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(+)-SKF-10,047 and dextromethorphan ameliorate conditioned fear stress through the activation of phenytoin-regulated σ_1 sites

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Abstract

Mice exhibited a marked suppression of motility when they were replaced in the same environment in which they had previously received an electric footshock. This psychological stress-induced motor suppression, known as conditioned fear stress, was dose dependently attenuated by (+)-*N*-allylnormetazocine ((+)-SKF-10,047) and by dextromethorphan, putative σ receptor agonists, but not by other σ receptor ligands, (+)-pentazocine and 1,3-di-(2-tolyl)guanidine (DTG). Unlike (+)-SKF-10,047 and dextromethorphan, the non-competitive NMDA receptor antagonists, phencyclidine and dizocilpine, attenuated the conditioned fear stress only at high doses that induced marked hypermotility in non-stressed mice. The effects of (+)-SKF-10,047 and dextromethorphan, but not phencyclidine and dizocilpine, on the conditioned fear stress were antagonized by the σ receptor antagonists, NE-100 (*N*,*N*-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl]-ethylamine monohydrochloride) and BMY-14802 (α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine-butanol hydrochloride). Interestingly, the effects of (+)-SKF-10,047 and dextromethorphan on the stress response were enhanced by combination with phenytoin, an anticonvulsant drug, whereas those of (+)-pentazocine, DTG, phencyclidine, and dizocilpine were not. These results suggest that activation of phenytoin-regulated type σ_1 receptors, but not of phencyclidine receptors, is involved in the ameliorating effects of (+)-SKF-10,047 and dextromethorphan on stress-induced motor suppression.

Keywords: Conditioned fear stress; σ Receptor; Phenytoin; Phencyclidine receptor; Motor suppression

1. Introduction

When rats or mice are replaced in the same environment in which they have previously received an electric footshock, they exhibit a marked suppression of motility (Kameyama and Nagasaka, 1982a, b; Yamada and Nabeshima, 1995). This motor suppression is regarded as a conditioned emotional response to the environment associated with previous footshock (Kameyama and Nagasaka, 1982a, b; Yamada and Nabeshima, 1995). Fanselow (1980) defined the same type of stress-induced motor suppression as conditioned fear stress. Our previous findings, that (±)-*N*-allylnormetazocine ((±)-SKF-10,047), a prototype σ receptor agonist (Martin et al., 1976), dose dependently attenuated the conditioned fear stress (Nabeshima et al., 1988) and that this effect was antagonized by BMY-14802 (α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine-butanol hydrochloride), a putative σ receptor antagonist (Kamei et al., 1994), indicate that the activation of σ receptors is responsible for the attenuation of conditioned fear stress. Furthermore, an increase in the binding capacity of σ receptors has been observed in brain synaptic membranes prepared from mice showing conditioned fear stress (Nabeshima et al., 1988). This change in σ receptors in the stressed group was restored to the control level by pretreatment with (±)-SKF-10,047. These observations suggest that the function of σ receptors may be altered in stress situations such as conditioned fear stress.

SKF-10,047 and the psychotomimetic agent, phencyclidine, were originally considered to act on the same site, designated as the σ/phencyclidine site. However, subsequent evidence has demonstrated that σ binding sites are distinct from phencyclidine binding sites on the NMDA receptor-ion channel complex (Gundlach et al., 1985, 1986;

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Quirion et al., 1987). Moreover, it has recently become apparent that there are at least two subtypes of σ receptors (Walker et al., 1990; Quirion et al., 1992). σ_1 Receptors are characterized by high affinity for (+)-SKF-10,047, (+)-pentazocine, and dextromethorphan, and σ_2 receptors have low affinity for these ligands. Both 1,3-di-(2-tolyl)guanidine (DTG) and haloperidol are non-selective drugs and bind with a similar high affinity to the two classes of sites. Additionally, binding studies with the anticonvulsant, phenytoin, have shown that σ_1 receptors can be differentiated into two different binding sites, phenytoin-sensitive and phenytoin-insensitive (McCann and Su, 1992; DeHaven-Hudkins et al., 1993). However, the functional role of these variant types of σ receptors in the conditioned fear stress is unclear.

In the present study, therefore, we examined the involvement of the different σ receptor subtypes in conditioned fear stress, using various σ receptor ligands, (+)-SKF-10,047, (+)-pentazocine, dextromethorphan, and DTG in combination with phenytoin. Further, since it is possible that some σ receptor ligands also interact with phencyclidine binding sites, we compared the effects of σ receptor ligands on the conditioned fear stress with the effects of the non-competitive antagonists of the NMDA receptor, phencyclidine and dizocilpine (a selective and potent agent; Wong et al., 1986).

2. Materials and methods

This study was approved by the Animal Care and Use Committee at Nagoya University.

2.1. Animals

Male ddY mice (Nihon SLC Co., Shizuoka, Japan), 7 weeks of age, were used. The animals were housed in a controlled environment (23 ± 1°C, 50 ± 5% humidity) and were allowed food and water ad libitum. The room lights were off between 8:00 p.m. and 8:00 a.m.

2.2. Drug treatment

The following drugs were used: (+)-*N*-allylnormetazocine hydrochloride ((+)-SKF-10,047, Research Biochemicals, Natick, MA, USA); (+)-pentazocine (Sterling Winthrop, USA); 1,3-di-(2-tolyl)guanidine (DTG, Research Biochemicals, Natick, MA, USA); dextromethorphan hydrobromide (Sigma Chemical Co., USA); phencyclidine hydrochloride (synthesized by Dr. H. Furukawa, a generous gift); dizocilpine maleate (Research Biochemicals, Natick, MA, USA); NE-100 (*N*,*N*-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl]-ethylamine monohydrochloride, Taisho Pharmaceutical Co., Saitama, Japan); BMY-14802 (α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine-butanol hydrochloride, Bristol-Myers Squibb, Wallinford, CT, USA); and phenytoin sodium salt (Research Biochemicals, Natick, MA, USA).

DTG and phenytoin were prepared as microsuspensions in Cremophor EL (Sigma Chemical Co., USA) 10% in 0.9% NaCl solution by ultrasonic vibration. BMY-14802 and (+)-pentazocine were initially dissolved in a minimum volume of 0.1 N HCl and were then diluted with 0.9% NaCl solution and with distilled water, respectively (the pH of the solutions was adjusted to about 4 with $NaHCO_3$). NE-100 was dissolved in distilled water. Other drugs were dissolved in 0.9% NaCl solution. The dose of each drug refers to the drug form listed above.

2.3. Schedule for conditioned fear stress

The experiments were carried out as previously described, the apparatus being a transparent acrylic rectangular cage (23 × 28 × 12 high cm) equipped with a metal wire floor (Nabeshima et al., 1983). The test cage was located in a sound-attenuated room and was illuminated with a 20-W bulb.

Each mouse was placed in the test cage and received electric shocks (0.1 Hz, 200 ms, 300 V DC) for 6 min through an isolated stimulator (Nihon Koden, Tokyo, Japan). When an animal was placed in the test cage, the current resistance varied between 100 and 250 kΩ. Therefore, each animal received electric shocks in a range of 1.2–3.0 mA. The test trial was carried out 24 h after the shock treatment; the animals were again placed into the same test cage, but no electric footshock was given. The spontaneous motility of the animal for 6 min in the test cage was determined with an automatic activity counter (Opto-Varimex, Columbus Instruments, OH, USA), equipped with photosensors, that surrounded the test cage. The non-shocked control group was prepared in exactly the same way, except for the absence of the electric footshock treatment. The mice aged 7 weeks and the shock intensity used in this method have been reported to be optimal to produce a stable response (motor suppression) (Kameyama and Nagasaka, 1982b; Nabeshima et al., 1983). Our ethical committee have approved this point.

All test drugs were administered before motility was measured in the test trial; (+)-SKF-10,047, (+)-pentazocine, dextromethorphan, and phencyclidine were administered subcutaneously (s.c.) 15 min before the test trial, dizocilpine and BMY-14802 were administered s.c. 30 min before, DTG and phenytoin were administered intraperitoneally (i.p.) 30 min before, and NE-100 was administered i.p. 45 min before the trial. Groups receiving no drugs were given an appropriate vehicle, i.e., a solution containing 0.1 N HCl or 10% Cremophor EL.

2.4. Effects on motility in habituated mice

To compare the effects of (+)-SKF-10,047 and dextromethorphan on motility of the stressed mice and the

motility-matched control mice, mice well-adapted to the test cage were prepared. The mice were placed individually into the test cage and 60 min later motility was measured for 6 min. Test drugs were administered 15 min before the measurement of motility. These animals showed motor-activity counts nearly equal to that of the shocked animals.

2.5. Statistical analysis

Statistical significance was determined by the Kruskal-Wallis test [non-parametric analysis of variance (ANOVA)] followed by a Dunn-type non-parametric test. Comparison of two sample means was performed with a Mann-Whitney U-test. P values less than 0.05 were taken to indicate statistically significant differences.

3. Results

3.1. Effects of σ receptor ligands and non-competitive NMDA receptor antagonists on conditioned fear stress

As shown in Figs. 1–3, the shocked mice (shocked groups) exhibited a marked suppression of motility; the shocked groups showed 10.0–15.4% of the motility exhibited by the non-shocked groups when returned to the same apparatus in which they had been given an electric shock. The shocked mice mostly froze and crouched in the corner of the test apparatus, while the non-shocked mice showed typical exploratory behaviors such as ambulation, sniffing and rearing.

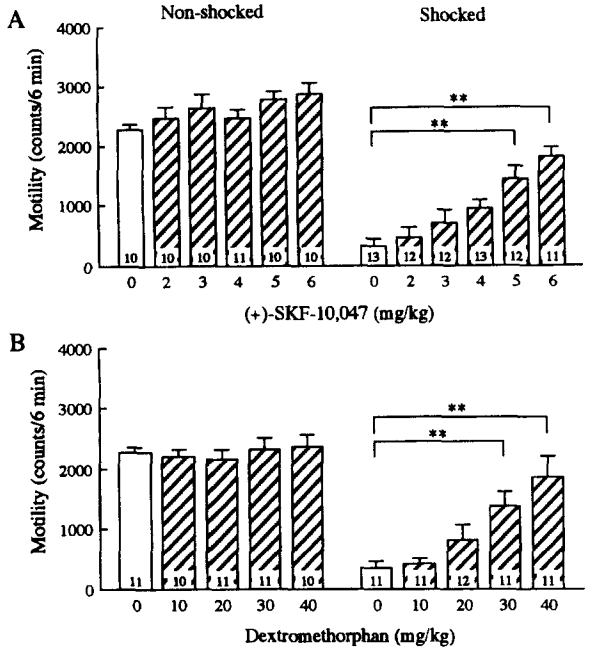

Fig. 1. Effects of (+)-SKF-10,047 (A) and dextromethorphan (B) on conditioned fear stress in mice. (+)-SKF-10,047 and dextromethorphan were administered s.c. 15 min before motility was measured. Values are means ± S.E.M. for the number of animals shown in each column. Results with ANOVA were: (A) non-shocked group, $H(5) = 7.266$ ($P > 0.05$); shocked group, $H(5) = 32.116$ ($P < 0.01$), (B) non-shocked group, $H(4) = 1.347$ ($P > 0.05$); shocked group, $H(4) = 22.002$ ($P < 0.01$). ** $P < 0.01$ compared to the vehicle-treated, shocked group (Dunn-type test).

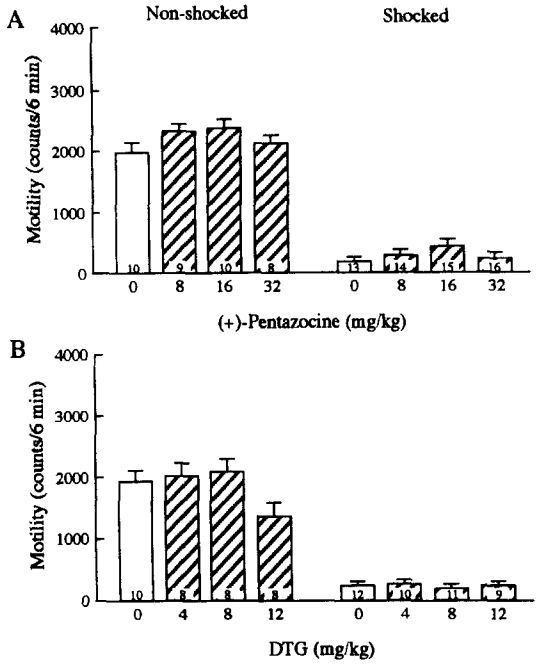

Fig. 2. Effects of (+)-pentazocine (A) and DTG (B) on conditioned fear stress in mice. DTG and (+)-pentazocine were administered i.p. 30 min and s.c. 15 min, respectively, before motility was measured. Other details are as shown in the legend to Fig. 1. Results with ANOVA were: (A) non-shocked group, $H(3) = 3.526$ ($P > 0.05$); shocked group, $H(3) = 5.913$ ($P > 0.05$), (B) non-shocked group, $H(3) = 6.087$ ($P > 0.05$); shocked group, $H(3) = 1.527$ ($P > 0.05$).

The suppression of motor activity in the shocked group was dose dependently attenuated by (+)-SKF-10,047, with no effect on motility in the non-shocked group; significant effects were observed at doses of 5 and 6 mg/kg (Fig. 1A). This effect of (+)-SKF-10,047 was more potent than that of its racemate observed in our previous study (Nabeshima et al., 1988). Like (+)-SKF-10,047, dextromethorphan (30 and 40 mg/kg) also significantly attenuated the motor suppression of the shocked group in a dose-dependent manner, without changing motility in the non-shocked group (Fig. 1B). The shocked mice given these drugs, like the non-shocked mice, exhibited exploratory behavior but moved slowly. Furthermore, we compared the effects of (+)-SKF-10,047 and dextromethorphan on motility of the shocked mice and of the motility-matched, non-shocked mice. Mice were allowed to adapt to the test cage for 60 min before the measurement of motility to equalize their motility with that of the shocked mice. These mice stopped exploring and stayed in a corner of the test apparatus. As shown in Table 1, the motility-matched mice, as well as the shocked mice, exhibited low motor activity. (+)-SKF-10,047 (5 mg/kg) and dextromethorphan (30 mg/kg) failed to increase motility

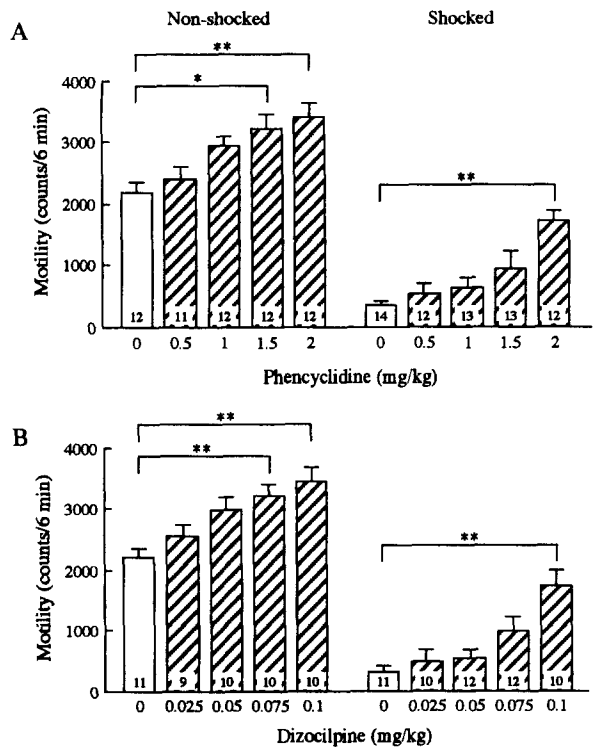

Fig. 3. Effects of phencyclidine (A) and dizocilpine (B) on conditioned fear stress in mice. Phencyclidine and dizocilpine were administered s.c. 15 min and 30 min, respectively, before motility was measured. Other details are as shown in the legend to Fig. 1. Results with ANOVA were: (A) non-shocked group, $H(4) = 19.425$ ($P < 0.01$); shocked group, $H(4) = 21.153$ ($P < 0.01$), (B) non-shocked group, $H(4) = 18.886$ ($P < 0.01$); shocked group, $H(4) = 17.469$ ($P < 0.01$). $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the corresponding vehicle-treated group (Dunn-type test).

in the motility-matched control mice. Therefore, (+)-SKF-10,047 and dextromethorphan acted predominantly on the stress-induced motor suppression.

Other σ receptor ligands, (+)-pentazocine (8, 16, and 32 mg/kg) and DTG (4, 8, and 12 mg/kg), had little effect on motility in either the non-shocked or the shocked groups (Fig. 2). A high dose (16 mg/kg) of DTG could not be tested because it induced ataxia.

Table 1
Effects of (+)-SKF-10,047 (SKF) and dextromethorphan (DM) on motility in motility-matched, non-shocked mice and shocked mice

Treatment (mg/kg)	Motility (counts/6 min)			
	Motility-matched	*n*	Shocked	*n*
Vehicle	173.8 ± 60.8	12	209.5 ± 99.7	10
SKF (5)	307.2 ± 97.6	12	1339.7 ± 316.1 [a]	10
DM (30)	188.8 ± 86.4	12	1250.1 ± 179.4 [a]	10

SKF and DM were administered s.c. 15 min before motility was measured. Each value is the mean ± S.E.M. for the number of animals shown by *n*. Results with ANOVA were: motility-matched, non-shocked group, $H(2) = 2.039$ ($P > 0.05$); shocked group, $H(2) = 13.058$ ($P < 0.01$). [a] $P < 0.01$ compared to the corresponding vehicle-treated group (Dunn-type test).

Unlike (+)-SKF-10,047 and dextromethorphan, the non-competitive NMDA receptor antagonists, phencyclidine (2 mg/kg) and dizocilpine (0.1 mg/kg), caused a significant increase in the motility of mice in both the non-shocked and shocked groups (Fig. 3). However, these drugs significantly increased motility at the higher doses, 2 mg/kg for phencyclidine and 0.1 mg/kg for dizocilpine, more so in the shocked group than in the non-shocked group. Phencyclidine and dizocilpine induced stereotyped sniffing accompanying hyperlocomotion at the high doses in both the non-shocked and shocked groups.

3.2. Antagonistic effects of σ receptor antagonists on the (+)-SKF-10,047- and dextromethorphan-induced attenuation of conditioned fear stress

To clarify whether the effects of (+)-SKF-10,047 and dextromethorphan on the motor suppression in the shocked mice were mediated through σ receptors, we investigated the antagonism of their effects with the selective σ receptor antagonists, BMY-14802 and NE-100. As shown in Fig. 4, both BMY-14802 (10 mg/kg) and NE-100 (5 mg/kg) significantly inhibited the (+)-SKF-10,047 (5 mg/kg)-induced attenuation of the motor suppression in

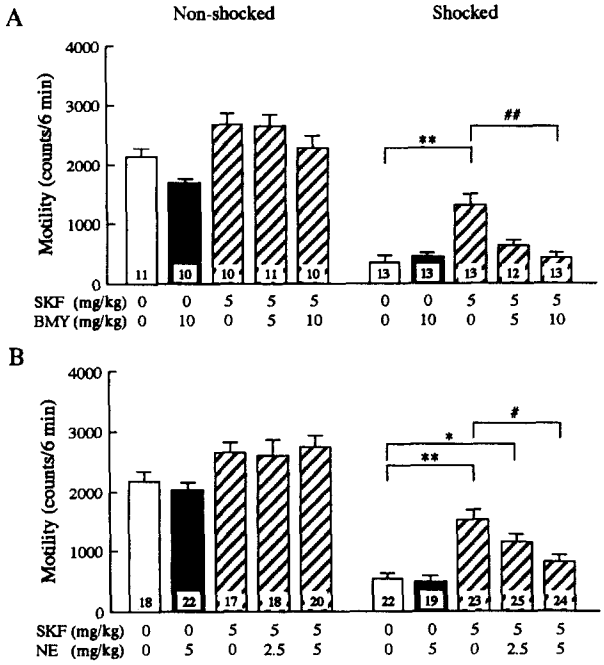

Fig. 4. Effects of BMY-14802 (BMY) (A) and NE-100 (NE) (B) on the (+)-SKF-10,047 (SKF)-induced attenuation of the conditioned fear stress in mice. SKF was administered s.c. 15 min before motility was measured. BMY and NE were administered s.c. 30 min and i.p. 45 min, respectively, before motility was measured. Other details are as shown in the legend to Fig. 1. Results with ANOVA were: (A) non-shocked group, $H(4) = 16.326$ ($P < 0.01$); shocked group, $H(4) = 23.587$ ($P < 0.01$), (B) non-shocked group, $H(4) = 11.359$ ($P < 0.05$); shocked group, $H(4) = 29.392$ ($P < 0.01$). $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the vehicle-treated, shocked group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to the SKF-treated, shocked group (Dunn-type test).

the shocked group. Similarly, the attenuating effect of dextromethorphan (30 mg/kg) on motor suppression was also significantly antagonized by both BMY-14802 (10 mg/kg) and NE-100 (5 mg/kg) (Fig. 5). On the other hand, the effects of phencyclidine (2 mg/kg) and dizocilpine (0.1 mg/kg) on motor suppression were not affected by either of the σ receptor antagonists (Table 2). BMY-14802 (10 mg/kg) and NE-100 (5 mg/kg) themselves had little effect on motility in either the non-shocked or the shocked groups (Figs. 4 and 5 and Table 2).

3.3. Effects of σ receptor ligands and non-competitive NMDA receptor antagonists in combination with phenytoin on conditioned fear stress

We investigated whether the effects of the σ receptor ligands and the non-competitive NMDA receptor antagonists were affected by combination with phenytoin. Phenytoin was used at a dose of 10 mg/kg, the ED_{50} value for the inhibition of maximal electroshock seizure in mice (Rogawski and Porter, 1990). This dose of phenytoin had little effect on motility in either the non-shocked or the shocked groups (Table 3). As shown in Table 3A, low doses of (+)-SKF-10,047 (2 mg/kg) and dextromethorphan (10 mg/kg), themselves, had little effect on motor suppression in the shocked group. However, when phenytoin (10 mg/kg) was coadministered with (+)-SKF-10,047 and with dextromethorphan, motor suppression was significantly attenuated, but motility in the non-shocked group was not changed. On the other hand, (+)-pentazocine (16 mg/kg) and DTG (8 mg/kg) in combination with phenytoin did not attenuate the motor suppression (Table 3B). Similarly, low doses of phencyclidine (1 mg/kg) and dizocilpine (0.05 mg/kg), which failed to increase motility in either the non-shocked or shocked groups, did not affect motor suppression when they were coadministered with phenytoin (Table 3C).

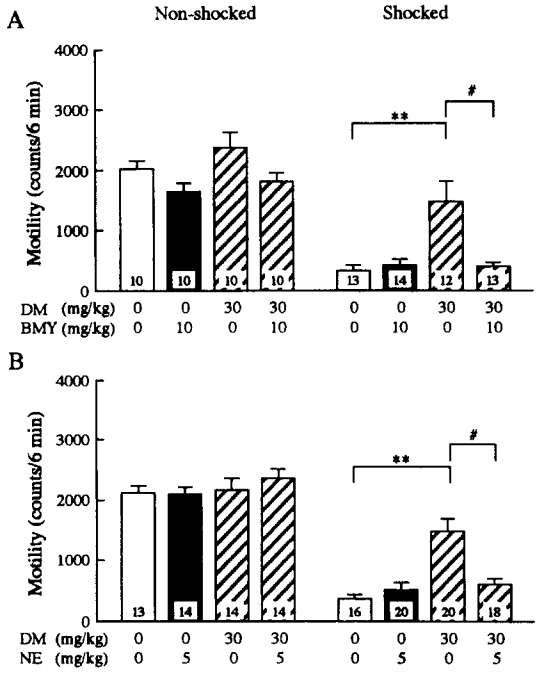

Fig. 5. Effects of BMY-14802 (BMY) (A) and NE-100 (NE) (B) on the dextromethorphan (DM)-induced attenuation of the conditioned fear stress in mice. DM was administered s.c. 15 min before motility was measured. BMY and NE were administered s.c. 30 min and i.p. 45 min, respectively, before motility was measured. Other details are as shown in the legend to Fig. 1. Results with ANOVA were: (A) non-shocked group, $H(3) = 6.814$ ($P > 0.05$); shocked group, $H(3) = 14.016$ ($P < 0.01$), (B) non-shocked group, $H(3) = 1.919$ ($P > 0.05$); shocked group, $H(3) = 21.413$ ($P < 0.01$). ** $P < 0.01$ compared to the vehicle-treated, shocked group, # $P < 0.05$ compared to the DM-treated, shocked group (Dunn-type test).

Table 2
Effects of BMY-14802 (BMY) and NE-100 (NE) on the motor response to phencyclidine (PCP) and dizocilpine (DIZ) in mice showing conditioned fear stress

Treatment	Motility (counts/6 min)			
(mg/kg)	Non-shocked	*n*	Shocked	*n*
(A)				
Vehicle	2205.5 ± 121.7	11	230.2 ± 69.1	10
BMY (10)	1881.1 ± 130.4	10	330.9 ± 77.6	10
PCP (2)	3449.9 ± 233.9 [a]	11	1557.5 ± 239.1 [b]	10
+ BMY (10)	2978.1 ± 282.0	11	1696.1 ± 285.3 [b]	11
(B)				
Vehicle	1887.3 ± 127.5	12	379.2 ± 130.3	12
NE (5)	1953.5 ± 121.2	10	275.5 ± 90.8	10
PCP (2)	2786.7 ± 176.6 [b]	10	1213.9 ± 238.8 [a]	10
+ NE (5)	2881.9 ± 121.2 [b]	10	1178.8 ± 236.3 [a]	10
(C)				
Vehicle	2268.1 ± 114.3	10	226.4 ± 77.3	13
BMY (10)	1824.9 ± 121.6	10	351.0 ± 60.4	14
DIZ (0.1)	3538.0 ± 167.0 [b]	10	1552.7 ± 177.2 [b]	13
+ BMY (10)	3006.1 ± 117.4	10	1502.5 ± 268.3 [b]	14
(D)				
Vehicle	2249.9 ± 120.9	10	480.3 ± 111.6	12
NE (5)	2285.1 ± 166.2	9	319.0 ± 110.1	13
DIZ (0.1)	3701.7 ± 207.9 [b]	10	1796.3 ± 361.3 [a]	14
+ NE (5)	3702.6 ± 217.4 [b]	10	1820.9 ± 287.1 [a]	14

PCP and DIZ were administered s.c. 15 min and 30 min, respectively, before motility was measured. BMY and NE were administered s.c. 30 min and i.p. 45 min, respectively, before motility was measured. Each value is the mean ± S.E.M. for the number of animals shown by *n*. Results with ANOVA were: (A) non-shocked group, $H(3) = 20.685$ ($P < 0.01$); shocked group, $H(3) = 27.060$ ($P < 0.01$), (B) non-shocked group, $H(3) = 21.629$ ($P < 0.01$); shocked group, $H(3) = 16.281$ ($P < 0.01$), (C) non-shocked group, $H(3) = 31.307$ ($P < 0.01$); shocked group, $H(3) = 33.404$ ($P < 0.01$), (D) non-shocked group, $H(3) = 23.960$ ($P < 0.01$); shocked group, $H(3) = 24.607$ ($P < 0.01$). [a] $P < 0.05$, [b] $P < 0.01$ compared to the corresponding vehicle-treated group (Dunn-type test).

4. Discussion

Mice exhibited a marked suppression of motility (low motor activity) when returned to the same apparatus in which they had been given an electric shock. Freezing and crouching behaviors were also observed. We have defined

Table 3
Effects of σ receptor ligands and non-competitive NMDA receptor antagonists in combination with phenytoin on conditioned fear stress in mice

Treatment	Motility (counts/6 min))			
(mg/kg)	Non-shocked	*n*	Shocked	*n*
(A)				
Vehicle	2271.0 ± 133.4	10	194.6 ± 51.1	12
PHE (10)	1920.2 ± 122.6	10	296.2 ± 90.9	11
SKF (2)	2270.8 ± 117.1	10	255.7 ± 86.2	12
+ PHE (10)	2163.1 ± 146.5	10	1108.5 ± 201.8 [a,b]	12
DM (10)	1933.0 ± 95.3	10	393.7 ± 81.6	11
+ PHE (10)	2155.1 ± 215.9	10	1156.1 ± 206.4 [a,c]	12
(B)				
Vehicle	2075.3 ± 125.8	10	192.0 ± 94.2	12
PHE (10)	2139.0 ± 159.2	10	153.1 ± 26.3	11
PTZ (16)	2125.9 ± 168.3	10	222.7 ± 57.2	12
+ PHE (10)	1955.8 ± 179.7	10	224.7 ± 100.0	12
DTG (8)	1993.7 ± 196.3	10	168.1 ± 63.6	12
+ PHE (10)	1870.7 ± 209.4	10	166.3 ± 73.6	12
(C)				
Vehicle	2176.8 ± 111.1	11	239.6 ± 75.7	13
PHE (10)	2263.5 ± 145.6	10	311.3 ± 70.0	12
PCP (1)	2690.9 ± 128.5	10	432.6 ± 137.0	13
+ PHE (10)	2762.5 ± 193.4	10	520.5 ± 109.3	13
DIZ (0.05)	2557.6 ± 202.7	10	435.1 ± 102.4	13
+ PHE (10)	2620.8 ± 207.7	10	592.5 ± 113.9	13

Dextromethorphan (DM), (+)-SKF-10,047 (SKF), (+)-pentazocine (PTZ), and phencyclidine (PCP) were administered s.c. 15 min before motility was measured. DTG and phenytoin (PHE) were administered i.p. 30 min before motility was measured. Dizocilpine (DIZ) was administered s.c. 30 min before motility was measured. Each value is the mean ± S.E.M. for the number of animals shown by *n*. Results with ANOVA were: (A) non-shocked group, $H(5) = 5.613$ ($P > 0.05$); shocked group, $H(5) = 27.796$ ($P < 0.01$), (B) non-shocked group, $H(5) = 2.094$ ($P > 0.05$); shocked group, $H(5) = 4.202$ ($P > 0.05$), (C) non-shocked group, $H(5) = 8.339$ ($P > 0.05$); shocked group, $H(5) = 9.686$ ($P > 0.05$). [a] $P < 0.01$ compared to the vehicle-treated, shocked group (Dunn-type test). [b] $P < 0.01$ compared to the animals treated with SKF alone, [c] $P < 0.01$ compared to the animals treated with DM alone (Mann-Whitney U-test).

this motor suppression of the shocked mice as conditioned fear stress. In the present study, to clarify the role of the different σ receptor subtypes in stressful situations, we evaluated the efficacy of four σ receptor ligands to attenuate the conditioned fear stress. (+)-SKF-10,047 and dextromethorphan attenuated the motor suppression of the shocked mice in a dose-dependent manner. At that time, the shocked mice given these drugs, like the non-shocked mice, exhibited exploratory behaviors. It is possible that the effects of (+)-SKF-10,047 and dextromethorphan on motor suppression in the shocked mice depend on the rate of motor activity; these drugs act mostly on a low level of motor activity (i.e., rate-dependent motor activation). However, rate-dependent motor activation was not found for either (+)-SKF-10,047 or dextromethorphan, because these drugs failed to induce hyperlocomotion in the low motility-matched, non-shocked mice. Therefore, (+)-SKF-10,047 and dextromethorphan predominantly reversed the stress-induced motor suppression. In contrast, other σ receptor ligands, (+)-pentazocine and DTG, did not attenuate the conditioned fear stress, even at the higher doses used here.

The differential potency of σ receptor ligands to ameliorate the conditioned fear stress could not be explained by their selectivity for the σ_1 receptor subtype, since (+)-pentazocine (Itzhak et al., 1991), as well as (+)-SKF-10,047 (Itzhak et al., 1991) and dextromethorphan (Rothman et al., 1990), have greater selectivity for the σ_1 receptor than for the σ_2 receptor, although DTG is non-selective with regard to the two subtypes (Hellewell and Bowen, 1990). Recent evidence from binding studies with the anticonvulsant, phenytoin, identified different populations of σ_1 binding sites (McCann and Su, 1992; DeHaven-Hudkins et al., 1993). Phenytoin acts as an allosteric modulator of the binding of [^{3}H]SKF-10,047 (Karbon et al., 1991) and [^{3}H]dextromethorphan (Musacchio et al., 1989). The binding affinity of (+)-SKF-10,047 and dextromethorphan to σ_1 sites is markedly increased by phenytoin, while that of (+)-pentazocine and DTG is unaffected by phenytoin (DeHaven-Hudkins et al., 1993). These findings suggest that σ_1 receptors can be differentiated into two different binding sites, phenytoin-sensitive and phenytoin-insensitive. The present results indicated that the effects of (+)-SKF-10,047 and dextromethorphan on the conditioned fear stress were enhanced by their combination with phenytoin, whereas the effects of (+)-pentazocine and DTG were not. This behavioral finding was closely related to the results obtained from the binding study with phenytoin referred to above, suggesting that the ameliorating effects of (+)-SKF-10,047 and dextromethorphan on the stress response may be mediated by phenytoin-regulated σ_1 sites.

Alternatively, it is possible that the effects of (+)-SKF-10,047 and dextromethorphan on the stress response are due to the involvement of the phencyclidine sites in the NMDA receptor-channel complex. In this regard, (+)-SKF-10,047, but not (+)-pentazocine (Costa et al., 1989) and DTG (Weber et al., 1986), bind to a phencyclidine receptor, although the affinity is low (Tam, 1983). However, evidence from an in vivo receptor binding study suggests that the majority of (+)-SKF-10,047 binding sites are associated with σ sites (Weissman et al., 1990). Thus, (+)-SKF-10,047 is considered to be primarily a σ receptor agonist. Dextromethorphan is rapidly metabolized to dextrorphan by microsomal enzymes in the liver (Misra, 1978). Dextrorphan has high affinity for phencyclidine binding sites (Murray and Leid, 1984). Little dextrorphan, however, is formed when dextromethorphan is given by the s.c. route (which avoids first-pass metabolism by the liver; Holtzman, 1994) as used in this present study. Therefore, it is unlikely that the behavioral effects of dextromethorphan observed here are related to the action of dextrorphan on the phencyclidine binding sites. Further,

the following findings from the present study suggest that the ameliorating effects of (+)-SKF-10,047 and dextromethorphan on the conditioned fear stress are mediated through σ receptors, but not through phencyclidine receptors. (1) The non-competitive NMDA receptor antagonists, phencyclidine and dizocilpine, attenuated the motor suppression induced by stress only at a high dose that produced marked hyperlocomotion in the non-stressed mice, whereas (+)-SKF-10,047 and dextromethorphan showed a predominant effect on the stress-induced motor suppression. (2) The σ (non-phencyclidine) receptor antagonists, BMY-14802 (Largent et al., 1988) and NE-100 (a highly selective and potent agent; Okuyama et al., 1993), antagonized the effects of (+)-SKF-10,047 and dextromethorphan on the stress response in the stressed mice, but did not affect the motor response elicited by phencyclidine and dizocilpine in these animals. In addition, unlike those of (+)-SKF-10,047 and dextromethorphan, the effects of phencyclidine and dizocilpine on the stress response were not affected by combination with phenytoin. Taken together, these results confirmed our hypothesis that phenytoin-regulated type σ_1 receptors, but not phencyclidine receptors, may be important in the ameliorating effects of (+)-SKF-10,047 and dextromethorphan on conditioned fear stress.

We have previously reported that stimulation of σ receptors by (±)-SKF-10,047 activated dopamine neuronal systems in the conditioned fear stress (Kamei et al., 1994). For instance, we had found in that study that the ameliorating effect of (±)-SKF-10,047 on stress-induced motor suppression was antagonized by pimozide, a dopamine receptor antagonist. When dopaminergic neurons were destroyed by pretreatment with 6-hydroxydopamine, the effect of (±)-SKF-10,047 on the stress response was also attenuated. Furthermore, (±)-SKF-10,047 dose dependently reversed the decrease in striatal dopamine turnover in the stressed group. Likewise, in the present study, the ameliorating effect of dextromethorphan on the conditioned fear stress was antagonized by dopamine receptor antagonists (data not shown). Therefore, we speculate that phenytoin-regulated σ_1 sites are closely connected with the dopamine neuronal systems. Further research, however, is needed to confirm this hypothesis.

In conclusion, results of the present experiments suggested that the activation of phenytoin-regulated type σ_1 receptors, but not of phencyclidine receptors, may be involved in the ameliorating effects of (+)-SKF-10,047 and dextromethorphan on the conditioned fear stress in mice.

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References

Costa B.R., W.D. Bowen, S.B. Hellewell, J.M. Walker, A. Thurkauf, A.E. Jacobson and K.C. Rice, 1989, Synthesis and evaluation of optically pure [^{3}H]-(+)-pentazocine, a highly potent and selective radioligand for σ receptors, FEBS Lett. 251, 53.

DeHaven-Hudkins D.L., F.Y. Ford-Rice, J.T. Allen and R.L. Hudkins, 1993, Allosteric modulation of ligand binding to [^{3}H]-(+)pentazocine-defined σ recognition sites by phenytoin, Life Sci. 53, 41.

Fanselow, M.S., 1980, Conditional and unconditional components of post-shock freezing, Pavlov J. Biol. Sci. 15, 177.

Gundlach A.L., B.L. Largent, S.H. Snyder, 1985, Phencyclidine and σ opiate receptors in brain: biochemical and autoradiographical differentiation, Eur. J. Pharmacol. 113, 465.

Gundlach A.L., B.L. Largent, S.H. Snyder, 1986, Autoradiographic localization of sigma receptor binding sites in guinea pig and rat central nervous system with (+)^{3}H-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine, J. Neurosci. 6, 1757.

Hellewell, S.B. and W.D. Bowen, 1990, A sigma-like binding site in rat pheochromocytoma (PC12) cells: Decreased affinity for (+)-benzomorphans and lower molecular weight suggest a different sigma receptor form from that of guinea pig brain, Brain Res. 527, 244.

Holtzman, S.G., 1994, Discriminative stimulus effects of dextromethorphan in the rat, Psychopharmacology 116, 249.

Itzhak, Y., I. Stein, S.-H. Zhang, C.O. Kassim and D. Cristante, 1991, Binding of sigma ligands to C57BL/6 mouse brain membranes: effect of monoamine oxidase inhibitors and subcellular distribution studies suggest the existence of sigma receptor subtypes, J. Pharmacol. Exp. Ther. 257, 141.

Kamei, H., T. Kameyama and T. Nabeshima, 1994, SKF-10,047 reverses stress-induced motor suppression: interaction with dopaminergic system, Eur. J. Pharmacol. 260, 39.

Kameyama, T. and M. Nagasaka, 1982a, Effects of apomorphine and diazepam in a quickly learned conditioned suppression in rats, Pharmacol. Biochem. Behav. 17, 59.

Kameyama, T. and M. Nagasaka, 1982b, The effect of analgesics on quickly learned conditioned suppression in mice, Neuropharmacology 21, 1283.

Karbon, E.W., K. Naper and M.J. Pontecorvo, 1991, [^{3}H]DTG and [^{3}H](+)-3-PPP label pharmacologically distinct σ binding sites in guinea pig brain membranes, Eur. J. Pharmacol. 193, 21.

Largent, B.L., H. Wikstrom, A.M. Snowman and S.H. Snyder, 1988, Novel antipsychotic drugs share high affinity for σ receptors, Eur. J. Pharmacol. 155, 345.

Martin, W.R., C.G. Eades, J.A. Thompson, R.A. Huppler and P.E. Gilbert, 1976, The effect of morphine and nalorphine-like drugs in non-dependent and morphine dependent chronic spinal dog, J. Pharmacol. Exp. Ther. 197, 517.

McCann D.J. and T.P. Su, 1992, Stimulation of σ ligand binding by phenytoin: apparent binding site and ligand specificity, in: Multiple Sigma and PCP Receptor Ligands: Mechanisms for Neuromodulation and Neuroprotection?, eds. J.-M. Kamenka and E.F. Domino (NPP Books, Ann Arbor) p. 295.

Misra, A., 1978, Factors affecting the action of narcotics, in: Metabolism of Opiates, eds. M.L. Adler, L. Manara and R. Samanin (Raven Press, New York) p. 297.

Murray, T.F. and M.E. Leid, 1984, Interaction of dextrorotatory opioids with phencyclidine recognition sites in rat brain membranes, Life Sci. 34, 1899.

Musacchio, J.M., M. Klein and J.J. Paturzo, 1989, Effects of dextromethorphan site ligands and allosteric modifiers on the binding of

(+)-[^{3}H]3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine, Mol. Pharmacol. 35, 1.

Nabeshima, T., K. Yamada and T. Kameyama, 1983, Effect of opiate agonists on the conditioned suppression in motility of mice, Neurosci. Lett. 39, 301.

Nabeshima, T., H. Kamei and T. Kameyama, 1988, A role played by sigma receptors in the conditioned suppression of motility in mice, Psycopharmacology 94, 515.

Okuyama, S., Y. Imagawa, S. Ogawa, H. Araki, A. Ajima, M. Tanaka, M. Muramatsu, A. Nakazato, K. Yamaguchi, M. Yoshida and S. Otomo, 1993, NE-100, a novel σ receptor ligand – in vivo tests, Life Sci. 53, 18.

Quirion R., P.C. Chicheportiche, K.M. Contreras, D. Johnson, S. Lodge, W. Tam, J.H. Woods and S.R. Zukin, 1987, Classification and nomenclature of phencyclidine and sigma receptor sites, Trends Pharmacol. Sci. 10, 444.

Quirion, R., W.D. Bowen, Y. Itzhak, J.L. Junien, J.M. Musacchio, R.B. Rothman, T.P. Su, S.W. Tam and D.P. Tayllor, 1992, A proposal for the classification of sigma binding sites, Trends Pharmacol. Sci. 13, 85.

Rogawski, M.A. and R.J. Porter, 1990, Antiepileptic drugs: pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage compounds, Pharmacol. Rev. 42, 223.

Rothman, R.B., A. Reid, A. Mahboubi, C. Kim, B.R. De Costa, A.E. Jacobson and K.C. Rice, 1990, Labeling by 1,2-di(2-[5-^{3}H]tolyl)guanidine of two high affinity binding sites in guinea pig brain: evidence for allosteric regulation by calcium channel antagonists and pseudoallosteric modulation by σ ligands, Mol. Pharmacol. 39, 222.

Tam, S.W., 1983, Naloxone-inaccessible σ receptor in rat central nervous system, Proc. Natl. Acad. Sci. USA 80, 6703.

Walker, J.M., W.D. Bowen, F.O. Walker, R.R. Matsumoto, B.D. Costa and K.C. Rice, 1990, Sigma receptors: biology and function, Pharmacol. Rev. 42, 355.

Weber, E., M. Sonders, M. Quarum, S. Pou and J.F.W. Keana, 1986, 1,3-Di(2-[5-^{3}H]tolyl)guanidine: a selective ligand that labels σ-type receptors for psychotomimetic opiates and antipsychotic drugs, Proc. Natl. Acad. Sci. USA 83, 8784.

Weissman A.D., E.P. Broussolle and E.D. London, 1990, In vivo binding of [^{3}H]d-*N*-allylnormetazocine and [^{3}H]haloperidol to sigma receptors in the mouse brain, J. Chem. Neuroanat. 3, 347.

Wong, E.H.F., J.A. Kemp, T. Priestly, A.R. Knight, G.N. Woodruff and L.L. Iversen, 1986, The anticonvulsant MK-801 is a potent *N*-methyl-D-aspartate antagonist, Proc. Natl. Acad. Sci. USA 83, 7104.

Yamada, K and T. Nabeshima, 1995, Stress-induced behavioral responses and multiple opioid systems in the brain, Behav. Brain Res. 67, 133.