Atypicality of Several Antipsychotics on the Basis of In Vivo Dopamine-D₂ and Serotonin-5HT₂ Receptor Occupancy

Tomiki Sumiyoshi, M.D., Kimiko Suzuki, M.D., Hiroshi Sakamoto, M.D.,
Nariyoshi Yamaguchi, M.D., Hirofumi Mori, M.D., Kazuhiro Shiba, Ph.D.,
and Koichi Yokogawa, Ph.D.

An in vivo receptor binding technique was used to evaluate the binding profiles of typical and atypical antipsychotic drugs to striatal dopamine-D₂ and frontal serotonin-5-HT₂ receptors in a rat brain using more specific ligands than those previously employed. [³H]-YM-09151-2 or [³H]-ketanserin was injected into the tail vein 10 minutes after administration of test drugs. One hour after the ligand injection, radioactivities in the striatum, frontal cortex, and cerebellum were counted to obtain receptor occupancies by the test drugs. Higher ratios of potency in occupying 5-HT₂ versus D₂ receptors

were found for clozapine, RMI-81512, and tiospirone compared to haloperidol and pimozide. Zotepine, mosapramine, and clocapramine produced ratios that fall between these two groups. Chlorpromazine was exceptional as a typical antipsychotic by these criteria. Relatively strong antagonism of 5-HT₂ receptors by atypical antipsychotics was confirmed by this in vivo measure of receptor binding using more selective ligands than those used in previous studies.

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KEY WORDS: Atypical antipsychotic drugs; D₂ receptors; 5-HT₂ receptors; Receptor binding study; In vivo

The mode of action of antipsychotic drugs has been discussed in relation to their potency to block central dopamine-D₂ receptors (Creese et al. 1976; Seeman et al. 1976). The so-called "typical" antipsychotic drugs have been shown to produce catalepsy in rats (Moore and Gershon 1989) and often, extrapyramidal side effects (EPS) in patients (Sovner and DiMascio 1978), by blocking the nigrostriatal dopamine system. "Atypical" antipsychotic drugs, on the other hand, are less

likely to produce disturbances of EPS or elevation of serum prolactin (Young and Meltzer 1980; Porsolt and Jafre 1981; Neale et al. 1983), and demonstrate favorable clinical efficacy for both positive and negative symptoms of patients who are resistant to treatment with typical antipsychotics (Kane et al. 1988).

Since a relatively high affinity for serotonin₂ (5-HT₂) receptors of atypical antipsychotic drugs was postulated by Meltzer et al. (1989a, b), a number of studies to test this hypothesis have been performed in laboratory animals (Leysen et al. 1992; Matsubara et al. 1992; Stockmeier et al. 1993) and in human PET measurement (Nordstrom et al. 1993; Nyberg et al. 1993). We have previously demonstrated that the atypical antipsychotic drugs clozapine, RMI-81582, and risperidone show relatively higher 5-HT₂ to D₂ ratios of in vivo receptor affinities than haloperidol, measuring time courses of a receptor occupancy by single doses of these drugs (Sumiyoshi et al. 1993, 1994a, b).

In the present study, we used [³H]-YM-09151-2 and

From the Department of Neuropsychiatry (TS, KS, HS, NY) Radioisotope Center (HM, KS) and Hospitals Pharmacy (KY) of Kanazawa, University School of Medicine, Kanazawa 920, Japan

Address correspondence to: Tomiki Sumiyoshi, M.D., Department of Psychiatry, University Hospitals of Cleveland, Hanna Pavilion, 11100 Euclid Ave, Cleveland, OH 44106.

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[³H]-ketaserin, selective ligands for D₂ and 5-HT₂ receptors, respectively (Laduron et al. 1982; Niznik et al. 1985; Terai et al. 1989), to measure the in vivo affinities of several antipsychotic drugs to these receptors by analyzing receptor occupancy. The test compounds include the new antipsychotic drug Y-516 (mosapramine) and clocapramine, iminodibenzyl derivatives that have been shown to exert favorable clinical effect on schizophrenic patients (Yamagami et al. 1988; Asano et al. 1992). The "atypicality" of these iminodibenzyls is also determined on the basis of in vivo D₂ and 5-HT₂ receptor occupancy in comparison with traditional typical and atypical antipsychotics.

MATERIALS AND METHODS

In Vivo Receptor Binding

Male Wistar rats (210 to 240 g; Sankyo Laboratory, Toyama, Japan) were housed in a temperature-controlled room with a 12-hour dark/light cycle (lights on at 8:30) and had free access to food and water. In vivo binding of [³H]-YM-09151-2 (specific activity 3.22 TBq/mmol) to D₂ receptors or [³H]-ketanserin (specific activity 2.22 TBq/mmol) to 5-HT₂ receptors was measured according to a method previously reported (Sumiyoshi et al. 1993, 1994a, b). For the kinetic study, [³H]-YM-09151-2 or [³H]-ketanserin (1540 to 1680 kBq/kg body weight) were injected into the lateral tail vein of rats that had received an intraperitoneal injection of a vehicle (dimethyl-sulfoxide, Wako Chemical Co., Osaka, Japan, 1 ml/kg), 10 minutes before. The rats were sacrificed by decapitation at 15, 30, 45, 60, 120, or 240 minutes after the injections of ligands. For competition studies, rats were pretreated with an intraperitoneal injection of varying doses of antipsychotic drugs or the same volume (0.21 to 0.24 ml) of the corresponding vehicle (dimethyl-sulfoxide), 10 minutes prior to the injection of [³H]-YM-09151-2 or [³H]-ketanserin. Based on results of the kinetic study, the rats were decapitated 60 minutes after the ligand injection. The brains were rapidly removed and dissected into cerebellum, striatum, frontal cortex, and the rest of the brain. After weighing, each region of the brain was solubilized with a tissue solubilizer (Soluene 350, Packard Co., CN) by incubation for 2 to 3 hours at 50°C. A scintillation cocktail (Aquasol 2, New England Nuclear) was added to the solubilized tissues adjusted to pH 7.0 with 0.5 N HCl solution (for inhibition of pseudo-fluorescence). After 12 to 24 hours, the radioactivity concentrations in the tissues were counted with a liquid scintillation counter (LSC-1000, Aloka Co., Tokyo, Japan) and the values, expressed as %dose/g tissue, were calculated. Receptor occupancy was determined by a modification of the method of former reports (Geoders and Kuhar 1985; Miller et al. 1987): percent occupancy $\Phi = [1 - (X_D -$

$X_{ND}) / (X_S - X_{NS})] \times 100$ (%), where each abbreviation represents the radioactivity (%dose/g tissue) of X_D, striatum, or frontal cortex of drug-treated rats; X_{ND}, cerebellum of drug-treated rats; X_S, striatum or frontal cortex of vehicle-treated rats; and X_{NS}, cerebellum of vehicle-treated rats.

Data Analysis

Dose-response competition curves were analyzed with the minimal-square method (Paalzow and Edlund 1979) and ED₅₀ (mg/kg) values reflecting binding potencies of the test drugs were calculated. The ED₅₀ values were then transformed into (−)log of the ED₅₀ (mol/kg) (pED₅₀) to compare the receptor binding potencies of the test drugs in the present study with those of Stockmeier et al. (1993).

Drugs

[³H]-YM-09151-2 (specific activity 3.22 TBq/mmol) and [³H]-ketanserin (specific activity 2.22 TBq/mmol) were purchased from DuPont New England Nuclear Corporation (Boston, MA). The following compounds were generously supplied by the manufacturers: haloperidol (Dainippon Pharmaceutical Co., Osaka, Japan); clozapine (Sandoz Ltd., Basel, Switzerland); RMI-81582 (Marion Merrill Dow Pharmaceuticals, Cincinnati, OH); tiospirone (Bristol-Myers-Squibb, Wallingford, CT); chlorpromazine, mosapramine, clocapramine (Yoshitomi Pharmaceutical Ltd., Osaka, Japan); zotepine, pimoziide (Fujisawa Pharmaceutical Co., Osaka, Japan).

RESULTS

The values of radioactivity in each brain region at the different time intervals after the injection of [³H]-YM-09151-2 are shown in Figure 1. A relatively large amount of [³H]-YM-09151-2 accumulates in the striatum as compared to the cerebellum. For [³H]-ketanserin-treated rats, the values in the frontal cortex continued to be higher than those in the cerebellum, as shown in Figure 2. In the following drug competition studies, rats were sacrificed 60 minutes after the injection of radioligand, based on stable receptor occupancies by drugs at this time point as reported in our previous studies (Sumiyoshi et al. 1993, 1994a, b). Figures 3 to 10 show the occupancy of D₄ and 5-HT₂ receptors following various doses of typical antipsychotic drugs, pimoziide and chlorpromazine; atypical antipsychotic drugs, clozapine, RMI-81582 and tiospirone; other drugs, zotepine, mosapramine and clocapramine. The dose-occupancy curves of haloperidol were reported elsewhere (Sumiyoshi et al. 1994a). Based on these occupancy curves, the ED₅₀ values (mg/kg) of the test compounds for the in vivo occupancy of striatal D₂ and

Figure 1. Kinetics of [^3H]-YM-09151-2 binding in vivo in rat brain. Rats were injected with [^3H]-YM-09151-2 (1540 to 1680 kBq/kg body weight) at time zero and killed at various times after injection. Values represent the mean $\pm$ SD of five rats at each time point.

frontal 5-HT₂ receptors are calculated (Table 1). The average ability of typical antipsychotic drugs to prevent the accumulation of [^3H]-YM-09151-2 in the striatum is more potent than for atypical antipsychotic drugs, except for tiospirone, revealing high affinity for [^3H]-YM-09151-2 labeled sites. In contrast, atypical antipsychotics are more potent than typical antipsychotics in preventing the accumulation of [^3H]-ketanserin in the frontal cortex. Chlorpromazine has a relatively high affinity for [^3H]-ketanserin labeled sites in the typical antipsychotic group. The ratios of ED₅₀ values of occupancy at 5-HT₂ versus D₂ receptors are also shown in the Table 1. Typical antipsychotics haloperidol and pimozide are slightly more potent in occupying D₂ than 5-HT₂ receptors. By contrast, atypical antipsychotics clozapine and RMI-81582 have a greater potency in occupying

Figure 2. Kinetics of [^3H]-ketanserin binding in vivo in rat brain. Rats were injected with [^3H]-ketanserin (1540 to 1680 kBq/kg body weight) at time zero and killed at various times after injection. Values represent the mean $\pm$ SD of five rats at each time point.

Figure 3. Occupancy ($\Phi\%$) of striatal D₂ and frontal 5-HT₂ receptors in rat brain with various doses of pimozide. [^3H]-YM-09151-2 or [^3H]-ketanserin (1540 to 1680 kBq/kg body weight) was injected into a tail vein at 10 minutes after administration (IP) with various doses of pimozide. Sixty minutes after the ligand injection, radioactivity in the striatum, frontal cortex, and cerebellum was counted. Each point represents the mean $\pm$ SE ($n = 4$ to 5). $\Phi = [1 - (X_D - X_{ND}) / (X_S - X_{NS})] \times 100 (\%)$, where each abbreviation represents radioactivity (%dose/g tissue) of X_D , striatum or frontal cortex of drug-treated rat; X_{ND} , cerebellum of drug-treated rat; X_S , striatum or frontal cortex of vehicle-treated rat; X_{NS} , cerebellum of vehicle-treated rat. The dose-response curves were analyzed with a minimal-square method. ●●, D₂; ○○, 5-HT₂ receptors. (ordinate, occupancy for D₂ and 5-HT₂ receptors; abscissa, dose of pimozide).

5-HT₂ than D₂ receptors. The ratios of occupancy at 5-HT₂ versus D₂ receptors for zotepine, mosapramine, and clocapramine fall somewhere between those two categories. Chlorpromazine and tiospirone are exceptional among typical and atypical antipsychotics: chlorpromazine has a four- to fivefold greater potency in occupying 5-HT₂ than D₂ receptors, and tiospirone has only a slightly higher affinity for 5-HT₂ than D₂ receptors within the same order.

Figure 4. Occupancy ($\Phi\%$) of striatal D₂ and frontal 5-HT₂ receptors in rat brain with various doses of chlorpromazine. ●●, D₂; ○○, 5-HT₂ receptors. (ordinate, occupancy for D₂ and 5-HT₂ receptors; abscissa, dose of chlorpromazine).

Figure 5. Occupancy ($\Phi\%$) of striatal D_2 and frontal 5-HT_2 receptors in rat brain with various doses of clozapine. ●●, D_2 ; ○○, 5-HT_2 receptors. (ordinate, occupancy for D_2 and 5-HT_2 receptors; abscissa, dose of clozapine).

Figure 8. Occupancy ($\Phi\%$) of striatal D_2 and frontal 5-HT_2 receptors in rat brain with various doses of zotepine. ●●, D_2 ; ○○, 5-HT_2 receptors. (ordinate, occupancy for D_2 and 5-HT_2 receptors; abscissa, dose of zotepine).

Figure 6. Occupancy ($\Phi\%$) of striatal D_2 and frontal 5-HT_2 receptors in rat brain with various doses of RMI-81582. ●●, D_2 ; ○○, 5-HT_2 receptors. (ordinate, occupancy for D_2 and 5-HT_2 receptors; abscissa, dose of RMI-81582).

Figure 9. Occupancy ($\Phi\%$) of striatal D_2 and frontal 5-HT_2 receptors in rat brain with various doses of mosapramine. ●●, D_2 ; ○○, 5-HT_2 receptors. (ordinate, occupancy for D_2 and 5-HT_2 receptors; abscissa, dose of mosapramine).

Figure 7. Occupancy ($\Phi\%$) of striatal D_2 and frontal 5-HT_2 receptors in rat brain with various doses of tiospirone. ●●, D_2 ; ○○, 5-HT_2 receptors. (ordinate, occupancy for D_2 and 5-HT_2 receptors; abscissa, dose of tiospirone).

Figure 10. Occupancy ($\Phi\%$) of striatal D_2 and frontal 5-HT_2 receptors in rat brain with various doses of clocapramine. ●●, D_2 ; ○○, 5-HT_2 receptors. (ordinate, occupancy for D_2 and 5-HT_2 receptors; abscissa, dose of clocapramine).

Table 1. ED₅₀ Values for Receptor Occupancy

	D2	5-HT ₂	D2/5-HT ₂
Haloperidol	1.4	1.7	0.82
Pimozide	1.5	6.4	0.23
Chlorpromazine	4.6	1	4.6
Clozapine	14.6	0.3	49
RMI-81582	53	0.2	265
Tiospirone	0.9	0.3	3
Zotepine	1.7	0.4	4.3
Mosapramine	5.2	0.7	7.4
Clocapramine	14.5	4.9	3

Values are expressed as mg/kg, IP.

To compare the present results with the former study (Stockmeier et al. 1993), the ED₅₀ values (mg/kg) for occupying D₂ and 5-HT₂ receptors and their ratios of haloperidol, chlorpromazine, clozapine, RMI-81582, tiospirone, and zotepine were converted into (–)log ED₅₀ (mol/kg) (pED₅₀, Table 2). The values in parentheses show the in vivo pED₅₀'s of Stockmeier et al. (1993). Strong correlations were obtained between the results of the two studies for D₂ receptor occupancy (Pearson's correlation coefficient, $R = 0.84$) and for the ratios of occupancy at 5-HT₂ versus D₂ receptors ($R = 0.71$), but not for 5-HT₂ receptor occupancy ($R = 0.31$).

DISCUSSION

Various studies have recently evaluated the in vivo receptor binding potency of antipsychotic drugs. Thus, Bischoff (1992), Leysen et al. (1992), and Stockmeier et al. (1993) have measured in vivo D₂ and 5-HT₂ receptor occupancy by typical and atypical antipsychotic drugs with laboratory animals. The latter two studies confirmed relatively strong 5-HT₂ receptor affinities of atypical antipsychotic drugs in comparison with typical ones in vivo. Moreover, central 5-HT₂ receptor occupancy of clozapine (Nordström et al. 1993) and risperidone (Nyberg et al. 1993) was recently reported even with human PET studies. These former studies utilized radio-labeled spiperone or its derivatives for labeling both the striatal D₂ and the cortical 5-HT₂ receptors at the same time. In contrast, we applied [³H]-YM-09151-2 and [³H]-ketanserin to label striatal D₂ and cortical 5-HT₂ receptors individually. As to the specificity of these ligands, radioactive derivatives of YM-09151-2 and ketanserin were reported to bind selectively in vivo to D₂ (Hatano et al. 1989) and 5-HT₂ (Laduron et al. 1982) receptors, respectively. Because the striatum is low in 5-HT_{1A} receptor sites, the binding of [³H]-YM-09151-2 in the striatum does not appear to represent a significant 5-HT_{1A} site. The effective radioactivities counted both in [³H]-YM-09151-2- and [³H]-ketanserin-treated rats brains (Figures 1 and 2)

Table 2. Comparisons of (–)log of the ED₅₀ Values (mol/kg)

	D2	5-HT ₂	5-HT ₂ /D ₂
Haloperidol	5.44 (6.5)	5.33 (5.4)	–0.11 (–1.1)
Chlorpromazine	4.89 (5.4)	5.57 (6.0)	0.68 (0.6)
Clozapine	4.35 (4.3)	6.0 (5.6)	1.63 (1.3)
RMI-81582	3.77 (4.4)	6.3 (5.5)	2.53 (1.1)
Tiospirone	5.76 (5.9)	6.21 (6.5)	0.44 (0.6)
Zotepine	5.29 (5.1)	5.89 (6.1)	0.60 (1.0)

Data from Stockmeier et al. (1993) in parentheses.

made a basis for calculating receptor occupancies by the test drugs for striatal D₂ and frontal 5-HT₂ receptors.

Atypical antipsychotic drugs clozapine, RMI-81582, and tiospirone revealed higher ratios of occupancy at 5-HT₂ versus D₂ receptors than haloperidol and pimozide in the present in vivo receptor binding method (Figures 3, 5, 6, 7; Table 1). The result supports previous reports by Meltzer et al. (1989a, b) suggesting that a series of atypical antipsychotics demonstrate relatively higher ratios of pK_i values for 5-HT₂ receptors to those for D₂ receptors than typical antipsychotics in vitro. Moreover, the present result seems to fit the in vivo D₂ and 5-HT₂ receptor occupation profiles of several typical and atypical antipsychotic drugs that were recently assessed by Stockmeier et al. (1993) using [³H]-N-methyl-spiperone as a ligand. This is supported by the finding that our results yielded a strong correlation with those of Stockmeier et al. (1993) regarding the pED₅₀ values for D₂ receptor occupancy and the ratios of potency for 5-HT₂ versus D₂ receptor occupancy by the six test drugs. The reason for the lack of a correlation of the pED₅₀ values for 5-HT₂ receptor occupation between the two studies is not readily apparent. It might be ascribed partly to the binding of [³H]-N-methyl-spiperone to the cortical D₂ sites in the previous study, the binding of [³H]-ketanserin to α_1 -adrenoceptors or tetrabenazine sites (Roth et al. 1987) in our study, and/or some other factors.

There is a slight difference between clozapine and RMI-81582 with regard to 5-HT₂/D₂ ratio of receptor occupancy (Table 2; the difference of pED₅₀ values, 2.53 RMI-81582 versus 1.63 for clozapine). In view of the fact that RMI-81582 has the same level of 5-HT₂/D₂ ratio of affinity with clozapine in vitro (Meltzer et al. 1989a) and in vivo (Stockmeier et al. 1993), this discrepancy is probably a result of the limited ability of both drugs to displace [³H]-YM-09151-2 from D₂ receptors (Figures 5 and 6; Table 1). In fact, [³H]-YM-09151-2 has a higher affinity for D₂ receptors than [³H]-spiperone (Terai et al. 1989).

Chlorpromazine acts differently from other typical antipsychotics in that we found a four- to fivefold greater potency in occupying 5-HT₂ than D₂ receptors, which is comparable to the atypical antipsychotic tiospi-

rone (Figures 4 and 7; Table 1). The considerable degree of in vivo 5-HT₂ receptor occupation by chlorpromazine was recently reported (Matsubara et al. 1992; Stockmeier et al. 1993). The latter study also showed relatively low selectivity of tiospirone for 5-HT₂ versus D₂ receptors in vivo (only fourfold) as compared to that in vitro (25-fold, by Meltzer et al. 1989a). This kind of discrepancy seems to indicate the importance of measuring affinities for receptors of drugs in vivo, which enables estimation of potency of drugs including reference to their bioavailability (Stockmeier et al. 1993). Nash et al. (1988) reported that clozapine and some other atypical antipsychotics, but not chlorpromazine, block MK-212 (a 5-HT₂ agonist)-induced hyperthermia and corticosterone secretion in the rat. Also, it should be noted that clozapine and other atypical antipsychotics bind to other subtypes of 5-HT receptors with high affinity such as 5-HT_{1C} receptors (Roth et al. 1992) in addition to the 5-HT₂ sites. Thus, some other aspects of serotonin receptor antagonism together with the affinity for 5-HT₂ receptors would help further explain the mode of action of atypical antipsychotic drugs that have less ability to cause EPS than chlorpromazine.

The possible relevance of serotonin and serotonin-dopamine interaction in the pathophysiology of schizophrenia, as well as the role of antagonism of 5-HT₂ receptors in the mode of action of atypical antipsychotic drugs such as clozapine and risperidone, have been discussed extensively (Meltzer 1991; Kahn and Davidson 1993; Leysen et al. 1993; Meltzer et al. 1993; Stockmeier et al. 1993). The hypothesis is supported by the clinical observation that ritanserin, a strong 5-HT₂ receptor antagonist, ameliorates neuroleptic-induced extrapyramidal signs (Bersani et al. 1986) and that 5-HT₂ receptor antagonists are effective for negative symptoms of schizophrenia (Gelders 1989; Wiesel et al. 1994). Our current data revealing relatively high 5-HT₂ versus D₂ receptor occupancy by the tested atypical antipsychotics in vivo extended and are in line with the above discussion.

With regard to the classification of antipsychotics, we applied the criterion by Deutch et al. (1991) in which tiospirone as well as clozapine and RMI-81582 is classified as atypical. As to zotepine, some authors classified it as atypical (Meltzer et al. 1989a; Stockmeier et al. 1993) and others did not (Matsubara et al. 1992). We have included this drug in the third group, together with two iminodibenzyls mosapramine and clocapramine. It should be noted that the present study tried, for the first time, to investigate in vivo D₂ and 5-HT₂ receptor occupancy by the two iminodibenzyls. With the exceptions of chlorpromazine and tiospirone as discussed above, zotepine, mosapramine, and clocapramine fall somewhere between typical and atypical group with regard to a ratio of potency in occupying 5-HT₂ versus D₂ receptors (Table 1). Some clinical

studies suggested zotepine is effective for psychiatric symptoms with less chance to cause extrapyramidal side effects than haloperidol (Fleischhacker et al. 1989; Barnas et al. 1992). In addition, it was reported that a low dose of zotepine is more efficacious in treating the negative symptoms in comparison with haloperidol (Barnas et al. 1992). Also, clinical trials revealed that two iminodibenzyl derivatives, mosapramine and clocapramine, have favorable clinical effects on negative symptoms in addition to some positive symptoms of schizophrenia (Kurihara et al. 1983; Yamagami et al. 1988; Asano et al. 1992). Indeed, clocapramine was shown to be superior to the atypical antipsychotic sulpiride in relieving negative symptoms as well as the hallucinations and delusions of chronic schizophrenic patients (Yamagami et al. 1988). If these clinical data are combined with the observation on the ratio of potency in occupying 5-HT₂ versus D₂ receptors of these drugs (Table 1), we can possibly say that mosapramine and clocapramine as well as zotepine have a certain degree of atypicality.

In conclusion, the current in vivo binding study measuring a receptor occupancy with selective ligands obtained results, which support the finding that atypical antipsychotic drugs generally possess a high affinity for 5-HT₂ receptors with a relatively low affinity for D₂ receptors. Moreover, some iminodibenzyls were shown to have a certain degree of pharmacological characteristic of atypical antipsychotic drugs by these criteria.

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