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Activities of Monoamine Oxidase (MAO) A and B in Discrete Regions of Rat Brain After Rapid Eye Movement (REM) Sleep Deprivation

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PEREZ, N. M. AND M. A. C. BENEDITO. *Activities of monoamine oxidase (MAO) A and B in discrete regions of rat brain after rapid eye movement (REM) sleep deprivation.* PHARMACOL BIOCHEM BEHAV **58**(2) 605–608, 1997.—Rapid eye movement (REM) sleep deprivation increases monoaminergic (noradrenergic and serotonergic) turnover and their metabolites in whole brain of rats. This increase in metabolites may indicate increased activity of the enzymes responsible for the inactivation of monoamines. To test this hypothesis, we assayed the activity of monoamineoxidases (MAOs) A and B in hippocampus, hypothalamus, brainstem and its divisions pons and medulla oblongata in rats deprived of REM sleep for 96h. REM sleep deprivation was carried out by the flower-pot technique. A control group remained in their home cages. MAO A was assayed by using [^{14}C]-5-hydroxytryptamine as the substrate (50 μM final concentration) and MAO B by using [^{14}C]-β-phenylethylamine (2 μM final concentration). The enzymes were assayed in the mitochondrial fraction.The results obtained showed that a significant decrease in the activity of MAO A was obtained in the brainstem and an increase in medulla oblongata and no statistical differences in the activity of MAO B in brainstem, pons and medulla oblongata and MAO A in pons; there were also no differences in the activities of both MAO A and B in hippocampus and hypothalamus. Although our results confirmed previous data regarding changes in MAO A activity in brainstem and medulla oblongata, they did not confirm our hypothesis that the increase in monamine turnover and metabolites in the brain would be the result of increased MAO activity. © 1997 Elsevier Science Inc.

REM sleep deprivation Monamine oxidases Brain regions

RAPID eye movement (REM) sleep deprivation induces a presynaptic increase in monoaminergic (noradrenergic and serotonergic) neurotransmission in the rat brain. This increase is shown by an increase in turnover measured by labeling the internal amine pools with tritiated monoamines or their precursors (6,13,18,19) or by measuring the increase in their metabolite levels (2,25). Higher presynaptic activity would require a change in neurotransmitter metabolism within the neuron to maintain it. Thus, REM sleep deprivation should induce a change in the rate of neurotransmitter synthesis and degradation and a change in the activity of the enzymes involved in these processes.

Monoamine oxidase (MAO) A and B are enzymes involved in the inactivation of noradrenaline (NA) and serotonin (5-HT) in the brain (5,8,28). Chevillard et al. (3) showed a diurnal variation in MAO activity in rat locus coeruleus (LC), indicating that the activity of these enzymes can be increased or lowered. Data in the literature show a concomitant increase in serotonin turnover rate and MAO activity induced by the administration of thallium in pons of rats (16). These data indicate that the activity of MAO can be up- or downregulated and that there is a relationship between monoamine turnover rate and MAO activity.

We examined whether the observed increase in monoamine turnover and metabolites could be the consequence of an increase in the activity of MAO A and/or B after REM sleep deprivation. There is only one report on MAO activity after REM sleep deprivation in the literature. Thakkar and Mallick (21) studied MAO activity in some brain regions after 48 or 96 h of REM sleep deprivation. They used a spectrophotometric method to assay enzyme activity. Therefore, by using a radioisotopic method (22) and by evaluating other brain

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regions, our study could add more information on MAO activity after REM sleep deprivation.

METHOD

Subjects

Wistar male rats, 3 months old, weighing 250–300 g, from our own breeding colony were used in the experiments. After weaning, rats were kept in wire cages (3 rats/cage) with free access to tap water and food (Purina lab chow) until they were killed. The rats were maintained in a room with controlled temperature (24°C) and light–dark cycle (lights on from 07:00 AM to 07:00 PM).

REM Sleep Deprivation

Two experimental groups were used. The control group consisted of rats kept individually housed for 96 h, and the REM-sleep-deprived group consisted of rats kept on top of a small platform (5.5 cm in diameter) surrounded by water up to 2 cm from the top (23). REM sleep deprivation started in the morning (around 09:00 AM), and all animals were killed 96 h later.

Brain Regions Assayed

The activity of MAO A and B was assayed in brain regions that receive monoaminergic inputs (hippocampus and hypothalamus). Brainstem and its anatomical divisions (pons and medulla oblongata) were included because MAO A and B were previously assayed in these brain regions after REM sleep deprivation (21) and because this is where noradrenergic and serotonergic nuclei are located. Moreover, they seem to have a modulatory role in REM sleep (7).

Homogenates Preparation

At the end of a 96-h deprivation period, the rats were transferred one at a time to another room and killed. Brains were excised out rapidly, and the regions to be examined were grossly dissected. Each brain region was weighed and transferred to a glass homogenizer tube containing 0.32 M sucrose buffered with Tris/HCl, pH 7. Tissue homogenates (10% W/V) were prepared by using a glass homogeneizing tube in a teflon pestle motor driven homogeneizer. Mitochondrial fraction was obtained according to De Robertis et al. (4). Homogenates were centrifuged at 900 *g* for 10 min at O°C. 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. Finally, after discarding the supernatant, the sediments (mitochondrial fraction) were kept at −20°C until they were analyzed. All materials used during homogenate preparation were kept at 4°C in an ice bath.

MAO A and B Assays

MAO A and B were assayed according to the method of Wurtman and Axelrod (26), with modifications. Tissue pellets were resuspended in 100 mM potassium phosphate buffer, pH 7.4 [^{14}C]-5-hydroxytryptamine, isotopically diluted with cold 5-HT, was used as substrate for MAO A activity (50 μM final concentration, s.a. 3.5 mCi/mmol) (1) and [^{14}C]-β-phenylethylamine (β-PEA), isotopically diluted with cold β-PEA, was used as substrate for MAO B activity (2 μM final concentration, s.a. 28 mCi/mmol) (27). For MAO A assay, 20 μl of homogenate were added to 100 μl of potassium phosphate buffer containing [^{14}C]-5-HT. For MAO B assay, 20 μl of [^{14}C]-β-PEA were added to 20 μl of homogenate mixed with 100 μl of buffer. Incubation time at 37°C in a water shaking bath lasted 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 tubes vortexed. MAO B metabolites were extracted with 3 ml of toluene. To extract metabolites, MAO A and MAO B samples were vortexed vigorously for exactly 10 s. MAO A and B samples were centrifuged at 600 *g* 0°C for 2 min and the organic layer was transferred to vials containing 10 ml of Aquasol after freezing the aqueous phase using dry ice. Samples were counted in a liquid scintillation counter with 95% efficiency for [^{14}C]. More than 90% of the metabolites were extracted in the first extraction (22). Blanks for both enzymes were run in duplicate and buffer was used instead of tissue homogenate. Enzyme activity samples were run in triplicate. Both assays for MAO A and MAO B were run in the linear range in relation to protein concentration and incubation time. Activity of the enzymes was expressed in picomoles of metabolites formed/milligram protein/minute. Proteins were determined according to Lowry et al. (12), using bovine albumin as standard. When needed, samples and reagents were kept at 4°C with crushed ice.

Reagents

All reagents used were of analytical grade and were obtained from Sigma Chemical Corporation (St. Louis, MO). Water was bidistilled in an all-glass apparatus. [^{14}C]-5HT (s.a. 53.5 mCi/mmol) and [^{14}C]-β-PEA (s.a. 56 mCi/mmol) were obtained from New England Nuclear (Boston, MA).

Statistical Analysis

Data were statistically analyzed using Student's *t*-test, with the level of significance set at $p \leqslant 0.05$.

RESULTS

REM sleep deprivation resulted in a decrease in MAO A activity in brainstem ($t = 2.10$, df = 10; $p < 0.05$) and an increase in medulla oblongata ($t = 1.83$, $df = 10$; $p < 0.05$) (Table 1). Enzyme activities of deprived and control animals were similar in pons, hypothalamus and hippocampus. There were no statistical differences in MAO B activity in any of the brain regions assayed after REM sleep deprivation (Table 2).

TABLE 1

MAO A ACTIVITY IN DISCRETE BRAIN REGIONS OF REM–SLEEP–DEPRIVED RATS

Brain Region	Controls (Home Cage)	Small Platform (REM Deprived)
Brainstem	765 ± 93 (6)	664 ± 73 (6)*
Pons	324 ± 48 (6)	317 ± 23 (6)
Medulla oblongata	176 ± 27 (6)	220 ± 52 (6)*
Hypothalamus	518 ± 24 (5)	523 ± 67 (6)
Hippocampus	431 ± 45 (6)	438 ± 37 (6)

MAO A activity is expressed in pmol [^{14}C] metabolite/mg protein/min. 5-HT final concentration in the assays was 50 μM. The results are expressed as mean ± SD. Number of rats is given in parentheses.

*$p < 0.05$.

TABLE 2

MAO B ACTIVITY IN DISCRETE BRAIN REGIONS OF REM–SLEEP–DEPRIVED RATS

Brain Region	Controls (Home Cage)	Small Platform (REM Deprived)
Brainstem	344 ± 76 (6)	328 ± 42 (6)
Pons	251 ± 49 (6)	281 ± 35 (6)
Medulla oblongata	201 ± 19 (6)	234 ± 46 (6)
Hypothalamus	504 ± 124 (6)	528 ± 111 (6)
Hippocampus	452 ± 58 (6)	469 ± 35 (6)

MAO B activity is expressed in pmol [^{14}C] metabolite/mg protein/min. The β-PEA final concentration in the assays was 2 μM. The results are expressed as mean ± SD. Number of animals is given in parentheses.

DISCUSSION

The data obtained in our study did not confirm our initial hypothesis of an overall increase in the activity of MAO A and/or B in the brain after REM sleep deprivation. However, we observed an increase in MAO A activity in the medulla oblongata, thus confirming previous data with a photometric assay (21).

The higher NA and 5-HT metabolite levels after REM sleep deprivation (2,25) were obtained in whole brain homogenates and we assayed MAO A and B activity in a mitochondrial fraction in some discrete brain regions. There is a paucity of information regarding monoamine metabolite levels in discrete brain regions after REM sleep deprivation, thus indicating the need of measuring them to detect whether the changes described in whole brain levels are restricted to some specific areas. This determination could help establish a possible link between metabolite levels and regional MAO activity after REM sleep deprivation.

MAO A and B may behave differently in vivo and in vitro. For instance, dopamine, which lacks specificity as a substrate for MAO in vitro (28), behaves as a MAO A substrate in striatum in vivo (9). Serotonin, which is a more specific substrate for MAO A in vitro (28), may behave differently in vivo, because raphe nuclei have very high MAO B levels (8,11,24). Therefore, enzyme compartmentalization in vivo might have been responsible for the lack of change observed in our in vitro study. Another point to be stressed is the enzyme saturability. If the enzymes are not saturated in vivo, an increase in metabolites, without change in enzyme activity or levels, would be likely.

In the brainstem, MAO A is preferentially located in the LC and MAO B in the raphe nuclei (8,11,24). Thus, we can cautiously assume that we measured MAO A activity mainly from LC and MAO B from raphe nuclei. We obtained a decrease in the activity of MAO A in brainstem and this result confirms the result of a previous report (21). During REM sleep, LC neurons cease firing (7), which could indicate a decrease in NA release suggests a decrease in MAO A activity. However, REM sleep deprivation would be expected to counteract the decrease in neuronal firing. This counteraction is shown biochemically by an increase in tyrosine hydroxylase activity in the upper brainstem (20) and in its mRNA in the LC (17). Therefore, decreased activity of MAO A in brainstem does not appear to agree with the increase in NA availability, reflected by an increase in tyrosine hydroxylase activity. However, an explanation for this apparent discrepancy may reside on the fact that although LC is the main noradrenergic nucleus, it is not the only supplier of noradrenergic innervation in the brain (15), nor it is the only nucleus with high MAO A activity (24). This fact raises the possibility that the decrease in MAO A activity, observed in brainstem, may not necessarily be due to changes in MAO A activity in LC but in other noradrenergic nuclei that are differently influenced by REM sleep deprivation.

A relevant issue in our study refers to the certainty that animals were deprived of REM sleep. Although we did not evaluate the degree of REM sleep deprivation to which rats were submitted, Mendelson et al. (14) showed that rats, weighing 200–225 g and kept on platforms 6.5 cm in diameter, present significantly less REM sleep after 96 h than controls. Because we used a similar platform (5.5 cm) and heavier rats, we suppose that our animals were indeed deprived of REM sleep.

A large platform control group for the stress of the deprivation procedure is usually included in the studies. We did not include such a group because Thakkar and Mallick (21) showed no changes in the activities of MAO A and B in rats kept over these platforms. Moreover, Landis (10) showed that rats kept over large platforms for 96 h are, to a lesser degree, also highly deprived of REM sleep. Therefore, at present, there seems to be no adequate control for the procedure of REM sleep deprivation and the stress effects on the enzymes have to be taken into account.

In conclusion, although we have confirmed changes reported by other investigators on MAO A activity in some brain regions after REM sleep deprivation, this procedure did not induce changes in other discrete brain regions receiving monoaminergic innervation.

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