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# Time Course of Dopamine<sub>1,2</sub> and Serotonin<sub>2</sub> Receptor Binding of Antipsychotics In Vivo

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SUMIYOSHI, T., H. KIDO, H. SAKAMOTO, K. URASAKI, K. SUZUKI, N. YAMAGUCHI, H. MORI AND K. SHIBA. *Time course of dopamine<sub>1,2</sub> and serotonin<sub>2</sub> receptor binding of antipsychotics in vivo.* PHARMACOL BIOCHEM BEHAV 49(1) 165-169, 1994.—An in vivo receptor binding technique was applied to evaluate the affinities of clozapine (20 mg/kg), RMI-81582 (20 mg/kg), and haloperidol (1 mg/kg) for dopamine D<sub>1</sub>, D<sub>2</sub> and serotonin 5-HT<sub>2</sub> receptors in rat brain with [<sup>3</sup>H]-SCH23390, [<sup>3</sup>H]-YM-09151-2, and [<sup>3</sup>H]-ketanserin as selective ligands. The time course study of receptor occupancy at 25 to 250 min after intraperitoneal administration of the drugs showed higher 5-HT<sub>2</sub> and lower D<sub>2</sub> receptor occupancies of clozapine and RMI-81582 than those of haloperidol both in the striatum and frontal cortex. The 5-HT<sub>2</sub>/D<sub>2</sub> ratios of receptor occupancy for clozapine and RMI-81582 were about 6 to 8 times higher than that for haloperidol. Stable occupancies of D<sub>1</sub> receptors were observed only with RMI-81582 and clozapine, the former demonstrating the higher occupancy. These findings are in agreement with the previous findings obtained under in vitro conditions and may account for some part of the properties of atypical antipsychotic drugs.

In vivo receptor binding    D<sub>1</sub>, D<sub>2</sub>, 5-HT<sub>2</sub> receptors    Clozapine    RMI-81582    Time course study

RECENT researches have concentrated on the development of antipsychotic drugs that are not accompanied by extrapyramidal symptoms (EPS) and have negative symptoms relieving efficacy. One of the clues is the concept of atypical antipsychotic drugs (AAPDs), defined as those that do not cause EPS, elevation of serum prolactin, or catalepsy in rats (17). Attempts have been made to characterize the pharmacology of AAPDs. High affinity for dopamine D<sub>1</sub> receptors (D<sub>1</sub>) (30), higher serotonin 5-HT<sub>2</sub> receptors (5-HT<sub>2</sub>)/dopamine D<sub>2</sub> receptors (D<sub>2</sub>) ratios of pKi values (18), or some other peculiarities were indicated. The majority of these kinds of receptor binding studies was performed under in vitro conditions. However, because of the discrepancies between the in vitro and in vivo findings about the blocking effects on D<sub>1</sub>, D<sub>2</sub> (2,25) and 5-HT<sub>2</sub> (20) receptors, more precise in vivo studies on receptor binding features of AAPDs are necessary.

Clozapine, a prototype of AAPDs, and its structurally related compound RMI-81582 were reported to exert their effects without causing EPS or elevation of serum prolactin levels in patients (31) and in laboratory animals (21,23).

Herein, the receptor binding properties of these AAPDs

were examined in comparison with a typical antipsychotic drug under in vivo conditions. Selective radioactive ligands for D<sub>1</sub>, D<sub>2</sub>, and 5-HT<sub>2</sub> receptors were intravenously injected into rats after administration of clozapine, RMI-81582, or haloperidol to follow the time course of the occupancy of the receptors in the brain by the drugs.

## METHOD

[<sup>3</sup>H]-SCH23390 (specific activity 2.78 TBq/mmol), [<sup>3</sup>H]-YM-09151-2 (specific activity 3.22 TBq/mmol), and [<sup>3</sup>H]-ketanserin (specific activity 2.22 TBq/mmol) were purchased from New England Nuclear Corporation. Haloperidol, clozapine, and RMI-81582 were obtained as gifts from Dainippon Pharmaceutical Ltd. (Japan), Sandoz Pharmaceutical Ltd. (Switzerland), and Marion Merrill Dow Research Institute (USA), respectively.

Male Wistar rats (220-240 g) were treated intraperitoneally with haloperidol 1 mg/kg, clozapine 20 mg/kg, RMI-81582 20 mg/kg, or the same volume of the corresponding vehicle (dimethyl sulfoxide), 10 min before the ligand injection. The

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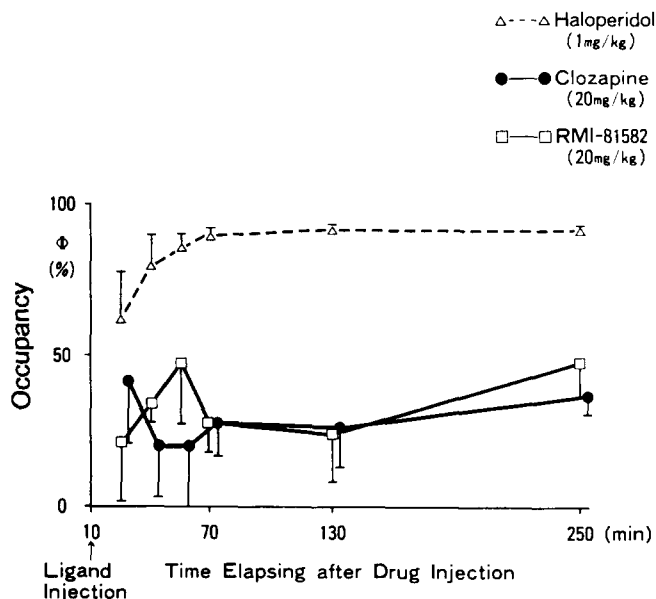


FIG. 1. Time course of  $D_2$  receptor occupancy ( $\Phi\%$ ) in rat striatum after single doses of clozapine (20 mg/kg IP), RMI-81582 (20 mg/kg IP), and haloperidol (1 mg/kg IP). [ $^3\text{H}$ ]-YM-09151-2 (1.54–1.68 MBq/kg body weight) was injected into a tail vein at 10 min after drug administration. Each point represents the mean  $\pm$  SD ( $n = 3$ –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 of drug-treated rat;  $X_{nD}$ , cerebellum of drug-treated rat;  $X_S$ , striatum of vehicle-treated rat;  $X_{nS}$ , cerebellum of vehicle-treated rat.

## RESULTS

Time courses of  $D_2$  receptor occupancy in the striatum by haloperidol (1 mg/kg IP), clozapine (20 mg/kg IP), and RMI-81582 (20 mg/kg IP) are shown in Fig. 1. Haloperidol demonstrated significantly higher occupancy than clozapine,  $F(1, 38) = 193.73$ ,  $p < 0.01$ , and RMI-81582,  $F(1, 41) = 201.83$ ,  $p < 0.01$ . In the frontal cortex also, haloperidol revealed higher  $D_2$  receptor occupancy than clozapine,  $F(1, 35) = 37.58$ ,  $p < 0.01$ , and RMI-81582,  $F(1, 42) = 11.88$ ,  $p < 0.01$  (Fig. 2). By contrast, 5-HT $_2$  receptor occupancy in the frontal cortex by clozapine,  $F(1, 31) = 129.05$ ,  $p < 0.01$ , and RMI-81582,  $F(1, 32) = 93.57$ ,  $p < 0.01$ , were significantly higher than those by haloperidol as described in Fig. 3 (comparisons made with data at 15–120 min after the injection of [ $^3\text{H}$ ]-ketanserin). Clozapine,  $F(1, 31) = 41.10$ ,  $p < 0.01$ , and RMI-81582,  $F(1, 31) = 30.73$ ,  $p < 0.01$ , revealed higher 5-HT $_2$  receptor occupancy than haloperidol also in the striatum (comparisons with data at 15–120 min after the injection of [ $^3\text{H}$ ]-ketanserin) (Fig. 4). Among the various time points (15, 30, 45, 60, 120, 240 min after the ligand injection), the 5-HT $_2$  receptor occupancy at 240 min were small and unstable in haloperidol treated groups in both of the regions (data points not shown in the figures). As for  $D_1$  receptors, only RMI-81582 and clozapine showed stable occupancies in the striatum and frontal cortex, whereas the  $D_1$  occupancies by haloperidol were small and unstable. RMI-81582 demonstrated significantly higher receptor occupancy than clozapine both in the striatum,  $F(1, 41) = 240.19$ ,  $p < 0.01$  (Fig. 5) and in the frontal cortex,  $F(1, 38) = 91.51$ ,  $p < 0.01$  (Fig. 6).

The results of these kinetic studies indicated that haloperi-

doses of the drugs were carefully chosen based on the in vitro affinities for  $D_2$  and clinical dosage. The ligands [ $^3\text{H}$ ]-SCH23390 for  $D_1$ , [ $^3\text{H}$ ]-YM-09151-2 for  $D_2$ , or [ $^3\text{H}$ ]-ketanserin for 5-HT $_2$  receptors were injected intravenously into the lateral tail vein (1.54–1.68 MBq/kg body weight). The rats were sacrificed by decapitation at 15, 30, 45, 60, 120, or 240 min after the injections of the ligands. 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 (Packard Soluene 350) by incubation (2–3 h at 50°C). A scintillation cocktail (New England Nuclear Aquasol 2) was added to the solubilized tissues adjusted to pH 7.0 with 0.5 N HCl solution (for inhibition of pseudofluorescence). After 12 to 24 h, the radioactivity concentrations in the tissues were counted with a liquid scintillation counter (Aloka LSC-1000) and the values, expressed as %dose/g tissue, were calculated. The receptor occupancy ( $\Phi\%$ ) were calculated as follows (11,19,27,28):  $\Phi = [1 - (X_D - X_{nD}) / (X_S - X_{nS})] \times 100(\%)$ , where each abbreviation represents the radioactivity 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 (if the radioactivity of  $X_D$  decreases as a result of the occupation of the specific region by the drugs,  $\Phi$  value will approach 100%; the radioactivities in the cerebellum are supposed to represent the nonspecific binding).

Receptor occupancies were compared by means of two-way ANOVAs followed by Tukey's multiple comparisons of the means.

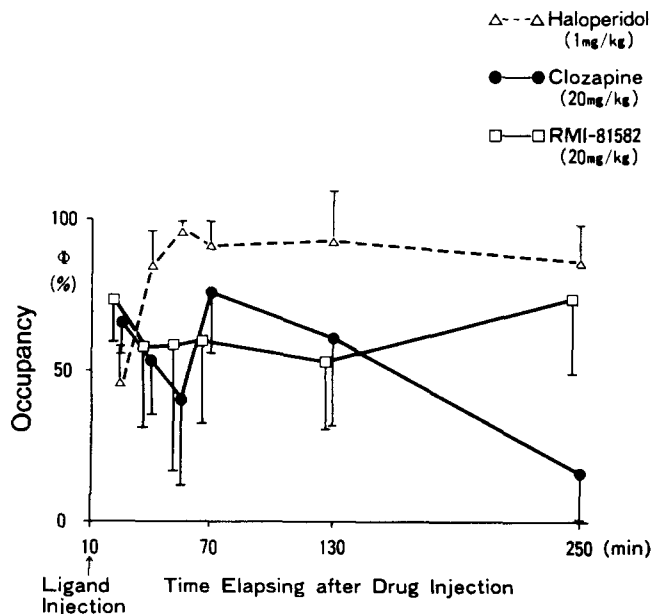


FIG. 2. Time course of  $D_2$  receptor occupancy ( $\Phi\%$ ) in rat frontal cortex after single doses of clozapine (20 mg/kg IP), RMI-81582 (20 mg/kg IP), and haloperidol (1 mg/kg IP). [ $^3\text{H}$ ]-YM-09151-2 (1.54–1.68 MBq/kg body weight) was injected into a tail vein at 10 min after drug administration. Each point represents the mean  $\pm$  SD ( $n = 3$ –5).  $\Phi = [1 - (X_D - X_{nD}) / (X_S - X_{nS})] \times 100(\%)$ , where each abbreviation represents radioactivity (% dose/g tissue) of,  $X_D$ , frontal cortex of drug-treated rat;  $X_{nD}$ , cerebellum of drug-treated rat;  $X_S$ , frontal cortex of vehicle-treated rat;  $X_{nS}$ , cerebellum of vehicle-treated rat.

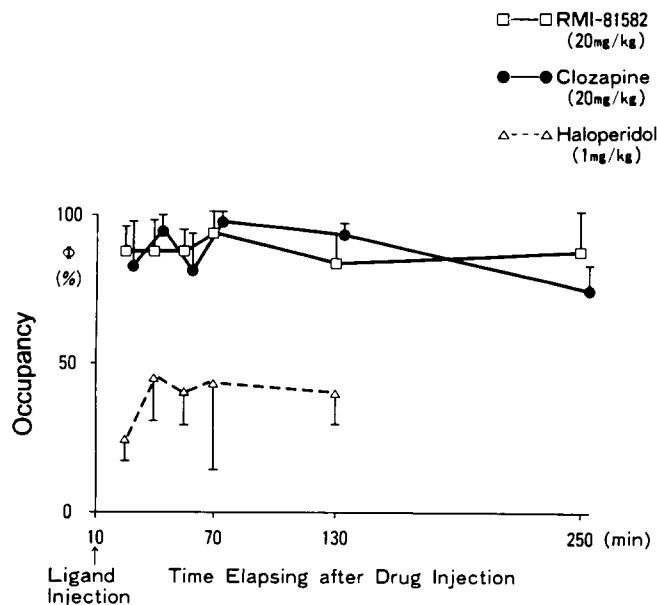


FIG. 3. Time course of 5-HT<sub>2</sub> receptor occupancy ( $\Phi$ %) in rat frontal cortex after single doses of clozapine (20 mg/kg IP), RMI-81582 (20 mg/kg IP), and haloperidol (1 mg/kg IP). [<sup>3</sup>H]-ketanserin (1.54–1.68 MBq/kg body weight) was injected into a tail vein at 10 min after drug administration. Each point represents the mean  $\pm$  SD ( $n = 3-5$ ).  $\Phi = [1 - (X_D - X_{nD}) / (X_S - X_{nS})] \times 100(\%)$ , where each abbreviation represents radioactivity (% dose/g tissue) of, X<sub>D</sub>, frontal cortex of drug-treated rat; X<sub>nD</sub>, cerebellum of drug-treated rat; X<sub>S</sub>, frontal cortex of vehicle-treated rat; X<sub>nS</sub>, cerebellum of vehicle-treated rat.

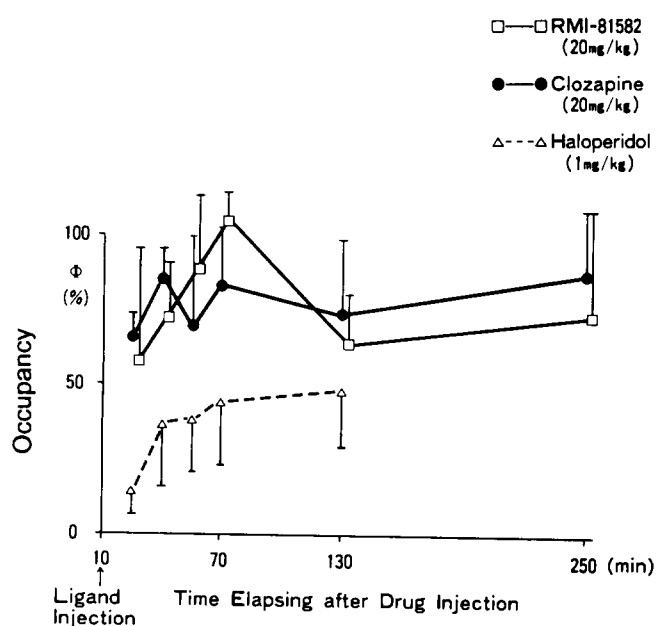


FIG. 4. Time course of 5-HT<sub>2</sub> receptor occupancy ( $\Phi$ %) in rat striatum after single doses of clozapine (20 mg/kg IP), RMI-81582 (20 mg/kg IP), and haloperidol (1 mg/kg IP). [<sup>3</sup>H]-ketanserin (1.54–1.68 MBq/kg body weight) was injected into a tail vein at 10 min after drug administration. Each point represents the mean  $\pm$  SD ( $n = 3-5$ ).  $\Phi = [1 - (X_D - X_{nD}) / (X_S - X_{nS})] \times 100(\%)$ , where each abbreviation represents radioactivity (% dose/g tissue) of, X<sub>D</sub>, striatum of drug-treated rat; X<sub>nD</sub>, cerebellum of drug-treated rat; X<sub>S</sub>, striatum of vehicle-treated rat; X<sub>nS</sub>, cerebellum of vehicle-treated rat.

dol failed to show stable 5-HT<sub>2</sub> receptor occupancies as late as 240 min after ligand injections (250 min after the drug administrations). Table 1 shows the means of the striatal D<sub>1</sub> and D<sub>2</sub> receptor occupancy including every time point, the means of the frontal 5-HT<sub>2</sub> receptor occupancy calculated without the data at 250 min after the drug administrations, and their ratios (5-HT<sub>2</sub>/D<sub>2</sub> ratios of occupancy) for haloperidol, clozapine, and RMI-81582. The latter two drugs marked about 6 to 8 times higher 5-HT<sub>2</sub>/D<sub>2</sub> ratios than haloperidol.

DISCUSSION

Little information has been available about the in vivo binding profiles of AAPDs to D<sub>1</sub>, D<sub>2</sub>, and 5-HT<sub>2</sub> receptors, obtained previously by studies (6,16,24) using the methodology applied in the current investigation.

The present trial differs from these studies in that a 5-HT<sub>2</sub> selective ligand [<sup>3</sup>H]-ketanserin, D<sub>1</sub> selective [<sup>3</sup>H]-SCH23390, and D<sub>2</sub> selective [<sup>3</sup>H]-YM-09151-2 were used. As to the specificity of the ligands, radioactive derivatives of YM-09151-2 and ketanserin have formerly been demonstrated to bind selectively in vivo to D<sub>2</sub> (13) and 5-HT<sub>2</sub> receptors (14), respectively. YM-09151-2 has been shown to have the same order of K<sub>i</sub> value for D<sub>4</sub> receptors as for D<sub>2</sub> receptors (29). Considering that the concentration of D<sub>4</sub> mRNA is one to two orders of magnitude lower than that of D<sub>2</sub> (29), the role played by D<sub>4</sub> sites in the total [<sup>3</sup>H]-YM-09151-2 binding seems to be negligible under the present condition.

[<sup>3</sup>H]-SCH23390 binding in vivo was also reported to retain the selectivity for D<sub>1</sub> receptors (2). SCH23390 has been indicated to bind also to 5-HT<sub>2</sub> receptors (5,15). Despite this fact,

brain sites labeled by [<sup>3</sup>H]-SCH23390, which clozapine and RMI-81582 displaced in the current trial, seem to reflect largely D<sub>1</sub> sites, at least in the striatum. One of the reasons for this is that RMI-81582 with a larger affinity for D<sub>1</sub> receptors

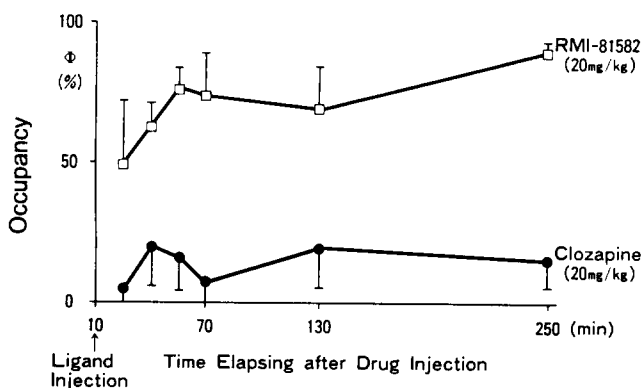


FIG. 5. Time course of D<sub>1</sub> receptor occupancy ( $\Phi$ %) in rat striatum after single doses of clozapine (20 mg/kg IP) and RMI-81582 (20 mg/kg IP). [<sup>3</sup>H]-SCH23390 (1.54–1.68 MBq/kg body weight) was injected into a tail vein at 10 min after drug administration. Each point represents the mean  $\pm$  SD ( $n = 4-5$ ).  $\Phi = [1 - (X_D - X_{nD}) / (X_S - X_{nS})] \times 100(\%)$ , where each abbreviation represents radioactivity (% dose/g tissue) of, X<sub>D</sub>, striatum of drug-treated rat; X<sub>nD</sub>, cerebellum of drug-treated rat; X<sub>S</sub>, striatum of vehicle-treated rat; X<sub>nS</sub>, cerebellum of vehicle-treated rat.

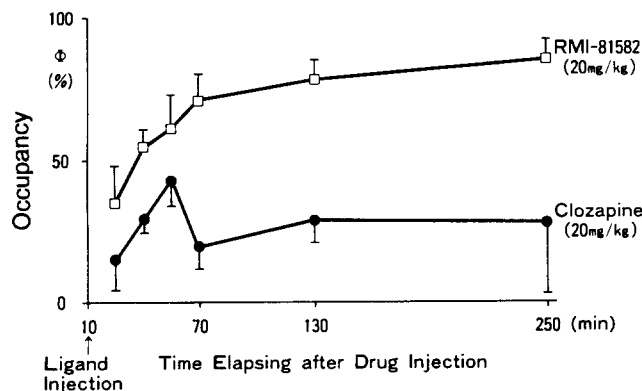


FIG. 6. Time course of  $D_1$  receptor occupancy ( $\Phi$ %) in rat frontal cortex after single doses of clozapine (20 mg/kg IP) and RMI-81582 (20 mg/kg IP). [ $^3$ H]-SCH23390 (1.54–1.68 MBq/kg body weight) was injected into a tail vein at 10 min after drug administration. Each point represents the mean  $\pm$  SD ( $n = 4-5$ ).  $\Phi = [1 - (X_D - X_{ND}) / (X_S - X_{NS})] \times 100(\%)$ , where each abbreviation represents radioactivity (% dose/g tissue) of,  $X_D$ , frontal cortex of drug-treated rat;  $X_{ND}$ , cerebellum of drug-treated rat;  $X_S$ , frontal cortex of vehicle-treated rat;  $X_{NS}$ , cerebellum of vehicle-treated rat.

than clozapine, although almost equipotent at 5-HT<sub>2</sub> receptors (18), demonstrated markedly higher occupancy than clozapine for the sites in the  $D_1$ -rich striatum (Fig. 5). Moreover, [ $^{11}$ C]-SCH23390 was used in PET studies to label the central  $D_1$  sites (9,12,26). One of the studies (26) also demonstrated that 1 mg/kg ketanserin does not affect the cortex binding of [ $^3$ H]-SCH23390 in vivo in an animal brain. Furthermore, a potent 5-HT<sub>2</sub> antagonist risperidone, revealing only a weak interaction with  $D_1$  receptors (16), failed to show an effective occupancy for striatal and frontal brain sites labeled by [ $^3$ H]-SCH23390 under the same condition as the current study (unpublished data).

Receptor occupancies at various time points during an in vivo competition period between the radioligand and the drugs were measured in the current investigation, because binding of ligands with receptors does not reach an equilibrium in vivo as concentrations of free ligands in brain tissue vary time dependently. This could explain that the percent occupation of the respective receptors by the tested drugs varied over the course of 4 h following the ligand injections (Figs. 1–6).

Clozapine 20 mg/kg and RMI-81582 20 mg/kg revealed higher occupancies for 5-HT<sub>2</sub> receptors and lower occupancies for  $D_2$  receptors than haloperidol 1 mg/kg. This finding is in line with our previous data with a larger dose of haloperidol and clozapine (27). The result that AAPDs displayed higher

TABLE 1  
MEANS OF STRIATAL  $D_1$ ,  $D_2$  AND FRONTAL  
5-HT<sub>2</sub> RECEPTOR OCCUPANCY BY ANTIPSYCHOTICS

	$D_1$ (%)	$D_2$ (%)	5-HT <sub>2</sub> (%)	5-HT <sub>2</sub> / $D_2$ Ratio
Haloperidol (1 mg/kg)	—	83	37	0.4
Clozapine (20 mg/kg)	13	29	91	3.1
RMI-81582 (20 mg/kg)	71	33	87	2.7

Means of occupancies are calculated with data at 25–250 min (for  $D_1$ ,  $D_2$  receptors) and those at 25–130 min (for 5-HT<sub>2</sub> receptors) after the drug administrations, based on the time course studies.

ratios of 5-HT<sub>2</sub> to  $D_2$  in occupancy than haloperidol well reflects the former achievements performed under in vitro (18) and in vivo (24) conditions. In fact, clozapine has recently been shown to occupy frontal 5-HT<sub>2</sub> receptors in schizophrenic patients in a PET study (22). Strong antagonism of 5-HT<sub>2</sub> receptors by AAPDs in vivo may give theoretical endorsement to the clinical view that addition of a 5-HT<sub>2</sub> antagonist to the treatment with antipsychotics like haloperidol reduces EPS (4) and that 5-HT<sub>2</sub> antagonists relieve negative symptoms (10).

It is also worthwhile to note that only clozapine and RMI-81582 showed stable occupancies for  $D_1$  receptors at the current doses tested, although the possibility remains that some part of [ $^3$ H]-SCH23390 binds to 5-HT<sub>2</sub> sites, especially in the frontal cortex. This finding seems to be in line with the observation that several AAPDs including clozapine and RMI-81582 show similar attitude toward release and metabolism of dopamine, to  $D_1$  receptor antagonists, suggesting that a mechanism related to  $D_1$  receptor antagonism may contribute to the action of AAPDs (1). The present in vivo binding property of these AAPDs to  $D_1$  receptors may support this proposition.

Although some other pharmacological profiles such as the interaction of clozapine and RMI-81582 with 5-HT<sub>3</sub> sites (3,8) or indirect effects of clozapine on nigral GABAergic mechanisms (7) should be fully considered to explain the therapeutic efficacy of each of these AAPDs, evaluation of in vivo receptor binding properties will help understand the mode of action of AAPDs.

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