Behavioral Effects of HR-592, a New Derivative of Indole

Yutaka GOMITA, Yasuyuki ICHIMARU\(^1\), Minehiro MORIYAMA\(^1\), Katsushi FURUNO, Katsuya SUEMARU, Fatima E. OSMAN\(^2\) and Yasunori ARAKI

Department of Hospital Pharmacy, Okayama University Medical School, Okayama 700, Japan
\(^1\)Department of Pharmacology, Daichi College of Pharmaceutical Sciences, Fukuoka 815, Japan

Accepted December 29, 1989

Abstract—Effects of HR-592 on various behaviors were investigated in rats and mice. 1) HR-592 at doses of 10–100 mg/kg, p.o., and chlorpromazine at doses of 2.5–20 mg/kg, p.o., suppressed dose-dependently spontaneous activities of mice. 2) In the mice treated with HR-592, 10 and 30 mg/kg, p.o., and with chlorpromazine, 1.25–5 mg/kg, p.o., the durations of loss of the righting reflex induced by thiopental-Na were extended in a dose-dependent manner. 3) In the mice and rats when HR-592 was administered at doses of 3–100 mg/kg, p.o., catalepsy was induced in a dose-dependent manner. 4) The incidence of catalepsy induced by haloperidol in mice was reduced dose-dependently after HR-592 administration (10–100 mg/kg, p.o.). 5) Dose-dependent suppressions of the slant of screen at which the mice slipped down were observed by HR-592 at 3–100 mg/kg, p.o., and chlorpromazine at 5–20 mg/kg, p.o. 6) The rota rod performance in mice was suppressed dose-dependently by HR-592, 3–100 mg/kg, p.o., and chlorpromazine, 5–20 mg/kg, p.o. 7) HR-592 at doses of 0.3–3 mg/kg, i.p., suppressed dose-dependently the turning behavior induced by methamphetamine in unilateral substantia nigra-lesioned rats. From these results and our previous data, it is considered that HR-592 has pharmacological properties as a major tranquilizer, although its behavioral effect is slightly weaker than that of chlorpromazine. Furthermore, these results imply that HR-592 has anti-cataleptogenic activity and might thereby alleviate the adverse effect of neuroleptics such as haloperidol.

Two representative compounds which contain an indole skeleton in their structures are reserpine, a Rauwolfia alkaloid, and LSD-25, a substance which induces psychological disturbances. Reserpine has an anti-hypertensive effect but induces depression. This agents is, therefore, not frequently used for the therapy of schizophrenia but is still used for that of hypertension. The mechanism of action of reserpine is thought to involve the depletion of catecholamines and serotonin in the central nervous system (1). On the other hand, LSD-25 is well-known as a potent hallucinogen (1). Furthermore, bufotenine, psilocybin, etc., other compounds which contain the indole skeleton in their structures, have unique pharmacological activities (2).

HR-592 is a new compound which also contains the indole skeleton with a CI radical at the 5-position of the indole ring, as shown in Fig. 1. Therefore, we are interested in the pharmacological activities of the new compound. We have found HR-592 to be pharmacologically similar to neuroleptics, in that it causes suppression of the conditioned avoidance response and intracranial self-stimulation behavior in rats (3). These findings indicate that HR-592 may have neurolep-
tic-like activity, but there has been no report concerning the detailed behavioral effects of this compound. For these reasons, we performed the present investigation in hopes of elucidating the actions of HR-592 on the central nervous system using behavioral techniques.

Materials and Methods

Animals: The animals used in the present study were male ddY mice and male Wistar rats. The body weights and the numbers of animals used are given in the description of each experiments. Twenty–25 mice and 3–4 rats per cage were housed in a breeding room maintained at 22±2°C and illuminated during 9:00–21:00. Food and water were provided ad libitum.

Drugs: Drugs used were HR-592 (powder, Hoechst Co.), chlorpromazine (powder, Yoshitomi Pharmaceutical Co.), haloperidol (for injection, Dainippon Pharmaceutical Co.), thiopental-Na (powder, Tanabe Pharmaceutical Co.) and methamphetamine (powder, Dainippon Pharmaceutical Co.). HR-592 was suspended in purified water containing 1% Tween 80; and chlorpromazine, which was used as a control drug, was dissolved in purified water. Other drugs were diluted with physiological saline. The concentration of each drug was adjusted so that a volume of 0.1 ml could be administered per 10 g of body weight to mice and per 100 g to rats. For the vehicle control groups in HR-592 and chlorpromazine-administration experiments, 1% Tween 80 solution and physiological saline were administered to the animals, respectively.

Gross behavior: Fourty-five male ddY mice weighing 19–24 g were used. The animals were randomly divided into 7 groups of 6–7 animals each to avoid the intergroup differences in body weights. HR-592 was orally administered at doses of 1, 3, 10, 30, 100 and 300 mg/kg, and then the general behaviors of each animal was observed for 4 hr in the home cage. The lethality was thereafter checked at 24, 48 and 72 hr after the administration.

Spontaneous activity: One hundred male ddY mice weighing 20–28 g were used. The spontaneous activity of the animals was measured by Hall’s open-field apparatus (4). This apparatus was shaped like a bucket, with a bottom diameter of 60 cm, a wall height of 50 cm and a diameter of 80 cm at the top. The inner surface of this apparatus was painted gray, and the bottom was divided into 19 blocks of practically equal area by black lines. During a test period (1 min), the ambulation (locomotor activity) was measured as the number of times an animal crossed a line. At the same time, other behaviors, such as rearing, preening, defecation and urination, were measured. The test was carried out once prior to the administration of the test drug, and the animals were randomly divided into 2 groups for the HR-592 and chlorpromazine administration experiments, and each group was divided into 6 and 5 groups for the former and the latter experiments, respectively, to avoid the intergroup differences in the mean ambulation values. Then, tests were carried out at 0.5, 1, 2, 4 and 24 hr after the HR-592 administration and at 0.5, 1, 2, 4, 6 and 24 hr after the chlorpromazine administration. These drugs were administered orally. The data obtained were statistically analyzed by a two-tailed t-test (5).

Potentiation of anesthetics: Eighty male ddY mice weighing 20–35 g were randomly divided into 2 groups for HR-592 and chlorpromazine administration experiments, and each group was divided into 4 and 5 groups for the former and the latter experiments, respectively, to avoid the intergroup differences in the body weights. At 1 hr after the oral administration of test drug, thiopental-Na (40 mg/kg) was administered intravenously. Then, the duration of the loss of the righting reflex was measured. The obtained data were statistically analyzed by the two-tailed Mann-Whitney U-test (5).

Cataleptogenic and anti-cataleptogenic activity: Ninety-two male ddY mice weighing 18–25 g were randomly divided into 6 groups...
of 15–16 animals each, and 24 male Wistar rats weighing 120–180 g were randomly divided into 6 groups. The cataleptogenic activity was evaluated by the method of Zetler et al. (6). In this test, the animal’s forelimbs were put on a stainless steel rod (2 mm in diameter) that was suspended at a height of 6.5 cm from the floor, and the cataleptogenic activity was judged by whether the animal could maintain this unusual posture for 30 sec or not. The test was carried out at 1 and 2 hr after the oral administration of test drug.

In addition, to investigate the effect of HR-592 on haloperidol-induced catalepsy, 107 male mice weighing 18–25 g were randomly divided into 6 groups of 17–18 animals each. At 30 min after the oral administration of a test drug haloperidol (1 mg/kg) was administered intraperitoneally, and the test was carried out at 0.5 and 1 hr after the administration of haloperidol. The obtained data were statistically analyzed by the two-tailed Fisher’s exact probability test (5).

Muscle-relaxation activity: One hundred male ddY mice weighing 20–28 g were used. For the measurement of muscle relaxation, an inclined screen test was used. The inclined screen apparatus consisted of canvas, and the slant of the canvas screen could be increased gradually. The animal was placed on the screen with its head facing downward, and the slant of the screen was increased at a rate of 4.5/sec, and the slant at which the animal slipped down was recorded. Each animal was tested triplicate, and the mean value of the slants was calculated. The test was carried out prior to the oral administration of the test drug. The animals were randomly divided into 2 groups for the HR-592 and chlorpromazine-administration experiments, and each group was further divided into 6 and 5 groups for the former and the latter experiments, respectively, to avoid the intergroup differences in the body weights. The obtained data were statistically analyzed by the two-tailed Fisher’s exact probability test (5).

Motor coordination: One hundred male ddY mice weighing 20–28 g were randomly divided into 2 groups for HR-592- and chlorpromazine-administration experiments, and then each group was further divided into 6 and 5 groups for the former and the latter groups, respectively, to avoid the intergroup differences in the body weights. For the measurement of the effects on motor coordination, a rotarod test was used. The diameter of the rod was 2.5 cm, and it was rotated at the speed of 20 rpm. The animal was placed on the rod facing against the revolution of the rod, and the effect on the motor coordination was evaluated by whether the animal fell off the rod within 3 min. This test was carried out at 0.5, 1, 2, 4 and 24 hr after the oral administration of HR-592 and at 0.5, 1, 2, 4, 6 and 24 hr after the chlorpromazine administration. The obtained data were statistically analyzed by a two-tailed Fisher’s exact probability test (5).

Turning behavior: To test the anti-dopaminergic activity of a test drug, the methamphetamine induced turning behavior in unilateral nigral lesioned rat was employed. Twenty-five male Wistar rats weighing 200–250 g (at surgery) were used. The animal’s head was placed on a stereotaxic apparatus under pentobarbital-Na anesthesia. According to the De Groot’s brain atlas (7), the unilateral substantia nigra (A: 2.2, L: 2.0, V: −1.7) was lesioned by the electric coagulation method (by 1 mA for 10 sec). The animal was allowed to recover from the surgery for at least one week. Then 25 min after the intraperitoneal administration of methamphetamine (5 mg/kg), the ipsilateral turning was measured for 10 min. The open-field apparatus described above was used for the test. Animals that turned 1/min or more by the administration of methamphetamine were employed for the experiments. A week later, the animals were randomly divided into 5 groups to avoid the intergroup differences in the mean numbers of turns. To determine the effect of a test drug, it was administered simultaneously with 5 mg/kg of methamphetamine by intraperitoneal injection. Then 25 min later, the ipsilateral turning was measured for 10 min. The obtained data were statistically analyzed by the two-tailed t-test (5).
Results

Gross behavior: There was no difference of the gross behaviors of the animals between the group treated with 1 mg/kg HR-592 and the control vehicle group. However, in the 3 mg/kg-treated group, sedation was observed in 4 of the 6 animals for 30 min after the administration. Otherwise, there was no difference from the control group. In the 10 mg/kg-treated group, sedation was observed in 5 of 6 animals for 1 hr after the administration, and 2 of 5 animals also showed marked ataxia. In the 30 mg/kg- and higher doses-treated groups, similar signs were observed; and in the latter 2 groups (treated with 100 and 300 mg/kg), all of the animals showed ataxia and no spontaneous activity, but no loss of the righting reflex. These effects persisted for 4 hr after the administration in these groups. However, abnormal behavior, such as convulsion, head twitch, etc., was not observed in the animal treated with any dose of HR-592. In addition, even in the highest dose-treated group, no lethality was observed within 72 hr after the administration.

Spontaneous activity: The effect of HR-592 on the spontaneous activity is shown in Fig. 2. Up to a dose of 3 mg/kg, the HR-592-treated group showed almost no difference from the control group, but the 10 to 100 mg/kg-treated groups showed a dose-dependent suppression of spontaneous activity. Compared with the control group, these effects were significant at 2 hr (P<0.01) and 4 hr (P<0.05) after the administration of 30 mg/kg, and from 1 to 4 hr (P<0.05, respectively) after 100 mg/kg.

The effect of chlorpromazine is shown in Fig. 3. A dose-dependent suppression of spontaneous activity was observed in the chlorpromazine-treated groups at the doses of 2.5–20 mg/kg. There were significant differences at 2 hr (P<0.05) after the 2.5 mg/kg administration; at 1 (P<0.01) and 2 hr (P<0.05) after the 5 mg/kg administration; at 0.5, 1 and 2 hr (P<0.01, respectively) after the 10 mg/kg administration; and at 0.5, 1 (P<0.01, respectively), 2 and 4 hr (P<0.05, respectively) after the 20 mg/kg administration.

Potentiation of anesthetics: Figure 4 shows the effects of HR-592 on the thiopental-Na induced anesthesia. In the control group, the disappearance of righting reflex was observed for approximately 6 min following the administration of thiopental-Na. In the group treated with 3 mg/kg of HR-592, there was no difference from the control group in the duration of loss of the righting reflex. In the treated groups with 10 and 30 mg/kg, the durations were extended to approximately 33 (P<0.05) and 47 min (P<0.01), respectively.

![Fig. 2. Effect of HR-592 on locomotor activity in mice. HR-592 was administered orally. The locomotor activity was measured for 1 min using Hall's open-field apparatus (4). HR: HR-592.](image-url)
Fig. 3. Effect of chlorpromazine on locomotor activity in mice. Chlorpromazine was administered orally. The locomotor activity was measured by the same method as used in the HR-592 experiment. CP: chlorpromazine.

Fig. 4. Effect of HR-592 on thiopental sleeping time in mice. Thiopental-Na at a dose of 40 mg/kg was administered intravenously 1 hr after the oral administration of HR-592. Numbers of animals used are given in parentheses. v: vehicle.

Figure 5 shows the effects of chlorpromazine on it. In the groups treated with 1.25, 2.5 and 5 mg/kg, the durations were extended to approximately 20, 30 (P<0.05, respectively) and 70 min (P<0.01), respectively.

Cataleptogenic activity: At 1 hr after the oral administration of HR-592 at the doses of 1 to 100 mg/kg, catalepsy was induced dose-dependently in mice (Fig. 6). The incidence of catalepsy was approximately 20% by the administration of 1 mg/kg and 33.3% (P<0.05) by 3 mg/kg. In addition, in the groups treated with higher doses of HR-592 (from 10 to 100 mg/kg), the incidences of catalepsy were 56, 3, 56.3 and 73.3%, respectively. Each of these values was significantly different (P<0.01, respectively) from the control value. At 2 hr after the administration, similar but higher incidences of catalepsy were observed at each dose of HR-592. These effects persisted until 4 hr after the administration.

Similar cataleptogenic activity of HR-592 was observed in rats (Fig. 7). At 1 hr after the administration, the incidences of catalepsy in the 30 and 100 mg/kg-treated groups were approximately 50 and 75%, respectively, while at 2 hr after, the incidences in the 10, 30 and 100 mg/kg-treated groups were 50, 100 and 100%, respectively. The latter two values were significantly different (P<0.05, respectively) compared with the control group.

Anti-cataleptogenic activity: Figure 8 shows the effect of HR-592 on the catalep-
Fig. 5. Effect of chlorpromazine on thiopental sleeping time in mice. Experimental procedure and abbreviations in figure are the same as those for the HR-592 experiment.

Fig. 6. Cataleptogenic activity of HR-592 in mice. The cataleptogenic activity was evaluated by the method of Zetler et al. (11). The test was carried out at 1 (left in figure) and 2 hr (right in figure) after the oral administration of HR-592. Numbers of animals used are given in parentheses. v: vehicle.

togenic activity of haloperidol. At 0.5 and 1 hr after the administration of haloperidol alone, catalepsy was observed in all of the animals. However, when HR-592 (10 mg/kg) was administered 30 min prior to haloperidol, the incidence of catalepsy induced by haloperidol was slightly reduced; and by the pretreatment of 30 and 100 mg/kg of HR-592, significant suppressions (P<0.01 at 1 hr after and P<0.05 and P<0.01 at 1.5 hr after, respectively) were observed. In Fig. 8, the time of 1 or 1.5 hr represents the time elapsed after the administration of HR-592.

Muscle-relaxation: Figure 9 shows the results of HR-592 on the inclined screen test in mice. HR-592 treatment caused a dose-
Fig. 7. Cataleptogenic activity of HR-592 in rats. Experimental procedures and abbreviations in this figure are the same as those in the experiments on mice.

Fig. 8. Effect of HR-592 on catalepsy induced by haloperidol in mice. Haloperidol (1 mg/kg) was administered intraperitoneally 30 min after the oral administration of HR-592. The test was carried out at 0.5 (left in figure) and 1 hr (right in figure) after the administration of haloperidol (HPD). Numbers of animals used are given in parentheses.

dependent suppression of the slant of the screen at which the animal slipped down. In the group treated with 1 mg/kg HR-592, no significant difference was observed, but in the 3 to 100 mg/kg-treated groups, significant differences were observed at 0.5, 1 and 2 hr (P<0.05, respectively) after the 3 mg/kg administration; at 0.5 hr (P<0.05) after the 10 mg/kg administration; at 0.5, 1 and 2 hr (P<0.01, respectively) after the 30 mg/kg
administration; and at 0.5 (P<0.01), 1 (P<0.05) and 2 hr (P<0.01) after the 100 mg/kg administration. These effects lasted from 1 to 4 hr after the administration of HR-592.

Figure 10 shows the results of chlorpromazine. A dose-dependent suppression of the slant of the screen was observed. Significant differences were observed at 1, 2 and 4 hr (P<0.01, respectively) after the 5 mg/kg administration; at 1 and 2 hr (P<0.01, respectively) after the 10 mg/kg administration; and at 0.5, 1, 2 (P<0.01, respectively) and 4 hr (P<0.05) after the 20 mg/kg administration.

**Motor coordination:** The effect of HR-592 on the motor coordination is shown in Fig. 11. In the 1 to 100 mg/kg of HR-592-treated groups, the animal's motor coordination was suppressed in a dose-dependent manner.
Moreover, in the 10 mg/kg or higher dose-treated groups, significant differences from the control group were observed at 1 and 2 hr (P<0.05, respectively) after the 10 mg/kg administration; at 0.5, 1, 2 (P<0.01, respectively) and 4 hr (P<0.05, respectively) after the 30 mg/kg administration; and at 0.5, 1, 2 and 4 hr (P<0.01, respectively) after the 100 mg/kg administration. Motor incoordination appeared in all of the animals at 1 and 2 hr after the administration.

Figure 12 shows the effects of chlorpromazine on it. In the 2.5 to 20 mg/kg of chlorpromazine-treated groups, the motor coordination was suppressed in a dose-dependent manner. Significant differences were observed at 0.5, 1, 2 (P<0.01, respectively), 4 and 6 hr (P<0.05, respectively) after the 10 mg/kg administration.
Fig. 13. Effect of HR-592 on methamphetamine-induced turning in unilateral substantia nigra lesioned rats. HR-592 was administered simultaneously with 5 mg/kg of methamphetamine by intraperitoneal injection. Then 25 min later, the ipsilateral turning was measured for 10 min. Numbers of animals used are given in parentheses. v: vehicle.

administration and at 0.5, 1, 2, 4 and 6 hr (P < 0.01, respectively) after the 20 mg/kg administration.

Turning behaviour: Figure 13 shows the effect of HR-592 on the methamphetamine-induced turning behavior in unilateral substantia nigra lesioned rats. In the control group, the number of turns during a 10 min period was approximately 70 following the administration of methamphetamine at 5 mg/kg. No difference of turning was observed in the 0.1 mg/kg, i.p.-treated group, but in the 0.3 mg/kg, i.p.-treated group, the turning was suppressed to about half of control value. In the 1 and 3 mg/kg, i.p.-treated groups, the turning was significantly suppressed (P < 0.05, respectively).

Discussion

The results of various experiments performed in rats and mice indicate that HR-592 has following activities: suppression of spontaneous activity, potentiation of thiopental-Na anesthesia, cataleptogenic activity, muscle relaxing activity, suppression of motor coordination and suppression of methamphetamine-induced turning. Considered from the present results and the previous data (3), the profile of action of HR-592 is similar to that of neuroleptics such as chlorpromazine or haloperidol (8).

In addition, except for the turning test in which the route of administration was different (i.p. vs. p.o.), significant differences were observed from a dose of 3 mg/kg of HR-592 in the cataleptogenic and muscle-relaxing activities and from a dose of 10 mg/kg in the suppressive effect of spontaneous activity and the potentiating effect of tiopental sleeping time. In the turning test, significant suppression was observed from a dose of 1 mg/kg, i.p. With regard to the anti-amphetamine activity of HR-592, it has been found that the oral administration requires nearly 3 times of the dosage as intraperitoneal administration (from C. Dumont, and our preliminary test). We concluded that an intraperitoneal dose of 1 mg/kg of HR-592 is roughly equivalent to an oral dose of 3 mg/kg. On the basis of these findings and influences, it can be concluded that the cataleptogenic, muscle-relaxing and anti-methamphetamine activities are the prominent activities of HR-592.

It is thought that the cataleptogenic and anti-methamphetamine activities are due to the blockade of dopaminergic receptors in the nigrostriatum (9) and limbic systems (10). It is also well-known that anti-dopaminergic activity is essential for neuroleptic activity (11). Because HR-592 has quite potent cataleptogenic and anti-methamphetamine activities, it is suggested that HR-592 has a neuroleptic-like activity. Furthermore, HR-592 showed anti-cataleptogenic activity against haloperidol-induced catalepsy. The mechanism involved in the anti-cataleptogenic activity of HR-592 is not clear at the present, but it may be due to it's potent muscle-relaxing activity.

Acknowledgments: The authors thank Mr. Y. Deguchi and Mr. H. Ono for their technical assistance; Dr. Wolfgang R. Ade, R & D Department, Roussel Japan for critical reading of this manuscript; and Hoechst Japan and Roussel Japan for supplying HR-592.

References

1 Weiner, N.: Drugs that inhibit adrenergic nerve-
ous and block adrenergic receptors. In *The Pharmacological Basis of Therapeutics*, Edited by Gilman, A.G., Goodman, L.S., Rall, T.W. and Murad, F., 7th ed., p. 181–214, MacMillan, New York (1985)

2 Jaffe, J.H.: Drug addiction and drug abuse. In *The Pharmacological Basis of Therapeutics*, Edited by Gilman, A.G., Goodman, L.S., Rall, T.W. and Murad, F., 7th ed., p. 532–581, MacMillan, New York (1985)

3 Ichimaru, Y., Gomita, Y., Araki, Y. and Moriyama, M.: Effects of HR-592, a new derivative of indole, on conditioned behavior. Acta Medica Okayama 44, 35–39 (1990)

4 Hall, S.S.: Emotional behavior in the rat: I. Defecation and urination as measures of individual differences in emotionality. J. Comp. Physiol. 18, 385–403 (1934)

5 Siegel, S.: *Nonparametric Statistics for Behavioral Sciences*, McGraw-Hill, New York (1956)

6 Zetler, G., Mahler, K. and Daniel, F.: Versuche zu einer pharmakologischen Differenzierung kataleptischer Wirkungen. Arch. Exp. Pathol. Pharmacol. 238, 486–501 (1960)

7 De Groot, J.: *The Rat Forebrain in Stereotaxic Coordinates*, N.V. Noord-Hollandsche Uitgevers Maatschappij, Amsterdam (1959)

8 Ueki, S., Ogawa, N., Watanabe, S., Gomita, Y., Araki, Y., Fujiwara, M., Kamei, C., Shimomura, K., Inoue, M., Ohishi, R., Ibib, N. and Tanaka, K.: Behavioral and electroencephalographic effects of clozapine (W-801). Folia Pharmacol. Japon. 69, 85–119 (1973) (Abs. in English)

9 Hornykiewicz, O.: Dopamine in the basal ganglia. Its role and therapeutic implications (including the clinical use of L-DOPA). Br. Med. Bull. 29, 172–178 (1973)

10 Dunnett, S.B., Bunch, S.T., Gage, F.H. and Bjorklund, A.: Dopamine-rich transplants in rats with 6-OHDA lesions of the ventral tegmental area. I. Effects on spontaneous and drug-induced locomotor activity. Behav. Brain Res. 13, 71–82 (1984)

11 Ohtsuki, S. and Sakakihara, K.: New trends in biological studies on schizophrenia. Japan. J. Neuropsychopharmacol. 5, 301–343 (1983)