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Potential Anxiolytic and Antidepressant-Like Activities of SNC80, a Selective δ-Opioid Agonist, in Behavioral Models in Rodents

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Abstract. In the present study, we investigated the antidepressant- and anxiolytic-like effects of (+)-4-[(a*R*)-a-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide (SNC80), a non-peptidic selective δ-opioid receptor agonist, in various animal models in rodents. SNC80 significantly reduced the duration of immobility in the forced swimming test. Furthermore, in the elevated plus-maze test, SNC80 dose-dependently and significantly increased the time spent in the open arms of the plus-maze. These effects were completely antagonized by a selective δ-opioid-receptor antagonist, naltrindole. In the conditioned fear stress test, which examines psychological stress-induced motor suppression, desipramine did not produce any significant effect on the conditioned suppression of locomotor activity. However, SNC80 completely attenuated the conditioned suppression of locomotor activity in the conditioned fear stress test. In conclusion, our results suggest that δ-opioid receptors may play an important role in the regulation of emotional responses. Furthermore, it is possible that δ-opioid-receptor agonists might be novel and potent antidepressants that also have anxiolytic-like effects.

Keywords: SNC80, δ-opioid receptor, forced swimming test, conditioned fear stress test, elevated plus-maze test

Introduction

Recently, it has been reported that δ-opioid receptors are associated with the regulation of emotional responses (1 – 3). Filliol et al. (2) reported that δ-opioid receptor-knockout mice exhibited depressive-like responses in the forced swimming test and anxiogenic-like responses in the elevated plus-maze test. On the other hand, Ragnauth et al. (3) reported that preproenkephalin-knockout mice displayed anxiogenic-like responses in the open field and light/dark tests. These transgenic findings strongly suggest that δ-opioid receptors naturally inhibit stress and anxiety. Furthermore, it is possible that δ-opioid-receptor agonists may have antidepressant and anxiolytic-like activity.

(+)-4-[(a*R*)-a-((2*S*,5*R*)-4-Allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethyl benzamide (SNC80) is a non-peptidic selective δ-opioid agonist. This compound shows a high affinity for δ-opioid receptors (K_i = 1.78 nM) in mouse whole-brain membranes, with 495-fold lower affinity at μ-opioid receptors and 248-fold lower affinity at κ-opioid receptors (4). Thus, SNC80 might be useful for elucidating the pharmacological and physiological functions of δ-opioid receptors. Several studies have reported that SNC80 has antinociceptive and anticonvulsant effects (4 – 6). However, the antidepressant- and anxiolytic-like effects of SNC80 have not yet been well examined.

Anxiety and depression have been studied in several animal models (7 – 9). For example, the forced swimming test can be used to detect a wide range of antidepressant-like activity (10). Thus, the forced swimming stress test has been widely used to screen antidepressant compounds. In the field of anxiety, the elevated plus-maze test has become a popular animal model, and the results are sensitive to benzodiazepine anxiolytic treatment (11). On the other hand, the conditioned fear stress model in mice examines psychological stress-induced

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motor suppression (12, 13). It has been recognized that the conditioned fear stress model might be a stress model reflecting the emotional abnormality including depressive and/or anxiety state (14, 15). Furthermore, it has been reported that conditioned fear stress-induced motor suppression is attenuated by antidepressants as well as anxiolytics (15 – 17).

The purpose of this study was to determine whether SNC80 has antidepressant and anxiolytic-like activity. Thus, we examined the antidepressant- and/or anxiolytic-like effects of SNC80 using the forced swimming test, elevated plus-maze test, and conditioned fear stress model.

Materials and Methods

Animals

Experiments of forced swimming stress were conducted with 4-week-old male ICR mice (Charles River Japan, Yokohama). In the conditioned fear stress model, again, 4-week-old male ICR mice (Charles River Japan) were used. On the other hand, in the elevated plus-maze test, 6-week-old male Lewis rats (Charles River Japan) were used. All animals had free access to food and water in an animal room that was maintained at 22 ± 1°C with a 12-h light-dark cycle. This study was carried out in accordance with the Declaration of Helsinki and the guidelines for the care and use of laboratory animals of Hoshi University, which is accredited by the Ministry of Education, Culture, Sports, Science, and Technology.

Forced swimming test

This test allows a depressed state to be induced in mice by forcing them to swim in a narrow cylinder from which they can not escape (10). We placed each mouse in a Plexiglas cylinder containing water to a depth of 15 cm (23°C), so that the tail of the mouse could not reach the bottom of the apparatus. After a brief period of vigorous activity, the mice adopt a characteristic immobile posture that is reversed by the administration of compounds with antidepressant-like activity.

For training, swimming sessions were conducted for a 10-min period on day 1 and 2. On day 3, the total duration of immobility, including small maintenance movements, was measured during the last 4 min of the test session.

Elevated plus-maze test

The elevated plus-maze consisted of a black Plexiglas apparatus with four arms set in a cross pattern from a neutral central square (11). Two opposite arms were delimited by vertical walls (closed arms), whereas the two other opposite arms had unprotected edges (open arms). The maze was elevated 70 cm above the ground and placed in indirect light (100 Lux). At the beginning of the 5-min test session, each rat was placed in the central neutral zone, facing one of the open arms. The total number of visits to the closed and open arms and the cumulative time spent (% time spent open arm) and visits (% open arm entry) in the open arms were then observed on a monitor through a video camera system. An arm visit was recorded when the rat moved all four paws into the arm.

Conditioned fear stress model

In the conditioned fear stress test, we exposed mice to an electric foot-shock session in an apparatus consisting of a box with a grid floor (conditioning session). When they were re-exposed to the box, even though no shocks were applied, mice showed reduced locomotor activity. This conditioned suppression is considered to be a conditioned emotional response associated with shocks (12, 13). In the present study, mice were subjected to electric foot shocks (1.3 mA, 0.3 s/10 s) by an isolated stimulator for 4 min. The next day, animals were re-exposed to the foot-shock box, but without shocks, and locomotor activity (motor activity counts) was measured for 4 min during re-exposure to the box.

Open field test

In order to discard non-specific actions of drug-treatments on general activity, mice were tested in the open field. The open field apparatus was made of a gray wooden box (50 × 50 × 50 cm) and the results in the open field test were determined automatically. An infrared beam sensor was installed on the wall to detect the numbers of rearing. The distance of movement (total locomotor activity (cm)) of mice was recorded by an overhead color CCD camera. The heads of the mice were painted yellow and the color CCD camera followed the center of gravity. Data from the CCD camera were collected through a custom-designed interface (CAT-10; Muromachi Kikai, Tokyo) as a reflection signal. For the open field experiments, each animal was placed in the center of the open field and allowed to freely explore the apparatus for 5 min. Total locomotor activity and numbers of rearing were recorded automatically. Each mouse was used only once.

Data analysis

To analyze the behavior and neurochemical data, one-way ANOVA followed by Dunnett's *t*-test was used for all experiments.

Drugs

The drugs used in the present study were desipramine and diazepam (Sigma Chemical Co., St. Louis, MO, USA) and SNC80, naltrindole, β-funaltrexamine, and nor-binaltorphimine (synthesized by Toray Industries, Inc.). Desipramine and SNC80 were dissolved in saline. Naltrindole, β-funaltrexamine, and nor-binaltorphimine were dissolved in distilled water. Diazepam, which was a solution dissolved in 40% benzyl alcohol, 10% ethanol, 1.5% propylene glycol, and 42.8 mg/ml benzoic acid, was diluted in saline. Naltrindole was injected 30 min before SNC80 administration. β-Funaltrexamine and nor-binaltorphimine were each injected 24 h before SNC80 administration.

Results

Effects of SNC80 in the forced swimming test in mice

SNC80 (0.3 – 3 mg/kg, s.c.) dose-dependently and significantly reduced the duration of immobility (Fig. 1). The effects of SNC80 were significantly reduced by naltrindole, a selective δ-opioid-receptor antagonist. However, pretreatment with either β-funaltrexamine (30 mg/kg, s.c.), a selective μ-opioid-receptor antagonist, or nor-binaltorphimine (10 mg/kg, s.c.), a selective κ-opioid-receptor antagonist, had no significant effect on the SNC80 (3 mg/kg, s.c.)-induced decrease in the duration of immobility (with vehicle, 67.5 ± 5.7 s; with β-funaltrexamine, 73.0 ± 5.8 s; with nor-binaltorphimine, 69.6 ± 7.0 s). Desipramine (10, 30 mg/kg, i.p.) also significantly reduced the duration of immobility. SNC80 (3 mg/kg, s.c.) reduced the duration of immobility to the same levels as observed in desipramine (30 mg/kg)-treated mice. Naltrindole (1 and 3 mg/kg) by itself had no significant effect on the duration of immobility.

Effects of SNC80 in the elevated plus-maze test in rat

SNC80 (1 – 10 mg/kg, s.c.)-treated rats showed a significant increase in the percentage of time spent and visits to the open arms (Fig. 2). The effects of SNC80 were significantly and dose-dependently reduced by naltrindole, a δ-opioid-receptor antagonist. However, as shown in Table 1, pretreatment with either β-funaltrexamine, a selective μ-opioid-receptor antagonist, or nor-binaltorphimine, a selective κ-opioid-receptor antagonist, had no significant effect on the SNC80 (3 mg/kg, s.c.)-induced increase in the percentage of time spent and visits to the open arms. On the other hand, diazepam (3 mg/kg, s.c.) also significantly increased the percentage of time spent and visits to the open arms. The total number of entries increased similarly in the groups treated with SNC80 and diazepam. The effects of SNC80 (10 mg/kg, s.c.) were similar to those observed in diazepam (3 mg/kg, s.c.)-treated rat. Lower doses of naltrindole (1 mg/kg) by itself had no significant effect on the percentage of time spent and visits to the open arms. However, higher doses of naltrindole (3 mg/kg) by iteself significantly decreased the percentage of time spent and visits to the open arms. On the other hand, neither β-funaltrexamine nor nor-binaltorphimine by itself had any significant effect on the percentage of time spent and visits to the open arms (Table 1).

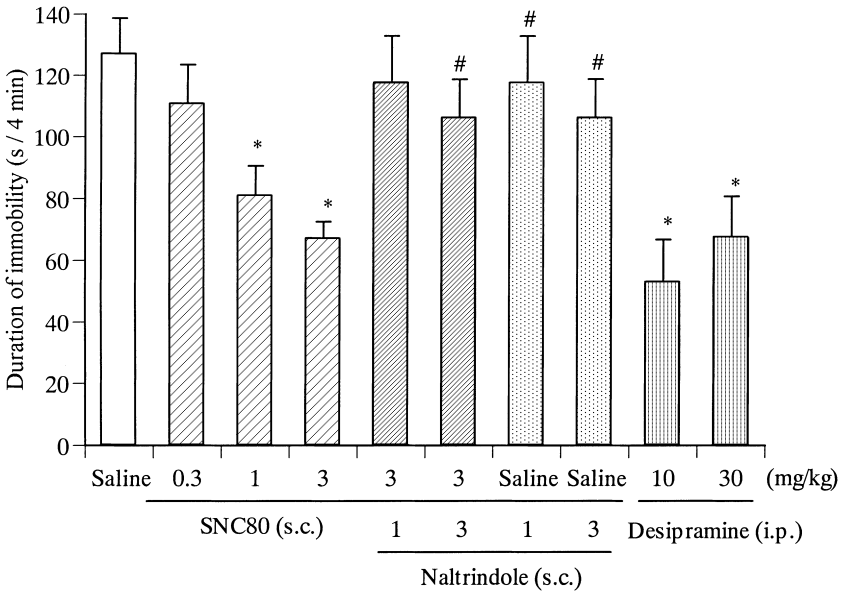

Fig. 1. Effects of SNC80 on the duration of immobility in the forced swimming test in mice. SNC80 was injected s.c. 30 min before the test. Naltrindole was injected s.c. 30 min before the administration of SNC80. Desipramine was injected i.p. 60 min before the test. Each column represents the mean with S.E.M. of 8 – 10 mice. *P<0.05 *vs* saline-treated group. $^{\#}P$<0.05 *vs* SNC80 (3 mg/kg)-treated group.

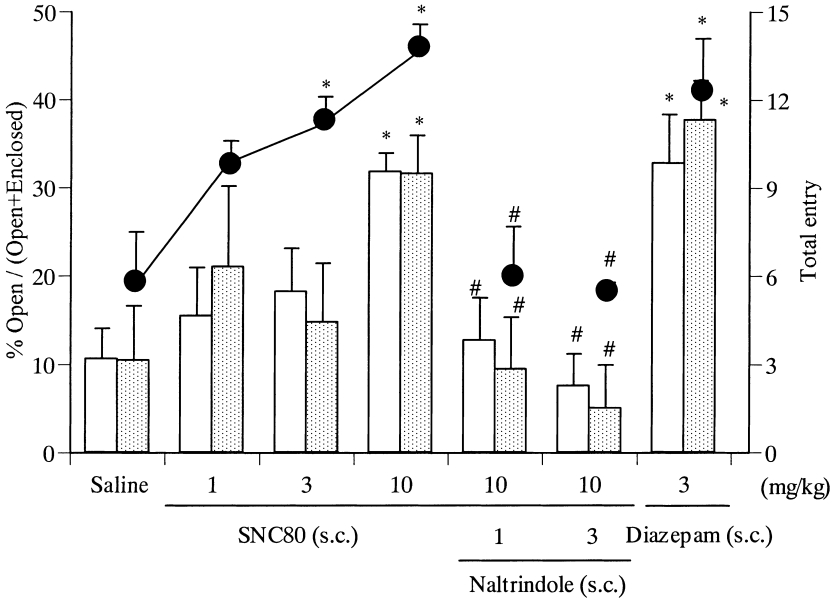

Fig. 2. Effects of SNC80 on the percentage of time spent and total visits to the open arms in the elevated plus-maze test in rats. SNC80 was injected s.c. 30 min before the test. Naltrindole was injected s.c. 30 min before the administration of SNC80. Diazepam was injected s.c. 30 min before the test. Each column represents the mean with S.E.M. of 6 – 8 rats. Open column: % time spent in an open arm. Closed column: % open arm visits. Closed circle: total visits. **P*<0.01 *vs* saline-treated group. #*P*<0.05 *vs* SNC80 (10 mg/kg)-treated group.

Table 1. Effects of naltrindole (NTI), nor-binaltorphimine (norBNI), and β-funaltrexamine (βFNA) in the percentage of time spent and visits to the open arms in the elevated plus maze test in rats

Antagonists	Dose (mg/kg)	Agonists	Dose (mg/kg)	% Time spent open arm	% Open arm entry	Total entry
Vehicle	—	Vehicle	—	7.0 ± 2.2	5.6 ± 5.6	6.5 ± 0.9
Vehicle	—	SNC80	10	20.3 ± 4.3	30.6 ± 4.2	10.8 ± 2.5
NorBNI	3	SNC80	10	27.2 ± 6.8	32.1 ± 5.6	10.3 ± 1.8
	10	SNC80	10	24.3 ± 2.8	35.7 ± 5.2	11.8 ± 0.8
βFNA	10	SNC80	10	31.0 ± 2.8	35.4 ± 5.2	13.3 ± 0.8
	30	SNC80	10	24.8 ± 5.1	30.2 ± 5.0	12.5 ± 0.6
Vehicle	—	Vehicle	—	10.4 ± 6.3	10.6 ± 3.5	5.8 ± 1.7
NTI	1	Vehicle	—	3.6 ± 3.6	6.9 ± 3.0	6.3 ± 0.8
	3	Vehicle	—	0 ± 0*	0 ± 0*	3.5 ± 1.9
NorBNI	3	Vehicle	—	3.6 ± 0.7	7.5 ± 0.3	5.5 ± 0.3
	10	Vehicle	—	11.1 ± 3.2	7.1 ± 7.1	6.3 ± 0.5
βFNA	10	Vehicle	—	6.5 ± 2.4	3.3 ± 2.3	7.8 ± 1.4
	30	Vehicle	—	4.9 ± 1.8	6.3 ± 6.3	2.8 ± 2.1

The elevated plus maze test was performed 30 min after the s.c. administration of SNC80 and/or vehicle. Each group consisted of 9 or 10 rats. Rats were treated with nor-binaltorphimine or β-funaltrexamine 24 h before drug treatment. Naltrindole was injected s.c. 30 min before the administration of drug. Values are expressed as the mean ± S.E.M. **P*<0.01 *vs* vehicle/vehicle-treated group.

Effects of SNC80 in the conditioned fear stress test in mice

As shown in Fig. 3, saline-treated shocked mice (closed column) exhibited a marked suppression of motility when they were returned to the same apparatus in which they had been given an electric shock. SNC80 (0.1 – 3 mg/kg, s.c.) significantly attenuated this locomotor suppression without affecting activity counts in the non-shocked group (open column). The effect of SNC80 was significantly reduced by naltrindole, a δ-opioid receptor antagonist, in a dose-dependent manner. Imipramine (30 mg/kg, i.p.) slightly, but not significantly, attenuated the suppression of locomotor activity. Naltrindole (1 or 3 mg/kg) by itself had no significant

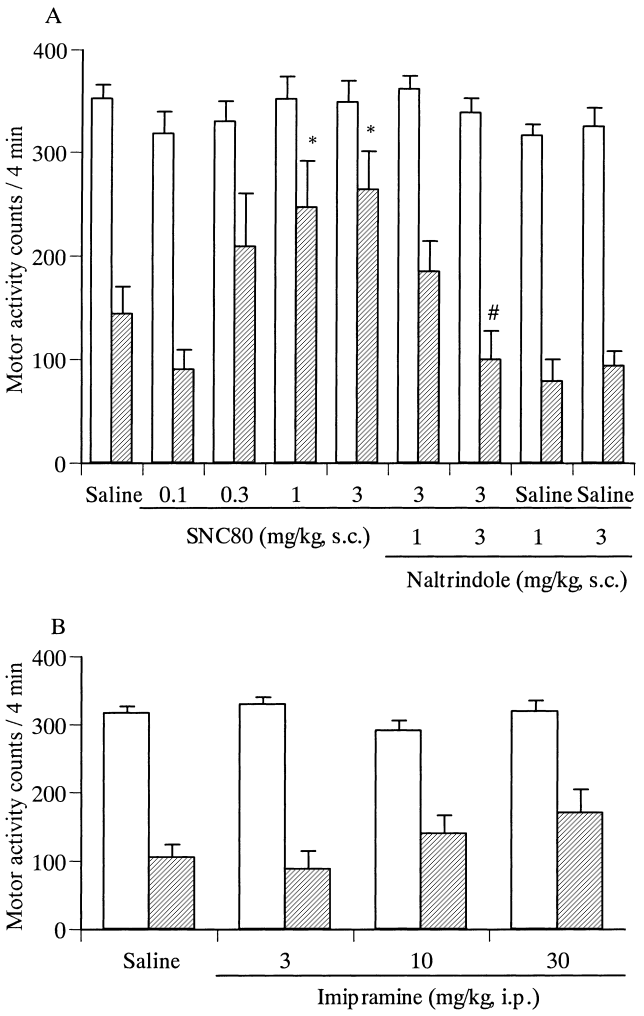

Fig. 3. Effects of SNC80 (A) and imipramine (B) on motor activity counts in the conditioned fear stress test in mice. SNC80 and imipramine were injected s.c. 30 min before the test. Naltrindole was injected s.c. 30 min before the administration of SNC80 and/or saline. Each column represents the mean with S.E.M. of 7 or 8 mice. Open column: non-stimulated mice. Closed column: electric foot shock (1.3 mA)-stimulated mice. *$P<0.05$ *vs* saline-treated control group. $^{\#}P<0.05$ *vs* SNC80 (3 mg/kg)-treated group.

effect on the locomotor activity in both shocked and non-shocked groups (Fig. 3A).

Effects of SNC80 on the spontaneous locomotor activity in the open field test

Table 2 shows the effects of SNC80 on the spontaneous locomotor activity of mice in the open field test. SNC80 (1 and 3 mg/kg) had no significant effect on total locomotor activity and rearing counts in the open field test (Table 2).

Table 2. Effects of SNC80 on the total locomotor activity and rearing counts of mice in the open field test

Drug	Dose (mg/kg)	Locomotion (cm)	Rearing counts
Vehicle	—	2778.3 ± 86.8	37.0 ± 3.0
SNC80	1	2720.0 ± 189.4	36.7 ± 1.5
	3	2819.1 ± 171.8	30.0 ± 2.7

The open field test was performed 30 min after the s.c. administration of SNC80. Each group consisted of 5 – 7 mice. Values are expressed as the mean ± S.E.M.

Discussion

In the present study, we observed that the δ-opioid receptor agonist SNC80 significantly reduced the duration of immobility in the forced swimming test. Furthermore, we also observed that SNC80 significantly increased the percentage of time spent and visits to the open arms in the elevated plus-maze test. In addition, we found that SNC80 completely attenuated the suppression of locomotor activity in mice in the conditioned fear stress test. These results suggest that SNC80 has antidepressant- and anxiolytic-like effects. Furthermore, these behavioral changes induced by SNC80 were significantly antagonized by naltrindole, a selective δ-opioid-receptor antagonist, but not by β-funaltrexamine, a selective μ-opioid-receptor antagonist, or nor-binaltorphimine, a selective κ-opioid-receptor antagonist. Thus, these results lead us to conclude that SNC80 produced its antidepressant- and anxiolytic-like effects via the activation of δ-opioid receptors.

The forced swimming test was developed by Porsolt et al. (10) using rats and mice. The widespread use of this model is largely a result of its ease of use, reliability across laboratories, and ability to detect a wide range of antidepressant-like activity (9). However, the major drawback of the forced swimming test is that it does not reliably detect the effects of selective serotonin reuptake inhibitors (SSRIs) (18, 19), which are the most widely prescribed antidepressant drugs today. However, in contrast to the forced swimming stress test, it has been reported that the conditioned fear stress-induced suppression of locomotor activity in mice was attenuated by pretreatment with SSRIs, such as fluvoxamine (20), whereas this suppression was less sensitive to imipramine (13). In our study, imipramine, a selective noradrenaline reuptake inhibitor, did not significantly reduce the conditioned fear stress-induced freezing behavior in mice. This finding is consistent with the observation of Kameyama et al. (13). Furthermore, this animal model is recognized to be a stress

model reflecting the emotional abnormality including the state of depression and anxiety, since conditioned fear stress-induced suppression of locomotor activity is attenuated by antidepressants as well as anxiolytics (16, 20). Considering our findings and previous reports, the effectiveness of SNC80 in two different types of stress models suggests that SNC80 may have the ability to inhibit emotional abnormality such as anxiety and/or depression produced by stress. Thus, it seems likely that δ-opioid-receptor agonists might have a wider range of antidepressant-like activity than preexisting drugs such as tricyclic antidepressant and SSRIs.

In the elevated plus maze test, the lower doses of naltrindole (1 mg/kg) by itself had no significant effect on the percentage of time spent and visits to the open arms. However, the rats treated with the higher dose of naltrindole (3 mg/kg) alone showed significant decreases in the percentage of time spent and visits to the open arms. Thus, this may suggest that the higher doses of naltrindole may produce the anxiogenic-like effects. These results are in agreement with previous studies (21, 22) and support the view that δ-opioid receptors are involved in the anxiety related responses. However, further studies are necessary before this possibility can be established with greater certainly.

Benzodiazepines are sometimes used together with antidepressants to treat patients with affective disorders such as major depressive disorder, neurotic depression, and anxious-depressive reactions. One reason for this drug combination may be the frequent co-morbidity of depression and anxiety. Benzodiazepines are usually co-administrated with antidepressants to reduce symptoms associated with depression, which do not respond sufficiently to antidepressants alone. Moreover, benzodiazepines are also given to control the release of psychomotor inhibition, which may occur at the onset of the action of antidepressants. In the present study, SNC80 produced not only a similar antidepressant-like effect as desipramine in the forced swimming test, but also a similar anxiolytic-like effect as diazepam in the elevated plus maze test. These findings suggest that δ-opioid-receptor agonists may be potent antidepressants that also have anxiolytic-like effects.

In this study, SNC80 was used at the dose of 0.1 – 0.3 mg/kg in the forced swimming test or conditioned fear stress test. On the other hand, relatively higher doses of SNC80 (1.0 – 10 mg/kg) were used for the elevated plus maze test. These differences in the dose-range used for behavioral experiments suggested that the antidepressant effect involves a mechanism different from that of the anxiolytic effect. The detailed mechanisms of these effects of SNC80 in behavioral models in rodents are not yet completely understood. Further studies are necessary to fully clarify the antidepressant and anxiolytic effects of SNC80.

A valid concern over the activity of drugs in the forced swimming test is whether increased locomotion can produce decreased immobility in this test. SNC80 has previously been shown to produce locomotor stimulation via a δ-opioid receptor-mediated mechanism (23). Those authors reported that SNC80 significantly affected locomotor activity at a dose of 10 mg/kg, s.c. On the other hand, in the present study, SNC80 produced a significant decrease in the duration of immobility in the forced swimming test at doses of 1 and 3 mg/kg, s.c. Furthermore, SNC80 (1 and 3 mg/kg) had no significant effect on the spontaneous locomotor activity and rearing counts of mice in the open field test. Thus, these results suggested that the antidepressant-like activity of SNC80 is due solely to its activation of locomotor activity.

Previous reports have shown that various types of δ-opioid receptor agonist produce a rewarding effect (24 – 26). However, Brandt et al. (27) recently reported that these were no behavioral signs of withdrawal after naltrindole administration in rhesus monkeys given chronic treatment with daily administration of 10 mg/kg SNC80. Furthermore, Negus et al. (28) also showed that SNC80 did not maintain responding in monkeys trained to self-administer cocaine. These results suggested that SNC80 might produce little or no rewarding effect as well as psychiatric dependence. Thus, we considered that SNC80 induced antidepressant-like and anxiolytic-like effects were not due to the rewarding effect.

In conclusion, these results suggest that SNC80 elicits antidepressant- and anxiolytic-like effects as a result of the activation of δ-opioid receptors. Furthermore, it seems that δ-opioid-receptor agonists might be novel and potent antidepressants that also have anxiolytic-like effects.

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