Binding Profile of SM-9018, a Novel Antipsychotic Candidate

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Abstract—The present study employed various receptor-binding assays to clarify the biochemical characteristics of SM-9018. SM-9018 possessed very high affinity for 5-HT2, D2 and 5-HT1A receptors (Kd=0.61, 1.4 and 2.9 nM, respectively), and it had moderate affinity for α1 and D1 receptors (Kd=17 and 41 nM, respectively). However, SM-9018 had only negligible affinity for α2, opiate, glutamate, phencyclidine, benzodiazepine and GABA receptors. These results suggest that SM-9018 may be a novel antipsychotic agent with binding affinity for 5-HT2 and 5-HT1A receptors.

SM-9018 (cis-2-[4-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl]butyl]hexahydro-1H-isoindole-1,3(2H)-dione hydrochloride) is a novel antipsychotic candidate with potent 5-hydroxytryptamine2 (5-HT2) and dopamine2 (D2) receptor blocking activity in behavioral tests. In spite of its potent D2 antagonism, SM-9018 has a very weak cataleptogenic activity in rodents (1). In the present study, the binding affinity of SM-9018 for various neurotransmitter- and drug-receptors was investigated in the rat brain.

Male Sprague-Dawley or Wistar rats weighing 180 g were used. Membrane fractions were prepared from rat brain, and radioligand binding experiments were carried out according to the methods summarized in Table 1. Briefly, membranes incubated with the respective radioligand were rapidly filtered under vacuum through Whatman GF/B glass fiber filters and washed two or three times with ice-cold Tris-HCl buffer. The filters were then placed in vials with scintillation fluid, and the radioactivity was measured by a liquid scintillation counter. Prazosin (0.02 μM) and 5-HT (0.1 μM) were included in the incubation medium to mask the binding of [3H]-ketanserin to α1 receptors and that of [3H]-WB 4101 to 5-HT1A receptors, respectively. SM-9018 was synthesized in our laboratory. All radioligands and other drugs were from commercial sources.

SM-9018 showed very high binding affinity for 5-HT2 and D2 receptors (Kd=0.61 and 1.4 nM, respectively) and its affinity for 5-HT2 receptors was 2.3 times higher than that for D2 receptors (Table 1 and Fig. 1). SM-9018 also possessed moderate affinities for α1, 5-HT1 and D1 receptors (Kd=17, 18 and 41 nM, respectively). However, its binding affinity for these receptors was 28, 30 and 67 times lower than that for 5-HT2 receptors, respectively. In addition, SM-9018 had only negligible affinities for α2, muscarinic, opiate, glutamate, phencyclidine, benzodiazepine and GABA receptors. Although all Hill coefficients of the displacement curves of 5-HT2, D1, D2, α1 and α2 receptors binding by SM-9018 were about 1.0, that of 5-HT1 receptors was 0.32±0.03. Therefore, a 5-HT1A receptor (one of 5-HT1 receptor subtypes) binding assay was undertaken. SM-9018 had a very high affinity for 5-HT1A receptors (Kd=2.9 nM) with a Hill coefficient of about 1.

We have previously reported that SM-9018 acts as a D2 antagonist in rodents. It inhibits hyperactivity induced by methamphetamine in rats (ED50 value=2.2 mg/kg, p.o.) and climbing behavior induced by apomorphine in mice (ED50 value=3.5 mg/kg, p.o.) (1). It is well-known that antipsychotics block postsynaptic D2 receptors in the frontal cortex and limbic areas and that there is a close correlation between their anti-D2 activity and
Table 1. Interaction of SM-9018 with various receptors in rat brain

| Receptor          | Tissue | $^3$H-Ligand (Ka value) | Nonspecific binding | Assay condition | $K_i$ value (nM)* |
|-------------------|--------|------------------------|---------------------|-----------------|-------------------|
| Serotonin 5-HT$_2^{ab}$ | CRT    | $^3$H-Ketanserin, 1 nM (1.5 nM) | Ketanserin, 10 μM | 37°C, 10 min | 0.61±0.11         |
| 5-HT$_1^{b}$      | CRT    | $^3$H-5-HT, 2 nM (1.5 nM) | 5-HT, 10 μM | 37°C, 10 min | 18±1             |
| 5-HT$_{1A}^{c}$   | HPC    | $^3$H-8-OH-DPAT, 0.15 nM (1.1 nM) | 8-OH-DPAT, 1 μM | 25°C, 30 min | 2.9±0.4          |
| Dopamine D$_2^{d}$ | STA    | $^3$H-Spiradone, 0.5 nM (0.11 nM) | Spiradone, 10 μM | 37°C, 60 min | 1.4±0.2          |
| D$_1^{e}$         | STA    | $^3$H-SCH 23390, 0.2 nM (0.31 nM) | (+) Butaclamol, 1 μM | 25°C, 45 min | 41±5             |
| Noradrenaline α$_1^{f}$ | CRT | $^3$H-WB 4101, 1.5 nM (0.94 nM) | Prazosin, 1 μM | 25°C, 30 min | 17±2.3           |
| α$_2^{g}$         | CRT    | $^3$H-Clonidine, 1.5 nM (1.6 nM) | Clonidine, 1 μM | 25°C, 30 min | 408±11           |
| Muscarine$h$      | CRT    | $^3$H-QNB, 0.15 nM (0.07 nM) | Atropine, 0.1 μM | 25°C, 60 min | >1000*           |
| Opiate$i$         | CRT    | $^3$H-Naloxone, 1 nM (1 nM) | Naloxone, 1 μM | 25°C, 40 min | >1000*           |
| Glutamate$j$      | WB     | $^3$H-L-Glutamate, 1.6 nM (437 nM) | L-Glutamate, 50 μM | 37°C, 10 min | >1000*           |
| PCP$k$            | CRT    | $^3$H-PCP, 10 nM (51 nM) | PCP, 0.1 μM | 4°C, 45 min | >1000*           |
| BZ$l$             | WB     | $^3$H-Flunitrazepam, 1 nM (4.4 nM) | Diazepam, 1 μM | 25°C, 60 min | >1000*           |
| GABA$_A^{m}$      | WB     | $^3$H-Muscimol, 1 nM (1.5 nM) | Muscimol, 10 μM | 0°C, 10 min | >1000*           |

*: $K_i$ values were derived from IC50/(1+S/Ka), where S is the concentration of $^3$H-ligand and Ka is its dissociation constant. #: IC50 values. Results are expressed as the mean±S.E. of three separate experiments, each of which was performed in duplicate. CRT: Cortex, HPC: Hippocampus, STA: Striatum, WB: Whole brain, PCP: Phencyclidine, BZ: Benzodiazepine, GABA: Gamma-amino butyric acid. $^a$: Domperid, Arch. Pharmacol. 328, 467 (1985); $^b$: Middleton, Eur. J. Pharmacol. 101, 289 (1984); $^c$: Hall, J. Neurochem. 44, 1685 (1985); $^d$: Seeman et al., Nature 261, 717 (1976); $^e$: Billard et al., Life Sci. 35, 1885 (1984); $^f$: Uprichard et al., Brain Res. 187, 143 (1980); $^g$: Uprichard et al., Mol. Pharmacol. 13, 454 (1983); $^h$: Luthin et al., J. Pharmacol. Exp. Ther. 228, 648 (1984); $^i$: Pasternak et al., Mol. Pharmacol. 11, 340 (1975); $^j$: Foster and Fagg, Eur. J. Pharmacol. 133, 291 (1987); $^k$: Zukin et al., Brain Res. 258, 177 (1983); $^l$: Speth et al., Life Sci. 24, 351 (1979); $^m$: Snodgrass, Nature 273, 392 (1978).
Fig. 1. Displacement of 5-HT\textsubscript{2}, D\textsubscript{2}, 5-HT\textsubscript{1A} and α\textsubscript{1} receptors binding by SM-9018 in rat cortex, striatum and hippocampus. To measure inhibition of radioligand receptor binding by SM-9018, this compound was added at concentrations between 10\textsuperscript{-10} and 10\textsuperscript{-6} M. In the absence or presence of SM-9018, the membrane was incubated with 1 nM [\textsuperscript{3}H]-ketanserin for 10 min at 37°C (—○—: 5-HT\textsubscript{2} receptor), 0.5 nM [\textsuperscript{3}H]-spiperone for 60 min at 37°C (—Δ—: D\textsubscript{2} receptor), 0.15 nM [\textsuperscript{3}H]-8-OH-DPAT for 30 min at 25°C (—□—: 5-HT\textsubscript{1A} receptor) and 1.5 nM [\textsuperscript{3}H]-WB 4101 for 30 min at 25°C (—●—: α\textsubscript{1} receptor). Inhibition curves were made by plotting the percentage of specific binding vs. the log values of SM-9018 concentrations. Results are expressed as the mean±S.E. of three separate experiments, each of which was performed in duplicate.

Clinical potency for improving positive symptoms of schizophrenia such as delusions, hallucinations, thought disorders and motor disturbances (2). Accordingly, it seems likely that SM-9018 improves these symptoms through D\textsubscript{2} blocking activity. It has also been reported that brain D\textsubscript{1} receptors are implicated in the antipsychotic effects (3). The ameliorative effects on the positive symptoms by SM-9018 may not be attributable to an interaction with D\textsubscript{1} receptors, because the affinity of SM-9018 for D\textsubscript{1} receptors was approximately 30 times lower than that for D\textsubscript{2} receptors.

In spite of its D\textsubscript{2} antagonistic activity, SM-9018 has a very weak cataleptogenic activity in rodents (ED\textsubscript{50} value=150 mg/kg, p.o. in rats, ED\textsubscript{50} value=57 mg/kg, p.o. in mice) (1). Thus, SM-9018 seems to belong to the atypical antipsychotics group of compounds which produce much weaker extrapyramidal side effects (EPS) than typical antipsychotics (e.g., haloperidol and chlorpromazine). However, the mechanism of the weak cataleptogenic activity of SM-9018 is unclear. Two major biochemical features have been suggested to explain this property of atypical antipsychotics: 1) An anticholinergic property (4) and 2) An interaction with 5-HT\textsubscript{2} receptors (5–7). Both anticholinergic and anti-5-HT\textsubscript{2} drugs (e.g., trihexyphenidyl and ritanserin, respectively) reduce the incidence of EPS induced by typical antipsychotics (8, 9). The present binding study demonstrated that SM-9018 has a negligible affinity for muscarinic receptors. In contrast, SM-9018 had the highest affinity for 5-HT\textsubscript{2} receptors, 2.3-fold higher than that for D\textsubscript{2} receptors. In addition, the ratio of the 5-HT\textsubscript{2}/D\textsubscript{2} affinity of SM-9018 was almost equal to that of an atypical antipsychotic agent (Zotepine: 2.1), whereas those of typical antipsychotics such as haloperidol and chlorpromazine were 0.02 and 0.30, respectively (1). These data are in agreement with previous studies in which the ratio of 5-HT\textsubscript{2}/D\textsubscript{2} affinity can differentiate atypical and typical antipsychotics (6, 7). Moreover, SM-9018 potently inhibits the tryptamine-induced bilateral clonic seizure in rats (ED\textsubscript{50} value=1.4 mg/kg, p.o.) (1). Thus, the binding of SM-9018 to the 5-HT\textsubscript{2} receptors as an antagonist may in part account for its weak cataleptogenic activity.

It has been recently reported that drugs with 5-HT\textsubscript{2} antagonistic action (e.g., setoperone and clozapine) improved the negative symptoms of schizophrenia such as flattening of affect, apathy and social withdrawal (10, 11). Since SM-9018 is a potent 5-HT\textsubscript{2} blocker as well as a D\textsubscript{2} blocker in rodents (1), it may be a novel atypical antipsychotic agent which may improve not only positive but also negative symptoms in schizophrenic patients.

In the present study, it was found that SM-9018 had a very high affinity for 5-HT\textsubscript{1A} receptors. SM-9018 might be a 5-HT\textsubscript{1A} receptor antagonist but not an agonist, because it failed to produce 5-HT\textsubscript{1A} receptor-mediated
behaviors such as flat body posture and forepaw treading in rats (T. Kato et al., unpublished data). Although the clinical relevance of 5-HT₁A receptor antagonism is unknown, it has been recently reported that 5-HT₁A receptors are significantly increased in the postmortem schizophrenic frontal cortex (12). In addition, some β-blockers (e.g., propranolol) with a 5-HT₁A antagonistic property are known to be effective in the treatment of schizophrenia and mania (13). Thus, this action of SM-9018 may provide an advantage in clinical therapy compared with other antipsychotics. Further studies are required to delineate the interaction of 5-HT₁A receptors and psychosis.

References

1 Hirose, A., Kato, T., Ohno, Y., Shimizu, H., Tanaka, H., Nakamura, M. and Katsube, J.: Pharmacological actions of SM-9018, a new neuroleptic drug with both potent 5-hydroxytryptamine₂ and dopamine₂ antagonistic actions. Japan. J. Pharmacol. 53, 321–329 (1990)

2 Seeman, P.: Dopamine receptor and the dopamine hypothesis of schizophrenia. Synapse 1, 133–152 (1987)

3 Hess, E.J., Bracha, H.S., Kleinman, J.E. and Creese, I.: Dopamine receptor subtype imbalance in schizophrenia. Life Sci. 40, 1487–1497 (1987)

4 Miller, R.J. and Hiley, C.R.: Anti-muscarinic properties of neuroleptics and drug-induced Parkinsonism. Nature 248, 596–597 (1974)

5 Alter, C.A., Wasley, A.M., Neale, R.F. and Stone, G.A.: Typical and atypical antipsychotic occupancy of D-2 and S-2 receptors: an autoradiographic analysis in rat brain. Brain Res. Bull. 16, 517–525 (1986)

6 Meltzer, H.Y., Matsubara, S. and Lee, J.-C.: The ratios of serotonin₂ and dopamine₂ affinities differentiate atypical and typical antipsychotic drugs. Psychopharmacol. Bull. 25, 390–392 (1989)

7 Meltzer, H.Y., Matsubara, S. and Lee, J.-C.: Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin₂ pKᵣ values. J. Pharmacol. Exp. Ther. 251, 238–246 (1989)

8 Feldman, R.S. and Quenzer, L.F.: Fundamentals of Neuro-Psychopharmacology. Sinauer Associates, Inc., Sunderland (1984)

9 Bersani, A., Grispini, S., Marini, A., Valducci, M. and Ciani, N.: Neuroleptic-induced extrapyramidal side effects. Clinical perspectives with ritanserin (R 55667), a new selective 5-HT₂ receptor blocking agent. Curr. Ther. Res. 40, 492–499 (1986)

10 Ceulemans, D.L.S., Gelder, S.Y.G., Hoppenbrouwers, M.-L.A., Reyntjens, A.J.M. and Janssen, P.A.J.: Effect of serotonin antagonism in schizophrenia: a pilot study with setoperone. Psychopharmacology (Berlin) 85, 329–332 (1985)

11 Kane, J., Honigfeld, G., Singer, J. and Meltzer, H.: Clozapine for the treatment-resistant schizophrenia. Arch. Gen. Psychiatry 45, 789–796 (1988)

12 Kitamura, T., Kajimoto, Y., Shirai, Y., Sakai, N., Hashimoto, K., Nakai, T., Nishino, N., Mitsuda, T. and Tanaka, C.: Changes of 8-OH-DPAT binding sites in schizophrenia. Bull. Japan. Neurochem. Soc. 28, 498–499 (1989) (in Japanese)

13 Middlemiss, D.N., Buxton, D.A. and Greenwood, D.T.: Beta-adrenoceptor antagonists in psychiatry and neurology. Pharmacol. Ther. 12, 419–437 (1981)