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(+)-SKF-10,047 and dextromethorphan ameliorate conditioned fear stress via dopaminergic systems linked to phenytoin-regulated σ_1 sites

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Abstract

Mice exhibited a marked suppression of motility when they were re-placed in the same environment in which they had previously received an electric footshock. (+)-SKF-10,047 ([2*S*-(2α,6α,11*R**)]-1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(2-propenyl)-2,6-methano-3-benzazocin-8-ol hydrochloride; (+)-*N*-allylnormetazocine hydrochloride) and dextromethorphan, putative σ receptor agonists, have been reported to reverse this psychological stress-induced motor suppression, defined as conditioned fear stress, through phenytoin-regulated type σ_1 receptors. In the present study, we investigated the involvement of dopaminergic neurons in the ameliorating effects of (+)-SKF-10,047 and dextromethorphan on conditioned fear stress. (+)-SKF-10,047 and dextromethorphan attenuated conditioned fear stress at low doses (4 and 20 mg/kg, respectively) when they were co-administered with phenytoin (10 mg/kg), an anticonvulsant drug. The effects were antagonized by the σ receptor antagonists, NE-100 (*N*,*N*-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl]-ethylamine monohydrochloride) and BMY-14802 (a-(4-fluoro-phenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine-butanol hydrochloride). Furthermore, the effects of (+)-SKF-10,047 or dextromethorphan in combination with phenytoin were blocked by the dopamine D_1 receptor antagonist, SCH 23390 (*R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine), and the dopamine D_2 receptor antagonist, (−)-sulpiride, and they were also attenuated by 6-hydroxydopamine-induced lesions of dopaminergic neurons. The ameliorating effects of (+)-SKF-10,047 and dextromethorphan on conditioned fear stress at high doses (5 and 30 mg/kg, respectively) were also blocked by both the dopamine receptor antagonists. These results suggest that the stress-induced motor suppression is restored by the activation of dopaminergic neuronal systems as a result of the stimulation of phenytoin-regulated type σ_1 receptors.

Keywords: Conditioned fear stress; σ Receptor; Phenytoin; Dopaminergic system; Motor suppression

1. Introduction

The functional role of σ receptors in the central nervous system has been investigated extensively. Several lines of evidence have demonstrated that σ binding sites are distinct from phencyclidine binding sites on the *N*-methyl-D-aspartate (NMDA) receptor-ion channel complex (Gundlach et al., 1985, 1986; 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 ([2*S*-(2α,6α,11*R**)]-1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(2-propenyl)-2,6-methano-3-benzazocin-8-ol hydrochloride; (+)-*N*-allylnormetazocine hydrochloride), (+)-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, it has been reported that phenytoin, an anticonvulsant drug, enhances the binding affinity of (+)-SKF-10,047 and dextromethorphan, but not of (+)-pentazocine and DTG, for the σ_1 sites (DeHaven-Hudkins et al., 1993), a finding which suggests that σ_1 receptors can be differentiated into two different binding sites, namely phenytoin-sensitive and phenytoin-insensitive sites. However, the physiological function of these variant types of σ receptors is unclear yet.

To date, we have tried to clarify the functional role of σ receptors in a stressful situation by using the conditioned fear stress response defined by Fanselow (1980). Mice or

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rats exhibit a marked suppression of motility when they are re-placed in the same environment in which they have previously received an electric footshock (Kameyama and Nagasaka, 1982a,b; Yamada and Nabeshima, 1995). This motor suppression has been regarded as a conditioned emotional response to the environment associated with previous footshock (Kameyama and Nagasaka, 1982a,b; Yamada and Nabeshima, 1995). The conditioned fear stress response is dose dependently attenuated by (+)-SKF-10,047 and dextromethorphan, but not by (+)-pentazocine and DTG (Kamei et al., 1996). These effects of (+)-SKF-10,047 and dextromethorphan are blocked by the selective σ receptor antagonists, NE-100 (*N*,*N*-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl]-ethylamine monohydrochloride) and BMY-14802 (a-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine-butanol hydrochloride) (Kamei et al., 1996). Furthermore, our preliminary study indicates that phenytoin enhances the effects of (+)-SKF-10,047 and dextromethorphan on the stress response, but it fails to enhance the effects of (+)-pentazocine and DTG (Kamei et al., 1996). This finding is consistent with the result obtained from the binding study described above, suggesting that the ameliorating effects of (+)-SKF-10,047 and dextromethorphan on the stress-induced motor suppression may be mediated by phenytoin-regulated type σ_1 receptors.

We have also shown that the stimulation of σ receptors by (±)-SKF-10,047 produces activation of dopaminergic neuronal systems involved in the conditioned fear stress response (Kamei et al., 1994): the ameliorating effect of (±)-SKF-10,047 on the stress-induced motor suppression is antagonized by pimozide, a dopamine receptor antagonist, as well as by BMY-14802; when dopaminergic neurons are destroyed by pretreatment with 6-hydroxydopamine, the effect of (±)-SKF-10,047 on the stress response is also attenuated; (±)-SKF-10,047 dose dependently re-

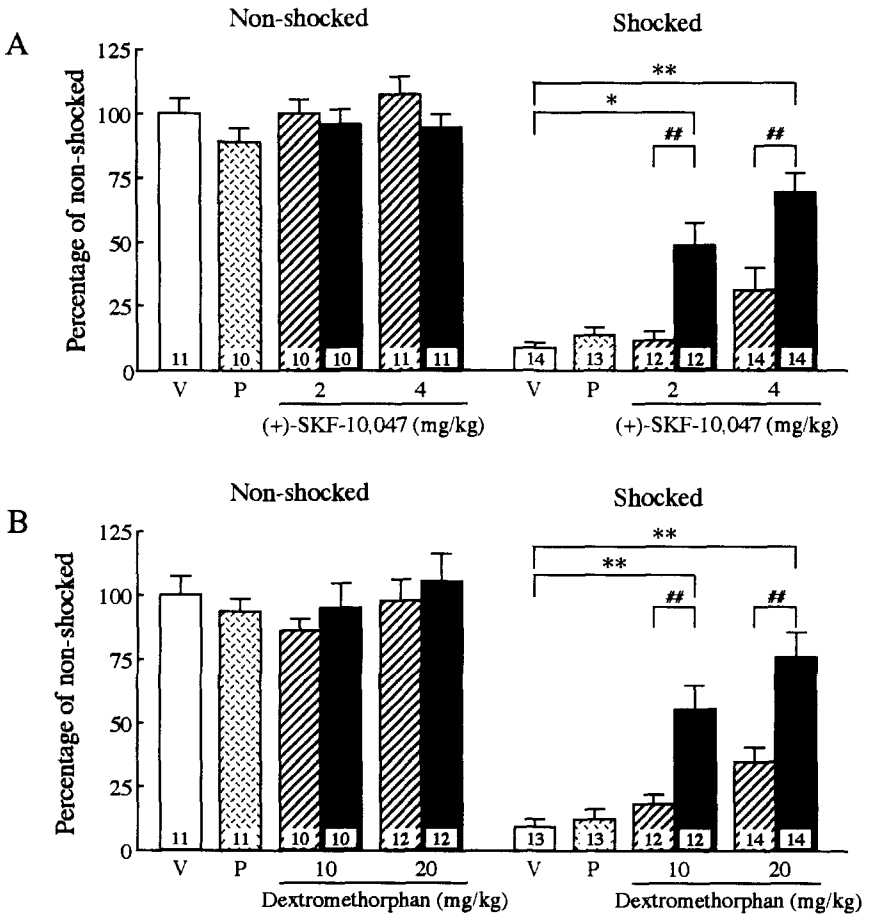

Fig. 1. Effects of (+)-SKF-10,047 (A) or dextromethorphan (B) in combination with phenytoin on conditioned fear stress in mice. (+)-SKF-10,047 and dextromethorphan were administered s.c. 15 min before motility was measured. Phenytoin was administered i.p. 30 min before motility was measured. Motility is expressed as a percentage of that of the vehicle-treated, non-shocked group (motility: (A) 2262.9 ± 135.8, (B) 2108.4 ± 150.3). Values are the means ± S.E.M. for the number of animals shown in each column. Results with ANOVA were: (A) non-shocked group, $H(5) = 5.101$ ($P > 0.05$); shocked group, $H(5) = 35.263$ ($P < 0.01$), (B) non-shocked group, $H(5) = 2.214$ ($P > 0.05$); shocked group, $H(4) = 42.107$ ($P < 0.01$). * $P < 0.05$, ** $P < 0.01$ compared to the vehicle-treated, shocked group (Dunn-type test). ## $P < 0.01$ compared to the corresponding animals treated with (+)-SKF-10,047 or dextromethorphan alone (Mann-Whitney *U*-test). (A) Open columns: vehicle (V); stippled columns: phenytoin alone (P; 10 mg/kg); hatched columns: (+)-SKF-10,047 alone; black columns: (+)-SKF-10,047 + phenytoin. (B) Open columns: vehicle (V); stippled columns: phenytoin alone (P; 10 mg/kg); hatched columns: dextromethorphan alone; black columns: dextromethorphan + phenytoin.

verses the decrease in striatal dopamine turnover in the stressed group. Taken together, these findings raise the possibility that phenytoin-regulated σ_1 sites are closely connected to dopaminergic neuronal systems in the conditioned fear stress response. In the present study, to clarify this hypothesis, we examined whether the effects of (+)-SKF-10,047 or dextromethorphan in combination with phenytoin on conditioned fear stress were regulated by dopaminergic neuronal systems.

2. Materials and methods

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

2.1. Animals

Male ddY mice (Nihon SLC, Shizuoka, Japan) at 7–8 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 20:00 and 08:00 h.

2.2. Drug treatment

The following drugs were used: (+)-SKF-10,047 ([2*S*-(2α,6α,11*R**)]-1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(2-propenyl)-2,6-methano-3-benzazocin-8-ol hydrochloride; (+)-*N*-allylnormetazocine hydrochloride; Research Biochemicals, Natick, MA, USA), dextromethorphan hydrobromide (Sigma Chemical, St. Louis, MO, USA), NE-100 (*N*,*N*-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)-phenyl]-ethylamine monohydrochloride; Taisho Pharmaceutical, Saitama, Japan), BMY-14802 (a-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine-butanol hydrochloride; Bristol-Myers Squibb, Wallinford, CT), phenytoin sodium salt (Research Biochemicals), SCH 23390 (*R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) maleate; Schering-Plough, USA), a dopamine D_1 receptor antagonist, (−)-

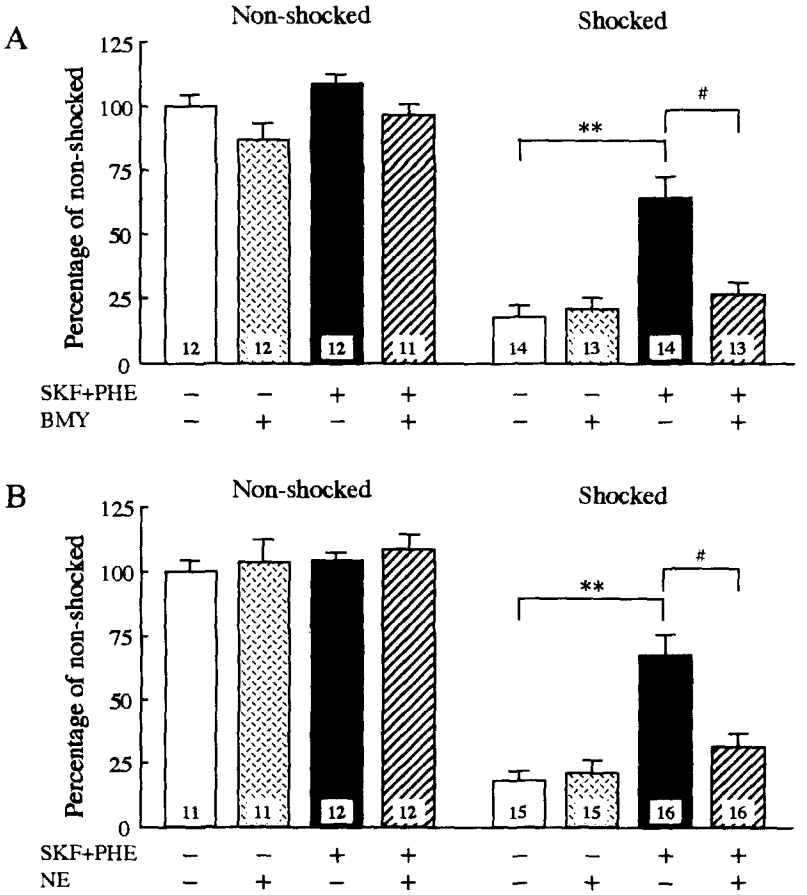

Fig. 2. Effects of BMY-14802 (BMY) (A) and NE-100 (NE) (B) on attenuation of the conditioned fear stress induced by (+)-SKF-10,047 (SKF) in combination with phenytoin (PHE) in mice. SKF (4 mg/kg) and PHE (10 mg/kg) were administered s.c. 15 min and i.p. 30 min, respectively, before motility was measured. BMY (10 mg/kg) and NE (5 mg/kg) were administered s.c. 30 min and i.p. 45 min, respectively, before motility was measured. Other details are as shown in the legend of Fig. 1. Motility in the vehicle-treated, non-shocked groups: (A) 2118.1 ± 97.9, (B) 2245.8 ± 100.2. Results with ANOVA were: (A) non-shocked group, $H(3) = 8.016$ ($P < 0.05$); shocked group, $H(3) = 19.307$ ($P < 0.01$), (B) non-shocked group, $H(3) = 1.614$ ($P > 0.05$); shocked group, $H(3) = 23.425$ ($P < 0.01$). ** $P < 0.01$ compared to the vehicle-treated, shocked group. $^{\#}$ $P < 0.05$ compared to the (SKF + PHE)-treated, shocked group (Dunn-type test).

sulpiride (Sigma Chemical), a dopamine D_2 receptor antagonist, 6-hydroxydopamine hydrobromide (Sigma Chemical), desmethylimipramine hydrochloride (Sigma Chemical).

NE-100 was dissolved in distilled water. 6-Hydroxydopamine was dissolved in 0.9% NaCl solution containing 0.1% ascorbic acid. Phenytoin was prepared as a microsuspension in Cremophor EL (Sigma Chemical) 10% in 0.9% NaCl solution by ultrasonic vibration. BMY-14802 and (−)-sulpiride 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 $NaHCO_3$). 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, 250 V DC) for 6 min through an isolated stimulator (Nihon Koden, Tokyo, Japan). Each animal received electric shocks in a range of 0.8-1.9 mA, because the current resistance with the animal in the test cage varied between 130 and 300 kΩ. The test trial was carried out 24 h after the shock treatment; the animals were again placed in 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 7–8 weeks and the shock intensity used in this method

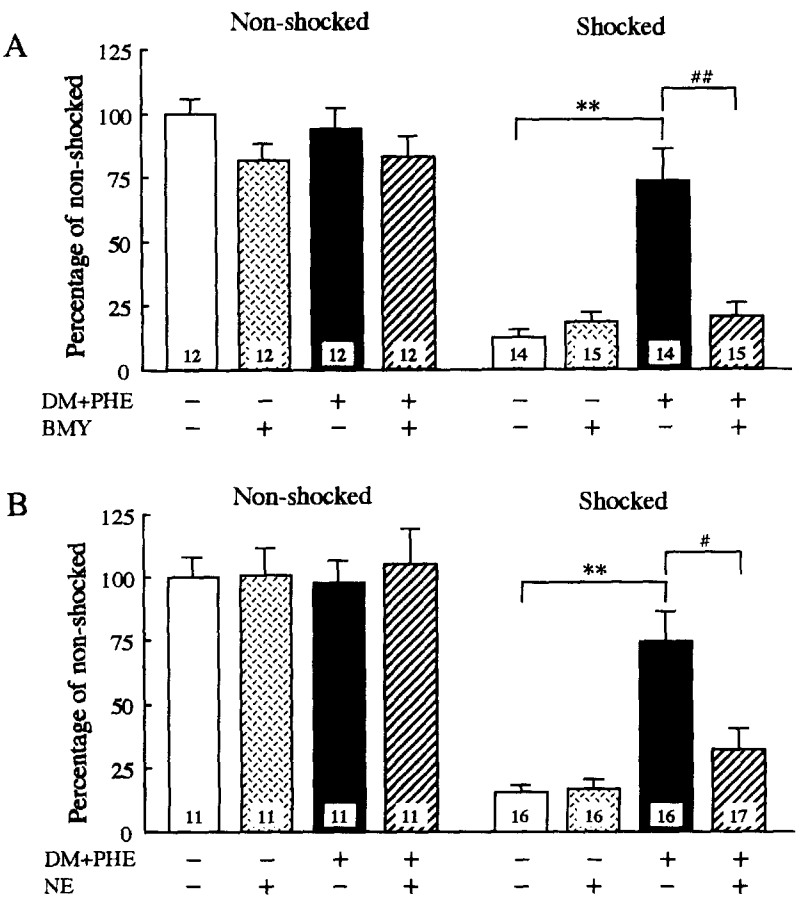

Fig. 3. Effects of BMY-14802 (BMY) (A) and NE-100 (NE) (B) on attenuation of the conditioned fear stress induced by dextromethorphan (DM) in combination with phenytoin (PHE) in mice. DM (20 mg/kg) and PHE (10 mg/kg) were administered s.c. 15 min and i.p. 30 min, respectively, before motility was measured. BMY (10 mg/kg) and NE (5 mg/kg) were administered s.c. 30 min and i.p. 45 min, respectively, before motility was measured. Other details are as shown in the legend of Fig. 1. Motility in the vehicle-treated, non-shocked groups: (A) 2188.2 ± 136.4, (B) 2066.8 ± 165.1. Results with ANOVA were: (A) non-shocked group, $H(3) = 4.629$ ($P > 0.05$); shocked group, $H(3) = 22.061$ ($P < 0.01$), (B) non-shocked group, $H(3) = 0.055$ ($P > 0.05$); shocked group, $H(3) = 22.078$ ($P < 0.01$). ** $P < 0.01$ compared to the vehicle-treated, shocked group, $^{\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$ compared to the (DM + PHE)-treated, shocked group (Dunn-type test).

have been reported to be optimal to produce a stable response (motor suppression) (Kameyama and Nagasaka, 1982b; Nabeshima et al., 1983). Our ethics committee have approved this point.

All test drugs were administered before motility was measured in the test trial; (+)-SKF-10,047 and dextromethorphan were administered subcutaneously (s.c.) 15 min before the test trial, BMY-14802 and SCH 23390 were administered s.c. 30 min before, phenytoin was administered intraperitoneally (i.p.) 30 min before, NE-100 and (−) sulpiride were administered i.p. 45 min and 90 min, respectively, before the trial. Ten days before the test trial, 6-hydroxydopamine (100 μg/10 μl per mouse, as a free base) was administered intracerebroventricularly under anesthesia with ether. According to our previous method (Kamei et al., 1994), desmethylimipramine (25 mg/kg) was administered i.p. 30 min before the treatment with 6-hydroxydopamine to protect against the lesion of noradrenergic neurons. With this schedule, we have demonstrated that 10 days after the treatment with 6-hydroxydopamine (100 μg/mouse), the dopamine content in the whole brain of mice is decreased by 68.5% of that of the vehicle-treated control (Kamei et al., 1994). Groups receiving no drugs were given an appropriate vehicle, i.e. solution containing 0.1 N HCl or 10% Cremophor EL.

2.4. Statical 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 (+)-SKF-10,047 or dextromethorphan in combination with phenytoin on conditioned fear stress

As shown in Figs. 1–6, the shocked mice (shocked groups) exhibited a marked suppression of motility; the

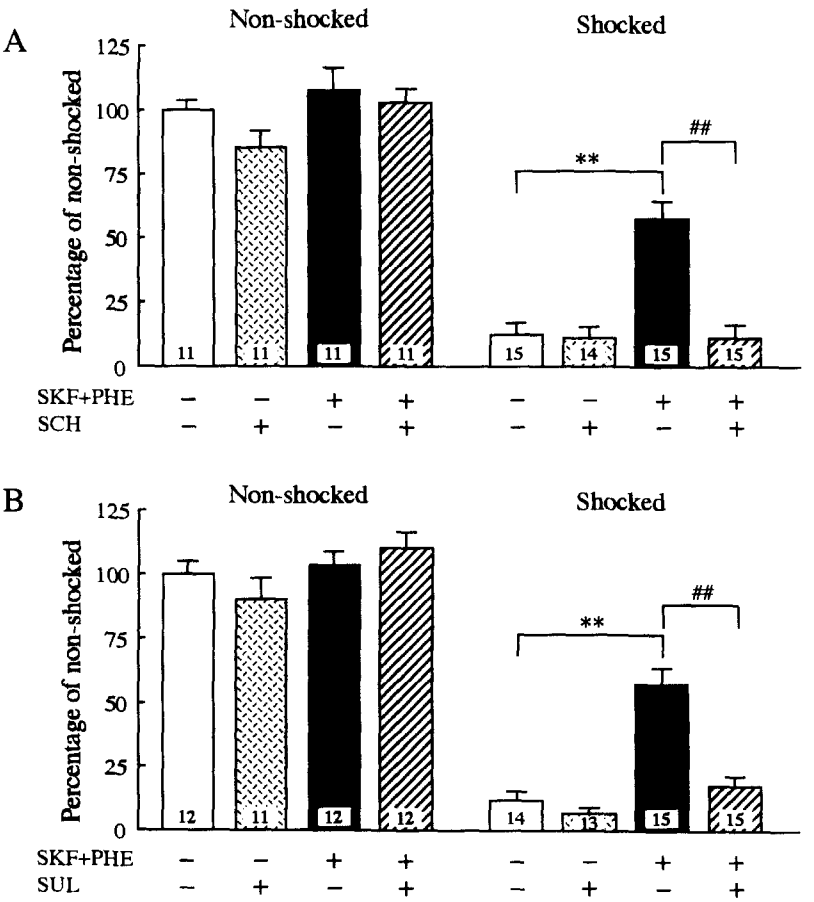

Fig. 4. Effects of SCH 23390 (SCH) (A) and (−)-sulpiride (SUL) (B) on attenuation of the conditioned fear stress induced by (+)-SKF-10,047 (SKF) in combination with phenytoin (PHE) in mice. SKF (4 mg/kg) and PHE (10 mg/kg) were administered s.c. 15 min and i.p. 30 min, respectively, before motility was measured. SCH (0.025 mg/kg) and SUL (10 mg/kg) were administered s.c. 30 min and i.p. 90 min, respectively, before motility was measured. Other details are as shown in the legend of Fig. 1. Motility in the vehicle-treated, non-shocked groups: (A) 2147.9 ± 85.1, (B) 2269.6 ± 120.2. Results with ANOVA were: (A) non-shocked group, $H(3) = 5.411$ ($P > 0.05$); shocked group, $H(3) = 24.999$ ($P < 0.01$), (B) non-shocked group, $H(3) = 5.957$ ($P > 0.05$); shocked group, $H(3) = 29.297$ ($P < 0.01$). ** $P < 0.01$ compared to the vehicle-treated, shocked group, $^{\#\#}$ $P < 0.01$ compared to the (SKF + PHE)-treated, shocked group (Dunn-type test).

shocked groups showed 8.8–18.3% 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 on the floor and crouched in the corner of test apparatus, while the non-shocked mice showed typical exploratory behaviors, such as ambulation, sniffing and rearing.

Fig. 1 shows the effects of (+)-SKF-10,047 (A) or dextromethorphan (B) in combination with phenytoin on the motor suppression in the shocked group. Phenytoin was used at a dose of 10 mg/kg, which is the ED_{50} for the inhibition of maximal electroshock seizure in mice (Rogawski and Porter, 1990), and it had little effect on motility in either the non-shocked or the shocked groups. When given alone, low doses of (+)-SKF-10,047 (2 and 4 mg/kg) and dextromethorphan (10 and 20 mg/kg) had little effect on motility in both groups. However, when phenytoin (10 mg/kg) was co-administered, (+)-SKF-10,047 and dextromethorphan significantly attenuated the motor suppression at these low doses without affecting motility in the non-shocked group. The shocked mice given (+)-SKF-10,047 or dextromethorphan in combination with phenytoin exhibited exploratory behaviors, like the non-shocked mice, but moved slowly.

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

To clarify whether the attenuating effects of (+)-SKF-10,047 or dextromethorphan in combination with phenytoin on motor suppression in the shocked mice were mediated by σ receptors, we investigated the antagonistic effects of the selective σ receptor antagonists, BMY-14802 and NE-100, on the effects of (+)-SKF-10,047 and dex-

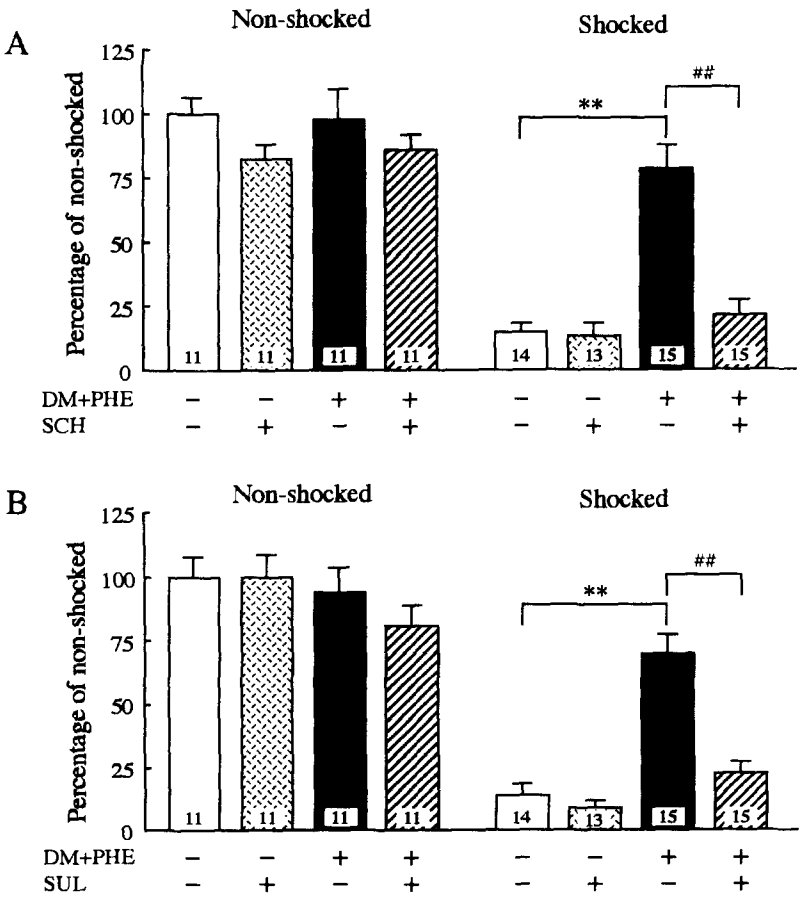

Fig. 5. Effects of SCH 23390 (SCH) (A) and (−)-sulpiride (SUL) (B) on attenuation of the conditioned fear stress induced by dextromethorphan (DM) in combination with phenytoin (PHE) in mice. DM (20 mg/kg) and PHE (10 mg/kg) were administered s.c. 15 min and i.p. 30 min, respectively, before motility was measured. SCH (0.025 mg/kg) and SUL (10 mg/kg) were administered s.c. 30 min and i.p. 90 min, respectively, before motility was measured. Other details are as shown in the legend of Fig. 1. Motility in the vehicle-treated, non-shocked groups: (A) 2083.9 ± 137.0, (B) 2009.4 ± 162.0. Results with ANOVA were: (A) non-shocked group, $H(3) = 3.055$ ($P > 0.05$); shocked group, $H(3) = 28.020$ ($P < 0.01$), (B) non-shocked group, $H(3) = 3.475$ ($P > 0.05$); shocked group, $H(3) = 28.355$ ($P < 0.01$). ** $P < 0.01$ compared to the vehicle-treated, shocked group, $^{\#\#}$ $P < 0.01$ compared to the (DM + PHE)-treated, shocked group (Dunn-type test).

tromethorphan. The σ receptor antagonists were used at doses that block the effects of high doses of (+)-SKF-10,047 (5 mg/kg) and dextromethorphan (30 mg/kg) on motor suppression (Kamei et al., 1996). As shown in Fig. 2, both BMY-14802 (10 mg/kg) and NE-100 (5 mg/kg) significantly inhibited the attenuation of the motor suppression induced by the co-administration of (+)-SKF-10,047 (4 mg/kg) and phenytoin. Similarly, the attenuating effect of the combination with dextromethorphan (20 mg/kg) and phenytoin on motor suppression was also significantly antagonized by both BMY-14802 (10 mg/kg) and NE-100 (5 mg/kg) (Fig. 3). 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. 2 and 3).

Table 1
Effects of SCH 23390 (SCH) and (−)-sulpiride (SUL) on the (+)-SKF-10,047 (SKF)- and dextromethorphan (DM)-induced attenuation of conditioned fear stress in mice

Treatment (mg/kg)	Motility (% of control value)			
	Non-shocked	n	Shocked	n
(A)				
Vehicle	100.0 ± 8.2	10	13.1 ± 3.6	11
SCH (0.025)	76.6 ± 4.9	11	5.8 ± 1.5	12
SKF (5)	119.8 ± 8.0	9	57.4 ± 5.9 [b]	13
+ SCH (0.0125)	100.1 ± 7.2	10	35.6 ± 8.0	12
+ SCH (0.025)	110.9 ± 8.9	9	14.8 ± 4.3 [d]	12
(B)				
Vehicle	100.0 ± 7.0	12	11.3 ± 3.7	12
SUL (10)	80.4 ± 5.2	12	9.4 ± 3.6	12
SKF (5)	121.7 ± 7.2	12	51.7 ± 8.4 [b]	12
+ SUL (5)	124.8 ± 7.2	9	29.6 ± 5.0	13
+ SUL (10)	107.4 ± 8.8	11	13.9 ± 2.0 [c]	12
(C)				
Vehicle	100.0 ± 8.0	11	17.9 ± 5.7	12
SCH (0.025)	80.1 ± 7.8	11	15.5 ± 5.5	13
DM (30 mg/kg)	108.1 ± 10.1	11	69.5 ± 15.4 [a]	12
+ SCH (0.025)	89.8 ± 9.8	11	20.1 ± 7.1 [e]	13
(D)				
Vehicle	100.0 ± 5.2	11	17.5 ± 5.6	12
SUL (10)	84.5 ± 5.5	11	10.9 ± 3.4	12
DM (30 mg/kg)	116.4 ± 6.6	12	59.6 ± 12.8 [a]	13
+ SUL (10)	94.6 ± 11.6	12	16.2 ± 3.8 [e]	13

SKF and DM were administered s.c. 15 min before motility was measured. SCH and SUL were administered s.c. 30 min and i.p. 90 min, respectively, before motility was measured. Other details are as shown in the legend of Fig. 1. Motility in the vehicle-treated, non-shocked group: (A) 2245.1 ± 183.5, (B) 2108.5 ± 148.3, (C) 1893.3 ± 150.9, (D) 2129.0 ± 110.9. Values are the means ± S.E.M. obtained from the number of animals shown by n. Results with ANOVA were: (A) non-shocked group, $H(4) = 15.049$ ($P < 0.01$); shocked group, $H(4) = 33.154$ ($P < 0.01$), (B) non-shocked group, $H(4) = 20.718$ ($P < 0.01$); shocked group, $H(4) = 27.478$ ($P < 0.01$), (C) non-shocked group, $H(3) = 4.058$ ($P > 0.05$); shocked group, $H(3) = 15.306$ ($P < 0.01$), (D) non-shocked group, $H(3) = 9.716$ ($P < 0.05$); shocked group, $H(3) = 24.607$ ($P < 0.01$). [a] $P < 0.05$, [b] $P < 0.01$ compared to the corresponding vehicle-treated group, [c] $P < 0.05$, [d] $P < 0.01$ compared to the SKF-treated, shocked group, [e] $P < 0.05$ compared to the DM-treated, shocked group (Dunn-type test).

Table 2
Effects of (+)-SKF-10,047 (SKF) and dextromethorphan (DM) on conditioned fear stress in vehicle- and 6-hydroxydopamine (6-OHDA)-pretreated mice

Pretreatment	Treatment (mg/kg)	Motility (% of control value)			
		Non-shocked	n	Shocked	n
Vehicle	Vehicle	100.0 ± 7.1	10	13.6 ± 6.3	10
	SKF (5)	120.4 ± 7.6	10	64.4 ± 12.7 [a]	12
	DM (30)	94.4 ± 8.3	10	65.0 ± 11.5 [a]	11
6-OHDA	Vehicle	94.7 ± 4.7	10	16.0 ± 6.8	11
	SKF (5)	106.8 ± 10.1	11	29.2 ± 10.1 [b]	12
	DM (30)	82.1 ± 9.9	10	30.2 ± 9.2 [c]	12

Schedule of pretreatment with 6-OHDA is described in Materials and methods. SKF and DM were administered s.c. 15 min before motility was measured. Other details are as shown in the legend of Fig. 1. Motility in the vehicle-treated, non-shocked group: 2374.8 ± 168.2. Results with ANOVA were: non-shocked group, $H(5) = 10.608$ ($P > 0.05$); shocked group, $H(5) = 23.208$ ($P < 0.01$). [a] $P < 0.01$ compared to the vehicle-treated, shocked animals with pretreatment of vehicle (Dunn-type test). [b] $P < 0.05$ compared to the SKF-treated, shocked animals with pretreatment of vehicle. [c] $P < 0.01$ compared to the DM-treated, shocked animals with pretreatment of vehicle (Mann-Whitney U-test).

3.3. Antagonistic effects of the dopamine receptor antagonists, SCH 23390 and (−)-sulpiride, on the attenuation of conditioned fear stress induced by (+)-SKF-10,047 and dextromethorphan in combination with phenytoin

In agreement with our previous results (Kamei et al., 1996), (+)-SKF-10,047 and dextromethorphan themselves significantly attenuated the motor suppression at higher doses (5 and 30 mg/kg, respectively) in the shocked group (Table 1). The effects were completely blocked by SCH 23390 (0.025 mg/kg), a dopamine D_1 receptor antagonist, and by (−)-sulpiride (10 mg/kg), a dopamine D_2 receptor antagonist. Similarly, the attenuating effects of combined administration of (+)-SKF-10,047 (4 mg/kg) and phenytoin (10 mg/kg) and of dextromethorphan (20 mg/kg) and phenytoin (10 mg/kg) were also antagonized by SCH 23390 (0.025 mg/kg) and (−)-sulpiride (10 mg/kg) (Figs. 4 and 5). SCH 23390 (0.025 mg/kg) and (−)-sulpiride (10 mg/kg) themselves had little effect on motility in either the non-shocked or the shocked groups (Table 1, Figs. 4 and 5).

3.4. Effect of 6-hydroxydopamine-induced lesions of dopaminergic neurons on the attenuation of conditioned fear stress induced by (+)-SKF-10,047 and dextromethorphan in combination with phenytoin

As shown in Fig. 6 and Table 2, both 6-hydroxydopamine-pretreated and non-pretreated, shocked mice exhibited motor suppression to a similar extent. The attenuating effects of (+)-SKF-10,047 (5 mg/kg) and dextromethorphan (30 mg/kg) on motor suppression were reduced in mice pretreated with 6-hydroxydopamine when compared

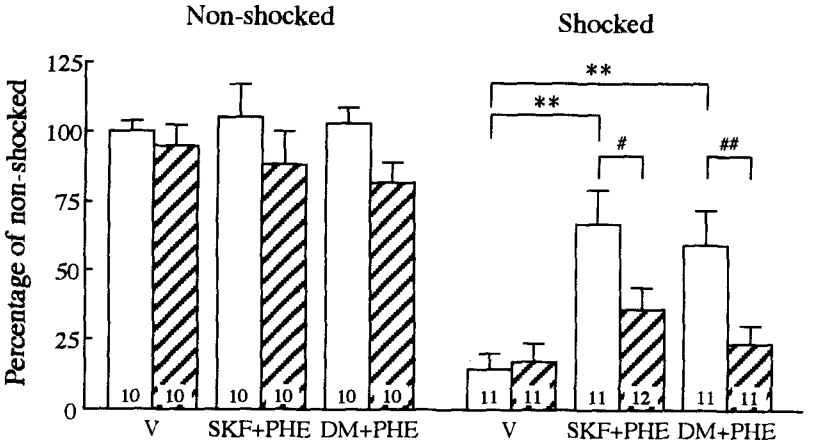

Fig. 6. Effects of (+)-SKF-10,047 (SKF) or dextromethorphan (DM) in combination with phenytoin (PHE) on conditioned fear stress in vehicle- and 6-hydroxydopamine-pretreated mice. Schedule of pretreatment with 6-hydroxydopamine is described in Materials and methods. SKF (4 mg/kg) and DM (20 mg/kg) were administered s.c. 15 min before motility was measured. PHE (10 mg/kg) was administered i.p. 30 min before motility was measured. Other details are as shown in the legend of Fig. 1. Motility in the vehicle-treated, non-shocked group: 2271.3 ± 90.2. Results with ANOVA were: non-shocked group, $H(5) = 5.356$ ($P > 0.05$); shocked group, $H(5) = 25.846$ ($P < 0.01$). ** $P < 0.01$ compared to the vehicle-treated, shocked group (Dunn-type test). $^{\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$ compared to the corresponding shocked animals with pretreatment of vehicle (Mann-Whitney U-test). V: vehicle; open columns: vehicle-pretreated group; hatched columns: 6-hydroxydopamine-pretreated group.

to those in non-pretreated mice (Table 2). Likewise, the attenuating effects of combined administration of (+)-SKF-10,047 (4 mg/kg) and phenytoin (10 mg/kg) and of dextromethorphan (20 mg/kg) and phenytoin (10 mg/kg) were also attenuated in mice pretreated with 6-hydroxydopamine (Fig. 6).

4. Discussion

As described in the Introduction, 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 this motor suppression as conditioned fear stress. Our previous study indicates that conditioned fear stress is dose dependently attenuated by (+)-SKF-10,047 and dextromethorphan, and that the effects are antagonized by the σ receptor antagonists, BMY-14802 (Largent et al., 1988) and NE-100 (a highly selective and potent agent; Okuyama et al., 1993) (Kamei et al., 1996). In the present study, the effects of (+)-SKF-10,047 and dextromethorphan on the stress response were enhanced by co-administration of phenytoin. The effects of (+)-SKF-10,047 or dextromethorphan in combination with phenytoin were also blocked by the σ receptor antagonists, BMY-14802 and NE-100. These results further support our hypothesis that the activation of phenytoin-regulated type σ_1 receptors is involved in the ameliorating effects of (+)-SKF-10,047 and dextromethorphan on the stress-induced motor suppression. Another possibility is that the effects of (+)-SKF-10,047 and dextromethorphan on the stress response occurred through the phencyclidine sites of the NMDA receptor-channel complex. The non-competitive NMDA receptor antagonists, phencyclidine and dizocilpine (Wong et al., 1986), attenuate the stress-induced motor suppression only at high doses that produce a marked hyperlocomotion in the non-stressed mice, whereas (+)-SKF-10,047 and dextromethorphan show a predominant effect on the stress-induced motor suppression (Kamei et al., 1996). Moreover, BMY-14802 and NE-100 antagonize the effects of (+)-SKF-10,047 and dextromethorphan on the stress response, but they fail to antagonize the effects of phencyclidine and dizocilpine on the stress response (Kamei et al., 1996). In addition, unlike (+)-SKF-10,047 and dextromethorphan, the effects of phencyclidine and dizocilpine on the stress response are not enhanced by combination with phenytoin (Kamei et al., 1996). Taken together, it is suggested that the ameliorating effects of (+)-SKF-10,047 and dextromethorphan on conditioned fear stress are not mediated by phencyclidine sites.

We have also reported that dysfunction of dopaminergic neuronal systems is involved in the conditioned fear stress response. For instance, stress-induced motor suppression is attenuated by dopamine receptor agonists, such as apomorphine or methamphetamine (Kameyama and Nagasaka, 1982a,1983; Nagasaka and Kameyama, 1983), whereas it is potentiated by the dopamine receptor antagonist, haloperidol (Kameyama and Nagasaka, 1983). Furthermore, a significant decrease in striatal dopamine turnover is observed in mice showing conditioned fear stress (Nabeshima et al., 1986). Combined treatment with the dopamine D_1 receptor agonist, SKF 38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1-*H*-3-benzazepine), and the dopamine D_2 receptor agonist, quinpirole, synergistically attenuates conditioned fear stress, although neither SKF 38393 nor quinpirole alone do so (Kamei et al., 1995), the

effects being inhibited by SCH 23390, a dopamine D_1 receptor antagonist, and (−)-sulpiride, a dopamine D_2 receptor antagonist. Thus, these findings suggest that the activation of both dopamine D_1 and D_2 receptors is necessary to attenuate conditioned fear stress. In the present study, SCH 23390 and (−)-sulpiride antagonized the ameliorating effects of (+)-SKF-10,047 and dextromethorphan on conditioned fear stress, and they also inhibited the effects of low doses of (+)-SKF-10,047 or dextromethorphan in combination with phenytoin. Furthermore, when dopaminergic neurons were destroyed by the pretreatment with 6-hydroxydopamine, the effects of (+)-SKF-10,047 and dextromethorphan on the stress response were reduced. The same results of the pretreatment with 6-hydroxydopamine were found when low doses of (+)-SKF-10,047 or dextromethorphan were used in combination with phenytoin. Additionally, (±)-SKF-10,047 reverses a decrease in the striatal dopamine turnover in stressed mice (Kamei et al., 1994). Taken together, the present results suggest that (+)-SKF-10,047 and dextromethorphan may elevate dopaminergic neurotransmission through the phenytoin-regulated σ_1 sites followed by activation of both dopamine D_1 and D_2 receptors to attenuate the conditioned fear stress response.

There is evidence that σ receptors modulate the activity of dopaminergic neurons: the existence of σ receptors on dopaminergic neurons has been demonstrated (Gundlach et al., 1985, 1986) and electrophysiological studies have shown that σ receptors are involved in the regulation of neuronal activity of midbrain dopaminergic neurons (Freeman and Bunney, 1984; Steinfels and Tam, 1989). Several investigators have demonstrated that the effects of (+)-SKF-10,047 on dopaminergic neurons differ from those of (+)-pentazocine and DTG. For instance, systemic administration of (+)-SKF-10,047, but not (+)-pentazocine, produces ipsilateral rotation in rats with unilateral lesion of the substantia nigra (Hepler et al., 1988), the effect being inhibited by blockade of σ receptors (Tam et al., 1992). In addition, (+)-SKF-10,047 increases the firing rate of A9 dopamine neurons (Freeman and Bunney, 1984), but (+)-pentazocine and DTG decrease it (Steinfels et al., 1989). However, it has been reported that (+)-pentazocine as well as (+)-SKF-10,047 increases dopamine release from mesolimbic and nigrostriatal dopaminergic neurons in rats (Iyengar et al., 1990; Patrick et al., 1993; Gudelsky, 1995). In a further study, we have found a decrease in dopamine turnover in the nucleus accumbens in rats showing conditioned fear stress, and this change in the stressed group is restored to the control level by (+)-SKF-10,047, but not by (+)-pentazocine (Kamei et al., in preparation). Therefore, it is unlikely that (+)-SKF-10,047 and (+)-pentazocine act on the dopaminergic neurons via a common mechanism at least in a stressful situation, although the reasons are not clear. To our knowledge, there is little evidence that dextromethorphan modulates dopaminergic neurons. In the present study, the data appear to support the possibility that dextromethorphan activates dopaminergic neurons when mice are stressed. In addition, we have reported that unlike (+)-SKF-10,047 and dextromethorphan, (+)-pentazocine and DTG cannot attenuate conditioned fear stress even at high doses (Kamei et al., 1996). Taken together, we speculate that the difference between σ ligands in the ability to ameliorate the conditioned fear stress response may be due to their different actions on the regulation site of dopaminergic neurons, which appears to be the phenytoin-regulated σ_1 site, in a stressful situation. However, further studies should be carried out to clarify the relationship between the activation of phenytoin-regulated σ_1 sites and the function of dopaminergic neuronal systems in conditioned fear stress.

In conclusion, the phenytoin-regulated σ_1 site may play an important role in the ameliorating effects of (+)-SKF-10,047 and dextromethorphan on conditioned fear stress, and this site may be closely connected to dopaminergic neuronal systems involved in this stress response.

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References

DeHave-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.

Freeman, A.S. and B.S. Bunney, 1984, The effects of phencyclidine and *N*-allylnormetazocine on midbrain dopamine neuronal activity, Eur. J. Pharmacol. 104, 287.

Gudelsky, G.A., 1995, Effects of σ receptor ligands on the extracellular concentration of dopamine in the striatum and prefrontal cortex of the rat, Eur. J. Pharmacol. 286, 223.

Gundlach, A.L., B.L. Largent and 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 and 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.

Hepler, D., S.W. Tam and V.J. DeNoble, 1988, Selective PCP and sigma receptor agonists produce differential effects in rats with unilateral lesions in the substantia nigra, Soc. Neurosci. Abstr. 14, 806.

Iyengar, S., V.M. Dilworth, S.J. Mick, P.C. Contreras, J.B. Monahan, T.S. Rao and P.L. Wood, 1990, Sigma receptors modulate both A9 and A10 dopaminergic neurons in the rat brain: functional interaction with NMDA receptors, Brain Res. 524, 322.

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.

Kamei, H., T. Kameyama and T. Nabeshima, 1995, Activation of both dopamine D1 and D2 receptors necessary for amelioration of conditioned fear stress, Eur. J. Pharmacol. 273, 229.

Kamei, H., T. Kameyama and T. Nabeshima, 1996, (+)-SKF-10,047 and dextromethorphan ameliorate the conditioned fear stress through the activation of phenytoin-regulated σ_1 sites, Eur. J. Pharmacol. 299, 21.

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.

Kameyama, T. and M. Nagasaka, 1983, Effects of apomorphine and methamphetamine on a quickly learned conditioned-suppression response in rats, Neuropharmacology 22, 813.

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

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., A. Katoh, M. Hiramatsu and T. Kameyama, 1986, A role played by dopamine and opioid neuronal systems in stress-induced motor suppression (conditioned suppression of motility) in mice, Brain Res. 398, 354.

Nagasaka, M. and T. Kameyama, 1983, Effect of diazepam, meprobamate, chlorpromazine and apomorphine on a quickly learned conditioned suppression in mice, J. PharmacobioDyn. 6, 523.

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.

Patrick, S.L., J.M. Walker, J.M. Perkel, M. Lockwood and R.L. Patrick, 1993, Increases in rat striatal extracellular dopamine and vacuous chewing produced by two σ receptor ligands, Eur. J. Pharmacol. 231, 243.

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.

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.

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.

Steinfels, G.F. and S.W. Tam, 1989, Selective σ receptor agonist and antagonist affect dopamine neuronal activity, Eur. J. Pharmacol. 163, 167.

Steinfels, G.F., S.W. Tam and L. Cook, 1989, Electrophysiological effects of selective σ-receptor agonists, antagonists, and the selective phencyclidine receptor agonist MK-801 on midbrain dopamine neurons, Neuropsychopharmacology 2, 201.

Tam, S.W., G.F. Steinfels, P.J. Gilligan, W.K. Schmidt and L. Cook, 1992, DUP 734 [1-(cyclopropylmethyl)-4-(2′(4″-fluorophenyl)-2′-oxoethyl)-piperidine HBr], a sigma and 5-hydroxytryptamine$_2$ receptor antagonist: receptor-binding, electrophysiological and neuropharmacological profiles, J. Pharmacol. Exp. Ther. 263, 1167.

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.

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.