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Involvement of Dopamine D_2 Receptor Mechanism in the REM Sleep Deprivation-Induced Increase in Swimming Activity in the Forced Swimming Test

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ASAKURA, W., K. MATSUMOTO, H. OHTA AND H. WATANABE. *Involvement of dopamine D_2 receptor mechanism in the REM sleep deprivation-induced increase in swimming activity in the forced swimming test.* PHARMACOL BIOCHEM BEHAV 48(1) 43–46, 1994.—Effects of monoamine synthesis inhibitors and dopamine antagonists on rapid eye movement sleep (REMs) deprivation treatment-induced increase in swimming activity were examined. Mice were deprived of REMs for 48 h by a small pedestal method. Swimming activity in REMs-deprived mice was significantly higher than those in group-housed or socially isolated animals used as the control. *dl*-α-Methyl-*p*-tyrosine methyl ester HCl (250 mg/kg, IP) decreased the swimming activity in REMs-deprived mice, whereas neither disulfiram (400 mg/kg, SC), a noradrenaline synthesis inhibitor, nor *dl*-*p*-chlorophenylalanine methyl ester HCl (300 mg/kg, IP) changed it. (+)-SCH23390 HCl (30 and 100 μg/kg, IP), a selective dopamine D_1 antagonist, did not affect the activity in REMs-deprived mice. (±)-Sulpiride (12.5 and 25 mg/kg, IP), a selective dopamine D_2 antagonist, dose-dependently decreased swimming activity in REMs-deprived mice, while it did not significantly change the swimming activity in the control animals. These results suggest that REMs deprivation treatment-induced increase in swimming activity is mainly due to the functional changes in the dopaminergic system rather than the noradrenergic or serotonergic system, and that dopamine D_2 but not D_1 receptor mechanism is involved in the increase in swimming activity in REMs-deprived animals.

REM sleep deprivation Forced swimming test Dopamine α-Methyl-*p*-tyrosine SCH23390 Sulpiride

RAPID eye movement sleep (REMs) deprivation has been clinically shown to improve certain types of depression in humans (23). In rodents, this treatment as well as antidepressant drug administration decreases immobility time (or increases swimming activity) in the forced swimming test (2,11,12, 17,18,22).

In the forced swimming test, catecholaminergic and serotonergic neuronal transmission in the brain appears to play important roles in immobility. Monoamine precursors such as dihydroxyphenylalanine, threo-dihydroxyphenylserine, and 5-hydroxytryptophan decrease immobility in mice (20). Sulpiride, a selective dopamine D_2 antagonist, and metitepine, a nonselective serotonin antagonist, block antidepressant drugs-induced decrease in immobility time (4,5,7,8,19). Moreover, α_2 and β adrenoceptor antagonists, yohimbine and propranolol, respectively, abolish the anti-immobility effect of antidepressant drugs (3,15,25). These results suggest that the functional change in catecholaminergic and/or serotonergic system is involved in the increase in the swimming activity following REMs deprivation. However, REMs deprivation-induced increase in swimming activity is not blocked by yohimbine, although REMs deprivation increases α_2 adrenoceptor-mediated anti-immobility effect of clonidine (2). These data suggest that α_2 adrenoceptor mechanism is not involved in REMs deprivation-induced increase in the activity.

In the present study, to clarify the monoaminergic mechanism(s) in the swimming activity increased by REMs deprivation, we examined the effects of several monoamine synthesis inhibitors in mice. Furthermore, we tested the effects of selective dopamine D_1 and D_2 antagonists, SCH23390 and sulpiride, respectively, on the REMs deprivation-induced increase in swimming activity.

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METHOD

Animals

Male 5-week-old ddY mice (Japan SLC, Inc., Hamamatsu, Japan) were used in the experiments. The animals were housed in groups of 20–25 per cage (35 × 30 × 16 cm) for at least 1 week before the start of the experiment, with free access to food and water. Housing conditions were thermostatically maintained at 24 ± 1°C, with a 12-h light : dark cycle (light on: 0730–1930).

REM Sleep Deprivation Treatment

According to the method described in our previous reports (1,2), the REM sleep (REMs) deprivation treatment was carried out. In brief, mice were individually placed on the small pedestal (4.5 cm high, 1.8 cm diameter) which was fixed at the center of a REM sleep deprivation chamber (20 × 15 × 21 cm) and was surrounded by water (3.5 cm deep). The animals were housed in the chamber for 48 h, and used as the REMs-deprived group. The other groups of animals were either housed in groups of four (group-housed mice) or housed individually (isolated mice) in a Plexiglas cage (25 × 18 × 12 cm) during the same period as the REMs deprivation treatment and used as the control groups.

The Forced Swimming Test

Each mouse was placed individually in a transparent glass cylinder (20 cm high, 8 cm diameter) containing fresh water (25°C, 8 cm deep), and was forced to swim for 15 min (pretest swimming). After a 20-min drying period, the animals were deprived of REMs for 48 h. Immediately after the termination of REMs deprivation treatment, the animals were placed in the cylinder for 5 min (test swimming). Swimming activity during the test was measured using an animal movement analyzing system, Scanet SV-10 (Toyo Sangyo Co. Ltd., Toyama, Japan), as described previously (2). In brief, this system consisted of a rectangular enclosure (40 × 38 cm), the side walls (12 cm) of which were equipped with 144 pairs of photosensors. Each pair of photosensors was set at a height of 8.8 cm above the floor, and was scanned every 0.1 s to detect animal movement. Swimming activity was calculated from the scanning data obtained.

Measurement of Locomotor Activity

A Scanet SV-10 system was used to measure locomotor activity. The photosensors were set at a height of 2.5 cm above the floor. Immediately after the termination of REMs deprivation, each mouse was placed individually in the Plexiglas cage (25 × 18 × 24 cm), which was fixed at the center of the Scanet SV-10 system. Locomotor activity was measured over a 30-min period and calculated from the scanning data obtained.

Drugs

dl-α-Methyl-*p*-tyrosine methyl ester HCl and tetraethylthiuram disulfide (disulfiram) (Nacalai Tesque Inc., Kyoto, Japan) were administered 4 h before the test swimming. *p*-Chlorophenylalanine methyl ester HCl (Sigma Chemical Co., St. Louis, MO) was administered 24 h before the test. (+)-SCH23390 HCl (Research Biochemicals Inc., Natick, MA) and (±)-sulpiride (Dogmatyl® Inj. 100 mg, Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan) were administered 30 and 60 min before the testing, respectively. All drugs, except disulfiram, were dissolved in or diluted with saline, and administered intraperitoneally using a constant volume (0.01 ml/g body weight). Disulfiram was suspended in saline containing 0.4% (v/v) Tween 80, and administered subcutaneously.

Statistical Analysis

Swimming activity and locomotor activity were analyzed by one-way analysis of variance (ANOVA) with completely randomized design followed by a two-tailed multiple Student's *t*-test for subsequent multiple comparisons between groups. Differences of $p < 0.05$ were considered significant.

RESULTS

The Effects of Monoamine Synthesis Inhibitors on REMs Deprivation-Induced Increase in Swimming Activity

As previously reported (2), the basal swimming activities in REMs-deprived mice were significantly higher than those in group-housed and isolated animals, $F(5, 84) = 10.5$, $p < 0.01$ (Table 1). α-Methyl-*p*-tyrosine (250 mg/kg), a catecholamine synthesis inhibitor, decreased the swimming activity in REMs-deprived mice ($p < 0.05$). It tended to reduce the swimming activity in the control groups, especially isolated animals, but changes in the activity were not significant

TABLE 1

EFFECTS OF MONOAMINE SYNTHESIS INHIBITORS ON RAPID EYE MOVEMENT SLEEP DEPRIVATION TREATMENT-INDUCED INCREASE IN SWIMMING ACTIVITY

Drugs	Doses	*n*	Swimming Activity (counts/5 min)
α-Methyl-*p*-tyrosine (mg/kg)			
Group-housed	0	15	450 ± 34
	250	15	328 ± 56^{NS}
Isolated	0	15	450 ± 34
	250	16	290 ± 30^{NS}
REMs-deprived	0	15	823 ± 96*†
	250	14	619 ± 70‡
Disulfiram (mg/kg)			
Group-housed	0	15	493 ± 59
	400	15	543 ± 80^{NS}
Isolated	0	15	483 ± 35
	400	15	498 ± 45^{NS}
REMs-deprived	0	15	905 ± 81*†
	400	15	826 ± 66^{NS}
p-Chlorophenylalanine (mg/kg)			
Group-housed	0	15	448 ± 48
	300	15	511 ± 37^{NS}
Isolated	0	15	540 ± 57
	300	15	511 ± 65^{NS}
REMs-deprived	0	15	791 ± 67*†
	300	15	867 ± 66^{NS}

Each value represent the mean ± SEM.
*$p < 0.01$ vs. saline (vehicle)-treated group-housed animals.
†$p < 0.01$ vs. saline (vehicle)-treated isolated animals.
‡$p < 0.05$ vs. saline-treated REMs-deprived animals.
NS: not significant, vs. respective saline (vehicle) control.

($p > 0.05$). Disulfiram (400 mg/kg), a noradrenaline synthesis inhibitor, did not affect the activity in all three groups ($p > 0.05$). Prior treatment with *p*-chlorophenylalanine (300 mg/kg), a 5-hydroxytryptamine synthesis inhibitor, did not alter the swimming activity in REMs-deprived or the control mice ($p > 0.05$).

The Effects of Dopamine Receptor Antagonists on REMs Deprivation-Induced Increase in Swimming Activity

As shown in Table 2, SCH23390 (30 and 100 μg/kg), a selective dopamine D_1 receptor antagonist, did not change the swimming activity in REMs-deprived mice or the control animals ($p > 0.05$). On the other hand, sulpiride (12.5 and 25 mg/kg), a selective dopamine D_2 receptor antagonist, dose-dependently decreased the REMs deprivation treatment-induced increase in swimming activity, $F(8, 134) = 9.43$, $p < 0.01$, but no significant change in swimming activity in the control animals was observed at the same dose range ($p > 0.05$).

The Effect of Sulpiride on Spontaneous Locomotor Activity

As shown in Table 3, spontaneous locomotor activities in group-housed, isolated, and REMs-deprived mice were in the range of 6500–8000 counts. Sulpiride (25 mg/kg) significantly decreased locomotor activity in the control animals, $F(5, 92) = 6.18$, $p < 0.01$, while it did not affect the locomotor activity in REMs-deprived animals ($p > 0.05$).

TABLE 2

EFFECTS OF DOPAMINE RECEPTOR ANTAGONISTS ON RAPID EYE MOVEMENT SLEEP DEPRIVATION-INDUCED INCREASE IN SWIMMING ACTIVITY

Drugs	Doses	*n*	Swimming Activity (counts/5 min)
(+)-SCH23390 (μg/kg)			
Group-housed	0	16	511 ± 73
	30	16	427 ± 32^{NS}
	100	16	345 ± 47^{NS}
Isolated	0	16	531 ± 52
	30	16	435 ± 49^{NS}
	100	16	410 ± 45^{NS}
REMs-deprived	0	16	761 ± 83*†
	30	16	803 ± 75^{NS}
	100	16	644 ± 87^{NS}
(±)-Sulpiride (mg/kg)			
Group-housed	0	16	534 ± 52
	12.5	16	410 ± 48^{NS}
	25	16	497 ± 48^{NS}
Isolated	0	15	521 ± 64
	12.5	16	444 ± 47^{NS}
	25	16	383 ± 44^{NS}
REMs-deprived	0	16	969 ± 88*‡
	12.5	16	781 ± 44§
	25	16	624 ± 99#

Each value represents the mean ± SEM.

*$p < 0.01$ vs. saline-treated group-housed animals. †$p < 0.05$, ‡$p < 0.01$ vs. saline-treated isolated animals. §$p < 0.05$, #$p < 0.01$ vs. saline-treated REMs-deprived animals. NS: not significant, vs. respective saline control.

TABLE 3

EFFECT OF SULPIRIDE ON LOCOMOTOR ACTIVITY

Drugs	Doses	*n*	Locomotor Activity (counts/30 min)
(±)-Sulpiride (mg/kg)			
Group-housed	0	16	6658 ± 440
	25	16	4324 ± 452*
Isolated	0	16	8055 ± 411
	25	16	5074 ± 448*
REMs-deprived	0	17	6991 ± 839
	25	17	6106 ± 462^{NS}

Each value represents the mean ± SEM. *$p < 0.01$ vs. respective saline-treated animals. NS: not significant, vs. respective saline-treated animals.

DISCUSSION

In the present study, α-methyl-*p*-tyrosine, a catecholamine synthesis inhibitor, significantly decreased the REMs deprivation treatment-induced increase in swimming activity in the forced swimming test without producing significant effect on the activity in the control groups, suggesting that endogenous dopamine and/or noradrenaline relates to the REMs deprivation treatment-induced increase in swimming activity. We found that disulfiram, a noradrenaline synthesis inhibitor, did not change the swimming activity in REMs-deprived animals at a dose capable of producing a significant effect on brain noradrenaline level in mice (13,24). These results suggest that the inhibitory effect of α-methyl-*p*-tyrosine on the swimming activity in REMs-deprived animals is due to synthesis inhibition of dopamine rather than noradrenaline, and that endogenous dopamine is involved in the effect of REMs deprivation treatment in the forced swimming test. Our previous findings that adrenoceptor antagonists such as yohimbine, phentolamine, and propranolol did not affect the swimming activity in REMs-deprived mice [(2), Asakura et al., unpublished data], further support a hypothesis that the REMs deprivation-induced increase in swimming activity is not mediated by noradrenergic mechanism. In contrast to our data, Van Luijtelaar and Coenen have reported that noradrenergic mechanism is involved in the anti-immobility effect of REMs deprivation in the forced swimming test (22). The reason for the discrepancy between their and our results remained unclear, but it may be due to the difference in animal species and/or method of REMs deprivation treatment. On the other hand, serotonergic system appears to relate to immobility in the forced swimming test (4,20). However, the present results that *p*-chlorophenylalanine, a serotonin synthesis inhibitor, did not change the swimming activity. The dose of *p*-chlorophenylalanine used in the present study has been shown to significantly decrease brain serotonin level in mice (6,14). Therefore, the present results suggest that serotonergic system in the brain does not play an important role in the REMs deprivation treatment-induced increase in swimming activity.

De Montis et al. (10) have reported that REMs deprivation increases dopamine D_1 receptor density in the mesolimbic area of rats. Moreover, Nikulina et al. (16) and Serra et al. (21) have shown that SKF38393, a selective D_1 agonist, has antidepressant-like effect in the forced swimming test. These findings suggest that the functional change of D_1 receptor may be involved in the REMs deprivation-induced increase in swim-

ming activity. In the present study, however, dopamine D_1 receptor-mediated mechanism does not play an important role in the increase in swimming activity induced by REMs deprivation treatment, since SCH23390 (30 and 100 μg/kg), a selective dopamine D_1 antagonist, did not affect the swimming activity in REMs-deprived animals. The doses used in the present study seem to be enough to determine whether this drug can antagonize the REMs deprivation treatment-induced increase in swimming activity, because 300 μg/kg SCH23390 significantly decreased swimming activity in the control groups as well as REMs-deprived animals (data not shown).

In the present study, sulpiride (25 mg/kg), a selective dopamine D_2 receptor antagonist, significantly inhibited the REMs deprivation treatment-induced increase in swimming activity, without affecting the swimming activity in the control animals. It is possible that such a preferential effect of sulpiride is attributed to difference in its sedative effect on REMs-deprived and the control animals. If this were the case, sedative effect of this dopamine antagonist would be expected to be potentiated by REMs deprivation. However, significant decrease in locomotor activity by sulpiride (25 mg/kg) was observed in the control groups but not in REMs-deprived animals. Therefore, the present findings indicate that dopamine D_2 receptor-mediated mechanism is involved in the increase in swimming activity induced by REMs deprivation treatment.

Cervo and his colleagues (7,8) reported that intraaccumbens injection of sulpiride abolished the anti-immobility effect of desipramine. Moreover, antidepressant drugs such as desipramine and imipramine can inhibit the uptake of dopamine in synaptosomal preparation from limbic system including the nucleus accumbens (9). Taken together, REMs deprivation enhances dopamine stimulation of D_2 receptor in the limbic region, resulting in an increase of the swimming activity. However, there was evidence that the increase in dopamine turnover in the nucleus accumbens of REMs-deprived mice was observed at the time that the REMs deprivation-induced increase in swimming activity returned to the control levels (1,2). Therefore, we need further investigation whether the REMs deprivation-induced increase in swimming activity is mediated by D_2 receptor in the nucleus accumbens or not.

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