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Rapid Eye Movement Sleep Deprivation Increases Chloride-sensitive Mg-ATPase Activity in the Rat Brain

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MALLICK, B. N. AND S. GULYANI. *Rapid eye movement sleep deprivation increases chloride-sensitive Mg-ATPase activity in the rat brain.* PHARMACOL BIOCHEM BEHAV 45(2) 359–362, 1993.—Rapid eye movement sleep deprivation is known to affect central neuronal excitability and responsiveness. Because chloride-sensitive Mg-ATPase is known to affect the neuronal transmembrane potential, this study was conducted to investigate if the enzyme activity might be affected on deprivation. The flower pot method was used for 2 and 4 days of deprivation and suitable control experiments were conducted. The enzyme activity was estimated in the microsomal preparation of the whole brain as well as in different areas of the brain in rats. The results suggested that the deprivation increased the enzyme activity although the chloride-insensitive Mg-ATPase activity remained unaffected. The increase in the enzyme activity is likely to reduce the neuronal hyperpolarization. The findings fit in with existing knowledge and help in explaining earlier observations.

Chloride-sensitive Mg-ATPase Chloride-insensitive Mg-ATPase Rapid eye movement sleep Deprivation Platform Brain

RAPID eye movement (REM) sleep deprivation is known to influence several physiological responses where the brain excitability is altered (2,12,13,15,20). More recently, the effect of REM deprivation has been found to affect the responsiveness of individual neurons (9,10). One of the possibilities of REM sleep deprivation-induced reduction in neuronal inhibitory response (9) could be due to a reduction in the neuronal hyperpolarization that may be affected, among several factors, by a change in the membrane bound ATPase activities. Because the effect of REM sleep deprivation on rat brain microsomal Na-K ATPase activity has recently been reported (3), its effect on the chloride ATPase activity, which may also affect the neuronal excitability, was investigated in this study. However, because the chloride ATPase is one of the two forms of Mg-ATPase (which is also known as chloride-sensitive Mg-ATPase), another being chloride-insensitive Mg-ATPase (5), the effect of REM sleep deprivation was studied on both the enzyme activities.

METHOD

The study was conducted on male albino rats (200–225 g) bred and maintained in the animal house. Experimental (E) animals were deprived of REM sleep by the flower pot technique (4,17,19). In brief, different groups of E animals were allowed to stay either for 2 (E2) or 4 (E4) days on a platform of 6.5 cm diameter surrounded by a pool of water. Free-moving (FM) rats were taken as normal. To rule out the effects of nonspecific surrounding factors, another group of rats was maintained on a little larger (12.5 cm) platform surrounded by a pool of water. To study the effect of stress factor due to movement restriction (MR) of rats on platforms in experimental rats, another group of rats was left in cages of 12.5 cm diameter in normal litter. In other control studies, two groups of rats were made to swim for 2 h (SW2) and 5 h (SW5) in a pool of water 28 ± 2 cm deep and 32 cm diameter. In another group, after the deprivation rats were maintained in their normal cages for at least an equivalent period as that of deprivation for a recovery study (R). However, because only the chloride ATPase was affected (and not the chloride-insensitive Mg-ATPase) on REM sleep deprivation the SW, MR, and R control studies were conducted on the former (chloride ATPase) only. The enzyme activities were estimated in the whole brain as well as in different areas of the brain viz. the cerebrum, cerebellum, and brainstem. Besides, because restricted regions within the brain stem are responsible for REM sleep (14) the effect of 2 days of deprivation was studied in different regions of the brain stem viz. the medulla, pons, and midbrain.

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Microsome Preparation

At the end of the deprivation or control period (as the case may be), rats were decapitated after spinal dislocation. Rat brains were removed and the different regions were dissected out within 2-3 min. For microsome preparation (16), rat brains were removed and homogenized in 10 vol of cold buffer containing 0.32 M sucrose, 12.5 mM Tris, and 1 mM EDTA. The homogenate was centrifuged for 5 min at 6,000 rpm ($g \times 1{,}000$). The supernatant was centrifuged for 20 min at 12,000 rpm ($g \times 10{,}000$) and 60 min at 40,000 rpm ($g \times 100{,}000$), respectively. The final pellet was suspended in 5 mM EDTA (pH 7.4 Tris), stirred for 30 min, and centrifuged at $10{,}000 \times g$ for 5 min. The supernatant fraction was then brought to 30% saturation by the addition of saturated ammonium sulfate (pH 7.4) with Tris. It was then stirred for 30 min and centrifuged for 30 min at 12,000 rpm ($g \times 10{,}000$). The precipitate was resuspended in 5 mM EDTA and stored at 4°C for 10-12 h. The microsomes so prepared were taken for the estimation of Cl-ATPase activity.

Estimation of Chloride ATPase Activity

The ATPase activity was determined by the spectrophotometric method (16). The reaction was carried out for 20 min at 37°C in 1 ml medium containing 100 mM NaCl, 20 mM KCl, 5 mM MgCl, 3 mM ATP, 50 mM Tris, and 20-30 μg membrane protein and 1 mM ouabain. The reaction was carried out with or without the presence of 0.3 mM ethacrynic acid in the reaction mixture. The Mg-ATPase activity in presence of 0.3 mM ethacrynic acid was taken as chloride-insensitive Mg-ATPase activity. The difference between the activities in the presence and absence of ethacrynic acid was taken as Cl-ATPase (chloride-sensitive Mg-ATPase) activity.

The protein concentration was estimated by the method of Lowry et al. (8). There were 6-10 rats in each group and the difference of activities between different groups were statistically analyzed. Values obtained from different groups of rats were compared by applying one-way analysis of variance (ANOVA). Those groups that showed a significant difference were subjected to Student's *t*-test for calculating the level of significance and are shown in Figs. 1 and 2.

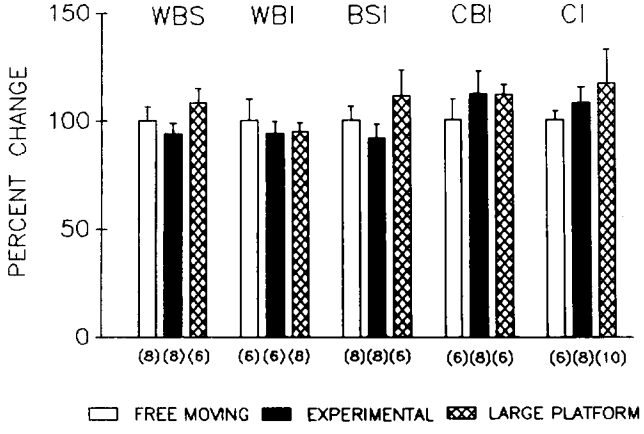

FIG. 1. The bar diagram shows the percent change (±SEM) of both Cl-sensitive (anion sensitive) and Cl-insensitive (anion insensitive) Mg-ATPase activities in whole brain as well as in different brain areas as compared to that of mean FM control taken as 100%. The numbers in parentheses show the number of rats used in each group. WBS-whole brain anion sensitive; WBI- whole brain anion insensitive; BSI-brain stem anion insensitive; CBI- cerebellum anion insensitive; CI-cerebrum anion insensitive.

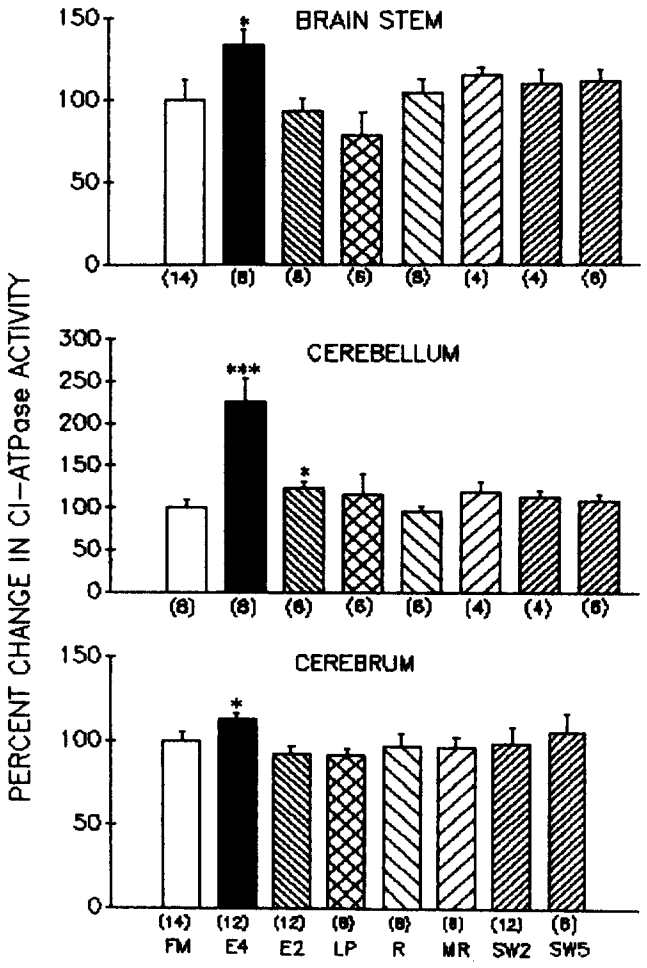

FIG. 2. The bars in this figure show the percent changes (±SEM) in Cl-ATPase (Chloride sensitive Mg-ATPase) activity in rat brain cerebrum, cerebellum, and brain stem in experimental and control rats as compared to FM control taken as 100%. The number in parentheses shows the number of rats studied in each group. The abbreviations are mentioned in the text. Significance levels are shown by (*) and (+) as compared to FM and LP controls, respectively. *, $+p < 0.05$, **$p < 0.02$ and ***, + + + $p0.01$.

RESULTS

Although no statistical test was done, after the deprivation behavioral observation of rats showed signs of REM deprivation (viz. rats were irritated, fought with each other if left together, extrasensitive to touch and noise, reduced grooming), and all those signs were proportional to the number of days of deprivation. Recovered and control rats did not show such signs. Some of the large-platform rats were seen sleeping on the edge of the platform and fell into the water presumably during REM. To avoid any discrepancy, only those rats were taken for study that were found sleeping comfortably on the large platform. The body weight of rats did not show a significant change on deprivation.

TABLE 1

SPECIFIC ACTIVITY OF Cl-ATPase (μmol Pi RELEASED/mg PROTEIN/h) IN MICROSOMAL PREPARATIONS OF BRAINSTEM REGIONS

	Midbrain	Pons	Medulla
FM	7.61 ± 2.25 (24)	1.73 ± 0.45 (12)	6.36 ± 2.05 (28)
E2	7.50 ± 1.83 (20)	1.48 ± 0.22 (12)	5.53 ± 2.52 (24)
LP	6.98 ± 3.09 (12)	1.65 ± 0.98 (6)	6.78 ± 2.90 (12)

Abbreviations mentioned in the text. Number of animals used in each set are indicated in parentheses.

Effect on Chloride-Insensitive Mg-ATPase

The REM sleep deprivation for 4 as well as 2 days was ineffective in inducing a significant alteration in the chloride-insensitive Mg-ATPase in the whole brain as well as in any of the regions of the brain (Fig. 1). The enzyme activity was not affected in any of the regions of the brain stem as well.

Effect on Chloride-Sensitive Mg-ATPase (Chloride ATPase)

The results suggest that 2 days of deprivation was ineffective in inducing a significant change in any of the regions of the brain stem, viz., the medulla, pons, and midbrain (Table 1). However, it was effective in significantly increasing (Table 2) the enzyme activity, as compared to the FM, in the cerebellum only (Fig. 2). Because the enzyme activity was not affected after 4 days of deprivation in the whole-brain preparation, it was not studied in the whole brain after 2 days of deprivation.

TABLE 2

SPECIFIC ACTIVITY OF Cl-ATPase (μmol Pi RELEASED/mg PROTEIN/h) IN MICROSOMAL PREPARATIONS OF DIFFERENT BRAIN REGIONS

	Brainstem	Cerebellum	Cerebrum
FM	7.18 ± 0.94 (14)	7.04 ± 0.71 (8)	12.5 ± 0.67 (14)
E4	10.50 ± 0.70 (8)*	14.11 ± 1.69 (8)†	14.10 ± 0.41 (12)*
E2	7.34 ± 0.57 (8)	9.73 ± 0.42 (8)*	11.53 ± 0.62 (12)
R	8.19 ± 0.66 (8)	6.02 ± 0.31 (6)	12.03 ± 1.00 (8)
LP	6.17 ± 1.00 (6)	7.22 ± 1.57 (6)	11.60 ± 0.93 (8)
MR	7.02 ± 1.40 (4)	7.46 ± 0.60 (4)	11.96 ± 0.75 (6)
SW2	7.81 ± 0.73 (4)	6.82 ± 0.43 (6)	12.24 ± 1.29 (12)
SW5	7.94 ± 0.65 (6)	6.51 ± 0.51 (6)	13.12 ± 1.39 (6)

Abbreviations mentioned in the text. Number of animals used in each set are indicated in parentheses.
*$p < 0.05$.
†$p < 0.01$.

Four days of deprivation was ineffective in inducing any significant change in the chloride ATPase activity in the whole-brain preparation (Fig. 1). However, 4 days of REM sleep deprivation was effective in inducing a significant increase in the chloride ATPase activity (chloride-sensitive Mg-ATPase) in different areas of the brain viz. the brain stem, cerebrum, and cerebellum (Table 2). The cerebellum was affected the most. The increase in the activity was significant as compared to both the FM as well as the LP control groups of rats (Fig. 2). The alteration in the chloride ATPase activity returned to a normal level after the recovery. Because the deprivation induced a significant increase in the chloride ATPase activity only (and not in the chloride-insensitive Mg-ATPase activity), that enzyme activity was studied in different control groups of rats. The enzyme activity remained unaffected in rats of all control groups, viz., MR, SW2, SW5, and LP animals.

DISCUSSION

The major finding of this study is that REM sleep deprivation increased selectively the chloride-sensitive Mg-ATPase (chloride ATPase) activity without affecting the chloride-insensitive Mg-ATPase activity in the rat brain. Two days of deprivation was probably the minimum period required to influence the enzyme activity significantly.

The nonspecific factors, which might have influenced E rats, were the environment surrounded by water, the raised platform, restricted movement on the platform, and probably higher muscular activity due to the loss of muscle atonia caused by REM deprivation and also due to the fact that rats spent more time sitting and standing. The nonspecific effect of the surrounding environment is unlikely to have influenced the results because the enzyme activity remained unaffected in LP control rats. Food, water, ambient temperature, etc. conditions remained identical for all groups of rats and hence are unlikely to have affected the enzyme activity. To rule out the possibilities of movement restriction and overactivity of the muscles on the platform affecting the enzyme activity, the MR as well as SW control experiments were performed and the enzyme activity remained unaffected in both groups of rats. Although it may be difficult to equate the stress, if at all, induced by REM deprivation and swimming, it may be argued that if the increase in the enzyme activity was at all due to stress it is likely that it would have been significantly different in the SW5 group as compared to that of the SW2 group (where the duration of swimming was less than half that of the SW5 group). Besides, it may also be argued that only the chloride-sensitive Mg-ATPase was affected, and the activity returned to basal level after recovery from deprivation. Because the flower pot method is well established as well as widely used for REM sleep deprivation and probably better (2-4,9-12,15,17-21), the method has been preferred in this study as well and the platform sizes were selected based upon earlier reports (11,21). Nevertheless, it is unlikely that the enzyme activity might have been affected due to the loss of insignificant amounts of nonREM sleep (11,18).

It is interesting to note that the REM sleep deprivation selectively affects the chloride-ATPase activity significantly and does not influence the chloride-insensitive Mg-ATPase activity. The increase in the chloride ATPase activity is likely to cause an increase in the extrusion of chloride ions out of the neurons (16), causing a reduction in the hyperpolarization

of the neurons. It has been suggested that ethacrynic acid, which blocks the chloride-sensitive Mg-ATPase (chloride ATPase) activity, enhances the noradrenergic neuronal anticonvulsive activity by stimulating the excitatory amino acid neurons (7). In this study, there has been an increase in the chloride ATPase activity. Thus, it is possible that an increase in the chloride ATPase activity might be the causative factor for REM deprivation induced alteration in the threshold of seizure (13), electroconvulsive shock (2), and paleocortical excitability (15), as well as a reduction in the firing rate of noradrenergic REM-off neurons (10). This article may help in explaining the underlying mechanism for norepinephrine-induced hyperpolarization of the locus coeruleus neurons (1).

It may be suggested that some of the factors, viz., alterations in the level of hormones including norepinephrine, small-molecular-weight proteins, especially the sleep factors, etc. that are known to be affected on deprivation (6) might induce such a change in the enzyme activity. Although the chloride ATPase activity and neuronal excitability are affected on REM sleep deprivation, this study does not allow to comment on the cause and effect or temporal relation between them, if any.

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