Pharmacological Properties of SM-3997: A New Anxiolytic Candidate

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Abstract—Pharmacological properties of SM-3997 (3 α ,4 β ,7 β ,7 α -hexahydro-2-(4-(4-(2-pyrimidinyl)-1-piperazinyl)-butyl)-4,7-methano-1H-isoindole-1,3(2H)-dione dihydrogen citrate) have been examined in rats and mice. SM-3997 showed a dose-related anticonflict activity in rats in a water lick conflict paradigm, and it had no effect on water consumption in a spontaneous water drinking test. The potency of SM-3997 appeared to be equal to that of buspirone and about one-half that of diazepam. No tolerance to the anticonflict activity of SM-3997 was observed following 5 and 10 consecutive days of treatment. Unlike diazepam, SM-3997 had no anticonvulsant effect and had very weak muscle relaxant and hypnotic effects. On the other hand, SM-3997 and buspirone exhibited dopamine antagonistic action, although the potency of SM-3997 was less than one fourth that of buspirone. These results show that SM-3997 is a new anxiolytic agent which is weaker than buspirone in the dopaminergic neuron system.

Benzodiazepines have been widely used for the treatment of anxiety. They, however, often produce undesirable effects such as drowsiness, ataxia and sedation at clinically effective doses. In addition, they adversely interact with other CNS depressants, particularly alcohol (1). As the occurrence of these side effects may be closely coupled to the mode of action involving benzodiazepine receptors, it is necessary to develop anxiolytics structurally unrelated to the benzodiazepines, which will not interact directly with benzodiazepine receptors.

The compound SM-3997 is a novel nonbenzodiazepine compound possessing potent anxiolytic properties in animal models (2). It does not have the other behavioral actions of benzodiazepines such as muscle relaxant and anticonvulsant activities (2). The present report describes the pharmacological profiles of SM-3997 in comparison with those of diazepam and buspirone, the recently synthesized nonbenzodiazepine anxiolytic (3).

Materials and Methods

Male Sprague-Dawley rats (200–300 g) and male ddY strain mice (20–30 g) were used in these studies. All experiments were performed in a room with a 12 hr day-night cycle (dark period: 20:00–08:00 hours) and a relatively constant environment (24 $\pm$ 1°C and 55 $\pm$ 5% relative humidity), and all animals received food and water ad libitum except when indicated. The ED₅₀ value was calculated according to the method of Litchfield and Wilcoxon (4). Drugs were administered in volumes of 10 ml/kg for all routes in rats and mice except in the conflict test. In the conflict test, drugs were administered in volumes of 5 ml/kg for all routes in rats.

1. Anticonflict activity

A modification of the method of Vogel et al. (5) was used. Rats were deprived of water for 24 hr prior to the first training session (unpunished session) consisting of a 3 min period. Each animal was then placed in a

plexiglass conflict test box (38×38×20 cm). A water bottle with a stainless steel spout was fitted to the middle of the outside of one of the sides so that the spout extended 1 cm into the box at a height of 2 cm above the floor. The rat was allowed to explore until it discovered the drinking spout and began to drink. The number of licks at the spout in 3 min was counted. Only rats making more than 300 licks during the 3 min unpunished session progressed to a further 24 hr deprivation of water. Twenty four hours after the unpunished session (48 hr after access to water), the rat was placed in the test box. The second session (pre-drug punished session) consisting of a 3 min period started automatically when the rat completed 20 licks and received the first mild electric shock (0.35 mA, 0.5 sec). After every 20 unpunished licks, subsequent licking was punished. Only rats showing suppression of licking (less than 260 licks) by punishment during the pre-drug punished session by comparison with the unpunished session were included in the study. Each animal was used only once.

Drugs were administered 30 min after the pre-drug punished session. Then the 3 min post-drug punished session was repeated after the administration of SM-3997, buspirone and diazepam.

1.1. Single administration test: The number of shocks taken in the 3 min post-drug punished session was measured at 1 hr after the oral or i.p. administration of SM-3997 and buspirone, and at 30 min after diazepam.

1.2. Repeated administration test: SM-3997 (10 mg/kg), buspirone (10 mg/kg) and diazepam (5 mg/kg) were injected i.p. continuously for 5 or 10 days, and the number of shocks taken in the 3 min post-drug punished session was measured at 1 hr (SM-3997 and buspirone) and 30 min (diazepam) after the last administration of each drug.

1.3. Spontaneous water drinking test: In order to measure water intake, the same apparatus in the conflict procedure was used. Rats were deprived of water for 24 hr prior to the first training session consisting of a 3 min period. Only rats making more than 300 licks during the 3 min training session progressed to a further 24 hr deprivation of water. Twenty four hours after the training

session (48 hr after access to water), the rat was placed in the test chamber. Water consumption sessions consisting of 3 min periods, starting automatically when the rat completed 20 licks, were repeated at 1 hr after the oral administration of SM-3997 and buspirone, and at 30 min after diazepam.

2. Muscle relaxant action

2.1. Traction test: This experiment was carried out according to the method described by Couvoisier et al. (6). Mice were hung on the horizontal wire (1 mm diameter, 30 cm in height) by the forepaws at 1 hr after the oral administration of SM-3997 and buspirone, and at 30 min after diazepam. If the hindpaws of the mouse did not touch the wire within 10 sec in 3 successive trials, a positive response was noted.

2.2. Rota-rod test: This experiment was carried out according to the method described by Dunham and Miya (7). Mice and rats were gently placed on the rota-rod (5 cm in diameter for mice and 8 cm in diameter for rats, 5 rpm) at 1 hr after the oral administration of SM-3997 and buspirone, and at 30 min after diazepam. If the animals fell from the rotating rod within 60 sec, a positive response was noted.

3. Potentiation of anesthesia

3.1. Potentiation of the hexobarbital-induced anesthesia: Hexobarbital at a dose of 140 mg/kg was injected i.p. at 1 hr after the oral administration of SM-3997 and buspirone, and at 30 min after diazepam. The anesthetic interval was recorded for each mouse by measuring the time between the loss and the recovery of the righting reflex. The ED₅₀ was calculated by determining the dose needed to prolong the anesthetic interval to a level two times the average value of the controls in 50% of the mice.

3.2. Potentiation of the ethanol-induced anesthesia: Ethanol at a dose of 3 g/kg was injected i.p. at 1 hr after the oral administration of SM-3997 and buspirone, and at 30 min after diazepam. At 45 min thereafter, the ED₅₀ value for causing the loss of the righting reflex was determined.

4. Effect on convulsion

Mice were challenged with pentylene-tetrazole (110 mg/kg, s.c.), bicuculline (0.6 mg/kg, i.v.) or maximum electroshock (MES;

Fig. 1. Chemical structure of SM-3997

15 mA, 0.2 sec duration) delivered through corneal electrodes at 1 hr after the oral administration of SM-3997 and buspirone, and at 30 min after diazepam. The dose which prevented clonic (pentylene-tetrazole) or tonic (bicuculline and MES) convulsions for 30 min following the injection of drugs or the application of maximum electric shock in 50% of the mice was defined as the ED50.

5. Effect on dopamine systems

5.1. Anti-apomorphine test in rats: Rats were challenged by apomorphine (0.5 mg/kg, i.v.) at 1 hr after the oral administration of SM-3997, buspirone and diazepam. If the rat showed no licking behavior for 20 min after the injection of apomorphine, a positive response was noted.

5.2. Anti-apomorphine test in mice: Mice were challenged by apomorphine (1 mg/kg, s.c.) at 1 hr after the oral administration of SM-3997 and buspirone. If the mouse showed no climbing behavior for 10 min at 10 min after the injection of apomorphine, a positive response was noted.

5.3. Anti-methamphetamine test: Mice were challenged by methamphetamine (1 mg/kg, i.p.) at 30 min after the oral administration of SM-3997 and buspirone. The dose which prevented spontaneous locomotor activity for 1 hr at 10 min after the injection of methamphetamine in 50% of the mice was defined as the ED50.

5.4. Conditioned avoidance test in a shuttle box: Rats were allowed to adapt to the shuttle box for 30 min on the first day and then given 6.5 min training sessions (13 trials/session) daily. The conditioned stimulus (CS) was the sound of a buzzer, and the unconditioned stimulus (UCS) was intermittent electric shock. The CS-UCS interval was 5 sec. The escape response was a movement from one compartment to the other after the onset of shock. The avoidance response was a similar movement made

during the 5 sec CS-UCS interval. Training was carried to a criterion of 90% avoidance responses in 13 trials. After reaching the criterion, the rats were injected with SM-3997 and buspirone. At 1 hr after this injection, the rats were returned to the shuttle box and tested for 6.5 min. A positive response was scored if the rat received 2 or more shocks within 10 trials after a 3 trial initiation period.

6. Influence on spontaneous locomotor activity (SLA)

A mouse was placed in a photocell activity cage at 1 hr after the oral administration of SM-3997 and buspirone or at 30 min after diazepam. The count during 10 min was taken as the spontaneous locomotor activity of a mouse. A positive response was scored if the locomotor activity of drug-treated mice decreased by 50% of the activity of the vehicle-treated control groups.

7. Drugs

SM-3997 citrate (Fig. 1), buspirone HCl and diazepam were synthesized at the Sumitomo Pharmaceuticals Co., Ltd. Other chemicals were obtained from standard commercial sources.

SM-3997, buspirone, pentylenetetrazole (Aldrich), apomorphine HCl (Sandoz) and methamphetamine HCl (Dainihon-Seiyaku) were dissolved in saline (0.9% NaCl). Diazepam was suspended in a 0.5% methylcellulose solution. Bicuculline (Sigma) was dissolved in 0.01 N HCl solution and hexobarbital (Teikoku-Kagaku) in 0.01 N NaOH solution. All control animals received the appropriate vehicle.

8. Statistical analysis

Dunnett's test was used for statistical analysis of the data.

Results

1. Anticonflict activity

1.1. Single administration test: SM-3997 produced a significant increase in the number

of shocks at doses of 5 and 10 mg/kg, i.p. or 20 and 40 mg/kg, p.o. Buspirone and diazepam also produced a dose-related and significant increase in the number of shocks (Fig. 2). The relative potency of SM-3997

appeared to be equal with that of buspirone and about one-half that of diazepam.

1.2. Repeated administration test: SM-3997 (10 mg/kg, i.p.), buspirone (10 mg/kg, i.p.) and diazepam (5 mg/kg, i.p.) produced

Fig. 2. Effects of SM-3997, buspirone and diazepam on the shock-induced suppression of the drinking test. The data are represented as mean values $\pm$ S.E.M. ($n=10$). The significance of the difference from the control is represented by asterisks (* $P<0.05$, ** $P<0.01$, by Dunnett's test).

Table 1. Anticonflict activity following single and repeated (5 and 10 days) administration of SM-3997, buspirone and diazepam in rats

Drugs	Days	Dose (mg/kg, i.p.)	Punished responding ¹			
			Single	(n) ²	Repeated	(n) ²
Control	5	0	2.1 $\pm$ 0.6	(10)	1.0 $\pm$ 0.5	(10)
SM-3997	5	10	28.3 $\pm$ 6.4**	(10)	25.2 $\pm$ 5.9**	(11)
Diazepam	5	5	21.5 $\pm$ 5.8**	(10)	24.7 $\pm$ 4.9**	(10)
Control	10	0	2.1 $\pm$ 0.6	(10)	3.1 $\pm$ 1.2	(12)
SM-3997	10	10	28.3 $\pm$ 6.4**	(10)	28.1 $\pm$ 2.8**	(10)
Buspirone	10	10	20.5 $\pm$ 5.1**	(10)	18.5 $\pm$ 3.1**	(10)

¹ Mean shocks $\pm$ S.E.M. in 3 min. ² Number of rats tested. ** $P<0.01$. Significantly different from the control (Dunnett's test).

Table 2. Effects of SM-3997, buspirone and diazepam on the spontaneous drinking behavior of rats

Drugs	Dose (mg/kg, p.o.)	Water drinking ¹
Control	0	25.6±3.1
SM-3997	20	32.3±3.6
	40	28.7±3.7
	80	28.6±3.7
	160	19.6±5.4
	20	30.6±2.3
Buspirone	40	25.4±3.5
	80	24.1±3.0
	160	5.0±2.7**
Control	0	24.7±3.7
Diazepam	5	34.9±3.0
	10	40.5±3.3**
	20	39.0±2.8**
	40	40.8±1.5**

¹ (Mean±S.E.M.) ×20 licks in 3 min. **P<0.01 Significantly different from the control.

a significant increase in the number of shocks following repeated administration for 5 or 10 days (Table 1). There was no significant difference among the single and 5 or 10 days repeated administration groups.

1.3. Spontaneous water drinking test: The comparative effects of SM-3997, buspirone and diazepam on spontaneous drinking behavior of rats are presented in Table 2. SM-3997 had no significant effect on the number of licks at doses up to 160 mg/kg, p.o. Buspirone, however, significantly decreased it at 160 mg/kg, p.o. In contrast, diazepam produced a significant increase in the number of licks at doses of 10 to 40 mg/kg, p.o.

2. Muscle relaxant action

The comparative muscle relaxant activities of SM-3997, buspirone and diazepam in the traction and rota-rod test are presented in Table 3. In the traction test, SM-3997, like buspirone, did not induce muscle relaxation at doses up to 300 mg/kg, p.o., whereas diazepam caused muscle relaxation at relatively low doses (ED₅₀: 3.9 mg/kg, p.o.) in the same test.

SM-3997 and buspirone impaired rota-rod performance at relatively high doses in mice and rats. The ED₅₀ values of SM-3997 in mice and rats were 207 mg/kg, p.o. and greater than 300 mg/kg, p.o., respectively, and those of buspirone in mice and rats were

172 mg/kg, p.o. and 125 mg/kg, p.o., respectively. In contrast, diazepam impaired rota-rod performance at relatively low doses. The ED₅₀ values were 2.1 mg/kg, p.o., in mice and 9.1 mg/kg, p.o., in rats.

3. Potentiation of anesthesia

SM-3997 and buspirone increased the duration of the hexobarbital-induced anesthesia at relatively high doses. The ED₅₀ value of SM-3997 was 212 mg/kg, p.o., and that of buspirone was 263 mg/kg, p.o. They, however, failed to increase the duration of the anesthesia induced by ethanol at doses up to 300 mg/kg, p.o. In contrast, diazepam produced a significant potentiation of the anesthesia induced by these two drugs at relatively low doses. The ED₅₀ values of diazepam were 1.8 mg/kg, p.o., in the hexobarbital test and 3.0 mg/kg, p.o., in the ethanol test (Table 3).

4. Effect on convulsion

SM-3997 and buspirone failed to inhibit the convulsion induced by pentylenetetrazole, bicuculline and MES at doses up to 300 mg/kg, p.o., in mice. In contrast, diazepam antagonized the convulsion induced by pentylenetetrazole, bicuculline and MES at relatively low doses. The ED₅₀ values of diazepam were 0.41 mg/kg, p.o., in the pentylenetetrazole test; 1.2 mg/kg, p.o., in the bicuculline test; and 5.9 mg/kg, p.o., in the MES test (Table 3).

Table 3. Comparison of the pharmacological actions of SM-3997, buspirone and diazepam in various behavioral tests in mice and rats

Test	Animal	SM-3997	Buspirone	Diazepam
		ED50 (mg/kg. p.o.) (95% Fiducial limits)		
Muscle relaxant action				
traction test	mouse	>300	>300	3.9 (2.9–5.2)
rota-rod test	mouse	207 (125–343)	172 (115–258)	2.1 (1.3–3.3)
	rat	>300	125 (103–152)	9.1 (6.3–13.2)
Potentiation of anesthesia				
hexobarbital test	mouse	212 (154–293)	263 (209–330)	1.8 (1.2–2.7)
ethanol test	mouse	>300	>300	3.0 (2.6–3.9)
Effect on convulsion				
anti-pentylenetetrazole test	mouse	>300	>300	0.41 (0.28–0.59)
anti-bicuculline test	mouse	>300	>300	1.2 (0.8–1.9)
anti-MES test	mouse	>300	>300	5.9 (5.2–6.7)
Effect on dopamine systems				
anti-apomorphine test	mouse	188 (65–545)	48 (27–85)	—
	rat	305 (262–357)	44 (36–54)	>100
anti-methamphetamine test	mouse	>300	150 (96–221)	—
inhibition of CAR	rat	>300	69 (45–106)	—
Inhibition of SLA	mouse	183 (144–233)	85 (62–115)	25 (14–42)

Ten mice and five rats were used at each dose; 3 to 5 doses of test drugs were tested. MES: maximum electroshock, CAR: conditioned avoidance response, SLA: spontaneous locomotor activity.

5. Effect on dopamine systems

5.1. Anti-apomorphine test in rats and mice, and anti-methamphetamine test in mice: SM-3997 exhibited the anti-apomorphine activity in mice and rats at relatively high doses (ED50: 188 mg/kg, p.o. and 305 mg/kg, p.o., respectively). SM-3997, however, failed to inhibit the hyperactivity induced by methamphetamine in mice at doses up to 300 mg/kg, p.o. In contrast, buspirone produced a significant inhibition in these three tests. The ED50 values of buspirone were 48 mg/kg, p.o., in the anti-apomorphine test in mice; 44 mg/kg, p.o., in the anti-apomorphine test in rats; and 150 mg/kg, p.o., in the anti-methamphetamine test in mice. Diazepam did not have an anti-apomorphine action in rats at doses up to 100 mg/kg, p.o. (Table 3).

5.2. Conditioned avoidance test in a shuttle box: SM-3997 failed to inhibit the conditioned avoidance response (CAR) in rats at doses up to 300 mg/kg, p.o. Buspirone, however, inhibited the CAR at relatively low doses (ED50: 69 mg/kg, p.o.) (Table 3)

6. Influence on spontaneous locomotor

activity (SLA)

SM-3997, buspirone and diazepam decreased the spontaneous locomotor activity of mice in a dose-related manner. Their ED50 values were 183 mg/kg, p.o., 85 mg/kg, p.o. and 25 mg/kg, p.o., respectively. The relative potency of SM-3997 was about one-half that of buspirone and about one seventh that of diazepam (Table 3).

Discussion

It is well-known that in Vogel's conflict procedure, the anticonflict action predicts clinical anxiolytic activity (8), and there have been many reports showing that benzodiazepines with clinical anxiolytic activity were effective in this test. The compound buspirone, whose structure and pharmacological profile are different from those of the benzodiazepines, has also been shown to induce anxiolytic activity in clinical studies (9, 10). It is, however, not clear that buspirone exhibits anticonflict activity in the conflict model. It has been reported that buspirone was active at doses of 20 to 80 mg/kg, p.o. (11) or 1 to 5 mg/kg, p.o. (12) in Vogel's conflict procedure, although Goldberg and

Finnerty (9) and Sanger et al. (13) have not found anticonflict activity of buspirone in the same test. Significant differences in the two methods may explain this discrepancy. In the present study, we have observed that buspirone exhibited clear effects in Vogel's conflict test at 20 to 40 mg/kg, p.o. or 5 to 10 mg/kg, i.p. Furthermore, the new compound SM-3997 was also effective in a dose-dependent fashion after i.p. and p.o. administration in this test. The efficacy of SM-3997 was equipotent to that of buspirone and was weaker than diazepam. This shows that SM-3997 will have clinically anxiolytic activity like diazepam and buspirone.

Since the conflict model in this study utilizes the primary drive of thirst to motivate the subject's behavior, it is possible that agents that affect the primary drive state could alter the animal's performance in such a procedure. For example, the hypertonic saline that increased water consumption exhibited significant anticonflict activity in the conflict model (14). Similarly, diazepam produced an increase in water consumption in rats. This result suggests that the effects of diazepam on the primary drive might be partly related to its anticonflict activity in this conflict model. On the other hand, SM-3997 failed to enhance water consumption in rats. This suggests that the anticonflict activity of SM-3997 is primarily due to its anxiolytic potential.

It is generally believed that tolerance to the anxiolytic activity of the benzodiazepines does not develop. We observed that tolerance to the anticonflict activity of diazepam did not develop, which is similar to the results reported by Margules and Stein (15). Furthermore, SM-3997 also exhibited the anticonflict activity following 5 or 10 daily administrations as well as single administration. These results suggest that there might be no development of tolerance to the putative anxiolytic activity of SM-3997.

Diazepam induced muscle relaxation and potentiation of anesthesia induced by hexobarbital and ethanol at doses close to anxiolytic doses. In contrast, SM-3997 showed a wide separation between anxiolytic activity and ability to cause muscle relaxation and was shown to potentiate anesthesia

induced by hexobarbital and ethanol. This indicates that SM-3997 might lack side effects like drowsiness, sedation and ataxia which were often produced by diazepam. Likewise, the interaction with alcohol and CNS depressants, compared with diazepam, may be less pronounced in the case of SM-3997.

SM-3997 and buspirone exhibited dopamine antagonistic action, although the potency of SM-3997 was less than one fourth that of buspirone. Wood et al. (16) have reported that buspirone produces a potent and dose-dependent increase in striatal DA metabolites (DOPAC and HVA) like haloperidol. Moreover, it has been reported that buspirone, due to its anti-dopamine activity, has weak neuroleptic activity and has shown extrapyramidal side effects in clinical study (17) and prolactin releasing activity in rats (18) and humans (19). It can be expected that SM-3997 is less likely to induce these actions than buspirone.

It is now generally accepted that the action of benzodiazepine anxiolytics is mediated via the benzodiazepine-GABA receptor complex in the CNS (20). SM-3997, however, did not bind to benzodiazepine receptors or GABA receptors, and it did not potentiate benzodiazepine or GABA binding to each receptor (21). The anticonflict action of SM-3997 was not inhibited by the benzodiazepine antagonist (e.g., Ro 15-1788 or CGS 8216) or a GABA antagonist (e.g., bicuculline) (2). Moreover, SM-3997 did not antagonize the convulsion induced by bicuculline. These results indicate that SM-3997 does not have a direct action on the benzodiazepine-GABA receptor complex and may have a mechanism of action which does not include the benzodiazepine-GABA receptor complex. On the other hand, we found that SM-3997 bound preferentially to a special subtype (5-HT_{1A} receptors) of 5-HT receptors, and it showed some effects on the 5-HT behavioral syndrome such as the flat body posture in rats (21). Moreover, in binding studies with radiolabelled SM-3997, it was indicated that ³H-SM-3997 bound selectively and with high affinity to 5-HT-1A receptors in rat brain, and it may be an

agonist for its binding sites (22). Therefore the pharmacological action of SM-3997 may be produced via the 5-HT neuron system, and is unrelated to the benzodiazepine-GABA receptor complex. Further work is in progress to clarify the mechanism of action of SM-3997 in more detail.

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