Pharmacological Studies on the Novel Antiallergic Agent HSR-609: Its Effects on Behavior in Mice and Electroencephalograms in Rabbits

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Received March 12, 1997 Accepted June 18, 1997

ABSTRACT—We studied the central nervous system (CNS) effects of HSR-609 (3-[4-(8-fluoro-5,11-dihydrobenz[b]oxepino[4,3-b]pyridin-11-ylidene)piperidino]propionic acid dihydrate), a novel amphoteric antiallergic agent having antihistaminic activity. Its effects on the behavior of mice and the electroencephalograms (EEG) of unanesthetized and unrestrained rabbits after oral administration were compared with those of typical antiallergic agents and the non-amphoteric basic compound PY-608 (8-fluoro-5,11-dihydro-11-(1-methyl-4-piperidylidene)benz[b]oxepino[4,3-b]pyridine), which has a chemical structure similar to that of HSR-609. HSR-609 (3–300 mg/kg) had no effect on general behavior, spontaneous locomotor activity, hexobarbital-induced sleeping time and reserpine-induced hypothermia in mice. HSR-609 (10–100 mg/kg) and terfenadine (100 mg/kg) had no effect on spontaneous EEG, sleep-wakefulness cycles and EEG power spectra in rabbits. On the other hand, cyproheptadine (3–30 mg/kg), ketotifen (30–100 mg/kg) and PY-608 (0.3–100 mg/kg) caused increases and/or decreases of spontaneous locomotor activity, prolongation of hexobarbital-induced sleeping time and antagonistic effects on reserpine-induced hypothermia in mice. These agents (30 mg/kg) increased slow wave sleep and enhanced EEG power spectra at low frequency bands such as δ and θ in rabbits. These findings suggest that HSR-609 has no inhibitory effect on the CNS due to its amphoteric chemical structure.

Keywords: Antiallergic agent, Central nervous system effect, Behavior (mouse), Electroencephalogram (rabbit), HSR-609

We have synthesized many amphoteric compounds through zwitter-ionization of classical tricyclic and basic antihistamines by conversion of N-alkyl groups into N-alkylenecarboxy groups to develop non-sedative antiallergic agents with potent antihistaminic activities (1–6). Our investigations of the potencies of these compounds as well as their depressant effects on the central nervous system (CNS) showed that the combination of zwitter-ionization and introduction of a pyridine component into the tricyclic antihistamines is important for attaining higher hydrophilicity, which is considered to be one of the factors reducing penetration into the CNS (6). Our efforts finally led to a new amphoteric compound, HSR-609 (3-[4-(8-fluoro-5,11-dihydrobenz[b]oxepino[4,3-b]pyridin-11-ylidene)piperidino]propionic acid dihydrate) (Fig. 1) (6). Various pharmacological studies using mice, rats, guinea pigs and dogs have shown that HSR-609 has potent antiallergic and antihistaminic activities in vivo (6, 7). HSR-609 had high affinity only for histamine H1-receptors and low affinity for muscarinic and serotonin 5-HT2-receptors (8, 9). HSR-609 displayed poor ability to penetrate into the CNS in mice and guinea pigs due to its amphoteric chemical structure as determined by the ex vivo displacement of [3H]mepyramine binding to H1-receptors (6, 8, 9).

As details of the effects of HSR-609 on the CNS had not yet been examined, we conducted various behavioral

![Fig. 1. Chemical structures of HSR-609 and PY-608.](image-url)
analyses with mice and electroencephalographic (EEG) analysis with rabbits to directly evaluate its effects on brain electrical activity. We then tried to evaluate the pharmacological difference and/or similarity on the CNS effects under the same experimental conditions for HSR-609 and various typical sedative antiallergic agents, such as cyproheptadine hydrochloride, ketotifen fumarate, azelastine hydrochloride and oxatomide, and also the non-sedative antiallergic agent terfenadine (10–14). Furthermore, we also tried to determine if the amphoteric chemical structure of HSR-609 is related to its lesser CNS effects, by comparing it with the non-amptheric basic compound PY-608 (8-fluoro-5,11-dihydro-11-(1-methyl-4-piperidylidene)benz[b]oxepino[4,3-b]pyridine), which has a similar chemical structure (Fig. 1).

MATERIALS AND METHODS

Animals
Male ICR mice (Charles River, Kanagawa) and male Japanese White rabbits (Kitayama Labes, Kyoto) were used. The animals were fasted for more than 16 hr before oral administration of the test compounds.

Effect on general behavior in mice
Mice (5-week-old) in groups of 4 animals each were used. Each mouse was put in a transparent plastic cage (W: 170 × D: 235 × H: 125 mm). The general behavior of each animal was observed at 0.5, 1, 2, 4, 6, 8 and 24 hr after oral administration of the test compounds according to the method of Irwin (15). Food and water were given 8 hr after the administration.

Effect on spontaneous locomotor activity in mice
Mice (5-week-old) in groups of 10 animals each were used. After oral administration of the test compounds, each mouse was immediately put into a transparent plastic cage (W: 295 × D: 345 × H: 175 mm). The spontaneous locomotor activity was measured continuously for 4 hr after the administration using SCANET (MV-10; Toyo Sangyo, Toyama), with a sensor of near infrared rays (870 nm) installed at 6-mm intervals.

Effect on hexobarbital-induced sleeping time in mice
Mice (5-week-old) in groups of 10 animals each were used. Mice were orally administered the test compounds and then were injected with hexobarbital (80 mg/kg, i.p.) 1 hr later. The sleeping time was measured from the time of loss to restoration of the righting reflex. The ID$_{50}$ values and their 95% confidence limits were calculated from the number of animals showing more than 50% inhibition at each dose by comparison with the mean sleeping time of the vehicle control according to the method of Tallarida and Murray (16).

Effect on reserpine-induced hypothermia in mice
Mice (5-week-old) in groups of 8 animals each were used. The mice were injected with reserpine (2 mg/kg, s.c.), and then were made to fast. After 18 hr, the test compounds were administered orally. The rectal temperature was measured with a thermometer (BAT-12; Physitemp Instruments, Clifton, NJ, USA) before and 0.5, 1, 2 and 4 hr after the administration.

Effect on spontaneous EEG, gross behavior and heart rate in rabbits
Preparation of chronic animals with indwelling brain electrodes: Under pentobarbital sodium anesthesia (35 mg/kg, i.v.), rabbits (2.5–3.4 kg) were fixed on a universal stereotaxic instrument (Summit Medical, Tokyo), and EEG electrodes were implanted according to the method of Yamamoto et al. (17). Bipolar silver ball electrodes (0.5–1.0 mm diameter, 2–3 mm apart) were placed on the epidural surface of the frontal cortex (FC) of the animals. Bipolar stainless-steel electrodes (0.25 mm diameter, 0.5 mm apart) insulated except for the tip of 0.5 mm (OKY91-042; Unique Medical, Tokyo) were inserted stereotaxically into the left amygdala (AMY: A; 2, L; 6, H; -6) and the left hippocampus (HIP: P; 2, L; 4, H; +5) according to the brain atlas of Sawyer et al. for rabbits (18). Each electrode was soldered to a rectangular connector (HDAB-15; Hirose Denki, Tokyo), secured to the skull and fixed with dental cement (Yata Polyset; Yata Chemical, Osaka). A recovery period of at least 2 weeks was allowed before the start of experiments. The animal was given penicillin G potassium (5 × 10$^4$ units, s.c.) prophylactically to reduce infection.

Polygraphic recording of EEG: The polygraphic recording was always carried out with an unanesthetized and unrestrained rabbit placed in a soundproof and electrically shielded room after the animal was sufficiently accustomed to the experimental environment. The EEG polygram, consisting of EEG, electromyogram (EMG) of the posterior neck muscle, and Lead II electrocardiogram (heart rate), was recorded on an electroencephalograph (EEG-5414; Nihon Kohden, Tokyo) and also on a magnetic tape recorder (A-69; Sony, Tokyo) for subsequent computer analysis of the EEG. After the control observation of behavior and EEG polygram for 1 hr, the test compound was administered orally to the animal. Recording of the EEG polygram usually started at 10 o’clock and continued for 7 hr. Behavior changes were observed macroscopically. The results were compared with those of the same animal given the vehicle only. Different test compounds were administered to the same
animal after an interval of at least 10 days.

**Gross behavior, EEG pattern and sleep-wakefulness cycles:** The observation of gross behavior and evaluation of EEG pattern were performed by the method of Yamamoto et al. (17). The EEG recordings were visually classified into the following five stages: awake, rest, slow wave light sleep (SWLS), slow wave deep sleep (SWDS) and rapid eye movement sleep (REMS). The diagrammatic representation of continuous changing of EEG levels is called the EEG sleep-wakefulness cycle (polysonmogram). The accumulated times of each EEG level for the initial 4 hr and each sleep-wakefulness cycle for 7 hr after the administration were compared with those of the vehicle control.

**EEG power spectral analysis:** Using an ATAC-450 system (Nihon Kohden), EEG power spectra were derived from the magnetically recorded EEG during the initial 4 hr after the test compound administration. The EEG was converted into a digital value with a resolution of 10 bits per 9.76 msec and processed by Fast Fourier Transformation. Each segment of the power spectral density for 30 sec in the frequency range of 0–30 Hz with a resolution of 0.4 Hz was added to obtain one power spectrum. The continuous plotted EEG power spectra, called the EEG power spectral array (17), was recorded on an XY plotter (7475A; Hewlett Packard, San Diego, CA, USA). The power spectra was classified into four frequency bands defined as δ (1.2–3.6 Hz), θ (4.0–7.6 Hz), α (8.0–12.8 Hz) and β (13.2–29.6 Hz). The individual and total frequencies of the power spectra were obtained from the initial 4 hr after the administration, and the relative potency of the power spectra was calculated in comparison with that of the vehicle control.

**Drugs**

The following agents were purchased: cyproheptadine (cyproheptadine hydrochloride; Pharmaceutia, Milan, Italy); ketotifen (ketotifen fumarate; Orion Chemicals, Espoo, Finland); terfenadine and imipramine (imipramine hydrochloride) (Sigma Chemical, St. Louis, MO, USA); scopolamine (scopolamine hydrobromide) and hexobarbital (Tokyo Kasei Kogyo, Tokyo); reserpine (Apoplon; Daiichi Pharmaceutical, Tokyo); pentobarbital sodium (Nembutal injection; Dainippon Pharmaceutical, Osaka); and penicillin G potassium (Meiji Seika, Tokyo). HSR-609, PY-608, azelastine (azelastine hydrochloride) and oxatomide were synthesized at the Research and Development Division, Hokuriku Seiyaku Co., Ltd. HSR-609, PY-608, cyproheptadine, oxatomide and terfenadine were suspended in 5% gum arabic, while ketotifen, azelastine, imipramine and scopolamine were dissolved in distilled water; all of the agents were administered orally.

**Statistical analyses**

All data are expressed as means±S.E. Statistical analyses were carried out using the Yukms Statistical Library (Yukms, Tokyo). Statistical differences among multiple groups were determined by Dunnett's multiple comparison test, while statistical differences between two groups were determined by the paired Student's t-test. P values less than 0.05 were considered statistically significant.

**RESULTS**

**Effect on general behavior in mice**

The effects on general behavior in mice after oral administration of HSR-609, PY-608 and typical antiallergic agents were evaluated. HSR-609, oxatomide and terfenadine at doses of 3–300 mg/kg displayed no effect on the general behavior during 24 hr after administration. On the other hand, PY-608 at doses of 10 and 30 mg/kg caused restlessness, and doses of more than 30 mg/kg caused depression of gross behavior and decrease of respiratory rate. At more than 100 mg/kg, flattened body posture, straub tail and tremor were observed. At a dose of 300 mg/kg, clonic convulsions occurred in all animals and one of four animals died at 129 min after the administration. Cyproheptadine at a dose of 3 mg/kg had no effect, while a dose of 10 mg/kg caused restlessness. Doses of more than 30 mg/kg caused depression of gross behavior, decrease of respiratory rate, flattened body posture, muscle relaxation, disturbance of gait and tremor. At 100 mg/kg, there was a decrease in the response to pain stimulation and the appearance of ptosis, hypothermia, salivation and cyanosis, and three of four animals died at 411–1440 min after the administration. At a dose of 300 mg/kg, clonic convulsions occurred in three of four animals and all animals died at 32–111 min after the administration. Ketotifen at doses of 3–30 mg/kg had no effect, while a dose of 100 mg/kg caused piloerection and depression of gross behavior, respiratory rate and response to pain stimulation. At a dose of 300 mg/kg, straub tail, tremor and clonic convulsions occurred, and all animals died at 11–46 min after the administration. Azelastine at doses of 3 and 10 mg/kg had no effect, while doses of more than 30 mg/kg caused depression of gross behavior and decrease of respiratory rate. At a dose of 100 mg/kg, flattened body posture, decrease of response to pain stimulation, muscle relaxation, disturbance of gait, ptosis, hypothermia, straub tail, tremor and clonic convulsions were observed. At a dose of 300 mg/kg, all animals died at 7–16 min after the administration.

**Effect on spontaneous locomotor activity in mice**

HSR-609 at doses of 10–300 mg/kg displayed no effect
on spontaneous locomotor activity in mice during 4 hr after the oral administration (Fig. 2). On the other hand, the activity increased for the initial 0.5 hr after the administration with PY-608 at doses of 3 - 10 mg/kg, for 0.5 - 1.5 hr with cyproheptadine at 10 mg/kg, for 2 - 4 hr with oxatomide at 100 and 300 mg/kg (data not shown) and for 0.5 - 1.5 hr with scopolamine at doses of 3 and 10 mg/kg. The activity decreased for the initial 0.5 - 1.5 hr after administration of PY-608 at a dose of 100 mg/kg, for 0.5 - 3.5 hr with cyproheptadine, for 0.5 - 1 hr ketotifen at 100 mg/kg, for 0.5 - 4 hr with azelastine at 30 - 100 mg/kg (data not shown) and 1.5 - 2 hr with terfenadine at 300 mg/kg.

**Effect on hexobarbital-induced sleeping time in mice**

One hour after oral administration of HSR-609 at doses of 10 - 300 mg/kg, there was no effect on the hexobarbital-induced sleeping time in mice, and the ID$_{50}$ value was > 300 mg/kg (Fig. 3). On the other hand, the sleeping time was prolonged by PY-608, cyproheptadine, ketotifen and terfenadine at doses of more than 30 mg/kg. It was also prolonged by azelastine and oxatomide at doses of more than 100 mg/kg (data not shown). The ID$_{50}$ values (95% confidence limits) of PY-608, cyproheptadine, ketotifen, azelastine, oxatomide and terfenadine were 14 mg/kg (4.9 - 41 mg/kg), 4.4 mg/kg (1.4 - 14 mg/kg), 21 mg/kg (13 - 35 mg/kg), 18

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**Fig. 2.** Time course of changes in spontaneous locomotor activity in mice after oral administration of HSR-609, PY-608, cyproheptadine, ketotifen, terfenadine and scopolamine. ○: vehicle control, △: 1 mg/kg, □: 3 mg/kg, ■: 10 mg/kg, ●: 30 mg/kg, ▲: 100 mg/kg, ■: 300 mg/kg. Each point represents the mean ± S.E. of 10 mice. *P<0.05, **P<0.01, compared with the vehicle control (Dunnett's multiple comparison test).
mg/kg (4.8–71 mg/kg), 41 mg/kg (18–94 mg/kg) and 72 mg/kg (27–190 mg/kg), respectively. Scopolamine at doses of 3–30 mg/kg had no effect on hexobarbital-induced sleeping time.

**Effect on reserpine-induced hypothermia in mice**

The rectal temperature in mice in each group ranged from 36.9±0.2 to 38.0±0.3°C, but 18 hr after the injection of reserpine, it had fallen to 29.7±0.5 to 31.3±0.7°C. HSR-609 at doses of 10–300 mg/kg did not show any influence on reserpine-induced hypothermia in comparison with that of the vehicle control (Fig. 4). On the other hand, the hypothermia was reversed with PY-608 at doses of 0.3–10 mg/kg, cyproheptadine at 3 and 10 mg/kg, ketotifen at 30 and 100 mg/kg, oxatomide at 30–300 mg/kg (data not shown) and imipramine at doses of 10 and 30 mg/kg. Azelastine at doses of 3–30 mg/kg (data not shown) and terfenadine at 30–300 mg/kg had no effect on the hypothermia.

**Effect on spontaneous EEG, gross behavior and heart rate in rabbits**

**EEG pattern, gross behavior and heart rate:** The effects on spontaneous EEG and gross behavior in unanesthetized and unrestrained rabbits with chronically indwelling brain electrodes were observed after oral administration...
of HSR-609, PY-608 and typical antiallergic agents. HSR-609 at doses of 10 and 100 mg/kg had no effect on EEG pattern, heart rate and gross behavior during the awake, rest, SWLS, SWDS and REMS episodes (Fig. 5). Ketotifen at a dose of 30 mg/kg and terfenadine at 100 mg/kg also had no effect on these parameters (data not shown). On the other hand, cyproheptadine at a dose of 30 mg/kg caused marked high amplitude slow waves of all EEG leads, particularly in the AMY lead, and depression of gross behavior (Fig. 6). These changes were also observed with PY-608 at a dose of 30 mg/kg and scopolamine at a dose of 10 mg/kg (data not shown). The heart rate for the vehicle control ranged from about 120 to 240 beats/min, while PY-608 at a dose of 30 mg/kg increased it in two of five rabbits, and the maximum value reached 310 beats/min. Cyproheptadine at a dose of 30 mg/kg increased the heart rate in two of five animals, and the maximum value reached 290 beats/min. Scopolamine also increased heart rate in three of five animals, and the maximum value reached 290 beats/min.

Sleep-wakefulness cycles: Even a large dose of HSR-609 such as 100 mg/kg had no obvious effect on the
Vehicle control

<table>
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<td>Behavior</td>
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HSR-609 100 mg/kg, p.o.

92 min 94 min 70 min 119 min

Lying Lying Lying Lying

Fig. 5. EEG patterns before and after oral administration of HSR-609 at 100 mg/kg in unanesthetized and unrestrained rabbit with chronically indwelling brain electrodes. AMY: amygdala, HIP: hippocampus, FC: frontal cortex, EMG: electromyogram at the posterior neck muscle, ECG: electrocardiogram lead II. SWLS: slow wave light sleep, SWDS: slow wave deep sleep, REMS: rapid eye movement sleep.

Vehicle control

<table>
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<tr>
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<th>Awake</th>
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<td>Behavior</td>
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Cyproheptadine 30 mg/kg, p.o.

162 min 86 min 26 min 306 min

Sitting Lying Lying Lying

Fig. 6. EEG patterns before and after oral administration of cyproheptadine at 30 mg/kg to a rabbit.
Fig. 7. Schematic representation of sleep-wakefulness cycles before and after oral administration of HSR-609 at 100 mg/kg to a rabbit.

Fig. 8. Schematic representation of sleep-wakefulness cycles before and after oral administration of PY-608 at 30 mg/kg to a rabbit.
Fig. 9. Schematic representation of sleep-wakefulness cycles before and after oral administration of cyproheptadine at 30 mg/kg to a rabbit.

Table 1. Effects of HSR-609, PY-608, scopolamine and typical antiallergic agents on EEG sleep-wakefulness cycles for the initial 4 hr in rabbits

<table>
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AW: awake + rest. Each value represents the mean±S.E. of 5 rabbits. (): Vehicle control. *P<0.05, **P<0.01, as compared with the vehicle control (paired Student's t-test).
periodic appearance of awake, rest, SWLS, SWDS and REMS episodes. No marked differences were seen in the sleep-wakefulness cycles between HSR-609 and the vehicle control for 7 hr after administration (Fig. 7). On the other hand, PY-608 and cyproheptadine at 30 mg/kg continuously and markedly increased the duration of SWDS and decreased the duration of awake, rest and REMS (Figs. 8 and 9). Table 1 presents the mean accumulated times of the four EEG levels (AW: awake + rest, SWLS, SWDS, REMS) obtained from five animals for the initial 4 hr after administration. HSR-609 at 10 and 100 mg/kg and terfenadine at 100 mg/kg had no effect on each EEG level in comparison with those of the vehicle control. On the other hand, PY-608 and cyproheptadine at doses of 10 and 30 mg/kg increased the duration of SWDS and decreased the duration of AW dose-dependently, and scopolamine at a dose of 10 mg/kg caused similar effects. Ketotifen at a dose of 30 mg/kg increased the duration of SWDS. A decrease of the duration of REMS was observed with PY-608 and cyprohep-
tadine at 30 mg/kg and scopolamine at 10 mg/kg.

**EEG power spectra:** To evaluate the objective and quantitative effect of antiallergic agents on EEG, analysis with EEG power spectra of all EEG leads was performed continuously for the initial 4 hr after the administration. HSR-609 at a dose of 100 mg/kg had no obvious effect on the power spectral array of all EEG leads in comparison with that of the vehicle control (Fig. 10). These results corresponded to previously described results of visual evaluation of EEG pattern and sleep-wakefulness cycles.

On the other hand, PY-608 at a dose of 30 mg/kg continuously and markedly enhanced the power spectra at low frequency bands (δ and θ) in all EEG leads (Fig. 11). These changes were also observed with cyproheptadine at a dose of 30 mg/kg and scopolamine at a dose of 10 mg/kg (data not shown).

Table 2 presents the relative changes of each EEG power spectra at four frequency bands (δ, θ, α and β) in the FC, which is said to be a typical indication of conscious levels, during 4 hr after administration of the test.

![Vehicle control](image1)

![PY-608 30 mg/kg, p.o.](image2)

**Fig. 11.** EEG power spectral arrays from the AMY, HIP and FC before and after oral administration of PY-608 at 30 mg/kg to a rabbit.
compounds in comparison with those of the vehicle controls. HSR-609 and terfenadine at a dose of 100 mg/kg had no effect on the power spectra at each frequency band. On the other hand, PY-608, cyproheptadine and ketotifen at a dose of 30 mg/kg and scopolamine at a dose of 10 mg/kg enhanced the power spectra at the low frequency bands (θ and δ) accompanied by an increase in the total frequency bands. As shown above, high amplitude slow waves of the EEG after administration of these agents was also confirmed by analysis of the EEG power spectra.

Table 2. Effects of HSR-609, PY-608, scopolamine and typical antiallergic agents on EEG power spectra of the frontal cortex for the initial 4 hr after administration in rabbits

<table>
<thead>
<tr>
<th>Dose (mg/kg, p.o.)</th>
<th>Increase of EEG power spectra (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>δ (1-3.6 Hz)</td>
</tr>
<tr>
<td>HSR-609 100</td>
<td>6 ± 6</td>
</tr>
<tr>
<td>PY-608 30</td>
<td>226 ± 21**</td>
</tr>
<tr>
<td>Cyproheptadine 30</td>
<td>93 ± 8**</td>
</tr>
<tr>
<td>Ketotifen 30</td>
<td>40 ± 9*</td>
</tr>
<tr>
<td>Terfenadine 100</td>
<td>−6 ± 7</td>
</tr>
<tr>
<td>Scopolamine 10</td>
<td>168 ± 56*</td>
</tr>
</tbody>
</table>

Change of each EEG power spectra were calculated by comparison with the mean response of the vehicle control, and the value represents the mean ± S.E. of 5 rabbits. *P < 0.05, **P < 0.01, as compared with the vehicle control (paired Student’s t-test).

DISCUSSION

In spite of the well-known depressant effects of sedative antiallergic agents and antihistamines on the CNS in humans, there have been few definite reports on systematic and comparative evaluation of the CNS effects of these agents using representative pharmacological methods such as general behavior, spontaneous locomotor activity, barbiturate-induced sleeping time and spontaneous EEG. Furthermore, some antihistamines such as diphenhydramine have both stimulant and depressant effects on the CNS, leading to different results with fine differences in experimental conditions, doses or route of administration (19, 20). Therefore, we considered what kind of pharmacological experimental methods would be the most suitable for objectively evaluating the CNS effects of antiallergic agents having antihistaminic effects. This led us to try to clarify the pharmacological characteristics of HSR-609 on the CNS in comparison with typical sedative and non-sedative antiallergic agents having antihistaminic effects under the same experimental conditions, using methods typically employed for behavioral analysis in mice and EEG analysis in unanesthetized and unrestrained rabbits with chronically indwelling brain electrodes.

The experimental results obtained from behavioral analysis with ICR mice are summarized in Table 3. HSR-609 displayed no effect on all spontaneous and induced behaviors. However, most of the sedative antiallergic agents showed stimulant and/or depressant effects on the general behavior and spontaneous locomotor activity, increased the hexobarbital-induced sleeping time and caused antagonistic effects on reserpine-induced hypothermia in mice. Our results using ICR mice also showed that cyproheptadine at low doses and oxatomide at high doses increased the spontaneous locomotor activity, in spite of cyproheptadine, ketotifen and azelastine at high doses decreasing it. Cyproheptadine was reported to have high affinity for muscarinic receptors in addition to high affinity for H1-receptors (9, 21). As scopolamine increased spontaneous locomotor activity in the mice, one of the reasons that cyproheptadine also increase it may be due to its antimuscarinic activity. Furthermore, all the tested sedative antiallergic agents prolonged hexobarbital-induced sleeping time, in agreement with previous reports (2, 20, 22). As in the case of imipramine, cyproheptadine and ketotifen showed antagonistic effects on reserpine-induced hypothermia, in agreement with previous reports (22, 23). This is the first evidence that oxatomide at high doses causes effects similar to these other agents. The sedative antiallergic agents having antihistaminic activity are known to cause sedative and stimulative side effects such as sedation, hypnosis, restlessness, insomnia, dizziness and headache at therapeutic doses (10-14). These findings indicated that most of the sedative antiallergic agents displayed depressant and/or stimulant effects in mice, and these effects seem to well reflect their clinical sedative and/or stimulant effects in humans. These side effects on the CNS may be due to the properties of their antimuscarinic and/or imipramine mimetic monoaminergic activities in addition to their antihistaminic activities.

Interestingly, terfenadine at doses of more than 30
mg/kg prolonged the hexobarbital-induced sleeping time and decreased the spontaneous locomotor activity at a dose of 300 mg/kg. As for the case of ddY mice, terfenadine has been reported to have no effect on the sleeping time induced by hexobarbital (50 mg/kg, i.p.) (24) or to prolong it (80 mg/kg, i.p.) (2). The latter results agreed with ours observed under the same experimental conditions with ICR mice. Terfenadine has been reported to be metabolized quickly and completely by hepatic cytochrome P450. However, it accumulates when given in overdose, used with hepatic compromise, or when given with cytochrome P450 inhibitors such as ketoconazole and erythromycin (25). Barbiturates are also well-known to interact with hepatic cytochrome P450 (26). Therefore, terfenadine may have prolonged the sleeping time from lower doses due to a metabolic interaction between it and hexobarbital. These findings seem to indicate that the evaluation using behavioral analysis in mice did not correctly reflect the absence of clinical CNS effects of terfenadine. These findings also warn us that a different evaluation might result between clinical and pharmacological effects on the CNS if the judgment is done with only a few pharmacological methods limited to small animals.

To accurately clarify the clinical effects of antiallergic agents on the CNS, we next examined the effect of HSR-609 on the spontaneous EEG in rabbits which is one of the convenient methods for directly evaluating brain electrical activity in comparison with those of typical antiallergic agents. The results are summarized in Table 4. HSR-609 even at the higher dose of 100 mg/kg had no effect on the EEG pattern, sleep-wakefulness cycles, gross behavior and heart rate. An absence of the CNS effect of HSR-609 was also confirmed objectively by EEG power spectral analysis. In previous studies, HSR-609 showed a potent antihistaminic effect against histamine-induced increase of the vascular permeability in mice at doses of more than 0.03 mg/kg, and the effect was stronger than those of cyproheptadine, ketotifen, azelastine, oxatomide and terfenadine (6, 9). In spite of the potent antihistaminic effect in mice, HSR-609 displayed no CNS effect on the behavior in mice and the EEG in rabbits. Terfenadine also showed no depressant effect on the CNS using EEG analysis, in agreement with previous reports in which it

Table 3. Summarized effects of HSR-609, PY-608 and typical antiallergic agents on spontaneous and induced behaviors in mice

<table>
<thead>
<tr>
<th>Agent</th>
<th>Gross behavior</th>
<th>Acute death dose (mg/kg, p.o.)</th>
<th>Spontaneous locomotor activity</th>
<th>Hexobarbital-induced sleeping time</th>
<th>Reserpine-induced hypothermia</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSR-609</td>
<td></td>
<td>(&gt;300)</td>
<td>(&gt;300)</td>
<td>(&gt;300)</td>
<td>(&gt;300)</td>
</tr>
<tr>
<td>FY-608</td>
<td>Restlessness</td>
<td>300</td>
<td>Increase (3) Decrease (100)</td>
<td>Prolongation (30)</td>
<td>Antagonism (0.3)</td>
</tr>
<tr>
<td>Cyproheptadine</td>
<td>Restlessness</td>
<td>100</td>
<td>Increase (10) Decrease (30)</td>
<td>Prolongation (30)</td>
<td>Antagonism (3)</td>
</tr>
<tr>
<td>Ketotifen</td>
<td>Sedation</td>
<td>300</td>
<td>Decrease (100)</td>
<td>Prolongation (30)</td>
<td>Antagonism (30)</td>
</tr>
<tr>
<td>Azelastine</td>
<td>Sedation</td>
<td>300</td>
<td>Decrease (30)</td>
<td>Prolongation (100)</td>
<td>(&gt;30)</td>
</tr>
<tr>
<td>Oxatomide</td>
<td></td>
<td>(&gt;300)</td>
<td>Increase (100)</td>
<td>Prolongation (100)</td>
<td>Antagonism (30)</td>
</tr>
<tr>
<td>Terfenadine</td>
<td></td>
<td>(&gt;300)</td>
<td>Decrease (300)</td>
<td>Prolongation (30)</td>
<td>(&gt;300)</td>
</tr>
</tbody>
</table>

—: No effect. ( ): Minimum effective dose (mg/kg, p.o.).

Table 4. Summarized effects of HSR-609, PY-608 and typical antiallergic agents on EEG in rabbits

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose (mg/kg, p.o.)</th>
<th>EEG sleep levels</th>
<th>EEG power spectra</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AW</td>
<td>SWDS</td>
</tr>
<tr>
<td>HSR-609</td>
<td>100</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PY-608</td>
<td>30</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Cyproheptadine</td>
<td>30</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Ketotifen</td>
<td>30</td>
<td>—</td>
<td>↑</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>100</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

—: No effect. ↓: Decrease. ↑: Increase.
had no effect on the EEG in cats (27, 28). These findings with terfenadine on the EEG should correspond to the absence of its clinical CNS effect, and it should be the same in the case of HSR-609. On the other hand, cyproheptadine and ketotifen caused slowing of the EEG, a continuous increase in the duration of SWDS, enhancement of the EEG power spectra at low frequency bands such as θ and δ, and depressant behavior. These results seem to agree with previous reports of ketotifen increasing the duration of slow wave sleep and the δ power spectra in cats and rats (20, 27). The slowing effect of cyproheptadine on the EEG in rabbits was more potent than that of ketotifen. Cyproheptadine decreased the duration of REMS and increased heart rate. Cyproheptadine and ketotifen have been reported to have high affinity for H1-receptors, while cyproheptadine also has higher affinity for muscarinic receptors than ketotifen (9, 21).

Our results with rabbits showed that scopolamine also causes slowing of EEG and decrease of the duration of REMS associated with an increase of heart rate, as reported previously for atropine (29, 30). Many endogenous sleep modulators and sleep substances have been reported (30). Among them, acetylcholine has been nominated as one of the most persuasive endogenous sleep substances regulating activation of EEG and the appearance of REMS episode (31, 32). Thus, the slowing of EEG and inhibition of REMS episode with cyproheptadine should result from its antimuscarinic activity in addition to antihistaminic activity. This hypothesis is supported by the report of an increase in the duration of SWDS, but no change in the REMS episode after a decrease of histamine content in the posterior hypothalamic activating system of the cat due to local and/or systemic administration of α-fluoromethylhistidine, α histidine decarboxylase inhibitor (33).

The non-amphoteric basic compound PY-608 appeared to cause excitement behavior in mice such as restlessness, increase of spontaneous locomotor activity, antagonistic effects on reserpine-induced hypothermia, similar to imipramine at low doses, and tremor and clonic convulsions at high doses. However, PY-608 caused depressant effects such as potentiation of hexobarbital-induced sleep at low doses and decrease of spontaneous locomotor activity at high doses. PY-608 caused slowing of EEG associated with an increase of heart rate, decrease of the duration of REMS and an enhancing of EEG power spectra at low frequency bands in rabbits similar to those of cyproheptadine. These findings seem to indicate that PY-608 has both stimulant and depressant effects on behavior in mice, but only has a depressant effect on the EEG in rabbits. PY-608 has been shown to have high affinity for both H1- and muscarinic receptors (9). Therefore, these stimulant and depressant effects of PY-608 on the CNS may be due to its antimuscarinic and imipramine mimetic monoaminergic activities in addition to antihistaminic activities, similar to those of cyproheptadine. As we previously reported, HSR-609 has been shown to have poor ability to penetrate into the CNS in mice and guinea pigs and high selectivity for the H1-receptor, which may be due to its amphoteric chemical structure (6, 9). The fine differences of chemical structure between HSR-609 and PY-608 appeared as obvious differences in their pharmacological effects on the CNS in mice and rabbits.

In conclusion, these findings suggest that if a new anti-allergic agent is thought to have a correct effect on the CNS, integrated synthetic evaluation such as direct investigation of brain electrical activity should be conducted in addition to pharmacological behavioral analysis in mice. HSR-609 appears to have no effect on the CNS from behavior analysis in mice and EEG analysis in rabbits, which may be due to its amphoteric chemical structure.

A part of these findings was presented at The 69th Annual Meeting of The Japanese Pharmacological Society in Nagasaki (March 20–23, 1996) (P-131).

Acknowledgments
The authors would like to thank Dr. K. Yamamoto (The Cell Science Research Foundation, Osaka) for his helpful advice on the manuscript. We also thank Dr. S. Yasuda, Dr. N. Iwasaki, Dr. Y. Iwanaga, Mr. N. Ohara, Mr. T. Kawaguchi and Mrs. M. Uno in our laboratories for their helpful suggestions and technical assistance, and Mrs. J. Noguchi for helping with the English editing of the manuscript.

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