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Effect of α_2-Adrenergic Drugs on REM Sleep Deprivation-Induced Increase in Swimming Activity

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ASAKURA, W., K. MATSUMOTO, H. OHTA AND H. WATANABE. *Effect of α_2-adrenergic drugs on REM sleep deprivation-induced increase in swimming activity.* PHARMACOL BIOCHEM BEHAV **46**(1) 111–115, 1993.—Effects of α_2-adrenergic agents on rapid eye movement sleep (REMs) deprivation-induced anti-immobility effect in the forced swimming test (FST) were investigated. Mice were deprived of REMs for 24–72 h by a small pedestal method. Animals that were either group housed or socially isolated during the same period as REMs deprivation were used as the control groups. REMs deprivation for 48 and 72 but not 24 h significantly increased swimming activity without increasing locomotor activity. Clonidine HCl (30–300 μg/kg, IP), an α_2-adrenoceptor agonist, dose-dependently increased swimming activity in group-housed, isolated, and REMs-deprived mice, but the effective doses of clonidine in REMs-deprived mice were lower than those in group-housed or isolated animals. Yohimbine HCl (5 mg/kg, IP), an α_2-adrenoceptor antagonist, blocked the clonidine (300 μg/kg)- but not the REMs deprivation-induced increase in swimming activity. These results suggest that REMs deprivation enhances the sensitivity of the α_2-adrenoceptor and that the increase in swimming activity by REMs deprivation may be mediated by other neuronal mechanisms rather than the α_2-adrenoceptor.

REM sleep deprivation Forced swimming test Swimming activity Clonidine Yohimbine
α_2-Adrenoceptor Locomotor activity Mice

RAPID eye movement sleep (REMs) deprivation has been shown to be clinically effective in depression therapy (27). In rats, this treatment decreases immobility time in the forced swimming test (FST) (4). In neurochemical studies, REMs deprivation increases noradrenaline and/or dopamine turnover in the brains of rats and mice (2,10,15,24,25,30) and decreases the density of the cortical β-adrenoceptor in rats (18). Moreover, clonidine- and apomorphine-induced sedation in rats can be prevented by REMs deprivation (17,26). However, whether the REMs deprivation-induced functional changes in the catecholaminergic system relate to its anti-immobility effect in the FST still remains unknown.

In the FST, noradrenergic systems in the brain, especially α_2-adrenoceptors, appear to play an important role in anti-immobility effect. Clonidine, an α_2-adrenoceptor agonist, was reported to decrease immobility time (7,14,22,28). α_2-Adrenoceptor antagonists, idazoxan and 1-(2-pyrimidinyl)piperazine, were shown to block desipramine-induced decrease in immobility time (6). These results suggest that the functional change of α_2-adrenoceptor may be involved in the REMs deprivation-induced antiimmobility effect.

In the present study, to clarify whether the noradrenergic α_2-mechanism is involved in the REMs deprivation-induced anti-immobility effect we examined the effects of clonidine, an α_2-adrenoceptor agonist, and yohimbine, an α_2-adrenoceptor antagonist, on swimming activity in REMs-deprived mice.

METHOD

Animals

Male ddY mice (5 weeks old, Japan SLC, Inc., Shizuoka, Japan) were used in the experiments. Animals were housed in groups of 20–25 per cage (35 × 30 × 16 cm) for at least 1 week before the experiments. Housing conditions were thermostatically maintained at 24 ± 1°C, with a 12 L : 12 D cycle (light on at 7:30 a.m.). Food and water were given ad lib.

REMs Deprivation

Mice were deprived of REMs by a small pedestal (platform) method as described previously (2). In brief, a small pedestal (4.5 cm high, 1.8 cm diameter) was fixed at the center of the REMs deprivation chamber (20 × 15 × 21 cm) and surrounded by water (3.5 cm deep). Animals were individually

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placed in the chamber and housed for 24–72 h with free access to food and water (REMs-deprived mice). Other groups of mice were either housed in groups of four per Plexiglas cage (25 × 18 × 12 cm) (group-housed mice) or individually (isolated mice) in the cage for the same period as the REMs deprivation.

Apparatus

Scanet SV-10 (Toyo Sangyo Co. Ltd., Toyama, Japan) was used to measure swimming activity in the FST and locomotor activity in mice. The system consisted of a rectangular enclosure (40 × 38 cm), the side walls of which were equipped with 144 pairs of photosensors. Each pair of photosensors was scanned every 0.1 s to detect animal movement. The photosensors were set 8.8 and 2.5 cm high from the floor in the FST and in the measurement of locomotor activity, respectively. Swimming activity and locomotor activity were calculated from the scanning data.

Measurement of Swimming Activity in the FST

A transparent glass cylinder (20 cm high, 8 cm diameter), which contains water (8 cm deep) maintained at 25°C, was fixed at the center of Scanet SV-10. Each mouse was individually placed in the cylinder and forced to swim for 15 min (pretest swimming). After drying, animals were deprived of REMs for 48 h except for specifically stated cases. Immediately after the termination of REMs deprivation, each animal was placed in the cylinder and swimming activity was measured for 5 min (test swimming).

Measurement of Locomotor Activity

A Plexiglas cage (25 × 18 × 24 cm) was fixed at the center of Scanet SV-10. Each mouse was deprived of REMs for 48 h except for specifically stated cases. Immediately after the termination of REMs deprivation, animals were individually placed in the cage and locomotor activity was measured for 30 min.

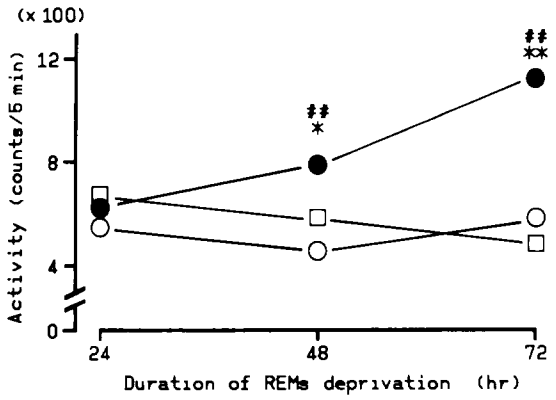

FIG. 1. Effect of rapid eye movement sleep (REMs) deprivation on swimming activity. After the pretest swimming, mice were either group housed (□), socially isolated (○), or deprived of REMs (●) for 24, 48, or 72 h. Immediately after the termination of REMs deprivation, the swimming activity was measured. Each point represents the mean swimming activity obtained from 15–16 animals. $^{**}p < 0.01$, $^{*}p < 0.05$ compared with group-housed mice. $^{\#\#}p < 0.01$ compared with isolated animals (multiple Student's *t*-test).

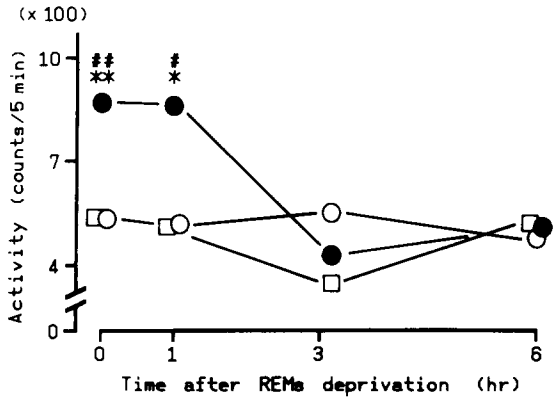

FIG. 2. Time course of the rapid eye movement sleep (REMs) deprivation-induced increase in swimming activity. Mice were either group housed (□), socially isolated (○), or deprived of REMs (●) for 48 h. Immediately or 1, 3, or 6 h after the termination of REMs deprivation, the swimming activity was measured. Each point represents the mean swimming activity obtained from 12 animals. $^{**}p < 0.01$, $^{*}p < 0.05$ compared with group-housed mice. $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ compared with isolated animals (multiple Student's *t*-test).

Drugs

The test drugs were dissolved in saline just before the experiments and IP administered at a constant volume (0.01 ml/g body weight). The drugs used were clonidine HCl (Sigma Chemical Co., St. Louis, MO) and yohimbine HCl (Nacalai Tesque, Inc., Kyoto, Japan).

Statistical Analysis

Swimming activity in the FST and locomotor activity were analyzed with one- or two-way analysis of variance (ANOVA). Significant main effects and treatment interactions were ana-

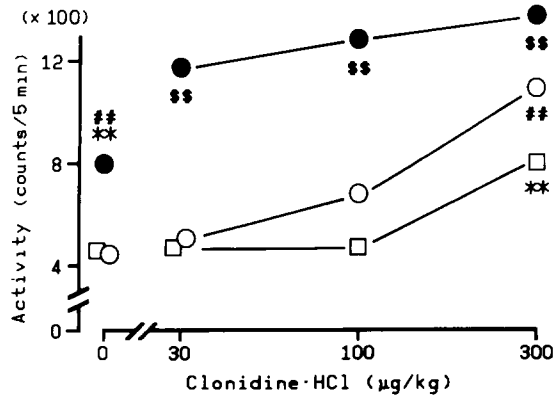

FIG. 3. Effect of clonidine on the rapid eye movement sleep (REMs) deprivation-induced increase in swimming activity. Clonidine HCl (30, 100, and 300 μg/kg) was injected 30 min before the test swimming and mice were either group housed (□), socially isolated (○), or deprived of REMs (●) for 48 h. Each point represents the mean value obtained from 14–16 animals. ANOVA three by four interaction, $F(6, 174) = 2.48$, $p < 0.05$. $^{**}p < 0.01$ and $^{\#\#}p < 0.01$ compared with saline-injected group-housed and isolated mice, respectively. $^{\$\$}p < 0.01$ compared with saline-injected REMs-deprived animals (multiple Student's *t*-test).

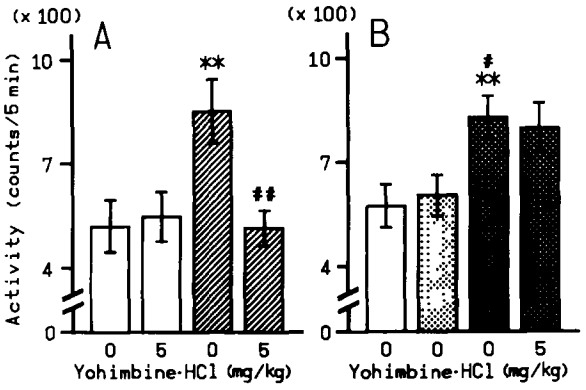

FIG. 4. Effects of yohimbine on the clonidine- and rapid eye movement sleep (REMs) deprivation-induced increase in swimming activity. Yohimbine HCl (5 mg/kg) was injected 60 min before the test swimming. (A). Open and hatched columns represent the data obtained from group-housed mice treated with saline and clonidine (300 μg/kg), respectively. Each value represents the mean swimming activity obtained from 12 mice, the vertical bar representing the SEM. ANOVA two by two interaction, $F(1, 44) = 5.89$, $p < 0.05$. $^{**}p < 0.01$ compared with mice treated with saline alone. $^{\#\#}p < 0.01$ compared with animals treated with clonidine alone (multiple Student's t-test). (B). Open, dotted, and hatched columns represent the data obtained from animals that were either group housed, isolated, or REMs deprived for 48 h, respectively. Each value represents the mean swimming activity obtained from 15-16 mice. $^{**}p < 0.01$ and $^{\#}p < 0.05$ compared with saline-injected group-housed and isolated mice, respectively (multiple Student's t-test).

lyzed further by two-tailed multiple Student's t-test. Differences with $p < 0.05$ were considered statistically significant.

RESULTS

Effect of REMs Deprivation on Swimming Activity

REMs deprivation for 24 h did not affect swimming activity. However, when examined after 48- or 72-h REMs deprivation the activity in REMs-deprived mice was significantly higher than that in group-housed and isolated animals. No significant difference of the activity was observed between group-housed and isolated mice (Fig. 1). When mice were tested immediately or 1 h after the termination of REMs deprivation for 48 h, swimming activity in REMs-deprived mice was significantly higher than that in group-housed and isolated animals. However, the activity in REMs-deprived mice did not differ from that in group-housed and isolated animals 3 or 6 h after the termination of REMs deprivation (Fig. 2).

Effect of Clonidine and Yohimbine on REMs Deprivation-Induced Increase in Swimming Activity

Clonidine (30 and 100 μg/kg), an α_2-adrenoceptor agonist, significantly enhanced the increase in swimming activity in REMs-deprived mice while it did not significantly affect the activity in group-housed or isolated mice. However, when tested at the dose of 300 μg/kg clonidine significantly increased the activity in group-housed and isolated mice (Fig. 3). Yohimbine (5 mg/kg), an α_2-adrenoceptor antagonist, inhibited clonidine (300 μg/kg)-induced increase in swimming activity in group-housed mice without affecting the basal swimming activity (Fig. 4A), while this antagonist did not affect the activity increased by REMs deprivation (Fig. 4B).

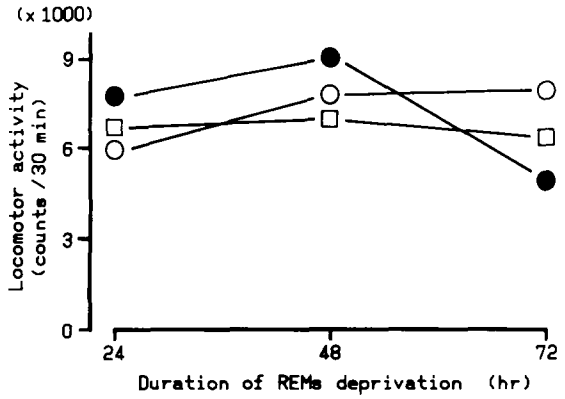

FIG. 5. Effect of rapid eye movement sleep (REMs) deprivation on spontaneous locomotor activity. Mice were either group housed (□), socially isolated (○), or deprived of REMs (●) for 24, 48, or 72 h. Immediately after the termination of REMs deprivation, locomotor activity was measured. Each point represents the mean locomotor activity obtained from 10 animals.

Effect of REMs Deprivation on Locomotor Activity

In group-housed mice, spontaneous locomotor activity for the 30-min observation period was about 7,000 counts. No significant difference of spontaneous locomotor activity among group-housed, isolated, and REMs-deprived mice was observed (Fig. 5). Clonidine (100 and 300 μg/kg) dose dependently and significantly decreased locomotor activity in mice (Fig. 6).

DISCUSSION

The present results clearly demonstrate that REMs deprivation increases swimming activity of mice in the FST. The method to measure the activity in the present FST is similar to that described by De Pablo et al. (9), who have shown that swimming activity negatively correlates with the duration of immobility time. Therefore, the present findings are consistent

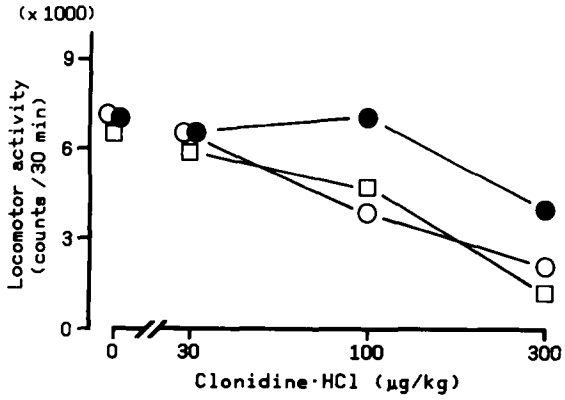

FIG. 6. Effect of clonidine on locomotor activity. Clonidine HCl (30, 100, and 300 μg/kg) was injected 30 min before the experiment, and mice were either group housed (□), socially isolated (○), or deprived of REMs (●) for 48 h. Each point represents the mean value obtained from 16 animals. ANOVA four by three interaction, $F(6, 168) = 1.60$, $p > 0.05$.

with the data reported by other groups that REMs deprivation reduces immobility time in the FST in rats (4).

A parameter in the FST, such as immobility time and/or swimming activity, can be affected by not only antidepressant drugs but also other factors that are not always related to antidepressive effects. For example, drugs such as caffeine and amphetamine that enhance locomotor activity or drugs such as scopolamine that impair memory acquisition and/or consolidation process are reported to have anti-immobility effects (8,21). These findings suggest that the REMs deprivation-induced increase in swimming activity may be ascribed to increase in locomotor activity and/or memory deficit. However, this does not seem to be the case because spontaneous locomotor activities in REMs-deprived mice were not different from those in the control groups. In addition, REMs-deprived mice seem to retain experience of the pretest swimming because the increase in swimming activity in REMs-deprived mice returned to the control levels 3 h after the termination of REMs deprivation.

The stressful manipulation has been reported to reduce immobility time in the FST (1,12,19,23), although these findings are controversial (11,20). Involvement of stress factors cannot be excluded in the present results because animals were limited in their movement within the pedestal area by soaking their tails during the 24- to 72 h-period. Such stressful manipulations as restraint, electric shock, forced shaking, or repeated cold stress are known to change the nociceptive response and/or barbiturate-induced sleeping time in rodents (3,5,13,16,29). However, our previous report (2) demonstrated that REMs deprivation does not affect the latency of nociceptive response in the hot-plate test or the duration of pentobarbital-induced sleep. Therefore, even if the REMs deprivation-induced increase in swimming activity could be due to stress the stress by REMs deprivation seems to have different characteristics from that which had been previously reported to decrease immobility time.

In agreement with previous reports (14), systemic administration of clonidine, an α_2-adrenoceptor agonist, dose dependently increased swimming activity in the FST. Clonidine further enhanced the increase in swimming activity in REMs-deprived mice at doses that did not affect swimming activity in group-housed or isolated animals. On the other hand, REMs deprivation tended to attenuate the sedative effect of clonidine. These results suggest that clonidine enhancement of swimming activity in REMs-deprived mice may be due to an apparent decrease in the sedative effect of clonidine on REMs-deprived mice. However, this does not seem to be the case because, at least at 30 μg/kg, clonidine significantly increased the swimming activity in REMs-deprived mice without inducing sedation in group-housed, isolated, or REMs-deprived animals.

It appears that the α_2-adrenoceptor is associated with anti-immobility effect in the FST. Therefore, it is likely that the REMs deprivation-induced increase in swimming activity in the FST may be mediated by α_2-adrenoceptor stimulation. However, this may be excluded by the facts that yohimbine, an α_2-adrenoceptor antagonist, did not affect the REMs deprivation-induced increase in swimming activity at a dose high enough to block the clonidine-induced increase in the activity. These results suggest that the REMs deprivation-induced increase in swimming activity may be mediated by other neuronal mechanisms rather than the α_2-adrenoceptor.

In conclusion, the present results demonstrate that REMs deprivation increases swimming activity without increasing locomotor activity in mice and enhances an α_2-adrenoceptor sensitivity in the brain. The REMs deprivation-induced increase in swimming activity may be mediated by the changes in other neuronal mechanisms rather than the α_2-adrenoceptor.

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