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Design of agents for stimulation of neostriatal dopaminergic mechanisms

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The structure-activity requirements for stimulation of striatal dopaminergic mechanisms were investigated using a series of phenethylamine derivatives, apomorphine and isoapomorphine, and the intracerebral injection technique for direct application into the striatum. Asymmetric body posturing (unilateral injections in saline- and haloperidol-pretreated rats) and hyperactive/stereotyped behaviour (bilateral injections in nialamide-pretreated rats) were used as indices of dopaminergic stimulation. Of 24 phenethylamine derivatives studied, 2-(3,4-dihydroxyphenyl)ethylamine (dopamine) possessed maximum activity. Alteration in length of the ethylamine side-chain, substitution of the α-C atom (α-methyldopamine) or hydroxylation of β-C atom (noradrenaline) markedly reduced or abolished activity. Activity was also decreased by *N*-methyl substitution but epinine was active. The catechol moiety appeared essential for dopaminergic stimulation since the 3- or 4-hydroxyphenethylamines (tyramine, *m*-tyramine) were inactive and 2-(3,4-methylenedioxyphenyl)ethylamine was only weakly active. The marked dopamine-like activity of the 2-(3,4-diacetoxyphenyl)ethylamine compounds was attributed to their non-enzymic hydrolysis to dopamine *in situ*. The stereoselectivity of the dopamine-induced effects was assessed using *trans* and *cis*-2-(3,4-methylenedioxyphenyl)cyclopropylamine. The *trans* conformer was active and the importance of this conformation was further indicated by the inactivity of isoapomorphine compared with apomorphine. It is suggested that the results provide a rational basis for the design of drugs required to stimulate striatal dopamine receptors and that the intrastriatal injection technique may be useful in the detection of potential antiparkinson agents.

The success of L-dopa in the treatment of Parkinson's disease, considered to result from its ability to enhance striatal dopaminergic activity, has stimulated interest in the 'design' of dopamine-like agents (Pinder, 1972, 1973). Models for the detection of such activity have relied upon peripheral administrations of drugs, while the developments of stereotyped or rotational behaviour patterns have been used as indices of striatal dopaminergic stimulation (Costall, Naylor & Wright, 1972; Ungerstedt, Avemo & others, 1973). However, an assessment of the optimal chemical structures for dopamine agonist activity from such studies is difficult. Metabolism may occur in the peripheral system to give either active or inactive compounds, while certain structures may have difficulty in passing the blood-brain barrier. Furthermore, it now appears probable that brain areas in addition to the striatum may be influenced to mediate the behavioural states used as indices of striatal dopaminergic stimulation (McKenzie, 1971; Divac, 1972; Costall & Naylor, 1973 a, b; Wolfarth, Grabowska & others, 1973). These difficulties were eliminated in the

present studies by application of drugs directly to striatal tissue and comparison with the behavioural effects elicited by intrastriatal dopamine.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley (C.F.E.) rats intracerebrally injected weighed 300 ± 25 g initially while those for peripheral drug administrations weighed 120–140 g.

Intracerebral injection technique

Stainless-steel guide cannulae were constructed and stereotaxically implanted into the caudate-putamen as described previously (Costall & Naylor, 1974). Briefly, guides were implanted such that the injection cannula, extending 1·5 mm beyond the tip of the guide, deposited drug at the centre of the caudate-putamen, A 8·0, vert. +1·5, lat. ± 3·0 (De Groot, 1959). Animals, first used 2 to 3 weeks after the operation, were manually restrained during the injection procedure in which drugs were administered intrastriatally in a volume of 1 or 2 μl over a 5 s period from an Agla micrometer syringe. 55 s were allowed for deposition of drug.

Histological techniques were those of Costall & Naylor (1973a).

Behavioural observations

Experiments were carried out between 09.00 and 21.00 h in a sound-proofed, diffusely illuminated room maintained at a temperature of 21 ± 1°. Immediately upon completion of an intracerebral injection rats were placed in an 'open field' and closely observed to determine any modification in behaviour/motor patterns. Contralateral asymmetric body posturing following unilateral intrastriatal injection and hyperactive/stereotyped behaviour after bilateral administration were characterized and assessed according to the scoring systems shown in Table 1.

In experiments to assess the stereotypic activity of drugs by peripheral administration, rats were placed in individual Perspex observation cages (30 cm × 20 cm × 15 cm high) 30 min before drug treatment to allow adaptation to the new environment and were observed 5, 10, 15, 30, 60 and 120 min after intraperitoneal administration for assessment of stereotyped behaviour (see Table 4).

Experimental design

Cannulated rats received saline, nialamide or haloperidol by the intraperitoneal route before the intrastriatal injection as detailed in the results. 4 to 8 rats were used at each dose level of intrastriatally administered agent. Each rat was used on no more than 4 occasions with the allowance of at least 14 days between treatments.

Drugs administered peripherally (Tables 1–3)

Nialamide was dissolved in a minimum quantity of hydrochloric acid made up to volume with distilled water, haloperidol in 1% lactic acid and compounds XVI to XX (hydrochlorides) in distilled water. All doses were calculated as the base and administered in a volume of 1 ml kg^{-1} intraperitoneally.

Drugs administered intrastriatally (Tables 1–3)

Compounds I to V, VII, XII to XVI, XIX to XXII and XXV (hydrochlorides),

Table 1. *Contralateral asymmetric behaviour and hyperactive/stereotyped behaviour induced by the intrastriatal administration of phenethylamine derivatives.*

Intrastriatal injection		Contralateral asymmetric behaviour								Hyperactive/stereotyped behaviour			
		Saline pretreatment				Haloperidol pretreatment (2 mg kg^{-1}, 30 min)				Nialamide pretreatmet (100 mg kg^{-1}, 2 h)			
Drug	Dose (μg μl^{-1})	Rats resp.	Onset (min)	Intensity*	Duration (h)	Rats resp.	Onset (min)	Intensity*	Duration (h)	Rats resp.	Onset (min)	Intensity*	Duration (h)
**1,2-Phenethylamine	100	0/4	—	0	—	0/4	—	0	—	0/4	—	0	—
II *p*-Tyramine	100	0/4	—	0	—	0/4	—	0	—	0/4	—	0	—
III *m*-Tyramine	100	0/4	—	0	—	0/4	—	0	—	0/4	—	0	—
IV Dopamine	100	4/4	15	+	2+	4/4		++	2+	4/4	82	+++	2+
	50	0/4	—	0	—	4/4		++	2+	4/4	66	+++	2+
	25					4/4	IM***	++	2+	4/4	77	+++	2+
	12·5					4/4		+	2+	4/4	90	+(+)	2+
	6·25					1/8		+	2+	2/8	110	(+)	2+
V Epinine	100	4/4	25	+	0·6	4/4	IM	++	2+	4/4	90	+	2+
	50	0/4	—	0	—	4/4	IM	+	1·1	4/4	95	(+)	2+
	25					0/4	—	0	—	0/4	—	0	—
VI *NN*-Dimethyl-2-(3,4-dihydroxyphenyl) ethylamine	100	0/4	—	0	—	4/4	IM	++	2+	4/4	121	+	2+
	50					4/4	IM	+	1·3	4/4	125	+	2+
	25					0/4	—	0	—	0/4	—	0	—
VII 3,4-Dihydroxybenzylamine	100	0/4	—	0	—	4/4	IM	+	2+	0/4	—	0	—
	50					0/4	—	0	—				
VIII 3-(3,4-Dihydroxyphenyl)propylamine	100	0/4	—	0	—	4/4	IM	+	2+	4/4	82	+	2+
	50					4/4	IM	+	0·3	4/4	90	+	2+
	25					0/4	—	0	—	0/4	—	0	—
IX 4-(3,4-Dihydroxyphenyl)butylamine	100	0/4	—	0	—	0/4	—	0	—	0/4	—	0	—
X Noradrenaline	100	0/4	—	0	—	0/4	—	0	—	0/4	—	0	—
XI α-Methyldopamine	100	0/4	—	0	—	4/4	28	+	2+	4/4	40	(+)	2+
	50					0/4	—	0	—	4/4	30	(+)	2+
	25									0/4	—	0	
XII 3-Hydroxy-4-methoxyphenethylamine	100	0/4	—	0	—	0/4	—	0	—	0/4	—	0	
XIII 4-Hydroxy-3-methoxyphenethylamine	100	0/4	—	0	—	0/4	—	0	—	0/4	—	0	—

All values represent the means of the number of animals responding.
*Scoring system used for estimation of the intensity of contralateral asymmetric behaviour: no asymmetry, 0; periodic holding of head to one side with movements of body in same direction when disturbed, able to move straight forward, +; head and body held continuously to one side, resistance to manual turning of body in opposite direction, ++. Scoring system used for estimation of the intensity of hyperactive/stereotyped behaviour: behaviour indistinguishable from control rats, 0; hyperactive exploration of cage, (+); repetitive head and front limb movements, periodic activity, +; repetitive head and front limb movements, infrequent biting movements and infrequent periods of activity, ++; continuous chewing or biting at the shavings, cage or body, +++.
**Compounds XIV and XV, *N*-methyl and *NN*-dimethyl-2-phenethylamine, were inactive in all experiments.
***Onset was apparent immediately following the period of "injection behaviour".

compounds XXIII and XXIV (sulphates), compounds VI, VIII, IX, XI, XVII and XVIII (hydrobromides) and compound X (hydrogen tartrate monohydrate) were dissolved in distilled water. Apomorphine hydrochloride and isoapomorphine hydrobromide were dissolved in nitrogen bubbled distilled water containing 0·1% sodium metabisulphite. All drug solutions were prepared immediately before use.

Synthesis of drugs

All compounds, excepting those described below, were synthesized by known methods or were obtained from commercial sources (apomorphine, Macfarlan Smith; (+)-amphetamine and nialamide, Sigma; dopamine, Koch-Light; ephedrine, Northern Pharmaceuticals; haloperidol, Janssen; noradrenaline, Hoechst; tranylcypromine, Smith, Kline and French).

Cis- (*and* trans) *2-(3,4-methylenedioxyphenyl) cyclopropane carboxyhydrazides.* A

Table 2. *Contralateral asymmetric behaviour and hyperactive stereotyped behaviour induced by the intrastriatal administration of phenethylamine derivatives.*

Intrastriatal injection		Contralateral asymmetric behaviour								Hyperactive/stereotyped behaviour			
		Saline pretreatment				Haloperidol pretreatment (2 mg kg^{-1}, 30 min)				Nialamide pretreatment (100 mg kg^{-1}, 2 h)			
Drug	Dose (μg μl^{-1})	Rats resp.	Onset (min)	Intensity*	Duration (h)	Rats resp.	Onset (min)	Intensity*	Duration (h)	Rats resp.	Onset (min)	Intensity*	Duration (h)
XVI 2-(3,4-Diacetoxyphenyl) ethylamine	100	4/4	IM***	+	2+	4/4		++	2+	4/4	31	++	2+
	50	0/4	—	0	—	4/4		++	2+	4/4	66	(+)	2+
	25					4/4	IM	++	2+	4/4	60	(+)	2+
	12·5					4/4		++	1·5	0/4	—	0	—
	6·25					6/8		+	0·4				
XVII *N*-Methyl 2-(3,4-diacetoxyphenyl) ethylamine	100	4/4	16	+	0·7	4/4	30	++	2+	4/4	82	+	2+
	50	0/4	—	0	—	4/4	28	++	2+	4/4	104	(+)	2+
	25					4/4	34	+	2+	6/8	120	(+)	2+
	12·5					0/4	—	0	—	0/4	—	0	—
XVIII *NN*-Dimethyl-2-(3,4-Diacetoxyphenyl) ethylamine	100	0/4	—	0	—	4/4	56	+	1·8	4/4	95	+	2+
	50					4/4	50	+	1·4	6/8	135	(+)	2+
	25					5/8	39	+	0·4	0/4	—	0	—
XIX α-Methyl-2-(3,4-methylenedioxyphenyl) ethylamine	100	0/4	—	0	—	4/4	IM	++	2+	4/4	12	++	1·1
	50					4/4	IM	+	2+	4/4	10	(+)	0·75
	25					0/4	—	0	—	0/4	—	0	—
XX 2-(3,4-Methylenedioxyphenyl)ethylamine	100	0/4	—	0	—	4/4	13	+	1·6	4/4	7	(+)	0·6
	50					0/4	—	0	—	4/4	18	(+)	0·5
XXI *trans*-2-(3,4-Methylenedioxyphenyl)cyclopropylamine	100	0/4	—	0	—	4/4	IM	+	2+	4/4	22	(+)	2+
	50					4/4	IM	+	2+	0/4	—	0	—
	25					6/8	10	+	2+				
XXII *cis*-2-(3,4-Methylenedioxyphenyl)cyclopropylamine	100****	0/4	—	0	—	0/4	—	0	—	0/4	—	0	—
XXIII Tranylcypromine	100	0/4	—	0	—	0/4	—	0	—	0/4	—	0	—
XXIV (+)-Amphetamine	100	0/4	—	0	—	0/4	—	0	—	4/4	29	(+)	2+
	50					0/4	—	0	—	1/6	35	(+)	2+

All values represent the means of the number of animals responding.
*Scoring systems indicated on Table 1.
**Compound XXV, ephedrine, was inactive in all experiments.
***Onset was apparent immediately following the period of "injection behaviour".
****Administered in a volume of 2 μl.

mixture of ethyl 2-(3,4-methylenedioxyphenyl) cyclopropane carboxylate (35 g, 0·15 mol) and hydrazine hydrate (150 ml) was heated under reflux for 2 h, when solution occurred. The orange solution was poured into cold water (1 litre), and the tan solid filtered and recrystallized from benzene, m.p. 145–146°, yield 15 g. This was the *trans*-isomer (lit.m.p. 142–144°; Kaiser, Lester & others, 1962).

The aqueous filtrate was evaporated to dryness and the residual oil crystallized from benzene to give the *cis*-isomer, m.p. 136–138°, yield 8 g. Found: C, 59·9; H, 5·4; N, 12·5. $C_{13}H_{12}N_2O_3$ requires C, 60·02; H, 5·5; N, 12·1%.

Cis (*and* trans) *2-(3,4-methylenedioxyphenyl)cyclopropylamines.* For example, a solution of 2·15 g (0·031 mol) of sodium nitrite in water (50 ml) was added dropwise to a stirred suspension of *cis*-2-(3,4-methylenedioxyphenyl) carboxhydrazide (6·8 g, 0·037 mol) in 5% HCl (150 ml) maintained at 0° and covered with 100 ml of diethyl ether. The layers were then separated, the ethereal layer dried ($MgSO_4$) and evaporated to dryness. The residual oil was heated under reflux for 4 h with 400 ml of toluene. This solution was again evaporated to dryness and the residual oil heated under reflux for 45 min with 150 ml of 10% H_2SO_4. The cooled solution was made alkaline (10% NaOH), extracted several times with ether, and dried ($MgSO_4$). The amine hydrochloride was prepared by addition of ethereal HCl, and recrystallized from propan-2-ol-ether. *Cis*-isomer:m.p. 183–184°, yield 31%. Found: C, 56·3; H, 5·9. $C_{10}H_{11}NO_2 \cdot HCl$ requires C, 56·2; H, 5·7%.

Trans-isomer: m.p. 206–207° (lit.m.p. 206–208°; Kaiser & others, 1962), yield 52%.

Table 3. *Contralateral asymmetric behaviour and hyperactive/stereotyped behaviour induced by the intrastriatal administration of apo and isoapomorphine.*

Intrastriatal injection		Contralateral asymmetric behaviour								Hyperactive/stereotyped behaviour			
		Saline pretreatment				Haloperidol pretreatment (2 mg kg^{-1}, 30 min)				Nialamide pretreatment (100 mg kg^{-1}, 2 h)			
Drug	Dose (μg μl^{-1})	Rats resp.	Onset (min)	Intensity*	Duration (h)	Rats resp.	Onset (min)	Intensity*	Duration (h)	Rats resp.	Onset (min)	Intensity*	Duration (min)
XXVI Apomorphine	50/2	0/4	—	0	—	4/4	IM**	++	2+	4/4	<5	++	36
	25/1					4/4		++	2+	4/4	16	++	20
	12·5/1					2/8		+	2+	0/4	—	0	—
XXVII Isoapomorphine	25/2	0/4	—	0	—	0/4	—	0	—	0/4	—	0	—

All values represent the means of the number of animals responding.
*Scoring systems indicated on Table 1.
**Onset was apparent immediately following the period of "injection behaviour".

RESULTS

Histological assessment of cannulae locations

Cannulae locations, determined in every 10th animal, were within the area of investigation and indistinguishable from those reported by Costall & Naylor (1974).

Behavioural assessments

Unilateral intrastriatal injections following peripheral pretreatment with saline or haloperidol. After peripheral saline pretreatment, insertion of the injection cannula, administration of 1 μl saline or solvent into the caudate-putamen invariably caused a rat to circle to the contralateral side. The duration of this "injection behaviour" never exceeded 90 s. Following haloperidol pretreatment the "injection behaviour" was more intense but its duration did not exceed 2 to 5 min. The behaviour was considered to result from physical disruption of the tissue and, as such, to be non-specific. The results expressed in Tables 1–3 refer to motor asymmetries which develop after the period of injection artifact.

The unilateral intrastriatal injection of dopamine in rats pretreated peripherally with saline caused a contralateral asymmetric response (Table 1). Only 2-(3,4-diacetoxyphenyl)ethylamine induced a comparable behaviour. *N*-Methyl-2-(3,4-dihydroxyphenyl)ethylamine and *N*-methyl-2-(3,4-diacetoxyphenyl)ethylamine were weakly active whilst the other agents tested were without effect (Tables 1–3). In animals pretreated with haloperidol peripherally, the contralateral asymmetric behaviour usually developed immediately following the injection artifact although the onset was significantly delayed for α-methyldopamine, 3,4-methylenedioxyamphetamine, *N*-methyl- and *N*,*N*-dimethyl-2-(3,4-diacetoxyphenyl)ethylamine. Doses of agents shown to be ineffective in saline-pretreated rats were frequently found to evoke a marked response in haloperidol-pretreated animals.

Bilateral intrastriatal injections following peripheral pretreatment with saline or nialamide. The bilateral injection of 1 μl saline, solvent or drugs frequently induced an immediate but brief period of injection artifact (2 to 7 min) characterized by hyperactivity with occasional biting or chewing. Bilateral intrastriatal injections were shown to induce hyperactive/stereotyped behaviour only in the presence of nialamide with the exception of (+)-amphetamine which caused weak stereotyped movements of the head approximately 15 min after injection (25 μg) in animals pretreated with saline

only. The intensity of this behaviour, which persisted for 30 to 45 min, was not increased with an increase in dose.

Following nialamide pretreatment, the hyperactive/stereotyped behaviour induced by the 2-(3,4-dihydroxyphenyl)ethylamine derivatives developed within 60 to 120 min of the intrastriatal injection, although the onset of action of α-methyldopamine was significantly shorter. In contrast, the onset of action after administration of the 2-(3,4-methylenedioxyphenyl)ethylamine derivatives and apomorphine was usually within 15 min. At lower doses, the phenethylamine derivatives invariably induced a hyperactive behaviour but this was not observed using apomorphine (Tables 1–3).

Stereotyped behaviour induced by peripherally administered drug. The intraperitoneal administration of 2-(3,4-diacetoxyphenyl)ethylamine, its *N*- and *NN*-dimethyl derivatives and 2-(3,4-methylenedioxyphenyl)ethylamine (25 and 50 mg kg^{-1}, 4 rats per dose) failed to induce any form of enhanced motor activity or stereotyped behaviour. 3,4-Methylenedioxyamphetamine induced a hyperactive/stereotyped behaviour as characterized in Table 4. This behaviour developed within 5 min of administration and lasted for 2+ h.

Table 4. *Hyperactive/stereotyped behaviour induced by 3,4-methylenedioxy amphetamine following administration by the peripheral route.*

Dose, mg kg^{-1} i.p.	Description of behaviour
1·56	Indistinguishable from saline treated rats.
3·13	Continuous locomotor activity; periods of repetitive head movements.
6.25	Continuous locomotor activity; frequent stereotyped movements of head and front limbs.
12·5, 25 or 50	Continuous locomotion but body posture modified such that animals appear to 'crawl'. Some repetitive head movements. Body tremor at 50 mg kg^{-1}.

DISCUSSION

Anatomical locus specificity and pharmacological rationale for the induction of contralateral asymmetric behaviour (CAB) and hyperactive/stereotyped behaviour (HSB)

Interpretation placed on the changes in motor function observed after an intrastriatal injection is based upon mediation of these changes within the striatum. Evidence for a striatal and, further, a nigrostriatal dopaminergic involvement with the CAB is considerable. Most important is that contraversive turning movements are observed after electrical stimulation of the substantia nigra (York, 1973) and that the CAB induced by intrastriatal dopamine is abolished following lesion of the substantia nigra (Costall & Naylor, 1974). Further, CAB is not observed after injection of dopamine into areas around the striatum (Ungerstedt, Butcher & others, 1969; Costall & Naylor, 1974). However, it would initially appear paradoxical that the dopamine-mediated CAB should be enhanced by peripheral pretreatment with haloperidol, a butyrophenone neuroleptic considered to cause a reduction in dopaminergic function by blockade of the dopamine receptor (Janssen, 1970). As observed in previous studies (Costall & Naylor, 1974), it was clear that such pretreatment not only enhanced the intensity of CAB but also allowed the initiation of this behaviour by compounds normally ineffective. It is possible that under normal conditions the dopaminergic mechanisms in the striata are balanced to modulate forward activity and posture and

must be capable of very rapid adjustment and compensation. Thus, the unilateral intrastriatal administration of dopamine may tend to induce an asymmetry which is effectively annulled by a compensatory increase in the activity of the other striatum. However, dopamine function is blocked in both striata following neuroleptic pretreatment and, although the injection of dopamine into one hemisphere may overcome the competitive neuroleptic blockade, the effect may not be compensated for by a neuronal release of dopamine in the other hemisphere.

It would appear probable that dopamine and dopamine-like agents induce CAB by a direct influence upon postsynaptic dopaminergic structures and this is supported by observations that dopamine can initiate asymmetries from the striatum even after reserpine pretreatment (Ungerstedt & others, 1969). However, an action at presynaptic nerve terminals must also be considered, especially for the development of HSB which could not be demonstrated in the absence of a monoamine oxidase inhibitory pretreatment. As monoamine oxidases have an intracellular location, the dopamine applied to striatal tissue presumably influences intraneuronal events, possible being taken up into the neuronal terminals and subsequently released and/or causing endogenous dopamine release on to dopamine receptive postsynaptic structures.

Since injections into areas around the striatum failed to induce HSB (Fog & Pakkenberg, 1971), changes in striatal events would appear to make a significant contribution to the development of this behaviour. However, recent observations that hyperactivity occurs following injection of dopamine into the associated nucleus accumbens septi deserves consideration (Pijnenburg & van Rossum, 1973).

It is speculative to consider whether CAB and hyperactivity are mediated by different dopaminergic mechanisms or simply reflect different degrees of activation within the same system. However, it is difficult to attribute the development of such different motor functions as general hyperactivity and stereotyped chewing, biting or licking to an activation of only one dopamine system. It is interesting to speculate that the former may represent the anti-akinetic effect and the latter the "orobuccolingual" dyskinesias observed after peripheral L-dopa treatment: this remains to be investigated.

Structural requirements for the induction of CAB and HSB.

The structures of the first series of compounds investigated were based on the 2-phenethylamine skeleton: this compound, *N*-methyl- and *NN*-dimethyl-2-phenethylamine were inactive. Hydroxylation at the 3-or 4-position of the phenyl ring (tyramine and *m*-tyramine) failed to induce activity although 2-(3,4-dihydroxyphenyl)-ethylamine (dopamine) was highly effective in inducing CAB and HSB. Dopamine homologues such as 3,4-dihydroxybenzylamine and 4-(3,4-dihydroxyphenyl)-butylamine were virtually inactive, and 3-(3,4-dihydroxyphenyl)propylamine was only weakly effective. Although activity decreased with increase in size of the nitrogen substituent, *N*-methyl-2-(3,4-dihydroxyphenyl)ethylamine (epinine) and *NN*-dimethyl-2-(3,4-dihydroxyphenyl)ethylamine still retained considerable activity. Substitution at the α-C atom markedly reduced activity; for example, compare dopamine and α-methyldopamine, 2-(3,4-methylenedioxyphenyl)ethylamine and l-methyl-2-(3,4-methylenedioxyphenyl)ethylamine. Hydroxylation of the β-C atom (noradrenaline) abolished all activity; ephedrine was also inactive. The importance of the 3,4-dihydroxyphenyl moiety for maximum activity was clearly shown by the

complete ineffectiveness of the isomeric 3-hydroxy-4-methoxyphenethylamine and 4-methoxy-3-hydroxyphenethylamine. It was interesting that even a 3,4-methylenedioxyphenyl substitution was only weakly active when compared to the 3,4 dihydroxylated function confirming the observed relation between apomorphine and its methylenedioxy derivative (Lal, Sourkes & others, 1972). Thus, it can be concluded that the molecular requirements to induce CAB and HSB are best represented by 2-(3,4 dihydroxyphenyl)ethylamine. This conclusion is in accord with studies of structure-activity relations for the stimulation of peripheral dopamine receptors in the canine renal vascular bed (Goldberg, Sonneville & McNay, 1968) or of those in the central nervous system of the snail *Helix aspersa* (Woodruff & Walker, 1969). Nevertheless, the flexible nature of the ethylamine side chain of dopamine allows the existence of several conformations, and the *trans-* and *gauche-* conformers apparently exist equally readily in solution with only a slight preference for the former (Bustard & Egan, 1971). These conformations can be "held" by incorporation of the dopamine skeleton into a more rigid molecule (Pinder, 1973), and we have studied stereoselectivity at dopamine receptors by the use of such compounds. Dopamine-like activity clearly resides in the *trans-* conformation. Thus, the *trans-* isomer of 2-(3,4-methylenedioxyphenyl)cyclopropylamine, but not the *cis*, is active in inducing dopamine-like effects in the striatum; the former corresponds in configuration to *trans-* dopamine whereas the latter resembles *gauche*-dopamine (Fig. 1), although it would clearly now be desirable to study the catechols analogous to dopamine itself (Pinder, 1973). This result was confirmed by the marked reduction in activity when the hydroxy groups of apomorphine are merely moved from the 10, 11 - to the 9, 10 - positions, that is to give isoapomorphine; this difference also prevails upon systemic administration (Neumeyer, McCarthy & others, 1973). Apomorphine corresponds closely in configuration to *trans*-dopamine (or, more correctly, *trans*-epinine) whereas isoapomorphine can only approximate to this configuration; its low activity reflects the importance of the 11-hydroxyl group in the aporphine series (Pinder, 1973) (Fig. 2).

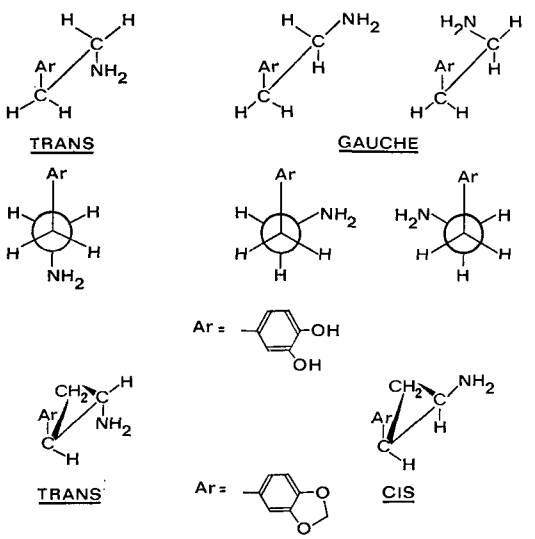

FIG. 1. Structural relation between dopamine conformers and 2-(3,4-methylenedioxyphenyl)-cyclopropylamine.

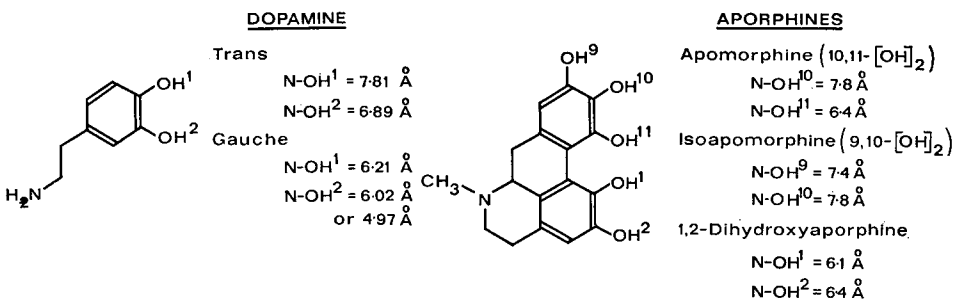

FIG. 2. Structural relation between dopamine conformers and some aporphine alkaloids.

It is significant that 1,2-dihydroxyaporphine, which corresponds more closely to *gauche*-dopamine than does either apomorphine or isoapomorphine, is even less active than the latter in producing dopamine-like effects upon systemic administration (Neumeyer & others, 1973). These results confirm our previous studies (Pinder, Buxton & Green, 1971; Pinder, Buxton & Woodruff, 1972) and those of others (Lal & others, 1972; Rekker, Engel & Nys, 1972; Neumeyer & others, 1973), and once more refute the molecular orbital calculations of Kier & Truitt (1970), although recent recalculations by Kier (1973) do predict a *trans*-preference for dopamine.

Detection of potential antiparkinson activity

The present studies indicate the basic structure and conformation necessary for an agent to cause stimulation of striatal dopaminergic mechanisms. Assuming that the behavioural effects observed as indices of dopaminergic stimulation can be related to the ability, in the clinic, of L-dopa and dopamine-like agents to increase motor function, then these observations could provide a rational basis for the design of antiparkinson agents. However, although the intrastriatal injection technique may be usefully employed to detect potential activity, an agent has clinical potential only if also effective by peripheral administration. Problems which may arise were clearly demonstrated by use of 2-(3,4-diacetoxyphenyl)ethylamine and its *N*-methyl and *NN*-dimethyl derivatives. Theoretically, lipophilic derivatives of dopamine which could undergo non-enzymatic hydrolysis to dopamine within the brain may usefully raise striatal dopamine levels. It has been suggested that the 3,4-diacetoxy derivatives may be such compounds (Pinder, 1970) and the present studies showed unequivocally that these agents are highly effective upon intrastriatal injection. However, upon peripheral administration they failed to induce stereotypy; presumably, hydrolysis to dopamine (with subsequent failure to pass the blood-brain barrier) is as effective peripherally as centrally. Such acetylated agents have been suggested as potential antiparkinson agents (Howes & Dalzell, 1972) and apparently show some effects claimed to be dopamine-like in nature when administered systemically (Borgman, McPhillips & others, 1973). Other, more bulky, alkoxy groups may possibly provide sufficient immunity from hydrolysis to allow entry into the brain before formation of the parent phenethylamine (Casagrande & Ferrari, 1973). In contrast, 3,4-methylenedioxyamphetamine exerted only weak dopamine-like effects upon intrastriatal injection but caused stereotyped behaviour patterns after peripheral administration. This is in agreement with other reports of the central stimulant action of this compound (Thiessen & Cook, 1973). The ability of a drug to gain access to central dopamine

systems is crucial and it is reasonable to conclude that even agents with relatively low intrastriatal dopamine-like activity should be considered as potential antiparkinson agents, a weak effect being overcome by an increase in dosage.

It has been suggested that the CAB induced by the unilateral intrastriatal application of agents considered to enhance catecholaminergic events is specific for dopamine (Costall & Naylor, 1974). The present studies support this hypothesis and indicate that the HSB resulting from bilateral intrastriatal administrations may also be usefully included as a test procedure for dopamine-like agents. The intrastriatal injection technique would, therefore, appear to be a valuable addition to the procedures employed for the detection of potential antiparkinson agents.

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