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Effect of REM Sleep Deprivation on Rat Brain Acetylcholinesterase

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THAKKAR, M. AND B. N. MALLICK. Effect of REM sleep deprivation on rat brain acetylcholinesterase. PHARMACOL BIOCHEM BEHAV 39(1) 211-214, 1991. — Acetylcholinesterase activity was compared in control, rapid eye movement sleep-deprived and recovered rat brain. The activity was estimated in the whole brain, cerebrum, brain stem and cerebellum. Flower pot technique was used for continuing deprivation for two, four and eight days. The results showed that the enzyme activity increased significantly in the deprived rat brain and it returned to control/nominal level on recovery. The enzyme activity increased first in the brain stem, while the activity in the cerebellum showed no significant change. Control experiments suggest that the increase was primarily caused by the deprivation. The finding fits well with existing knowledge and would possibly help in explaining earlier observations.

Acetylcholinesterase Rapid eye movement sleep Deprivation Platform

THE role of the cholinergic mechanism in the regulation of rapid eye movement (REM) sleep has been put forward by several workers (12, 13, 18, 28, 32). Acetylcholine (ACh) and its agonists have been reported to increase (1,16,18), while antagonists decrease (12,18) REM. Level of ACh in the brain increases (11, 14, 17) during REM, while it decreases (4) on REM deprivation. Brain stem 'REM-on' neurons are probably cholinceptive and cholinomimetic (27,30) and increase their firing rate on REM deprivation (21). Another line of evidence in support of the cholinergic mechanism of REM sleep is that the cholinesterase (ChE) inhibitors have been reported to enhance REM (2, 5, 8, 18). In addition, REM sleep is affected in diseased states where the ACh or ChE is affected (6, 10, 24). Though the effect of REM deprivation on brain ACh was studied, its effect on acetylcholinesterase (AChE), which may also affect ACh levels, was not known. Hence, before further investigation of the relationship between REM and AChE and their mechanism of action, it was probably necessary to investigate if the brain AChE activity is at all affected by REM sleep deprivation. It was hypothesized that REM deprivation may increase the levels of brain AChE, which in turn possibly would precipitate deprivation-induced reduction in the level of ACh.

METHOD

Experiments were conducted on male albino rats weighing between 220-280 g. The animals were maintained in the animal house under 12:12-h light and dark cycle. REM sleep deprivation was continued for two, four and eight days by the flower pot technique (15, 21-23, 31, 33-35). Experimental (E) rats were maintained on a 6.5 cm diameter island projecting above a pool of water. Food and water were supplied ad lib. In addition to free moving control (FMC), where rats were maintained in the cages, two other control experiments were performed. First, large platform control (LPC) where rats were maintained for 8 days on a large circular island of 13.5 cm diameter projecting above a pool of water, i.e., a condition similar to the E rats except that the platform size was a little larger; and second, where rats were maintained individually on normal litter for 8 days in cages of 12.5 cm diameter so that the movement was restricted (RM) but the rats did not undergo possible stress induced by raised platform surrounded by water used in the E situation. In another set of experiments after the rats had spent eight days under E conditions they were allowed to spend three days in normal cages for recovery study (R). The plan of the experiments including number of rats used in each group is summarised in Table 1.

Brains were removed after decapitation (35), and the activity of AChE was measured in whole brain as well as in different areas of the brain. Whole brain and its different regions, viz. cerebrum, cerebellum and brain stem, were dissected out within two to three min and homogenized in 1 M saline buffer containing 1% Triton X-100 (v/v). For AChE estimation (9) the reaction mixture contained 0.168 M phosphate buffer (pH 8.0), 0.01 mM DTNB (Sigma) and 0.01 mM acetylthio-choline (Sigma) and increase in the absorbance was observed spectrophotometrically (Shimadzu UV 260) at 412 nm for 5 min. Protein concentration was esti-
cholinesterase activity in the rat whole brain homogenate in the control, experimental and recovery conditions as compared to that of mean free moving control taken as 100%. Experimental rats were subjected to eight days deprivation. Number of rats in each group is shown in parentheses. Abbreviations are mentioned in the text. ***p<0.01.

RESULTS

The rats could stand and sit on the smaller platform but could not assume the posture for REM sleep without falling into the water resulting in waking. On the other hand, rats on larger platforms could assume the posture required for REM during sleep. Some of the rats were found to sleep at the edge of large platform. Those were seen occasionally to fall into the water presumably during REM sleep. To avoid loss of REM in LPC rats, only those rats were taken which were found to sleep comfortably in curled posture on large platform.

The body weight of the rats did not change significantly as compared to that of respective predeprivation. Though no scoring was done, behavioral observations revealed that the E rats became irritable and fought with other rats if left together, decreased grooming and quite often became extra sensitive to noise and touch while handling. Recovered and other control rats did not show appreciable change in those behaviors.

Effect of REM Sleep Deprivation on Whole Brain AChE Activity

Results showed that four days deprivation did not show any significant change in the activity of AChE. However, eight days REM deprivation increased AChE activity significantly in E rats as compared to all the control rats Table 1. Percent increase in the AChE activity as compared to FMC values is shown in Fig. 1. The activities of LPC and RM were comparable to each other as well as to FMC rats. The AChE activity in the recovered (R) animals was also comparable to that of FMC values.

Effect of REM Sleep Deprivation on AChE Activity in Different Areas of the Brain

Results showed that four days deprivation induced a significant increase in AChE activity in brain stem only. Eight days REM deprivation, on the other hand, increased AChE activity significantly in brain stem and the cerebrum of E rats as compared to FMC and R animals. The activity in the cerebellum was never affected. Percent increase in the AChE activity in different areas of the brain as compared to FMC values, after four and eight days deprivation, is shown in Figs. 2 and 3, respectively.

As four days deprivation affected the AChE activity in the brain stem only, the effect of two days deprivation was estimated in that region only. The brain stem AChE activity was not significantly affected in the two days REM deprived rat brain.

DISCUSSION

The results of this study suggest REM deprivation (E) causes an increase in the brain AChE activity. The increase is expressed first in the brain stem even before it is expressed in the cerebrum or in any other portion of the brain. Though four days deprivation was effective in inducing an increase in the brain

FIG. 1. The bar diagram represents percentage increase in mean acetylcholinesterase activity in the rat whole brain homogenate in the control, experimental and recovery conditions as compared to that of mean free moving control taken as 100%. Experimental rats were subjected to eight days deprivation. Number of rats in each group is shown in parentheses. Abbreviations are mentioned in the text. ***p<0.01.

FIG. 2. The bar diagram represents percentage increase in mean acetylcholinesterase activity in brain stem, cerebellum and cerebrum of rat brain in control and experimental conditions as compared to that of mean free moving control taken as 100%. Experimental rats were subjected to four days deprivation. Number of rats in each group is shown in parentheses. Abbreviations are mentioned in the text. ***p<0.01.

FIG. 3. The bar diagram represents percentage increase in mean acetylcholinesterase activity in brain stem, cerebellum and cerebrum of rat brain in control, experimental and recovery conditions as compared to that of mean free moving control taken as 100%. Experimental rats were subjected to eight days deprivation. Number of rats in each group is shown in parentheses. Abbreviations are mentioned in the text. ***p<0.01.
stem, the increase was maximum in the cerebrum (after eight days deprivation) and the enzyme activity did not change in the cerebellum. The increase was unlikely due to restriction of movement or stress caused by the experimental set up and the alteration was reversed on recovery.

For REM deprivation study, suitable control experiments and achieving REM deprivation are basic methodological criticisms which can reasonably be raised. The flower pot method for REM deprivation, which is most widely used (4, 15, 21-23, 31, 33-35), has been preferred in this study. Nevertheless, the following observations support that the results obtained were primarily due to REM deprivation. First, for E and LPC experiments, platform sizes were chosen as per criteria suggested by earlier workers (15, 22, 35) and the activity did not increase in the latter situation which served as a control for stress (7). Second, the enzyme activity did not increase in RM group of rats. Third, enzyme activity in the R rats was comparable to FMC.

REM deprivation induced a significant increase in AChE activity in the rat brain and may be explained as follows: First, the deprivation might have a direct effect to increase the level of AChE in the brain. Though the mechanism of increase is yet to be investigated, it may be supported by earlier report that cholinesterase inhibitor, which is likely to reduce the activity of AChE, have shown to increase the REM sleep (1, 5, 8, 12, 16, 18). Second, the decrease in ACh in REM deprived rat brain (4) may be due to an increase in the AChE activity. Third, increase in the heart rate and energy expenditure (3,19) on REM deprivation may be due to reduced level of ACh as the result of an increase in the activity of AChE. Thus REM deprivation may be a withdrawal of parasympathetic effect. Fourth, the present finding may explain earlier observations that REM sleep is disturbed in pathological states where ACh or AChE levels are affected (6, 10, 24). Fifth, this finding supports the cholinergic mechanism in REM sleep (12,18).

One of the interesting observations is that the enzyme activity increases significantly first in the brain stem and then in the cerebrum though it remains unaffected in the cerebellum. Increase in the cerebrum fits well with the earlier findings that ACh level decreases in cerebrum on REM deprivation (4). The increase in AChE activity in the brain stem may have relevance to deprivation induced increase (21) in the firing rate of 'REM-on' neurons which are probably cholinceptive and cholinergic (27,30). The increase in the enzyme activity first in the brain stem fits well with the concept of cholinergic mechanisms involved in generation of REM sleep.

Though it may be said that the increase in the enzyme activity was primarily because of AChE (9), the possible increase in different forms of ChE cannot be commented on. The enzyme is present in the red blood cell (RBC) membrane also (25). It is unlikely that such a significant increase may take place due to the small number of RBC remained trapped in the brain. Changes in the behavior of the E rats due to deprivation has been reported earlier (19,34). It is difficult to comment from this study if those behavioral changes have any relevance to an increase in the brain AChE activity. The finding of this study would probably form the basis for further investigation regarding changes in different forms and kinetics of the enzyme activity on REM sleep deprivation as well as the mechanism for inducing such a change. As the entire brain stem may not be absolutely necessary for induction of REM sleep (26,29), it would probably be worth studying the enzyme activity in different regions of the brain stem. Since 4 days deprivation affected AChE activity in the brain stem region and not in the whole brain homogenate, it is possible that though 2 days deprivation did not affect the enzyme activity in the entire brain stem, localized area in the brain stem may get affected. We expect that the enzyme activity may not change uniformly in different regions of the brain stem.

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Short-term REM sleep deprivation increases acetylcholinesterase activity in the medulla of rats

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Involvement of cholinergic ponto-medullary brainstem mechanism regulating rapid eye movement (REM) sleep is known. Recently it was found that though short term REM deprivation influenced brainstem neuronal excitability, the activity of the brainstem acetylcholinesterase was not affected until after 96 h deprivation. Therefore, it was hypothesized that short-term REM deprivation might influence acetylcholinesterase in a restricted brainstem region. Results of this study show that the enzyme activity increased only in the medulla after 24 and 48 h REM deprivation. The flower pot technique was used for depriving the experimental rats of REM sleep. Suitable control experiments were conducted to rule out the possibility of non-specific effects. Thus, the medullary cholinergic mechanism probably is more important for REM.

The brainstem mechanism for rapid eye movement (REM) sleep is well documented [9, 13, 17, 24--26, 29]. It is difficult to comment on which of the REM related neurons, REM-on or REM-off, are more important for REM generation. The importance of the ponto-medullary region [16, 24--26, 29] and/or, an interaction between the brainstem cholinergic and aminergic neurons [13, 14] has been put forward. The role of acetylcholine (ACh) in relation to REM sleep has been extensively studied [1--3, 5, 7--10, 12, 15, 17, 28, 32, 35]. Recently, we have shown that the acetylcholinesterase (AChE) level increases first in the brainstem of REM sleep-deprived rats [30]. Though 48 h REM deprivation did not affect the brainstem AChE activity [30], only 20 h deprivation was sufficient to affect the brainstem single neuronal activity [19, 20]. It was hypothesized that if the brainstem cholinergic mechanism does play a significant role in REM sleep, the level of AChE might increase first at the site of the REM generator. In an attempt to shed light on this matter, the effect of 24 and 48 h REM sleep deprivation was investigated on midbrain, pons and medulla AChE activity.

Experiments were conducted on male albino rats weighing between 220 and 280 g. The animals were maintained in the animal house under 12:12 h light-dark cycle and food and water ad libitum. REM deprivation was continued on experimental (E) group of rats for 24 and 48 h by the flower pot technique [33]. Free moving control (FMC), large platform control (LPC) and recovery (R) experiments were performed. Details of experimental procedures are mentioned in a previous report [30]. In brief, FMC rats were maintained in rat cages in the same room along with E, LPC and R rats. LPC and E rats were subjected to similar environmental conditions. Each rat of the former group was maintained on a 6.5 cm diameter island while that of the latter group on an island of 13.5 cm diameter projecting above a pool of water. For the R group, rats were subjected to a treatment similar to that of the E group (48 h) followed by maintaining them in normal rat cages for 48 h.

Brains were removed after decapitation. Medulla, pons and midbrain were separately dissected out and homogenized in 1 M saline buffer containing 1% Triton X-100 (v/v). The AChE activity was estimated by the method of Ellman et al. [6] and the protein concentration was estimated by the method of Lowry et al. [18] as reported earlier [30]. In short, for AChE estimation the reaction mixture contained 0.168 M phosphate buffer (pH 8.0), 0.01 mM DTNB (Sigma) and 0.01 mM acetylthio-choline (Sigma) and increase in the absorbance was observed spectrophotometrically (Shimadzu UV 260) at 412 nm for 5 min. For protein estimation the reaction mixture contained 5 ml of Lowry's reagent (48 ml of 2% Na2CO3 in 0.1 N NaOH + 1 ml 0.5% CuSO4 + 1 ml of 1% Na/K tartarate) and 0.5 ml of Folin's reagent (SRL, India). The mixture was incubated for 30 min at room temperature and the colour developed was read at
700 nm. Bovine serum albumin was used as a standard. Data were collected from 6–9 rats in each group and the significance level of difference in the mean specific activity of AChE between different groups of rats was statistically analyzed by applying a t-test.

REM sleep deprivation for 24 and 48 h induced a significant increase in rat brain AChE activity in the medulla only and neither in the pons nor in the midbrain. Percent increase in the mean AChE activity in different areas of the brainstem, compared to that of the FMC values, after 24 h and 48 h deprivation, is shown in Figs. 1 and 2, respectively. LPC and R values also did not show any significant changes, as compared to FMC, in any of the 3 regions of the brainstem. Since the AChE activity did not show any significant change after 48 h in the LPC group and returned back to baseline in the R group after 48 h REM deprivation, those two groups were not studied after 24 h deprivation.

The significant finding of this study is that (1) even 24 h REM sleep deprivation increased AChE activity and (2) the increase was restricted to the medulla only and not in other parts of the brainstem. The validity of the results would depend on (a) if REM deprivation was achieved and (b) if the effect was not due to non-specific reasons viz. stress, etc. Thus, after commenting on the validity of the observation, its physiological significance will be discussed.

The flower pot method was used for depriving the rats of REM sleep. As the method has been used reliably and most extensively [3, 4, 11, 19–22, 30, 31, 33, 34], the same has been adopted in this study without questioning its validity. The size of the platforms was selected as

![Fig. 1. Bar diagram representing percent (± S.D.) increase in mean acetylcholinesterase activity in medulla, pons, and midbrain in E, LPC and R rats compared to that of mean FMC taken as 100%. Experimental rats were subjected to 24 h REM deprivation. Number of rats in each group is shown in parentheses. Abbreviations are explained in the text. ***p < 0.01.](image1)

![Fig. 2. Bar diagram representing percent (± S.D.) increase in mean acetylcholinesterase activity in medulla, pons, and midbrain in E, LPC and R rats compared to that of mean FMC taken as 100%. Rats were left on the platforms for 48 h. Number of rats in each group is shown in parentheses. Abbreviations are explained in the text. ***p < 0.01.](image2)

reported earlier [21] and the method has been used by one of the authors on cats [19, 20]. Nevertheless, to rule out the possibility of non-specific effects, which might have influenced the results in addition to deprivation, LPC and R experiments were conducted. One of the non-specific factors might be movement restriction of the experimental animals during REM deprivation. During an earlier experiment [30] 8 days' movement restriction had not affected the AChE activity in the brain, and therefore it was not repeated in this study. Therefore, the observations were primarily due to REM deprivation and unlikely to be due to non-specific factors.

Results of this study may be a step forward in settling the issue regarding the precise area of the brainstem responsible for generation of REM sleep signs through the cholinergic mechanism [1, 2, 9, 12, 16, 17, 24–26, 28, 32, 35]. This study indicates that the medullary cholinergic region/mechanism is probably more important. An increase in ACh during REM [7, 10, 15], a decrease during REM deprivation [3] and alteration in the AChE activity with changes in the levels of ACh [23] have been reported. Thus, regarding the mechanism of REM deprivation-induced increase in AChE activity the following two possibilities may be postulated: (1) due to REM deprivation there might have been a tendency for an increase in the level of ACh (possibly due to REM pressure) which led to an increase in AChE activity: or, (2) the deprivation might cause an induced increase in the AChE which did not allow the ACh level to increase. Which possibility is true, if any at all, is at present unclear and therefore it is difficult to comment on the source of ACh in the
medullary region, cholinergic cell bodies or the terminals, responsible for REM.

Our finding that AChE activity increased in the medulla only and not in the pons or the midbrain is consistent with evidence that REM-related atonia and EEG desynchronization are mediated by a medullary cholinergic mechanism [24, 27]. REM sleep deprivation for a comparable duration is reported [19, 20] to modulate brainstem neuronal activity/responsiveness. However, it is yet to be confirmed if the two phenomena, viz. the altered brainstem neuronal activity/responsiveness. However, it is yet to be confirmed if the two phenomena, viz. the activity, could be correlated. It is interesting that an increase in the AChE activity in the whole brain could be observed after 8 days and not 4 days deprivation; in the brainstem after 4 days and not 2 days deprivation [30]; and in the medulla after 1 and 2 days deprivation. It is tempting to hypothesize that REM deprivation induced an increase in the level of AChE starting at the medulla and spreading to other regions of the brain. Though the spreading of AChE in different regions of the brain needs to be confirmed, the non-significant change in the activity of AChE in the cerebellum, as reported earlier [30], probably does not fit in with such a hypothesis. At this stage it is difficult to comment on the kinetics and form of AChE which gets altered on REM deprivation. This study could form the basis for future study of REM sleep deprivation-induced changes in the AChE activity by subjecting animals to REM deprivation for 24 h only which would be less stressful.

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