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Effects of Rapid Eye Movement Sleep Deprivation on the Properties of Striatal Dopaminergic System

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HAMDI, A., J. BROCK, K. ROSS AND C. PRASAD. *Effects of rapid eye movement sleep deprivation on the properties of striatal dopaminergic system.* PHARMACOL BIOCHEM BEHAV 46(4) 863–866, 1993.—Using the water tank procedure, we have examined the effects of rapid eye movement (REM) sleep deprivation and associated stress on the properties of striatal dopaminergic system. While stress decreased the number of D_1 and D_2 dopamine receptors, a combination of REM sleep deprivation attenuated the decrease. The ratio of D_1 to D_2 densities, however, increased on both the stress and REM sleep deprivation groups. In contrast, the number of dopamine uptake sites remained unchanged. The enhanced behavioral responses to dopaminergic stimulants after REM sleep deprivation are discussed.

Rapid eye movement sleep deprivation Rat Dopamine receptors Dopamine uptake sites Striatum

RAPID eye movement (REM) sleep deprivation has been reported to precipitate a variety of behavioral changes in both animals and humans. For example, REM sleep deprivation in rats has been shown to induce increased aggression (14,22), motor activity (1), sexuality (20), self-stimulation (23), and appetite (17). While the neurochemical bases for the above behavioral changes following REM sleep deprivation are not clear, many observations suggest a major role for central dopamine in these processes. These include hyperresponsiveness to dopaminergic agonists (both direct and indirect) after REM sleep deprivation (11,12,27–29) and modulation of REM sleep (decrease and increase) by D_1 receptor agonists and antagonists, respectively (7,25,26). The implication is that REM sleep deprivation-induced facilitation of dopaminergic activity involves a change at the level of the dopamine receptors (5). Therefore, to further understand the role of dopamine in this process, we have studied the effect of REM sleep deprivation on striatal dopaminergic system by examining the properties (B_{max} and K_d) of D_1 dopamine ([^{3}H]SCH-23390 binding) and D_2 dopamine ([^{3}H]YM-09151-2 binding) receptors, and dopamine uptake sites ([^{3}H]GBR-12935 binding) from individual rats.

METHOD

Male, Sprague–Dawley rats (211–270 g; Harlan, Sprague–Dawley) were housed individually at controlled temperature (22–23°C) and 12L : 12D cycles (light on at 7:00 a.m., both before and during the sleep deprivation protocol) with free access to food and water. On the day of the experiment, the rats were randomly divided into four groups (n = 6 rats per group) as follows: A) normal cage control; B) stress-control group, in which the rats were placed on a large pedestal (15 cm in diameter) for 96 h; C) rats kept on a large pedestal for 72 h then moved to a small pedestal (6.5 cm in diameter) for 24 h; and D) the rats were placed on a small pedestal for 96 h. All rats continued to have free access to food and water during the treatments. At the end of the treatments, rats were sacrificed by decapitation during the light cycle, between 11:00 a.m. and 12:00 noon. Brains were removed, frozen on dry ice, and stored at −80°C until the binding assays were performed.

Binding of [^{3}H]YM-09151-2 to D_2 dopamine receptor (24) and [^{3}H]GBR-12935 to dopamine uptake sites (2) was carried out using striatal membranes as described previously (10,13). The striatal membranes were also used to measure [^{3}H]SCH-23390 binding to D_1 dopamine receptor according to the method of Andersen et al. (3) with the following modifications. Briefly, striatal tissue from each rat was homogenized separately in 5 ml of 10 mM imidazole and 1 mM ethylenediamine tetraacetic acid (EDTA) (pH 7.4 at 25°C), using a VirTishear setting at 50 for 20 s and centrifuged at 48,000 × g for 20 min at 4°C. The homogenization and centrifugation was repeated twice. The final pellet was resuspended in 2 ml of 2 mM imidazole and 2 mM ethylene glycol-bis(B-amino-

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ethylether)*N,N',N'*-tetraacetic acid (EGTA) (pH 7.4 at 25°C). The binding assay was performed in a polystyrene tube (12 × 75 mm) containing 16.67 mM imidazole (pH 7.4 at 25°C), 16.67 mM theophylline, 1 mM EGTA, 1 mM $MgSO_4$, striatal homogenate (150 μg protein), and [^{3}H]SCH-23390 (75 to 4800 pM) in a total volume of 1 ml by incubating at 30°C for 90 min. Rapid filtration under vacuum through Whatman GF/F filters separated the membrane-bound radioactivity from the free. The filters were rinsed two times with 10 ml of ice-cold 0.9% NaCl and counted in 10 ml of Scintiverse II scintillation fluid in a Beckman liquid scintillation counter at an efficiency of 55–60%. The nonspecific binding was determined in the presence of 1 μM *cis*-flupentixol.

The specific binding data from each treatment group were analyzed separately using a nonlinear regression analysis (the computer program GraphPAD, ISI Software, Philadelphia, PA) to give the estimates of the maximal density (B_{max}) and the affinity (K_d) values. Protein determinations were performed using bicinchoninic acid (BCA) protein assay kit (Pierce Chemical Co.).

To examine the effect of REM sleep deprivation on B_{max} and K_d of the receptors, the data were analyzed statistically by the analysis of variance (ANOVA) followed by nonpaired *t*-test for group comparison. All data are presented as mean + SD.

The "inverted flower pot" or "platform" technique, which consists of keeping the animals on a small platform (6.5 cm diameter) surrounded by water (20°C), is the most widely used method for REM sleep deprivation in rats (8,15,21), without the need for EEG monitoring (30). It has been shown by electroencephalographic (EEG) recording that rats residing in the water tank on a small platform experienced significantly less REM sleep after 96 h than rats residing on a large platform, whereas the large platform animals experienced REM sleep that was not different from baseline levels (19). While this procedure is specific for REM sleep deprivation (16), it also causes stress that results from isolation, motor restriction, novel environment, and falling into water. Therefore, it is essential to control for the stress response when examining the effect of REM sleep deprivation on neurochemical changes. To this end, we have introduced a stress-control group (treatment B) where animals are housed on a pedestal (15 cm diameter) large enough not to interfere with REM sleep. In the present study, we also included a group of rats that resided on large pedestals for the first 72 h of the 96-h period, then were transferred to small pedestals for the remaining 24 h. This was done to determine whether the effects of more acute REM sleep deprivation could be detected if the animals were allowed more time to acclimate to the novelty of the water tank environment.

RESULTS

The specific [^{3}H]SCH-23390, [^{3}H]YM-09151-2, and [^{3}H]GBR-12935 binding to D_1 and D_2 dopamine receptors and dopamine uptake sites, respectively, was of high affinity and saturable in all groups. Saturation curves were analyzed by nonlinear regression and modeled first to a one-site and then a two-site model. The two-site model was accepted only if the addition of the second site reduced the residual sums of squares of the deviations from regression, as judged by a significant *F* statistic. The above procedures and Scatchard analysis revealed only a single class of binding sites in all radioligand assays in all groups.

The data presented in Table 1 show that the stress of being housed on a large pedestal with no REM sleep deprivation (treatment B) led to a significant decrease in the densities of both D_1 and D_2 dopamine receptors when compared to the cage control (treatment A) group. Since the decrement in the B_{max} of D_1 receptor (−9%) was much smaller than D_2 receptor (−34%), the D_1 to D_2 ratio increased by 38% in the stress group. However, the density of [^{3}H]GBR-12935 binding that labels presynaptic dopamine uptake sites did not change in response to stress. The affinity of D_2 receptor increased by a factor of at least 2 (decreased K_d) in the stressed group with no apparent changes in the affinity for the other two binding sites (Table 1).

Groups C and D (24 and 96 h of REM sleep deprivation, respectively), compared with group B, showed a significant increase in the densities of both D_1 and D_2 dopamine receptors

TABLE 1

EFFECT OF REM SLEEP DEPRIVATION ON THE PROPERTIES OF STRIATAL DOPAMINERGIC NEURONS

Binding Sites		Treatment Groups			
		A	B	C	D
[^{3}H]GBR-12935	B_{max}	26.4 ± 3.0	28.8 ± 2.1	26.7 ± 2.0	27.0 ± 2.3
	K_d	7.4 ± 1.2	6.4 ± 0.7	8.0 ± 0.6†	8.7 ± 1.0†
[^{3}H]SCH-23390	B_{max}	1285 ± 14	1172 ± 10*	1527 ± 18*†	1296 ± 33†
	K_d	824 ± 13	800 ± 19	891 ± 22*†	771 ± 27*
[^{3}H]YM-09151-2	B_{max}	606 ± 14	402 ± 12*	450 ± 11*†	426 ± 13*†
	K_d	86 ± 8	41 ± 2*	54 ± 4*†	47 ± 2*†
D1/D2 ratio	B_{max}	2.12 ± 0.05	2.92 ± 0.09*	3.40 ± 0.05*†	3.04 ± 0.06*

All data are presented as mean ± SD (n = 6). The units for B_{max} and K_d are pmol/mg protein and nM, respectively, for [^{3}H]GBR-12935 binding sites only. For all other binding sites, however, the units for B_{max} and K_d are fmol/mg protein and pM, respectively. The treatment groups A, B, C, and D are described in detail in the text. Group A, nonstressed, no REM sleep deprivation; group B, stressed, no REM sleep deprivation; group C, stressed, REM sleep deprived for 1 day; group D, REM sleep deprived for 96 h. The effect of different treatments on the properties of different binding sites was analyzed by ANOVA.

*†The ANOVA followed by nonpaired *t*-test for the comparison between two treatments: $p < 0.05$ compared to groups A and B, respectively.

(Table 1). However, the B_{max} for dopamine uptake sites remained unchanged. While the affinity of D_2 dopamine receptors and dopamine uptake sites decreased in both groups (C and D), the affinity of D_1 dopamine receptors decreased in group C only, compared with group B.

DISCUSSION

In the present study, while 24 h of REM sleep deprivation (group C) increased both B_{max} and K_d of the striatal D_1 and D_2 dopamine receptors, as well as the K_d of the dopamine uptake sites (compared to stress group B), the 96 h of REM sleep deprivation (group D, compared to group B) increased the B_{max} for D_1 and D_2 dopamine receptors, as well as the K_d for D_2 dopamine receptors and the dopamine uptake sites (Table 1). Previous investigators reported an increase in the B_{max} of striatal D_2 dopamine receptors after 24 h of sleep deprivation (31). Although an increase in K_d was recently reported for the rat striatal D_2 dopamine receptors after 24 h of sleep deprivation (compared to 96-h stress group) (33), a comparison to the present data is difficult because our 24-h REM sleep-deprived rats resided on 15-cm diameter (control) pedestals for 72 h before being placed on the small pedestals for REM sleep deprivation. Another study using [^{3}H]spiroperidol to label striatal D_2 dopamine receptors reported that B_{max} and K_d for these receptors were unchanged after 96 h of REM sleep deprivation (9). The high concentrations of [^{3}H]spiroperidol that had been used by binding to sites other than the D_2 dopamine receptors could be the reason for the apparent discrepancy.

The decrease in affinity (increase in K_d) for dopamine uptake sites observed in the REM sleep-deprived rats (groups C and D) seems to be specific for REM sleep deprivation, since the K_d in the stress-control group (B), which had normal amounts of sleep, was not different from the K_d in the home cage control (group A). The mechanism(s) that underly changes in receptor affinity are not known, and the biological significance of slight changes in receptor or reuptake site affinity remains unclear. However, a decrease in affinity for dopamine uptake sites may be expected to enhance dopaminergic transmission.

Several studies have suggested a complex functional interaction between D_1 and D_2 dopamine receptors in the expression of many dopamine-mediated behaviors [for review see (6)]. The change in the ratio of D_1 to D_2 dopamine receptors, for example, may determine the type and the intensity of dopaminergic behaviors in animals (4). The most important observations made from the present study were the decreases in the affinities of D_2 and the increases in B_{max} for D_1 and D_2 dopamine receptors after 24 h (group C) and 96 h (group D) of REM sleep deprivation when compared to the stress-control group (group B). These data confirm the hypothesis that was originally proposed by others (5,29), and are consistent with the behavioral effects of dopaminergic stimulants in the REM sleep-deprived animals (11,12,27–29). Furthermore, increasing the ratio of D_1 to D_2 receptor densities after 24 h (group C) of REM sleep deprivation in comparison to the stress-control (group B), as shown in the present study, will enhance dopaminergic transmissions, and thus facilitate the expression of dopaminergic behavior (18). However, it also likely that additional changes in the dopaminergic neurotransmission at the postreceptor level in response to REM sleep deprivation may be responsible for enhancement in the dopaminergic transmission. REM sleep deprivation is also known to induce changes in other neurotransmitter systems (noradrenergic and serotonergic) (32), which in turn may modulate dopaminergic transmission in an indirect or direct fashion.

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