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

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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.

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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-

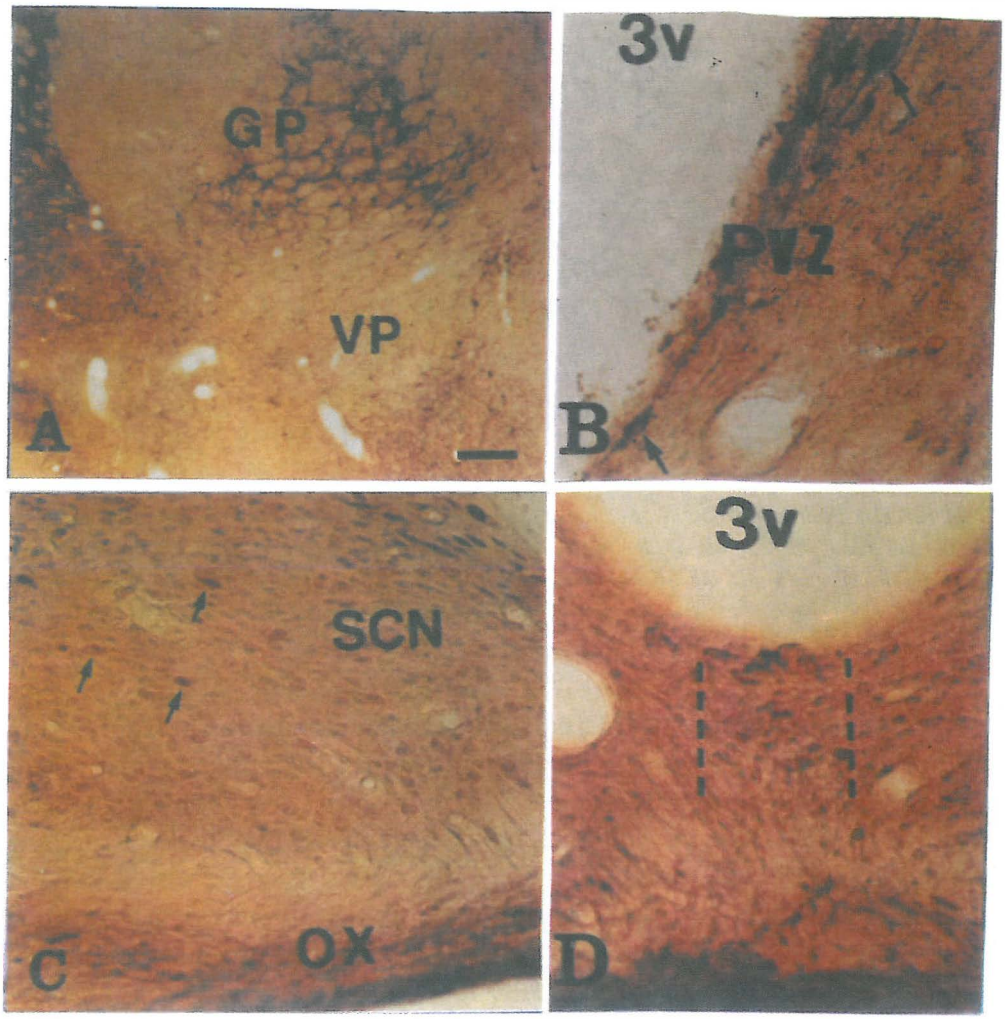

Fig. 1. Distribution of acetylcholinesterase-reactive perikarya in rat forebrain. A: Positive reaction to the enzyme in the striatal and pallidal regions showing sensitivity of staining; B: AchE containing neurons (arrows) in the periventricular zone. Some of them are located close to the ventricular 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 method[17] 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 non-specific 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, pH 7.4. The brains were removed rapidly

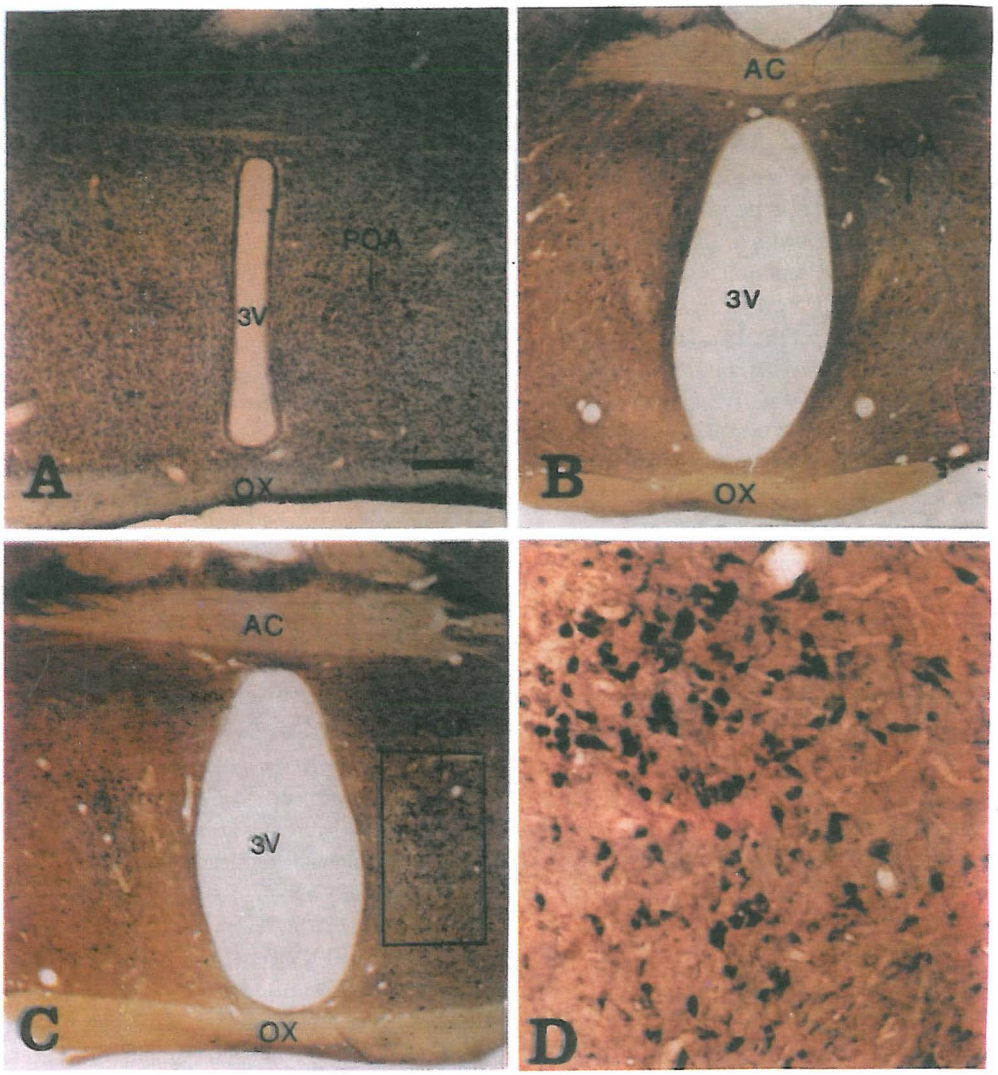

Fig. 2. Comparison of AchE activity between control and experimental animals in the preoptic area. A: Cresyl violet-stained section representing the preoptic area; B: AchE reactivity in the preoptic area in control animals. A few AchE-positive neurons were detected in this region; C: A considerable increase 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% $AgNO_3$ solution for intensification of the staining. Following a brief rinse in distilled water, sections were finally transferred to physiological saline and mounted on gelatine-coated 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

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 non-cholinergic (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 protein[23] attempting to regulate biorhythmical processes during REM sleep deprivation.

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