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Decreased Activity of Striatal Monoamine Oxidase B After Rapid Eye Movement (REM) Sleep Deprivation in Rats

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PEREZ, N. M., R. MATTEI AND M. A. C. BENEDITO. *Decreased activity of striatal monoamine oxidase B after Rapid Eye Movement (REM) sleep deprivation in rats.* PHARMACOL BIOCHEM BEHAV **60**(1) 33–37, 1998.—The striatum seems to be the main brain region involved in stereotyped behavior induced by dopaminergic agonists. Rapid eye movement (REM) sleep deprivation increases dopaminergic agonist-induced stereotypy and produces biochemical changes in striatal dopaminergic neurotransmission. However, the mechanism underlying the increased dopaminergic sensitivity induced by REM sleep deprivation has not been elucidated. In an attempt to determine some of the biochemical changes in striatal dopaminergic neurotransmission that could contribute to REM sleep deprivation effects, we measured the activity of monoamine oxidase (MAO) A and B, the enzymes responsible for dopamine and beta-phenylethylamine (beta-PEA) deamination in striatum. Male adult rats were deprived of REM sleep for 96 h by the flower-pot technique. MAO A and B were assayed radioisotopically in the mitochondrial fraction by standard laboratory procedures, using [^{14}C]-5-hydroxytryptamine (5-HT) and [^{14}C]-beta-phenylethylamine (beta-PEA), as substrates for MAO A and MAO B, respectively. The results showed no significant statistical differences in striatal MAO A activity, whereas a significant decrease in MAO B activity was observed. The results are discussed in terms of the possible involvement of beta-PEA, a striatal endogenous trace amine, which potentiates dopaminergic neurotransmission and may participate in the increased dopaminergic sensitivity observed after REM sleep deprivation. © 1998 Elsevier Science Inc.

REM sleep deprivation Striatum Monoamine oxidases Tyrosine hydroxylase

IN rats, rapid eye movement (REM) sleep deprivation induces an increase in stereotypy produced by apomorphine, a mixed D_1-D_2 dopaminergic agonist (24). This effect of REM sleep deprivation on dopaminergic neurotransmission may occur at the level of the striatum, because stereotyped behavior seems to be induced by dopaminergic stimulation of this brain area (10).

There have been several attempts to establish a relationship between REM sleep deprivation and dopaminergic neurotransmission. These studies include the demonstration of increased levels of striatal dopamine (DA) (7) and dihydroxyphenylacetic acid (DOPAC), a DA metabolite (6) after REM sleep deprivation. There is, however, a negative report (25) showing no change in total brain levels of DOPAC and homovanillic acid (HVA), another DA metabolite. No change was observed in tyrosine hydroxylase (L-tyrosine, tetrahydropteridine: oxygen oxidoreductase E.C. 1.14.16.2) activity (6), the limiting step in DA synthesis (15). The data on striatal dopaminergic receptors after REM sleep deprivation are controversial. Thus, decrease (29), increase (18), or no change (6) in D_2 receptor binding have been described. These available biochemical data show that the exact mechanisms underlying the higher behavioral dopaminergic sensitivity, reported to be induced by REM sleep deprivation, needs further experimentation.

Monoamine oxidase (MAO) (monoamine: oxygen oxidoreductase E.C. 1.4.3.4) A and B are enzymes involved in the inactivation of DA in vitro (1,27). In rat striatum it was shown in vivo that DA is preferentially metabolized by MAO A (3,5,9), whereas MAO B metabolizes beta-phenylethylamine (beta-PEA) (2), an endogenous striatal trace amine that potentiates dopaminergic neurotransmission [for review, see (19)]. Therefore, decreased activity of MAO A and B

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would result in higher availability of DA and/or beta-PEA, which could lead to an increase in dopaminergic neurotransmission.

The objective of the present study was to test the hypothesis that REM sleep deprivation induces a decrease in striatal MAO activity, thus increasing dopaminergic striatal neurotransmission. We also assayed striatal tyrosine hydroxylase because an increase in the activity of this enzyme could also contribute to a higher intracellular DA availability to be released at the synaptic cleft (15).

METHOD

Subjects

Three-month-old male Wistar rats (250–300 g) from our own colony were used in the experiments. After weaning, rats were kept in wire-mesh cages (three rats/cage) and had free access to food (Purina lab chow) and tap water. The rats were kept in a room with controlled temperature (24°C) and light–dark cycle (lights on from 0700 to 1900 h).

REM Sleep Deprivation

Rats were deprived of REM sleep by the flower-pot technique as implemented in our laboratory (24,29). Three groups of rats (six animals/group) for each assay were used in the experiments: a control group that consisted of rats kept in their home cages, a large platform control group (14 cm in diameter, surrounded by water up to 2 cm from the top) to control the stress of the procedure of REM sleep deprivation, and a REM sleep-deprived group. REM sleep deprivation was performed by keeping the rats over a small platform (6.5 cm in diameter) surrounded by water up to 2 cm from the top. During the deprivation period all groups were kept in the same room.

All rats had free access to food and water until they were sacrificed. REM sleep deprivation started in the morning (0900 h) and all rats were sacrificed 96 h later.

Preparation of Homogenates

At the end of the 96-h period of REM sleep deprivation, one rat at a time was brought to another room and then sacrificed by decapitation. The brains were excised rapidly and striatum dissected over a Petri dish, kept cold with crushed ice.

The homogenates for the tyrosine hydroxylase assay were prepared in 10 mM potassium phosphate buffer pH 7.0 (10 mg tissue/300 μl) using an Ultraturrax apparatus. After homogenization, the samples were centrifuged for 5 min at 900 × *g* at 0°C and the supernatant collected, and kept at −70°C until assayed. All steps were carried out with materials kept at 4°C.

The homogenates for MAO A and B assays were prepared in a glass tube using cold 0.32 M sucrose buffered with Tris/HCl (pH 7.0). Striatum homogenates (10% W/V) were obtained using a glass homogenizer tube and a motor-driven Teflon pestle. The mitochondrial fraction was obtained according to De Robertis et al. (4). Homogenates were centrifuged at 900 × *g* for 10 min at 0°C. The sediment was discarded and the supernatant was centrifuged at 11,500 × *g* for 20 min at 4°C. After discarding the supernatant the pellet was resuspended in sucrose and centrifuged again. The sediment (mitochondrial fraction) was kept at −20°C until it was analysed.

Tyrosine Hydroxylase Assay

Evaluation of tyrosine hydroxylase activity was carried out according to the method of Reinhard et al. (21) as modified by Sonsalla et al. (22). The method is based on the measurement of the [^{3}H]-H_2O formed after tyrosine hydroxylation. Forty microliters of homogenate and 50 μl of buffer-substrate containing 0.3 μCi of [^{3}H-3,5]-l-tyrosine, 1.5 μl of l-tyrosine hydrochloride 1 mM, 10 μl of 1 M potassium phosphate buffer pH 6.1, 1 μl of catalase (43 mg/ml), 1 μl of 0.5 M dithiothreitol, and 36.2 μl of bidistilled water were preincubated at 37°C in a water shaking bath for 5 min. Thereafter, 10 μl of 6(R)-L-erythro-5,6,7,8-tetrahydrobiopterin (BH4) 10 mM was added and the incubation continued for 20 min. Blanks were obtained by substituting water for BH4. After incubation, 1 ml of an acidified charcoal suspension was added to the tubes and they were vigorously vortexed. Tubes were centrifuged at 900 × *g* for 5 min and an aliquot from the supernatant (500 μl) was transferred to scintillation counting vials and 5 ml of Aquasol II (NEN, Boston, MA) were added.

MAO A and B Assays

MAO A and B were assayed according to the method of Wurtman and Axelrod (26) with modifications. Tissue pellets (mitochondrial fraction) were resuspended in 100 mM potassium phosphate buffer pH 7.4. [^{14}C]-5-hydroxytryptamine (5-HT), isotopically diluted with cold 5-HT, was used to evaluate MAO A activity (50 μM final concentration, s.a. 3.5 mCi/mmol), and [^{14}C]-beta-phenylethylamine (beta-PEA), isotopically diluted with cold beta-PEA, was used as substrate for MAO B (2 μM final concentration, s.a. 28 mCi/mmol). For MAO A assay, 20 μl of homogenate was added to 100 μl of potassium phosphate buffer containing [^{14}C]-5-HT. For MAO B assay, 20 μl of [^{14}C]-beta-PEA was added to 20 μl of homogenate mixed with 100 μl of buffer. Incubation time at 37°C in a water shaking bath was 20 min for MAO A and 5 min for MAO B. Incubation was interrupted by adding 20 μl of 3 M HCl to MAO A and B samples. MAO A metabolites were extracted in 1 ml of a mixture of toluene:ethylacetate [1:1] saturated with water after the acid was added and the tubes vortexed. MAO B metabolites were extracted with 3 ml of toluene. To extract metabolites, MAO A and B samples were vortexed vigorously for exactly 10 s. MAO A and B samples were centrifuged at 600 × *g* at 0°C for 2 min and the organic layer was transferred to scintillation counting vials containing 10 ml of Aquasol II after freezing the aqueous phase using dry ice.

Blanks for all enzymatic assays were run in duplicate and for MAO A and B buffer was used instead of tissue homogenate. All enzyme activity samples were run in triplicate and in the linear range in relation to protein concentration and incubation time. Samples were counted in a Beckman LS-300 liquid scintilation counter with 45% efficiency for [^{3}H] and 95% for [^{14}C]. Specific activity of the enzymes were expressed in pmol of [^{3}H]-H_2O formed/mg protein/min for tyrosine hydroxylase and in pmol of metabolite formed/mg protein/min for MAO A and B.

Protein levels were determined according to Lowry et al. (14) using bovine serum albumin as standard.

Reagents

All reagents used were of analytical grade and were obtained from Sigma Chemical Corporation (St. Louis, MO). Bidistilled water was used to prepare reagents. [^{3}H-3,5]-l-tyrosine (s.a. 50 Ci/mmol), [^{14}C]-5-HT (s.a. 53.5 mCi/mmol) and [^{14}C]-beta-PEA (s.a. 56 mCi/mmol) were from New England Nuclear (Boston, MA).

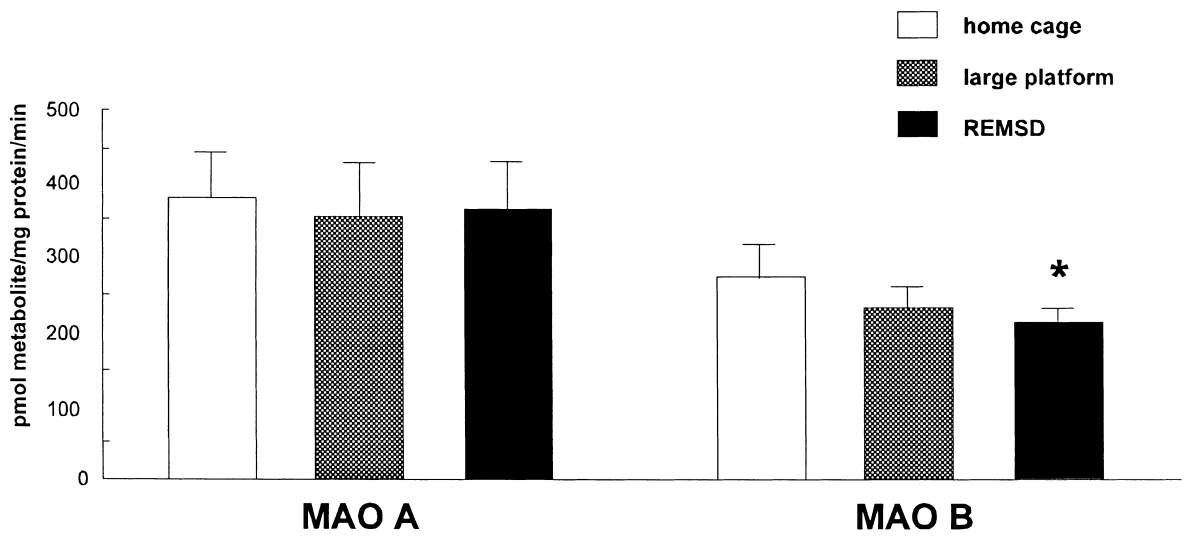

FIG. 1. Striatal MAO A and B activity in REM sleep-deprived rats (REMSD). The results are expressed as mean ± SD from six rats in each group. *Differs from home cage, $p < 0.05$.

Statistical Analysis

One-way analysis of variance (one-way ANOVA) followed by post hoc Scheffe's test were used for statistical analysis. The level of significance was set at a $p \leq 0.05$, one tailed.

RESULTS

As can be seen in Fig. 1, there was a statistically significant change in striatal MAO B activity [one-way ANOVA, $F(2, 15) = 4.03$, $p < 0.05$]. Further statistical analysis showed that 96 h of REM sleep deprivation induced a significant decrease in the activity of striatal MAO B (Scheffe's test, $p < 0.05$) when compared only to the home cage control group. There was no change in the activity of striatal MAO A [one-way ANOVA, $F(2, 15) = 0.91$, $p > 0.05$]. REM sleep deprivation also did not result in a significant change of the activity of striatal tyrosine hydroxylase (Fig. 2) [one-way ANOVA, $F(2, 15) = 2.07$, $p > 0.05$].

DISCUSSION

The results obtained in this study showed that 96 h of REM sleep deprivation did not change the activity of striatal tyrosine hydroxylase, the rate-limiting step in the synthesis of dopamine (15). This result is in accordance with previous data using a different assay method (6). An increase in total striatal DA levels after REM sleep deprivation (7) could suggest an increase in DA synthesis. Striatal tyrosine hydroxylase has distinct forms (soluble and membrane bound) having different affinities for the substrate and its cofactor (11,12). We assayed tyrosine hydroxylase in total striatal homogenates and have not run kinetic studies. Therefore, in view of the com-

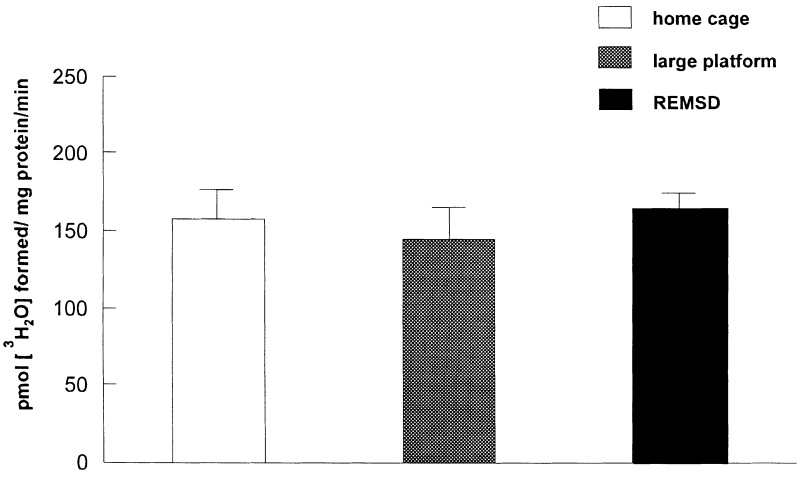

FIG. 2. Striatal tyrosine hydroxylase activity in REM sleep-deprived rats (REMSD). The results are expressed as mean ± SD from six rats in each group.

plexity of this enzyme we can not completely rule out a possible change after REM sleep deprivation. We also failed to observe a significant difference in the activity of striatal MAO A after 96 h of REM sleep deprivation. In the striatum and in vivo, DA is preferentially metabolized by MAO A and to a lesser extent by MAO B (3,5,9). Thus, the lack of alteration in striatal MAO A activity parallels previous data showing no change in whole-brain DOPAC and HVA levels after REM sleep deprivation (25). However, previously reported was an increase in striatal DA and DOPAC levels after REM sleep deprivation (6,7). This increase in substrate (DA) availability and metabolite (DOPAC) levels could indicate a higher MAO A activity in vivo. The lack of change in enzyme activity in vitro obtained in our study may suggest that in vivo the number of available MAO A molecules exceeds those needed for deamination of the increased levels of DA. We used 5-HT as the substrate for striatal MAO A activity instead of DA because in vitro, DA shows no specificity for MAO A (1,27); moreover, we assayed MAO A using a 5-HT concentration near the apparent K_m to avoid a contribution of MAO B to 5-HT deamination. Taking this into account, striatal MAO A activity after REM sleep deprivation does not seem to be changed.

REM sleep deprivation induced a significant decrease in the activity of striatal MAO B. In the striatum, MAO B preferentially deaminates beta-PEA (1), an endogenous substance that appears to possess a modulatory role in striatal dopaminergic neurotransmission [see (19) for recent review]. Several studies suggest a strong relationship between striatal beta-PEA levels and dopaminergic function, indicating that beta-PEA potentiates dopaminergic neurotransmission (19). Therefore, a decrease in MAO B activity induced by REM sleep deprivation could lead to an increase in striatal beta-PEA levels, which would, in turn, increase dopaminergic neurotransmission. It is worth mentioning that beta-PEA alone or in combination with a MAO B inhibitor induces stereotyped behavior in rats (17). Thus, the decrease in striatal MAO B activity observed after REM sleep deprivation together with these data strenghtens our assumption of an involvement of beta-PEA in the higher sensitivity to dopaminergic agonists induced by REM sleep deprivation. This is a possibility to be explored in further experiments in an attempt to understand the mechanisms underlying the increased behavioral dopaminergic sensitivity (24) and decreased MAO B activity observed in REM sleep deprived rats (present results).

Dopamine seems to control striatal beta-PEA synthesis (8), and this effect is mediated by presynaptic D_1 and D_2 receptors, because antagonists of these receptors increase beta-PEA synthesis as a consequence of an increase in aromatic L-amino acid decarboxylase activity, the rate-limiting step in the synthesis of beta-PEA (28). Therefore, it is possible that the decrease in D_2 receptors (29), in addition to the decrease in MAO B activity (present results) after REM sleep deprivation could induce higher beta-PEA striatal levels. Increased striatal beta-PEA levels might, in turn, be involved in the higher sensitivity to dopaminergic agonists observed after REM sleep deprivation (24).

REM sleep deprivation involves quite a high degree of stress and the inclusion of a control group of animals, kept over large platforms, has been used to overcome this problem. Mendelson et al. (16) showed that rats kept over large platforms are also deprived of REM sleep at the beginning of the procedure, but later showed no significant difference from the home cage controls. Recently, however, Landis (13) demonstrated that after 96 h of REM sleep deprivation, rats kept over large platforms are also deprived of REM sleep through the entire period of deprivation compared to baseline values, although less than the rats kept over small platforms. These data indicate that the large platform group included in our experimental design does not seem to constitute a suitable control for the procedure to induce REM sleep deprivation and also may explain the lack of difference in MAO B activity between large platform and REM-sleep deprived group observed in our study (Fig. 1).

Thakkar and Mallick (23) measured MAO A and B activity in some regions (cerebrum, cerebellum, brainstem) of the rat's brain after 96 h of REM sleep deprivation. They used a spectrophotometric method to estimate MAO activity and specific inhibitors to differentiate MAO A from MAO B. They included in their study a large platform control group, as we did in the present study, and their results showed no significant differences in MAO B activity in the large platform control group and REM sleep-deprived rats when compared to the home cage control group. We have also measured MAO A and B activity in several discrete rat brain areas (brainstem, pons, medulla oblongata, hypothalamus, and hippocampus) after 96 h of REM sleep deprivation using a radioisotopic method (20). We have also not found any significant difference in MAO B activity in the brain areas assayed. Taken together, these results may indicate a specific effect of REM sleep deprivation on striatal MAO B activity.

In conclusion, REM sleep deprivation induces a decrease in striatal MAO B activity, and this decrease may lead to an increase in striatal beta-PEA levels that could potentiate dopaminergic neurotransmission after REM sleep deprivation.

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