Anxiolytic Activity of SC-48274 Compared with Those of Buspirone and Diazepam in Experimental Anxiety Models

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ABSTRACT—The present study was performed to assess the anxiolytic activity of the novel anxiolytic SC-48274 in comparison with those of buspirone and diazepam. Drugs were administered p.o. The experimental anxiety models included gastric lesions of mice induced by socio-psychological stress in the communication box (CB) and the passive avoidance paradigm in rats. In the CB experiments, non-foot-shocked mice (responder) exposed to the emotional responses of foot-shocked mice (sender), 3 hr per day for 3 days, developed gastric lesions. Single treatments of diazepam (1, 2, 5 mg/kg) and SC-48274 (25, 50 mg/kg) prevented gastric lesion formation, but buspirone at 2.5 -10 mg/kg did not. A 3-day treatment with SC-48274 at doses over 5 mg/kg prevented gastric lesions; and a 3-day treatment with buspirone at 2, 5 and 10 mg/kg prevented the lesions with a U-shaped dose-response. Diazepam also prevented gastric lesion formation at the doses of 1 and 2 mg/kg. In the passive avoidance response study, rats which had a single acquisition trial for 2 days were used. The step-down latency for rats to enter from the illuminated compartment to the dark one was recorded. Single treatments of SC-48274 (25 mg/kg), diazepam (5, 10 mg/kg) or buspirone (25 mg/kg) shortened the delayed latency. These results suggest that SC-48274 has anxiolytic activity of the same potency as buspirone and repeated-dose administration is needed to induce anxiolytic activity.

Keywords: Anxiety, SC-48274, Buspirone, Diazepam, Communication box

SC-48274, (±)-3-[3-(dimethylamino)propyl]-3-(3-methoxyphenyl)-4,4-dimethyl-2,6-piperidinedione, monohydrochloride, is a candidate anxiolytic drug with the chemical structure shown in Fig. 1. SC-48274 was found to inhibit the activation of tryptophan hydroxylase (1), an enzyme that undergoes activation under depolarizing or phosphorylating conditions (2). An inhibitor of this activation reduced the turnover of serotonin in the serotonergic system (2). Therefore, according to a new theory for treating depression, as proposed by Aprison et al. (3), SC-48274 was clinically evaluated in depressed patients, and the results suggested that it was an effective antidepressant (4).

Buspirone, a 5-HT1A partial agonist that is clinically effective as an anxiolytic drug (5, 6), reduces the turnover rate of serotonin (7) and the firing rate of the serotonergic neurons (8). Accordingly, it is possible that SC-48274 may have anxiolytic activity. Other pre-clinical tests of SC-48274 suggested that this drug may represent a novel, non-sedative anxiolytic agent that is orally active over a wide dose range (9). Therefore, the present study examined the anxiolytic activity of SC-48274 in mice with the communication box method and in rats by using the passive avoidance task in comparison with the activities of buspirone and diazepam.

The communication box method was designed to induce “experimental anxiety” by employing intraspecies emotional communication (10). The inside of the communication box was divided into foot-shock and non-foot-shock compartments by transparent plastic boards. The animals, which were individually placed into each compartment, were unable to make physical contact with one another, but were able to receive other cues such as visual, auditory and olfactory sensations. During the foot shock period, the animals placed in the non-foot-shock compartments were exposed to the emotional cues from foot-shocked animals such as shrieks, smell of feces or urine, and jumping response. Accordingly, non-foot-shocked animals were assumed to be in fear or in a state of anxiety. The foot-shocked animals were designated as
“senders”, since they are senders of emotional cues, while the non-foot-shocked animals receiving emotional cues from the “senders” were designated as “responders”. The previous study using mice indicated that responders with a 3-day exposure to the emotional response of senders revealed gastric lesions with a high incidence (14). In studies using rats, the repeated exposure to emotional responses of senders induced an increase of plasma corticosterone in the responders (11, 12). Thus, the communication box method is considered to be a beneficial tool for setting “experimental anxiety” socio-psychologically.

The passive avoidance task also has been used to induce experimental anxiety. Prolongation of the time taken for animals to enter the dark compartment after the foot shock experience was considered to be based on conditioned fear (13–15).

The present study consisted of the following two experiments: 1) Effects of drugs on gastric lesions of responder mice in the communication box method (Experiment 1) and 2) effects of drugs on a passive avoidance task in rats (Experiment 2).

MATERIALS AND METHODS

Experiment 1. Communication box study

Animals: Male ICR mice (7–8 weeks old) were used. Prior to the experiment, the animals were housed in laboratory cages with 10 animals per cage and maintained in an air-conditioned room (23 ± 1 °C) with a 12 hr:12 hr light-dark cycle (lights on 07:00–19:00).

Apparatus: The communication box for mice was used.

The floor of the communication box was equipped with grids for electric shock. The inside was divided into small compartments (10 × 10 cm), consisting of foot-shock compartments with a grid floor and non-foot shock compartments with a grid floor covered by transparent plastic boards. The foot shock compartments were arranged such as to surround the non-foot shock compartments. The inside temperature of the communication box was maintained at 23 ± 1 °C throughout the experiment.

Procedures: The experimental groups consisted of the following 3 groups: sender group, responder group, and food-yoked group to responder. Sender animals received a foot-shock of 10-sec duration at intervals of 50 sec for 3 hr. The electric current for the shock was increased stepwise from 1.6 mA to 2.0 mA at the rate of 0.2 mA per 1 hr. Responder animals were exposed daily to the emotional responses of sender animals, 3 hr per day for 3 days. Sender animals were changed daily to naive mice to prevent a reduced emotional response to foot shock based on adaptation or learned helplessness due to repeated exposure. The 3-hr foot shock period was started at 19:00. Both sender and responder animals were placed individually into each compartment in the communication box 15 min before beginning the shock period. On Day-1, responder animals were returned to their home cages after the 3-hr foot shock period. On Day-2, after completing the foot-shock period, they were transferred to the metal cages (14.5 × 24.5 × 17 cm), and they were housed in the cages with 4 animals per cage under the food-deprivation condition. Food-yoked control animals were maintained to the metal cage during the foot-shock period under the

Fig. 1. Chemical structure of SC-48274 in comparison with those of buspirone and diazepam.
aggregated housing condition (5 animals each), and then they were returned to their home cages after the foot-shock period. From beginning of the Day-2 experiment to completion of the Day-3 experiment, they were maintained in the metal cages under aggregated housing. On Day-3, just after completing the foot-shock period, the responders were sacrificed by chloroform, and their stomachs were removed. The stomachs were visually inspected for lesions.

**Experiment 2. Passive avoidance response study**

**Animals:** Male Wistar strain rats (7- to 8-week-old) were used. They were housed in laboratory cages, 4 rats per cage, and maintained in an air-conditioned room with a 12 hr:12 hr LD cycle (lights on 07:00–19:00) and fed ad libitum.

**Apparatus:** The step-down type of passive avoidance apparatus was used. This consisted of light and dark compartments. The light compartment (10 x 30 x 25 cm) was illuminated with 400 lux by a lamp and separated from the dark compartment (25 x 30 x 31 cm) by a wall with an opening (8 x 10 cm). The floor of the dark compartment was equipped with grids for foot shock. When placed in the light compartment, a rat could enter the dark compartment through the opening.

**Procedure:** The animals were randomly divided into 5 groups: saline-, 0.5% CMC-, SC-48274-, buspirone- and diazepam-treated groups. They received 3 pretraining trials on Day-1 of the experiment. During the pretraining trials, the rat was placed at the end of the light compartment. The time taken for the animal to enter the dark compartment was recorded and defined as the step-down latency. On Day-2 and Day-3, a single acquisition trial was given. This was similar to the pretraining trial except that the animals received a scrambled foot-shock (3 mA for 5 sec) upon entering the dark compartment. Rats could move to the light compartment through the opening to escape from foot-shock whenever they wanted. On Day-4, a single test trial was given. When a rat did not enter the dark compartment within 180 sec, it was taken from the light compartment and a score of 180 sec was assigned.

**Drugs**

In the present study, SC-48274 (G.D. Searle), buspirone (Bristol-Myers Squibb) and diazepam (Nippon Roche) were used. SC-48274 and buspirone were dissolved in saline. Diazepam was suspended in 0.5% carboxymethylcellulose (CMC). In the communication box study, the administration volume was 0.1 ml per 10 g body weight. Effects of drugs on gastric lesion formation were examined after either single or 3-day treatment. In the single treatment study, drugs were administered p.o. 30 min before beginning the foot-shock period on Day-3. In the 3-day treatment study, drugs were administered 30 min before the foot shock period once a day for 3 days.

In the passive avoidance study, drugs were administered p.o. 1 hr before a single test trial was given on Day-4 with an injection volume of 0.1 ml per 100 g body weight.

**Statistics**

In the communication box study, the data are reported as the incidence of mice with gastric lesions, since the gas-
tric lesions of responders were characterized by slight erosions or bleeding (11). The data were analyzed by Fisher’s exact probability test. In the passive avoidance study, the results were analyzed by the one-tailed Mann-Whitney U-test.

RESULTS

Experiment 1. Communication box study

Effects of SC-48274, buspirone and diazepam on gastric lesions in responder mice with 3-Day exposure to the emotional responses of sender mice in the communication box are shown in Figs. 2, 3 and 4, respectively.

As shown in Figs. 2, 3 and 4, the food-yoked group revealed gastric lesions, but the incidence was less than
30%. The saline-treated responder group developed gastric lesions with significantly higher incidence compared with the food-yoked group.

A single treatment with SC-48274 significantly reduced the incidence of mice with gastric lesions at doses over 25 mg/kg compared with the saline treatment, while a 3-Day treatment with SC-48274 significantly suppressed gastric lesion formation at doses over 5 mg/kg (Fig. 2).

A single treatment with buspirone had no preventative effect on gastric lesion formation (Fig. 3). The 3-Day treatment with buspirone suppressed gastric lesion formation at similar doses of 1 and 2 mg/kg to the single treatment, but the dose response curve was not a U-shape, different from the behavior of buspirone (Fig. 4).

A single treatment with diazepam significantly suppressed gastric lesion formation at doses of 1, 2 and 5 mg/kg compared with 0.5% CMC (Fig. 4). The 3-Day treatment with diazepam also suppressed gastric lesion formation at doses over 2 mg/kg (Fig. 4).

DISCUSSION

The present study assessed the anxiolytic activity of a novel anxiolytic, SC-48274, using two animal models of anxiety, the communication box method and a passive avoidance response model.

In the communication box study, the gastric lesion for-
anxiolytic activity. The difference in potency between the single treatment with buspirone is weaker than the response at doses over 2 mg/kg. The result indicates that a higher dose of buspirone, over 25 mg/kg, is needed for anxiolytic action. On the other hand, buspirone and diazepam shortened the step-down latency in the passive avoidance task (Fig. 6). These findings suggest that the experimental models used in the present study can assess anxiolytic activity.

A single treatment with SC-48274 prevented gastric lesion formation in the communication box study in mice at doses over 25 mg/kg (Fig. 2), while the single treatment showed anxiolytic activity at a dose of 25 mg/kg in the passive avoidance study of rats (Fig. 6). Since the escapable foot-shock was used in the passive avoidance study, the anxiety level in the experimental situation may be mild. The results suggest that a large dose of SC-48274 is needed for anxiolytic action. On the other hand, a single treatment with diazepam in the passive avoidance task in rats showed anxiolytic activity at doses of 5 and 10 mg/kg (Fig. 6), while in the communication box study in mice, it prevented gastric lesion formation at 1, 2 and 5 mg/kg with inverse dose-dependency (Fig. 4). The inverse dose-dependency of diazepam on gastric lesion formation may be affected by hypothermia induced by the higher dose (16), aggravating gastric lesions. A single treatment with buspirone in the passive avoidance task showed anxiolytic activity at a dose 25 mg/kg (Fig. 6), and it did not prevent gastric lesion formation in the communication box study, although the incidence of gastric lesions in the saline control group in the single treatment study of buspirone was lower than in the other studies (Fig. 3). These findings suggest that a higher dose of buspirone, over 25 mg/kg, is needed for anxiolytic action with a single p.o. treatment. Thus, the results in the single treatment study suggest that the anxiolytic activity of SC-48274 is as potent as that of buspirone, but weaker than that of diazepam.

Three-day treatment with SC-48274 in the communication box study markedly prevented gastric lesion formation at doses over 5 mg/kg (Fig. 2). The difference in potency between single and 3-day treatments with SC-48274 on the prevention of gastric lesion formation suggests that chronic SC-48274 treatment can enhance its anxiolytic activity, and the chronic treatment with a large dose is needed to elicit anxiolytic activity.

Three-day treatment with buspirone markedly prevented gastric lesion formation, but with a U-shaped dose response at doses over 2 mg/kg. The result indicates that a single treatment with buspirone is weaker than the chronic treatment or a single treatment of diazepam in anxiolytic activity. The difference in potency between the single and 3-day treatment with buspirone may be due to the pharmacokinetic properties of buspirone which cause its concentration to be markedly affected by first-pass metabolism after oral administration (6, 17). Regarding the U-shaped dose response curve of buspirone found in the present study, it has been reported that buspirone at low doses attenuated and at higher doses potentiated gastric lesion formation induced by cold-restraint stress (18). This biphasic effect seems to resemble the U-shaped dose response of buspirone found in the present study. The aggravation effect at higher doses seems to be based upon the hypothermic effect (19) or corticosterone-releasing effect (20) of buspirone, since these effects are aggressive gastric ulcer formation factors (21, 22).

Three-day treatment with diazepam markedly suppressed gastric lesion formation at doses of 1 and 2 mg/kg (Fig. 4), but this was not dose-dependent. However, the results in the present study suggest that diazepam has a preventative effect on gastric lesion formation. The discrepancy between buspirone and diazepam in the 3-day treatment study may be attributable to the effects of buspirone and diazepam on the gastro-intestinal tract as well as the central nervous system. Buspirone suppresses mental stress- and corticotrophin-releasing factor (CRF)-stimulated cecal motility (23), and the effects of stress and CRF on ceco-colonic motility persist in hypophysectomized rats (24). However, the preventative effect of diazepam on the cold-restraint gastric lesion is considered to result from a combination of sedative, anxiolytic and antisecretory actions (25). Therefore, the difference of the dose-response between buspirone and diazepam in the prevention of gastric lesion formation in the present study may be due to a difference in their pharmacological actions on the central nervous system or the gastrointestinal tract.

The present study suggests that SC-48274 has anxiolytic activity similar to buspirone. The anxiolytic activity of buspirone is considered to be based on the reduction of serotonergic neuron activity via a partial agonistic action at somatodendritic 5-HT1A receptors (8). Since SC-48274 is a tryptophan hydroxylase inhibitor (1), the anxiolytic activity is also considered to be based on suppression of presynaptic serotonergic neuron activity (2). However, no additional biochemical study on SC-48274 has been done yet. Hence, the mode of action of this drug is still unclear, although the anxiolytic action may be related to suppression of the serotonergic neurons (2). Therefore, additional studies to clarify the mode of action of SC-48274 are needed.

In conclusion, the results of the present study suggest that SC-48274 has an anxiolytic activity, but the chronic or large dose treatment is needed to elicit the anxiolytic activity. In addition, SC-48274 is effective across a broader range of doses than either buspirone or diazepam, pos-
sibly due to the lack of side-effects.

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