Pharmacology Biochemistry & Behavior, Vol. 39, pp. 211–214. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

BRIEF COMMUNICATION

Effect of REM Sleep Deprivation on Rat Brain Acetylcholinesterase

MAHESH THAKKAR AND BIRENDRA NATH MALLICK[1]

School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067, India

Received 24 May 1990

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/normal 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 cholinoceptive 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-

[1]Requests for reprints should be addressed to B. N. Mallick.

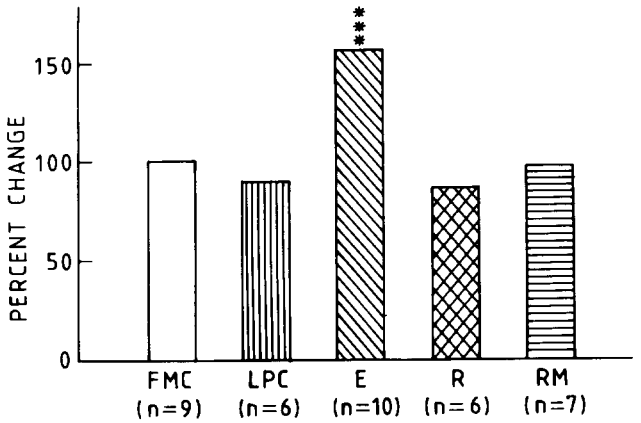

FIG. 1. The bar diagram represents percentage increase in mean acetylcholinesterase activity in the rat whole brain homogenate in the control, experimental and recovery conditions as compared to that of mean free moving control taken as 100%. Experimental rats were subjected to eight days deprivation.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

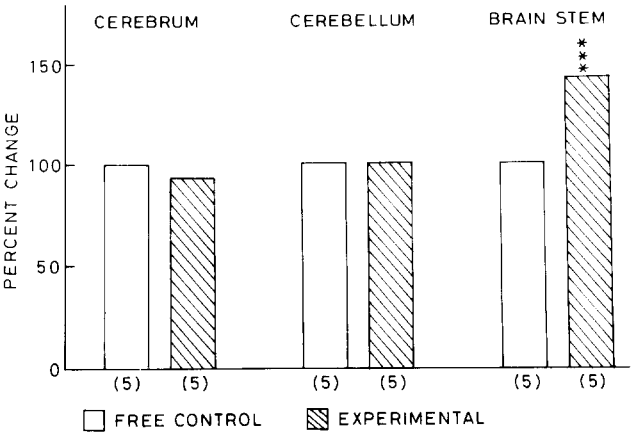

FIG. 2. The bar diagram represents percentage increase in mean acetylcholinesterase activity in brain stem, cerebellum and cerebrum of rat brain in control and experimental conditions as compared to that of mean free moving control taken as 100%. Experimental rats were subjected to four days deprivation.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

mated by the method of Lowry et al. (20). Data was collected from 5–10 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 *t*-test.

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 rats 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 rat 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

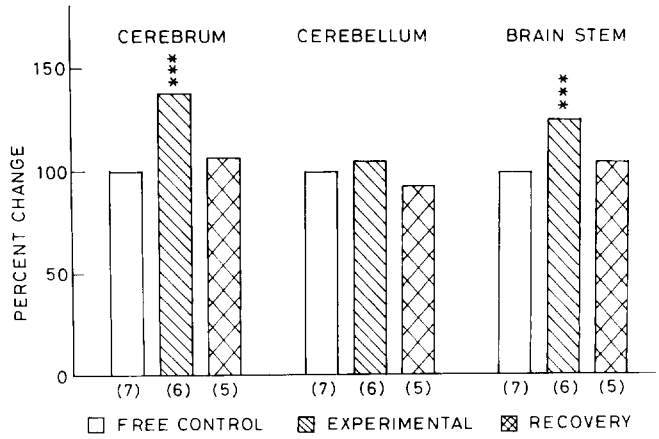

FIG. 3. The bar diagram represents percentage increase in mean acetylcholinesterase activity in brain stem, cerebellum and cerebrum of rat brain in control, experimental and recovery conditions as compared to that of mean free moving control taken as 100%. Experimental rats were subjected to eight days deprivation. Number of rats in each group is shown in parentheses. Abbreviations are mentioned in the text. ***$p<0.01$.

TABLE 1

ACETYLCHOLINESTERASE ACTIVITY (μmoles/min/mg protein) IN THE RAT BRAIN IN DIFFERENT GROUPS OF ANIMALS

Groups	Specific Activity of AChE in Whole Brain	Cerebrum	Cerebellum	Brain Stem
FMC	0.095 ± 0.009 (9)	0.140 ± 0.015 (7)	0.041 ± 0.008 (7)	0.082 ± 0.011 (7)
E (8 days)	0.138 ± 0.017† (10)	0.193 ± 0.017† (6)	0.043 ± 0.01 (6)	0.110 ± 0.013† (6)
E (4 days)	0.118 ± 0.009 (5)	0.130 ± 0.024 (5)	0.040 ± 0.013 (5)	0.116 ± 0.027* (5)
R	0.089 ± 0.005 (6)	0.150 ± 0.009 (5)	0.038 ± 0.006 (5)	0.086 ± 0.014 (5)
LPC	0.091 ± 0.025 (6)	—	—	—
RM	0.095 ± 0.016 (7)	—	—	—

No. of animals is shown in parentheses under respective group; *$p<0.02$; †$p<0.01$.

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 cholinoceptive 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.

ACKNOWLEDGEMENT

The financial support received from the Indian Council of Medical Research to carry out the research project is duly acknowledged by the authors.

REFERENCES

1. Amatruda, T. T.; Black, D. A.; McCarley, R. W.; Hobson, J. A.; McKenna, T. M. Sleep cycle control and cholinergic mechanisms: Differential effects of carbachol injections at pontine brain stem sites. Brain Res. 98:501–515; 1975.
2. Baghdoyan, H. A.; McCarley, R. W.; Hobson, J. A. Cholinergic manipulation of brain stem reticular systems: Effects on desynchro-

nized sleep generation. In: Waquier, A.; Gaillard, J. M.; Monti, J. M.; Radulovacki, M., eds. Sleep: neurotransmitters and neuromodulators. New York: Raven Press; 1985:15–28.
3. Bergmann, B. M.; Everson, C. A.; Freedman, D.; Fang, V. S.; Leitch, C. A.; Schoeller, D. A.; Refetoff, S.; Rechtschaffen, A. Sleep deprivation in rat: V. Energy used and mediation. Sleep 12: 31–41; 1989.
4. Bowers, M.; Hartmann, E.; Freedman, D. Sleep deprivation and brain acetylcholine. Science 153:1416–1417; 1966.
5. Bowers, M. B.; Goodman, E.; Sim, V. M. Some behavioral changes in man following acetylcholinesterase administration. J. Nerv. Ment. Dis. 138:383–389; 1964.
6. Casaldo, V. Down's syndrome: a study of sleep patterns related to levels of mental retardation. Am. J. Ment. Defic. 74:187–190; 1969.
7. Coll-Andreu, M.; Ayora-Mascarell, L.; Trullas-Oliva, R.; Morgado-Bernal, I. Behavioral evaluation of the stress induced by the platform method for short-term paradoxical sleep deprivation in rats. Brain Res. Bull. 22:825–828; 1989.
8. Domino, E. F.; Yamamoto, K.; Dren, A. T. Role of cholinergic mechanisms in states of wakefulness and sleep. In: Bradley, P. B.; Fink, M., eds. Progress in brain research. Amsterdam: Elsevier; 1968:113–133.
9. Ellman, G. L.; Courtney, D. K.; Andres, V.; Featherstone, R. M. A new and rapid calorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7:88–95; 1961.
10. Feinberg, I.; Kresko, R. L.; Heller, N. EEG patterns as a function of normal and pathological aging in man. J. Psychiatry Res. 5:107–144; 1967.
11. Gadea-Circia, M.; Stadler, H.; Lyod, K.; Bartholini, G. Acetylcholine release within cat striatum during sleep wakefulness cycle. Nature 243:518–519; 1973.
12. Gillin, J. C.; Sitaraman, N.; Janowosky, D.; Risch, C.; Huey, L.; Storch, F. I. Cholinergic mechanisms in REM sleep. In: Wauquier, A.; Gaillard, J. M.; Monti, J. M.; Radulovacki, M., eds. Neurotransmitters and neuromodulators. New York: Raven Press; 1985: 153–164.
13. Gnadt, J. W.; Pergram, V. Cholinergic brainstem mechanisms of REM sleep in the rat. Brain Res. 384:29–41; 1986.
14. Hanranth, P. S. R. K.; Venkatakrishna-Bhatt, H. Release of acetylcholine from perfused ventricles in unanaesthetized dogs during waking and sleep. Jpn. J. Physiol. 23:241–248; 1973.
15. Hicks, R. A.; Okuda, A.; Thomsen, D. Depriving rats of REM sleep: The identification of a methodological problem. Am. J. Psychol. 90:95–102; 1977.
16. Hobson, J. A.; Goldberh, M.; Vivaldi, E.; Riew, D. Enhancement of desynchronized sleep signs after pontine microinjection of muscarinic agonist bethanacol. Brain Res. 275:127–136; 1983.
17. Jasper, H. H.; Tessier, J. Acetylcholine liberation from cerebral cortex during paradoxical (REM) sleep. Science 172:601–602; 1971.
18. Jouvet, M. Cholinergic mechanisms and sleep. In: Waser, P. G., ed. Cholinergic mechanisms volume. New York: Raven Press; 1975: 455–476.
19. Kushida, A. C.; Bergmann, B. M.; Rechtscaffen, A. Sleep deprivation in rat: iv. paradoxical sleep deprivation. Sleep 12:22–30; 1989.
20. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with Folin-phenol reagent. J. Biol. Chem. 193: 265–275; 1951.
21. Mallick, B. N.; Siegel, J. M.; Fahringer, H. Changes in pontine unit activity with REM sleep deprivation. Brain Res. 515:94–98; 1989.
22. Mendelson, W. B.; Guthrie, R. D.; Fredrick, G.; Wyatt, R. J. The flower pot technique of rapid eye movement (REM) sleep deprivation. Pharmacol. Biochem. Behav. 2:553–556; 1974.
23. Oniani, T. N.; Lortkipanidze, N. D.; Mgaloblishvili, M. M.; Maisuradze, L. M.; Oniani, L. T.; Babilodze, M. R.; Gvasalia, M. G. Neurophysiological analysis of paradoxical sleep deprivation. In: Oniani, T., ed. Neurobiology of sleep wakefulness cycle. Tbilisi: Metsniereba; 1988:19–42.
24. Papazian, D. Rapid eye movement sleep alteration in myasthenia gravis. Neurology 26:311–316; 1976.
25. Rosenberry, T. L. Acetylcholinesterase. Adv. Enzymol. 43:103–218; 1975.
26. Sakai, K. Anatomical and physiological basis of paradoxical sleep. In: McGinty, D. J.; Drucker-Colin, R. R.; Morrison, A.; Parmeggiani, P. L., eds. Brain mechanisms of sleep. New York: Raven Press; 1985:111–137.
27. Sakai, K. Executive mechanisms of paradoxical sleep. Arch. Ital. Biol. 126:239–257; 1988.
28. Shiromani, P. J.; Gillin, J. C.; Henricksen, S. J. Acetylcholine and regulation of REM sleep: basic mechanisms and clinical implications for affective illness and narcolepsy. Annu. Rev. Pharmacol. Toxicol. 27:136–156; 1987.
29. Siegel, J. M. Brain stem mechanisms generating REM sleep. In: Kryger, M. H.; Roth, T.; Dement, W. C., eds. Principles and practice of sleep medicine. Philadelphia: Saunders; 1989:104–120.
30. Steriade, M.; Hobson, J. A. Neuronal activity during the sleep waking cycle. Prog. Neurobiol. 6:155–376; 1976.
31. Van Luijtelaar, E. L. J. M.; Coenen, A. M. L. Electrophysiological evaluation of three paradoxical sleep deprivation techniques in rats. Physiol. Behav. 36:603–609; 1986.
32. Vanni-Mercier, G.; Sakai, K.; Lin, J. S.; Jouvet, M. Mapping of cholinoceptive brainstem structures responsible for the generation of paradoxical sleep in cat. Arch. Ital. Biol. 127:133–164; 1989.
33. Vimont-Vicary, P.; Jouvet-Mounier, D.; Delorme, F. Effects EEG et comportmentaux des privations de sommeil paradoxal chez le chat. EEG Clin. Neurophysiol. 20:439–449; 1966.
34. Vogel, G. W. A review of REM sleep deprivation. Arch. Gen. Psychiatry 32:749–761; 1975.
35. Yanik, G.; Radulovacki, M. REM sleep deprivation up-regulates adenosine A1 receptors. Brain Res. 402:362–364; 1987.