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Dopamine antagonistic effects of a series of analogues of oxiperomide and spiroxatrine measured behaviourally in the rodent

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The activity spectra of oxiperomide, spiroxatrine and analogues were determined in two experimental models of abnormal peri-oral movements (induced by intrastriatal dopamine and subcutaneously administered 2-(*NN*-dipropyl)amino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene (*NN*-diPr-5,6-diOHATN) in the guinea-pig), and in a stereotypy test (induced by subcutaneous apomorphine in the guinea-pig); the ability of the test compounds to induce catalepsy or catatonia in the rat was also determined. The parent compounds oxiperomide and spiroxatrine possessed optimal activity in all tests, although responses to the series of compounds allowed clear differentiation between an ability to antagonize the peri-oral movements (dopamine- or *NN*-di Pr-5,6-diOHATN induced) and an ability to antagonize apomorphine stereotypy. However, all compounds that antagonized the abnormal peri-oral movements also caused catalepsy/catatonia. The results are considered in terms of the selection of suitable agent(s) for the treatment of peri-oral dyskinesias.

It has been suggested that cerebral dopamine mechanisms may be differentially classified into those that cause increased locomotor behaviour and stereotypy when stimulated, and those that cause the development of abnormal involuntary movements when stimulated (dyskinesias, including peri-oral dyskinesias) (Costall, Naylor & Pinder, 1975; Costall & Naylor, 1976; Costall, Naylor & Owen, 1977b). Thus, dopamine antagonists should theoretically be able to inhibit locomotion (causing catalepsy), inhibit stereotypies and antagonize dyskinesias. However, although neuroleptic agents have been found to be highly effective as cataleptogenic and antistereotypic agents (Janssen, Niemegeers & Schellekens, 1965), they generally exhibit little ability to modify dyskinesias induced in animals (Costall & Naylor, 1975). Dyskinesias have been induced in the rodent by intrastriatal dopamine and intrastriatally or peripherally administered 2-(*NN*-dipropyl)amino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene (*NN*-diPr-5,6-diOHATN): in these tests two agents, oxiperomide and spiroxatrine, were found to be particularly effective (Costall & Naylor, 1975; Costall & others, 1977b). The lack of clinically useful antidyskinetic agents therefore prompted the present examination of the activity spectra of a series of analogues of oxiperomide and spiroxatrine to determine their relative effectiveness to antagonize abnormal involuntary movements, stereotypies and general locomotor function as indicated by catalepsy production.

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MATERIALS AND METHODS

Animals

Male Dunkin-Hartley (DHP/Lac) guinea-pigs, 200–300 g for the peripheral studies or 400–500 g at the time of operation, and male Sprague–Dawley (CFE) rats, 250–275 g, were used.

Intracerebral injection technique

Bilateral stainless steel guide cannulae (0·65 mm diameter) were stereotaxically implanted for injections into the caudate-putamen of guinea-pigs at Ant. 10·5, Vert. +6·6, Lat. ±3·5, the co-ordinates being determined with respect to the zero of the Kopf stereotaxic instrument with the incisor bar raised 5 mm. Injection cannulae (0·3 mm diameter) were made to extend 2·5 mm beyond the guide tips. The operative procedures have been detailed previously (Costall & Naylor, 1975). Animals were used once 14 days after operation and were manually restrained for the intracerebral injection. The location of the guide cannulae was confirmed histologically in every 5th guinea-pig.

Behavioural studies

All behavioural experiments were carried out between 08.00 and 18.00 h in a sound-proofed, diffusely illuminated room maintained at 21 ± 1°. For observation, animals were housed in screened Perspex cages (25 × 15 cm and 15 cm high).

Assessment of stereotypy/peri-oral movements

The stereotyped behaviour patterns induced by subcutaneous (s.c.) injection of apomorphine and the peri-oral movements induced by s.c. *NN*-diPr-5,6-diOHATN in the guinea-pig were characterized almost exclusively by biting, gnawing or licking; sniffing and repetitive head and limb movements were rarely observed. The peri-oral effects observed after intrastriatal dopamine were accompanied by increased locomotor activity. A dose of 2 mg kg^{-1}, s.c. apomorphine, 0·05 mg kg^{-1}, s.c. of the ATN compound and 100 μg bilateral intrastriatal dopamine (1 μl volume 2 h after 75 mg kg^{-1}, i.p. nialamide) were selected for drug antagonism studies because they were the lowest doses to induce a continuous biting behaviour in 100% of animals tested. The putative antagonists were injected peripherally when biting behaviour was established at maximum intensity (15 min after apomorphine or the ATN compound, and 2 h after dopamine), and the intensity of biting assessed at 10–15 min intervals for the duration of effect. The system used to indicate the relative effectiveness of the various antagonists was 0 no block, (+) inconsistent block, + partial block, ++ complete block.

Measurement of catalepsy

Rats were placed in observation cages, equipped with a 10 cm high horizontal bar, 30 min before drug treatment to allow adaptation to the new environment. Animals were tested for the presence of catalepsy by placing both front limbs over the horizontal bar, a cataleptic animal maintaining this position for a time dependent on the degree of catalepsy (see below). To account for animals maintaining the cataleptic position for an 'infinite' time, a scoring system was used to indicate the intensity of catalepsy: 0 = no catalepsy, 1 = 0·1–2·5 min, 2 = 2·6–5.0 min, 3 = 5·1–10·0 min, 4 = 10·1–20·0 min, 5 = 20·1–∞. With some agents used, catalepsy was accompanied by muscle rigidity, a state of immobility frequently described as catatonia. In this report the term 'catalepsy' does not exclude a catatonic immobility.

Drugs

For intrastriatal injection dopamine HCl (Aldrich) was dissolved in nitrogen bubbled distilled water neutralized with sodium bicarbonate. For injection by the peripheral route nialamide (Sigma) and clothiapine (Wander) were dissolved in the minimum quantity of hydrochloric acid and made up to volume with distilled water, fluphenazine HCl (Squibb), piperoxan HCl (Roche), (±)-propranolol HCl (ICI) and atropine SO_4 (Sigma) were dissolved in distilled water, aceperone (Janssen) and methysergide hydrogen maleinate (Sandoz) in the minimum quantity of *NN*-dimethyl formamide made up to volume with distilled water. Apomorphine HCl (Macfarlan Smith) and *NN*-diPr-5,6-diOHATN HBr (Cannon) were dissolved in nitrogen bubbled distilled water containing 0·1% sodium metabisulphite. Haloperidol and the Janssen compounds indicated in Figs 1 and 2 were dissolved in the minimum quantity of tartaric acid and made up to volume with distilled water. With the exception of R5812, R6537, R7577, R5880 (oxalate) and R9614 (HCl), the compounds indicated in Figs 1 and 2 were supplied as the base.

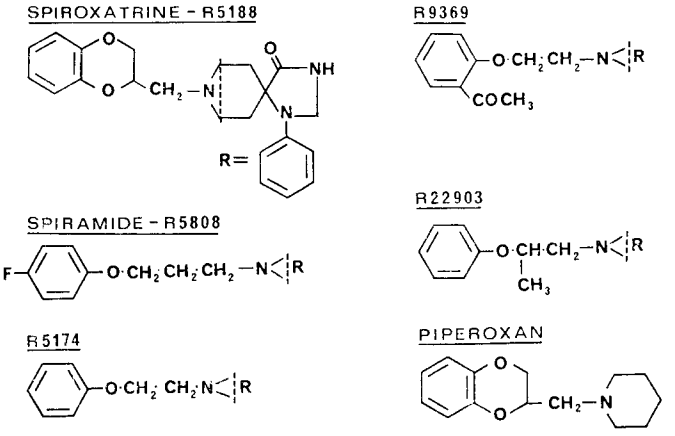

FIG. 1. Spiroxatrine and analogues. With the exception of piperoxan the grouping R is common to all compounds.

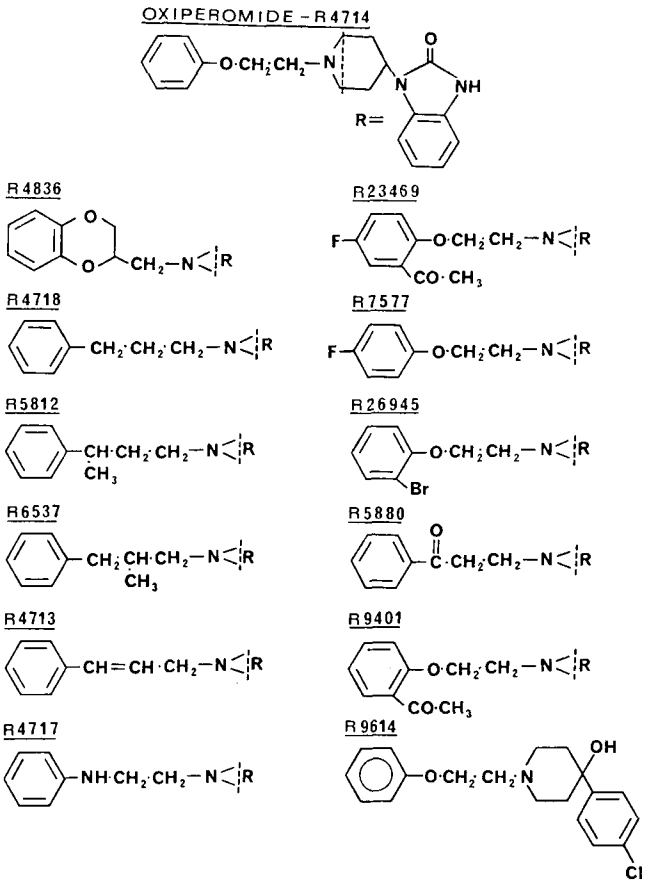

FIG. 2. Oxiperomide and analogues. With the exception of R9614 the grouping R is common to all compounds.

RESULTS

Typical neuroleptic agents such as haloperidol, fluphenazine and clothiapine failed to antagonize dopamine-induced dyskinesias in doses up to 16 mg kg^{-1}, whereas doses of 0·5 or 1 mg kg^{-1} of these agents completely antagonized the peri-oral movements induced by *NN*-diPr-5,6-diOHATN. Apomorphine stereotypy was also antagonized, but larger doses were generally required, and each neuroleptic agent caused a dose-dependent catalepsy (Table 1). Acceperone, 1–5 mg kg^{-1}, propranolol, 0·5–5 mg kg^{-1}, atropine, 0·5–5 mg kg^{-1} and methysergide, 0·1–5 mg kg^{-1}, were inactive in all tests.

Spiroxatrine, spiramide and R5174 completely antagonized dopamine-induced dyskinesias in the guinea-pig, spiroxatrine being the most potent. R9369 only partially inhibited the dopamine dyskinesias and R22903 and piperoxan were inactive in doses up to 16 mg kg^{-1}. Those agents that antagonized the dopamine effects also antagonized the ATN dyskinesias and apomorphine stereotypy, and caused dose-dependent catalepsy. Generally, the test compounds were most effective in antagonizing the ATN dyskinesias and least effective against the dopamine dyskinesias (Table 2).

Oxiperomide, R4836 and R6537 effectively antagonized dopamine dyskinesias, the ATN dyskinesias and apomorphine stereotypy, and induced catalepsy. Again, the ATN effects were most readily antagonized (Table 3). R5812 was also shown to completely antagonize the dopamine dyskinesias, but whilst this agent also antagonized the ATN dyskinesias and induced catalepsy, doses up to 16 mg kg^{-1}, i.p. failed to antagonize apomorphine stereotypy (Table 3). R4718 and R4713 were only weakly active or inactive in all tests (Table 3). R23469 was inactive in both dyskinesia models although it effectively antagonized apomorphine stereotypy and caused catalepsy (Table 3). R7577 inhibited dopamine dyskinesias and caused catalepsy

Table 1. *Modification of peri-oral dyskinesias and stereotyped biting, and the induction of catalepsy by typical neuroleptic agents.* Abnormal peri-oral movements were induced in the guinea-pig by 100 μg bilateral intrastriatal dopamine (following nialamide pretreatment, 75 mg kg^{-1}, i.p. 2 h) and by 0·05 mg kg^{-1}, s.c. *NN*-diPr-5,6-diOHATN. Stereotypy was induced in the guinea-pig by 2 mg kg^{-1}, s.c. apomorphine. Putative antagonists were injected peripherally when biting behaviour was established at maximum intensity (15 min after apomorphine or the ATN, 2 h after dopamine). The effectiveness of the antagonists was characterized as: no block = 0, inconsistent block = (+), partial block = +, complete block = ++. Catalepsy was assessed in the rat according to a scoring system representing an intensity range of 0-5 (see methods section). n = 6–8. s.e.s on catalepsy >15% means.

	Antagonism of			
Neuroleptic (mg kg^{-1}, i.p.)	Dopamine dyskinesias	ATN dyskinesias	Apomorphine stereotypy	Catalepsy induction (score)
Haloperidol				
0·13		0		
0·25		+		1·0
0·5		++	0	1·5
1	0	++	+	3·0
2	0		++	5·0
4	0			
8	0			
16	0			
Fluphenazine				
0·13		0		
0·25		+		
0·5		++	0	1·0
1	0	++	+	2·5
2	0		++	4·0
4	0			5·0
8	0			
16	0			
Clothiapine				
0·13		0		
0·25		+	0	
0·5		++	+	0·5
1	0		++	2·1
2	0			3·8
4	0			4·5
8	0			
16	0			

in the same dose range: an insufficiency of this drug was available for inclusion in all tests. Similarly, R9401 was only tested in the dopamine dyskinesia model in which it was only partially effective at 16 mg kg^{-1} (Table 3). R26945, R5880 and R9614, 4–16 mg kg^{-1}, i.p., were inactive in this dyskinesia model. R4717 was inactive in all test situations (see legend, Table 3).

DISCUSSION

Spiroxatrine and oxiperomide are equipotent in inhibiting the peri-oral movements induced by intrastriatal dopamine injection in the guinea-pig.

Table 2. *Modification of peri-oral dyskinesias and stereotyped biting, and the induction of catalepsy by spiroxatrine and analogues.* See Table 1 for experimental details. n = 6–8. s.e.s on catalepsy <18% means.

	Antagonism of			
Agent (mg kg^{-1}, i.p.)	Dopamine dyskinesias	ATN dyskinesias	Apomorphine stereotypy	Catalepsy induction (score)
Spiroxatrine (R5188)				
0·015		0		
0·03		+		
0·06		++		
0·13		++		
0·25		++		
0·5	0	++	0	1·0
1	++		+	2·0
2	++		++	4·0
4				5·0
Spiramide (R5808)				
0·25		++	+	1·0
0·5		++	++	2·8
1				4·5
2				5·0
4				
8	0			
16	++			
R5174				
0·13		+		
0·25		++	0	
0·5		++	+	1·0
1			++	2·5
2	0		++	3·7
4	++			4·5
8	++			
R9369				
0·06		0		
0·13		++	0	
0·25		++	(+)	
0·5		++	+	
1		++	++	0
2	0		++	1·0
8	0			2·2
16	(+)			3·0

R22903 and piperoxan, 4–16 mg kg^{-1}, i.p., failed to antagonize dopamine-induced dyskinesias and, therefore, these agents were not included in the other tests.

A superficial similarity in their structures is the presence of a $-O-CH-CH_2-N\langle$ function, a function absent from other types of neuroleptic agent from the phenothiazine, butyrophenone and dibenzazepine series which are ineffective in reducing dopamine-induced peri-oral movements. The present studies indicate this to be an essential requirement for optimal effect against the abnormal oral movements induced by dopamine. Thus, the inclusion of a further methyl group in or on the chain (spiramide, R22903), or substitution of the oxygen function by CH_2 (R4718), $CH-CH_3$ (R5812), $CH_2-CH-CH_3$ (R6537), $CH=CH$ (R4713) and NH (R4717) all reduce the antagonistic action.

Further important features which affect antagonistic abilities against the dopamine-induced peri-oral movements are the phenoxy grouping in the oxiperomide series and the incorporation of the oxygen into a benzodioxane structure in the

Table 3. *Modification of peri-oral dyskinesias and stereotyped biting, and the induction of catalepsy by oxiperomide and analogues.* See Table 1 for experimental details. n = 6–8. s.e.s on catalepsy <15% means.

Agent Dose (mg kg^{-1}, i.p.)	Antagonism of: Dopamine dyskinesias	ATN dyskinesias	Apomorphine stereotypy	Catalepsy induction (scores)
Oxiperomide (R4714)				
0·016		0		
0·03		+		
0·06		+		
0·13		++		
0·25		++		
0·5	0	++	0	10·
1	++		+	1·5
2	++		++	2·5
4	++			4·5
R4836				
0·16		0		
0·31		+		
0·63		+		
1·25		++	0	
2·5		++	+	
4	0		++	0
8	+			0·3
16	++			2·3
R4718				
4	0	0		
8	0	+	0	0
16	+	+	+	0·5
R5812				
1		0		
2		+		
4	0	+	0	0·7
8	+	++	0	1·7
16	++		0	3·3
32				Toxic
R6537				
0·5		0		
1		+	0	
2		+	0	
4	0	++	(+)	
8	++	++	+	1·0
16	++		++	2·0
32				Toxic
R4713				
4	0	0	0	0
8	0		0	0
16	+		(+)	
R23469				
4	0	0	0	0
8	0	0	+	0
16	0	0	++	1·3
32			++	4·7
R7577				
2	0			0·8
4	++			1·0
8	++			1·5
16				2·4
R9401				
4	0			
8	0			
16	(+)			

R26945, R5880 and R9614, 4–16 mg kg^{-1}, i.p., failed to antagonize dopamine-induced dyskinesias and, therefore, these agents were not included in the other tests. R4717 was inactive in all tests in doses of 4–16 mg kg^{-1}, i.p. Certain effects of larger doses of some agents are omitted, this indicates insufficient drug.

spiroxatrine series. Rather surprisingly, it was found that whilst the phenoxyethyl grouping confers activity in the oxiperomide series, this grouping reduces activity in the spiroxatrine series (R5174) and the benzodioxane moiety similarly reduces activity in the oxiperomide series (R4836). However, it is clear that the presence of either the benzodioxane or phenoxyethyl groups alone is not sufficient to confer activity (piperoxan, R9614), and that a third feature is the importance of the bulky benzimidazolidinone or phenylimadolidinone groups for the receptor interaction, R9614 (which lacks these groupings) failing to antagonize the peri-oral movements induced by dopamine.

As a second model of peri-oral movements we have used *NN*-diPr-5,6-diOHATN. Recent studies have shown that the abnormal peri-oral movements induced by this compound are specifically blocked by neuroleptic agents (Costall & others, 1977), and that it is the most potent 'dopamine agonist' known, as assessed by its ability to displace a neuroleptic ligand in receptor binding assays (Cannon, Costall & others, 1978; Leysen, Gommeren & Laduron, 1978). Further, *NN*-diPr-5,6-diOHATN will reduce dopamine turnover which is also indicative of dopamine agonist properties (Costall & others, unpublished data). Nevertheless, it would appear unlikely that this ATN derivative acts via precisely the same mechanisms/sites as dopamine to induce abnormal peri-oral movements since these are reduced by some agents (e.g. compounds enhancing GABA activity) which are ineffective against dopamine (Costall & others, 1977), and unlike dopamine the ATN fails to induce a hyperactivity on intrastriatal injection (Costall, Naylor & others, 1977a). In the ATN model, spiroxatrine and oxiperomide were approximately 10 times as potent as the classical neuroleptic agents fluphenazine, haloperidol and clothiapine, although in other tests for neuroleptic activity (catalepsy induction, antagonism of apomorphine stereotypy) both oxiperomide and spiroxatrine were equipotent with the classical agents. The analogues of oxiperomide and spiroxatrine gave a good correlation of ability to antagonize the peri-oral effects induced by dopamine and those induced by the ATN. The presence of the $-O-\underset{|}{CH}-CH_2-N\langle$, benzodioxane/phenoxyethyl and benzimidazolidinone/phenylimadolidinone groups were essential for optimal antagonistic activity. Whilst the peri-oral movements induced by the ATN compound cannot, therefore, be used to qualitatively delineate absolutely between haloperidol-like neuroleptics and the oxiperomide/spiroxatrine derivatives, the results emphasize the value of the compound as a tool for investigating the mechanisms involved in peri-oral movements and their antagonism.

The third experimental procedure assessed the ability of the test agents to antagonize apomorphine-

induced stereotyped behaviour (identical in appearance, at the dose used, to the peri-oral movements mentioned above), a test routinely used to detect 'neuroleptic' activity. Generally, apomorphine stereotypy was more resistant to neuroleptic inhibition than the peri-oral movements described, although compounds that were shown to antagonize the abnormal peri-oral effects induced by dopamine and the ATN also antagonized apomorphine stereotypy. But, the converse was not necessarily correct since some compounds that antagonized apomorphine stereotypy, including haloperidol, related agents and R23469, did not antagonize the peri-oral movements induced by dopamine. R23469 also showed an unusual spectrum of activity in failing to antagonize the peri-oral movements induced by the ATN, and R5812 abolished all peri-oral movements but not apomorphine stereotypy. It would appear that apomorphine causes stereotypies through mechanisms/sites that differ from those involved in the production of peri-oral movements. It is also apparent that an ability or failure to inhibit apomorphine stereotypy cannot be taken as an absolute index of drug action on cerebral dopamine systems.

The fourth test, catalepsy induction, was used to determine an antagonistic effect on dopamine systems, in particular (although not exclusively) an action on extrapyramidal dopamine systems. All the test compounds that were shown to antagonize the peri-oral movements induced by dopamine and the ATN, and stereotypy induced by apomorphine, caused a cataleptic/catatonic immobility in the rat. Further, catalepsy was produced by agents which failed to antagonize the peri-oral movements induced by dopamine. Thus, it is suggested that a cataleptic potential may reflect gross inhibitory effects on dopamine mechanisms and is not necessarily indicative of an ability to antagonize peri-oral movements. Nevertheless, it remains clear that oxiperomide, spiroxatrine and analogues which antagonize peri-oral movements can also induce catalepsy/catatonia.

Drug-induced movement disorders, particularly the abnormal peri-oral movements that develop after therapy with dopamine agonists, or long-term treatment with neuroleptic agents, are considered to reflect an enhanced dopaminergic activity (Marsden, 1976). The present study indicates that oxiperomide, spiroxatrine and certain analogues are particularly efficacious in inhibiting such movements in animals. Preliminary data suggest a similar effectiveness for oxiperomide in the clinic (Brugman, personal communication; Bédard, Parkes & Marsden, 1978) although careful dosing is required to avoid a more general retardation of psychomotor function. It is therefore suggested that in present and future studies an examination of the ratio of doses required to inhibit peri-oral movements or induce catalepsy may be valuable in the selection of a suitable drug for use in the clinic.

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