Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 1, pp. 19-23, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)E0083-T

Evaluation of Bradykinesia Induction by SM-9018, a Novel 5-HT_2 and D_2 Receptor Antagonist, Using the Mouse Pole Test

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Received 29 November 1993

OHNO, Y., K. ISHIDA, K. IKEDA, T. ISHIBASHI, K. OKADA AND M. NAKAMURA. *Evaluation of bradykinesia induction by SM-9018, a novel 5-HT_2 and D_2 receptor antagonist, using the mouse pole test.* PHARMACOL BIOCHEM BEHAV **49**(1) 19-23, 1994.—Induction of bradykinesia by SM-9018, a novel 5-HT_2 and D_2 antagonist, was compared with that of other neuroleptics using the pole test in mice. Neuroleptics including SM-9018, haloperidol, chlorpromazine, and thioridazine dose dependently induced bradykinesia in the pole-descending behavior of mice with relative potencies consistent with those for catalepsy induction. SM-9018 was about 70 times weaker than haloperidol and twice as weak as thioridazine in inducing bradykinesia. Other CNS drugs such as barbiturates and antidepressants had no effects. Haloperidol-induced bradykinesia was significantly attenuated by a cholinergic muscarinic antagonist (i.e., trihexyphenidyl) and 5-HT_2 antagonists (i.e., ritanserin and cyproheptadine) whereas that caused by SM-9018 was relatively resistant to the 5-HT_2 antagonists. These findings suggest that SM-9018 is weaker than other neuroleptics in inducing extrapyramidal side effects and that the 5-HT_2 blocking activity of SM-9018 may contribute to its atypical neuroleptic property.

SM-9018 D_2 receptor antagonist 5-HT_2 receptor antagonist Neuroleptics Bradykinesia Extrapyramidal side effects

THE ventral tegmental area and substantia nigra (pars compacta) are the two main origins of dopamine neurons in the mesencephalon and form the mesocortical/limbic and nigrostriatal dopaminergic pathway, respectively (13). The D_2 receptor blockade in the cerebral cortex and/or limbic structures by neuroleptics has been implicated in their antipsychotic activities, whereas their actions in the striatum have been suggested to be related to their extrapyramidal side effects (EPS) (2,6,17,18). Although conventional neuroleptics such as haloperidol and chlorpromazine are still widely used in the treatment of schizophrenia, they often produce EPS such as akathisia, parkinsonism, and dystonia in humans.

Catalepsy behavior is widely used as a simple measurement of the EPS associated with neuroleptic treatments in rodents. The cataleptogenic activities of neuroleptics are relatively consistent with their EPS in humans though some drugs other than neuroleptics are also known to induce catalepsy (5). On the other hand, Ogawa et al. (14) developed a pole test to quantitatively evaluate bradykinesia in mice treated with the dopaminergic neurotoxin, MPTP. MPTP selectively reduced the dopamine content in the striatum and induced bradykinesia as revealed by a marked delay of the pole-descending behavior of mice. In addition, MPTP-induced bradykinesia in mice was alleviated by an antiparkinsonian agent L-DOPA in a dose-dependent manner (15). Thus, the pole test also seems to be useful to assess the EPS associated with the neuroleptic treatment.

SM-9018 (*cis*-2-(4-(4-(1,2-benzisothiazol-3-yl)-1-piperazinyl)-butyl)hexahydro-1H-isoindole-1,3(2H)-dione HCl) is a potential neuroleptic that has both 5-HT_2 and D_2 receptor blocking activity (7,8,10). It showed high affinities both for 5-HT_2 (K_i = 0.61 nM) and D_2 receptors (K_i = 1.41 nM), and potently blocked various behaviors mediated by these receptors (7). In addition, SM-9018 was weaker than the typical neuroleptics, haloperidol and chlorpromazine, in inducing catalepsy in rats and mice, implying that SM-9018 is an atypical neuroleptic.

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To further evaluate the EPS of SM-9018, its ability to induce bradykinesia in mice was compared with that of other neuroleptics using the pole test.

METHOD

Animals

Male ddY mice (Japan SLC, Inc., Shizuoka, Japan) each weighing 17–29 g were used. The animals were kept in air-conditioned rooms at 23 ± 2°C and 55 ± 10% relative humidity under a 12 h light/dark cycle (dark period: 20:00–08:00 hours).

Pole Test

The pole test was performed 1 h after oral administration of drugs according to the method of Ogawa et al. (14) with slight modifications. The mice were placed head-upward at the top of a wooden pole (8 mm in diameter and 45 cm in height), and then the time required for the animal to turn downward completely (T_{turn}) and to descend to the floor (T_{total}) was measured. Ten to 30 mice were used at each dose level and bradykinesia was evaluated by prolongation of T_{turn} and T_{total} values with upper limits at 90 s. The T_{total} values were also expressed as a percentage of the control value to construct the dose-response curves, and the dose of each drug to increase the T_{total} value by 50% was determined by the least squares regression analysis. Only mice that showed the T_{total} value < 10 s in the pretest trial (usually performed 3–4 h before the test trial) were subjected to the experiment.

Catalepsy Test

The catalepsy of mice was evaluated 1 h after oral administration of the drugs according to the method described previously (7). Briefly, animals were tested for the presence of catalepsy by placing both front limbs over a horizontal bar positioned 5-cm above the bench surface. Catalepsy was judged to be positive if the animal maintained the imposed posture for more than 30 s. Eight to ten mice at each dose level were used to determine the ED_{50} value that induces catalepsy in 50% of the animals tested.

Apomorphine Climbing Test

Apomorphine-induced climbing behavior of mice was evaluated in individual cylindrical stainless-steel wire cages (12 cm in diameter and 14 cm in height) (7). Apomorphine (1 mg/kg) was subcutaneously injected 50 min after oral administration of the test drugs. The climbing behavior was observed during 10–30 min after apomorphine injection and was scored on a four ranked intensity scale as follows: 0, absent; 1, slight; 2, moderate; 3, pronounced. Five to ten mice at each dose level were used to determine the ED_{50} value that reduced the climbing score by 50%.

Drugs

SM-9018, haloperidol, clozapine, and diazepam were synthesized in our laboratory. These drugs and chlorpromazine hydrochloride (Sigma Chem. Co., St. Louis, MO), zotepine (extracted and purified from LODOPIN, Fujisawa, Osaka, Japan), thioridazine (extracted and purified from MELLERIL, Sankyo, Tokyo, Japan), desipramine hydrochloride (Res. Biochem. Int.), imipramine hydrochloride (Res. Biochem. Int., Natick, MA), phenobarbital (PHENOBAL, Dainippon, Osaka, Japan), hexobarbital (CYCLOPAN, Nagase Iyakuhin, Osaka, Japan), all were suspended in 0.5% methylcellulose solution and orally administered 1 h before the behavioral tests. In the experiments with trihexyphenidyl (Sigma Chem.), ritanserin (Res. Biochem. Int.) and cyproheptadine (Res. Biochem. Int.), animals received an IP injection of these drugs followed by oral administration of haloperidol or SM-9018 at 1 h before the pole test. Apomorphine hydrochloride (Sandoz, Basel, Switzerland) was dissolved in physiological saline and injected subcutaneously. Control animals received equivalent volume of vehicle alone.

Statistics

Statistical significance of differences among T_{turn} or T_{total} values was determined by one-way ANOVA followed by Duncan's multiple comparison test. Student's *t*-test was used only for the comparison between two groups.

RESULTS

Control animals placed head-upward at the top of the pole readily rotate downward and descend to the floor within 14 s. The mean T_{turn} and T_{total} values of the control group were 3.17 ± 0.30 and 8.57 ± 0.46 s, respectively. Figure 1 shows the effects of SM-9018 and other neuroleptics on the T_{turn} and T_{total} values in the pole test. Oral administration of SM-9018 (30–100 mg/kg), haloperidol (0.3–10 mg/kg), chlorpromazine (3–30 mg/kg), thioridazine (10–30 mg/kg) all increased both T_{turn} and T_{total} values in a dose-dependent manner (Fig. 1). The relative potency of neuroleptics to increase the T_{total} value were as follows, haloperidol > chlorpromazine > thioridazine > SM-9018 (Table 1). The rank order was consistent with that for catalepsy induction in mice, and a good relationship was found among the dosage of neuroleptics to induce bradykinesia and catalepsy (Fig. 2). The therapeutic indices of the neuroleptics, as revealed by their potency ratios for the antagonism of apomorphine-induced climbing behavior (D_2 block-

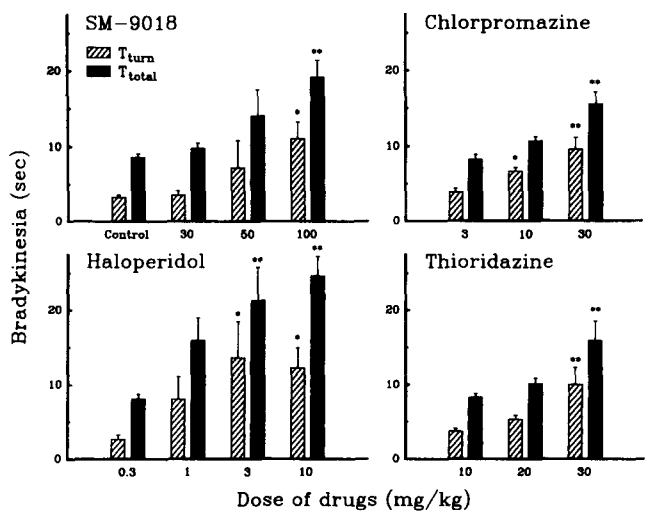

FIG. 1. Effects of SM-9018 and other neuroleptics on T_{turn} and T_{total} values in the mouse pole test. Each column shows the mean ± SEM of 10–30 animals. Drugs were orally administered 1 h before the pole-test. $*p < 0.05$, $**p < 0.01$. Significantly different from the control value (one-way ANOVA and Duncan's test).

TABLE 1

COMPARISON OF SM-9018 WITH OTHER NEUROLEPTICS FOR INDUCTION OF BRADYKINESIA AND ANTAGONISM OF APOMORPHINE-INDUCED CLIMBING BEHAVIOR IN MICE

Drugs	Bradykinesia(BK)* (mg/kg)	Antiapomorphine(APO)† (mg/kg)	BK/Anti-APO‡ Ratio
SM-9018	44.2 (1)	3.50 (1)	12.6
Haloperidol	0.66 (67)	0.67 (5)	0.99
Chlorpromazine	14.9 (3)	4.20 (0.8)	3.55
Thioridazine	23.3 (2)	7.00 (0.5)	3.33

The pole test and APO-induced climbing test were performed 1 hr after administration of drugs. *Value shows the dose that produced 50% increase of the T_{total} value in the pole test. †Value shows the dose that reduces the score of APO-induced climbing behavior by 50%. ‡Potency ratio of drugs for antagonism of APO-induced climbing behavior to bradykinesia induction. Values in parentheses indicate the potency of drugs relative to that of SM-9018.

ing activity) to the bradykinesia induction, were SM-9018 > chlorpromazine = thioridazine > haloperidol (Table 1). Clozapine and zotepine produced only weak bradykinesia at doses up to 30 and 50 mg/kg, respectively (Table 2). The increases in T_{total} and T_{turn} values with clozapine and zotepine were considerably smaller than those with haloperidol, chlorpromazine and thioridazine. However, their effective dosage could not be determined since both compounds at higher doses produced marked muscle relaxation in most of the animals tested.

To clarify the specificity of the actions of the neuroleptics in the pole test, several CNS drugs were also examined. As shown in Table 2, subanesthetic doses of hexobarbital did not significantly change either the T_{turn} or T_{total} values at doses up to 50 mg/kg. Similarly, phenobarbital at 10–50 mg/kg failed to induce bradykinesia while a slight decrease in the T_{total} value was observed at 30 mg/kg. Tricyclic antidepressants, desipramine and imipramine, and an anxiolytic diazepam also did not affect the T_{turn} and T_{total} values although 60% of the animals treated with diazepam could not be tested because of muscle relaxation.

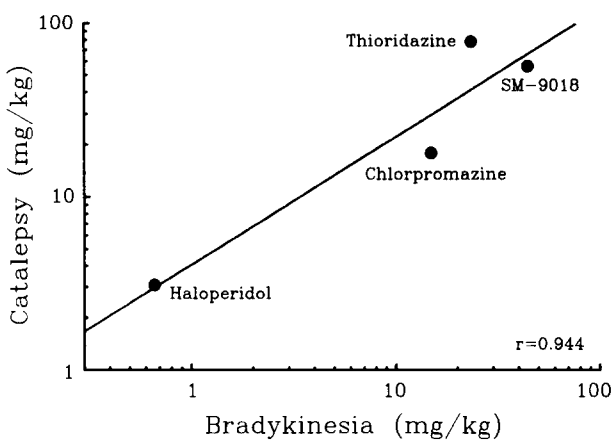

FIG. 2. Relationship among potencies of SM-9018 and other neuroleptics in inducing bradykinesia and catalepsy in mice. Ordinate shows the dose that induces catalepsy in 50% of animals tested. Abscissa represents the dose that increases the T_{total} value by 50% in the pole test.

We next examined the effects of trihexyphenidyl (a cholinergic muscarinic antagonist), ritanserin and cyproheptadine (5-HT_2 antagonists) on haloperidol (3 mg/kg)- or SM-9018 (100 mg/kg)-induced bradykinesia. As shown in Fig. 2, haloperidol-induced bradykinesia was markedly attenuated either by trihexyphenidyl (0.3–3 mg/kg, IP), ritanserin (0.1–3 mg/kg, IP) or cyproheptadine (1–3 mg/kg, IP) in a dose-dependent manner (Fig. 2). The T_{total} value increased with 3 mg/kg of haloperidol (21.3 ± 4.49 s) was significantly reduced to 8.10 ± 0.50, 9.70 ± 0.72 and 9.25 ± 0.94 s by treatment with 3 mg/kg of trihexyphenidyl, ritanserin and cyproheptadine, respectively. Bradykinesia induced by SM-9018 (100 mg/kg) was also significantly reduced by trihexyphenidyl, but weakly by ritanserin or cyproheptadine (Fig. 3).

DISCUSSION

The present study demonstrated that bradykinesia induced by neuroleptics in mice could be assessed by the pole test originally used in the study of the MPTP-treated parkinsonian model by Ogawa et al. (14,15). In their studies, the dopaminergic neurotoxin MPTP selectively reduced the dopamine content in the striatum and induced bradykinesia in the pole-descending behavior of mice 1–2 weeks after the treatment. In addition, the bradykinesia caused by MPTP was dose dependently attenuated by treatment with L-DOPA, suggesting that MPTP-induced bradykinesia resulted from degradation of the nigrostriatal dopaminergic system (15). In the present study, most of the neuroleptics that block striatal dopamine D_2 receptors also prolonged the T_{turn} and T_{total} values in a dose-dependent manner. The relative potencies of the neuroleptics in inducing bradykinesia were consistent with their cataleptogenic activities in mice. In addition, an antimuscarinic agent trihexyphenidyl, which is widely used as a remedy for neuroleptic-induced EPS and Parkinson's disease in humans, markedly attenuated the neuroleptic-induced bradykinesia. Our findings suggest that neuroleptic-induced bradykinesia also resulted from the blockade of the nigrostriatal dopaminergic system by neuroleptics, and that the pole test is of value not only in the study of MPTP-induced parkinsonian model but also in evaluating the EPS associated with neuroleptics. Furthermore, CNS drugs other than neuroleptics such as depressants (i.e., barbiturates and diazepam) and antidepressants, did not significantly alter the T_{turn} and T_{total} values, implying that neuroleptic-induced bradykinesia was not simply due to

TABLE 2

EFFECTS OF VARIOUS CNS DRUGS ON T_{turn} AND T_{total} VALUES IN THE MOUSE POLE TEST

Drugs	Dose (mg/kg)	n	Bradykinesia (sec) T_{turn}	Bradykinesia (sec) T_{total}
Clozapine	10	20	4.65 ± 0.59	9.90 ± 0.75
	30	18*	6.78 ± 1.45‡	10.6 ± 1.40
Zotepine	10	20	4.45 ± 0.50	8.95 ± 0.64
	30	20	4.90 ± 0.39	8.20 ± 0.51
	50	18*	6.89 ± 1.17‡	10.0 ± 1.19
Hexobarbital	10	10	2.50 ± 0.27	7.60 ± 0.72
	30	10	4.00 ± 0.56	8.20 ± 0.44
	50	10	3.60 ± 0.50	8.50 ± 0.73
Phenobarbital	10	20	4.30 ± 0.48	9.65 ± 0.75
	30	20	3.15 ± 0.35	6.50 ± 0.45†
	50	10	5.00 ± 1.59	6.90 ± 1.57
Desipramine	3	10	2.60 ± 0.27	6.60 ± 0.31
	10	10	3.80 ± 0.47	8.40 ± 0.64
	30	10	3.90 ± 0.84	8.20 ± 1.02
Imipramine	3	10	4.30 ± 0.79	8.50 ± 0.85
	10	10	3.40 ± 0.60	7.70 ± 0.84
	30	10	3.20 ± 0.53	7.40 ± 0.58
Diazepam	10	4*	4.50 ± 2.18	5.75 ± 2.43

Values show the mean ± SEM. Drugs were orally administered 1 hr before the pole test. *Twelve, two, and six animals treated with clozapine (30 mg/kg), zotepine (50 mg/kg), and diazepam (10 mg/kg), respectively, were unable to be tested due to prominent muscle relaxation with the drug treatments. †$p < 0.05$, ‡$p < 0.01$. Significantly different from the control value (one-way ANOVA and Duncan's test).

their nonspecific CNS depressant actions. However, it should be noted that some neuroleptics which produced prominent muscle relaxation (e.g., clozapine and zotepine), were not eligible to be tested in the pole test.

We previously demonstrated that SM-9018 was weaker than the typical neuroleptics, haloperidol and chlorpromazine, in inducing catalepsy in rodents (7). In the pole test, SM-9018 was about 70 times weaker than haloperidol and 3 times as weak as chlorpromazine in inducing bradykinesia. In addition, when the potency ratio of the drugs is compared between dopamine receptor antagonism (anti-apomorphine actions) and bradykinesia induction, SM-9018 exhibited the highest ratio among the neuroleptics examined. These findings suggest that SM-9018 belongs to the atypical neuroleptics characterized by the fewer EPS in humans and has a favorable safety margin. Clozapine and zotepine, which are reportedly atypical neuroleptics (11), were also much weaker than haloperidol and chlorpromazine in inducing bradykinesia at relatively high doses (30–50 mg/kg). Although we could not determine the effective dose of these drugs because of their muscle relaxant activity, clozapine and zotepine seemed to behave as atypical neuroleptics in the pole test. Regarding the action of thioridazine, there is still some controversy as to whether it belongs to atypical neuroleptics (11). In our study, thioridazine induced bradykinesia at higher doses than did typical neuroleptics, but showed a therapeutic (bradykinesia/antiapomorphine) ratio similar to that of chlorpromazine. Thus, we were unable to confirm its atypical neuroleptic property. Our results are consistent with previous work that described a borderline nature of thioridazine between typical and atypical neuroleptics (11). Since thioridazine is known to act at least in part through its active metabolite, mesoridazine, its action may vary among animal species and the experimental conditions used, in that thioridazine failed to induce catalepsy in rats even at high doses (ED_{50} > 300 mg/kg) (7).

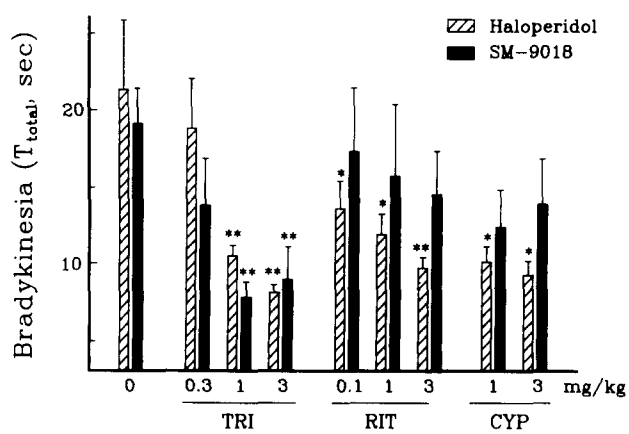

FIG. 3. Effects of trihexyhenidyl (TRI), ritanserin (RIT), and cyproheptadine (CYP) on haloperidol-induced and SM-9018-induced bradykinesia in the mouse pole test. Each column indicates the mean ± SEM of 20–30 animals. The animals received an IP injection of TRI, RIT, and CYP followed by oral administration of SM-9018 (100 mg/kg) or haloperidol (3 mg/kg) at 1 h before the pole test. *$p < 0.05$, **$p < 0.01$. Significantly different from the value with haloperidol or SM-9018 alone (one-way ANOVA and Duncan's test).

Meltzer et al. (11,12) have shown that the ratio of 5-HT_2 to D_2 receptor affinity can discriminate typical and atypical neuroleptics, in that, the compounds including SM-9018 (12), which more potently interact with 5-HT_2 receptors, produce fewer EPS. However, the typical neuroleptics are more potent D_2 antagonists than 5-HT_2 antagonists. Furthermore, several studies have shown that the blockade of 5-HT_2 receptors may counteract the effects of D_2 receptor blockade in the nigrostriatal dopaminergic system. In fact, 5-HT_2 receptor antagonists (e.g., ritanserin and ICI 169369) reportedly attenuate D_2 receptor antagonist-induced catalepsy and/or parkinsonism in rodents (1,16), monkeys (9) and humans (3,4). In this study, both 5-HT_2 antagonists (ritanserin and cyproheptadine) significantly attenuated haloperidol-induced bradykinesia in a dose-dependent manner. These findings support the hypothesis (11) that the blockade of 5-HT_2 receptors has a role in reducing EPS associated with neuroleptic treatment. The bradykinesia induced by SM-9018 was also reduced by ritanserin and cyproheptadine, but to a lesser extent than that induced by haloperidol. SM-9018 is a potent antagonist for 5-HT_2 receptors (7). Its affinity to 5-HT_2 receptors is about 6 times that of ketanserin (a 5-HT_2 antagonist) and about 200 times that of haloperidol. In addition, SM-9018 negligibly interacts with cholinergic muscarinic receptors (8). Thus the 5-HT_2 blocking activity of SM-9018 might contribute to its reduced activity in inducing bradykinesia.

ACKNOWLEDGEMENTS

We thank Mrs. Yoko Ueda, Miss Hitomi Matsuno, and Mr. Kenji Matsumoto for their skillful technical assistance.

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