these "D_1-like" agonists are distinct from those of "D_2-like" agonists, though their effects are subject to "D_1-like":"D_2-like" interactions (41–43). Both the isochromans (15) and the phenanthridines (35) show efficacy in the MPTP nonhuman primate model of Parkinson's disease.

Given the importance now attached to the functional role of the "D_1-like" family of receptors and the therapeutic potential of full-efficacy agonists, but with an indication of some divergence in psychopharmacological profile between these two novel but chemically distinct full-efficacy "D_1-like" agonists, we have conducted the first systematic behavioural comparison between them; this involved the construction of an ethogram for each drug followed by evaluation of the relative sensitivity of resultant responses to the selective "D_1-like" antagonist SCH 23390 vs. the selective "D_2-like" antagonist YM 09151-2 (11).

METHOD

Behavioural Studies

Young adult male Sprague–Dawley rats (250–470 g; UCD, Dublin, Ireland) were housed in groups of five per cage with food and water available ad lib and were maintained at 21 ± 1°C on a 12 L:12 D cycle (lights on at 0600 h). 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 as described previously (10,11). Immediately before and at intervals after injection of drug or vehicle, animals were assessed using a rapid, time-sampling behavioural checklist technique. For this procedure, each rat was observed individually for 5-s periods at 1-min intervals over 15 consecutive minutes, using a behavioural checklist. 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 onto any physical material), and chewing (directed onto any physical material); the presence of forepaw myoclonus or any other unusual behaviour was also noted. After this 15-min assessment with the behavioural checklist, animals were evaluated for 30 s each using 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, and 6 = continuous licking or gnawing. This cycle of assessment by behavioural checklist 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 1 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.

Radioligand Binding Studies

Using methods described previously (10,11), striata from male Sprague–Dawley rats were homogenised in 30 volumes of 50 mM Tris-HCl buffer, pH 7.6, at 25°C, and centrifuged at 10,000 × *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 in Tris-HCl buffer containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 0.2 mM $Na_2S_2O_5$ (as antioxidant), and 10 μM pargyline (as monoamine oxidase inhibitor).

The binding of [^{3}H]SCH 23390 to "D_1-like" receptors was determined by incubating 0.5 ml membrane suspension (approximately 4 mg/ml) with 0.5 nM ligand plus unlabelled drugs at 37°C for 20 min in a total volume of 1 ml; specific binding was defined as that displaced by 100 nM piflutixol and typically represented >90% of total binding. 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, using an LKB 1214 Rackbeta counter with 45–51% counting efficiency for tritium.

The binding of [^{3}H]spiperone to "D_2-like" receptors was determined using membranes prepared as above. Incubations contained 0.5 ml membrane suspension (approximately 8 mg/ml) with 0.2 nM ligand plus unlabelled drugs in a total volume of 5 ml; specific binding was defined as that displaced by 1 μM domperidone and typically represented >75% of total binding. Incubation and filtration were as described above.

Drugs

The following investigational agents were used: the "D_1-like" agonist A 68930 ([1*R*,3*S*]-1-aminomethyl-5,6-dihydroxy-3-phenylisochroman; Abbott, USA) was dissolved in dilute acetic acid and made up to volume with distilled water; the "D_1-like" agonist dihydrexidine (Research Triangle Institute/NIMH, USA) was dissolved in 0.1% ascorbic acid and made up to volume with distilled water; the "D_2-like" agonist RU 24213 (*N*-*n*-propyl-*N*-phenylethyl-*p*-3-hydroxyphenylethylamine; Roussel-UCLAF, France) and the "D_1-like" antagonist SCH 23390 ([*R*]-7-chloro-8-hydroxy-2,3,4,5-tetrahydro-3-methyl-1-pheny l-1H-3-benzazepine; Schering-Plough, USA) were dissolved in distilled water; and the "D_2-like" antagonist YM 09151-2 ([*cis*]-*N*-(1-benzyl-2-methyl-pyrolidin-3-yl)-5-chloro- 2-methoxy-4-methylaminobenzamide; Yamanouchi, Japan) was dissolved in 0.1 N HCl and made up to volume with distilled water. All investigational agents were injected subcutaneously into the flank in a volume of 2 ml/kg, with antagonists or respective vehicles given 30 min before agonist challenge.

Data Analysis

From application of the behavioural checklist, the total "counts" for each individual behaviour were 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 ± SEM for 8–24 animals per group; 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 nonparametric ANOVA (as *H*-values), followed by Student's *t*-test or Mann–Whitney *U*-test, respectively.

For radioligand binding studies, data were analysed by using an iterative curve-fitting procedure (3) 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 (10).

RESULTS

Behavioural Profile of A 68930

As a deliberate consequence of the prolonged habituation period, baseline levels of activity in vehicle-injected animals were low; some three-quarters of observation "windows" were populated by stillness, between which were interpolated only episodes of sniffing and, occasionally, of grooming in the essential absence of any other form of behaviour (Fig. 1).

Administration of A 68930 (0.0625–4.0 mg/kg) resulted in a substantial reduction in episodes of stillness. When given at 0.25–1.0 mg/kg, A 68930 readily induced grooming, to which the major contributory component was intense grooming [$F(4, 35) = 11.73$, $p < 0.001$]; these responses diminished at the highest dose of A 68930, with the emergence of more prominent sniffing and rearing. Lower doses of A 68930 (0.0625–0.25 mg/kg) induced vacuous chewing [$F(4, 35) = 5.07$, $p < 0.001$], which was transformed into chewing of cage bedding and/or faecal pellets at intermediate doses (0.25–1.0 mg/kg); no form of chewing was evident following the highest dose of A 68930 administered, at which prominent sniffing and rearing were evident. No locomotion was induced by any dose of A 68930 (Fig. 1).

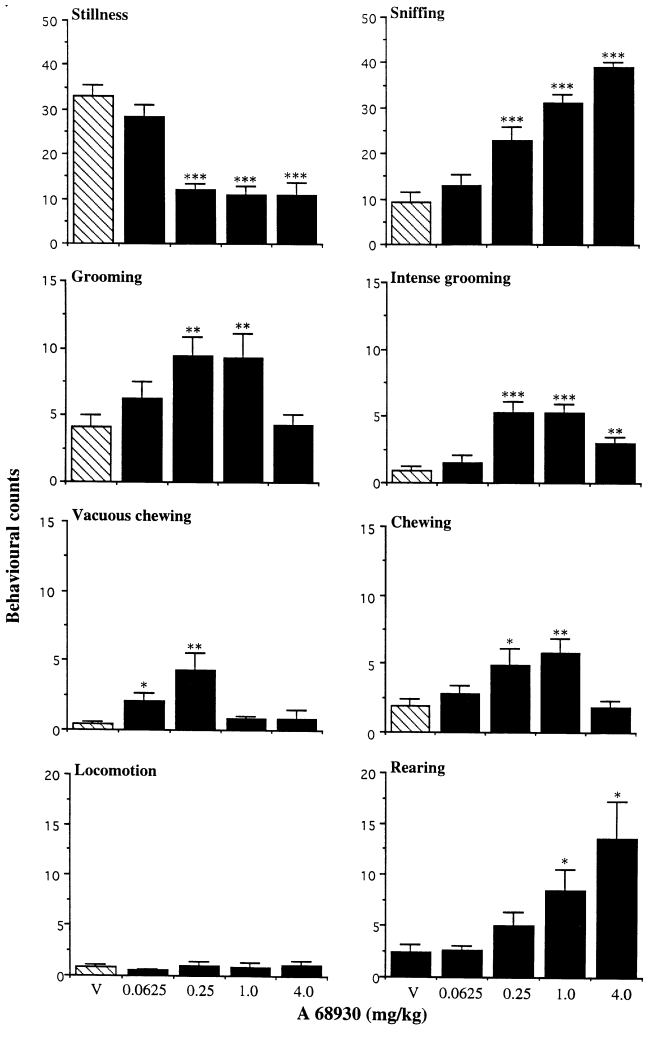

FIG. 1. Behavioural responses to challenge with 0.0625–4.0 mg/kg A 68930. Data are mean counts over 1 h for each behaviour indicated ± SEM for eight animals per group. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ vs. challenge with vehicle (V).

All behaviours induced by A 68930 were episodic, discontinuous, and interpolated in nature. Using the 0–6 scale, mean stereotypy scores for each dose of A 68930 were as follows ($H = 21.81$, $p < 0.001$): vehicle, 0.1 ± 0.1; 0.0625 mg/kg, 0.7 ± 0.2 ($p < 0.05$); 0.25 mg/kg, 1.3 ± 0.1 ($p < 0.01$); 1.0 mg/kg, 1.6 ± 0.3 ($p < 0.01$); 4.0 mg/kg, 1.5 ± 0.2 ($p < 0.01$). Thus, the individual responses described above were confirmed to be manifested in a nonstereotyped manner.

Behavioural Profile of Dihydrexidine

Administration of dihydrexidine (0.25–16.0 mg/kg) resulted in the essential abolition of episodes of stillness. When given at 1.0–4.0 mg/kg, dihydrexidine readily induced grooming, though intense grooming [$F(4, 33) = 7.47$, $p < 0.001$] was only a minor contributory component of this response (Fig. 2); in particular, the peak grooming response to dihydrexidine was greater than, whereas the peak intense grooming response was less than, that to A 68930. No dose of dihydrexidine induced significant vacuous chewing [$F(4, 33) = 1.60$], whereas chewing of cage bedding and/or faecal pellets was progressively induced over the dose range administered. The higher doses of dihydrexidine (4.0–16.0 mg/kg) induced sniffing with occasional episodes of rearing, but no locomotion was evident at any dose (Fig. 2).

All behaviours induced by dihydrexidine were episodic, discontinuous, and interpolated in nature. Using the 0–6 scale, mean stereotypy scores for each dose of dihydrexidine were as follows ($H = 23.90$, $p < 0.001$): vehicle, 0.4 ± 0.2; 0.25 mg/kg, 0.2 ± 0.1; 1.0 mg/kg, 0.9 ± 0.8; 4.0 mg/kg, 2.4 ± 0.8 ($p < 0.01$); 16.0 mg/kg, 2.0 ± 0.6 ($p < 0.01$). Thus, the individual responses described above were confirmed to be manifested in the absence of compulsive stereotypy.

Effect of "D_1-like" Antagonism on Responses to A 68930 and Dihydrexidine

SCH 23390 (0.01–1.0 mg/kg) (11) readily blocked both the grooming and intense grooming responses to 0.5 mg/kg A 68930 [$F(3, 26) = 23.14$, $p < 0.001$]; the sniffing and chewing responses were blocked similarly, with restoration of prominent episodes of stillness. However, no dose of SCH 23390 exerted any influence [$F(3, 26) = 0.23$] on the vacuous chewing response to A 68930 (Fig. 3). These doses of SCH 23390 also blocked the grooming and intense grooming responses to 4.0 mg/kg dihydrexidine; the sniffing and chewing responses were blocked similarly, with restoration of prominent episodes of stillness. Although dihydrexidine failed to induce vacuous chewing when given alone (Fig. 2), the highest dose of SCH 23390 (1.0 mg/kg) when given prior to dihydrexidine was associated with the emergence of some vacuous chewing (Fig. 3).

Effect of "D_2-like" Antagonism on Responses to A 68930 and Dihydrexidine

YM 09151-2 (0.005–0.5 mg/kg) (11) attenuated but did not block the grooming response to 0.5 mg/kg A 68930, whereas intense grooming was not influenced significantly [$F(3, 28) = 2.91$]; the chewing response was blocked, but sniffing was attenuated only by a midrange dose of YM 09151-2 (0.05 mg/

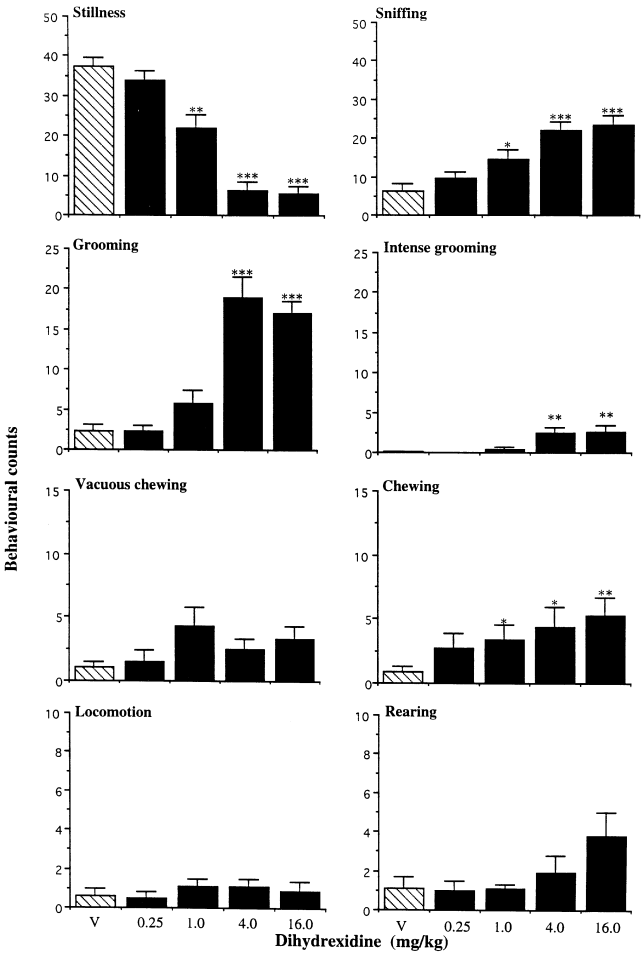

FIG. 2. Behavioural responses to challenge with 0.25–16.0 mg/kg dihydrexidine. Data are mean counts over 1 h for each behaviour indicated ± SEM for eight animals per group. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ vs. challenge with vehicle (V).

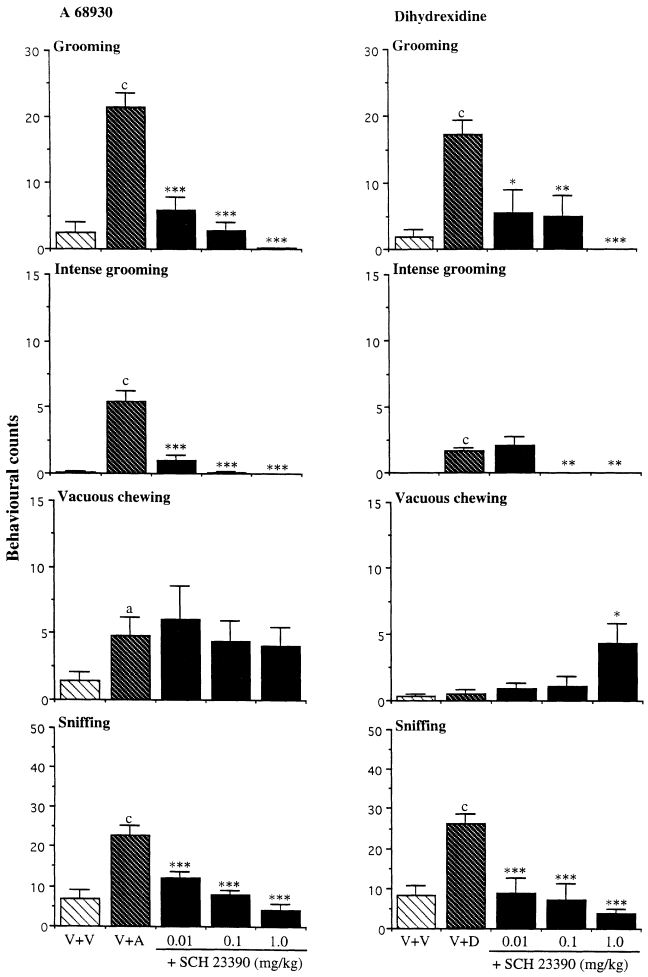

FIG. 3. Behavioural responses to challenge with 0.5 mg/kg A 68930 or 4.0 mg/kg dihydrexidine following pretreatment with 0.01–1.0 mg/kg SCH 23390 or vehicle. Data are mean counts over 1 h for each behaviour indicated ± SEM for 8–24 animals per group. $^{a}p < 0.05$, $^{b}p < 0.01$ and $^{c}p < 0.001$ vs. challenge with vehicle (V); $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ vs. challenge with A 68930 (A) or dihydrexidine (D).

kg), with only partial restoration of episodes of stillness. The vacuous chewing response to A 68930 was attenuated [$F(3, 28) = 3.14$, $p < 0.05$] by a low dose of YM 09151-2 (0.005 mg/kg), whereas this effect was lost at higher doses (0.05–0.5 mg/kg), which tended to release additional episodes of vacuous chewing (Fig. 4). These doses of YM 09151-2 readily blocked both the grooming and intense grooming responses to 4.0 mg/kg dihydrexidine; the chewing and sniffing responses were blocked similarly, with restoration of prominent episodes of stillness. Although dihydrexidine failed to induce vacuous chewing when given alone (Fig. 2), a midrange dose of YM 09151-2 (0.05 mg/kg) when given prior to dihydrexidine was associated with the emergence of vacuous chewing (Fig. 4).

Radioligand Binding Studies

A 68930 demonstrated high affinity and 70-fold selectivity, whereas dihydrexidine demonstrated more modest affinity and only 12-fold selectivity for "D_1-like" over "D_2-like" receptors (Table 1); SCH 23390 and YM 09151-2 were confirmed to show high affinity and selectivity for "D_1-like" and "D_2-like" receptors, respectively (11).

DISCUSSION

In this first systematic comparison of the two principal series of novel full-efficacy, selective "D_1-like" agonists identified to date, a number of important differences in psychopharmacological profile were evident; these are considered firstly in terms of the ethogram, for each agonist given alone, and secondly in terms of the sensitivity of these responses to selective "D_1-like" vs. "D_2-like" antagonists.

Our finding that low doses of A 68930 readily induced a grooming response that included the prominence of intense grooming together with vacuous chewing and directed chewing, but that declined at higher doses in response-competition with the emergence of sniffing and rearing but not locomotion, is consistent with our initial description of its ethogram (6). The induction of such grooming, particularly intense grooming, constitutes the most widely accepted model of "D_1-like" receptor activation (21,22,38,41), whereas the induction of vacuous chewing/perioral dyskinesia remains a more controversial model thereof (5,25,29,30,41). Although dihydrexidine also

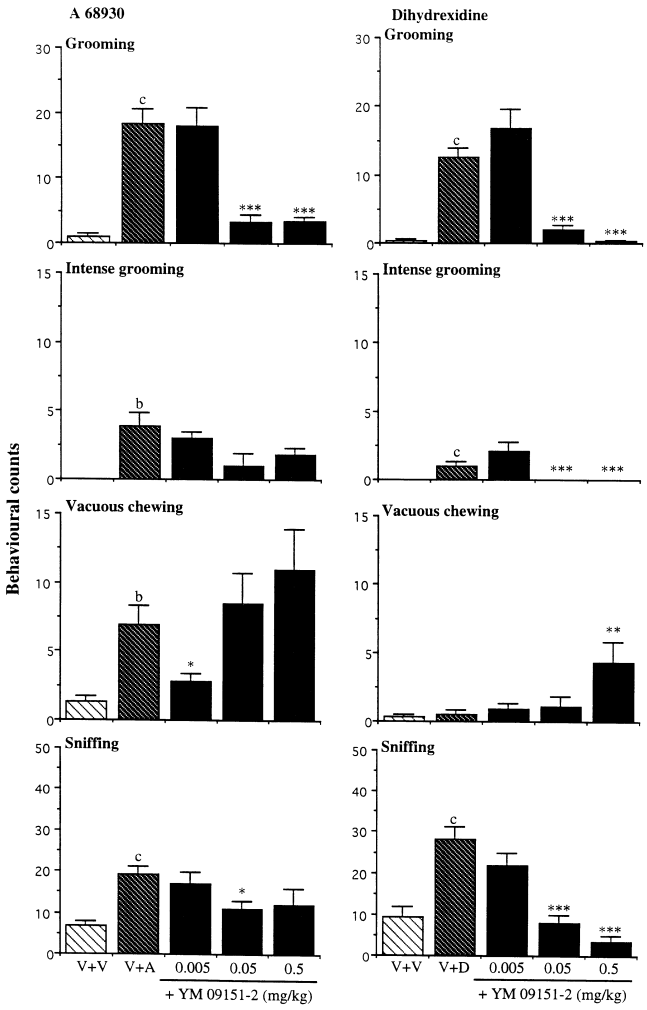

FIG. 4. Behavioural responses to challenge with 0.5 mg/kg A 68930 or 4.0 mg/kg dihydrexidine following pretreatment with 0.005–0.5 mg/kg YM 09151-2 or vehicle. Data are mean counts over 1 h for each behaviour indicated ± SEM for 8–24 animals per group. $^{a}p < 0.05$, $^{b}p < 0.01$ and $^{c}p < 0.001$ vs. challenge with vehicle (V); $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ vs. challenge with A 68930 (A) or dihydrexidine (D).

readily induced grooming, intense grooming was only a minor contributor to this overall response, and the maximal intense grooming response was prominently less than that to A 68930; furthermore, although higher doses of dihydrexidine induced sniffing and chewing but little rearing and no locomotion, it failed to induce vacuous chewing at any dose.

In the only other available study (7), a similar range of doses of dihydrexidine (0.3–30.0 mg/kg) induced grooming, and the qualitative description of the grooming response appeared consistent with the presence of at least some episodes of intense grooming, though this element of behaviour was not resolved specifically; at higher doses of dihydrexidine, the induction of grooming was lost with the emergence of prominent sniffing and locomotion, but no form of chewing was reported at any dose. Thus, the major differences in behavioural responsivity to dihydrexidine between the previous report (7) and the present study involved our failure to find any induction of locomotion and our noting of a somewhat less prominent and dose-dependent sniffing response; this would be consistent with our finding of less diminution in the overall grooming response at higher doses of dihydrexidine. The previous study (7) used the same strain, gender, and size of rats, which were assessed using a similar time-sampling procedure but following a rather shorter period of habituation; however, as a lower baseline level of spontaneous behaviour following the present prolonged habituation period should *favour* the detection of locomotor stimulation, it is not clear how this parameter could account for our finding *no* such locomotion. Although the previous study (7) did not report any induction by dihydrexidine of chewing directed onto cage bedding and/or faecal pellets, this may be explained by the absence of such "target" materials through the use of a wire mesh cage floor; the basis of the remaining differences in findings remains unclear.

On direct, systematic comparison, the present differences in ethogram between A 68930 and dihydrexidine involve dihydrexidine inducing a less prominent intense grooming response and failing to induce vacuous chewing; thus, these differences centre on the two primary behaviours proposed as indices of selective "D_1-like" receptor stimulation. Both A 68930 and dihydrexidine stimulate the activity of striatal adenylyl cyclase to an extent indistinguishable from DA itself and in a manner sensitive to blockade by SCH 23390, indicating "full" agonist efficacy at "D_1-like" receptors, but A 68930 appears more potent by an order of magnitude (9,23). At the receptor level, A 68930 shows high affinity and is selective by some two orders of magnitude for "D_1-like" over "D_2-like" receptors [70–250-fold: (6,9) and present data], whereas dihydrexidine shows rather less affinity and is only selective by one

TABLE 1

DISPLACEMENT OF [^{3}H]SCH 23390 AND [^{3}H]SPIPERONE FROM STRIATAL "D_1-LIKE" AND "D_2-LIKE" RECEPTORS, RESPECTIVELY, BY INVESTIGATIONAL AGENTS

	K_i (nM)		
	[^{3}H]SCH 233090 ("D_1-like")	[^{3}H]Spiperone ("D_2-like")	"D_1-like"/"D_2-like"
Dihydrexidine	85	1,005	0.084
A 68930	6.8	464	0.015
SCH 23390	0.18	189	0.001
YM 09151-2	2,636	0.09	29,290

Values are geometric means of at least three independent determinations, each performed in duplicate.

order of magnitude for "D_1-like" over "D_2-like" receptors [(23) and present data]. Other than dihydrexidine also having significant affinity for D_3 receptors (44), any interactions of either A 68930 or dihydrexidine with members of the "D_2-like" receptor family or within the "D_1-like" family have yet to be specified. Both A 68930 and dihydrexidine demonstrate little affinity for nondopaminergic receptors, with each showing weak affinity for α_2, and A 68930 showing some residual affinity for 5-HT_{2A} receptors (9,23). On this basis, the most prominent mechanistic difference between these two agents would appear to be the greater "D_2-like" affinity and reduced "D_1-like"/"D_2-like" selectivity of dihydrexidine. This interpretation would seem complementary to the reduced activities of dihydrexidine relative to A 68930 in the two putative behavioural indices of "D_1-like" receptor stimulation.

The grooming and intense grooming responses both to A 68930 and to dihydrexidine were each blocked by SCH 23390, although antagonism of A 68930 evidenced a more typical dose dependency than did the antagonism of dihydrexidine. In agreement with our initial report (6), vacuous chewing in response to A 68930 was not blocked by SCH 23390; indeed, SCH 23390 (but not other selective "D_1-like" antagonists) *induces* vacuous chewing (but not other behaviours) when given alone (5,6,10,11), but the basis of the effect is not clear. This phenomenon does not appear readily explained by a nondopaminergic mechanism, although this remains a possibility, and may involve a partial agonist action of SCH 23390 at an as yet unspecified but behaviourally relevant "D_1-like" site (10); there are both neurochemical (20) and electrophysiological (39) data consistent with such an action. Although dihydrexidine failed to induce vacuous chewing, thus precluding determination of any sensitivity to SCH 23390, this behaviour became evident when SCH 23390 was given prior to dihydrexidine. Given the action of SCH 23390 to induce vacuous chewing, the most parsimonious explanation would be that this effect is attributable to SCH 23390, though some form of synergism with dihydrexidine cannot be excluded incontrovertibly.

The grooming response to A 68930 was attenuated but not blocked, and the intense grooming response spared, by the selective "D_2-like" antagonist YM 09151-2. This partial attenuation of grooming is in accordance with cooperative/synergistic "D_1-like":"D_2-like" interactions known to regulate such *typical* dopaminergic behaviours (6,11,42), although some contribution from general motor depression has been suggested (38). Conversely, YM 09151-2 readily blocked both grooming and intense grooming induced by dihydrexidine; we could find no evidence for any significant action of selective "D_2-like" antagonism to release additional episodes of grooming to dihydrexidine, as noted previously using remoxipride (7). A low dose of YM 09151-2 attenuated but did not block A 68930-induced vacuous chewing, whereas at higher doses this attenuation was lost and release of additional vacuous chewing was evident. Given our previous finding that YM 09151-2 released a significant excess of vacuous chewing to A 68930, in accordance with oppositional "D_1-like":"D_2-like" interactions known to regulate such *atypical* dopaminergic behaviours, the basis of this present low-dose attenuation effect remains unclear (6,11,30,42). Although dihydrexidine failed to induce vacuous chewing, this behaviour became evident when YM 09151-2 was given prior to dihydrexidine. Because YM 09151-2 fails to induce vacuous chewing (10), the most parsimonious explanation is the release of this behaviour through oppositional "D_1-like":"D_2-like" interactions, in a manner similar to its release of vacuous chewing to A 68930 at comparable doses.

On considering the overall antagonist profiles of responses to A 68930 and dihydrexidine (Figs. 3, 4), it would appear that: a) typical responses to each agonist are blocked by selective "D_1-like" antagonism, with antagonism of A 68930 following a conventional dose–response relationship but that of dihydrexidine being somewhat less "smooth"; and b) typical responses to A 68930 are either partially attenuated or uninfluenced by selective "D_2-like" antagonism, whereas those to dihydrexidine are readily blocked. These antagonist behavioural profiles suggest that responses to dihydrexidine involve action(s) additional to "D_1-like" agonism and may have a functionally significant "D_2-like" component in their genesis, whereas A 68930 appears more specific in its "D_1-like" activity. Such a conclusion would be consonant with that derived above from the ethogram for each agonist and their respective receptor profiles.

However, alternative explanatory schemas must be considered. Firstly, we have found A 68930 to share with the classical benzazepine "D_1-like" agonists an action to induce prominent grooming but to be much more active than the benzazepines in inducing vacuous chewing. Furthermore, whereas the benzazepine "D_1-like" antagonists SCH 23390 and its isoquinoline counterpart BW 737C (28) each readily block these grooming responses, only BW 737C but not SCH 23390 blocks the vacuous chewing response to A 68930 (6,25). On conventional pharmacological grounds, this could suggest that grooming is mediated by a "D_1-like" receptor that recognises all known chemical classes of "D_1-like" compounds, whereas vacuous chewing is mediated by a subtype of "D_1-like" receptor that recognises preferentially the isochromans and the isoquinolines (6,41). Were this to be the case, dihydrexidine could have some activity at the "D_1-like" receptor mediating grooming but have little or no activity at that mediating vacuous chewing. Secondly, we have reported (10) the induction of grooming and vacuous chewing by SK&F 83959, a benzazepine derivative that shows *no* activity to stimulate adenylyl cyclase and readily *inhibits* the stimulation of adenylyl cyclase by DA (2); that is, it shows all the defining characteristics of a "D_1-like" antagonist. These findings challenge the presumption that "D_1-like" receptors involved in the regulation of behaviour are all coupled to adenylyl cyclase, and are complementary to an emerging body of evidence indicating the existence of "D_1-like" receptors that appear coupled to additional or alternative transduction mechanisms, particularly phosphoinositide hydrolysis (1,12,14,17–19,25,31,36,37,41). In such circumstances, the full efficacies of A 68930 and of dihydrexidine to stimulate adenylyl cyclase need not be relevant to all aspects of their behavioural effects; dihydrexidine may be less active at such non-cyclase-coupled "D_1-like" receptors than is A 68930. It may be relevant that whereas A 68930 is a relatively flexible molecule, dihydrexidine exhibits considerably greater conformational rigidity (24), which might hinder interaction with certain "D_1-like" sites.

In the present era of receptor subtype designation on the basis of molecular biological criteria, the more traditional basis for such subtyping in functional considerations should not be overlooked; A 68930 and dihydrexidine, as well as differing in their relative selectivity for "D_1-like" over "D_2-like" receptors to a functionally meaningful extent, may also differ in their interactions with putative "D_1-like" subtypes that are indicated by behavioural and other physiological considerations.

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