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Role of (+)-SKF-10,047-sensitive sub-population of σ_1 receptors in amelioration of conditioned fear stress in rats: association with mesolimbic dopaminergic systems

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Abstract

Rats exhibited a marked suppression of motility when they were re-placed in the same environment as that in which they had previously received an electric footshock. We examined the behavioral and neurochemical effects of (+)-*N*-allylnormetazocine hydrochloride ((+)-SKF-10,047) and (+)-pentazocine, putative σ_1 receptor ligands, on this psychological-stress-induced motor suppression, defined as a conditioned fear stress. (+)-SKF-10,047 (3 and 6 mg/kg) dose-dependently attenuated the conditioned fear stress, whereas (+)-pentazocine failed to do so even at a higher dose (32 mg/kg). In rats showing the conditioned fear stress, dopamine turnover (i.e., the ratio of dopamine metabolites/dopamine contents) was decreased in the nucleus accumbens and was increased in the medial prefrontal cortex, but remained unchanged in the striatum. (+)-SKF-10,047 (3 and 6 mg/kg) dose-dependently reversed the decreased dopamine turnover in the nucleus accumbens without changing the increased dopamine turnover in the medial prefrontal cortex. (+)-Pentazocine (32 mg/kg) did not affect the stress-induced changes in dopamine turnover in these brain regions. Thus, the decreased dopamine turnover in the nucleus accumbens appears to be involved in the conditioned fear stress. These results suggest that (+)-SKF-10,047 ameliorates the conditioned fear stress by reversing the psychological stress-induced dysfunction in the mesolimbic dopaminergic systems, and that the (+)-SKF-10,047-sensitive sub-population of σ_1 receptors may play an important role in this stress response.

Keywords: Conditioned fear stress; σ Receptor; Dopaminergic system; Nucleus accumbens; Motor suppression; (Rat)

1. Introduction

The functional role of σ receptors in the central nervous system has been investigated extensively. Recent evidence has suggested that σ receptors are divided into at least two subtypes, σ_1 and σ_2 receptors (Walker et al., 1990; Quirion et al., 1992): σ_1 receptors are characterized by a high affinity for (+)-*N*-allylnormetazocine hydrochloride ((+)-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 an anticonvulsant, phenytoin, have shown that σ_1 receptors can be differentiated into two different binding sites, namely phenytoin-sensitive and phenytoin-insensitive sites (McCann and Su, 1992; De-Haven-Hudkins et al., 1993). However, the physiological function of these variant types of σ receptors is not yet well understood.

We have been trying to clarify the functional role of σ receptors in a stressful situation by using the conditioned fear stress defined by Fanselow (1980). Mice or rats exhibit a marked suppression of motility when they are re-placed in the same environment as that in which they had 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;

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Yamada and Nabeshima, 1995). We have observed that (+)-SKF-10,047 dose-dependently attenuates the conditioned fear stress in mice, the effect being blocked by the selective σ receptor antagonists, NE-100 (*N*,*N*-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl]-ethylamine monohydrochloride; Okuyama et al., 1993) and BMY-14802 (α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine-butanol hydrochloride; Largent et al., 1988) (Kamei et al., 1996a). However, (+)-pentazocine fails to affect the conditioned fear stress in mice (Kamei et al., 1996a).

We have also shown that stimulation of σ receptors by (+)-SKF-10,047 produces activation of dopaminergic neuronal systems involved in the conditioned fear stress response in mice (Kamei et al., 1996b). The ameliorating effect of (+)-SKF-10,047 on the stress response is antagonized by SCH 23390 (*R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine), a dopamine D_1 receptor antagonist, and (−)-sulpiride, a dopamine D_2 receptor antagonist; when dopaminergic neurons are destroyed by pretreatment with 6-hydroxydopamine, the effect of (+)-SKF-10,047 on the stress response is also attenuated. There is evidence indicating that σ receptors modulate the activity of dopaminergic neurons: the existence of σ receptors on the 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 the midbrain dopaminergic neurons (Freeman and Bunney, 1984; Steinfels and Tam, 1989). However, the effects of σ receptor ligands on the dopaminergic neurons are inconsistent. 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), whereas (+)-pentazocine and DTG decrease it (Steinfels et al., 1989). These controversial results may be due to the presence of multiple types of σ receptors as described above. Taken together, these findings suggest that the difference between the ability of (+)-SKF-10,047 and of (+)-pentazocine to ameliorate the conditioned fear stress may be due to their different actions on the dopaminergic neurons involved in a stressful situation.

To clarify this hypothesis, we now attempted to compare the behavioral and neurochemical effects of (+)-SKF-10,047 and (+)-pentazocine on the conditioned fear stress in rats. Dopamine metabolism was assessed in the medial prefrontal cortex, nucleus accumbens and striatum, which are the primary targets of the mesocortical, mesolimbic and nigrostriatal dopaminergic systems, respectively, because these areas are well known to be affected by stressful situations (Herman et al., 1982; Roth et al., 1988; Abercrombie et al., 1989; Rossetti et al., 1993)

2. Materials and methods

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

2.1. Animals

Male Kbl Wistar rats (Oriental Bioservice, Kyoto, Japan) weighing 290–350 g 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. The animals were habituated to handling for 1 week before being used for experiments.

2.2. Drugs

(+)-*N*-Allylnormetazocine hydrochloride ((+)-SKF-10,047, Research Biochemicals Inc., Natick, MA) was dissolved in 0.9% NaCl solution. (+)-Pentazocine (Dainippon Pharmaceutical, Osaka, Japan) was initially dissolved in a minimum volume of 0.1 M HCl and was then diluted with distilled water (the pH of the solutions was adjusted to about 4 with $NaHCO_3$). The dose of each drug refers to the drug form listed above. Other compounds were purchased from commercial sources.

2.3. Schedule for conditioned fear stress

The experiments were carried out in a transparent acrylic rectangular cage (25 × 30 × 47 high cm) equipped with a metal wire floor. The test cage was located in a sound-attenuated room and was illuminated with a 20-W bulb.

Each rat was placed in the test cage and received electric shocks (0.1 Hz, 200 ms, 100 V DC) for 10 min through an insulated stimulator (Nihon Koden, Tokyo, Japan). Each animal received electric shocks in a range of 0.2–0.5 mA, because the current resistance with the animal in the test cage varied between 200 and 500 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 10 min in the test cage was measured with an automatic activity counter (Opto-Varimex, Columbus Instruments, Columbus, 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.

(+)-SKF-10,047 and (+)-pentazocine were administered subcutaneously (s.c.) 20 min before motility was measured in the test trial. Groups receiving no drugs were given an appropriate vehicle (i.e., solution containing 0.1 M HCl).

2.4. Biochemistry

Immediately after the measurement of motility, each rat was decapitated. The brain was rapidly removed and the

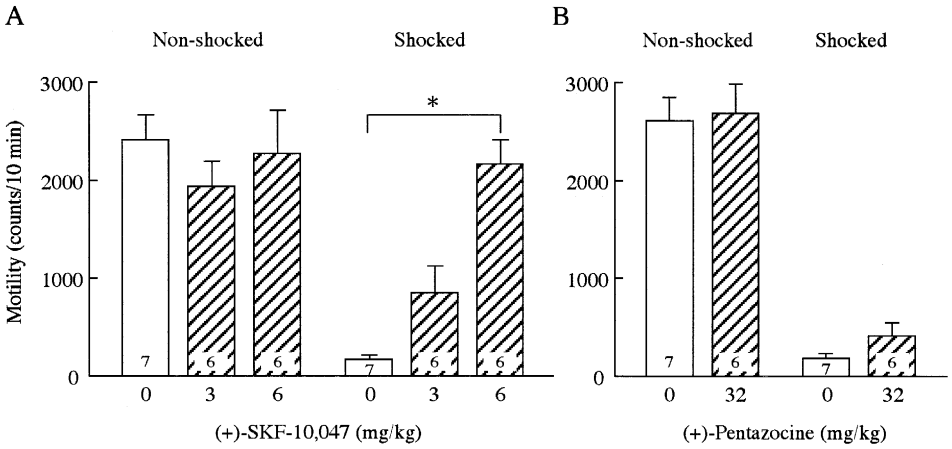

Fig. 1. Effects of (+)-SKF-10,047 (A) and (+)-pentazocine (B) on conditioned fear stress in rats. (+)-SKF-10,047 and (+)-pentazocine were administered s.c. 20 min before motility was measured. Values are means ± S.E.M. for the number of animals shown in each column. Results with ANOVA in (+)-SKF-10,047 group were: non-shocked group, $H(2) = 1.336$ ($P > 0.05$); shocked group, $H(2) = 14.968$ ($P < 0.01$). * $P < 0.01$ compared to the vehicle-treated, shocked group (Dunn-type test).

medial prefrontal cortex, nucleus accumbens and striatum were dissected out on an ice-cold plate after 2-mm thick coronal slices had been made, according to the method described by Herman et al. (1982). The dissected tissues were rapidly frozen and stored at −80°C until assayed.

The contents of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the three brain regions were determined using high-performance liquid chromatography with electrochemical detection according to a modification of the method of Nitta et al. (1992). Each frozen tissue sample was weighed and homogenized with an ultrasonic cell disruptor (160 W, Model UCD-200TM, Cosmo Bio, Tokyo, Japan) in 80 μl of 0.2 M perchloric acid for the medial prefrontal cortex and in

Table 1
Effects of (+)-SKF-10,047 (SKF) and (+)-pentazocine (PTZ) on the content of dopamine, DOPAC and HVA in the nucleus accumbens (NAC, A), medial prefrontal cortex (MPFC, B) and striatum (STR, C) of rats showing conditioned fear stress

Region	Treatment (mg/kg)	Dopamine		DOPAC		HVA	
		Non-shocked	Shocked	Non-shocked	Shocked	Non-shocked	Shocked
(A) NAC	Vehicle	8069.5 ± 909.9	7282.8 ± 827.4	2141.6 ± 243.2	1609.5 ± 184.1	895.3 ± 58.6	646.0 ± 65.7 [a]
	SKF (3)	8352.4 ± 300.6	7018.7 ± 457.9	2326.4 ± 144.5	1913.3 ± 128.4	942.4 ± 66.7	745.8 ± 64.1
	SKF (6)	8449.0 ± 482.6	6309.5 ± 480.7	2508.8 ± 216.9	2220.7 ± 232.8	1144.0 ± 125.9	951.6 ± 91.7 [b]
	Vehicle	7095.2 ± 990.4	6890.8 ± 756.1	2001.5 ± 255.9	1611.1 ± 201.8	807.8 ± 60.1	623.2 ± 56.5
	PTZ (32)	6991.8 ± 155.9	6798.4 ± 595.9	2357.8 ± 77.9	1884.0 ± 207.4	1108.8 ± 56.2 [c]	736.0 ± 66.2
(B) MPFC	Vehicle	93.2 ± 13.5	74.8 ± 8.0	44.9 ± 4.9	52.7 ± 4.6	125.2 ± 11.0	139.2 ± 5.8
	SKF (3)	82.0 ± 7.9	83.3 ± 8.3	49.2 ± 6.7	72.5 ± 7.1	119.1 ± 9.0	133.6 ± 16.2
	SKF (6)	89.6 ± 10.0	80.7 ± 9.6	69.5 ± 7.3 [b]	68.1 ± 7.3	152.9 ± 11.6	148.6 ± 7.5
	Vehicle	91.6 ± 9.9	76.6 ± 6.1	44.5 ± 4.5	58.4 ± 4.7	116.0 ± 12.9	134.8 ± 15.5
	PTZ (32)	71.5 ± 7.4	73.9 ± 5.8	55.4 ± 5.3	68.8 ± 4.6	125.3 ± 11.1	149.9 ± 15.0
(C) STR	Vehicle	10946.4 ± 932.0	11652.9 ± 705.1	1638.5 ± 136.4	1812.3 ± 98.8	1090.4 ± 124.8	1014.6 ± 71.2
	SKF (3)	11427.3 ± 487.1	11178.1 ± 726.6	1634.7 ± 70.1	1800.0 ± 187.3	979.4 ± 68.3	988.5 ± 121.4
	SKF (6)	11838.5 ± 420.7	10857.1 ± 711.4	1849.2 ± 94.1	1858.2 ± 178.1	1060.9 ± 68.0	1032.9 ± 114.3
	Vehicle	10435.8 ± 372.1	11335.4 ± 622.6	1686.8 ± 43.1	1835.6 ± 91.4	992.0 ± 39.5	1010.0 ± 85.2
	PTZ (32)	10520.2 ± 227.7	11143.3 ± 848.8	2405.5 ± 36.5 [c]	2619.7 ± 132.7 [c]	1441.2 ± 53.3 [c]	1375.9 ± 45.1 [c]

Values are expressed as ng/g wet weight and are the means ± S.E.M. of 7 (vehicle-treated groups) or 6 (SKF- and PTZ-treated groups) rats, which showed behavioral changes as described in Fig. 1 SKF and PTZ were administered s.c. 20 min before motility was measured. The rats were decapitated immediately after the measurement of motility for 10 min, and the content of dopamine and its metabolites (DOPAC and HVA) in the discrete brain regions was determined. Results with ANOVA on the content of dopamine, DOPAC and HVA in the SKF group were: (A) Non-shocked group, $F(2,16) = 0.092$ ($P > 0.05$), 0.772 ($P > 0.05$) and 2.323 ($P > 0.05$), respectively; shocked group, $F(2,16) = 0.610$ ($P > 0.05$), 2.730 ($P > 0.05$) and 4.437 ($P < 0.05$), respectively. (B) Non-shocked group, $F(2,16) = 0.265$ ($P > 0.05$), 4.365 ($P < 0.05$) and 2.736 ($P > 0.05$), respectively; shocked group, $F(2,16) = 0.264$ ($P > 0.05$), 2.646 ($P > 0.05$) and 0.435 ($P > 0.05$), respectively. (C) Non-shocked group, $F(2,16) = 0.424$ ($P > 0.05$), 1.241 ($P > 0.05$) and 0.357 ($P > 0.05$), respectively; shocked group, $F(2,16) = 0.323$ ($P > 0.05$), 0.04 ($P > 0.05$) and 0.046 ($P > 0.05$), respectively. [a] $P < 0.05$ compared to the corresponding non-shocked group (Student's *t*-test); [b] $P < 0.05$, [c] $P < 0.01$ compared to the corresponding vehicle-treated group (Dunnett's test or Student's *t*-test).

350 μl for the nucleus accumbens and striatum, containing isoproterenol (internal standard). The homogenate was placed in ice for 30 min and then centrifuged at 18 500 × *g* for 15 min at 4°C. The supernatant was mixed with 1 M sodium acetate to adjust the pH to 3.0 and then injected into a liquid chromatography system equipped with a reversed-phase ODS column (4.6 × 150 mm, Eicompak MA-5ODS, Eicom, Kyoto, Japan) and an electrochemical detector (Model ECD-100, Eicom). The column temperature was maintained at 25°C and the detector potential was set at +750 mV. The mobile phase was 0.1 M citric acid and 0.1 M sodium acetate, pH 3.9, containing 11–14% methanol, 200 mg /l sodium-l-octanesulfonate and 5 mg/l EDTA; the flow rate was 1 ml/min.

2.5. Statistical analysis

Statistical significance of behavioral data was determined by means of the Kruskal-Wallis test (non-parametric analysis of variance (ANOVA)) followed by a Dunn-type non-parametric test; biochemical data was evaluated by one-way analysis of variance (parametric ANOVA) followed by Dunnett's multiple range test. Comparison of two sample means was performed with a Mann-Whitney *U*-test for behavioral data and Student's *t*-test for biochemical data. *P*-values less than 0.05 were taken to indicate statistically significant differences.

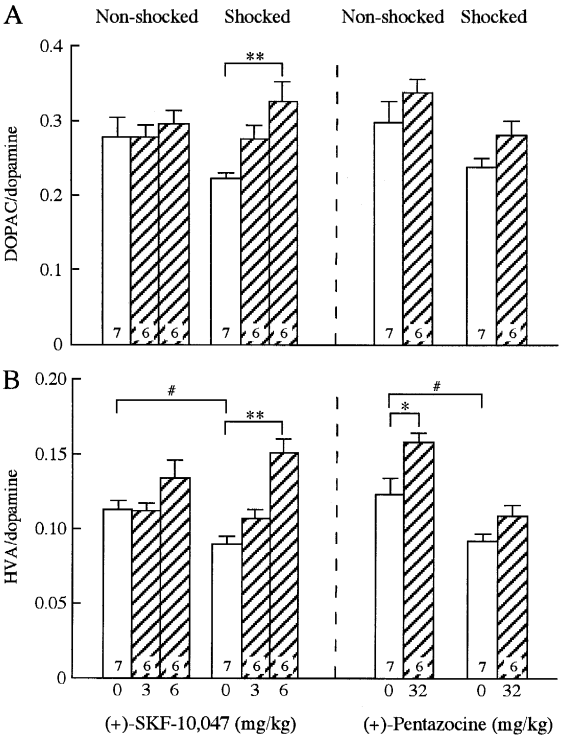

Fig. 2. Effects of (+)-SKF-10,047 and (+)-pentazocine on the ratios of DOPAC/dopamine (A) and HVA/dopamine (B) in the nucleus accumbens of rats showing conditioned fear stress. Values are means ± S.E.M. for the number of animals shown in each column. Other details are as shown in the legend of Table 1. Results with ANOVA in (+)-SKF-10,047 group were: (A) Non-shocked group, $F(2,16) = 0.296$ ($P > 0.05$); shocked group, $F(2,16) = 8.331$ ($P < 0.01$). (B) Non-shocked group, $F(2,16) = 2.470$ ($P > 0.05$); shocked group, $F(2,16) = 21.628$ ($P < 0.01$). $^{\#}$ $P < 0.01$ compared to the corresponding non-shocked group (Student's *t*-test), * $P < 0.05$, ** $P < 0.01$ compared to the corresponding vehicle-treated group (Dunnett's test or Student's *t*-test).

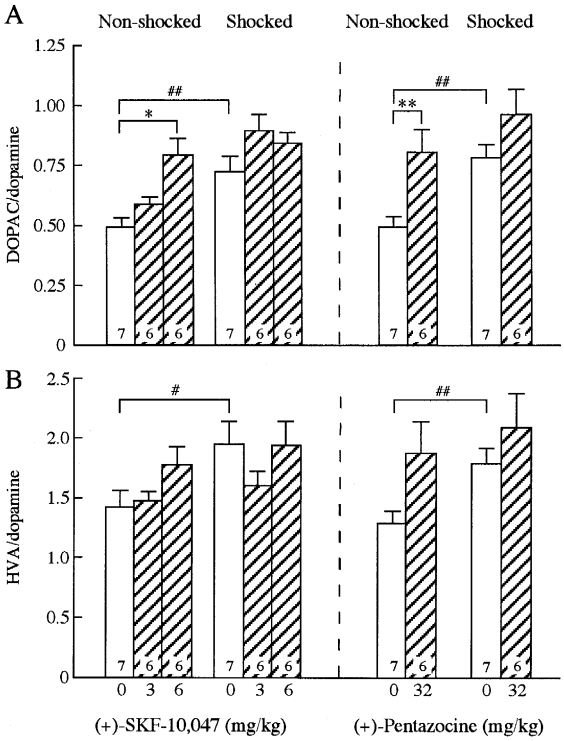

Fig. 3. Effects of (+)-SKF-10,047 and (+)-pentazocine on the ratios of DOPAC/dopamine (A) and HVA/dopamine (B) in the medial prefrontal cortex of rats showing conditioned fear stress. Values are means ± S.E.M. for the number of animals shown in each column. Other details are as shown in the legend Table 1. Results with ANOVA in (+)-SKF-10,047 group were: (A) Non-shocked group, $F(2,16) = 11.693$ ($P < 0.01$); shocked group, $F(2,16) = 1.975$ ($P > 0.05$). (B) Non-shocked group, $F(2,16) = 2.136$ ($P > 0.05$); shocked group, $F(2,16) = 1.293$ ($P > 0.05$). $^{\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$ compared to the corresponding non-shocked group (Student's *t*-test), ** $P < 0.01$ compared to the corresponding vehicle-treated group (Dunnett's test or Student's *t*-test).

3. Results

3.1. Effects of (+)-SKF-10,047 and (+)-pentazocine on conditioned fear stress

The shocked rats treated with vehicle (shocked group) exhibited a marked suppression of motility; the shocked group showed 7% of the motility exhibited by the non-shocked group when returned to the same apparatus in which they had been given an electric shock, in agreement with our previous results (Fig. 1) (Kameyama and Nagasaka, 1982a, 1983). The shocked rats mostly froze on the floor and crouched, while the non-shocked rats showed typical exploratory behaviors such as ambulation, sniffing and rearing.

Fig. 1 shows the effects of (+)-SKF-10,047 (A) and (+)-pentazocine (B) on motor suppression in the shocked group. Doses for both ligands were selected on the basis of our previous study with mice (Kamei et al., 1996a). (+)-SKF-10,047 (3 and 6 mg/kg) dose-dependently attenuated the motor suppression of the shocked group without changing motility in the non-shocked group; a significant effect was observed at a dose of 6 mg/kg which restored the extent of motor suppression to the non-shocked level (Fig. 1A). The shocked rats given this drug, like the non-shocked rats, exhibited exploratory behavior. On the other hand, (+)-pentazocine, at a dose of 32 mg/kg which is the highest dose tested in mice (Kamei et al., 1996a), had little effect on motility in either the non-shocked or the shocked groups (Fig. 1B).

3.2. Effects of (+)-SKF-10,047 and (+)-pentazocine on dopamine metabolism in the brain regions of rats showing the conditioned fear stress

The contents of dopamine, DOPAC and HVA in the discrete brain regions in the non-shocked and shocked groups used in the behavioral experiments are summarized in Table 1. The ratios of DOPAC/dopamine and HVA/dopamine (which are used as indices of dopamine turnover) in the nucleus accumbens, medial prefrontal cortex, and striatum in both groups are also shown in Figs. 2–4, respectively. Significant decreases in the HVA content and the HVA/dopamine ratio in the nucleus accumbens (Table 1 and Fig. 2, respectively) and significant increases in the DOPAC/dopamine and HVA/dopamine ratios in the medial prefrontal cortex (Fig. 3) were observed in the vehicle-treated, shocked group, compared with those in the vehicle-treated, non-shocked group. However, there were no significant changes in dopamine metabolism in the striatum (Table 1 and Fig. 4) when the vehicle-treated, non-shocked and shocked groups were compared.

In the nucleus accumbens, (+)-SKF-10,047 (3 and 6 mg/kg) dose-dependently restored the HVA content and the HVA/dopamine ratio in the shocked group to their control level. A significant effect was observed at a dose of 6 mg/kg, with little effect on dopamine metabolism in the non-shocked group (Table 1 and Fig. 2). On the other hand, (+)-pentazocine (32 mg/kg) did not significantly affect the decreased dopamine metabolism in the shocked group, although it produced significant increases in the HVA content and the HVA/ dopamine ratio in the non-shocked group (Table 1 and Fig. 2).

In the medial prefrontal cortex, neither (+)-SKF-10,047 (3 and 6 mg/kg) nor (+)-pentazocine (32 mg/kg) had any significant effect on the increases in the DOPAC/dopamine and HVA/dopamine ratios in the shocked group (Fig. 3). In the non-shocked group, both ligands induced a significant increase in dopamine metabolism; (+)-SKF-10,047 (6 mg/kg) increased the DOPAC content and the DOPAC/dopamine ratio, and

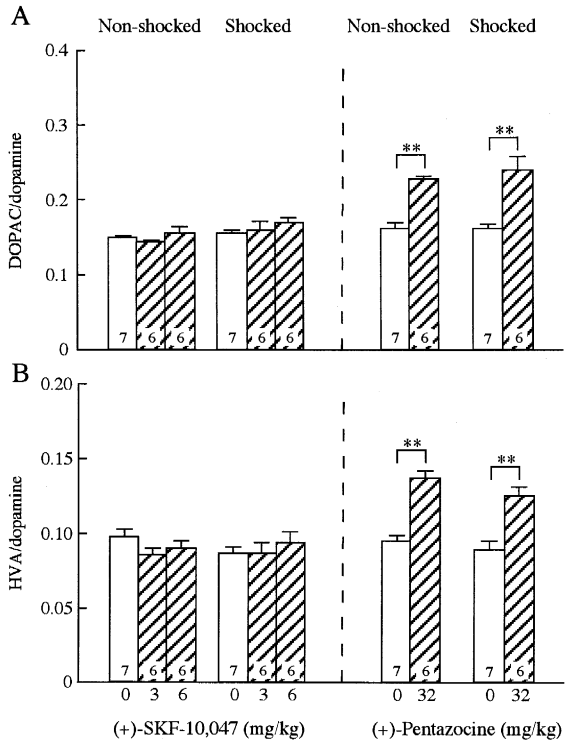

Fig. 4. Effects of (+)-SKF-10,047 and (+)-pentazocine on the ratios of DOPAC/dopamine (A) and HVA/dopamine (B) in the striatum of rats showing conditioned fear stress. Values are means ± S.E.M. for the number of animals shown in each column. Other details are as shown in the legend of Table 1. Results with ANOVA in (+)-SKF-10,047 group were: (A) Non-shocked group, $F(2,16) = 2.117$ ($P > 0.05$); shocked group, $F(2,16) = 0.642$ ($P > 0.05$). (B) Non-shocked group, $F(2,16) = 1.330$ ($P > 0.05$); shocked group, $F(2,16) = 0.457$ ($P > 0.05$). ** $P < 0.01$ compared to the corrsponding vehicle-treated group (Student's *t*-test).

(+)-pentazocine (32 mg/kg) increased the DOPAC/dopamine ratio (Table 1 and Fig. 3).

In the striatum, (+)-SKF-10,047 (3 and 6 mg/kg) had no effect on dopamine metabolism in either the non-shocked or the shocked groups, while (+)-pentazocine (32 mg/kg) significantly increased the DOPAC and HVA contents and the DOPAC/dopamine and HVA/dopamine ratios in both groups (Table 1 and Fig. 4).

4. Discussion

As described in Section 1, rats exhibited a marked suppression of motility (low motor activity) when returned to the apparatus in which they had been given an electric shock. Freezing and crouching were also observed. We have defined this motor suppression as a conditioned fear stress. Our previous findings from a behavioral pharmacological study have suggested that dysfunction of dopaminergic neuronal systems is involved in the conditioned fear stress: this stress response 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). In the present study, different changes in dopamine neuronal activity in discrete brain areas of rats showing the conditioned fear stress response were observed. Dopamine turnover, as measured by the ratios of DOPAC/dopamine and HVA/dopamine, was decreased in the nucleus accumbens and was increased in the medial prefrontal cortex, but remained unchanged in the striatum. Thus, the decrease in dopamine turnover in the nucleus accumbens appears to support further our hypothesis of dysfunction of dopaminergic neuronal systems in the stress response. However, a study using in vivo brain microdialysis showed that the extracellular dopamine levels in the striatum of rats showing conditioned fear stress are lower than those in the non-stressed rats although those in other brain areas were not determined (Katoh et al., 1996). This discrepancy may be due to the differences in measurement procedures, although we cannot provide a plausible explanation. In addition, our observations differed from a report by Herman et al. (1982) in that the conditioned fear test results in increased levels of DOPAC in the medial prefrontal cortex, but fails to affect dopamine metabolism in any other brain region, including the nucleus accumbens and striatum. The reason for the discrepancy between our results and those of Herman et al. (1982) is not clear, but may be attributable to differences in the intensity and duration of the footshock used in each experimental design (0.2–0.5 mA at 10-s intervals for 10 min versus 1.5 mA at 8-s intervals for 20 min, respectively). Alternatively, our results may be consistent with the hypothesis that the mesocortical dopaminergic neurons exert an inhibitory role on dopamine transmission in the nucleus accumbens (Pycook et al., 1980; Louilot et al., 1989). In fact, when dopamine turnover in the medial prefrontal cortex was increased in the stressed group, the opposite effect was observed in the nucleus accumbens. Our preliminary studies indicated that there was no change in dopamine turnover rate in the three brain regions studied between non-shocked and shocked rats which were never placed in the apparatus where they had previously received an electric footshock (data not shown). Therefore, it is conceivable that the changes in dopaminergic neuronal activity in the medial prefrontal cortex and nucleus accumbens described above are related specifically to the conditioned fear stress response.

Further, we examined the effect of (+)-SKF-10,047 on the motor suppression and changes in dopamine metabolism induced by stress, comparing it with the effect of (+)-pentazocine. The stress-induced motor suppression was dose-dependently attenuated by (+)-SKF-10,047, but not by (+)-pentazocine even at a higher dose. These behavioral results were consistent with our previous results in mice (Kamei et al., 1996a). Additionally, (+)-SKF-10,047 dose-dependently restored the decreased dopamine turnover in the nucleus accumbens in the stressed group to the control level, as indicated by the increase in HVA/dopamine ratio, whereas (+)-pentazocine failed to do so. On the other hand, the increased dopamine turnover in the medial prefrontal cortex in the stressed group was not affected by either ligand. Thus, the neurochemical effect of (+)-SKF-10,047 observed in the nucleus accumbens appears to be closely related to its attenuating effect on the conditioned fear stress. In the non-stressed group, a higher dose of (+)-pentazocine resulted in an increased dopamine turnover in the all regions determined, in agreement with other reports (Iyengar et al., 1990; Gudelsky, 1995), while (+)-SKF-10,047 at the doses used here increased dopamine turnover only in the medial prefrontal cortex. Therefore, it is considered that (+)-SKF-10,047 shows a predominant effect on the neurochemical change in the nucleus accumbens involved in the stress response. These findings suggest that (+)-SKF-10,047 prevents the stress-induced reduction in dopaminergic function in the nucleus accumbens to ameliorate the conditioned fear stress response, while (+)-pentazocine has little effect on the stress-induced motor suppression and changes in dopaminergic function. Therefore, the dysfunction in the dopaminergic neurons in the nucleus accumbens may be responsible for the development of conditioned fear stress.

The differential effect of (+)-SKF-10,047 and (+)-pentazocine on the conditioned fear stress could not be explained by their selectivity for the σ_1 receptor subtype, since (+)-pentazocine, as well as (+)-SKF-10,047, has greater selectivity for the σ_1 receptor than for the σ_2 receptor (Itzhak et al., 1991). Recent evidence from binding studies with an anticonvulsant, phenytoin, has identified different populations of σ_1 binding sites (McCann and Su, 1992; DeHaven-Hudkins et al., 1993). The binding affinity of (+)-SKF-10,047 and dextromethorphan for 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 (i.e., phenytoin-sensitive and phenytoin-insensitive sites). We have found that the ameliorating effects of (+)-SKF-10,047 and dextromethorphan on conditioned fear stress in mice are enhanced by their combination with phenytoin, whereas the effects of (+)-pentazocine and DTG are not (Kamei et al., 1996a). The previous behavioral findings appear to be 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 are mediated by phenytoin-regulated σ_1 sites. Furthermore, we have shown that phenytoin-regulated σ_1 sites are closely connected to dopaminergic neuronal systems involved in the stress response. The ameliorating effects of (+)-SKF-10,047 or dextromethorphan in combination with phenytoin on the conditioned fear stress are blocked by either SCH 23390, a dopamine D_1 receptor antagonist, or (−)-sulpiride, a dopamine D_2 receptor antagonist, as well as by selective σ receptor antagonists

such as NE-100 and BMY-14802, and they are also attenuated by 6-hydroxydopamine-induced lesions of dopaminergic neurons (Kamei et al., 1996b). Taken together with our previous findings, the present results suggest that (+)-SKF-10,047, unlike (+)-pentazocine, elevates dopaminergic neurotransmission in the nucleus accumbens through the activation of phenytoin-regulated σ_1 sites 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 the mesolimbic dopaminergic systems in conditioned fear stress.

Another possibility is that the effect of (+)-SKF-10,047 on the stress response occurred through the phencyclidine binding sites of the NMDA receptor–channel complex, which is known to interact with dopaminergic neuronal systems (Hiramatsu et al., 1989; Rao et al., 1990), since (+)-SKF-10,047 binds to phencyclidine sites, although this affinity is low (Gundlach et al., 1985; Weissman et al., 1990). In this regard, we have reported that in mice, the non-competitive NMDA receptor antagonists, phencyclidine and dizocilpine (Wong et al., 1986), attenuate the conditioned fear stress only at high doses that produce a marked hyperlocomotion in the non-stressed group, whereas (+)-SKF-10,047 shows a predominant effect on the conditioned fear stress over the same dose range as used in the present study (Kamei et al., 1996a). Moreover, NE-100 and BMY-14802 antagonize the effect of (+)-SKF-10,047 on the stress response, but fail to antagonize the effects of phencyclidine and dizocilpine on the stress response (Kamei et al., 1996a). In addition, unlike those of (+)-SKF-10,047, the effects of phencyclidine and dizocilpine on the stress response are not enhanced by combination with phenytoin (Kamei et al., 1996a). Thus, it is unlikely that the ameliorating effect of (+)-SKF-10,047 on the conditioned fear stress is not mediated by phencyclidine binding sites.

We have also reported that the conditioned fear stress can be attenuated by treatment with antidepressants such as imipramine and desipramine (Kameyama et al., 1985), but not by anxiolytics such as diazepam and chlordiazepoxide (Kameyama and Nagasaka, 1982a; Nagasaka and Kameyama, 1983), suggesting that this stress response is associated with depressive states. Rossetti et al. (1993) have indicated that the dysfunction of the mesolimbic dopaminergic systems may be correlated to depressive states, since chronic pretreatment with imipramine prevents a decrease in the dopamine release from mesolimbic dopaminergic neurons induced by the forced swimming stress. Likewise, in the present study, the ameliorating effect of (+)-SKF-10,047 on the conditioned fear stress was accompanied by a reversal of the dysfunction in mesolimbic dopaminergic neurons. Therefore, we speculate that (+)-SKF-10,047 may have an antidepressant property.

In conclusion, results of the present behavioral and biochemical experiments using rats suggest that the functional reduction in the mesolimbic dopaminergic neurons is involved in the expression of conditioned fear stress, and that (+)-SKF-10,047, unlike (+)-pentazocine, ameliorates this stress response by reversing the dysfunction in mesolimbic dopaminergic neurons. It is therefore possible that (+)-SKF-10,047 acts on a sub-population of σ_1 sites (probably phenytoin-regulated σ_1 site), which are closely connected to the dopaminergic neurons involved in the stress response, although further studies are needed to verify this point.

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