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Neurosteroids Ameliorate Conditioned Fear Stress: An Association with Sigma$_1$ Receptors

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Mice exhibited a marked suppression of motility (conditioned fear stress) when placed in an environment in which they had previously received an electric footshock. This conditioned fear stress response was dose-dependently attenuated by neurosteroids such as dehydroepiandrosterone sulfate (DHEAS; 25 and 50 mg/kg, s.c.) and pregnenolone sulfate (PREGS; 10–50 mg/kg, s.c.), and by a putative sigma$_1$ receptor agonist, (+)-N-allylnormetazocine ((+)-SKF-10,047; 3 and 6 mg/kg, s.c.). However, progesterone (PROG; 10–50 mg/kg, s.c.) and allopregnanolone (5 and 20 mg/kg, s.c.) had no effect on this stress response. The attenuating effects of DHEAS (50 mg/kg, s.c.), PREGS (50 mg/kg, s.c.), and (+)-SKF-10,047 (6 mg/kg, s.c.) were reversed by NE-100 (5 mg/kg, i.p.), a sigma$_1$ receptor antagonist and PROG (5 or 10 mg/kg, i.p.). When DHEAS (25 mg/kg) was co-administered with (+)-SKF-10,047 (3 mg/kg) at doses that do not affect the conditioned fear stress response by themselves, motor suppression was significantly attenuated. In mice showing the conditioned fear stress response, the serum concentration of DHEAS was lower than that in non-shocked mice. These results suggest that the attenuating effects of DHEAS and PREGS on the conditioned fear stress response are mediated via sigma$_1$ receptors and that PROG has a sigma$_1$ receptor antagonistic property. Further, the endogenous DHEAS may be involved in the expression of conditioned fear stress response in mice. ***[Neuropsychopharmacology 23:276–284, 2000]***

KEY WORDS: *Neurosteroids; Sigma$_1$ receptors; Conditioned fear stress; DHEAS*

Neurosteroids are synthesized in the brain, either de novo from cholesterol or from steroid hormone precursors, and accumulate in the nervous system to levels that are at least in part independent of steroidogenic gland secretion (Baulieu 1981). Neurosteroids, such as progesterone (PROG), pregnenolone (PREG), dehydroepiandrosterone (DHEA), and their respective sulfate ester (PREGS or DHEAS), are involved in regulating the imbalance between excitation and inhibition in the CNS (Wu et al. 1991). The neurosteroids, allopregnanolone, allotetrahydrodeoxycorticosterone, PREGS, and DHEAS have been shown to possess antistress, anxiolytic, and antiamnesic properties in experimental animal models (Bitran et al. 1991; Brot et al. 1997; Maurice et al. 1997, 1999; Reddy and Kulkarni 1998; Reddy et al. 1998; Urani et al. 1998; Wieland et al. 1991). Recent evidence suggests that DHEAS and PREGS also play an important role in depression. Interestingly, decreased levels of DHEA, DHEAS, and PREGS have been associated with depression, cognitive dysfunction, aging, and other neurological conditions (Orentreich et al. 1984; Vallée et al. 1997), and DHEA improves the depression score in patients (Wolkowitz et al. 1997). However, the

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mechanism underlying the beneficial effects of neurosteroids is not yet known.

Neurosteroids have been shown to affect the activity of neurotransmitter systems, which are involved in a variety of neuropsychiatric illnesses. DHEAS and PREGS are negative allosteric modulators of the γ-aminobutyric acid $(GABA)_A$ receptors (Majewska 1992), and positive modulators of N-methyl-D-aspartate (NMDA) receptor-mediated responses (Bowlby 1993; Irwin et al. 1992, 1994). Evidence is mounting that DHEAS and PREGS act as agonists at central sigma receptors, especially sigma$_1$ sites, and exert facilitatory actions on NMDA-mediated glutamatergic and noradrenergic neurotransmission (Maurice and Lockhart 1997; Monnet et al. 1995). Some neurosteroids such as PREGS and PROG have been shown to have an affinity for sigma$_1$ receptors (Su et al. 1988; Walker et al. 1990). Further, Maurice et al. (1996) have reported that exogenous administration of neurosteroids leads to dose-dependent inhibition of in vivo binding of [^{3}H]-(+)-N-allylnormetazocine ((+)-SKF-10,047) to sigma$_1$ receptors. Thus, it is possible that neurosteroids are the main endogenous modulator of sigma$_1$ receptors, and that the pharmacological effects of neurosteroids described above are mediated, at least partly, through the sigma$_1$ receptors.

To date, we have tried to clarify the functional role of sigma$_1$ receptors in a stressful situation by using the conditioned fear stress response defined by Fanselow (1980). Rats and mice exhibit a marked suppression of motility when they are re-placed in an environment in which they have previously received an electric footshock (Kameyama and Nagasaka 1982, 1983; Yamada and Nabeshima 1995). This motor suppression is regarded as a conditioned emotional response to the environment associated with previous footshock (Kameyama and Nagasaka 1982, 1983; Yamada and Nabeshima 1995). We have found that (+)-SKF-10,047 and dextromethorphan, prototype sigma$_1$ receptor agonists, dose dependently attenuate the conditioned fear stress (Kamei et al. 1994, 1996a) and that this effect was antagonized by NE-100, a sigma$_1$ receptor antagonist stress (Kamei et al. 1994, 1996a), indicating that the activation of sigma$_1$ receptors is responsible for the attenuation of conditioned fear stress response. These observations suggest that the sigma$_1$ receptors play an important role in stress responses. However, the functional role of neurosteroids having affinity for sigma$_1$ receptors, in the conditioned fear stress response is unclear.

In the present study, therefore, we examined the effects of neurosteroids on the conditioned fear stress response in mice, in comparison with those of the sigma$_1$ receptor ligand, (+)-SKF-10,047 and discussed the relationship between neurosteroids and sigma$_1$ receptors in the stress response.

METHODS

Animals

Male mice of the ddY strain (Japan SLC, Inc., Shizuoka, Japan), at 7–8 weeks of age were used. The animals were housed in plastic cages and were kept in a regulated environment (23 ± 1°C, 50 ± 5% humidity), with a 12/12 hr light-dark cycle (lights on at 7:00 A.M.). Food (CE2; Clea Japan Inc., Tokyo, Japan) and tap water were available *ad libitum.*

All experiments were performed in accordance with the Guidelines for Animal Experiments of the Nagoya University School of Medicine. The procedures involving animals and their care conformed with the international guidelines set out in 'Principles of Laboratory Animal Care' (NIH publication no. 85–23; revised 1985).

Drugs

The following drugs were used: (+)-N-allylnormetazocine hydrochloride ((+)-SKF-10,047; Research Biochemicals Inc., Natick, MA, USA), dehydroepiandrosterone sulfate (DHEAS; Sigma, St. Louis MO, USA), pregnenolone sulfate (PREGS; Sigma), allopregnanolone (Sigma), and progesterone (PROG; Sigma). NE-100 (N, N-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl]-ethylamine monohydrochloride) was kindly supplied by Taisho Pharmaceutical Co. Ltd. (Saitama, Japan). DHEAS radioimmunoassay kit was obtained from Japan DPC Co. (Chiba, Japan).

(+)-SKF-10,047 and NE-100 were dissolved in saline and distilled water, respectively. DHEAS, PREGS, allopregnanolone, and PROG were solubilized in 7% dimethylsulfoxide (Sigma). All compounds were administered in a volume of 0.1 ml/10 g body weight.

Schedule for Conditioned Fear Stress

The experiments were carried out according to the method of Kamei et al. (1994, 1996a,b), with a minor modification. A transparent acrylic rectangular test cage (25.5 x 31.0 x 14.0 cm) equipped with a metal wire floor was used. 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, 1 mA) for 6 min through a shock generator-scrambler (Neuroscience Inc., Tokyo, Japan). 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 (shocked group). The spontaneous motility of the animal was determined for 6 min in the same test cage by using Scanet SV-10 (Toyo Sangyo, Toyama, Japan) equipped with photosensors. The non-shocked control

group was prepared exactly the same way except without electric footshock (non-shocked group).

All test drugs were administered before measuring motility in the test trial; (+)-SKF-10,047, DHEAS, PREGS, allopregnanolone, and PROG were administered subcutaneously (s.c.) 30 min before the test trial. In the antagonistic experiments, NE-100 and PROG were administered intraperitoneally (i.p.) 45 min before the test trial. Groups receiving no drugs were given an appropriate vehicle.

Determination of DHEAS Concentration in the Serum

Immediately after the measurement of motility, each mouse was decapitated and the blood was rapidly collected. The blood was centrifuged at 15,000g for 5 min and the serum was extracted. The DHEAS concentration in the serum was quantified with a radioimmunoassay kit.

Statistical Analysis

Statistical significance was determined by a Dunn-type non-parametric test. Comparisons of two sample means were performed with the Mann-Whitney U-test and Student t-test. *P*-values less than .05 were taken to indicate statistically significant differences.

RESULTS

Effects of Neurosteroids and (+)-SKF-10,047 on the Conditioned Fear Stress Response in Mice

The shocked mice treated with vehicle (shocked group) exhibited a marked suppression of motility; the shocked group showed 4.3–10.1% of the motility exhibited by the non-shocked group when returned to the apparatus in which they had been given an electric shock (Figure 1 and Table 1A), in agreement with our previous results (Kamei et al. 1994, 1996a,b). The shocked mice mostly froze on the floor and crouched, while the non-shocked mice showed typical exploratory behavior such as ambulation, sniffing and rearing.

Figure 1 and Table 1A show the effects of neurosteroids and (+)-SKF-10,047, respectively, on motor suppression in the shocked group. DHEAS (25 and 50 mg/kg), PREGS (10–50 mg/kg) and (+)-SKF-10,047 (3 and 6 mg/kg) partially attenuated the motor suppression of the shocked group in a dose-dependent manner, without changing motility in the non-shocked mice (Figure 1 and Table 1A); significant effects of DHEAS, PREGS, and (+)-SKF-10,047 were observed at a dose of 50, 50, and 6 mg/kg, respectively.

Allopregnanolone (5 and 20 mg/kg) and PROG (10–50 mg/kg) had no effects on motility in the shocked and non-shocked groups (Figure 1).

Antagonistic Effects of Sigma$_1$ Receptor Antagonist and PROG on the Attenuation of Conditioned Fear Stress Induced by DHEAS, PREGS, and (+)-SKF-10,047

To clarify whether the attenuating effects of DHEAS and PREGS on motor suppression in the shocked mice were mediated by sigma$_1$ receptors, we investigated the antagonistic effects of a selective sigma$_1$ receptor antagonist, NE-100 and a sigma$_1$ receptor antagonistic neurosteroid, PROG, on the effects of DHEAS and PREGS.

NE-100 and PROG were used at doses that could block the attenuating effect of sigma$_1$ receptor ligands on the conditioned fear stress (Kamei et al. 1996a) and on scopolamine-induced learning impairment (Urani et al. 1998), respectively. As shown in Figures 2 and 3, NE-100 (5 mg/kg) and PROG (5 mg/kg) significantly inhibited the attenuation of the motor suppression induced by DHEAS

Table 1. Effect of (+)-SKF-10,047 on the Conditioned Fear Stress in Mice

Treatment	Dose (mg/kg)	Motility (counts/6 min)	
		Non-shock	Shock
A			
Vehicle		2679.4 ± 184.7 (10)	230.9 ± 90.8 (10)
(+)-SFK-10047 (s.c.)	3	N.D.	285.8 ± 67.4 (11)
	6	2920.1 ± 295.1 (10)	824.7 ± 132.0 (11)[b]
B			
Vehicle		N.D.	182.7 ± 9.8 (10)
(+)-SFK-10047 (s.c.)	6	N.D.	554.0 ± 7.0 (10)[a]
+NE-100 (i.p.)	5	N.D.	195.7 ± 90.5 (10)[c]

(+)-SKF-10,047 and NE-100 were administered 30 and 45 min, respectively, before motility was measured. The results are expressed as the mean ± S.E.M. for the number of animals shown in parenthesis. N.D. not determined.

[a]$p < .05$ and [b]$p < .01$ compared to the corresponding vehicle-treated, shocked group.

[c]$p < .05$ compared to the (+)-SKF-10,047-treated, shocked group.

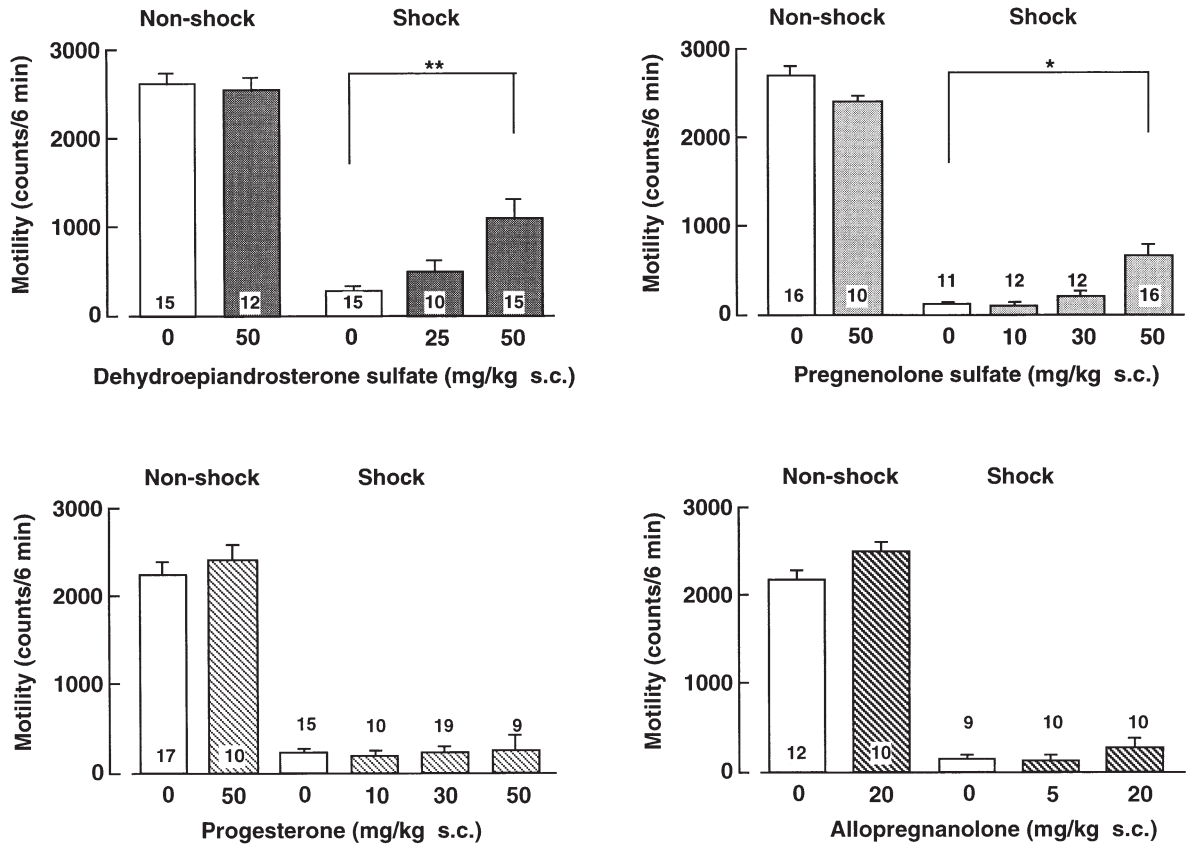

Figure 1. Effects of neurosteroids on the conditioned fear stress in mice. Dehydroepiandrosterone sulfate (DHEAS), pregnenolone sulfate (PREGS), progesterone (PROG), and allopregnanolone were administered s.c. 30 min before motility was measured. The results are expressed as the mean ± S.E.M. for the number of animals shown in each column. *$p < .05$ and **$p < .01$ compared to the corresponding vehicle-treated, shocked group (Dunn-type non-parametric test).

(50 mg/kg) and PREGS (50 mg/kg). Similarly, the attenuating effect of (+)-SKF-10,047 (6 mg/kg) on motor suppression was significantly antagonized by NE-100 (5 mg/kg) and PROG (10 mg/kg) (Table 1B and Figure 3).

Effects of (+)-SKF-10,047 in Combination with DHEAS on Conditioned Fear Stress

Figure 4 shows the effects of (+)-SKF-10,047 in combination with DHEAS on the motor suppression in the shocked group. Low doses of DHEAS (25 mg/kg) and (+)-SKF-10,047 (3 mg/kg) themselves had no effects on motility in the shocked group. However, when DHEAS was co-administered with (+)-SKF-10,047, motor suppression was significantly attenuated, but not the motility in the non-shocked groups. The shocked mice given (+)-SKF-10,047 in combination with DHEAS exhibited exploratory behavior.

The Serum Concentration of DHEAS in Mice Showing the Conditioned Fear Stress Response

The serum concentrations of DHEAS in the non-shocked and shocked groups are shown in Figure 5. In mice showing the conditioned fear stress response, the serum concentration of DHEAS was significantly decreased compared with that in the non-shocked mice. However, the serum DHEAS concentration in mice, which did not place into the same test cage 24 h after shock treatment, was unchanged (data not shown).

DISCUSSION

Sigma$_1$ receptors have been demonstrated to play an important role in conditioned fear stress response (Kamei et al. 1994, 1996a,b, 1997). For example, (+)-SKF-10,047 and dextromethorphan, putative sigma$_1$ receptor ligands attenuate the conditioned fear stress-induced motor suppression in mice, the effects being antagonized by NE-100, a selective sigma$_1$ receptor antagonist (Kamei et al. 1994, 1996a). In the present study, (+)-SKF-10,047 (6 mg/kg), neurosteroids, DHEAS (50 mg/kg) and PREGS (50 mg/kg) could partially attenuate the conditioned fear stress-induced motor suppression in mice. The effects of both neurosteroids and (+)-SKF-10,047 were antagonized by NE-100, a selective sigma$_1$ receptor antagonist. Several steroids including PROG, PREG, PREGS, testosterone,

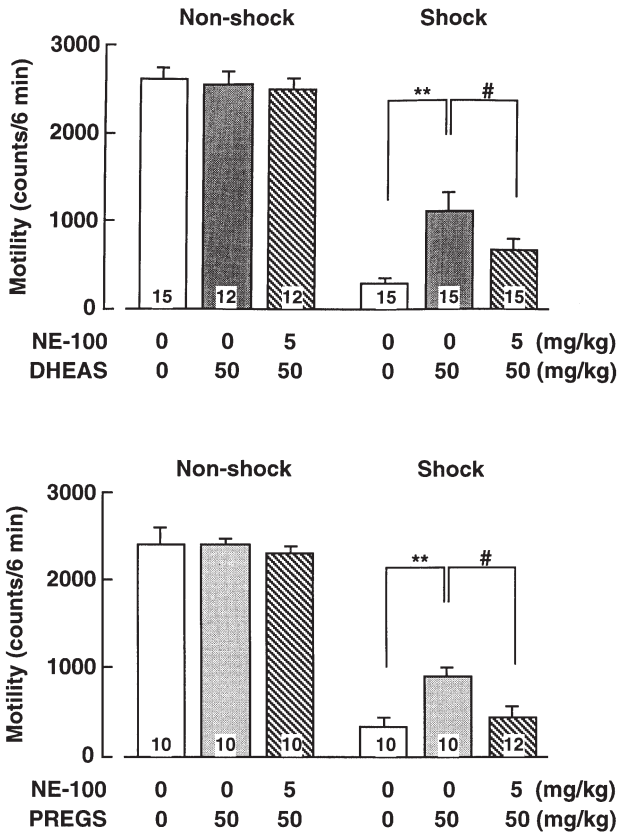

Figure 2. Effect of NE-100 on dehydroepiandrosterone sulfate (DHEAS)- and pregnenolone sulfate (PREGS)-induced attenuation of conditioned fear stress in mice. DHEAS and PREGS were administered s.c. 30 min before motility was measured. NE-100 was administered i.p. 45 min before motility was measured. The results are expressed as the mean ± S.E.M. for the number of animals shown in each column. $^{**}p < .01$ compared to the corresponding vehicle-treated, shocked group (Mann-Whitney U-test). $\#p < .05$ compared to the DHEAS- or PREGS-treated, shocked group (Mann-Whitney U-test).

and 17β-estradiol have been shown to inhibit the in vitro binding of the $sigma_1$ receptor radioligands [^{3}H]-SKF-10,047, [^{3}H]-dextromethorphan, [^{3}H]-3-PPP, and [^{3}H]-haloperidol to rat brain, splenocytes, plasma membranes, and liver microsomes (Klein and Musacchio 1994; McCann and Su 1991; Ross 1991; Schwartz et al. 1989; Su et al. 1988; Yamada et al. 1994). DHEAS and PREGS dose-dependently inhibited the in vivo haloperidol-sensitive [^{3}H]-(+)-SKF-10,047 binding to $sigma_1$ receptor in the mouse hippocampus and cortex (Maurice et al. 1996). Taken together, these findings suggest that the attenuating effects of neurosteroids on the conditioned fear stress are mediated at least partly through the $sigma_1$ receptors. That there is interaction between DHEAS and $sigma_1$ receptors is further supported by the finding that the combination of DHEAS with (+)-SKF-10,047 had an additive effect to attenuate the motor suppression in the conditioned fear stress.

Recent evidence has suggested that sigma receptors are divided into at least two subtypes, $sigma_1$ and $sigma_2$ receptors (Walker et al. 1990; Quirion et al. 1992). Additionally, binding studies with an anticonvulsant, phenytoin, have shown that $sigma_1$ receptors can be differentiated into two different binding sites, namely phenytoin-sensitive and phenytoin-insensitive sites (DeHaven-Hudkins et al. 1993). The binding affinity of (+)-SKF-10,047 and dextromethorphan to $sigma_1$ sites is markedly increased by phenytoin while that of (+)-pentazocine (a $sigma_1$ receptor ligand) and DTG (a $sigma_1$/$sigma_2$ receptor ligand) is unaffected by phenytoin (DeHaven-Hudkins et al. 1993). The activation of phenytoin-regulated type $sigma_1$ receptors is involved in the attenuation of (+)-SKF-10,047 and dextromethorphan on conditioned fear stress, since (+)-pentazocine and DTG failed to attenuate its effect.

In the present study, DHEAS and PREGS, as well as (+)-SKF-10,047, attenuated the conditioned fear stress in mice. Further, the attenuating effects of both neurosteroids were antagonized by a selective $sigma_1$ receptor antagonist. Thus, we speculate that the attenuating effects of DHEAS and PREGS may be mediated via $sigma_1$ receptors, particularly, phenytoin-regulating $sigma_1$ site, but not $sigma_2$ receptors. Further research, however, is needed to confirm this hypothesis. In addition, allopregnanolone at the dose of 50 mg/kg did not attenuate the conditioned fear stress response. It is unlikely that the doses of allopregnanolone are insufficient, since allopregnanolone less than 20 mg/kg, as well as diazepam, has anxiolytic effects in numerous anxiety paradigms, including the Vogel conflict, open field light/dark transition and elevated plus maze tasks in rodents (Bitran et al. 1991; Brot et al. 1997; Wieland et al. 1991). The anxiolytic actions of this drug have been proposed to be related to an augmentation of $GABA_A$ receptor function, although the mechanisms underlying these effects are yet unclear (Bitran et al. 1991; Brot et al. 1997; Wieland et al. 1991). It has been demonstrated that anxiolytics such as diazepam or chlordiazepoxide do not attenuate the motor suppression in conditioned fear stress (Kameyama and Nagasaka 1982; Nagasaka and Kameyama 1983). Thus, it is likely that the conditioned fear stress is resistant to $GABA_A$ mimic neurosteroids and agents such as allopregnanolone and benzodiazepines.

A crossed pharmacology between the effects of $sigma_1$ receptor ligands and neurosteroids was recently described for neuronal responses, DHEAS and PREGS behaving as agonists and PROG as an antagonist (Bergeron et al. 1996; Monnet et al. 1995). In the present study, PROG at 10–50 mg/kg, which inhibit the in vivo haloperidol-sensitive [^{3}H]-(+)-SKF-10,047 binding to $sigma_1$ receptor in the mouse hippocampus and cortex (Maurice et al. 1996), had no effect on the conditioned fear stress response in mice. Like NE-100, PROG be-

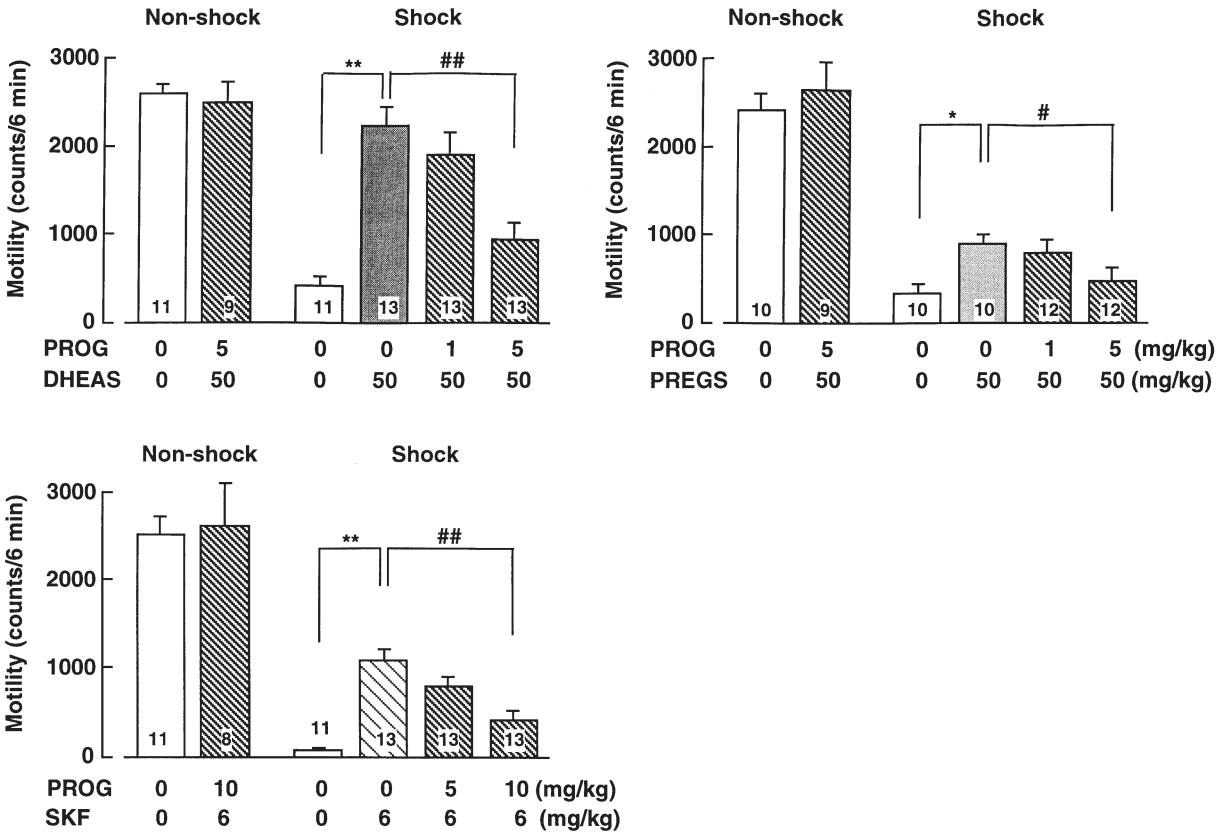

Figure 3. Effect of progesterone (PROG) on dehydroepiandrosterone sulfate (DHEAS)-, pregnenolone sulfate (PREGS)-, and (+)-SKF-10,047 (SKF)-induced attenuation of conditioned fear stress in mice. DHEAS, PREGS, and SKF were administered s.c. 30 min before motility was measured. PROG was administered i.p. 45 min before motility was measured. The results are expressed as the mean ± S.E.M. for the number of animals shown in each column. *$p < .05$ and **$p < .01$ compared to the corresponding vehicle-treated, shocked group (Mann-Whitney U-test). #$p < .05$ and ##$p < .01$ compared to the DHEAS-, PREGS-, or SKF-treated, shocked group (Dunn-type non-parametric test).

haved as a potent sigma$_1$ receptor antagonist in the conditioned fear stress response, since it antagonized the attenuating effects of (+)-SKF-10,047, DHEAS, and PREGS on the response. Such an effect of PROG was observed in other experiments, that is the attenuating effects of DHEAS and sigma$_1$ receptor agonists on immobility in the Porsolt forced swim test and on memory impairment were antagonized by PROG (Maurice et al. 1998; Reddy et al. 1998; Urani et al. 1998). Thus, these results suggest that PROG acts as a potent sigma$_1$ receptor antagonist, whereas DHEAS and PREGS act as sigma$_1$ receptor agonists.

PROG antagonized the attenuating effects of neurosteroids (DHEAS and PREGS) at smaller doses compared to those of (+)-SKF-10,047. One possible explanation is that this may be due to difference of affinities for sigma$_1$ receptors between (+)-SKF10,047 and neurosteroids used since the affinity of (+)-SKF-10,047 for sigma$_1$ receptors is higher than that of DHEAS, PREGS, and PROG. However, this point must be considered with caution, as other neuropharmacology of sigma ligands and neurosteroids remains to be clarified.

Interestingly, we found that the serum DHEAS concentration in mice showing the conditioned fear stress response was lower than that in the non-shocked mice, but the serum DHEAS concentration in mice which did not place into the same test cage 24 h after shock treatment, was unchanged (data not shown). This finding suggests that changes in the serum level of DHEAS are involved in the stress-induced physiopathological changes such as anxiety and depression. A recent clinical study suggests that DHEA is involved in depression: when DHEA was openly administered to patients with major depression and low plasma levels of DHEA and DHEAS for four weeks, depression ratings were significantly improved with the increase in DHEA and DHEAS plasma levels (Wolkowitz et al. 1997). Further, when DHEA was administered to patients with treatment-resistant depression for six months, a marked improvement in depression ratings was observed (Wolkowitz et al. 1997).

The conditioned fear stress has been regarded as an animal model of treatment-resistant psychoses, since anxiolytics and/or antidepressants did not attenuate the mo-

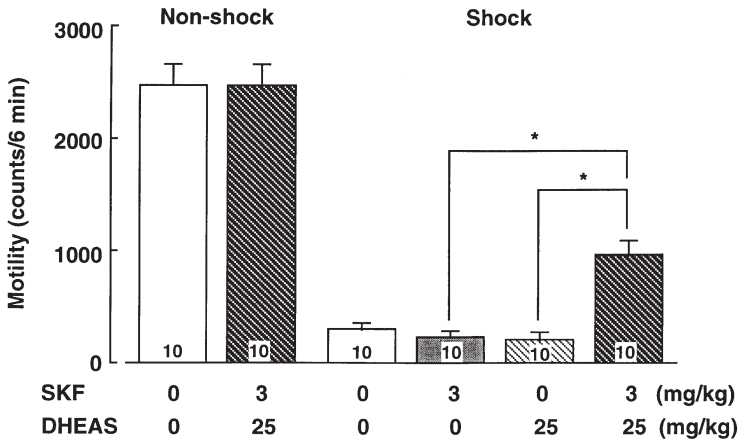

Figure 4. Effect of (+)-SKF-10,047 (SKF) in combination with dehydroepiandrosterone sulfate (DHEAS) on conditioned fear stress in mice. SKF and DHEAS were administered s.c. 30 min before motility was measured. The results are expressed as the mean ± S.E.M. for the number of animals shown in each column. *$p < .05$ compared to the (+)-SKF-10,047 or DHEAS alone-treated, shocked group (Mann-Whitney U-test).

tor suppression in conditioned fear stress (Kameyama and Nagasaka 1982; Nagasaka and Kameyama 1983). Taken together, these findings suggest that the decrease in the serum levels of DHEAS is related to the development of conditioned fear stress response and neurosteroids might be useful anti-psychotics for treatment-resistant psychoses.

Conditioned fear stress has been shown to affect central neurotransmitters such as the dopamine (Herman et al. 1982; Inoue et al. 1994; Katoh et al. 1996; Nabeshima et al. 1986), norepinephrine (Tsuda et al. 1986), and serotonin (Inoue et al. 1993, 1994) neuronal systems, which are well known to be involved in stress responses. In this regard, our previous behavioral and neurochemical experiments suggest that the reduction of dopamine release from dopaminergic terminals in the nucleus accumbens is responsible for the development of the motor suppression in conditioned fear stress, and that (+)-SKF-10,047 attenuates the conditioned fear stress-induced motor suppression by reversing the conditioned fear stress-induced dysfunction in the mesolimbic dopaminergic systems (Kamei et al. 1996b, 1997). A preliminary experiment showed that DHEAS, like (+)-SKF-10,047, restored the decreased dopamine turnover in the nucleus accumbens of shocked mice to control levels, as indicated by the increase in DOPAC/dopamine and HVA/dopamine (data not shown).

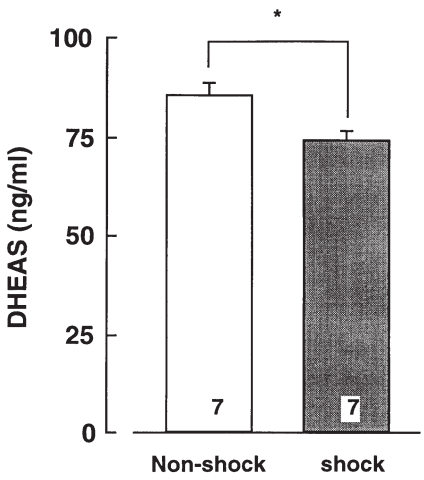

Figure 5. Dehydroepiandrosterone sulfate (DHEAS) levels in the serum of mice showing conditioned fear stress response. The results are expressed as the mean ± S.E.M. for the number of animals shown in each column. *$p < .05$ compared to the vehicle-treated, non-shocked group (Student's t-test).

There is evidence that $sigma_1$ receptors modulate the activity of dopaminergic neurons: the existence of $sigma_1$ receptors on the dopaminergic neurons has been demonstrated (Gundlach et al. 1986) and electrophysiological studies have shown that these receptors are involved in the regulation of neuronal activity of the midbrain dopaminergic neurons (Freeman and Bunney 1984; Steinfels and Tam 1989). Further, the in vivo brain microdialysis technique has demonstrated that sigma ligands as (+)-SKF-10,047 (Volonte et al. 1995) and (+)-pentazocine (Patrick et al. 1993) increase the endogenous dopamine release in rat brain. In conclusion, we speculate that DHEAS and PREGS, as well as $sigma_1$ receptor agonists, reverse the reduction of dopamine release in the nucleus accumbens via activation of presynaptic $sigma_1$ receptors of the mesolimbic dopaminergic system and, as a result, conditioned fear stress response is attenuated. In the present study, neurosteroids and (+)-SKF-10,047 did not totally attenuate the conditioned fear stress response in mice. Previously, we have found that opioid μ-agonists also attenuate the conditioned fear stress response via dopaminergic systems. Thus, the partial attenuating effects of DHEAS, PREGS, and (+)-SKF-10,047 on conditioned fear stress response in mice, may be due to involvement in non-sigma-mediated mechanisms. However, further studies are needed to verify this point.

At present, much evidence suggests that there is a common mechanism of action between neurosteroids and $sigma_1$ receptor agonists in stress-induced disor-

ders such as anxiety and depression (Reddy et al. 1998; Wolkowitz et al. 1997). However, this mechanism has still to be examined, particularly as concerns the different neurotransmission systems involved in the pathological state. We believe that the use of neurosteroids is a novel therapeutic approach for at least some mood disorders including treatment-resistant depression.

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REFERENCES

Baulieu EE (1981): Steroid hormones in the brain: several mechanisms? In Fuxe K, Gustafson JA, Wettenberg L (eds), Steroid Hormone Regulation of the Brain. Oxford, Pergamon Press, pp 3–14

Bergeron R, De Montigny C, Debonnel G (1996): Potentiation of neuronal response induced by dehydroepiandrosterone and its suppression by progesterone: Effects mediated via sigma receptors. J Neurosci 16:1193–1202

Bitran D, Hilvers RJ, Kellogg CK (1991): Anxiolytic effects of 3α-hydroxy-5-α [β]-pregnan-20-one: Endogenous metabolites of progesterone that are active at the GABA$_A$ receptor. Brain Res 561:157–161

Bowlby MR (1993): Pregnenolone sulfate potentiation of N-methyl-D-aspartate receptor channels in hippocampal neurons. Mol Pharmacol 43:813–819

Brot MD, Akwa Y, Purdy RH, Koob GF, Britton KT (1997): The anxiolytic-like effects of the neurosteroid allopregnanolone: Interactions with GABA$_A$ receptors. Eur J Pharmacol 325:1–7

DeHaven-Hudkins DL, Ford-Rice FY, Allen JT, Hudkins RL (1993): Allosteric modulation of ligand binding to [^{3}H](+)pentazocine-define σ recognition sites by phenytoin. Life Sci 53:41–48

Fanselow MS (1980): Conditional and unconditional components of post-shock freezing. Pavlov J Biol Sci 15:177–182

Freeman AS, Bunney BS (1984): The effects of phencyclidine and N-allylnormetazocine on midbrain dopamine neuronal activity. Eur J Pharmacol 104:287–293

Gundlach AL, Largent BL, Snyder SH (1986): Autoradiographic localization of σ receptor binding sites in guinea pig and rat central nervous system with (+)^{3}H-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine. J Neurosci 6:1757–1770

Herman JP, Guillonneau D, Dantzer R, Scatton B, Semerdjian-Rouquier L, Le Moal M (1982): Differential effects of inescapable footshocks and of stimuli previously paired with inescapable footshocks on dopamine turnover in cortical and limbic areas of the rat. Life Sci 30:2207–2214

Inoue T, Koyama T, Yamashita I (1993): Effect of conditioned fear stress on serotonin metabolism in the rat brain. Pharmacol Biochem Behav 24:115–119

Inoue T, Tsuchiya K, Koyama T (1994): Regional changes in dopamine and serotonin activation with various intensity of physical and psychological stress in the rat brain. Pharmacol Biochem Behav 49:911–920

Irwin RP, Lin SZ, Rogawski MA, Purdy RH, Paul SM (1994): Steroid potentiation and inhibition of N-methyl-D-aspartate receptor-mediated intracellular Ca^{2+} responses: Structure-activity studies. J Pharmacol Exp Ther 271:677–682

Irwin RP, Maragakis NJ, Rogawski MA, Purdy RH, Farb DH, Paul SM (1992): Pregnenolone sulfate augments NMDA receptor mediated increases in intracellular Ca^{2+} in cultured rat hippocampal neurons. Neurosci Lett 141:30–34

Kamei H, Kameyama T, Nabeshima T (1994): SKF-10,047 reverses stress-induced motor suppression: Interaction with dopaminergic system. Eur J Pharmacol 260:39–46

Kamei H, Kameyama T, Nabeshima T (1996a): (+)-SKF-10,047 and dextromethorphan ameliorate the conditioned fear stress through the activation of phenytoin-regulated σ_1 sites. Eur J Pharmacol 299:21–28

Kamei H, Kameyama T, Nabeshima T (1996b): (+)-SKF-10,047 and dextromethorphan ameliorate conditioned fear stress via dopaminergic systems linked to phenytoin-regulated σ_1 sites. Eur J Pharmacol 309:149–158

Kamei H, Noda Y, Kameyama T, Nabeshima T (1997): 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. Eur J Pharmacol 319:165–172

Kameyama T, Nagasaka M (1982): Effects of apomorphine and diazepam in a quickly learned conditioned suppression in rats. Pharmacol Biochem Behav 17:59–63

Kameyama T, Nagasaka M (1983): Effects of apomorphine and methamphetamine on a quickly learned conditioned-suppression response in rats. Neuropharmacology 22:813–817

Katoh A, Nabeshima T, Kuno A, Wada M, Ukai R, Kameyama T (1996): Changes in striatal dopamine release in stress-induced conditioned suppression of motility in rats. Behav Brain Res 77:219–221

Klein M, Musacchio JM (1994): Effects of cytochrome P-450 ligands on the binding of [^{3}H]dextromethorphen and sigma ligands to guinea-pig brain. In Itzhak Y (ed), Sigma Receptors. San Diego, CA, Academic Press, pp 243–262

Majewska MD (1992): Neurosteroids: Endogenous bimodal modulators of the GABA$_A$ receptor. Mechanism of action and physiological significance. Prog Neurobiol 38:379–395

Maurice T, Lockhart BP (1997): Neuroprotective and anti-amnesic potentials of sigma (σ) receptor ligands. Prog Neuro-Psychopharmacol Biol Psychiatry 21:69–102

Maurice T, Junien JL, Privat A (1997): Dehydroepiandrosterone sulfate attenuates dizocilpine-induced learning impairment in mice via σ_1 receptors. Behav Brain Res 83:159–164

Maurice T, Phan VL, Noda Y, Yamada K, Privat A, Nabeshima T (1999): The attenuation of learning impairments induced after exposure to CO or trimethyltin in mice by sigma (σ) receptor ligands involves both σ_1 and σ_2 sites. Br J Pharmacol 127:335–342

Maurice T, Roman FJ, Privat A (1996): Modulation by neurosteroids of the in vivo (+)-[^{3}H]SKF-10,047 binding to σ_1 receptors in the mouse forebrain. J Neurosci Res 46:734–743

Maurice T, Su TP, Private A (1998): Sigma$_1$ (σ_1) receptor agonists and neurosteroids attenuate $\beta_{25\text{-}35}$-amyloid peptide-induced amnesia in mice through a common mechanism. Neuroscience 83:413–428

McCann DJ, Su TP (1991): Solubilization and characterization of haloperidol-sensitive (+)-[^{3}H]SKF-10,047 binding sites (sigma sites) from rat liver membranes. J Pharmacol Exp Ther 257:547–554

Monnet FP, Mahe V, Robel P, Baulieu EE (1995): Neurosteroids, via σ receptors, modulate the [^{3}H]norepinephrine release evoked by N-methyl-D-aspartate in the rat hippocampus. Proc Natl Acad Sci USA 92:3774–3778

Nabeshima T, Katoh A, Hiramatsu M, Kameyama T (1986): A role played by dopamine and opioid neuronal systems in stress-induced motor suppression (conditioned suppression of motility) in mice. Brain Res 398:354–360

Nagasaka M, Kameyama T (1983): Effect of diazepam, meprobamate, chlorpromazine and apomorphine on a quickly learned conditioned suppression in mice. J Pharmacobio-Dyn 6:523–526

Orentreich N, Brind JL, Rizer RL, Vogelman JH (1984): Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. J Clin Endocrinol Metab 59:551–555

Patrick SL, Walker JM, Perkel JM, Lockwood M, Patrick RL (1993): Increases in rat striatal extracellular dopamine and vacuous chewing produced by two sigma receptor ligands. Eur J Pharmacol 231:243–249

Quirion R, Bowen WD, Itzhak Y, Junien JL, Musacchio JM, Rothman RB, Su TP, Tam SW, Taylor DP (1992): A proposal for the classification of sigma binding sites. Trends Pharmacol Sci 13:85–86

Reddy DS, Kulkarni SK (1998): The effects of neurosteroids on acquisition and retention of a modified passive-avoidance learning task in mice. Brain Res 791:108–116

Reddy DS, Kaur G, Kulkarni SK (1998): Sigma (σ_1) receptor mediated antidepressant-like effects of neurosteroids in the Porsolt forced swim test. NeuroReport 9:3069–3073

Ross SB (1991): Heterogeneos binding of σ radioligands in the rat brain and liver: Possible relationship to subforms of cytochrome P-450. Pharmacol Toxicol 68:293–301

Schwartz S, Ohl P, Zzhou GZ (1989): Steroid binding at σ-"opioid" receptors. Science 246:1635–1637

Steinfels GF, Tam SW (1989): Selective σ receptor agonist and antagonist affect dopamine neuronal activity. Eur J Pharmacol 163:167–170

Su TP, London ED, Jaffe JH (1988): Steroid binding at σ receptors suggests a link between endocrine, nervous, and immune systems. Science 240:219–221

Tsuda A, Tanaka M, Ida Y, Tsujimaru S, Ushijima I, Nagasaki N (1986): Effects of preshock experience on enhancement of rat brain noradrenaline turnover induced by psychological stress. Pharmacol Biochem Behav 24:115–119

Urani A, Privat A, Maurice T (1998): The modulation by neurosteroids of the scopolamine-induced learning impairment in mice involves an interaction with sigma$_1$ (σ_1) receptors. Brain Res 799:64–77

Vallée M, Mayo W, Darnaudéry M, Corpéchot C, Young J, Koehl M, Le Moal M, Baulieu EE, Robel P, Simon H (1997): Neurosteroids: Deficient cognitive performance in aged rats depends on low pregnenolone sulfate levels in the hippocampus. Proc Natl Acad Sci USA 94:14865–14870

Volonte M, Ceci A, Borsini F (1995): Effect of the 5-hydroxytryptamine3 receptor antagonist itasetron (DAU 6215) on (1)-N-allylnormetazocine-induced dopamine release in the nucleus accumbens and in the corpus striatum of the rat: An in vivo microdialysis study. J Pharmacol Exp Ther 275:358–367

Walker JM, Bowen WD, Walker FO, Matsumoto RR, De Costa B, Rice KC (1990): Sigma receptors: Biology and function. Pharmacol Rev 42:355–402

Wieland S, Lan NC, Mirasedeghi S, Gee KW (1991): Anxiolytic activity of the progesterone metabolite 5α-pregnan-3 α -ol-20-one. Brain Res 565:263–268

Wolkowitz OM, Reus VI, Roberts E, Manfredi F, Chan T, Raum WJ, Ormiston S, Johnson R, Canick J, Brizendine L, Weingartner H (1997): Dehydroepiandrosterone (DHEA) treatment of depression. Biol Psychiatry 41:311–318

Wu FS, Gibbs TT, Farb DH (1991): A positive allosteric modulator at the N-methyl-D-aspartate receptor. Mol Pharmacol 40:333–336

Yamada K, Nabeshima T (1995): Stress-induced behavioral responses and multiple opioid systems in the brain. Behav Brain Res 67:133–145

Yamada M, Nishigami T, Nakasho K, Nishimoto Y, Miyaji H (1994): Relationship between sigma-like site and progesterone-binding site of adult male rat liver microsomes. Hepatology 30:1271–1280