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Activation of both dopamine D_1 and D_2 receptors necessary for amelioration of conditioned fear stress

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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. This psychological stress-induced motor suppression, known as conditioned fear stress, was dose dependently attenuated by apomorphine, a non-selective dopamine receptor agonist. 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, also synergistically attenuated the conditioned fear stress although, alone, neither SKF 38393 nor quinpirole did so at the doses used. The effects of apomorphine and of the coadministration of SKF 38393 and quinpirole on the conditioned fear stress were completely 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 by the dopamine D_2 receptor antagonist, (−)-sulpiride. These results suggest that a dysfunction in the dopaminergic neuronal system is responsible for the conditioned fear stress, and that the activation of both dopamine D_1 and D_2 receptors is necessary to attenuate this stress-induced motor suppression.

Keywords: Conditioned fear stress; Conditioned suppression; Dopamine D_1 receptor; Dopamine D_2 receptor; Motor suppression

1. Introduction

When rats or mice are re-placed in the same environment in which they previously received an electric footshock, they exhibit a marked suppression of motility (Kameyama and Nagasaka, 1982a,b). This motor suppression has been considered to be a conditioned emotional response (conditioned suppression) to an environment associated with previous footshock (Kameyama and Nagasaka, 1982a,b). The same type of stress-induced motor suppression was defined as conditioned fear stress by Fanselow (1980). Our previous findings indicated that dysfunction of dopaminergic neuronal systems is involved in conditioned fear stress. For instance, the stress-induced suppression of motility was attenuated by dopamine receptor agonists such as apomorphine or methamphetamine (Kameyama and Nagasaka, 1982a, 1983; Nagasaka and Kameyama, 1983), whereas it was potentiated by the dopamine receptor antagonist, haloperidol (Kameyama and Nagasaka, 1983). Furthermore, a decrease in striatal dopamine turnover was observed in mice showing conditioned fear stress (Nabeshima et al., 1986).

Dopamine receptors were originally subclassified into D_1 and D_2 subtypes (Kebabian and Calne, 1979; Stoof and Kebabian, 1981). Recently, behavioral studies have demonstrated that a functional interaction exists between the two subtypes of dopamine receptors (e.g. review by Waddington, 1989). However, the interaction between dopamine D_1 and D_2 receptor subtypes in the development of the conditioned fear stress is not clear.

Therefore, in the present study we examined the role of dopamine D_1 and D_2 receptor subtypes in conditioned fear stress, using selective dopamine D_1 and D_2 receptor agonists.

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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 Co., Shizuoka, Japan) at 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: SKF 38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1-H-3-benzazepine) hydrochloride (Research Biochemicals, USA), a dopamine D_1 receptor agonist; 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; quinpirole hydrochloride (Research Biochemicals, USA), a dopamine D_2 receptor agonist; (−)-sulpiride (Sigma Chemical Co., USA), a dopamine D_2 receptor antagonist, and apomorphine hydrochloride (Sigma Chemical Co., USA), a mixed dopamine D_1/D_2 receptor agonist.

SKF 38393, quinpirole and SCH 23390 were dissolved in 0.9% NaCl solution. Apomorphine was dissolved in 0.9% NaCl solution containing 0.1% ascorbic acid. (−)-Sulpiride was initially dissolved in a minimum volume of 0.1 N 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.

2.3. Schedule for conditioned fear stress

The experiments were carried out as previously described with 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 insulated 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 then carried out 24 h after shock treatment; the animals were again placed into the same test cage, but no electric footshocks were given. The spontaneous motility of the animal was determined for 6 min in the same test cage surrounded by an automatic activity counter (Opto-Varimex, Columbus Instruments, Ohio, USA) equipped with photosensors. The non-shocked control group was prepared exactly the same way except for the absence of the electric footshock.

All test drugs were administered before measuring motility in the test trial; apomorphine was adminis-

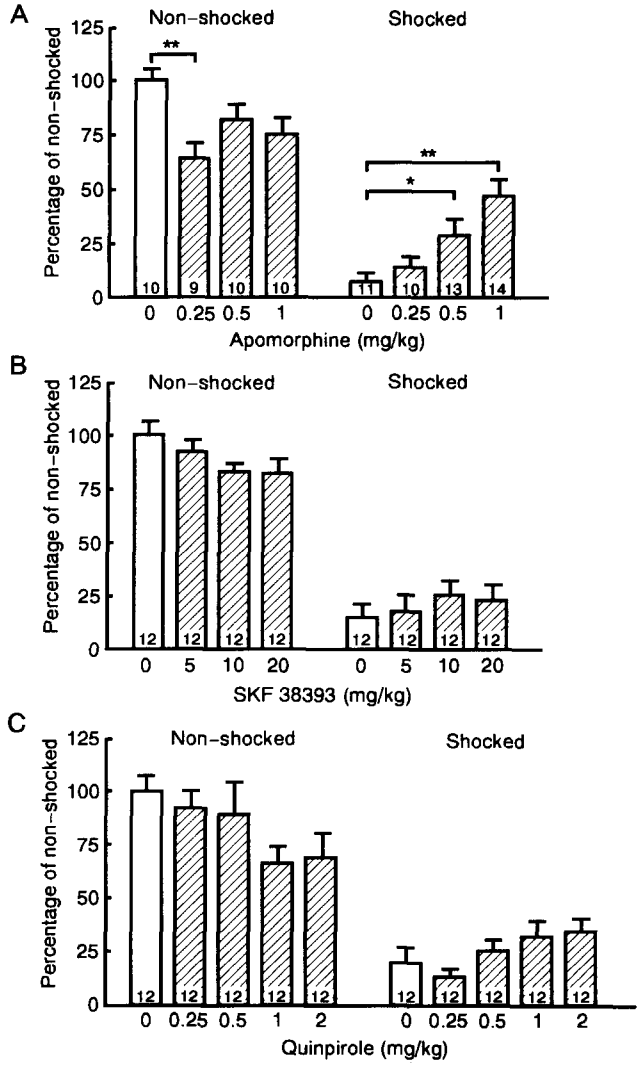

Fig. 1. Effects of apomorphine (A), SKF 38393 (B) and quinpirole (C) on conditioned fear stress in mice. Apomorphine was administered s.c. 20 min before measuring motility. SKF 38393 and quinpirole were administered i.p. and s.c., respectively, 30 min before measuring motility. Motility is expressed as a percentage of that of the vehicle-treated, non-shocked group (motility: (A) 1538.6 ± 77.9, (B) 1953.8 ± 115.9, (C) 1932.3 ± 133.3). Values are the mean ± S.E.M. for the number of animals shown in each column. ANOVA as follows: (A) non-shocked group, $H(3) = 13.103$ ($P < 0.01$); shocked group, $H(3) = 22.719$ ($P < 0.01$), (B) non-shocked group, $H(3) = 6.093$ ($P > 0.05$); shocked group, $H(3) = 3.146$ ($P > 0.05$), (C) non-shocked group, $H(4) = 10.534$ ($P > 0.05$); shocked group, $H(4) = 11.475$ ($P > 0.05$). $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the corresponding vehicle-treated group (Dunn-type test).

tered subcutaneously (s.c.) 20 min before, SKF 38393 and quinpirole were administered intraperitoneally (i.p.) and s.c., respectively, 30 min before, and SCH 23390 and (−)-sulpiride were administered s.c. 40 min and i.p. 90 min, respectively, before the trial. Groups receiving no drugs were given an appropriate vehicle, i.e. solutions containing 0.1 N HCl or 0.1% ascorbic acid.

2.4. Statistical analysis

Statistical significance was determined by means of 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.

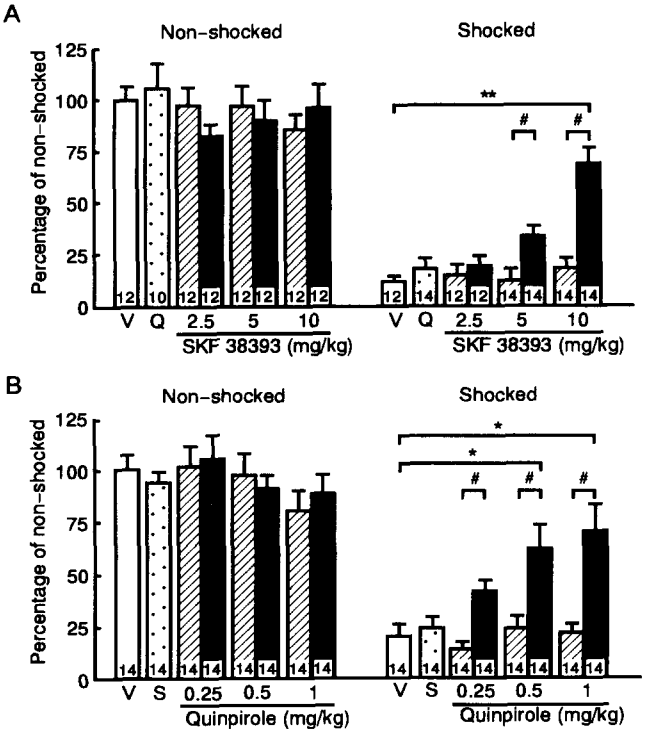

Fig. 2. Effects of SKF 38393 administered in combination with quinpirole on conditioned fear stress in mice. The schedule for dosage, and other details, are as shown in Fig. 1. Motility in the vehicle-treated, non-shocked groups: (A) 1802.1 ± 114.7, (B) 1682.0 ± 119.5. ANOVA as follows: (A) non-shocked group, $H(7) = 7.219$ ($P > 0.05$); shocked group, $H(7) = 41.250$ ($P < 0.01$), (B) non-shocked group, $H(7) = 7.430$ ($P > 0.05$); shocked group, $H(7) = 38.641$ ($P < 0.01$). $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the vehicle-treated, shocked group (Dunn-type test), $^{\#}P < 0.01$ compared to the corresponding animals treated with SKF 38393 or quinpirole alone (Mann-Whitney *U*-test). (A) Open columns: vehicle (V); stippled columns: quinpirole alone (Q; 1 mg/kg); hatched columns: SKF 38393 alone; black columns: SKF 38393 + quinpirole. (B) Open column: vehicle (V); stippled columns: SKF 38393 alone (S; 10 mg/kg); hatched columns: quinpirole alone; black columns: quinpirole + SKF 38393.

Table 1
Effects of SCH 23390 (SCH) and (−)-sulpiride (SUL) on the motor response to apomorphine (APO) or coadministration of SKF 38393 (SKF) and quinpirole (QUI) in mice showing conditioned fear stress

Treatment (mg/kg)	Motility (percent of control value)			
	Non-shocked	*n*	Shocked	*n*
(A)				
Vehicle	100.0 ± 6.9	10	16.2 ± 6.4	10
SCH (0.025)	83.1 ± 4.9	10	7.2 ± 2.2	10
APO (1)	82.8 ± 7.6	10	64.6 ± 12.3 [a]	10
+ SCH (0.0125)	78.2 ± 12.1	10	38.2 ± 8.0	11
+ SCH (0.025)	69.0 ± 7.2	11	17.3 ± 5.8 [b]	10
(B)				
Vehicle	100.0 ± 5.5	10	17.2 ± 5.9	10
SUL (20)	92.0 ± 8.6	10	21.6 ± 6.5	11
APO (1)	83.3 ± 8.4	10	69.2 ± 9.5 [a]	12
+ SUL (10)	73.7 ± 8.6	10	45.1 ± 8.6	10
+ SUL (20)	71.4 ± 11.0	11	21.0 ± 2.8 [c]	11
(C)				
Vehicle	100.0 ± 8.4	10	16.8 ± 5.6	13
SCH (0.025)	84.3 ± 9.3	10	12.4 ± 4.4	12
(SKF (10) + QUI (1))	91.1 ± 9.0	10	55.0 ± 6.0 [a]	14
+ SCH (0.0125)	91.1 ± 14.4	11	42.3 ± 10.7	14
+ SCH (0.025)	71.5 ± 12.4	11	21.7 ± 4.1 [d]	14
(D)				
Vehicle	100.0 ± 8.2	10	14.8 ± 4.6	12
SUL (20)	80.0 ± 9.9	11	22.6 ± 6.6	11
(SKF (10) + QUI (1))	78.2 ± 8.8	10	49.4 ± 5.9 [a]	13
+ SUL (10)	73.6 ± 4.8	10	28.2 ± 5.2	11
+ SUL (20)	65.2 ± 6.1	11	11.9 ± 3.2 [e]	11

SCH and SUL were administered 40 (s.c.) and 90 (i.p.) min, respectively, before measurement of motility. Other details are as shown in Fig. 1. Motility in the vehicle-treated, non-shocked group: (A) 1770.4 ± 121.8, (B) 1676.2 ± 92.2, (C) 1751.2 ± 146.5, (D) 1535.5 ± 126.1. Each value is the mean ± S.E.M. obtained from the number of animals shown by *n*. ANOVA as follows: (A) non-shocked group, $H(4) = 8.648$ ($P > 0.05$); shocked group, $H(4) = 24.487$ ($P < 0.01$), (B) non-shocked group, $H(4) = 10.222$ ($P < 0.05$); shocked group, $H(4) = 24.244$ ($P < 0.01$), (C) non-shocked group, $H(4) = 4.665$ ($P > 0.05$); shocked group, $H(4) = 26.286$ ($P < 0.01$), (D) non-shocked group, $H(4) = 8.437$ ($P > 0.05$); shocked group, $H(4) = 21.530$ ($P < 0.01$).
[a] $P < 0.01$ compared to the corresponding vehicle-treated, shocked group, [b] $P < 0.05$, [c] $P < 0.01$ compared to the APO-treated, shocked group, [d] $P < 0.05$, [e] $P < 0.01$ compared to the (SKF + QUI)-treated, shocked group (Dunn-type test).

3. Results

3.1. Effects of apomorphine, SKF 38393 and quinpirole on conditioned fear stress

As shown in Fig. 1 and 2 (shocked group), mice exhibited marked suppression of motility (conditioned fear stress: 7.4–20.2% of the non-shocked groups) when returned to the same apparatus in which they had been given an electric shock.

In agreement with previous results (Nagasaka and Kameyama, 1983), the mixed dopamine D_1/D_2 receptor agonist, apomorphine (0.5 and 1 mg/kg), significantly attenuated the conditioned suppression of motility in a dose-dependent manner (Fig. 1A). Conversely, at a lower dose (0.25 mg/kg), apomorphine significantly decreased motility in the unshocked group (Fig. 1A).

Unlike apomorphine, neither the dopamine D_1 receptor agonist, SKF 38393 (5–20 mg/kg), nor the dopamine D_2 receptor agonist, quinpirole (0.25–2 mg/kg), affected conditioned fear stress (Fig. 1B and C). However, when SKF 38393 and quinpirole were administered in combination, the conditioned fear stress was attenuated synergistically. When quinpirole was coadministered at a dose of 1 mg/kg, SKF 38393 dose dependently attenuated the conditioned fear stress; a significant effect was observed at a dose of 10 mg/kg (Fig. 2A). Conversely, when SKF 38393 was coadministered at a dose of 10 mg/kg, quinpirole (0.5 and 1 mg/kg) significantly attenuated the conditioned fear stress in a dose-dependent manner (Fig. 2B). Coadministration of SKF 38393 and quinpirole, however, produced no change in the motility of the non-shocked group (Fig. 2).

3.2. Antagonistic effects of the dopamine receptor antagonists, SCH 23390 and (−)-sulpiride, on the dopamine receptor agonist-induced amelioration of conditioned fear stress

As shown in Table 1A and B, SCH 23390 (0.025 mg/kg), a dopamine D_1 receptor antagonist, and (−)-sulpiride (20 mg/kg), a dopamine D_2 receptor antagonist, completely blocked the apomorphine (1 mg/kg)-induced amelioration of conditioned fear stress. The ameliorative effect of the combined administration of SKF 38393 (10 mg/kg) and quinpirole (1 mg/kg) on conditioned fear stress was also antagonized by SCH 23390 (0.025 mg/kg) and (−)-sulpiride (20 mg/kg) (Table 1C and D).

SCH 23390 (0.025 mg/kg) and (−)-sulpiride (20 mg/kg) themselves had little effect on motility in both the non-shocked and shocked groups (Table 1).

4. Discussion

In the present study, neither SKF 38393, a dopamine D_1 receptor agonist, nor quinpirole, a dopamine D_2 receptor agonist, when administered alone, affected conditioned fear stress. However, coadministration of these agonists caused significant attenuation of the conditioned fear stress in a synergistic manner, an effect which was comparable to that of apomorphine, a mixed dopamine D_1/D_2 receptor agonist. These findings suggest that the activation of both dopamine D_1 and D_2 receptors is necessary to attenuate conditioned fear stress. Similar synergistic interactions of dopamine D_1 and D_2 receptors have been observed in other behavioral studies. For example, Arnt et al. (1987) reported that, only when given in combination to rats, did SKF 38393 and quinpirole produce intensive stereotyped behaviors such as licking and biting similar to those seen with apomorphine.

In normal mice well-habituated to the test cage, quinpirole (1 mg/kg) induced hypermotility (data not shown), although it failed to do so in non-shocked mice. Therefore, it is likely that quinpirole at the doses used here sufficiently stimulates dopamine D_2 receptors. It has been suggested that behavioral responses produced by selective D_2 agonists are dependent on the presence of endogenous dopamine to stimulate D_1 receptors. For instance, the depletion of brain dopamine by α-methyl-*p*-tyrosine, a tyrosine hydroxylase inhibitor, inhibits quinpirole-induced responses such as stereotyped behavior and circling behavior in rats with a unilateral striatal lesion, an effect which is reversed by coadministration of SKF 38393 (Walters et al., 1987; Barone et al., 1986). Comparable results have also been observed in electrophysiological studies (Carlson et al., 1987; Walters et al., 1987). Our previous findings suggested that dysfunction of dopaminergic neurotransmission in the striatum is responsible for conditioned fear stress because, in mice showing this response, the dopamine turnover rate in the striatum was significantly reduced compared to that in the non-shocked controls (Nabeshima et al., 1986). In addition, striatal dopamine release in the shocked rats was lower than that in the unshocked rats in a study using in vivo brain microdialysis (Katoh et al., in preparation). Since a decrease in dopamine release in the nucleus accumbens is observed in stressful situations such as prolonged immobilization (Puglisi-Allegra et al., 1991) and forced swimming (Rossetti et al., 1993), it is possible that this brain area also plays a role in the conditioned fear stress. Taking the present results together with our previous results, we suggest that the inability of quinpirole to attenuate the stress-induced motor suppression is due to dysfunction in the dopaminergic neuronal systems, resulting in insufficient stimulation of dopamine D_1 receptors by endogenous dopamine. This hypothesis may be supported by the present findings that the conditioned fear stress was effectively attenuated by quinpirole only when administered in combination with SKF 38393. In addition, both the dopamine D_1 receptor antagonist, SCH 23390, and the dopamine D_2 receptor antagonist, (−)-sulpiride, administered singly counteracted the effects of the dopamine receptor agonists on conditioned fear stress. Thus, it seems that the functional interaction between dopamine D_1 and D_2 receptors in the stress-induced motor suppres-

sion is similar to that observed in non-stressed normal animals, although dopamine neuronal activity is appreciably suppressed.

However, most dopamine D_2 receptor agonists and antagonists also bind to D_3 receptors (Sokoloff et al., 1990). In the future, it will be necessary to examine the involvement of these D_3 receptors in the development of stress-induced motor suppression.

In conclusion, the results of the present behavioral study suggest that a functional reduction in dopaminergic neuronal systems may be responsible for the development of stress-induced motor suppression, although the functional interaction of dopamine D_1 and D_2 receptors is still intact.

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