PROFILES OF THE AFFINITY OF ANTIPSYCHOTIC DRUGS FOR NEUROTRANSMITTER RECEPTORS AND THEIR CLINICAL IMPLICATION

Kimie Yonemura, Kazuo Miyanaga, and Yukiteru Machiyama

Abstract: The binding affinities of 12 different neurotransmitter receptors were measured using a radio-labeled receptor assay.

The profiles of the pharmacological affinities of 20 antipsychotic drugs were assessed. Based on the Ki values for the D₂ receptor, 5HT₂ receptor, and α₁ receptor, the antipsychotic drugs were classified into five groups (i.e., three anti-DA activity predominant groups and two anti-5HT activity predominant groups).

A theoretical basis for drug choice in clinical settings is also proposed. We recommend that the groups of the drugs having predominantly anti-DA activity be used in the treatment of hallucinations and delusions and that the drugs in the anti-5HT activity predominant group be used to treat hypobulia. If it is necessary to administer two or more of these drugs together, we propose that they should be drugs with different profiles. Extrapyramidal symptoms, side effects of antipsychotic drugs, were interpreted in terms of interaction among the anti-D₂ activity, anti-5HT₂ activity, and anti-Ach activity.

Key words: Antipsychotic drugs, Anti-DA action, Anti-5HT action, Anti-adrenergic action, Extrapyramidal symptoms.

INTRODUCTION

A variety of antipsychotic agents have been developed for clinical use since 1952, when the effectiveness of chlorpromazine in treating schizophrenia was first demonstrated. However, selection of antipsychotic drugs in individual cases has often been empirical, without any definite theoretical or pharmacological guidelines. This is corroborated by the tendency for psychiatrists in different countries to prescribe different drugs for the same mental symptom and there are also differences between university hospitals and other hospitals within Japan.

As a result of recent advances in pharmacological research on neurotransmitter receptors in the brain, the mechanism of action of antipsychotic drugs has been increasingly elucidated, and it now seems possible to argue for the establishment of theoretical foundations for the selection of drugs to treat mental symptoms. A number of reports on the affinity of antipsychotic agents for receptors have been published both in Japan and other countries. The reports, however, have often dealt with drugs that are seldom used or not used at all in Japan.

The present study was undertaken to assess the affinity for receptors of clinically popular antipsychotic drugs, and to obtain comprehensive profiles of their pharmacological actions, with the ultimate goal of providing a theoretical basis for selecting drugs in individual clinical cases.

MATERIALS AND METHODS

Nineteen antipsychotic drugs on the market in Japan and one that is currently being developed were investigated by radiolabeled receptor assays in regard to their affinity for the receptors of 12 different neurotransmitters.

Materials and methods (³H ligands, membrane
preparation, buffers, incubation conditions and the drugs to test for nonspecific binding) are summarized in Table 1.

1. Membrane preparation

A. Dopamine 1 (D1) receptor and dopamine 2 (D2) receptor

Striatum of male Wistar rat (200-250g) was homogenized in 30 volumes of 50mM Tris/HCl (pH 7.5) with an ultrasonic homogenizer (Nihonseiki Factory). The homogenate was centrifuged at 50,000 × g for 20 min and the pellet was resuspended in 30 volumes of 50mM Tris/HCl (pH7.5), and the same procedure was repeated two more times. The resulting pellet was then suspended in 3 volumes of the same buffer. In the binding assay, the suspension was diluted to an appropriate concentration and used as the crude membranes fraction for the binding assay.

B. Dopamine 3 (D3) receptor and Dopamine 4 (D4) receptor

The membranes from Chinese hamster ovary (CHO) cells expressing the cloned human dopamine D3 receptor and D4 receptor were used. These materials were obtained from the Yamanouchi Pharmaceutical Co., Ltd. and DuPont, respectively. They were stored frozen at -70°C until used. Before the binding study, the membranes were diluted in 7.5 volumes of 50mM Tris/HCl (120mM NaCl, 5mM MgCl2, 5mM KCl, 1.5mM CaCl2, 5mM EDTA : pH7.4) and in 25 volumes of 50mM Tris/HCl (120mM NaCl, 5mM KCl, 5mM MgCl2, 1mM EDTA : pH7.4), respectively.

C. Serotonin 1A (5HT1A) receptor

Hippocampus of the male Wistar rat (200-250g) was used. The method of homogenation was the same procedure described in the paragraph of dopamine 1 receptor and dopamine 2 receptor.

D. Serotonin 3 (5HT3) receptor

Cerebral cortex of male Wistar rat (200-250g) was used. The method of homogenation was the same procedure described in the paragraph of dopamine 1 receptor and dopamine 2 receptor, except for the kind of buffer (50mM HEPES : pH7.4).

E. Serotonin 2 (5HT2) receptor, adrenaline ß1 (ß1) receptor, adrenaline ß2 (ß2) receptor, and acetylcholine (Ach) receptor

Cerebral cortex of male Wistar rat (200-250g) was used. The method of homogenation was the same procedure described in the paragraph of dopamine 1 receptor and dopamine 2 receptor.

F. Histamine 1 (H1) receptor

Brain stem of male Wistar rat (200-250g) was used. The method of homogenation was the same procedure described in the paragraph of dopamine 1 receptor and dopamine 2 receptor.

Table 1. Materials and methods

<table>
<thead>
<tr>
<th>Receptors</th>
<th>¹H-ligand conc.(nM)</th>
<th>Brain tissue</th>
<th>Buffer</th>
<th>Incubation time temp. (min°C)</th>
<th>Nonspecific binding (µM)</th>
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<tr>
<td>D1</td>
<td>SCH22390 (0.2)</td>
<td>Rat striatum</td>
<td>50mM Tris/HCl (120mM NaCl, 5mM KCl, 1mM MgCl2, 1mM CaCl2)</td>
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<td>Fluphenazine (10)</td>
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<td>D2</td>
<td>Spiperone (0.5)</td>
<td>Rat striatum</td>
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<td>Sulpiride (10)</td>
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<tr>
<td>D3</td>
<td>YM-09151-2 (0.2)</td>
<td>CHO-cells*</td>
<td>50mM Tris/HCl (120mM NaCl, 5mM MgCl2, 5mM KCl, 1.5mM CaCl2, 5mM EDTA)</td>
<td>60 25</td>
<td>Quiniprol (10)</td>
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<tr>
<td>D4</td>
<td>Spiperone (5)</td>
<td>CHO-cells**</td>
<td>50mM Tris/HCl (120mM NaCl, 5mM MgCl2, 5mM KCl, 1mM EDTA)</td>
<td>120 25</td>
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<td>5HT1A</td>
<td>8-OH-DPAT (0.6)</td>
<td>Rat hippocampus</td>
<td>50mM Tris/HCl (0.1% Ascorbic acid, 10µM Pargyline)</td>
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<td>Ketanserin (1.0)</td>
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<td>50mM Tris/HCl</td>
<td>20 37</td>
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<td>5HT3</td>
<td>BRL-43694 (2.6)</td>
<td>Rat cortex</td>
<td>50mM HEPES</td>
<td>40 25</td>
<td>Tropisetron (10)</td>
</tr>
<tr>
<td>a1</td>
<td>Prazosin (0.6)</td>
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<tr>
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<td>Ach</td>
<td>QNB (0.07)</td>
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<td>60 25</td>
<td>QNB (0.5)</td>
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<td>H1</td>
<td>Mepyramine (3.2)</td>
<td>Rat brain stem</td>
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<td>60 25</td>
<td>Promethazine (500)</td>
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<tr>
<td>σ</td>
<td>SKF10047 (2.0)</td>
<td>Rat whole brain</td>
<td>50mM Tris/HCl</td>
<td>60 4</td>
<td>SKF10047 (30)</td>
</tr>
</tbody>
</table>

( ) : Final concentration
* : Membranes from CHO-cells expressing the cloned human dopamine D1 receptor
** : Membranes from CHO-cells expressing the cloned human dopamine D4 receptor
G. Sigma (σ) receptor

Whole brain of male Wistar rat (200-250g) was used. The method of homogenation was the same procedure described in the paragraph of dopamine 1 receptor and dopamine 2 receptor.

All suspensions were stored frozen at -70°C. Before the 5HT1A receptor and σ receptor binding assays, the suspension was incubated for 20 min at 37°C and 60 min at 37°C, respectively. The protein concentration was measured by the method of Lowry, with bovine serum albumin as the protein standard.

II. Antipsychotic drugs

The antipsychotic drugs used in this study and their sources were as follows: Chlorpromazine HCl, levomepromazine maleate, propraziazone, perphenazine maleate, fluphenazine maleate, clozapine, haloperidol, bromperidol, carpipramine HCl, clozapamine HCl and mosapramine, from Yoshitomi Pharmaceutical Ind., Ltd.; thioridazine HCl, from Sandoz Pharmaceuticals Ltd.; zotepine, from Fujisawa Pharmaceutical Co., Ltd.; timiperone, from Daiichi Pharmaceutical Co., Ltd.; sulpiride, from Dainippon Pharmaceutical Co., Ltd.; nemonapride, from Yamanouchi Pharmaceutical Co., Ltd.; and SM-9018, from Sumitomo Pharmaceutical Co., Ltd.

Most of the drugs used as displacing drugs were dissolved in distilled water, but properciazone, clotiapine, zotepine, haloperidol, timiperone, bromperidol, and pimozide were dissolved in 0.1% lactic acid.

III. Radio Receptor Assays

A. DA receptor

1. D1 receptor

The receptor binding assay was performed according to the method of Billard et al.8). The binding assay consisted of 100μl of 2nM [3H]SCH23390 solution (DuPont-NEN), 100μl of displacing drugs (antipsychotic drugs), 700μl of 50mM Tris/HCl (120mM NaCl, 5mM KCl, 1mM MgCl2, 1mM CaCl2; pH7.5), and 100μl of resuspended homogenate (0.1mg protein/tube). Incubation conditions were 37°C for 30 min. Nonspecific binding was determined in the presence of 10μM sulpiride (SIGMA).

2. D2 receptor

The receptor binding assay was performed according to the method of Tada et al.11). The binding assay consisted of 100μl of 5nM [3H]spiperone solution (DuPont-NEN), 100μl of displacing drugs (antipsychotic drugs), 700μl of 50mM Tris/HCl (120mM NaCl, 5mM MgCl2, 5mM KCl, 1.5mM CaCl2, 5mM EDTA; pH7.4), and 100μl of membrane suspension (8μg prot./tube). Incubation conditions were 25°C for 30 min. Nonspecific binding was determined in the presence of 10μM Quinpirol (RBI).

3. D3 receptor

The receptor binding assay was performed according to the method of Van Tol et al.12). The binding assay consisted of 100μl of 50nM [3H]spiperone solution (DuPont-NEN), 100μl of displacing drugs (antipsychotic drugs), 700μl of 50mM Tris/HCl (120mM NaCl, 5mM MgCl2, 5mM KCl, 1.5mM CaCl2, 5mM EDTA; pH7.4), and 100μl of membrane suspension (22μg prot./tube). Incubation conditions were 25°C for 60 min. Nonspecific binding was determined in the presence of 10μM nemonapride (Yamanouchi Pharmaceutical Co., Ltd.).

B. 5HT receptor

1. 5HT1A receptor

The receptor binding assay was performed according to the method of Peroutka et al.13). The binding assay consisted of 100μl of 6nM [3H]8-OH- DPAT solution (DuPont-NEN), 100μl of displacing drugs (antipsychotic drugs), 100μl of 0.1% ascorbic acid, 100μl of 10nM pargyline (SIGMA), 500μl of 50mM Tris/HCl (pH7.5), and 100μl of resuspended homogenate (0.1mg protein/tube). Incubation conditions were 30°C for 20min. Nonspecific binding was determined in the presence of 10μM serotonin (SIGMA).

2. 5HT2 receptor

The receptor binding assay was performed according to the method of Leysen et al.14). The binding assay consisted of 100μl of 10nM [3H]ketanserin solution (DuPont-NEN), 100μl of displacing drugs (antipsychotic drugs), 700μl of 50mM Tris/HCl (pH7.5), and 100μl of resuspended homogenate (0.1-0.2mg prot./tube). Incubation conditions were 37°C for 20min. Nonspecific binding was determined
in the presence of 1μM ketanserin (RBI).

(3) 5HT3 receptor

The receptor binding assay was performed according to the method of Nelson et al.15 and Sakamori et al.16.

The binding assay consisted of 100μl of 26nM [3H] BRL-43694 solution (DuPont-NEN), 100μl of displacing drugs (antipsychotic drugs), 600μl of 50mM HEPES (pH7.5), and 200μl of resuspended homogenate (0.3mg prot./tube). Incubation conditions were 25°C for 40min. Nonspecific binding was determined in the presence of 10μM tropisetron (Smith-Kline Beecham Consumer).

C. α adrenaline (α-Ad) receptor

(1) α1 receptor

The receptor binding assay was performed according to the method of Peroutka et al.17.

The binding assay consisted of 200μl of 3nM [3H] prazosin solution (Dupont-NEN), 100μl of displacing drugs (antipsychotic drugs), 40μl of 0.01% ascorbic acid, 500μl of 50mM Tris/HCl (pH7.5), and 100μl of resuspended homogenate (0.15mg prot./tube). Incubation conditions were 25°C for 30min. Nonspecific binding was determined in the presence of 10μM prazosin (SIGMA).

(2) α2 receptor

The receptor binding assay was performed according to the method of U’Prichard et al.18.

The binding assay consisted of 100μl of 1nM [3H] clonidine solution (DuPont-NEN), 100μl of displacing drugs (antipsychotic drugs), 50μl of 50mM Tris/HCl (pH7.5), and 100μl of resuspended homogenate (0.2mg prot./tube). Incubation conditions were 25°C for 60min. Nonspecific binding was determined in the presence of 10μM clonidine (SIGMA).

D. Ach receptor

The receptor binding assay was performed according to the method of Yamamura et al.19.

The binding assay consisted of 100μl of 0.7nM [3H] QNB solution (DuPont-NEN), 100μl of displacing drugs (antipsychotic drugs), 700μl of 50mM Tris/HCl (pH7.5), and 100μl of resuspended homogenate (0.2mg prot./tube). Incubation conditions were 25°C for 60min. Nonspecific binding was determined in the presence of 0.5μM QNB (SIGMA).

E. Histamine 1 receptor

The receptor binding assay was performed according to the method of Hill et al.19.

The binding assay consisted of 100μl of 32nM [3H] pyrilamine solution (DuPont-NEN), 100μl of displacing drugs (antipsychotic drugs), 700μl of 50mM Tris/HCl (pH7.5), and 100μl of resuspended homogenate (0.25–0.30mg prot./tube). Incubation conditions were 25°C for 60min. Nonspecific binding was determined in the presence of 500μM promethazine (Yoshitomi Pharmaceutical Ind. Ltd.).

F. Sigma receptor

The receptor binding assay was performed according to the method of Zukin et al.20 and Tam et al.22.

The binding assay consisted of 100μl of 20nM [3H] (+) SKF10047 solution (DuPont-NEN), 100μl of displacing drugs (antipsychotic drugs), 700μl of 50mM Tris/HCl (pH7.5), and 100μl of resuspended homogenate (0.2–0.3mg prot./tube). Incubation conditions were 4°C for 60min. Nonspecific binding was determined in the presence of 30μM (−) SKF10047 (RBI).

After incubation, 5ml of ice-cold buffer was added to the assay mixtures. The mixtures were then rapidly filtered under a vacuum through Whatman GB/F filters. After washing with 2×5ml of ice-cold buffer, the filter was dried by heating at 90°C for 2h. Radioactivity was measured with a liquid scintillation counter (Aloka LSC-700) in 5 ml of Aquasol (AQUA SOL-2 ; DuPont). In the D3 receptor and σ receptor experiments, the Whatman GB/F filter was soaked in 0.5% polyethyleneimine (SIGMA) for 60min.

Saturation experiments were performed with 5 to 8 [3H] ligand concentrations. Data are reported as the mean values of triplicate assays performed in each experiment.

IV. The analysis of results of the binding assays

The dissociation constant (Kd) and maximal number of binding sites (Bmax) were determined by Scatchard plots of data from the saturation experiments for each receptor. The affinity constant (Ki) of each antipsychotic drug was obtained from the inhibition curve of the displacement experiment data, i.e., Ki values were calculated as follows: Ki = IC50/(1+C/Kd), where IC50 is the concentration that inhibits 50% of [3H] ligand binding; Kd is dissociation constant; and C is the concentration of [3H] ligand.

RESULTS

Fig.1 shows the results of the D3 binding assay as example. Fig.1(a) shows the results of the saturation experiment. Fig.1(b) is the Scatchard plot and shows the Kd value and Bmax. Fig.1(c) shows the displacement curve of haloperidol for the D3 receptor and the IC50. The experiments showed sufficient reproducibility. Similar plots were obtained for the other receptors and the other antipsychotic drugs. All Ki values obtained in this study are summarized in Table 2.
The affinity of antipsychotic drugs for receptors

A lower Ki value indicates greater ability of a drug to bind to receptors and to block the binding of radioactive ligands to the receptors. Thus, higher affinity for a receptor is associated with a more potent pharmacologic action.

In this report, the affinity of drugs for receptors is rated on a four-point scale: high affinity (drugs with a Ki value on the order of 1nM or less), moderate affinity (Ki on the order of 10nM), relatively low affinity (Ki on the order of 100nM) and low affinity (Ki over 1μM).

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   A. **DA receptors**
      
      (1) **D₁ receptors**

      The affinity of the following drugs for D₁ receptors (i.e., the ability to block D₁ receptors) was high (Ki on the order of 1nM): fluphenazine, perphenazine, levomepromazine, and chlorpromazine. The Ki of these drugs for D₁ receptors ranged between 2.5nM and 6.3nM. Moderate affinity (Ki on the order of 10nM) was recorded for propericiazine, clozapramine, clozapine, zotepine, cariprazine, oxyperazine, and mosapramine. Relatively low affinity (Ki on the order of 100nM) was shown by thioridazine, timiperone, haloperidol, and bromperidol. Low affinity (Ki over 1μM) was shown by pimozide, sulpiride, sulphotride, nemonapride, and SM–9018. All phenothiazines except thioridazine were found to have high affinity for D₁ receptors.

      (2) **D₂ receptors**

      High affinity for D₂ receptors was found for timiperone, perphenazine, nemonapride, clozapramine, bromperidol, pimozide, mosapramine, SM–9018, haloperidol, perphenazine, levomepromazine, and zotepine. Moderate affinity for D₂ receptors was shown by chlorpromazine, cariprazine, sulpiride, sulphotride, oxypertine, propericiaine, and clozapine. Relatively low affinity was shown by thioridazine.

      (3) **D₃ receptors**

      High affinity for D₃ receptors was shown by timiperone, pimozide, nemonapride, SM–9018, fluphenazine, mosapramine, perphenazine, clozapramine, and chlorpromazine. Moderate affinity was shown by zotepine, haloperidol, levomepromazine, bromperidol, propericiaine, sulphotride, thioridazine, clozapine, and cariprazine. Relatively low affinity was shown...
(4) D₄ receptors

High affinity for D₄ receptors was shown by timiperone, haloperidol, bromperidol, nemonapride, and clotiapine. Moderate affinity was shown by SM-9018, clozapine, pimozide, timiperone, thioridazine, chlorpromazine, levomepromazine, and propracaine. Relatively low affinity was shown by meprobamate, fluphenazine, carpiramine, perphenazine, and oxyperine. Low affinity was shown by sulpiride and sulotroide, i.e., by all benzamides tested except nemonapride.

### Table 2. Ki values of antipsychotics

<table>
<thead>
<tr>
<th>Ki (nM)</th>
<th>D₁</th>
<th>D₂</th>
<th>D₃</th>
<th>D₄</th>
<th>5HT₁A</th>
<th>5HT₂</th>
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<td>Chlorpromazine</td>
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<td>5.9</td>
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Ki values were calculated by using the formula: IC₅₀/(1+C/Kd), where IC₅₀ is the concentration of the drug that half maximally displaces the ³H-ligand, C is the concentration of ³H-ligand, and Kd is its dissociation constant.

The data printed in boldface could not be found in any report except ours.

### B. 5HT receptors

(1) 5HT₁A receptors

As a whole affinity for 5HT₁A receptors tended to be low. High affinity was shown by SM-9018. None of the antipsychotic agents currently on the market showed high affinity for this receptor. Moderate affinity was shown by nemonapride. Relatively low affinity was shown by zotepine, oxyperine, pimozide, timiperone, etc. All benzamides except nemonapride had low affinity (Ki over 1μM).

(2) 5HT₂ receptors

As a whole affinity for 5HT₂ receptors tended to be high.

Levomepromazine, SM-9018, clozapine, carpi-
The affinity of antipsychotic drugs for receptors

pramine, perphenazine, propericiazine, chlorpromazine, timiperone, mosapramine, fluphenazine, clotiapine, zotepine, thioridazine, and pimozide had high affinity (Ki on the order of 10^{-2} nM with the first two drugs). Moderate affinity was shown by oxypertine, haloperidol, and bromperidol. Low affinity was shown by benzamides.

3) 5HT_{3} receptors
Most test drugs had a Ki value over 100nM, that is, low affinity for 5HT_{3} receptors. Relatively high affinity for this receptor was shown by perphenazine (470nM), chlorpromazine (600nM), and levomepromazine (800nM).

C. Ad receptors
(1) \( \alpha_{1} \) receptors
High affinity for \( \alpha_{1} \) receptors was shown by thioridazine, SM-9018, levomepromazine, chlorpromazine, timiperone, zotepine, propericiazine, bromperidol, and oxypertine. Moderate affinity was shown by perphenazine, haloperidol, clotiapine, pimozide, mosapramine, fluphenazine, and carpiramine. Among the benzamides, nemonapride had a Ki of 620nM for this receptor, but the other drugs in the family had low affinity (Ki over 1\mu M).

(2) \( \alpha_{2} \) receptors
High affinity was shown by clozapramine. Moderate affinity was shown by carpiramine, fluphenazine, and perphenazine. Relatively low affinity was shown by SM-9018, chlorpromazine, zotepine, thioridazine, pimozide, levomepromazine, propericiazine, mosapramine, and nemonapride. The other drugs showed low affinity.

D. Ach receptors
High affinity was shown by chlorpromazine and levomepromazine. Moderate affinity was shown by oxypertine, clotiapine, thioridazine, zotepine, clozapramine, perphenazine, and timiperone. Relatively low affinity was shown by fluphenazine, mosapramine, bromperidol, propericiazine, and pimozide. Low affinity was shown by carpiramine, sulpiride, sulproide, nemonapride, and SM-9018.

E. Histamine receptors
The Ki for H_{1} receptors was on the order of 10nM or higher with all of the drugs tested. Moderate affinity for H_{1} receptors was shown by mosapramine, chlorpromazine, perphenazine, and propericiazine. Relatively low affinity was shown by pimozide, thioridazine, clotiapine, timiperone, fluphenazine, levomepromazine, SM-9018, zotepine, and clozapramine. Low affinity was shown by haloperidol, bromperidol, carpiramine, oxypertine, nemonapride, sulpiride, and sulproide.

F. Sigma receptors
High affinity for \( \sigma \) receptors was shown by haloperidol, perphenazine, fluphenazine, clozapramine, chlorpromazine, and levomepromazine. Moderate affinity was shown by carpiramine, timiperone, clotiapine, zotepine, pimozide, bromperidol, thioridazine, propericiazine, and oxypertine. Low affinity was shown by mosapramine, SM-9018, and the benzamides.

II. Ki-based classification of antipsychotic drugs
According to the report by Otsuki et al.\(^{23}\), on the relationship between clinical symptoms and neurotransmitters (DA, 5HT and Ad), the pharmacologic actions of antipsychotic agents are primarily explained by anti-D_{2} activity, anti-5HT_{2} activity and anti-\( \alpha_{1} \) activity\(^{23}\). Based on their report, we attempted to classify antipsychotic agents. First, the drugs were classified according to the strength of anti-5HT_{2} activity, and anti-D_{2} activity, and then subclassified according to the strength of anti-\( \alpha_{1} \) activity. This yielded five groups, i.e., three DA receptor blockade dominant groups and two 5HT receptor blockade dominant groups, as shown in Table 3.

A. DA receptor blockade dominant groups
(1) Group A
The anti-D_{2} activity is the major activity and there is hardly any anti-5HT_{2} activity (anti-D_{2} activity/anti-5HT_{2} activity \leq 100). Sulpiride, which has no anti-\( \alpha_{1} \) activity, and nemonapride, which has an anti-\( \alpha_{1} \) activity, belong to this group.

(2) Group B
The anti-D_{2} activity is the major activity, and a weak anti-5HT_{2} activity is also observed (100 > anti-D_{2} activity/anti-5HT_{2} activity \geq 10). Haloperidol and bromperidol, members of the butyrophenone family, belong to this group.

(3) Group C
The anti-D_{2} activity is slightly stronger than the anti-5HT_{2} activity (10 > anti-D_{2} activity/anti-5HT_{2} activity \geq 1). Fluphenazine, timiperone, and pimozide belong to this group.

B. 5HT receptor blockade dominant groups
(1) Group D
The anti-5HT_{2} activity is the major activity (anti-5HT_{2} activity/anti-D_{2} activity \geq 10). This group can be divided into three subgroups: (1) drugs with little anti-\( \alpha_{1} \) activity (carpiramine), (2) drugs with anti-\( \alpha_{1} \) activity comparable to their anti-D_{2} activity (chlorpromazine, levomepromazine, clotiapine, perpropicin, perfenazine, and SM-9018), and (3) drugs with strong anti-\( \alpha_{1} \) activity (thioridazine).

(2) Group E
The anti-5HT_{2} activity is slightly stronger than the
anti-D\(_2\) activity (10> anti-5HT\(_2\) activity/anti-D\(_2\) activity  ¨' 1). This group can be divided into two subgroups: (1) drugs with little anti-\(\alpha_1\) activity (mosapramine and clocapramine) and (2) drugs with anti-\(\alpha_1\) activity comparable to their anti-D\(_2\) activity (zotepine and oxypertine).

### DISCUSSION

#### I. Affinity of antipsychotic agents

##### A. DA receptors

In 1976, Seeman et al.\(^24\) showed that the therapeutic doses of antipsychotic agents were inversely correlated with their ability to bind to DA receptors. Since then, blockade of DA receptors has been considered a major mechanism of antipsychotic actions.

As a result of recent advances in pharmacology and molecular biology studies, it has been found that DA receptors can be divided into five subtypes. These five subtypes are grouped into two families on the basis of their pharmacologic profiles: the D\(_1\) family (D\(_1\) and D\(_5\)) and the D\(_2\) family (D\(_2\), D\(_3\) and D\(_4\)). The present study dealt with D\(_1\), D\(_2\), D\(_3\) and D\(_4\).

(1) D\(_1\) family

D\(_1\) receptors are distributed in the corpus striatum, the substantia nigra, the limbic system, and the ventral tegmental area. Behavioral pharmacology studies have shown that simultaneous stimulation of D\(_1\) and D\(_2\) receptors results in stereotyped behavior\(^{25,26}\). Oral dyskinesia has also been reported when D\(_1\) receptors were blocked after blocking D\(_2\) receptors\(^27\). These findings suggest that blockade of D\(_1\) receptors is also involved in antipsychotic actions.

In the present study, high affinity for D\(_1\) receptors (Ki on the order of 1nM) was shown by phenothiazines such as fluphenazine, perphenazine, levomepromazine, and chlorpromazine. The affinity of butyrophenones for D\(_1\) receptors was lower (Ki on the order of 100nM). Data concerning anti-D\(_1\) action have been reported by Hyttel et al.\(^28\) and Kinoshita\(^29\). The IC\(_{50}\) reported for antipsychotic agents by Hyttel et al. was about 10 times lower than that obtained in the present study. This discrepancy is probably due to the difference in ligands used in the two studies. Consistent with previous reports, the anti-D\(_1\) receptor activity of phenothiazines tended to be higher than that of butyrophenones in the present study. No reports prior to the present study have dealt with the affinity of levomepromazine, propricizine, clothiapine, zotepine, timiperone, carpipramine, oxypertine, or sultopride for D\(_1\) receptors.

(2) D\(_2\) family

The same as D\(_1\) receptors, D\(_2\) receptors are dis-
The affinity of antipsychotic drugs for receptors

D₄ receptors are mainly distributed in the limbic system. They serve as the autoreceptors of DA neurons and are involved in mental functions such as cognition and emotion. The anti-D₄ receptor activity of chlorpromazine, thiioridazine, and clozapine is higher than their anti-D₂ receptor activity. Anti-D₃ receptor activity has also been reported by Tada et al. and Sokoloff et al. The data obtained in the present study was similar to their data. No reports prior to the present study have dealt with the affinity of propericiazine, or clothiapine for D₄ receptors.

D₃ receptors are distributed in the cerebral cortex and the limbic system. Clozapine has been shown to be useful in the treatment of chronic schizophrenia characterized by negative symptoms (e.g., avolition, affective flattening, and social withdrawal) and treatment-resistant schizophrenia, and to block D₄ receptors. On the basis of such findings, a relationship between anti-D₄ receptor activity and successful treatment of negative symptoms was hypothesized. In recent years, however, it has been reported that only a small number of D₄ receptors are present in the brain. Thus, the validity of this hypothesis has not yet been confirmed. In the present study, butyrophenones and SM-9018 (an atypical antipsychotic agent) were found to have relatively high anti-D₄ receptor activity. The data obtained in the present study concerning the potency of anti-D₄ receptor activity of antipsychotic agents was similar to that reported by Van Tol et al. No reports prior to the present study have dealt with the affinity of levomepromazine, propericiazine, perphenazine, clothiapine, timiperone, carpipramine, oxypertine, or sulotropride for D₄ receptors.

D₅ receptors are distributed in the corpus striatum, the substantia nigra, the limbic system, the ventral tegmental area. Blockade of D₅ receptors is a pharmacologically important action that suppresses hallucinations and delusions. Consistent with previous reports, butyrophenones were found to have higher affinity for D₅ receptors than phenothiazines in the present study. A number of reports on anti-D₅ receptor activity have been published, but the data for the potency of this activity differ by more than 10 fold among the different reports. Thus, the exact potency of this activity is unknown. The potency of this activity obtained in the present study was within the range of this parameter reported in the past. No reports prior to the present study have dealt with the affinity of propericiazine, or clothiapine for D₅ receptors.

B₁ 5HT receptors

5HT has been reported to be involved in mood disorders and symptoms of schizophrenia such as anxiety, depression, obsession, and aggressiveness. At present, 7 families (14 subtypes) of 5HT receptor are known. The present study dealt with 5HT₁A, 5HT₂, and 5HT₃ receptors.

5HT₁A receptors are distributed in the limbic system, the raphe nuclei and the hippocampus. They are involved in body temperature and blood pressure regulation, eating behavior, anxiety, and aggressiveness. In the present study, the affinity of all antipsychotic agents for 5HT₁A receptors was on the 100nM order. Affinity for 5HT₁A receptors was low with all agents except SM-9018 and nemonapride. Nishizaki and Zolan et al. have reported the affinity of antipsychotic agents for this type of receptor. The data obtained in the present study were similar to the data in their report. No reports prior to the present study have dealt with the affinity of levomepromazine, propericiazine, perphenazine, clothiapine, timiperone, pimozide, carpipramine, clozapramine, oxypertine, or sulotropride for 5HT₁A receptors.

5HT₂ receptors are distributed widely in the cerebral cortex and the basal ganglia. They are involved in stereotypic behavior, anxiety, depression, obsession, and aggressiveness. The affinity for this receptor was high with levomepromazine, clozapramine, carpipramine, perphenazine and propericiazine. All of these drugs except levomepromazine are often used to treat patients with negative symptoms or avolition, providing clinical evidence of the importance of 5HT₂ receptor blockade in treating these symptoms. Hamon and Harada reported that antipsychotic drugs have affinity for 5HT₂ receptors. The data obtained in the present study on the affinity of antipsychotic drugs for this receptor is similar to theirs. No reports prior to the present study have dealt with the affinity of levomepromazine, propericiazine, clothiapine, timiperone, carpipramine, or sulotropride for 5HT₂ receptors.

5HT₃ receptors are distributed in the cerebral cor-
tex, the hippocampus and the amygdala. They are reported to be involved in anxiety, memory, cognition, vomiting, and promotion of the release of DA and GABA. More recently, it has been reported that 5HT$_3$ antagonists manifest antianxiety activity. In the present study, the Ki was between 100nM and 1µM or higher, indicating low affinity for 5HT$_3$ receptors. Phenothiazines showed relatively high affinity for this receptor, suggesting that 5HT$_3$ receptors are partially related to the sedative actions of drugs. A few reports on anti–5HT$_3$ receptor activity have been published, but the data for the potency of this activity differ by more than 3–4 fold among the different reports. This discrepancy is probably due to the difference in ligands and tissues used in these studies. No reports prior to the present study have dealt with the affinity of thioridazine, perphenazine, clotiapine, timiperone, pimozide, carpipramine, oxypertine, sulpiride or sulotropride for 5HT$_3$ receptors.

C. Ad receptors

Adrenaline (Ad) serves as a neurotransmitter of the sympathetic nervous system and the adrenergic neurons of the central nervous system. Central adrenergic nerves, which can be divided into locus coeruleus system and lateral tegmental system, are involved in level of consciousness, anxiety, memory, and learning. Ad receptors can be classified into alpha and beta families. The former excites the nervous system, while the latter inhibits it. Two subtypes of the alpha family were investigated in the present study.

$\alpha_1$ receptors are distributed in the thalamus, cerebral cortex, vagal nerve nuclei, and raphe nuclei. Otsuki et al. reported that blockade of $\alpha_1$ receptors resulted in sedation, reduction of anxiety, and reduction of the level of consciousness or attention. In the present study, anti–$\alpha_1$ activity was found to be high with thioridazine, chlorpromazine, and levomepromazine. Peroutka et al. reported on the anti–$\alpha_1$ receptor activity of drugs. Their data is similar to that obtained in the present study. No reports prior to the present study have dealt with the affinity of propericiazine, clotiapine, timiperone, or sulotropride for $\alpha_1$ receptors.

$\alpha_2$ receptors are autoreceptors of noradrenergic neurons of the locus coeruleus. Stimulation of this receptor is said to suppress the neuron activity. In the present study, the Ki of most drugs for $\alpha_2$ receptors was between 10 nM and 100nM. Clozapramine, carpipramine, fluphenazine, and perphenazine had relatively high affinity (Ki below 100nM). These drugs are reported to improve negative symptoms, without reducing the level of consciousness. They seem to exert this activity by blocking the $\alpha_2$ receptor.

Prior to the present study, Setoguchi et al. and Hasegawa et al. reported on the affinity of drugs for $\alpha_2$ receptors. Our data is similar to that reported by Setoguchi et al., but not similar to that reported by Hasegawa et al. The Ki value reported by Hasegawa et al. was several times higher than that obtained in the present study and this discrepancy is unclear. No reports prior to the present study have dealt with the affinity of clotiapine, zotepine, timiperone, or sulotropride for $\alpha_2$ receptors.

D. Muscarinic acetylcholine receptor

Ach receptors are distributed in the cerebral cortex, the corpus striatum, and the lateral nucleus accumbens. Blockade of Ach receptors is associated with autonomic nervous system symptoms (thirst, constipation, anuresis, intraocular hypertension, etc.) and with reduction of cognitive function. At present, acetylcholinergic drugs are being developed for use in treating memory disturbances in demented patients. This means that the Ach receptor blocking action of antipsychotic agents can induce cognitive disturbances as adverse reactions. Blockade of Ach receptors in the corpus striatum suppresses the emergence of extrapyramidal symptoms induced by blockade of D$_2$ receptors. The results of the present study suggest that butyrophenones have lower anti-Ach receptor activity and are more likely to induce extrapyramidal symptoms than phenothiazines, which is consistent with our clinical experience.

Data reported in the past concerning anti-Ach receptor activity have varied greatly among reports, and thus detailed analysis is difficult. No reports prior to the present study have dealt with the affinity of propericiazine, clotiapine, timiperone, carpipramine, or sulotropride for Ach receptors.

E. Histamine receptors

Histamine receptors are distributed in the cerebral cortex and the limbic system. This type of receptor is reported to be associated with sleep-wake schedule, suppression of convulsions, and regulation of appetite and body temperature. Histamine receptors can be divided into three types: H$_1$, H$_2$ and H$_3$. Drowsiness is induced by blockade of H$_1$ receptors.

In the present study, mosapramine and perphenazine showed high affinity for H$_1$ receptors. This probably explains the high incidence of drowsiness during mosapramine therapy. The affinity of phenothiazines for H$_1$ receptors was higher than the affinity of butyrophenones. This is not surprising in view of the fact that phenothiazines were originally developed as antihistaminics.

According to past reports on the incidence of convulsions during antipsychotic drug therapy, treat-
ment with chlorpromazine, levomepromazine, and zotepine is associated with high incidences. In the present study, mosapramine and perphenazine showed high affinity for H₁ receptors, the same as chlorpromazine. However, few cases of convulsions during treatment with mosapramine or perphenazine have been reported. Thus, the results of the present study concerning H₁ receptor blockade are not consistent with the clinical incidence of convulsion. It therefore seems likely that the convulsions induced by antipsychotic agents involve some mechanism other than blockade of H₁ receptors.

Prior to the present study, Hill et al. reported on the anti-histamine action of various drugs. Their data are similar to those obtained in the present study. No reports prior to the present study have dealt with the affinity of clotiapine, zotepine, timiperone, carpipramine, or sulitopride for H₁ receptors.

F. Sigma receptors

Sigma receptors are distributed in the cerebral cortex and the hippocampus. This receptor as well as μ and κ receptors had been hypothesized to be opioid receptors, but at present, it is considered to be nonopioid receptor. Blockade of σ receptors has been considered to have an antipsychotic effect for the following reasons: (1) antipsychotic agents have affinity for σ receptors; and (2) some opiates serve as σ agonists and induce psychosis. A recent report, however, states that mental symptoms induced by opiates are mediated by κ receptors. Thus, the relationship between σ receptors and antipsychotic actions is unclear.

In the present study, haloperidol and fluphenazine were found to have high affinity for σ receptors, consistent with previous reports. Despite a previous report that atypical antipsychotic agents have high affinity for σ receptors, the affinity of SM-9018 for this type of receptor was found to be low in the present study.

The data we obtained concerning anti-σ action were similar to those reported by Tam et al., except that the affinity of chlorpromazine and perphenazine was lower than found by Tam et al. in guinea pig brains, and that in the present study the affinity of chlorpromazine was higher than that of pimozide and thioridazine. These two differences in results between the present study and the study by Tam et al. are probably due to the species difference (rat and guinea pig). No reports prior to the present study have dealt with the affinity of clotiapine, zotepine, or oxypertine for σ receptors.

II. Clinical applications

The same as behavioral-pharmacology experiments, the performance of receptor binding experiments is a routine procedure in the process of developing of psychotropic agents. A great deal of data have been accumulated in receptor binding experiments. In Japan, however, few reports of receptor binding experiments carried out on currently used drugs under the same experimental conditions have been published. In other countries, a number of reports of such experiments involving antipsychotic agents have been published, but many of these experiments have not involved any drugs used in Japan. With this in mind, we performed receptor binding experiments on many drugs used in Japan under identical experimental conditions. Although the present study did not examine all types of receptors identified, the data obtained in this study will be clinically useful in assessments of the major effects of antipsychotic agents. Clinical application of the data obtained will be discussed in terms of the three aspects below.

A. Classification of antipsychotic agents

The classification shown in Table 3 overlaps the classification based on chemical structures, except for fluphenazine. This classification will be useful in the following clinical settings.

1) When selecting drugs according to the stage of the disease

1) Acute stage

Major symptoms seen in this stage are positive symptoms such as hallucinations and delusions. Drugs with high affinity for D₂ receptors should be selected to suppress these symptoms. Drugs belonging to the three anti-DA receptor activity dominant groups (Group A through Group C) are recommended. These include haloperidol, bromperidol, fluphenazine, timiperone, and pimozide.

2) Chronic stage

Major symptoms in this stage are negative symptoms such as avolition and affective flattening. Drugs with high affinity for D₂ receptors should be selected to suppress these symptoms. Drugs belonging to Group D (one of anti-5HT receptor activity dominant groups) are recommended. These include caripramine, chlorpromazine, levomepromazine, clotiapine, propriciazine, perphenazine and thioridazine.

2) Subacute to subchronic stage (mixed stage)

Hallucinations and delusions have subsided, and anxiety, hypochondriasis, and depressed mood are the major symptoms in this stage. Drugs with high affinity for D₂ and 5HT₂ receptors should be selected to suppress these symptoms. Drugs belonging to Group E (one of anti-5HT receptor activity dominant groups)
are recommended. These include mosapramine, cloca-
pramine, zotepine, and oxypertine.

(2) **When selecting combined drug therapy**

It seems rational to select drugs from different
groups for combined therapy. Drugs belonging to the
same group have similar pharmacologic profiles, and
combined use of these drugs will have no effect other
than to increase the dose level of the drug initially
used.

(3) **When using a combination of drugs with the
same affinity profile as unapproved drug.**

The affinity profile of a given drug can be re-
produced by combining two or more other drugs
having similar profiles. For example, the profile of an
unapproved drug can be reproduced by combining
approved drugs with similar profiles. In fact, we have
succeeded in treating some cases by using a cocktail of
drugs whose profile resembles that of olanzapine. This
method will become more widely applicable when data concerning antidepressants, etc., as well as
antipsychotic agents, are included.

B. **Drug therapy based on the concept of SDA for
the treatment of chronic or intractable cases of
schizophrenia in which negative symptoms pre-
dominate**

Pharmacologic studies of risperidone (an atypical
antipsychotic drug) have revealed a clinical phenome-
non in which negative mental symptoms are reduced
by strong blockade of 5HT₂ receptors. This finding led
to proposal of the concept of serotonin-dopamine
antagonists (SDA). Matsubara et al. used the
ratio of the affinity for 5HT₂ to affinity for D₂ re-
ceptors (pKi) to distinguish typical antipsychotic
agents from atypical antipsychotic agents (pKi > 1.2). If
the data obtained in the present study are used, the
5HT₂/D₂ ratio is less than 1 for the butyrophenones
(haloperidol, bromperidol, timiperone) and fluphen-
azine, and greater than 1.2 for levomepromazine, thio-
ridazine, peropericiazine, and clotiapine (Table 4).

### Table 4. pKi value ratios (5HT₂/D₂)

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<tr>
<td>SM9018</td>
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Although the present study did not involve any
atypical antipsychotic agents, the drugs mentioned
above can be regarded as SDA defined by Matsubara et al. The 5HT₂/D₂ ratio of antidepressants also
rued between 0.8 and 1.5, as we reported elsewhere.
It can therefore be concluded that antidepressants as
well as the drugs in Group D may be useful in treating
cases with negative symptoms.

C. **Side effects of antipsychotic agents**

The type and dose level of drugs, the background
variables of the patient (age, sex), and other factors are
known to be associated with the onset of extrapy-
ramidal symptoms (EPS). This paper will summarize
the potency of individual antipsychotic agents in in-
ducing EPS. EPS is thought to involve not only the
balance between anti-DA and anti-Ach actions, but
anti-5HT actions. This view is based on the report
that DA transmission in the nigrostriatal pathway is
improved by blockade of 5HT₂ receptors and stimu-
lation of 5HT₁A receptors. It has not yet been fully
clarified how antipsychotic agents act on 5HT₁A recep-
tors, and thus this summary will include D₂, Ach, and
5HT₂ receptors, but exclude 5HT₁A receptors.

Fig. 2 shows the total affinity for 5HT₂ and Ach
receptors of drugs that suppresses EPS (positive, i.e.,
upward part) and the affinity for D₂ receptors that
induces EPS (negative or downward part) in graph
form. The downward part is marked for fluphenazine,

### Antidepressants

<table>
<thead>
<tr>
<th>Antidepressants</th>
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<th>Authors</th>
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<td>Setipitline</td>
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</table>

Atypical antipsychotics have higher ratios than typical anti-
psychotics; i.e., the former have a stronger affinity for 5HT₂
receptors.
The affinity of antipsychotic drugs for receptors

Fig. 2 Profiles of affinity for receptors thought to be related to EPS

![Graph showing profiles of affinity for receptors](image)

Total affinity for 5HT₂ receptors and Ach receptors were considered to be the potency for the anti-extrapyramidal symptoms (EPS).

Tendency to cause EPS were identified by comparing this potency with ant-D₂ action.

timiperone, haloperidol, bromperidol, and benzamides. These drugs are likely to cause EPS and need to be carefully used in clinical cases. Phenothiazines usually show high affinity for 5HT₂ and Ach receptors and suppress EPS. However, only fluphenazine in this family seems likely to cause EPS, because its affinity for 5HT₂ is lower than for D₂ receptors. This is corroborated by the clinical report of an EPS incidence close to 50% among patients treated with fluphenazine. Fig.2 suggests that butyrophenones are likely to induce EPS, but the incidence of EPS in clinical cases was not high (between 10% and 50%). This discrepancy is probably attributable to some other factors such as dose level.

EPS are the most important adverse reaction that needs to be borne in mind when using antipsychotic agents. To avoid this adverse reactions, it is recommended that Fig.2 be consulted when selecting antipsychotic agents.

CONCLUSION

Radioceptor assays were performed on antipsychotic agents that are frequently used in Japan. The affinity of these drugs for 12 receptors was examined to provide pharmacologic spectra. The receptors examined were D₁, D₂, D₃, D₄, 5HT₂, 5HT₃, 5HT₁A, α₁, α₂, Ach, H₁ and σ receptors. The antipsychotic agents examined were chlorpromazine HCl, levomepromazine maleate, thioridazine HCl, propranolol, perphenazine maleate, fluphenazine maleate, clotiapine, zotepine, haloperidol, timiperone, bromperidol, pimozide, caripipramine HCl, clonaparine HCl, oxypertine, sulpiride, sulpropride HCl, mosapramine, nemonapride, and SM-9018.

On the basis of the results of the assays, the drugs were classified into three anti-DA receptor activity dominant groups and two anti-5HT receptor activity dominant groups. This classification is expected to provide a theoretical basis for selection of drugs in clinical cases. Extrapyramidal symptoms, which are often observed as adverse reactions to antipsychotic drugs, have been discussed in relation to the affinity for relevant receptors.

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REFERENCES


26) Braum AR, Chase TN. Obligatory D-1/D-2 receptor interactions in the generation of
The affinity of antipsychotic drugs for receptors


