Behavioral Effects of Brotizolam, a New Thienotriazolodiazepine Derivative

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Abstract—The behavioral effect of brotizolam was investigated in mice and rats, in comparison with those of diazepam, nitrazepam and estazolam. Locomotor activity of rats in an open field situation was slightly increased with smaller doses of brotizolam and estazolam and with larger doses of nitrazepam, while it was decreased with large doses of brotizolam and estazolam. The anticonflict effect of brotizolam in rats was approximately as potent as that of diazepam and was augmented following chronic administration for 10 days. In suppressing hyperemotionality and muricide of olfactory bulbectomized rats, brotizolam was more potent than diazepam, being approximately equipotent to nitrazepam and estazolam. Brotizolam, diazepam, nitrazepam and estazolam prevented both maximal electroshock and pentetrazol convulsions in mice, the effects on the latter being much more potent than those on the former. In impairing rotarod performance, brotizolam was as potent as estazolam and nitrazepam and was much more potent than diazepam in mice, but was less potent than estazolam and nitrazepam in rats. These results indicate that brotizolam possesses pharmacological properties characteristic to benzodiazepines and that the activity is more potent than that of diazepam and approximately as potent as those of nitrazepam and estazolam.

Brotizolam is a new thienotriazolodiazepine, 2-bromo-4-[(2-chlorophenyl)-9-methyl-6 H-thieno [3, 2-f] [1, 2, 4] triazolo [4, 3-a] [1, 4] diazepine, which has been found to have a marked sedative, anticonvulsant and taming effects, but to scarcely provoke coma even at large doses at the institute of Boehringer Ingelheim, West Germany (1). The chemical structure of the drug is shown in Fig. 1.

Clinically it is reported that in healthy volunteers, the drug exerts marked sedative and hypnotic effects with EEG findings indicating an increase in the β and δ activities and a decrease in the α activity, and these effects are about 100 times as potent as those of flurazepam (2–4). Valasco et al. reported, on the other hand, that the drug decreased the duration of wakefulness and the number of arousal episodes, decreased the latency of slow wave sleep in healthy

Fig. 1. Chemical structures of brotizolam, diazepam, nitrazepam and estazolam.
volunteers, and increased the duration of sleep also in insomniac patients (5).

As the toxicity is reported to be extremely low in animal studies (1), the drug may be clinically useful as an anxiolytic hypnotic. In the present study, therefore, the authors performed animal experiments on the behavioral pharmacology of brotizolam in an attempt to clarify the pharmacological characteristics of the drug in comparison with nitrazepam, estazolam and diazepam.

Materials and Methods

1. Animals
   Male ddY mice (Seiwa Institute of Experimental Animals), male CF1 mice (Kyushu University Institute of Laboratory Animals), male Wistar rats (Kuroda and Kyudo Experimental Animals) and male Wistar King A rats (Kyushu University Institute of Laboratory Animals) were used. All the animals were housed in an air-conditioned room at 23±1 °C, with a 12 hr light-dark schedule (lights on at 07:00). All experiments were carried out in laboratories under the same conditions as the animal’s room.

2. Drugs
   Brotizolam (pure powder, Boehringer Ingelheim), nitrazepam (Hokuriku Pharmaceutical), estazolam (Boehringer Ingelheim) and diazepam (Takeda Pharmaceutical) were suspended in a 0.5% aqueous solution of carboxymethylcellulose (CMC) and given orally. The control animals received 0.5% CMC solution in a volume of 0.1 ml per 10 g and 100 g body weight in mice and rats, respectively. Pentetrazol sodium and thiopental sodium were also used in the experiments.

3. Experiment on open field activities
   Male Wistar rats weighing 160–200 g were tested for ambulation and rearing using a Hall’s open field apparatus (6), a bucket-shaped chamber (60 cm floor diameter, 50 cm wall height and 80 cm upper brim diameter) with a greyish white painted surface. The numbers of ambulation and rearing in a 3 min period after placing the rats in the chamber were recorded. Rats were divided into groups of 8 each according to the ambulation which was measured before drugs, so that differences between average scores were minimal. The tests were made 0.5, 1, 2, 4, 8 and 24 hr after the single administration of drugs.

   The effect of repeated administration of brotizolam was investigated in the rats weighing 220–310 g. The animals were divided into three groups: 1-day administration group (n=5), 5-day group (n=12) and 10-day group (n=13). Brotizolam at a dose of 5 mg/kg was orally administered once daily for consecutive respective days. Ab- 

4. Experiment on conflict behavior in rats
   Male Wistar rats weighing 300 to 350 g were used. The test was performed using a Skinner box (24×31×27 cm; Tech-Servo) equipped with a lever, speaker and milk dipper, with a stainless steel grid floor by which an electric foot shock with an alternative current is given to the animal via an electrostimulator. Rats of which body weight was reduced to approximately 80% of the usual weights by restriction of water intake (20 ml/day) were trained for lever pressing on a fixed ratio schedule (FR20) (milk reward, 0.02 ml) of reinforcement at 2- to 3-day intervals. After attaining a constant level of lever pressing on the FR20 schedule of reinforcement, the animals were subjected to further training as follows: In the unpunished period (7 min) followed by the punished period (3 min), milk rewards were delivered on FR20. In the punished period which is signaled by a monotone, 1850 Hz, 70 db, and pilot lamp equipped just above the lever, every lever pressing was rewarded with milk and also punished with electric shock to the floor grid. The training was repeated at intervals of 2 to 3 days until the number of lever-pressings became less than 10 in each 3 min punished period. Only rats attaining this criterion were selected for the drug experiment. Brotizolam and diazepam were orally administered at doses of 10 and 20 mg/kg, once daily for successive 10 days, respectively, and the conflict tests were performed on days 1, 4, 7 and 10.

5. Experiment on aggressive behavior
   Male Wistar King A rats weighing 200 to 250 g at the time of surgery were used. The animals’s head was fixed on a stereotaxic
instruments (Narishige Scientific Instruments) under pentobarbital (40 mg/kg, i.p.) anesthesia, and brain lesions were performed.

Olfactory bulbectomized rats (OB rats): According to the method of Ueki et al. (7), the olfactory bulbs were bilaterally removed, and the animals were maintained in individual cages (18×17×17 cm) following the surgery. Emotionality was measured with respect to the following 3 responses to given stimuli:

1. Mouse-killing (muricide),
2. Startle response to blowing with a constant volume of air onto the back of the animal,
3. Attack response to a rod presented in front of the snout of the animal.

Muricide was regarded as positive if the rat bit a mouse to death within 3 min after a mouse was introduced into the rat's home cage. All responses of the rat except muricide were graded on a scale from 0 to 4, employing the criteria reported by Ueki et al. (7). Only rats exhibiting muricide and total scores of emotionality being greater than 6 in tests (2) and (3) were subjected to the following experiment.

Midbrain raphe lesioned rats (raphe rats): The midbrain raphe nuclei were lesioned according to the method of Yamamoto and Ueki (8). After the surgery, the animals were housed in individual cages and the emotional response assessed only with respect to muricide by the same test procedures as in the OB rat. Only rats showing muricide were selected and subjected to the following experiment. The animals were divided into groups of 8 each, and the test for emotionality were performed 0.5, 1, 2, 4, 8 and 24 hr after the drug was given.

6. Experiment on convulsion

Male CF1 mice weighing 22 to 28 g, divided into groups of 8 to 10, were used.

Maximal electroshock convulsion (MES): A constant current of 60 Hz, 50 mA, was given to the animal for 0.2 sec via corneal electrodes placed on both eyes, using the apparatus described by Woodbury and Davenport with some modifications (9). Mice were pre-tested once and only mice which had been resuscitated by artificial respiration from an electric shock were used in the experiment. The anti-MES effect of drugs was regarded as positive when tonic extension (TE) disappeared. All drugs were administered 1 hr before an electroshock.

Pentetrazol convulsion: Animals were injected s.c. with 85 mg/kg of pentetrazol 60 min after the test drugs and were individually placed into a glass beaker (capacity, 2 liter). The observation for clonic convulsion (CL) was made over a 30 min period following pentetrazol injection. The anti-convulsant effect was taken as positive when clonic convulsion disappeared.

7. Experiment on thiopental anesthesia

Male ddY mice weighing 22 to 28 g were used in groups of 10 each. Drugs were administered 1 hr prior to the injection of 40 mg/kg of thiopental sodium into the tail vein. The duration of anesthesia was measured taking restoration of righting reflexes as an indication of recovery from anesthesia.

8. Rotarod test

Male ddY mice weighing 20 to 33 g and male Wistar rats weighing 250 to 300 g were used. The rotarod test in mice was performed in accordance with the procedure reported by Dunham and Miya (10). The animal was placed on a linen-coated wooden rod (2.5 cm in diameter) rotating at 30 r.p.m. with its head against the direction of rotation. Mice were pre-selected for those which stayed for at least 5 min on the rod and divided into groups of 10 each. When the mice fell off the rotarod within 3 min in two consecutive tests, the drug effect was taken as positive. The tests were performed 0.5, 1, 2, 4, 8 and 24 hr after single administration and 0.5, 1 and 2 hr after final administration when brotizolam was repeatedly administered at a dose of 5 mg/kg, p.o., once daily, for respective days in 3 groups: 1-day administration group (n=10), 5-day group (n=14) and 10-day group (n=10).

Rats were tested according to the procedure described by Sofia (11), using a rotarod (5 cm in diameter) rotating at 5 r.p.m. The procedure was identical with the method for mice. The drug effect which caused falling of the animals within 1 min was taken as positive. Rats were pre-selected for those which stayed on the rotarod for more than 1 min and divided into groups of 8 each. The tests were performed at 0.5, 1, 2, 4, 8 and 24 hr after medication.

9. Inclined screen test
Ten male ddY mice in each group, weighing 22 to 30 g, were used. The muscle relaxant activity was measured using an inclined screen of which the angle of inclination was freely adjustable. The animal was placed on the screen with its head downward, and the gradient was gradually increased at a rate of 4.5 degrees per second to determine the minimal angle of inclination at which the animal slipped down from the screen. The measurement was repeated three times on each animal, and the mean values were calculated. Mice were tested 0.5, 1, 2, 4, 6 and 8 hr after test drugs.

Results
1. Effects on open field activities
1) Single administration
The animals in the control group came to adapt themselves to the open-field situation as tests were repeated, with a progressive decrease of ambulation. Brotizolam at a dose of 0.5 mg/kg caused a slight increase of ambulation without significance. Brotizolam at doses of 1–2 mg/kg did not exert significant effects, but significantly suppressed ambulation at 5 mg/kg, the effect reaching a peak 30–60 min after administration and lasting for about 2 hr (Fig. 2A).

Nitrazepam at a dose of 1 mg/kg displayed a slight effect, but significantly decreased ambulation at a dose of 2 mg/kg 2 and 4 hr after. However, nitrazepam at a dose of 20 mg/kg significantly increased the ambulation 2 hr after administration. The effect of nitrazepam persisted for about 8 hr.

Estazolam at doses of 0.5–1 mg/kg induced a significant increase of ambulation 30 min after administration, which was almost restored to the control level in 1 hr. At a dose of 10 mg/kg, estazolam did not produce any significant changes in ambulation.

Rearing was increased by brotizolam at a dose of 1 mg/kg, but decreased at doses of 2–5 mg/kg. Rearing was inhibited dose-dependently by nitrazepam at doses of 2–20 mg/kg and by estazolam at doses of 1–10

Fig. 2. Effects of single and repeated administration of brotizolam on ambulation of the mice in an open-field test (3 min period). A: single administration, B: repeated administration.
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2) Repeated administration
In the 1-day (n=5), 5-day (n=12) and 10-day (n=13) administration groups, ambulation was significantly inhibited 30 min after the final administration of brotizolam, but the inhibitions were not significant after 1 and 2 hr (Fig. 2B). No significant difference was observed among 1-day, 5-day and 10-day administration groups, implying that there was no alteration of drug effect by repeated administration.

2. Effect on conflict behavior
1) Single administration
Brotizolam at a dose of 5 mg/kg, p.o. significantly increased the number of lever-pressing during the punished period, whereas that during the unpunished period slightly decreased. At a dose of 10 mg/kg, no marked change was observed in the punished response, whereas a marked inhibition (P<0.001) was observed in the unpunished response (Fig. 3A). The number of lever-pressing during the punished period increased dose-dependently at doses of 20 and 50 mg/kg of brotizolam, with almost the same increase at 50 and 100 mg/kg. The lever-pressing during the unpunished period was markedly inhibited at any doses larger than 10 mg/kg. Figure 4 shows representative cumulative records of lever-pressings in rats administered with 20 mg/kg of brotizolam. The unpunished response was markedly inhibited 10–70 min after administration, whereas the punished response was markedly enhanced 30 and 40 min after medication. The lever-pressings during both the unpunished and punished periods almost returned to the control level 2 hr after medication. Complete return was observed 24 hr after administration. Thus, brotizolam exhibits a marked anti-conflict action at doses larger than 5 mg/kg.

Diazepam at a dose of 5 mg/kg caused no significant change in the lever-pressings during either the unpunished or punished periods. At doses of 10 and 20 mg/kg, the lever-pressings during the unpunished period remained almost unchanged, whereas those during the punished period were markedly

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Fig. 3. Effects of single and repeated administration of brotizolam and diazepam on conflict behavior in rats. A: single administration, B: repeated administration.
enhanced. Diazepam at a dose of 50 mg/kg produced a marked decrease in the unpunished response and a significant increase in the punished response.

2) Repeated administration

On day 1 of daily administration of brotizolam at 10 mg/kg, the number of lever-pressings during the punished period was not increased, whereas that during the unpunished period was markedly decreased. Such findings continued up to day 7 of administration. On day 10, the number of punished responses was significantly increased (P<0.05) (Fig. 3B), while that of the unpunished responses remained inhibited as seen on day 1.

Diazepam at a dose of 20 mg/kg caused no significant change in the lever press responses during the punished period on day 1 of repeated administration, but slightly increased the lever-pressings on day 4 and thereafter. The lever pressings during the unpunished period, on the other hand, were markedly inhibited on day 1, and similar inhibition persisted up to day 10.

3. Effects on the experimentally induced aggressive behavior

1) OB rat

As shown in Fig. 5A, brotizolam at a dose of 5 mg/kg inhibited muricide in 3 out of 8 rats 2 and 4 hr after administration, without significance. At doses larger than 10 mg/kg, brotizolam significantly inhibited muricide 1–4 hr after administration, the effect reaching maximum between 2 and 4 hr and recovering 8 hr after administration. The ED50 values of brotizolam for inhibition of muricide at the peak time was 7.0 (3.6–13.4) mg/kg. Figure 5B shows the effect of brotizolam on the attack and startle responses. Both the attack and startle responses were also suppressed by brotizolam at doses larger than 5 mg/kg when determined at peak effect, the suppression being more marked in the startle response than in the attack response.

Diazepam showed no marked influence on muricide at a dose of 20 mg/kg, but significantly inhibited it at doses larger than 40 mg/kg. The effect reached a peak 30 min after administration and lasted for 4 hr at doses of 40–60 mg/kg and 8 hr at 80 mg/kg. The ED50 value of diazepam for muricide inhibition at peak time was 40.0 (27.4–58.4) mg/kg. Both the attack and startle responses of OB rats were dose-dependently inhibited by diazepam at doses larger than 40 mg/kg.

Nitrazepam did not affect significantly muricide at doses smaller than 5 mg/kg, but inhibited it at doses larger than 10 mg/kg. The effect reached a peak 2 hr after administration and lasted for about 4 hr. The ED50 value of nitrazepam for muricide inhibition at peak time was 8.8 (2.5–31.0) mg/kg. Both the attack and startle responses were significantly inhibited at doses larger than
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5 mg/kg. The effect reached its peak 1 hr after administration.

Estazolam markedly inhibited muricide at doses larger than 5 mg/kg. The inhibitory effect reached a peak 1 hr after administration and lasted for about 2 and 4 hr at doses of 8 and 10 mg/kg, respectively. The ED50 value of estazolam for inhibition of muricide at peak time was 6.4 (3.7–11.1) mg/kg. Both attack and startle responses were significantly inhibited by estazolam at doses larger than 1 mg/kg, the inhibition of the attack response being almost complete at 10 mg/kg.

2) Raphe rats

As seen in Fig. 6, brotizolam produced inhibition of muricide at doses of 10, 20 and 40 mg/kg, which reached its peak 1 hr after administration and lasted for about 2 hr. The ED50 value for inhibition at peak time was calculated to be 25.0 (13.6–46.0) mg/kg.

Fig. 5. Effects of brotizolam on hyperemotionality and muricide in olfactory bulbectomized rats. A: muricide, B: hyperemotionality (attack, startle).

Fig. 6. Effect of brotizolam on muricide in midbrain raphe lesioned rats.
Diazepam also induced inhibition of muricide at doses larger than 40 mg/kg, which reached a peak 1 hr after medication and lasted for about 2 hr, the ED50 value being 40.0 (26.3–60.8) mg/kg.

Nitrazepam caused inhibition of muricide at doses larger than 10 mg/kg, which reached its peak 1 hr after medication and lasted for about 4 hr at doses of 10–20 mg/kg, the ED50 value being 12.5 (7.4–21.0) mg/kg.

Estazolam inhibited without significancy the muricide in 3 out of 8 rats at a dose of 5 mg/kg, but brought about significant inhibition at doses of 7 and 10 mg/kg, which reached a peak 1 hr after medication and lasted for about 4 hr. The ED50 value was 7.4 (4.1–13.5) mg/kg.

4. Effects on convulsion in mice

1) Maximal electroshock convulsion (MES)

Brotizolam inhibited TE and CL in a dose-dependent manner at doses larger than 10 mg/kg (Table 1), but tended to enhance tonic flexion with prolonging of its duration. Both nitrazepam and estazolam also significantly inhibited TE at doses larger than 7 mg/kg and 10 mg/kg, respectively.

The ED50 values of brotizolam, nitrazepam and estazolam for preventing the maximal electroshock convulsions calculated according to the method of Litchfield-Wilcoxon are 16.0 (9.3–27.6), 7.5 (5.7–9.9) and 10.5 (8.8–12.5) mg/kg, respectively.

2) Pentetrazol convulsion

As shown in Tables 1 and 2, the ED50 values of brotizolam, nitrazepam and estazolam for prevention of pentetrazol-induced convulsion were 0.10 (0.06–0.18), 0.17 (0.09–0.30) and 0.45 (0.30–0.67) mg/kg, respectively, suggesting that brotizolam possesses the strongest anticonvulsant activity among the drugs tested.

5. Effects on thiopental anesthesia

When the duration of anesthesia was found to be more than twice as that in the CMC-treated control group in which the duration of anesthesia was 8.4±0.8 min (mean±S.E.), anesthesia potentiation was considered to be positive. The ED50 values of brotizolam, estazolam and nitrazepam determined according to the method of Litchfield-Wilcoxon were 0.50 (0.41–0.60), 0.46 (0.25–0.83) and 0.70 (0.54–0.90) mg/kg, respectively.

6. Effects on rotarod performance in mice and rats

1) Mice

Single administration: Brotizolam, 0.5 mg/kg, did not have an effect, but it inhibited the rotarod performance in a dose-dependent manner at doses larger than 1 mg/kg. The inhibition reached its peak 30 min and

<table>
<thead>
<tr>
<th>(A) Drug</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Inhibition of clonic convulsion</th>
<th>(B) Drug</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Incidence of TE loss</th>
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<td>CMC</td>
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<td></td>
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*a: Number of mice showing the inhibition of clonic convulsion/the number of mice tested.  b: Number of mice exhibiting TE loss/the number of mice tested.  *P<0.05.  ***P<0.005.
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<td>5.2 (2.2–12.1)</td>
</tr>
<tr>
<td>Inclined screen test</td>
<td>mouse</td>
<td>8.6 (6.7–11.0)</td>
<td>25.5 (17.0–38.2)</td>
<td>2.3 (0.7–7.1)</td>
<td>4.3 (2.4–7.7)</td>
</tr>
</tbody>
</table>

↑, ↑↑, ↑↑↑: slight, moderate (P<0.05), marked increase (P<0.01). ↓, ↓↓, ↓↓↓: slight, moderate (P<0.05), marked decrease (P<0.01). The dosage range indicated is in parenthesis.
recovered 2 and 4 hr after medication at doses of 2 and 5 mg/kg, respectively (Fig. 7A).

Both diazepam and nitrazepam inhibited in a dose-dependent fashion the rotarod performance at doses larger than 5 mg/kg and 2 mg/kg, respectively, and the effects reached a peak at 30 min after administration. Estazolam showed marked inhibition of rotarod performance at doses larger than 1 mg/kg, and the peak effect was found 30 min after administration. However, the duration of action of estazolam was quite short, as the performance completely returned to normal 1 hr after administration. The ED50 values of brotizolam, diazepam, nitrazepam and estazolam for impairing the rotarod performance at peak time of the effects calculated according to the method of Litchfield and Wilcoxon were 1.30 (0.93–1.69), 5.2 (4.4–6.2), 1.9 (1.0–3.3) and 1.2 (1.1–1.3) mg/kg, respectively.

Repeatead administration: At 5 mg/kg brotizolam, the rotarod performance was markedly inhibited 30 min after in the 1-day administration group. Though the performance gradually recovered, the inhibition was significant even 2 hr later. The rotarod performance was also inhibited 30 min after the 5- and 10-day administration; however, the performance inhibition recovered more rapidly than in the 1-day administration group and was not significant 2 hr after administration (Fig. 7B).

2) Rats

The rotarod performance was not influenced after brotizolam at 5 mg/kg, but was impaired in 2 out of 8 rats and 5 out 8 rats 1 hr after brotizolam at 10 and 30 mg/kg, respectively. The effect of brotizolam reached a peak 1 hr after administration and lasted for about 2 hr.

Diazepam and nitrazepam caused dose-dependent impairment of rotarod performance at doses larger than 10 and 5 mg/kg, respectively, which reached a peak at 30 min after administration and lasted for about 2 hr. Estazolam did not produce any significant
Behavioral Effects of Brotizolam

A study was conducted to evaluate the behavioral effects of brotizolam, diazepam, nitrazepam, and estazolam in rats and mice. The effects were assessed through various tests such as rotarod performance, inclined screen tests, and conflict tests.

**Behavioral Effects of Brotizolam**

- **Rotarod Performance**: Brotizolam had a mixed effect on rotarod performance in rats at doses of 1–5 mg/kg, with an inhibition of performance at a dose of 10 mg/kg, which reached a peak at 30 min after administration and lasted for about 1 hr.
- **Inclined Screen Tests**: The ED50 values of brotizolam, diazepam, nitrazepam, and estazolam for impairment of rotarod performance in rats at the peak time of the effect were 17.5 (11.4–27.0), 25.0 (12.3–50.8), 10.2 (6.4–16.2) and 5.2 (2.2–12.1) mg/kg, respectively.

**Discussion**

Behavioral effects of brotizolam, diazepam, nitrazepam, and estazolam in rats and mice are summarized in Table 2. These results clearly indicate that brotizolam possesses a typical anxiolytic profile which qualitatively resembles those of benzodiazepines. Though varying to a certain extent in each test, the activity of brotizolam generally is far more potent than that of diazepam and slightly less potent than those of estazolam and nitrazepam.

In the open-field test in rats, brotizolam increased, though not significantly, ambulation at a low dose of 0.5 mg/kg, but inhibited ambulation at a dose of 5 mg/kg. The same tendency was observed with estazolam. Nitrazepam, on the other hand, inhibited ambulation at smaller doses of 1 and 2 mg/kg, but significantly accelerated it at a large dose of 20 mg/kg, being just vice versa with brotizolam. These results indicate that these drugs tested possess a facilitatory effect on ambulation following single administration at certain doses. The underlying mechanisms involved in the facilitation of ambulation by certain benzodiazepines have not yet been elucidated. The ambulation of rats in the open field represents an exploratory behavior which the animals exhibit when introduced into a new environment. In the animals which are in a state of freezing due to an intense fear to the environment, the increase of ambulation can be easily induced by the administration of benzodiazepines. Therefore, the increase of ambulation induced by benzodiazepines may reflect a disinhibitory action of these drugs that is related to the clinical anxiolytic effect.

Warner (12) reported that with repeated administration of oxazepam, the depressant side effects such as drowsiness and sedation tended to disappear, though the anxiolytic action persisted. In the present experiment, no significant change in the inhibitory effect of brotizolam on the ambulation was observed following repeated administration of 5 mg/kg for 10 days.

In the conflict tests for the single administration of brotizolam and diazepam, the facilitatory effect of brotizolam on the punished responses was found to be almost equipotent to that of diazepam. On the other hand, the inhibitory effect of brotizolam on the unpunished responses was far more potent than that of diazepam, that is, brotizolam significantly inhibited the unpunished responses even at a dose of 10 mg/kg and diazepam at a dose as large as 50 mg/kg.

The facilitatory effects of brotizolam and diazepam on the punished responses were augmented following repeated administration.
for 10 days, whereas their inhibitory effects on unpunished responses were not altered. As Warner’s proposal (12) mentioned above, Margules and Stein (13) found that oxazepam markedly inhibited the lever pressings during the unpunished period in the experiment on conflict behavior and produced a slight increase in the lever pressings during the punished period at an early stage of the repeated administration. As the administration was repeated, however, the inhibitory effect of oxazepam on the unpunished responses was gradually decreased due to the development of tolerance, whereas the lever pressing during the punished period was increased. Based on these findings, they suggested that the facilitatory effect of oxazepam on the punished responses was well correlated with its clinical anxiolytic effect. This proposal is also supported by Cook and Davidson’s report (14) with the anticonflict activity of benzodiazepines.

The facilitatory effects of brotizolam and diazepam on the punished responses were here potentiated following the repeated administration in the same way as reported by Margules and Stein (13), whereas their inhibitory effects on the unpunished responses were not changed throughout the experimental period. Therefore, it is conceivable that the augmentation of the facilitatory effect on the punished responses following the repeated administration of brotizolam and diazepam was due to a reduction in their effects of inhibiting the unpunished responses (i.e., decrease in their sedative and muscle relaxant activities), but was due to an enhancement of the effects of increasing the punished response. This was further confirmed by the findings that the inhibitory effect of brotizolam on ambulation in the open field test and on the rotarod performance was not changed following repeated administration in the present experiment. Since brotizolam showed a marked anticonflict effect both in single and repeated administrations, it can be expected that the drug will exert a marked anxiolytic effect in clinical practice.

Brotizolam suppressed the hyperemotionality of the OB rats, being far more potent than diazepam and slightly less potent than nitrazepam and estazolam. The inhibitory effect of brotizolam on muricide of the OB rats was far more potent than that of diazepam and almost equipotent to those of estazolam and nitrazepam. The anti-muricidal activity of brotizolam in the raphe rats was far more potent than that of diazepam and less potent than those of nitrazepam and estazolam. The anti-muricidal effects of diazepam, nitrazepam and estazolam in the OB rats were not significantly different from those in the raphe rats, whereas that of brotizolam in the OB rats was far more potent than that in the raphe rats. It is well known that muricide of the rat is selectively inhibited by antidepressants (15–17). Muricide of the OB rats is inhibited well by desipramine, a potent noradrenaline uptake inhibitor, while that of the raphe rats is inhibited preferentially by chlorimipramine, a potent serotonin uptake inhibitor, and 5-HTP (8). Taking these facts into account, it may be considered that brotizolam exerts a more pronounced effect on the catecholaminergic systems in the brain as compared with diazepam, nitrazepam and estazolam, though the underlying mechanisms are still obscure.

Regarding the anticonvulsant action, the ratio of the ED50 values of brotizolam, nitrazepam and estazolam for protecting the maximal electroshock convulsion to those for inhibiting the pentetrazol-induced convulsion were 1:160, 1:44 and 1:23, respectively. This result indicates that brotizolam possesses a particularly strong activity in preventing the pentetrazol convulsion in comparison with that in preventing the maximal electroshock convulsion.

The effect of brotizolam in potentiating thiopental anesthesia is quite strong, being approximately as potent as those of nitrazepam and estazolam. Therefore, the general central depressant activity of brotizolam is considered to be quite potent.

In impairing rotarod performance in mice, brotizolam was approximately as potent as estazolam and nitrazepam and about 4 times as potent as diazepam. In rats, on the other hand, the effect of brotizolam was more potent than that of diazepam, but less potent than those of nitrazepam and estazolam, being 1/2 and 1/3 times as potent as those
of nitrazepam and estazolam, respectively. Brotizolam showed a great difference in the potency of this effect by animal species, the effect in mice being 13 times as potent as that in rats. Though both nitrazepam and estazolam also showed the same tendency, the difference in the potency between rats and mice was found to be the most significant in the case of brotizolam. The potency of brotizolam in impairing rotarod performance did not show any changes following repeated administration, whereas the duration of action was shortened, suggesting that the rate of metabolism and excretion of the drug may be increased with repeated administration. The muscle relaxant effect of brotizolam measured by the inclined screen test was not so strong, being 3 times more potent than that of diazepam and less potent than those of nitrazepam and estazolam.

The present results indicate that brotizolam possesses pharmacological properties characteristic to benzodiazepines and that the activity is far more potent than that of diazepam and slightly less potent than those of nitrazepam and estazolam. Concerning the taming effect, the anti-muricidal activity of brotizolam is quite potent, especially in the OB rats. In addition, brotizolam increased the exploratory behavior in the open field test at relatively small doses and showed potent anticonflict and anesthesia potentiating effects. Based on these findings, brotizolam is expected to exert strong anxiolytic, sedative and hypnotic effects in clinical practice.

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