RESEARCH ARTICLES

Affinity of Neuroleptics for D_1 Receptor of Human Brain Striatum

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We determined the inhibition-dissociation constant (Ki) of a number of neuroleptics for D_1 receptors of normal human brain tissue using [^{3}H]SCH23390 [R-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3[benzazepine-7-ol]. SCH23390 had the highest affinity with a Ki of 0.76 nM. Among clinically used drugs, propericiazine showed the highest affinity with a Ki of 10 nM. When neuroleptics were classified according to chemical structures, the Ki values were as follows. Phenothiazines ranged from 10 nM to 250 nM. Butyrophenones ranged from 45 nM to 250 nM. Thioxanthenes ranged from 12 nM to 340 nM. Orthopramines were more than 10,000 nM. The Ki values for the binding site of this study were significantly correlated with those reported in studies using animal brain. The possible relationship between D_1 receptors and negative symptoms is discussed.

Key Words: dopamine D_1 receptors, negative symptoms, neuroleptics, human brain, radioreceptor assay

INTRODUCTION

Antipsychotic effects of neuroleptics have been ascribed primarily to blockade of dopamine D_2 receptors (D_2 receptors). This is based on the strong correlation between affinities of neuroleptics for D_2 receptors and their average clinical dosages (Creese et al 1976; Seeman et al 1976; Richelson and Nelson 1984). Although most of the currently available neuroleptics block D_2 receptors, these compounds also significantly block dopamine D_1 receptors (D_1 receptors). Specific D_1 receptor antagonists may have neuroleptic properties with less adverse neurological effects (Weddington 1987; Chipkin et al 1988). Moreover, recent findings indicate that D_1 receptors and D_2 receptors operate by a mutual interaction rather than by independent mechanisms (Seeman et al 1989). In addition, several lines of evidence suggest that D_1 receptors are involved in the pathogenesis of negative symptoms (Lynch 1992).

Therefore, the data on the affinities of neuroleptics for D_1 receptors would provide clinicians with valuable information. The affinities of the neuroleptics for D_1 receptors have

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previously been determined by radioligand binding assay (RBA). However, these studies used receptors from animal brain which have an unknown relationship to human brain receptors. Indeed, we showed differences between animal brain and human brain in the way that their receptors bind certain drugs (Kanba and Richelson 1984). In this paper, we report the affinities of 30 clinically available neuroleptics for D_1 receptors of normal human brain tissue obtained by autopsy and discuss the possible relevance of the blockade of D_1 receptors to their clinical effects.

METHODS

Normal human brain and tissue preparation

Normal human brain tissues were obtained at the time of autopsy from seven individuals (three females, four males) as described previously (Kanba et al 1988). All the brains were grossly judged to be normal by a neuropathologist. The causes of death were either cardiovascular disease or cancer. None of them had neuropsychiatric disease or brain damage prior to death and none had received psychotropic medication. The interval between death and autopsy (mean ± sd) was 7.0 ± 4.0 h (range 1.0 h to 13.0 h). The mean ± sd age of the individuals was 63.0 ± 8.0 years old (range 55 years to 76 years).

The tissues were quickly dissected into individual regions and stored at -180° C in a liquid nitrogen refrigerator until they were homogenized using a Brinkmann Polytron PT-45 (45 s on channel 8) in 10 volumes of ice-cold 50 mM Tris-HCl buffer (pH = 7.7 at 25° C) containing no NaCl or KCl. Striata collected from seven brains were used for this study. After centrifugation at 38,000 g for ten minutes, the pellets were resuspended in fresh Tris-HCl buffer and the centrifugation was repeated. The final pellets were resuspended in 50 mM Tris-HCl buffer (pH = 7.7 at 25° C) and the suspension was diluted to a volume which would provide the appropriate amount in 0.75 ml aliquots. The homogenates were stored at -33° C until assay.

Radioligand binding assay

The same procedures as those for the previous human brain binding study were used (Kanba et al 1988). One hundred microlitres of [^{3}H]SCH23390 (1 nM, 90 Ci/mmol), competing drug solution (100 µl of variable concentrations) and 50 mM Tris-HCl buffer (700 µl) containing no NaCl or KCl were placed in glass tubes. The solutions were stirred using a vortex mixer. One hundred microlitres of membrane preparation (2.5 mg wet tissue/tube) were added and the solution was again stirred using a vortex mixer. Solutions were incubated for 30 min at 37° C. Incubations were terminated by rapid filtration under vacuum using Whatman GF/B filters (24 mm diameter). The tubes and filters were rinsed with 6 ml ice-cold saline. The filters were punched out and placed in a 20 ml glass vial with 10 ml scintillation cocktail. After the samples stood six hours, radioactivities were measured using a Beckman LS 7800 liquid scintillation counter. The specific bound was defined as the total amount of [^{3}H]SCH23390 bound (zero unlabelled ligand) minus the nonspecific amount bound (binding in presence of 1 µM SCH23390). Ki values were obtained from the LIGAND program (Munson and Rodbard 1980). All of the data were obtained in at least three independent experiments, each determined in triplicate. The following data are expressed as mean ± sem. The statistical test used was the Student's t-test.

RESULTS

In human brain striata, SCH23390 had the highest affinity for the SCH23390 binding site with a Ki of 0.76 ± 0.05 nM and a Hill coefficient nearly equal to one (0.92 ± 0.12). Thus, SCH23390 bound to single site with high affinity. We examined several compounds as competitors of this site of [^{3}H]SCH23390 (1 nM). Specific binding accounted for about 70% of total binding in these experiments. The Ki values for the D_1 receptors yielded covered a broad range (see Table 1).

The recent discovery of several new dopamine receptors revealed that SCH23390 has the similar affinity for dopamine D_5 receptors (D_5 receptors). However, D_5 receptors would not be measured in our assays, since it is expressed at low or non-existent levels in the extrapyramidal systems, the source of our tissue (Sunahara et al 1991).

When neuroleptics were classified according to chemical structures, the Ki values were as follows. Phenothiazines were 57 ± 69 (10 nM to 250 nM) nM. Butyrophenones were 90 ± 90 (45 nM to 250 nM) nM. Thioxanthenes were 180 ± 230 (12 nM to 340 nM) nM. Benzamides were more than 10,000 nM.

There was no significant correlation between the log of the Ki values and that of the chlorpromazine equivalent doses for each drug based on calculations mostly by Davis et al (1976) and by ourselves (data not shown). Also, there was no significant correlation between the log of the Ki values and that of the average clinical daily doses of each drug reported by Creese et al (1976) (data not shown).

The Ki values for D_1 receptors of this study were significantly correlated with those obtained from studies with animal brain (see Figure 1). There was a strong correlation between our data and those of rat striatal tissue reported by Billard (1984) ($r = 0.93$, p 0.001) and by Anderson and Nielsen (1986) ($r = 0.97$, p 0.001).

DISCUSSION

This article presents the data of the affinities of a large number of the clinically available neuroleptics for D_1 receptors of human striatum. The Ki values of the neuroleptics were similar to those for the rat striatum reported by others (Billard et al 1984; Andersen and Nielsen 1986). There was

Table 1

Inhibition-dissociation constants of neuroleptics for dopamine D_1 and D_2 receptors of human and animal brain striatum

Drugs	Human brain Ki (mean ± sem) (nM)			Animal brain Ki (mean ± sem) (nM)		
	D_1	D_2[a]	D_2/D_1	D_1[b]	D_2[b]	D_2/D_1
Benzazepine						
SCH23390	0.76 ± 0.05			0.14	895	6392
Phenothiazines, aliphatic						
Levomepromazine	25 ± 8					
Chloropromazine	56 ± 2	19 ± 2	0.34	25	4.6	0.18
Zotepine	29 ± 8					
Phenothiazines, piperidine						
Propericiazine	10 ± 8					
Thioridazine	13 ± 2	26 ± 8	2.0	21	9.5	0.45
Mesoridazine	46 ± 12	19 ± 2	0.41			
Phenothiazines, piperazine						
Fluphenazine	15 ± 2	0.8 ± 0.1	0.05	4.5	0.84	0.19
Perphenazine	28 ± 6	1.4 ± 0.2	0.05	44	0.95	0.02
Trifluoperazine	69 ± 1	2.6 ± 0.3	0.04			
Perazine	89 ± 160					
Prochlorperazine	250 ± 34	7 ± 1	0.03			
Butyrophenones						
Bromperidol		25 ± 24				
Haloperidol	45 ± 12	4 ± 1	0.09	76	2.6	0.03
Moperone	50 ± 16					
Timiperone	81 ± 24					
Spiperone	250 ± 68	0.16 ± 0.02	0	360	0.11	< 0.0001
Thioxanthenes						
Chloroprothixene	12 ± 9	8 ± 2	0.67			
Thiothixene	340 ± 130	0.45 ± 12	0			
Diphenylbutyl Piperidines						
Pimozide	1,000 ± 870			1,420	0.38	< 0.0001
Dibenzothiazepines						
Clotiapine	23 ± 8					
Clozapine	38 ± 1	180 ± 5	4.8			
Benzamides						
Sulpiride	10,000 ± 9300			> 15,000	34	< 0.0001
Sultopride	> 30,000					
Others						
Butaclamol-D	6.3 ± 0.2	0.86 ± 0.06	0.14	0.95	0.9	0.95
Oxypertine	47 ± 10					
Clocapramine	76 ± 15					
Carpipramine	100 ± 33					
Butaclamol-L	2,900 ± 1,330			> 9,000	6,700	< 0.74
Buspirone	> 10,000					
Tiapride	> 30,000					

[a]The values of Ki for D_2 receptor of human brain tissue are from Richelson and Nelson (1984). [b]The values of Ki for D_1 and D_2 receptors of animal brain tissue are from Anderson and Neilson (1986).

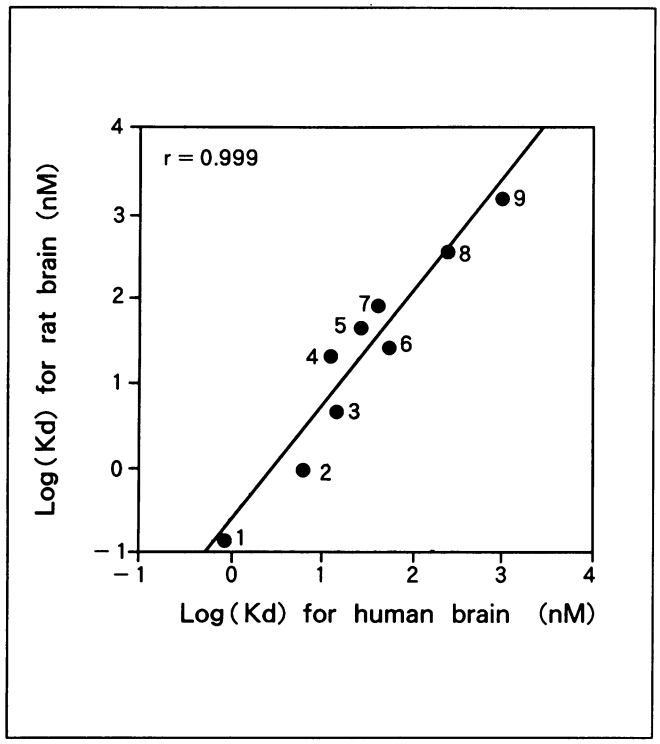

Fig. 1. Correlation between the Ki values of several compounds for the SCH 23390 binding site of this study and those obtained with animal brain (Billard 1984; Anderson and Nielsen 1986) (r = 0.999, p < 0.001, t-test). 1=SCH23390; 2=butaclamol-D; 3=fluphenazine; 4=thioridazine; 5=perphenazine; 6=chlorpromazine; 7=haloperidol; 8=spiperone; 9=pimozide.

a significant correlation between the Ki values for human striatum and those for rat striatum. Therefore, there may be little species variation of the Ki values for D_1 receptors.

Hess et al (1987) reported that, when only phenothiazines and neuroleptics with somewhat similar structure were considered, there was a high correlation between their affinities for D_1 receptors and the average clinical dosages. However, no correlation was found between the affinities of any subclasses of the neuroleptics and their average clinical daily doses or their chlorpormazine equivalent doses.

Clozapine (Kane et al 1988) and phenothiazines (Goldberg 1985) are more effective to treat negative symptoms of schizophrenia than are other subclasses of neuroleptics. It is interesting that the affinities of clozapine and phenothiazines for D_1 receptors are relatively higher than the others. Most neuroleptics have relatively high affinities for other receptors, so it should not be concluded that the clinical effects on the negative symptoms are based primarily on the blockade of D_1 receptors. However, in another study, we investigated the relationship between change of negative symptoms, plasma homovanillic acid level and that of plasma anti-D_1 receptor activities in medicated patients with schizophrenia. We obtained the results suggesting that the improvement in negative symptoms and the change in plasma homovanillic acid level may be caused by the blockade of D_1 receptors rather than that of D_2 receptors (Suzuki et al 1992). Behavioral, biochemical, and clinical data indicates that D_1 receptors in the prefrontal cortex are involved in pathogenesis of negative symptoms as extensively reviewed by Lynch (1992). Thus, the clinical significance of the SCH23390 binding site blockade by neuroleptics needs further investigation.

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