Behavioral Analysis of Zopiclone on the Basis of Their Discriminative Stimulus Properties in the Rat

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Abstract—Zopiclone is a new cyclopyrrolone derivative which exerts pharmacological activities similar to those of benzodiazepines in behavioral and biochemical studies. In order to clarify the discriminative stimulus properties of zopiclone, 8 rats were trained to discriminate the interoceptive stimulus induced by zopiclone (3.2 mg/kg, i.p.) from those of saline. Following discrimination acquisition, administration of zopiclone resulted in drug-appropriate responding with an ED50 of 1.3 (1.0–1.8) mg/kg. The zopiclone discriminative stimulus generalized to the benzodiazepines diazepam (1.8 mg/kg), nitrazepam (10 mg/kg) and alprazolam (10 mg/kg). A non-benzodiazepine, suriclone, at 3.2 mg/kg, generalized to the zopiclone stimulus in 5 out of 7 rats, but meprobamate, hydroxyzine, tracazolate and muscimol did not. The benzodiazepine antagonist Ro 15-1788 (1 mg/kg) completely blocked zopiclone stimulus. In contrast, however, bicuculline and pentetrazol failed to antagonize it. The serotonin antagonist cinanserin and ritanserin neither generalized to the zopiclone stimulus nor did they exhibit antagonism. These results suggest that the zopiclone discriminative stimulus is mediated by binding to benzodiazepine receptors and appears not to be related to GABAergic or serotonergic system.

The drug discrimination paradigm has been shown to be a sensitive and specific tool in CNS pharmacology for clarifying pharmacological properties of drugs (1–5). Many psychoactive drugs, including benzodiazepines and barbiturates, are known to produce discriminative stimuli which reliably control operant responding for reinforcement (6).

Various studies have shown that barbiturates, meprobamate and benzodiazepines, which are chemically diverse, have a number of pharmacological actions in common and exhibit similar and very specific discriminative stimulus properties. Zopiclone is a newly-developed cyclopyrrolone derivative whose chemical structure is quite different from those of barbiturates and benzodiazepines (Fig. 1). Yet, zopiclone exerts many activities similar to those of the benzodiazepines and, to a lesser extent, the barbiturates in laboratory animals (7–11). It has been shown that the binding activity of zopiclone to benzodiazepine receptors is as high as those of diazepam and nitrazepam in vitro and that zopiclone also inhibits the binding of [3H]-flunitrazepam in vivo (8). Clinically, zopiclone has been recognized as a useful sleep-inducer, comparable in efficacy to nitrazepam (12).

It was of interest to investigate the discriminative stimulus properties of zopiclone. Al-
though Julou et al. (13) already demonstrated that zopiclone belongs to the same group as benzodiazepines and barbiturates in the drug discrimination paradigm, there are few reports about the discriminative properties of zopiclone in detail. Therefore, it was the aim of the present study to investigate systematically the pharmacological specificity of the discriminative stimulus effects of zopiclone by generalization testing with benzodiazepines (diazepam etc.), non-benzodiazepines (suriclone) that bind to the benzodiazepine-recognition site, and other drugs with anxiolytic effects similar to those of benzodiazepines (muscimol etc.) and by antagonism testing with the selective benzodiazepine antagonist Ro 15-1788, the GABA<sub>\alpha</sub> antagonist bicuculline, the Cl-channel blocker pentetrazol and the serotonin antagonist ritanserin etc.

**Materials and Methods**

**Subjects:** The experimental animals were eight male Slc:Wistar-KY rats (purchased from Shizuoka Laboratory Animal Center), weighing 210–280 g at the beginning of the experiments. They were housed four per cage in an air-conditioned colony room illuminated between 7:00 a.m. and 7:00 p.m. Throughout the study, all rats had free access to tap water, while food (Lab. MR Stock: Nihon-kosan Co.) was limited to about 15 g per rat per day.

**Apparatus:** Eight identical operant Skinner boxes (Gerbrands Co., 23.5 x 30.5 x 26.5 cm) were used. Each chamber contained two levers mounted 5.5 cm above the grid floor, one houselight, and one small light above each lever. Between the two levers, a food-pellet receptacle was placed 2.5 cm above the cage floor. Scheduling of contingencies and recording of data were achieved with a combination of electromechanical and solid-state programming equipment.

**Drugs:** Drugs used in this experiment were zopiclone (Rhône-Poulenc, Vitry-sur-Seine, France), diazepam (Cercine injection, Take-da), suriclone (Rhône-Poulenc, Vitry-sur-Seine, France), sodium pentobarbital (Somnopentyl injection, Pitman-Moore), meprobamate (Atraxin, Daichi), hydrazine HCl (Pfizer Taito), tracazolate, muscimol (Sigma), cinanserin HCl, ritanserin (Janssen Kyowa), Ro 15-1788 (Roche Japan) and sodium pentetrazol (Sigma).

Zopiclone, suriclone, meprobamate and tracazolate were suspended in 0.5% carboxymethylcellulose. Muscimol, cinanserin and pentetrazol were dissolved in distilled water. Ritanserin and diazepam were dissolved in 20% and 40% polyethylene glycol, respectively. Ro 15-1788 was suspended 1% Tween 80.

The volume injected intraperitoneally (i.p.) was 0.1 ml/100 g.

**Training procedure:** Rats were initially trained to respond on a fixed-ratio one schedule (FR 1) of reinforcement on both of the two levers. The schedule of reinforcement was gradually increased from FR 1 to FR 10 on lever independently. Lever press training on the FR 10 schedule continued until rats of responding stabilized (approximately 40–50 responses/min) and then drug discrimination training began.

Rats were injected i.p. at a constant volume of 0.1 ml/100 g body weight with zopiclone (3.2 mg/kg) or saline 30 min before each session. Immediately after injection, the rats were placed in the chamber. For half of the rats, responding on the right lever was reinforced after zopiclone administration; responding on the opposite lever was reinforced for the other half after zopiclone administration. After saline administration, responding on the opposite lever was reinforced.

After every consecutive tenth press (FR 10) on the appropriate lever, a 50 mg food pellet was delivered through the food dispenser. Responses on the incorrect lever (i.e., saline lever after zopiclone and drug lever after saline) were recorded, but did not result in the delivery of reinforcement. Training sessions were 30 min long or until rats took 50 pellets. After the session, the rat was removed and placed in its living cage and was allowed to feed (about 15 g x 4 rats/cage). For initial training, zopiclone or saline was administered on alternate days. When the number of incorrect responses did not exceed 15 for 2 consecutive training sessions, the sequence of drug-saline injections was changed (i.e., drug-saline-saline-drug-saline or saline-drug-drug-saline-drug). Discriminative learning was continued for each rat until the num-
ber of incorrect responses equalled 15 or less for at least 4 consecutive training sessions. Following this, it was determined whether saline and the training drug were correctly discriminated in a test schedule. This schedule was the same as in the training session, but both levers were available; i.e., either lever would deliver one food pellet every 10 consecutive presses. The criterion for acquisition of discrimination was 2 consecutive test probes (saline and drug) with 80% or more correct responding. If rats failed to respond on the correct lever under both saline- and drug-test schedules, further training sessions were given before testing was reinstated.

**Drug test procedure:** After the rats reached the criterion level of performance, they were repeatedly used in generalization and antagonism tests. The results were expressed as the percent responding on the drug lever (i.e., number of responses on zopiclone designated lever/total number of response \( \times 100 \)). Responses on both levers during the entire session were recorded. The generalization and antagonism test for various drugs was performed on a random basis, but in each case, the same animals were used to study the different doses of the test drugs. The sequence in which the rats received the various test treatments was randomized for each rat.

Each rat was placed in the Skinner box immediately (30 min before the session) after injection of the test drug (generalization test) or after injection of the training drug zopiclone, which was preceded (40-50 min before the session) by injection of the antagonist test drug (antagonism test). The results were analyzed for statistical significance by comparing the percentage responding on the drug lever and response rate (lever-presses/min) after drug injection with those observed after saline injection using the two-tailed Student's t-test (variances seem to be equal) or Cochran-cox test (variances seem not to be uniform). The ED50 was calculated according to the method of Litchfield and Wilcoxon.

### Results

The acquisition of the discrimination is shown in Fig. 2 for the eight rats trained to discriminate between zopiclone at the dose of 3.2 mg/kg (i.p.) and saline. By session 50, the drug-lever percent response (drug appropriate response) of rats in the first 3 min averaged 95.4±1.8% (average±S.E.) when given zopiclone and 4.2±1.5% when given saline. After 60 training sessions, discriminative performance was stable under both zopiclone and saline conditions.

The zopiclone dose-response determination showed that the drug appropriate response was increased in a dose-dependent manner (Fig. 3, left panel). The ED50 calculated according to the method of Litchfield-Wilcoxon was 1.3 (1.0–1.8) mg/kg. The response rate for various doses of zopiclone was almost the same (63 responses/min; Fig. 3, right panel).

The non-benzodiazepine suriclone at doses over 1.0 mg/kg generalized significantly to the zopiclone stimulus (Table 1). At 3.2 mg/kg, 5 out of 7 rats showed generalization to zopiclone stimulus without producing significant changes in response rate. At 10 mg/kg, however, 2 out of 7 rats showed inhibition of lever-pressing and in general, all rats displayed a significantly reduced response rate (Table 1).

The benzodiazepine diazepam was generalized to the zopiclone stimulus in a dose-dependent manner (Table 1). Diazepam at a dose of 3.2 mg/kg was able to generalize to the zopiclone stimulus in all rats, but in contrast to zopiclone, showed a significantly reduced response rate. Nitrazepam at doses of 3.2 and 10 mg/kg was generalized to zopiclone stimulus without producing changes in responses rate. Alprazolam at 10 mg/kg was generalized completely to zopiclone, although the response rate was significantly decreased.

Table 2 shows the result of generalization studies of non-benzodiazepine agents, which possess anti-conflict effects, in zopiclone-trained rats. Tracazolate at 10 mg/kg was generalized to zopiclone in only 1 out of 5 rats. Tracazolate at 32 mg/kg produced suppression in 4 out of 6 rats and failed to generalize to the zopiclone stimulus. Meprobamate at 100 mg/kg produced about 50% generalization with a significant inhibition of response rate. At a large dose of 180 mg/kg, 4 out of 6 rats showed suppression and only one rat showed zopiclone appropriate re-
Fig. 2. Acquisition of a discrimination between 3.2 mg/kg zopiclone and saline in rats (upper panel, N=8). ○—○, saline-training session; ●—■, zopiclone-training session. Values are the mean (±S.E.M.) percent drug appropriate responding during 3 min following initiation of the training period. The response rate is expressed as the mean number of lever press responses per min (lower panel). Vertical lines represent ±S.E.M.

Fig. 3. Dose-response curve for rats trained to discriminate 3.2 mg/kg zopiclone from saline administered 30 min before the test (left panel). All points are the mean of 8 rats responding on the zopiclone lever during the test period (30 min long or taking 50 pellets). The response rate is expressed as the mean number of lever press response per min (right panel). Vertical lines represent ±S.E.M.

Responding. Hydroxyzine at 10 and 18 mg/kg also failed to be generalized to zopiclone stimulus. At 18 mg/kg, one of 4 rats was suppressed, and the average response rate in the 3 responding rats was markedly decreased. This dose also caused the rats to press the
Immediately after the i.p. injection of test drug, each rat was placed in the Skinner box (house light off) and allowed to respond with the levers from 30 min following the injection (house light on). Values represent the mean percentage of responses on the zopiclone-appropriate lever ± S.E.M. during the test period. When the lever-press response was decreased below 50 responses/30 min (suppression), the data were not included in the values of generalization percentage. Number of rats selecting the zopiclone lever (more than 80%) divided by the number of rats tested. Significant difference from saline, *P<0.05, **P<0.01, ***P<0.001.

Table 1. Generalization to the zopiclone discriminative stimulus by suriclone and benzodiazepines in rats

| Test drug | Dose (mg/kg, i.p.) | Zopiclone appropriate response %a (mean±S.E.M.) | Incidence of generalizationb | Response/min (mean±S.E.M.) |
|-----------|--------------------|-----------------------------------------------|-----------------------------|---------------------------|
| Saline    | --                 | 0.7±0.3                                      | 0/8                         | 62.2±4.1                  |
| Zopiclone | 3.2                | 99.4±0.3                                     | 8/8                         | 65.1±3.1                  |
| Suriclone | 0.32               | 1.4±1.3                                      | 0/7                         | 69.3±1.4                  |
|           | 1.0                | 55.9±18.3*                                   | 4/7                         | 57.7±2.3                  |
|           | 3.2                | 69.6±18.3**                                  | 5/7                         | 45.5±8.4                  |
|           | 10                 | 70.1±18.6**                                  | 3/7                         | 29.6±8.6**                |
| Diazepam  | 1.0                | 0                                             | 0/5                         | 61.2±2.4                  |
|           | 1.6                | 80.0±17.9*                                   | 4/5                         | 50.2±2.0*                 |
|           | 3.2                | 98.1±0.9***                                  | 5/8                         | 39.4±3.8**                |
| Nitracepam| 0.1                | 0                                             | 0/4                         | 60.8±3.0                  |
|           | 0.32               | 39.9±21.9                                    | 2/5                         | 57.0±3.0                  |
|           | 1.0                | 37.3±18.2                                    | 3/7                         | 59.2±3.3                  |
|           | 3.2                | 62.1±18.2*                                   | 5/7                         | 48.3±6.1                  |
|           | 10                 | 73.9±15.7**                                  | 6/7                         | 46.7±6.6                  |
| Alprazolam| 0.32               | 0                                             | 0/6                         | 61.0±4.1                  |
|           | 1.0                | 66.1±20.9*                                   | 4/6                         | 56.0±3.3                  |
|           | 3.2                | 49.7±22.2                                    | 3/6                         | 49.3±6.0                  |
|           | 10                 | 96.3±3.3***                                  | 5/5                         | 37.4±5.6**                |

Immediatly after the i.p. injection of test drug, each rat was placed in the Skinner box (house light off) and allowed to respond with the levers from 30 min following the injection (house light on). Values represent the mean percentage of responses on the zopiclone-appropriate lever ± S.E.M. during the test period. When the lever-press response was decreased below 50 responses/30 min (suppression), the data were not included in the values of generalization percentage. Number of rats selecting the zopiclone lever (more than 80%) divided by the number of rats tested. Significant difference from saline, *P<0.05, **P<0.01, ***P<0.001.

saline lever. The GABA agonist muscimol failed to produce a significant increase in drug-appropriate responding (Table 2). Suppression in all rats occurred at a dose of 3.2 mg/kg. The serotonin antagonist ritanserin at 10 mg/kg and cinanserin at 3.2 and 10 mg/kg also failed to substitute for zopiclone, although both drugs did not produce a significant decrease in response rate.

The results of antagonism tests of the zopiclone response are shown in Fig. 4. A benzodiazepine antagonist, Ro 15-1788, produced dose-related antagonism of the generalization response. In particular, the administration of a relatively low dose of Ro 15-1788 (1 mg/kg. N=5) was sufficient to block generalization without affecting the response rate (68.6±7.1 response/min, Fig. 4). In contrast, however, pentetrazol at subconvulsive doses up to 32 mg/kg (N=5) produced no attenuation of the zopiclone stimulus, but produced a significant decrease in response rate (39.6±2.8 response/min).

Discussion

Specific receptors in the central nervous
Table 2. A number of compounds failed to generalize to zopiclone stimulus

| Test drug   | Dose (mg/kg, i.p.) | Zopiclone appropriate response | Response/min (mean±S.E.M.) |
|-------------|--------------------|--------------------------------|---------------------------|
|             |                    | % (mean±S.E.M.) | incidence of |                                     |
|             |                    |                  | generalization |                                     |
| Tracazolate| 10                 | 20.5±17.5        | 1/5           | 63.8± 5.4                           |
|            | 32                 | 50.5±35.4        | 1/5           | 13.1±10.9**                         |
| Meprobamate| 10                 | 0                | 0/6           | 55.6± 2.0                           |
|            | 32                 | 16.4±14.9        | 1/6           | 59.9± 3.9                           |
|            | 100                | 46.7±19.1        | 3/6           | 47.5± 4.0*                          |
|            | 180                | 50.0±35.4        | 1/6           | 19.1±10.9**                         |
| Hydroxyzine| 10                 | 0                | 0/4           | 47.8± 3.2*                          |
|            | 18                 | 0                | 0/4           | 26.4± 8.6**                         |
| Muscimol   | 0.32               | 10.0± 8.9        | 0/5           | 63.3± 4.8                           |
|            | 1.0                | 31.8±16.4        | 1/5           | 45.9± 2.5**                         |
|            | 3.2                | —                | 0/3           | 0                                    |
| Ritanserin | 10                 | 0                | 0/5           | 63.2± 2.6                           |
| Cinanserin | 3.2                | 3.6± 3.1         | 0/4           | 55.2± 3.9                           |
|            | 10                 | 0                | 0/4           | 53.9± 2.3                           |

Significant difference from saline, *P<0.05, **P<0.01. —: no response (supression). Details are the same as for Table 1.

Fig. 4. The effects of Ro 15-1788 and pentetrazol in combination with the training dose (3.2 mg/kg) of zopiclone. Ro 15-1788 and pentetrazol were injected 20 and 10 min prior to zopiclone administration; i.e., 50 and 40 min prior to test sessions, respectively. D: zopiclone, 3.2 mg/kg alone.
system which selectively recognize pharmacologically active benzodiazepines, but not sedative-hypnotics such as barbiturates, are thought to mediate many of the pharmacological effects of benzodiazepines (14, 15).

Many benzodiazepines and barbiturates produce discriminative stimuli. The present study, using a 2-lever operant apparatus, revealed that zopiclone, like benzodiazepines, may serve as a discriminative stimulus. The non-benzodiazepine suriclone, like zopiclone, belongs to the chemical family of cyclopyrrolones and possesses a pharmacological profile close to that of the benzodiazepines (16,17). Suriclone also possesses the pharmacological properties of zopiclone (18). In the generalization test, suriclone was generalized to the zopiclone stimulus. However, suriclone failed to produce complete generalization to the zopiclone stimulus. Specific binding studies have shown that the affinities of suriclone in the hippocampus and cerebellum is stronger than that of zopiclone (18). Furthermore, suriclone has therapeutic activity as an anxiolytic, but zopiclone acts as a hypnotic (18). It is suggested from these results that the mechanism of action of zopiclone might not be exactly the same as that of suriclone. This may be the reason why suriclone did not generalize completely to the zopiclone stimuli. Zopiclone binds to benzodiazepine receptors, and its affinity is comparable to those of diazepam and nitrazepam (8, 19). In the present studies, diazepam, nitrazepam and alprazolam were also generalized to the zopiclone stimulus. Therefore, the discriminative stimulus produced by zopiclone appears to be similar to that of the benzodiazepines, agreeing with the results obtained by Julou et al. (13). On the other hand, Sanger et al. (9) reported that rats trained to discriminate chloridiazepoxide from saline responded on the chloridiazepoxide-appropriate lever at doses of zopiclone lower than those producing reductions in response rates. As shown here, zopiclone at 3.2 mg/kg (i.p., N=5) perfectly generalized to the diazepam discriminative stimulus (training dose: 1.0 mg/kg, i.p.) without producing any significant changes in response rate (T. Yamamoto et al., unpublished data). Taken together we conclude that cross-drug (symmetrical) generalization occurs between zopiclone and benzodiazepines such as chloridiazepoxide and diazepam.

The 4 drugs which generalized to the zopiclone stimulus produce anti-conflict effects in common. From this point, it is possible to consider that the zopiclone stimulus is related to an anxiolytic activity. However, the pyrazolopyridine derivative tracazolate, which has an anxiolytic profile in a punished drinking test (20, 21), failed to generalize to the zopiclone stimulus. In binding studies, tracazolate increased the affinity of benzodiazepines for their binding sites, probably through an action at the picrotoxin site (20). Meiners and Salama (22) also reported that tracazolate enhances benzodiazepine and GABA binding. On the other hand, mepromamine and hydroxyzine, which produce anxiolytic activities, are also not directly bound to the BDZ receptor. These non-benzodiazepines were not generalized to the zopiclone stimulus. Therefore, based on these findings, the zopiclone discriminative stimulus is independent of the presence of the benzodiazepine structure and probably mediated by binding to the benzodiazepine receptor. Further support is provided by the fact that Ro 15-1788, a potent benzodiazepine-receptor antagonist (23), completely blocked the zopiclone stimulus at a relative low dose of 1 mg/kg. Patel et al. (24) reported that the anticonflict activity of agents ('typical' anxiolytics) that bind to benzodiazepine receptors is blocked by a benzodiazepine antagonist (i.e., Ro 15-1788, CGS 8216), whereas the activity of those ('atypical' anxiolytics) that do not bind to these receptors is not inhibited. Based on these results, it is suggested that the zopiclone stimulus cue may be related to the anti-conflict mechanism induced by 'typical' anxiolytics.

Cananzi et al. (25) have suggested that GABAergic neurons take part in the anti-conflict effects of benzodiazepines because an anti-conflict action was shown by intraventricular injection of muscimol, a GABA_A agonist. Moreover, Shibata et al. (26) showed that micro-injection of GABA and muscimol into the amygdala centrals produced an anti-conflict effect. In addition, a GABAergic
system activator such as the GABA precursor progabide (27) and the GABA transaminase inhibitor sodium valproate (28) also possess potent anti-convulsive activity. Furthermore, it is suggested that anxiolytic action is mediated by GABA_A receptors (29). However, the zopiclone discriminative stimulus was neither generalized to the GABA_A agonist muscimol nor antagonized by bicuculline [which is a competitive inhibitor of GABA binding at the GABA_A recognition site (30)] or pentetrazol (which inhibits GABAergic neuron activity by Cl-channel blocking). It is evident from these results that the GABA receptor is not involved in the discriminative stimulus by zopiclone. This hypothesis receives further support from the fact that zopiclone has weak anti-convulsive action in comparison with those of diazepam and nitrazepam (10, 11).

On the other hand, it is known that anti-conflict action can be induced by administration of anti-5-HT agents (e.g., methysergide and cinanserin), 5-HT synthesis blockers (e.g., p-chlorophenylalanine) and 5-HT neurotoxins (e.g., 5,6-dihydroxytryptamine) (31–33). Microinjection of cyproheptadine, another anti-5-HT agent, into the amygdala centralis, also produced an anti-conflict effect (34). Ritanserin, which is an extremely potent and centrally active 5-HT_2 antagonist (35), also exerts putative anxiolytic effects in an open-field test (36). Based on these results, there is a possibility that the discriminative stimulus of zopiclone could be due to an anti-5-HT action. However, ritanserin failed to generalize to the zopiclone stimulus. Furthermore, the non-specific 5-HT antagonist cinanserin also failed to substitute for the zopiclone stimulus and to antagonize it. From these results, it is suggested that serotonergic mechanisms do not seem to be major components in the zopiclone stimulus.

In conclusion, it is found that zopiclone produces a discriminative stimulus in rats and that this discriminative stimulus is mediated by binding to the benzodiazepine receptor and appears not to be primarily related to GABAergic or serotonergic systems.

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