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SYNTHESIS OF NEW HALOPERIDOL ANALOGUES AND CHARACTERIZATION OF THEIR INTERACTIONS WITH α-ADRENOCEPTORS IN RAT PAROTID SLICES AND HUMAN PLATELET MEMBRANES

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1 The synthesis of several butyrophenone analogues of haloperidol is described.

2 The effects of these compounds on α-adrenoceptors were evaluated by examining their ability to reduce α_1-stimulated K^+ release from rat parotid slices and to displace [^{3}H]-phentolamine from human platelet membrane α_2-adrenoceptors.

3 The affinity of haloperidol and its analogues for α_1-receptors was found to be 1 to 2 orders of magnitude greater than that for α_2-adrenoceptors. These observations suggest that most of the α-adrenoceptor activity of butyrophenones results from their interaction with α_1-adrenoceptors.

4 The relatively high affinity of the butyrophenones for α_1-adrenoceptors suggests that they may be useful as probes in studies of α_1-adrenoceptors in these and other tissues.

Introduction

The widely used butyrophenone drugs are known to interact specifically and bind with high affinity to dopamine binding sites in the central nervous system (Van Rossum, 1966; Janssen & Allewijn, 1969; Clement-Cormier, Kebabian, Petzgold & Greengard, 1974; Karobath & Leitich, 1974) and kidney (Nahajama, Natolii & Kuruma, 1977). Recent reports (Laduron, Janssen & Leysen, 1978; Leysen, Niemegeers, Tollenacre & Laduron, 1978; Peroutka & Snyder, 1980) have indicated that neuroleptic drugs, such as spiroperidol, can bind to 5-hydroxytryptamine (5-HT) receptors in the central nervous system. Furthermore, displacement of radiolabelled α-adrenoceptor antagonists by antipsychotic drugs (Greenberg, U'Prichard & Snyder, 1976; Williams & Lefkowitz, 1976; U'Prichard, Greenberg & Snyder, 1977) suggested that the butyrophenones might also interact with α-adrenoceptors. Thus, the complex pharmacological effects of neuroleptic drugs may be due to their interaction with at least three types of neuroreceptors, and at least part of their clinical side effects may be due to these interactions. In this paper we describe studies which have characterized the interaction of haloperidol with α_1- and α_2-adrenoceptors. The former have been evaluated by measuring the butyrophenone-induced inhibition of α-stimulated potassium efflux from rat parotid slices, a response that is believed to be of the α_1 sub-type (Fain & García-Sáinz, 1980). The effects of butyrophenones on α_2-adrenoceptors were evaluated by measuring their ability to displace [^{3}H]-phentolamine from human platelets (Newman, Williams, Bishopric & Lefkowitz, 1978; Steer, Knorana & Galgoci, 1979). In addition to haloperidol, several analogues whose synthesis is described, have been studied. These studies indicate that haloperidol and its analogues bind with high affinity and preferentially to α_1-adrenoceptors.

Methods

Syntheses

A *Amine-haloperidol analogue: 4-[4-(ethylamine)-4-piperidino]-4′-fluoro-butyrophenone* (see Figure 1) α-Chloro-*p*-fluorobutyrophenone (10.0 mmol) is slowly added to 4-aminoethyl-piperidine (20.0 mmol). An immediate white precipitate, the hydrochloride salt of 4-aminoethyl-piperidine, is formed. The reaction mixture is allowed to stand overnight at 30°C to complete the precipitation of the salt, which is then filtered on sintered glass and washed with ethyl acetate. The residue is separated on a Kisselgel-type 60 column (48 × 2.5 cm) with the organic phase of the mixture *n*-butanol/acetic acid/H_2O (4:1:4 by volume). The amine analogue

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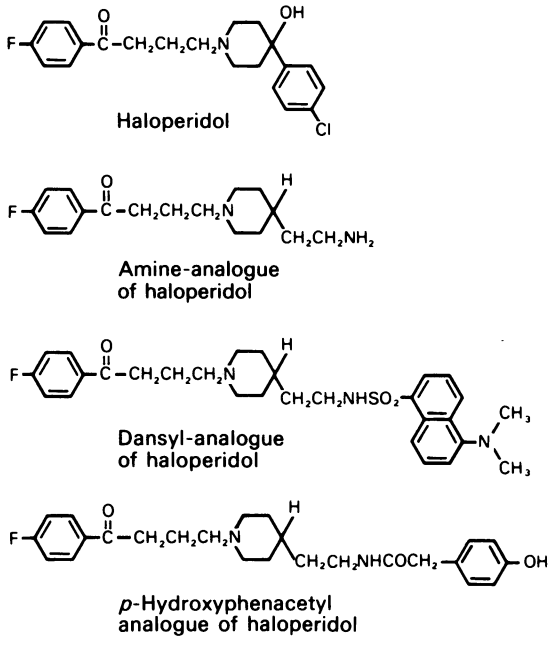

Figure 1 The structures of new butyrophenone analogues used in the present study.

obtained (yield 30%) had an $R_F = 0.33$ in the above solvent system. Analysis %N = 7.7 (calculated 7.95).

B t-*Boc-tyrosine-haloperidol: 4-[4-(N-ethyl-N'-(tetrabutyloxyaminotyrosine)-4-peperidino]-4-fluorobutyrophenone.* The amine-haloperidol (see (A) 1 mmol) is dissolved in 0.5 ml of dimethylformamide. Triethylamine (1 mmol) is added to form the free base of the amine. The *t*-boc-tyrosine-*o*-benzyl succinimide ester (1.1 mmol) is then added and the reaction mixture is stirred at room temperature overnight. The product is precipitated in water, washed and dried. The dried compound is dissolved in methanol (90%) with 10% water, and the hydrogenation with molecular hydrogen at atmospheric pressure is carried out in the presence of platinum (10% on active charcoal) for 8 h. Then the catalyst is filtered and the product is recovered from the methanolic solution.

C *Dansyl analogue of haloperidol: 4-[4-(N-dansyl-N'-ethylamine)-4-piperidino]-4'-fluorobutyrophenone* The amine analogue of haloperidol (1.0 mmol) (see (A)), dissolved in 2.0 ml of dry dioxane, is mixed with triethylamine (2 mmol). Dansyl-chloride (1.1 mmol), dissolved in 1.0 ml dry dioxane, is added to the free amine. After 4 h at 30°C, the reaction mixture is concentrated under vacuum and the residue is extracted with ethyl acetate. The dansyl analogue of haloperidol is obtained in the hydrochloride form after acidification with hydrochloric acid, followed by precipitation with ether. The rate of flow was determined on thin layer plates coated with silica gel, using *n*-butanol/acetic acid/H_2O (4:1:4 by volume) as a solvent system ($R_F = 0.7$). The structure of dansyl-haloperidol analogue is shown in Figure 1. Analysis: %N = 6.39 (calculated + $2H_2O$, 6.64%). The molar extinction coefficient of the dansyl analogue of haloperidol was found to be 2720 M^{-1} cm^{-1}, as determined in 90% methanol.

D p-*Hydroxy-phenacetyl analogue of haloperidol: 4-[4-(N-ethyl-N'-p-hydroxy phenacetyl)-4-piperidino]-4'-fluorobutyrophenone* The amine analogue of haloperidol (1.0 mmol) is dissolved in 2 ml of dry dioxane and traces of dimethylformamide by slight heating. Triethylamine (2.0 mmol) is added and the reaction is stirred to the appearance of crystals of triethylammonium chloride salt. *p*-Hydroxyphenacetyl succinimide ester (prepared by *p*-hydroxyphenyl acetic acid and N-hydroxysuccinimide ester in the presence of equimolar amounts of DCC) is added, and the reaction mixture is vigorously stirred at room temperature (25°C) for 24 h. The urea derivative, dicyclohexylurea (DCU), is filtered, and the filtrate evaporated under reduced pressure, extracted with ethyl acetate, dried under reduced pressure, and precipitated from traces of methanol and ether (absolute solvents). The rate of flow, $R_F = 0.45$, was determined in the same system used for the dansyl analogue (see section (C) above).

Potassium release in rat parotid slices

Rat parotid slices were prepared and assayed according to a procedure previously described by Friedman & Selinger (1978). Slices from 12 to 16 glands were pooled and incubated for 5 min, washed with fresh medium, and then divided into 12 to 16 aliquots. Slices were incubated in 2 ml of a modified Krebs-Ringer buffer in which bicarbonate was replaced with 25 mM HEPES buffer, pH 7.4, and gassed with 100% O_2 (KRH medium). The potassium released into the medium was measured by atomic absorption spectroscopy of aliquots of the incubation mixture. In each experiment, the release of potassium by (−)-adrenaline was determined, using several concentrations of (−)-adrenaline in the presence and absence of various concentrations of haloperidol and its analogues. The ability of these ligands to inhibit K^+ release induced by stimulation of muscarinic cholinoceptors by carbachol was also evaluated.

Binding of [^{3}H]-phentolamine to human platelet membranes

The ability of the haloperidol analogues (Figure 1) to inhibit binding of [^{3}H]-phentolamine to α-adrenoceptors on human platelets was evaluated by

the method of Steer *et al.* (1979). Binding studies were performed with platelet membranes obtained by the glycerol-lysis purification technique, and experiments were performed immediately after membrane preparation from freshly obtained blood. Assays were performed in a final volume of 0.1 ml, containing the following agents: Tris-HCl 50 mM, pH 7.6, EGTA 1 mM, bovine serum albumin 0.025%, dithiothreitol 0.1 mM, $MgCl_2$ 5 mM, 1 to 2 mg/ml membrane protein and [^{3}H]-phentolamine 20 nM. After incubation (5 min, 30°C), samples were rapidly (<15 s) filtered through Whatman GF/A filters and washed three times with 3.5 ml of the above buffer. The filters were dissolved by incubation (30 min, 60°C) in 1.3 ml 'Protosol' (New England Nuclear), neutralized by addition of 50 μl glacial acetic acid, and counted in a Packard liquid scintillation counter after addition of 10 ml 'Econofluor' (New England Nuclear). Non-specific binding was determined using samples incubated in the presence of 1×10^{-6} M non-radioactive phentolamine or 1×10^{-4} M (−)-adrenaline, and the value for non-specific binding was subtracted from that obtained in the absence of excess non-radioactive phentolamine or excess (−)-adrenaline to determine the specific binding. Specific binding was 30 to 50% of the total binding.

Materials

[^{3}H]-phentolamine (23.0 Ci/mmol) was synthesized and kindly donated by Dr Wigger, Radiosynthesis Laboratory, Ciba-Geigy Pharmaceuticals, Basel, Switzerland. (−)-Adrenaline and dopamine were purchased from Sigma. α-Chloro-*p*-fluorobutyrophenone and 4-aminoethyl-4-piperidine were purchased from Aldrich. WB-4101 was a generous donation by Dr Green of Ward Blenkinsop & Co., England. Haloperidol was a gift from the Janssen Pharmaceutical Co. Clonidine was a gift of Boehringer Ingelheim, and phentolamine (Rogitine HCL) was agift of Ciba-Geigy. Protein was determined according to Lowry, Rosebrough, Farr & Randall (1951), using bovine serum albumin as the standard.

Results

Interaction of haloperidol analogues with x-adrenoceptors in rat parotid slices

Haloperidol and each of its analogues (Figure 1) were found to be competitive inhibitors of the adrenaline-induced potassium release. Apparent dissociation constants for the different ligands (K_I) were calculated from the dose-response curve, using the relationship:

$$K_D' = \frac{K_D}{\left(1 + \frac{[I]}{K_I}\right)}$$

where K_D is the apparent dissociation constant for (−)-adrenaline in the absence of inhibitor, K_D' the apparent dissociation constant for (−)-adrenaline in the presence of the butyrophenone inhibitor at a concentration [I], and K_I the apparent dissociation constant of the inhibitor for the α-adrenoceptor. The apparent dissociation constants for the different butyrophenone analogues are summarized in Table 1.

Table 1 The affinity of haloperidol and its analogues for α-adrenoceptors

Compound	*Dissociation constant*	
	Rat parotid slices[a] nM	*Platelet membranes*[b] nM
Haloperidol	500 ± 100	5400 ± 500
Amine-haloperidol[c]	3.0 ± 2	4000 ± 400
Dansyl-haloperidol[c]	30 ± 10	200 ± 20
t-Boc-tyrosine-haloperidol[c]	50 ± 10	5000 ± 10
p-Hydroxy-haloperidol analogue[c]	—	400 ± 100
(−)-Adrenaline	5000 ± 1000	440 ± 70
Clonidine[d]	110 ± 50	20 ± 3
Phentolamine	28 ± 20	12 ± 3

[a]Dissociation constants determined by measuring inhibition of adrenaline-induced potassium release.
[b]Dissociation constants determined from binding studies measuring displacement of [^{3}H]-phentolamine.
[c]See Figures 1 and 2.
[d]Clonidine was found to inhibit adrenaline-induced K^+ release from parotid slices. Similar observations were reported for dispersed acinar cells of rat parotid (Davis & Maury, 1978).
Values are given with their standard deviations of three independent determinations.

The different analogues of haloperidol as well as haloperidol itself were tested for their ability to inhibit carbamylcholine (carbachol, 10^{-5} M)-stimulated potassium release in the rat parotid system. Concentrations up to 10^{-5} M of either haloperidol or of the other butyrophenones were found to be devoid of any muscarinic activity, *i.e.*, they did not alter carbachol-induced K^+ release.

Interaction of haloperidol and its analogues with α-adrenoceptors of human platelet membranes

Haloperidol as well as other butyrophenones inhibit the binding of [^{3}H]-phentolamine to human platelet membranes and the dissociation constants for the different ligands, calculated from the displacement curves, are summarized in Table 1. A typical displacement is depicted in Figure 2.

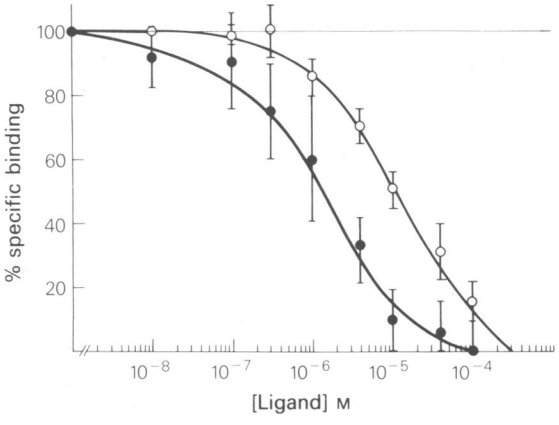

Figure 2 Displacement of [^{3}H]-phentolamine by amin-haloperidol and *p*-hydroxy-phenacetyl haloperidol analogues. Platelet membranes (1 to 2 mg/ml) were incubated with 20 nM [^{3}H]-phentolamine for 5 minutes at 30°C in the presence of varying concentrations of either one of the analogues (see Methods). Specific binding was determined by subtracting the binding observed in the presence of 1×10^{-4} M (−)-adrenaline from the total binding obtained in the absence of (−)-adrenaline. Maximal specific binding was found to be 0.165 ± 0.060 pmol/mg protein. The dissociation constants were calculated from the $S_{0.5}$ (50% displacement) according to the equation $K_{diss} = \frac{S_{0.5}}{1 + \frac{[S]}{K_m}}$ where $S_{0.5}$ is the concentration of the ligand needed for displacement of 50% of the specific binding, [S] is the concentration of [^{3}H]-phentolamine used in the assay, and K_m is the apparent dissociation constant observed for [^{3}H]-phentolamine. The K_{diss} for the amine analogue is $(4.0 \pm 0.5) \times 10^{-6}$ M (○) and for the *p*-hydroxyphenacetyl analogue of haloperidol $(4.0 \pm 1.0) \times 10^{-7}$ M (●). The dissociation constants for the various ligands are summarized in Table I. All the values are given with their standard deviations of three independent determinations.

Discussion

The ability of butyrophenones such as haloperidol to bind to α-adrenoceptors has been noted previously in binding experiments using the α-antagonists [^{3}H]-WB-4101 (Greenberg *et al.*, 1976; U'Prichard *et al.*, 1977), [^{3}H]-dihydroergokryptine (Williams & Lefkowitz, 1976; Newman *et al.*, 1978), and [^{3}H]-phentolamine (Steer *et al.*, 1979), as well as the α-agonist [^{3}H]-clonidine (Greenberg *et al.*, 1976; U'Prichard *et al.*, 1977). In the present studies we have further characterized the α-adrenoceptor activity of haloperidol and a few of its newly synthesized analogues by evaluating their ability to inhibit adrenaline-induced potassium release in rat parotid slices (a typical α_1-response) and to inhibit [^{3}H]-phentolamine binding to human platelet membranes (a purely α_2-response) (Wood, Arnett, Clarke, Tasi & Lefkowitz, 1979).

The amine-haloperidol (see Figure 1) was found to be the most effective among the butyrophenones tested in inhibiting adrenaline-induced potassium release [$K_I = (3 \pm 2) \times 10^{-9}$ M] in rat parotid slices. It is also apparent (Table 1) that any further modification of the amino group (Figure 1) reduces the potency of the ligand as inhibitor of the adrenaline-dependent potassium release. Indeed, the apparent infinity of haloperidol itself for the receptor site is lower [$(5 \pm 2) \times 10^{-7}$ M] than that noted for the other haloperidol analogues. This is a biochemical signal which, in addition to binding studies, demonstrates that haloperidol and other butyrophenone analogues have high affinity for peripheral α-adrenoceptors.

The α_2-adrenoceptor actions of the new ligands were also studied by evaluating their ability to displace [^{3}H]-phentolamine from human platelet membranes (Figure 2). The affinities of several haloperidol analogues for the platelet α_2-receptor are lower than their affinity for the α-receptor of the rat parotid gland (Table 1). On the other hand, clonidine, an α_2-adrenoceptor agonist, has ten times more affinity for the platelet α-receptor than for the rat parotid α-receptor. These results indicate that butyrophenones exhibit higher affinity for the α_1-type of receptors (i.e., parotid K^+ release) than for α_2-adrenoceptors (for example, platelet [^{3}H]-phentolamine binding).

The high affinity for α_1-receptors noted for the newly synthesized compounds, suggests that they might serve as potential probes in studies directed at characterizing both haloperidol binding sites and α_1-receptors. The amine analogue could be further modified to obtain an affinity label which would enable

direct labelling of the receptors; the dansyl analogue could be used for fluorescent localization of the receptors, both *in vivo* and *in vitro*. The *t*-boc-tyrosine analogue could be radio-iodinated and be used as a probe in direct binding studies.

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