PHARMACOLOGICAL STUDIES OF 5-METHYL-8-ETHYL-SULFONYL-10-(2-DIMETHYLAMINOETHYL)-5H-DIBENZO [B,E] [1,4] DIAZEPINE-11 (10)-ONE (SM-307), AN ANTIDEPRESSIVE SUBSTANCE

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Since the introduction of dibenzepine (Noveril®) in the medical practice, this compound has been used for the management of depression. Stille et al. (1) have suggested that dibenzepine should be placed somewhere between imipramine and desipramine.

In our laboratory we were engaged in an investigation on the influence of substitution on the tricyclic group on the biological activities of this type of compounds and found that the new compound, 5-methyl-8-ethylsulfonyl-10-(2-dimethylaminoethyl)-5H-dibenzo [b, e] [1, 4] diazepine-11 (10)-one (SM-307) was as potent as dibenzepine with regard to antagonism of reserpine induced hypothermia and had lower toxicity than dibenzepine. The present report serves to confirm some of the antidepressive effects of SM-307 compared with imipramine and other antidepressive substances.

MATERIALS AND METHODS

1) Antagonism of the hypothermia produced by reserpine in rats

Male Wistar rats weighing 250-300 g were housed in individual cages at a room temperature of 22°C and a relative humidity of 60%. Reserpine was administered intraperitoneally at a dose of 5 mg/kg 5 hours before the intraperitoneal injection of test drugs. Body temperature of rats was determined by an electrical thermometer inserted into the rectal cavity for 5 hours after the injection of test drugs.

2) Effects on hyperthermia induced by noradrenaline in rats

Unanesthetized male Wistar rats weighing 200-250 g were placed in a supine position. The experiments were carried out at a room temperature of 22°C. Body temperature was determined by an electrical thermometer inserted into the rectal cavity. Noradrenaline was infused at a dose of 60 μg/animal in 10 minutes into the femoral vein at a speed of 0.1 ml/min. The test drugs were injected intraperitoneally 30 minutes before the infusion of noradrenaline.
3) Effects on the pressor response of noradrenaline or adrenaline in pithed rats

Male Wistar rats of the average weight of 250 g were anesthetized with ether and one of the carotid arteries was cannulated to record the blood pressure by means of an electromanometer connected to the recorder. Rats were then pithed by the method of Brown et al. (2) and maintained 30 minutes before the intravenous injection of noradrenaline and adrenaline.

4) Effects on the behavior of monkeys pretreated with reserpine

Male Rheusus monkeys (2.0-2.5 kg) were injected with reserpine at a dose of 1 mg/kg intravenously. Thirty minutes after reserpine, SM-307 or imipramine was administered subcutaneously at a dose of 20 and 10 mg/kg, respectively and changes of the monkeys' behavior were observed for 3 hours.

5) Effect on the amine oxidase activity

Effect of SM-307 on the amine oxidase activity was examined in vitro. Mitochondria fractions were prepared from rat liver homogenates by the method of Malherbe et al. (3). Incubation mixture contained 1 ml of 0.004% tyramine as a substrate, 1 ml of liver mitochondria fraction and 0.5 ml of 0.5 M phosphate buffer PH 7.4 which contained SM-307 or nialamide was made to a final volume of 3 ml with water and incubations were carried out for one hour at 37 °C. The amount of tyramine was determined spectrophotometrically by the procedure of Udenfriend et al. (4).

6) Effects on dopamine and noradrenaline levels in normal and reserpine treated rat brains

Male Wistar rats weighing 150-250 g were used in a group of 5 animals. SM-307 (20 mg/kg, p.o.) or imipramine (10 mg/kg, p.o.) was injected once a day for 10 days and reserpine (5 mg/kg/day, i.p.) was injected in the first and the fifth day. After 10 days, the rats were sacrificed by decapitation. The brains were removed immediately and were homogenized in 0.4 N perchloric acid. The noradrenaline and the dopamine contents in whole brain were determined fluorometrically by the method of Anton et al. (5, 6).

7) Anticonvulsant activities in mice

Maximal electroshock seizures were induced by the technique of Swinyard et al. (7). Tests were carried out in groups of 5 mice at four dosage levels. The dose of drug which prevented the appearance of limb extensor phase in 50 per cent of the mice was calculated as the ED50 by the method of Weil (8).

8) Potentiation of anesthetic effect of barbital

Groups of 5 mice weighing 18-20 g were injected i.p. with drugs at various doses 15 minutes after the intravenous injection of 100 mg/kg of barbital sodium. ED50 was calculated as the dose which produced the loss of righting reflex in half of the mice for more than 30 seconds.

9) Effects on the blood pressure and respiration

Male cats weighing 3-4 kg were anesthetized with an intraperitoneal injection of 40 mg/kg of pentobarbital. Carotid blood pressure and respiration were recorded on the paper by means of a mercury manometer and Marey's tambour.
10) Spasmolytic activity in the isolated guinea pig intestine

Smooth muscle effects were studied using strips of guinea pig ileum. A portion of ileum about 2 cm in length was removed from a freshly killed guinea pig and was suspended in 30 ml of Tyrode solution at 30°C. SM-307 and other drugs were added 2 minutes before adding a submaximal dose of acetylcholine (ACh) or histamine (His). ED₅₀ is the dose of drug causing 50% reduction in the spasm produced by ACh or His. The values express the final concentration of drug in the muscle bath in g/ml.

11) Acute toxicity in mice

Male adult mice weighing 18-22 g were injected with drugs i.v., i.p. and p.o. in groups of 5 for each of four dosage levels. LD₅₀ was calculated as the dose which killed half of the mice in 48 hours according to the method of Weil (8).

RESULTS

15) Antagonism of the hypothermia produced by reserpine in rats

When rats were injected with reserpine at a dose of 5 mg/kg intraperitoneally, the body temperature of rats fell gradually and in 5–10 hours after the injection they reached the lowest degree. SM-307 protected the reserpine induced hypothermia in rats. The effect of 10 mg/kg of SM-307 was similar to that elicited by 5 mg/kg of imipramine (Fig. 2).

![Fig. 2. Influence of SM-307 and the other antidepressive substances on the body temperature of rats. Rats were injected with reserpine (5 mg/kg, i.p.) 5 hours before the experiment and kept at a room temperature of 22°C. Drugs were given by intraperitoneal injection. − −, ○, ⋅, ⋅ and ⋅ ⋅ represent 0, 1, 5, 10 and 50 mg/kg. The number of rats used is 10 for each dose.](image-url)
Effect on hyperthermia induced by noradrenaline in rats

Noradrenaline infused in rats at a concentration of 60 μg/animal 10 min induced an elevation of body temperature which returned gradually to normal level after termination of the infusion. Rats pretreated with SM-307 or imipramine showed a higher rise and particularly a more sustained increase of the body temperature than noradrenaline alone (Fig. 3).

Effects on the pressor response of noradrenaline or adrenaline in pithed rats

Injected intravenously at doses of 0.05 μg and 0.1 μg, noradrenaline and adrenaline...
increased the blood pressure by 10 to 30 mmHg. There was twofold in pressor responses to noradrenaline and adrenaline when 5 mg/kg of SM-307 was injected intravenously 30 minutes before. Imipramine exhibited promptly the potentiating action for both noradrenaline and adrenaline at a dose of 2.5 mg/kg i.v. (Figs. 4 and 5).

4) Effect on behavior of monkeys pretreated with reserpine

Behavioral changes of monkey were usually evident within 30 minutes after intravenous injection of 1 mg/kg of reserpine. Hostile and active monkeys became more quiet and less aware of external stimuli. When SM-307 was injected at a dose of 20 mg/kg subcutaneously 30 minutes before the administration of reserpine, the sedative effect of reserpine was suppressed by SM-307 especially in respect to reactivity and stupor. Three hours after reserpine, responses of SM-307 treated monkey to visual and auditory stimuli were still more prominent than in the monkey treated with reserpine alone. On the other hand imipramine enhanced the sedation induced by reserpine for the first 2 hours but 3 hours after imipramine the animal recovered from the semistupor.

Fig. 6 shows the behaviors of monkeys treated with reserpine alone and of monkeys treated with SM-307 or imipramine followed by reserpine.

5) Effect on the amine oxidase activity

As shown in Fig. 7, marked inhibition of the metabolism of tyramine was produced by the previous administration of niatalamide in vitro. On the other hand, SM-307 could not produce any inhibition up to the final concentration of $10^{-5}$ g/ml.

6) Effect on dopamine and noradrenaline levels in normal and reserpine treated rat brains

In Table I are shown the data obtained by daily injections of SM-307 and imipramine for 10 days. SM-307 and imipramine did not change the amine levels of rats significantly. In reserpine treated rats, which were injected with reserpine twice on the first and the fifth day in the experiment, the amounts of dopamine and noradrenaline were decreased signifi-
Fig. 6. Effects of SM-307 and imipramine on the behavior in reserpine treated monkeys. Reserpine (1 mg/kg, i.v.) was injected 30 minutes before the test drugs.

Fig. 7. Tyramine metabolism in the rat liver mitochondria and inhibition of the metabolism by SM-307 and nialamide. All incubations were performed for 1 hour as described in the text.
TABLE 1. Changes in brain amine levels produced by reserpine, SM-307 and imipramine.

| Compound          | Daily dose (mg/kg) | Amine levels (μg/g) | Noradrenaline | Dopamine |
|-------------------|--------------------|---------------------|---------------|----------|
| Control           |                    |                     |               |          |
| SM-307            | 20                 | 0.57 ± 0.06         | 0.87 ± 0.04   |
| SM-307 & Reserpine| 20 & 5             | 0.21 ± 0.02         | 0.35 ± 0.03   |
| Reserpine         | 5                  | 0.22 ± 0.02         | 0.33 ± 0.03   |
| Imipramine        | 10                 | 0.56 ± 0.06         | 0.86 ± 0.07   |
| Imipramine        | 10                 | 0.20 ± 0.02         | 0.31 ± 0.03   |

SM-307 (20 mg/kg/day, p.o.) and imipramine (5 mg/kg/day, p.o.) were given for 10 days. Reserpine (5 mg/kg, i.p.) was injected twice on the first and the fifth day. Each value is the average and its standard error for 10 rats.

Significantly. The decreases of brain catecholamines induced by reserpine were not influenced when SM-307 or imipramine was injected for 10 days.

7) Anticonvulsant activities in mice

SM-307 could not protect the maximal electroshock seizure up to 200 mg/kg, i.p. in mice. Dibenzepine also had no effect at doses of 12.5 to 25 mg/kg but at a higher dose of 50 mg/kg dibenzepine protected the seizure by about 60%. Imipramine and amitriptyline protected the seizure and ED₅₀ were 30.8 mg/kg and 15.4 mg/kg, respectively.

8) Potentiation of anesthetic effect of barbital

SM-307 at subcutaneous doses of 50 to 225 mg/kg did not potentiate the effect of barbital sodium. On the other hand, imipramine and amitriptyline showed a prolonged effect on sleeping time and ED₅₀ of imipramine and amitriptyline were 28.3 mg/kg and 18.6 mg/kg, respectively.

9) Effect on blood pressure and respiration

Intravenous administration of 1 mg/kg of SM-307 did not produce any marked changes in carotid blood pressure and respiration of cats. Five to 10 mg/kg of SM-307 caused
temporary fall of the blood pressure with a slight increase of the respiratory rate. At a larger dose, 20 mg kg of SM-307 caused about 30 mmHg fall of the blood pressure accompanied with a slight suppression of respiration (Fig. 8).

9. Spasmolytic activity in the isolated guinea pig intestine

Administration of SM-307 before ACh or His protected the contraction of the smooth muscle. E D₉₅ for SM-307 against ACh spasm was 2.3 × 10⁻² g ml and those for imipramine and amitriptyline were 6.4 × 10⁻² g ml and 5.5 × 10⁻² g ml respectively. Anti-ACh activity of SM-307 was approximately 1/40 that of imipramine and 1/400 that of amitriptyline. E D₉₅ for SM-307 against His spasm was 1.4 × 10⁻² g ml and those of imipramine and amitriptyline were 1.8 × 10⁻² and 4.5 × 10⁻² g ml respectively. Anti-His activity of SM-307 was considerably weaker than those of imipramine or amitriptyline.

11. Acute toxicity in mice

Table 2 shows LD₅₀ values with 95% confidence limits of SM-307 and the other antidepressive substances in mice. On any routes, SM-307 revealed the lowest toxicity among the test drugs. Mice did not exhibit any marked change in the spontaneous activity at doses of 25 to 300 mg kg, i.p. of SM-307, but 15 minutes after 250 mg kg, i.p., the spontaneous activities of mice were increased and a moderate staggering gait was observed for about 30 minutes. At a dose of 500 mg kg, i.p., animals showed excitement followed by twitch and clonic convulsion and all mice died within 2 hours.

**Table 2. Acute toxicity in mice.**

| Compound | LD₅₀ (mg kg) |
|----------|--------------|
|          | i.v.         | i.p.         | p.o.         |
| SM-307   | 73.9         | 253.5        | 857.4        |
|          | (60.1-91.9)  | (253-447)    | (610-1204)   |
| Imipramine| 24.0         | 122.7        | 447.5        |
|          | (14.0-41.3)  | (98-153)     | (138-527)    |
| Desipramine| 38.9         | 122.7        | 447.5        |
|          | (33.1-45.7)  | (55-275)     | (381-527)    |

LD₅₀ values with 95% confidence limits. Five mice were used for each dose. Mice were observed for 48 hours.

**DISCUSSION**

A combination of several procedures has been used for screening of tricyclic antidepressive substances: antagonism of temperature fall and ptosis produced by reserpine (9), potentiation of certain peripheral pharmacological action of noradrenaline (10) and (+)-amphetamine (11), etc.

In rats, the hypothermia caused by reserpine was clearly prevented by SM-307 of an intraperitoneal dose of 10 mg kg to the same extent as 5 mg kg of intraperitoneal injection of imipramine. SM-307 markedly potentiated the pressor effect of noradrenaline and adrenaline in pithed rats and also enhanced the hyperthermic effect of adrenaline. SM-307 antagonized reserpine induced sedation in monkeys. SM-307 did not influence the
monoamine oxidase activity. Repeated injection of SM-307 or imipramine led to no change in dopamine and noradrenaline levels in the rat brain and did not suppress the decrease of the two amine levels induced by reserpine. These results supported the findings of Sulser et al. (12) that imipramine could not change the contents of brain catecholamines. In these respects this compound should be regarded as being indetical with those of tricyclic antidepressive substances.

SM-307, however, differed in several pharmacological effects from the other tricyclic antidepressive substances such as imipramine and amitriptyline. Thus, the duration of sleep evoked by barbital sodium in mice was not influenced by SM-307, though imipramine and amitriptyline prolonged the sleeping time markedly. SM-307 did not protect the mice from the maximal electroshock seizure but imipramine and amitriptyline showed a clear inhibition against maximal electroshock seizure. In the guinea pig ileum the anti-ACh and anti-His activities of SM-307 were the weakest in the test drugs.

SUMMARY

The pharmacological properties of 5-methylsulfonyl-10-(2-dimethylaminoethyl)-5H-dibenzo [b, e] [1, 4] diazepine-11 (10)-one (SM-307) were studied. SM-307 showed no change in spontaneous activities and low toxicity in mice. SM-307 showed some properties corresponding to those of tricyclic antidepressive substances. SM-307 was active against the effects of reserpine (body temperature and motor activity) and enhanced the effects of catecholamines (body temperature and blood pressure). The peripheral anti-ACh and anti-His activities were very weak (1 40 1 100 of imipramine activity). SM-307 did not influence amine levels of brain and monoamine oxidase activity. Cardiovascular and respiratory effects of SM-307 were very weak.

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