[Downloaded from mjiri.iums.ac.ir on 2025-05-19]

Basic Science In Medicine

RAPID EYE MOVEMENT SLEEP DEPRIVATION INDUCES ACETYLCHOLINESTERASE ACTIVITY IN THE PREOPTIC AREA OF THE RAT BRAIN

MOHAMMAD HOSSEIN NOYAN ASHRAF, Ph.D., GILA BEHZADI,* Ph.D., AND BEHNAM JAMEIE,* Ph.D.

From the Department of Anatomy, Faculty of Medicine, Guilan University of Medical Sciences, Rasht-Gazvin Road, Rasht, Guilan, I.R. Iran, (e-mail: noyan@cd. gu. ac. ir), and the *Department of Physiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Evin, Tehran, I.R. Iran.

ABSTRACT

Acetylcholinesterase (AchE) is a large glycoprotein that, aside from its known cholinolytic activity, co-exists with other transmitter systems and possesses other functions. In the present study, the effects of short-term rapid-eye-movement sleep deprivation (REM-SD) on AchE activity in the anterior hypothalamic area have been investigated.

Using the flower-pot method, adult male albino rats were deprived of REM sleep for a period of 72, 96, and 120 h and perfused brains were then sectioned with a vibratome and stained histochemically for AchE.

In comparison to control animals, marked positive AchE activity was observed in neurons located in the preoptic area in the 120SD group only.

Results of this study have shown that AchE could be involved in some unknown functions related to REM sleep physiology.

MJIRI, Vol. 14, No. 1, 47-51, 2000

Keywords: Sleep deprivation, Acetylcholinesterase, Preoptic area, Rat brain.

INTRODUCTION

Sleep is a unique reversible phenomenon and has attracted the attention of scientists and philosophers alike since ancient times. ¹² Sleep deprivation (SD) is a potentially useful strategy for studying the function of sleep. Since the identification of rapid eye movement (REM) sleep by Aserinsky and Kleitman in 1953, ¹ the functional and physiological significance of this active stage of sleep is not yet understood.

REM sleep deprivation (REM-SD) has been reported to induce several behavioral and physiological changes such as alterations in immediate early gene expression, 12,15

receptor density,^{10,26} level of some neurotransmitters,^{2,4,24} or enzymes responsible for their synthesis^{3,20} or degradation,^{5,11,25} and changes in neuronal membrane properties.^{8,13}

Biochemical estimations showed stepwise enhancement in the level of acetylcholine degrading enzyme, i.e. acetylcholinesterase (AchE), in different parts of the rat brain following REM-SD.¹¹ AchE also co-exists in non-cholinergic neurons and has some non-cholinolytic functions.^{9,22} With regards to AchE expression in non-cholinergic neurons of the hypothalamus,²¹ and the significant role of this center in sleep homeostasis,⁶ in the present study, we attempted to evaluate the effects of short-

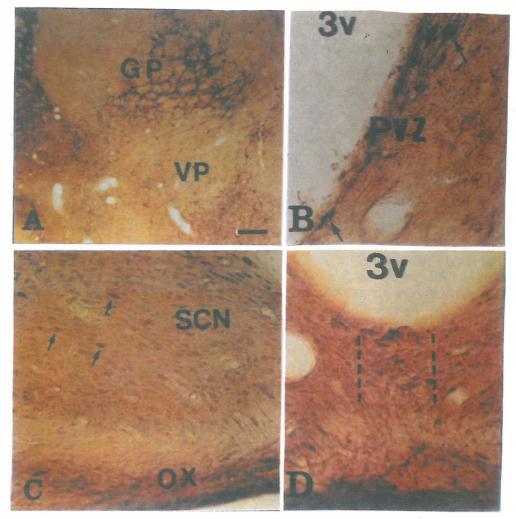


Fig. 1. Distribution of acetylcholinesterase-reactive perikarya in rat forebrain. A: Positive reaction to the enzyme in the striatal and pallidal regions showing sensitivity of staining; B: AchE containing neurons (arrows) in the periventricular zone. Some of them are located close to the ventricular epithelium. C: Small fusiform AchE-positive neurons (arrows) in the suprachiasmatic nucleus. D: AchE containing neurons in the inter-SCN region. GP, Globus pallidus; VP, Ventral pallidum; PVZ, Periventricular zone; SCN, Suprachiasmatic nucleus; OX, Optic chiasma; 3V, Third ventricle. Scale bar: A: 245 μm, B-D: 62.5 μm.

term REM-SD on AchE activity in the anterior hypothalamic area.

MATERIALS AND METHODS

Adult male albino rats (200-230 g/w) were deprived of REM-sleep by the flower-pot method¹⁷ for a period of 72, 96, and 120 hours. Food and water were supplied *ad libitum*. Experimental animals (n=10, each group) were maintained on circular disks, 6 cm in diameter, projecting above a pool of water. The height of the disk from the bottom of the cage was 4 cm, so that animals could move freely to avoid the possible effects of restriction of

movement. In this condition, the animal could stand, sit and move but did not have enough space to assume the posture for REM sleep. Therefore, at the onset of REM, rats fall into the surrounding water due to muscle atonia and are awakened leading to loss of REM sleep. Suitable control experiments, including cage controls and larger platform (dia., 15 cm) controls, were conducted to rule out the possibility of nonspecific effects.

Following deep anesthesia with sodium pentobarbital (50mg/kg,i.p.), the animals were perfused via the ascending aorta with 0.9% heparinized saline followed by a solution of 4% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer, pH7.4. The brains were removed rapidly

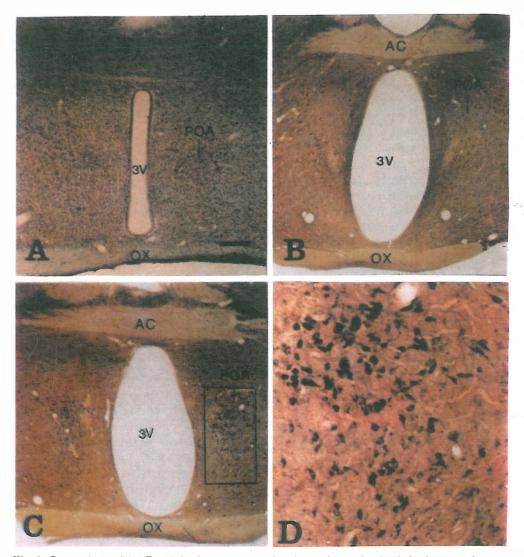


Fig. 2. Comparison of AchE activity between control and experimental animals in the preoptic area. A: Cresyl violet-stained section representing the preoptic area; B: AchE reactivity in the preoptic area in control animals. A few AchE-positive neurons were detected in this region; C: A considerable increase in the number and reactivity of neurons is visible in this area following REM-SD for 120 h (BOX), and is shown in part D. AC, Anterior commissure; POA, Preoptic area; OX, Optic chiasma; 3V, Third ventricle. Scale bar: A-C: 245 μm, D: 231 μm.

and postfixed at 4°C, overnight. Then 40 micrometer coronal sections of the forebrain were prepared using a vibratome and then stained for AchE according to Mesulam. 18

Briefly, sections were kept in reaction solution containing 72 mg ethopropazine (Sigma), 750 mg glycine, 500 mg copper sulfate, 1200 mg acetylthiocholine (Sigma) and 6800 mg sodium acetate in 1000 mL of distilled water, pH 5, for 1hr. Sections were then rinsed and developed for 1 minute in 19.2 g of sodium sulphite in 500 mL of 0.1 N HCl. After rinsing, the sections were transferred to 1% AgNO₃ solution for intensification of the staining. Following a brief rinse in distilled water, sections were finally

transferred to physiological saline and mounted on gelatinecoated slides, air-dried, dehydrated and coverslipped. The selected sections were photographed using a Zeiss light microscope.

RESULTS

With increasing periods of REM-SD, changes in mood were observed, including nervousness, irritability, aggressiveness and sleepiness. SD rats showed a progressively debilitated appearance manifested in disheveled, clumped, yellowing fur. Despite an increase in

Increase in AchE Activity by REM Sleep Deprivation

food intake, there was a progressive marked decrease in weight during REM-SD.

The histochemical procedure used in this study resulted in the accumulation of granular dark brown AchE reaction products within neurons. Based on the intensity of staining, i.e. the extent of deposition of AchE reaction product, several types of neurons could be identified. Moderate to highly staining intensity was seen in neurons belonging to different regions of the hypothalamic area.

The AchE activity within the striatum, ventral pallidum and substantia innominata was used as a control for the sensitivity of the histochemical method utilized (Fig.1, A). Some AchE positive neurons with moderate to high reactivity were identified in the periventricular region (Fig.1, B). These neurons are fusiform and multipolar in shape and are located close to the ependymal layer of the third ventricle (Fig.1, B).

Small, fusiform moderately stained neurons were also found in the mid-region of the suprachiasmatic nucleus (SCN, Fig.1, C). A few AchE positive neurons were visible in the region between the two SCNs (Fig.1,D).

Compared to control animals, no changes in AchE activity were detected in the mentioned hypothalamic area in SD groups, but marked positive AchE activity was observed in neurons located in the preoptic area (POA) in the 120 SD group only. These AchE positive neurons were bipolar (fusiform), elliptical, and multipolar in shape and showed different staining intensity from mild to high reactivity as shown in Fig. 2.

DISCUSSION

Previous biochemical studies have shown many noncholinergic (presumably cholinoceptive) cell groups including certain thalamic and hypothalamic nuclei which have been shown to be rich in AchE.^{7,21}

AchE is also widely associated with neurons using catecholamines, GABA and enkephalin as neurotransmitters.²² It has also been reported that the preoptic area in the basal forebrain containing GABA-ergic and norepinephrinergic neurons is of potential importance for sleep-wake regulation.^{11,14,19,23}

Using the flower-pot technique for induction of REM-SD and biochemical measurement, Thakkar and Mallick (1991) have reported a significant increase in AchE activity in the rat brain.²⁴ They emphasized that the alteration in enzyme activity is dependent on the duration of SD and the different regions of the brain.²⁵

In the present investigation, AchE histochemical staining permitted us to verify the enzyme activity in different forebrain regions where the neurons contain AchE but are non-cholinergic in nature. Among these areas, the POA of the hypothalamic complex showed an intense AchE activity in the majority of its neuronal cell

bodies comparing to controls. Since POA, as a whole, influences the regulation and/or maintenance of sleep and wakefulness states, ^{14,19} and regarding the non-catalytic functions of AchE in proteolysis, peptidase activity and promotion of synaptogenesis, ¹⁶ we can hypothesize that the induced increase of AchE activity in POA neurons may act as a neuromodulatory secretory protein²³ attempting to regulate biorhythmical processes during REM sleep deprivation.

ACKNOWLEDGEMENT

This work was supported by a grant from the Shahid Beheshti University of Medical Sciences, Tehran, I.R. Iran.

REFERENCES

- Aserinsky E, Kleitman N: Regularly occurring periods of eye motility and concomitant phenomena during sleep. Science 118: 273-274, 1953.
- Asikainen M, Topila J. Alanko L, et. al: Sleep deprivation increases brain serotonin turnover in the rat. Neuroreport 8(7): 1577-82, 1997.
- Basheer R, Magner M, McCarley RW, Shiromani PJ: Rapid eye movement sleep deprivation increases the levels of tyrosine hydroxylase and norepinephrine transporter mRNA in the locus coeroleus. Brain Res Mol Brain Res 57(2): 235-40, 1998.
- Bergmann BM, Seiden LS, Landis CA, et al: Sleep deprivation in the rat: XVIII. Regional brain levels of monoamines and their metabolites. Sleep 17(7): 583-589, 1994.
- Camarini R, Benedito MA: Rapid eye movement sleep deprivation reduces rat frontal cortex acetylcholinesterase (FC 3.1.1.7) activity. Braz J Med Biol Res 30(5): 641-7, 1997.
- Carlson NR: Physiology of behavior. Massachusetts: Allyn and Bacon Press, 5th ed., pp. 327-35, 1994.
- Geula C, Mesulam MM, Tokuno H, et al: Developmentally transient expression of acetylcholinesterase within cortical pyramidal neurons of the rat brain. Dev Brain Res 76: 23-31, 1993.
- Gulyani S, Mallick BN: Possible mechanisms of REM-sleep deprivation induced increase in Na-K ATPase activity. Neurosci 64(1): 255-260, 1995.
- Hawkings CA, Greenfield SA: Noncholinergic actions of exogenous acetylcholinesterase in the rat substantia nigra.
 II. Long term interactions with dopamine metabolism.
 Behav Brain Res 84: 159-163, 1992.
- Jimenez-Anguiano A, Garcia-Garcia F, Mendosa-Ramirez JL, et al: Brain distribution of VIP receptors following rapid eye movement sleep deprivation. Brain Res 728: 37-46, 1996.
- 11. Mallick BN, Thakkar M: Short-term sleep deprivation

- increases acetylcholinesterase activity in the medulla of rats. Neurosci Lett 130: 221-224, 1991.
- 12. Mallick BN, Singh R (eds.): Environment and Physiology. New Delhi: Narosa Publishing House, pp-196-203, 1994.
- 13. Mallick BN, Thakkar M, Gangabhagirathi R: Rapid eye movement sleep deprivation increases membrane fluidity in the rat brain. Neurosci Res 22: 117-122, 1995.
- Mallick BN, Joseph MM: Adrenergic and cholinergic inputs in the preoptic area of rats interact for sleep-wake thermoregulation. Pharmacol Biochem Behav 61(2): 193-9, 1998.
- Maloney KJ, Mainville L, Jones BE: Differential c-FOS expression in cholinergic, monoaminergic, and GABAergic cell groups of the pontomesencephalic tegmentum after paradoxical sleep deprivation and recovery. J Neurosci 19(8): 3057-72, 1999.
- Massoulie J, Pezzement L, Bon S, et al: Molecular and cellular biology of cholinesterases. Prog Neurobiol 41: 31-91, 1993.
- 17. Mendelson WB, Guthrie RD, Fredrick G, Wyatt RJ: The flower-pot technique of rapid eye movement (REM) sleep deprivation. Pharmacol Biochem Behav 2: 553-56, 1974.
- 18. Mesulam MM: Tracing neural connections with HRP. IBRO series, New York: John Wiley and Sons, pp. 132-3, 1982.
- 19. Nooralam M, Mallick BN: Differential acute influence of

- medial and lateral preoptic areas on sleep-wakefulness in freely moving rats. Brain Res 525: 242-248, 1990.
- Porkka-Heiskannen T: Noradrenergic activity in rat brain during REM sleep deprivation and rebound sleep. Am J Physiol 55(3): 677-683, 1995.
- 21. Satoh K, Armstrong DM, Fibiger HC: A comparison of the distribution of central cholinergic neurons as demonstrated by acetylcholinesterase pharmacohistochemistry and cholinacetyltransferase immunohistochemistry. Brain Res Bull 11: 693-720, 1983.
- 22. Shen ZX: Acetylcholinesterase provides deeper insights into Alzheimer's disease. Med Hypoth 43: 21-36, 1994.
- 23. Szymusiak R: Magnocellular nuclei of the basal forebrain: substrates of sleep and arousal regulation. Sleep 18(6): 478-500, 1995.
- Thakkar M, Mallick BN: Effect of REM-sleep deprivation on rat brain acetylcholinesterase. Pharm acol Biochem Behav 39: 211-214, 1991.
- Thakkar M, Mallick BN: Effect of REM-sleep deprivation on rat brain monoamine oxidases. Neurosci 55(3): 677-683, 1993.
- 26. Tsai LL, Bergmann BM, Perry B, Rechtschaffen A: Effects of chronic sleep deprivation on central cholinergic receptors in the rat brain. Brain Res 62: 95-103, 1994.