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Neuroleptic Activity of the 5-Aryltetrahydro-γ-carboline Series

Conformational Requirements for Interaction with Central Dopamine Receptors

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SUMMARY

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A series of novel 5-aryltetrahydro-γ-carboline neuroleptics is described. Their interaction with the dopamine receptor is demonstrated by their potent and long-lasting blockade of amphetamine-induced stereotyped behavior in rats and by the displacement of bound ^{3}H-spiroperidol *in vitro*. The 5-aryl-γ-carboline nucleus appears to be primarily responsible for receptor interaction while the side chain serves to extend duration, presumably by altering metabolism and/or tissue distribution. The conformation of the semirigid 5-aryl-γ-carboline nucleus approximates that of the previously proposed active conformation of the open-chain diphenylbutylpiperidine neuroleptics. Comparison of the crystal structure of CP-36,584 with those of apomorphine and (+)-dexclamol suggests a common, conformationally restricted phenethylamine moiety as the species interacting with the receptor. Findings with the γ-carboline neuroleptics coalesce previously disparate proposals for the dopamine receptor interactions of butaclamol, diphenylbutylamines, and piperidylidene thioxanthenes.

INTRODUCTION

Neuroleptic drugs are believed to exert their primary effect by blocking dopamine receptors in the brain (1) and at least two conformations have been proposed to account for this blockade. One, based on the solid state conformation of chlorpromazine (2), overlaps nearly perfectly with an extended form of dopamine (3). A conformationally restricted phenethylamine moiety in the potent neuroleptic (+)-butaclamol (4) supports the dopamine overlap hypothesis, although the phenethylamine rotamer postulated for the active conformer of butaclamol differs from the anti conformation of dopamine that overlaps solid state chlorpromazine. A second conformation, which was proposed by Janssen (5), derives from the "S-shaped" arrangement of the 4 atom sequence that links the aromatic ring to the basic nitrogen in many open-chain neuroleptics. The potent neuroleptic activity of a series of piperidylidene thioxanthenes that incorporate a semirigid "S-shaped" conformation, but are incapable of adopting the conformation of solid state chlorpromazine, is consistent with the Janssen hypothesis (6). We have discovered that 5-*p*-F-phenyl substituted 1,2,3,4-tetrahydro-γ-carboline derivatives, which contain both a conformationally rigid phenethylamine moiety and an S-shape arrangement of atoms, exhibit high affinity for the dopamine receptor. These compounds are potent and long-acting neuroleptic agents in rats.

METHODS

8-Fluoro-5-(4-fluorophenyl)-2-[4-hydroxy-4-(4-fluorophenyl)butyl]-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole[1] (VII) and related 5-aryl derivatives were synthesized as previously described in U.S. Patent 4,001,263. Compounds II, IV, and V were synthesized by methylation (II), reduction, (IV) or methylation and reduction (V) of commercially available I (Aldrich Chemical Co.).

The crystal structure of VII[2] was determined from crystals of the racemic free base. Pertinent data are: a = 26.412, b = 7.846, c = 11.752 Å; β = 111.58°; space group: PZ_1/c with Z = 4, D_0 = 1.31, D_c = 1.321 g/cm^3.

Antagonism of (+)-amphetamine-induced symptoms in rats. Neuroleptic effects *in vivo* were estimated by the blockade of amphetamine stereotypy. Rats were placed

[1] The common name for the pyrido[4,3-*b*]indole ring system is γ-carboline, which is used herein.

[2] Bordner, J., J. J. Plattner, and W. M. Welch, unpublished observations.

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individually in covered plastic compartments; after a brief period of acclimation in the cages the rats in groups of five were treated intraperitoneally with compounds at doses separated by 0.5 log units (i.e., ... 1, 3.2, 10, 32, ... mg/kg). They were subsequently treated 1, 5, and 24 hr later with *d*-amphetamine sulfate, 5 mg/kg ip. One hour after each amphetamine challenge, each rat was assessed for its most characteristic behavior on a 6-point scale (7). These ratings represent increasing degrees of drug effect (8) and the time of rating chosen coincides with the peak effect of amphetamine (9). Scores were dichotomized (cf. (7)), and approximate ED_{50}'s were determined, based on the quantal data. Doses are expressed in terms of the hydrochloride salts.

^{3}H-Spiroperidol binding to dopamine receptor. The method was adapted from that of Burt, Creese, and Snyder (10). Rats (Sprague–Dawley CD males, 250–300 g, Charles River Laboratories, Wilmington, Mass.) were decapitated, and brains were immediately dissected to recover the corpus striatum. The latter was homogenized in 40 vol of ice-cold 50 mM Tris (tris[hydroxymethyl]-aminomethane)·HCl buffer, pH 7.7 with a Brinkmann Polytron PT-10. The homogenate was centrifuged twice at 50,000*g* for 10 min at 0–4° with rehomogenization of the intermediate pellet in fresh Tris buffer (same volume) in the Polytron. The final pellet was gently resuspended in 90 vol of cold 50 mM Tris·HCl buffer, pH 7.6, containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid, and 10 μM pargyline. The tissue suspension was placed in a 37° water bath for 5 min and kept ice cold until use. The incubation mixture consisted of 0.02 ml inhibitor solution or vehicle, 1.0 ml tissue preparation, and 0.10 ml ^{3}H-spiroperidol (New England Nuclear, 23.6 Ci/mmol) prepared so as to obtain 0.5 nM final concentration. Tubes were incubated in sequence for 10 min at 37° in groups of three, after which 0.9 ml from each incubation tube was filtered through Whatman GF/B filters with vacuum. After washing twice with 5 ml of cold tris·HCl buffer, pH 7.7, each filter was placed in a scintillation vial with 10 ml Aquasol-2 (New England Nuclear), and each vial was vortexed. Samples were kept at room temperature overnight before determination of radioactivity in a liquid scintillation counter. Binding was calculated as fmoles of ^{3}H-spiroperidol bound per milligram of protein. Controls (vehicle or 10^{-7} M 1-butaclamol), blank (10^{-7} M *d*-butaclamol) and inhibitor solutions (four concentrations) were run in triplicate. The concentration that reduced binding by 50% (IC_{50}) was estimated on semilog paper. The IC_{50} values in Table 1 represent means of two or three runs. Insoluble drugs were dissolved in ethanol (1–2% ethanol in final incubation mixture).

RESULTS

The data are shown in Table 1. In series A, the 5-*p*-F-phenyl carboline derivative (III) was considerably more potent than the 5-H and 5-methyl congeners. Compound I, which has previously been reported to elicit analgesic and neuroleptic effects (11), was effective in the dosage range of 3.2–10 mg/kg ip, but its effect decreased markedly by 5 and 24 hr, as did the effect of the 5-methyl congener, II. On the other hand, the 5-*p*-F-phenyl analog III was about three times more potent than I at 1 hr, and its effect continued unabated for at least 24 hr, at which time the potency difference was greater than 30. In series B, compounds VI and VII exhibited marked potency and duration increments compared with the 5-H and 5-CH_3 congeners IV and V. As indicated by comparison of the activities of VI and VII at 24 hr, the F substituent in the pendant phenyl extended the duration of effect. Compound VIII, which has the diphenylbutylpiperidine side chain found in pimozide and penfluridol, failed to block amphetamine at the doses tested.

The potency of chlorpromazine was relatively modest at 1 hr and it was essentially inactive at later times. The marked activity of haloperidol at 1 and 5 hr diminished considerably by 24 hr (Table 1).

Results obtained from *in vitro* inhibition of ^{3}H-spiroperidol binding generally agree closely with the *in vivo* antiamphetamine results (Table 1). The lower IC_{50} values of compounds III and VII compared with those of I and IV, respectively, indicate that 5-aryl substituents enhance affinity for the dopamine receptor. That aralkyl side chain substitution is favored at N-2 is indicated by the lower IC_{50} values of III and VII compared with compound IX. The very low *in vitro* activity of VIII is consistent with its lack of *in vivo* activity. Values for chlorpromazine, haloperidol, and *d*-butaclamol are listed in Table 1 for comparison.

DISCUSSION

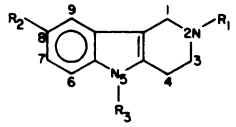
The 5-aryl-1,2,3,4-tetrahydro-γ-carbolines exert marked anti-dopaminergic activity both *in vitro* and *in vivo*. The structure–activity correlations point to the importance of the 5-aryl substituted nucleus as a major determinant of potency. Although the butyrophenone and reduced butyrophenone moieties at position N-2 contribute to potency and duration of action, neuroleptic activity appears not to derive from this portion of the molecule, as it does for haloperidol, since compound VIII, containing the diphenylbutylpiperidine side chain found in pimozide and penfluridol, was essentially inactive. The activity of the methyl congener (IX) further suggests that the butyrophenone or related side chain at N-2 is not requisite for potent neuroleptic activity.

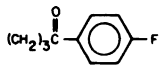
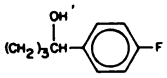
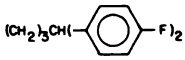
Because the 5-aryl-1,2,3,4-tetrahydro-γ-carbolines contain a semirigid nucleus they help to further clarify the structural requirements for dopamine receptor blockade. These compounds are fixed in a conformation approximating that of the postulated (5) active S-shaped conformation of mobile, open-chain neuroleptics. Viewed in this way, the 5-arylcarbolines are analogous to long-acting diphenylbutylpiperidine neuroleptics such as pimozide and penfluridol (Fig. 2). The enhanced potency in both series deriving from *p*-F-aryl substitution and the similarly prolonged neuroleptic activity are consistent with this analogy.

The γ-carboline nucleus also contains an extended phenethylamine moiety, depicted in Fig. 1. Two parameters commonly used to describe the conformation of this system are the distance between the basic nitrogen and the aromatic ring, and the distance between basic nitrogen and the plane of the aromatic ring. An X-ray crystallographic study of compound VII demonstrates that

TABLE 1

Anti-amphetamine activity of tetrahydro-γ-carboline derivatives in rats



Series	Compound[c]	R_1	R_2	R_3	Anti-amphetamine activity[a] 1 hr	5 hr	24 hr	Inhibition of ^{3}H-spiroperidol binding[b] IC_{50}
					(approximate ED_{50}, mg/kg ip)			*(nM)*
A	I		F	H	3.2–10	10–32	>32	51
	II		F	CH_3	10–32	>32		71
	III		F		1.0–3.2	1.0–3.2	1.0–3.2	26
B	IV		F	H	3.2–10	>10	>10	103
	V		F	CH_3	10–32	10–32	>32	101
	VI		F		0.1–0.32	0.1–0.32	3.2–10	31
	VII[d]		F		0.1–0.32	0.1–0.32	0.32–1.0	14
C	VIII		F		>32	>32	>32	753
D	IX	CH_3	F		1.0–3.2	3.2–10	>32	83
	Chlorpromazine				3.2–10	>17.8	>56	51
	Haloperidol				0.1–0.32	0.1–0.32	>3.2	9
	d-Butaclamol				0.32–1.0[e]	0.32–1.0	>3.2	13

[a] Entries are ranges within which fall the ED_{50} values for blocking hyperactivity and stereotypy induced by amphetamine. Details are given in the text.

[b] IC_{50} values were estimated graphically using four drug concentrations separated by 0.5 log unit. Entries are means of two or three determinations. For details, see the text.

[c] Chemical and physical properties of compounds I–IX may be obtained from the pertinent references cited in the text.

[d] Pfizer code number CP-36,584.

[e] The anti-amphetamine data were obtained using *d,l*-butaclamol.

the distance between the center of aromatic ring A and N-2 (cf. Fig. 2) resembles published X-ray data for (+)-dexclamol[3] and apomorphine, i.e., 5.16 Å for VII vs 5.10 Å for (+)-dexclamol (12) and 5.12 Å for apomorphine (13). It should be noted, however, that an additional, more remote aromatic ring binding site has been proposed for isobutaclamol (14), suggesting that distances in excess of 5.2 Å may be permissible in certain cases.

The distance of N-2 out of the plane defined by ring A is +0.6 Å or −0.6 Å for the two conformers of VII, which are presumed to be equally populated. (+)-Dexclamol and apomorphine likewise have two conformations, but in these molecules the out-of-plane distances are nonequivalent, being 0.19 and −0.90 Å in (+)-dexclamol and −0.90 and −1.23 Å in apomorphine. It is interesting to note that in none of the semirigid structures discussed above does the nitrogen out of plane distance approach

[3] Dexclamol is the isopropyl analog of butaclamol. The absolute configuration of (+)-dexclamol and (+)-butaclamol, the biologically active enantiomer of butaclamol, are identical (cf. Refs. 4, 12).

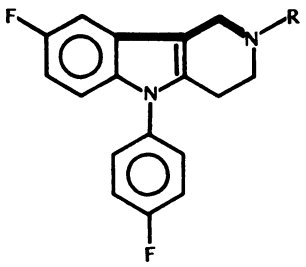


FIG. 1. *Extended phenethylamine moiety in the tetrahydro-γ-carboline nucleus*

the 1.61 Å value reported for crystalline dopamine (15). These data plus the approximate equivalence of VII and (+)-butaclamol in the *in vitro* ^{3}H-spiroperidol binding assay (IC_{50} values = 14 vs 13 nM, respectively) suggest that potent neuroleptic activity is achieved when the nitrogen out-of-plane distance is in the range 0.6–0.9 Å.

Interaction of dopamine agonists and antagonists with the dopamine receptor appears to be achieved by their

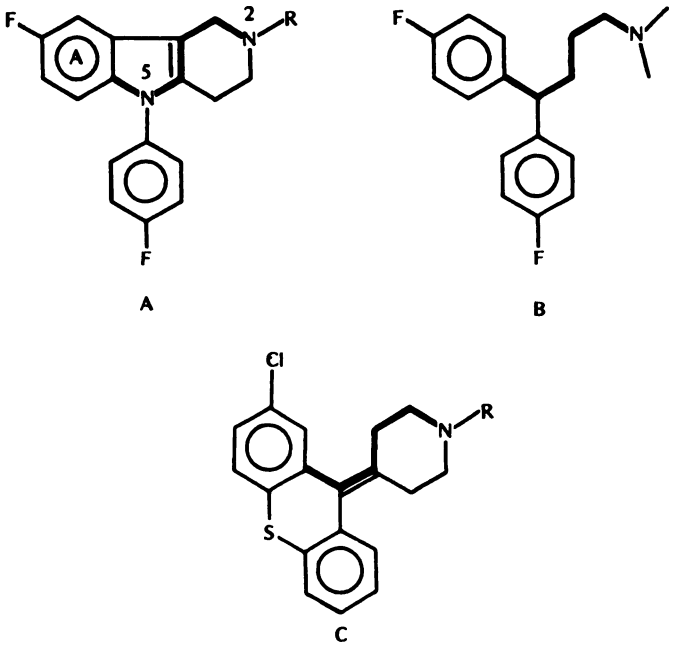


FIG. 2. *S-shaped conformations of 5-aryl-1,2,3,4-tetrahydro-γ-carbolines (A), diphenylbutylamine neuroleptics (B), and piperidylidene thioxanthene neuroleptics (C)*

R is lower alkyl or as defined in the text.

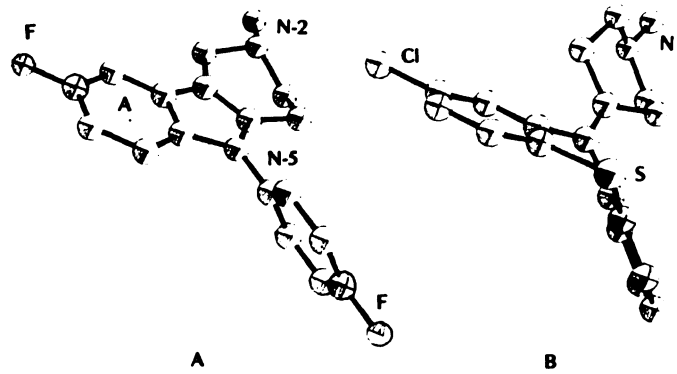


FIG. 3. *(A) Lowest energy conformation of 2-methyl substituted γ-carboline nucleus (compound IX, Table I); (B) lowest energy boat conformation of N-methyl-2-chloropiperidylidene thioxanthene analog*

Hydrogens absent to facilitate depiction of features of molecular overlap.

ability to position an aryl ring and a basic nitrogen atom in a spatial relationship approximating ring A and N-2 of the tetrahydro-γ-carbolines. It seems likely that the flexible diphenylbutylpiperidine and butyrophenone neuroleptics assume this relative positioning of aromatic ring and basic nitrogen at the dopamine receptor, and thereby adopt an S-shape (Fig. 2).

Using Dreiding molecular models, it can be shown that the carboline nucleus also achieves good overlap of N-2, aromatic ring A and aromatic substituents with the piperidylidene thioxanthenes (6) (Fig. 3), providing the piperidine ring is in one of the three possible boat conformations that direct the N atom toward the plane of the substituted aromatic ring. Calculations using Allinger's force field program (16)[4] indicate that the lowest energy boat conformation (Fig. 3) is about 4 kcal/mole[5] above the lowest energy chair conformation, an energy difference that could be overcome by interaction with the receptor.[6] Thus, the two postulated active conformations of neuroleptics, phenethylamine and S-shaped, seem to be compatible and can be reconciled by a conformation closely resembling the tetrahydro-γ-carboline nucleus. That the aromatic ring to basic nitrogen atom distance of the piperidylidene thioxanthenes is somewhat greater than that of dexclamol and VII may reflect the existence of an additional aromatic ring binding site (14).

Compound VII (CP-36,584), displays a full range of neuroleptic effects in animals, including selective blockade of conditioned avoidance behavior, blockade of apomorphine symptoms in several species, and marked enhancement of dopamine turnover in brain. These results, as well as more complete structure–activity research and details of synthesis in this series, will be reported subsequently.

CONCLUSIONS

On the basis of the above, we suggest that: (1) the conformationally restrained phenethylamine portion of 5-aryl-1,2,3,4-tetrahydro-γ-carbolines is the major contributor to the dopamine receptor blocking activity of this series; (2) the optimum nitrogen out-of-plane distance is in the range 0.6–0.9 Å based on values for VII and butaclamol; (3) the phenethylamine conformations contained in butaclamol and the tetrahydro-γ-carbolines are better models for the conformation of dopamine at the receptor than the *trans* conformation of crystalline dopamine and, by extension, the solid state conformation of chloropromazine; (4) diphenylbutylamine and butyrophenone neuroleptics most likely interact with the dopamine receptor in a folded, S-shaped conformation that approximates the spatial arrangement of the tetrahydro-γ-carboline nucleus; and (5) piperidylidene thioxanthenes may interact with the dopamine receptor with the piperidine ring in a boat conformation.

REFERENCES

1. Carlsson, A. and M. Lindqvist. Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. *Acta Pharmacol. Toxicol.* **20**: 140–144 (1963).
2. McDowell, J. J. H. Crystal and molecular structure of chlorpromazine. *Acta Crystallogr. Sect. B Struct. Crystallogr. Cryst. Chem.* **25**: 2175–2181 (1969).
3. Horn, A. S. and S. H. Snyder. Chlorpromazine and dopamine: conformational similarities that correlate with the antischizophrenic activity of phenothiazine drugs. *Proc. Nat. Acad. Sci. USA* **68**: 2325–2328 (1971).
4. Humber, L. G., F. T. Bruderlein and K. Voith. Neuroleptic agents of the benzocycloheptapyridoisoquinoline series. A hypothesis on their mode of interaction with the central dopamine receptor. *Mol. Pharmacol.* 11: 833–840 (1975).
5. Janssen, P. A. J. Chemical and pharmacological classification of neuroleptics, in *Modern Problems in Pharmacopsychiatry*, Vol. 5: *The Neuroleptics* (D. P. Bobon, P. A. J. Janssen and J. Bobon, eds.). S. Karger, Basel 33–44 (1970).
6. Kaiser, C., R. J. Warren and C. L. Zirkle. Analogs of phenothiazines. 6.

[4] The calculations were made using program number 318 obtained from the Quantum Chemistry Program Exchange (QCPE), University of Indiana, Bloomington. cf. Allinger, N. L., *QCPE*, **11**, 318, 1976.

[5] We thank Dr. Beryl Dominy of Pfizer Central Research for conducting the energy calculations.

[6] Assuming most neuroleptics bind to the dopamine receptor with $K_i = 10^{-7}$–10^{-9} M, the ΔG is roughly in the range 10–13 kcal/mol.

Stereochemical assignment of isomeric aminoalkylidene derivative of xanthenes and thioxanthenes with neuropharmacological activity. *J. Med. Chem.* **17:** 131–133 (1974).
7. Weissman, A., B. K. Koe and S. S. Tenen. Antiamphetamine effects following inhibition of tyrosine hydroxylase. *J. Pharmacol. Exp. Ther.* **151:** 339–352 (1966).
8. Quinton, R. M. and G. Halliwell. Effects of α-methyl DOPA and DOPA on the amphetamine excitatory response in reserpinized rats. *Nature (London)* **200:** 178–179 (1963).
9. Weissman, A. Psychopharmacological effects of thiothixene and related compounds. *Psychopharmacologia* **12:** 142–157 (1968).
10. Burt, D. R., I. Creese and S. H. Snyder. Properties of ^{3}H-haloperidol and ^{3}H-dopamine binding associated with dopamine receptors in calf brain membranes. *Mol. Pharmacol.* **12:** 800–812 (1976).
11. Kimura, E. T., P. W. Dodge, P. R. Young and R. P. Johnson. Pharmacologic studies with A-30360, an analgesic–tranquilizer, and its analogues. *Arch. Int. Pharmacodyn. Ther.* **190:** 124–134 (1971).
12. Bird, P. H., F. T. Bruderlein and L. G. Humber. Crystallographic studies on neuroleptics of the benzocycloheptapyridoisoquinoline series. The crystal structure of butaclamol hydrochloride and the absolute configuration and crystal structure of dexclamol hydrochloride. *Can. J. Chem.* **54:** 2715–2722 (1976).
13. Giesecke, J. The crystal and molecular structure of apomorphine hydrochloride hydrate. *Acta Crystallogr. Sect. B Struct. Crystallogr. Cryst. Chem.* **29:** 1785–1791 (1973).
14. Philipp, A. H., L. G. Humber and K. Voith. Mapping the dopamine receptor. 2. Features derived from modifications in the rings A/B region of the neuroleptic butaclamol. *J. Med. Chem.* **22:** 768–773 (1979).
15. Bergin, R. and D. Carlström. Structure of pyrocatecholamines. II. Crystal structure of dopamine hydrochloride. *Acta Crystallogr. Sect. B Struct. Crystallogr. Cryst. Chem.* **24:** 1506–10 (1968).
16. Allinger, N. L. and J. T. Sprague. Calculation of the structures of hydrocarbons containing delocalized electronic systems by the molecular mechanics method. *J. Amer. Chem. Soc.* **95:** 3893–3907 (1973).

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