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Binding of [^{3}H]Haloperidol to Dopamine D_2 Receptors in the Rat Striatum

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Abstract—The present study was designed to examine the properties of [^{3}H]haloperidol binding to dopamine D_2-receptors in rat striatum membranes, displacement potencies of various chemicals and differences between the affinities of various chemicals and two new 5-hydroxytryptamine (5-HT_2) receptor antagonists, MCI-9042, (±)-2-(dimethylamino)-1-[[*o*-(*m*-methoxyphenetyl)phenoxy]methyl]ethyl hydrogen succinate hydrochloride and one of its metabolites. The plots of specific binding for the striatum membranes obtained from the Scatchard analysis using [^{3}H]haloperidol were monophasic when non-specific binding was determined with 10 μM chlorpromazine, and the K_d and B_{max} values were 7·42 ± 1·03 nM and 1·58 ± 0·20 pmol (mg protein)$^{-1}$ (n = 10), respectively. The displacement potencies of D_2 receptor, 5-HT_2 receptor, histamine H_1-receptor, and adrenoceptor antagonists were characterized by [^{3}H]haloperidol binding to D_2 receptors. The pK_i values of a new antiplatelet agent, MCI-9042, and its metabolite were 5·02 and 5·53, respectively, and these values were lower than those of the D_2-receptor antagonists, fluphenazine, spiperone, haloperidol, prochlorperazine, chlorpromazine, thioridazine, and sulpiride. They were also lower than the pK_i values of the 5-HT_2-receptor antagonists, pirenperone, ketanserin, methysergide, and mianserin. We conclude that the binding site of [^{3}H]haloperidol in the rat striatum is the D_2 receptor, that MCI-9042 and its metabolite have lower affinities for D_2 receptors than for 5-HT_2 receptors, and that this radioreceptor assay is useful for assessing the affinities of various agents.

Dopamine D_2-receptor antagonists are clinically effective antipsychotic agents (Creese et al 1976a, b; Seeman et al 1976). The affinities of the antagonists for D_2 receptors correlate well with the average daily dose (Creese et al 1976a, b; Seeman et al 1976), inhibition of apomorphine and amphetamine stereotypy (Creese et al 1976a), and inhibition of apomorphine-induced emesis (Creese et al 1976a).

Dopamine D_2-receptor binding has been demonstrated in various tissue membranes by labelling the receptors with [^{3}H]haloperidol (Creese et al 1975, 1976a, b; Leysen et al 1977; Seeman et al 1975, 1976) or [^{3}H]spiperone (Hamblin et al 1984; Quik et al 1987; Zahniser & Dubocovich 1983). Spiperone has a much greater affinity for D_2 receptors than haloperidol, but spiperone also has much greater affinities for D_3, D_4, 5-HT_2 and adrenergic α_1-receptors than haloperidol (Leysen et al 1978; Morgan et al 1984; Sokoloff et al 1990; Van Tol et al 1991). The results of studies in which [^{3}H]haloperidol was used as a radioligand indicate that this radioreceptor assay is useful for assessing the affinities of antipsychotic agents for D_2 receptors (Creese et al 1976a, b; Seeman et al 1976).

A new anti-platelet agent, MCI-9042, and its major metabolite, M-1, can inhibit collagen-induced platelet aggregation and displace [^{3}H]ketanserin binding at low concentrations (Kikumoto et al 1990). We have also found that these agents are much more selective for 5-HT_2 receptors in the rat brain (Maruyama et al 1991) and rabbit platelet (Tsuchihashi et al 1991) than they are for 5-HT_1-ergic, α_1-, α_2- and β-adrenergic and muscarinic receptors in the brain (Maruyama et al 1991).

The purpose of the present paper is to examine the characteristics of [^{3}H]haloperidol binding to D_2 receptors in the rat striatum, to assess the affinities of MCI-9042 and M-1 and to compare these affinities with those of 5-HT_2, D_2 and H_1 receptors and adrenoceptor antagonists.

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Materials and Methods

Materials

[^{3}H]Haloperidol (8·9 Ci mmol^{-1}; 329·3 GBq mmol^{-1}) was purchased from New England Nuclear/Dupont Ltd. MCI-9042, (±)-2-(dimethylamino)-1-[[*o*-(*m*-methoxyphenetyl)phenoxy]methyl]ethyl hydrogen succinate hydrochloride, and its metabolite, M-1, (±)-3-dimethylamino-1-[*o*-(*m*-methoxyphenetyl)phenoxy]-2-propanol, were donated by Mitsubishi Kasei Corporation.

Preparation of membrane-enriched fraction

Male Wistar rats, 250–350 g, were used. After the brain was removed the striatum was immediately frozen and stored at −80°C until use. Membrane-enriched fractions were prepared as follows. Tissues were defrosted at room temperature (21°C) and minced with small scissors in 10 vol of buffer I (0·25 M sucrose, 10 mM Tris-HCl buffer, pH 7·4). The suspensions were homogenized in a glass homogenizer. Homogenates were filtered through 4 layers of gauze. The filtrate was centrifuged at 40 000 *g* for 30 min at 4°C. The resultant pellet was immediately rinsed and homogenized in buffer II (120 mM Tris-HCl, 40 mM $MgCl_2$, pH 7·4), in a glass homogenizer. Portions were taken for protein determination (Lowry et al 1951). The membrane-enriched fraction was then frozen in liquid nitrogen, stored at −80°C, and diluted to appropriate concentrations immediately before use. There was no observable decrease in binding of the membrane-enriched fraction after 2 months of storage, when compared with the fraction which was frozen and thawed.

Binding assay

The membrane suspension (0·1 mg protein) was incubated for 45 min at 23°C in a 0·5 mL solution of 60 mM Tris-HCl, 20 mM $MgCl_2$ (pH 7·4). In the saturation experiment, nine [^{3}H]haloperidol concentrations were used (0·5–13 nM). In the displacement experiments, the concentration of [^{3}H]haloperidol was 1·5 nM. At the end of the incubation period, the incubation medium was immediately filtered through GF/C glass fibre and was washed with the incubation buffer as described previously (Tsuchihashi et al 1985). The radioactivity of the filter was counted with a Packard 2200 Tri-Carb Scintillation Analyser. The specific binding was determined by subtracting the non-specific binding in the presence of 10 μM chlorpromazine from the total.

Kinetic analysis

All kinetic analyses were carried out on a computer, with an iterative nonlinear regression program (Tsuchihashi & Nagatomo 1987; Tsuchihashi et al 1989a, b, 1990). The fit between the data and a model that accounts for only one receptor subtype was compared with the fit between the data and a 2-receptor-subtype model (Tsuchihashi & Nagatomo 1987; Tsuchihashi et al 1989a, b, 1990). Most of the K_i values of various chemicals are expressed as pK_i ($-\log K_i$). To quantify the model of saturation and the displacement, Hill numbers for Scatchard analysis and slope factors for displacement curves were determined as described previously (Tsuchihashi & Nagatomo 1987; Tsuchihashi et al 1989a, b, 1990).

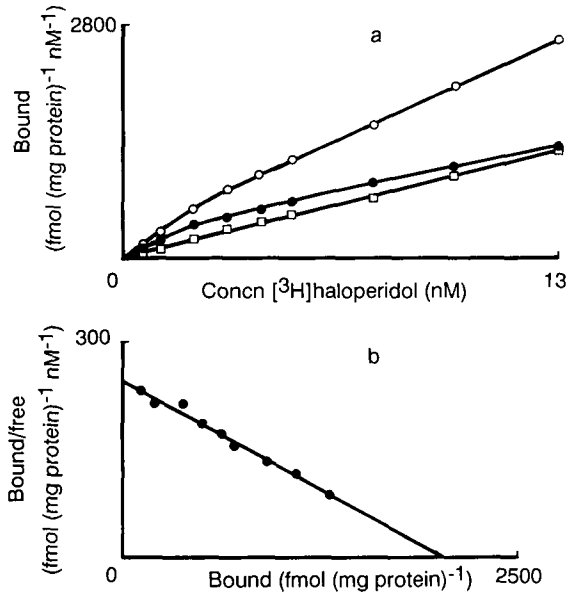

FIG. 1. Results of saturation experiments (a) and a Scatchard plot (b) of [^{3}H]haloperidol binding to rat striatum membrane. The values of K_d and B_{max} were 8·38 nM and 2·04 pmol (mg protein)$^{-1}$, respectively. The data were obtained from one experiment performed twice. The points show total (○), specific (●), and non-specific (□) binding. Specific binding was determined by subtracting the non-specific binding that remained in the presence of 10 μM chlorpromazine from the total.

Results

A typical result from the saturation experiments of binding of [^{3}H]haloperidol to rat striatum membranes is shown in Fig. 1a, with Scatchard plots of the same data in Fig. 1b. Specific binding accounted for 50–90% of the total radioactivity bound to the membranes. In striatum membranes from 10 male rats, the values of K_d and B_{max} were 7·42 ± 1·03 nM and 1·58 ± 0·20 pmol (mg protein)$^{-1}$ (n = 10), respectively. The Hill coefficients were 1.

Fig. 2 shows the typical displacement curves which were obtained for [^{3}H]haloperidol binding to rat striatum membranes for three D_2-receptor antagonists, fluphenazine, thioridazine, and prochlorperazine. All displacement curves were monophasic when fluphenazine, thioridazine, and prochlorperazine were used as the competitors. Table 1 shows the pK_i values of various 5-HT_2, D_2 and H_1 receptors, and adrenoceptor antagonists. The pK_i values of fluphenazine and spiperone were higher than those of the other chemicals used. The pK_i values of MCI-9042 and M-1 were 5·02 and 5·53, respectively. These values were lower than those of all of the D_2-receptor antagonists tested. They were also lower than the pK_i values of four 5-HT_2-receptor antagonists, pirenperone, ketanserin, methysergide, and mianserin. The slope factors of all drugs were 1 except for dopamine (0·74 ± 0·07) and apomorphine (0·85 ± 0·05).

The correlations between pK_i values measured in the present study and those for D_1 and D_2 receptors in the calf caudate (Creese et al 1975), those for cloned D_1 (Dearry et al 1990), D_2 and D_3 (Sokoloff et al 1990), D_4 (Van Tol et al 1991), and D_5 receptors (Sunahara et al 1991) were computed. The affinities for D_2 receptors measured in the present study correlated well with those for D_2 receptors in the calf caudate ($r = 0·98$, $P < 0·001$) and in Chinese hamster ovary cells expressed by cloned cDNA of D_2 receptors ($r = 0·95$, $P < 0·01$). There was no significant correlation with D_1

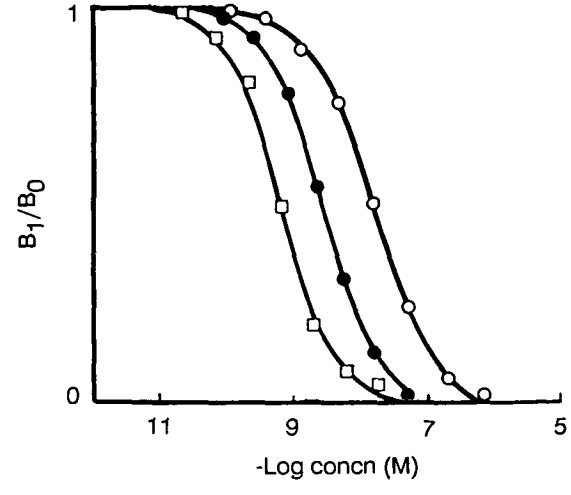

FIG. 2. Displacement curves of fluphenazine (□), prochlorperazine (●) and thioridazine (○) for [^{3}H]haloperidol binding to rat striatum membrane. The concentration of [^{3}H]haloperidol was 1·5 nM and specific binding was determined by subtracting the non-specific binding that remained in the presence of 10 μM chlorpromazine from the total. B_1 and B_0 are the concentrations of the radioligand bound with and without the cold ligand, respectively. The data were obtained from one experiment performed twice.

Table 1. pK_i values of various agents.

	pK_i	
Dopamine D_2-receptor antagonists		
Fluphenazine	9·34 ± 0·26	(3)
Spiperone	9·12 ± 0·22	(3)
Haloperidol	8·62 ± 0·19	(3)
Prochlorperazine	8·54 ± 0·21	(4)
Chlorpromazine	8·23 ± 0·07	(3)
Thioridazine	7·85 ± 0·16	(3)
Sulpiride	7·78 ± 0·16	(3)
Histamine H_1-receptor antagonists		
Cyproheptadine	8·68 ± 0·11	(3)
Promethazine	6·64 ± 0·05	(3)
Meclizine	6·61 ± 0·10	(3)
Cinnarizine	6·02 ± 0·05	(3)
(+)-Chlorphenylamine	4·96 ± 0·04	(3)
Diphenhydramine	4·70 ± 0·12	(3)
5-HT_2-receptor antagonists		
Pirenperone	7·41 ± 0·04	(3)
Ketanserin	6·37 ± 0·07	(3)
Methysergide	5·99 ± 0·17	(3)
Mianserin	5·87 ± 0·05	(3)
M-1	5·53 ± 0·21	(3)
Cinanserin	5·09 ± 0·08	(3)
MCI-9042	5·02 ± 0·25	(3)
Adrenoceptor antagonists		
Phentolamine	5·26 ± 0·07	(3)
Yohimbine	4·91 ± 0·13	(3)
(±)-Propranolol	4·35 ± 0·20	(3)
Bunazosin	4·04 ± 0·21	(4)
Prazosin	3·84 ± 0·08	(6)
Others		
Apomorphine	6·96 ± 0·12	(3)
Dopamine	6·00 ± 0·22	(4)
8-OH-DPAT	4·82 ± 0·24	(3)
5-HT	4·65 ± 0·29	(3)
Atropine	4·43	(1)
Pirenzepine	< 3	(1)
Ranitidine	< 3	(1)

Values in parenthesis show the numbers of experiments. Data are mean values ± s.e.

receptors in the calf caudate (r = 0·36), cloned D_1 (r = 0·75), D_3 (r = 0·59), D_4 (r = 0·45) or D_5 receptors (r = 0·34).

Discussion

The antagonists of D_2 receptors are clinically effective antipsychotic agents (Creese et al 1976a, b; Seeman et al 1976) and the affinities of these agents correlate well with average daily dose (Creese et al 1976a, b; Seeman et al 1976), inhibition of apomorphine and amphetamine stereotypy (Creese et al 1976a), and inhibition of apomorphine-induced emesis (Creese et al 1976a). As expected, all of the antipsychotic agents (fluphenazine, spiperone, haloperidol, prochlorperazine, chlorpromazine, thioridazine and sulpiride) have high affinities for [^{3}H]haloperidol binding sites in the rat striatum. The results of the present study are similar to those found in a study of the calf caudate (Creese et al 1975). Furthermore, there were strong correlations between pK_i values measured in the present study and those for D_2-receptors, but not for D_1, D_3, D_4, or D_5 receptors (Creese et al 1975; Dearry et al 1990; Sokoloff et al 1990; Sunahara et al 1991; Van Tol et al 1991). These suggests that [^{3}H]haloperidol binding sites corresponded to D_2 receptors.

A new anti-platelet agent, MCI-9042, and its major metabolite, M-1, inhibited the collagen-induced platelet aggregation and displaced [^{3}H]ketanserin binding at low concentrations (Kikumoto et al 1990). We also found that the displacement potencies of these agents for 5-HT_2-ergic receptors in rat brain (Maruyama et al 1991) and rabbit platelet (Tsuchihashi et al 1991) were much greater than those for 5-HT_1-ergic, α_1-, α_2-, and β-adrenergic and muscarinic receptors in the brain (Maruyama et al 1991). MCI-9042 and M-1 had lower affinities for D_2 receptors than for 5-HT_2-receptors in the present study. MCI-9042 and M-1 bound 100–200 times more strongly to 5-HT_2 receptors in the rat brain (pK_i values; 7·15 and 7·82) and in rabbit platelets (pK_i values; 7·19 and 7·59) as measured by [^{3}H]ketanserin binding (Maruyama et al 1991; Tsuchihashi et al 1991) than to D_2 receptors measured in the present study. The pK_i values of these new agents were lower than those of all of the D_2-receptor antagonists tested. They were also lower than the pK_i values of four 5-HT_2-receptor antagonists, pirenperone, ketanserin, methysergide, and mianserin. Among the D_2 receptor antagonists, spiperone and fluphenazine had high affinities for this binding site while the displacement potencies of other agents were weak. These results suggest that MCI-9042 and M-1 were more selective to 5-HT_2 receptors than to 5-HT_1-ergic, α_1-, α_2-, and β-adrenergic, muscarinic, and D_2 receptors.

Although cyproheptadine was thought to act as an antagonist of 5-HT_2 and H_1 receptors, the pK_i value of cyproheptadine (8·68) for D_2 receptors as measured in this study was higher than those reported previously for 5-HT_1 (5·83 in the brain), 5-HT_2 (7·83 in the brain and 5·61 in platelets), α_1- (7·01), α_2- (5·78), β- (4·64) and muscarinic (7·69) receptors (Maruyama et al 1991; Tsuchihashi et al 1991). These characteristics of cyproheptadine, which is a dibenzo [a,d]cycloheptene are similar to those of chlorpromazine, which is a phenothiazine derivative. The structural similarity between cyproheptadine and chlorpromazine may account for the similarities in their affinities for various receptors.

Dopamine D_2-receptor binding has been demonstrated in various tissue membranes by labelling the receptors with [^{3}H]haloperidol, [^{3}H]spiperone, [^{3}H]raclopride, or [^{3}H]YM 09152-2. Spiperone and YM 09152-2 have similar affinities for D_2 and D_4 receptors (Van Tol et al 1991) and raclopride has a similar affinity for D_2 and D_3 receptors (Sokoloff et al 1990). The affinity of haloperidol for D_2-receptors was much greater than for D_3 (22-fold, Sokoloff et al (1990)), D_4 (10-fold, Van Tol et al (1991)), 5-HT_2- (220-fold, Leysen et al (1978)) and α_1-receptors (9-fold, unpublished data), but the affinity of spiperone for D_2-receptors was similar to the affinity for D_3 (9-fold, Sokoloff et al (1990)), D_4 (2-fold, Van Tol et al (1991)), 5-HT_2 (16-fold, Leysen et al (1978)) and α_1-receptors (0·7-fold, unpublished data). The present results are also consistent with previous reports that [^{3}H]haloperidol can be used to assess the affinities of antipsychotic agents for D_2 receptors (Creese et al 1976a, b; Seeman et al 1976).

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