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Changes in cAMP concentration in the rat preoptic area during synchronized and desynchronized sleep [1]

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Summary. cAMP concentration was found to be significantly lower during desynchronized sleep than during synchronized sleep in the preoptic area of rats kept at normal laboratory temperature. No significant changes in cerebral cortex cAMP concentration were observed in the same experimental conditions.
Key words. cAMP; sleep; preoptic area.

Sleep is a behavioral state characterized by two contrasting sets of functional events, occurring in cycles of ultradian periodicity. A reductionist classification of sleep is based on electroencephalographic (EEG) criteria, namely: synchronized (high amplitude, low frequency EEG waves) sleep (SS) and desynchronized (low amplitude, high frequency EEG waves) sleep (DS). SS is a stage of behavioral quiescence in which homeostasis is maintained at a lower level of energy expenditure than in wakefulness. DS is characterized by an impairment of the hypothalamic-preoptic control of homeostatic regulation [3]. In this respect, SS and DS are two opposite functional states. This dichotomy may be underlied by specific changes in cellular activity, as suggested by the finding that the responsivity of hypothalamic-preoptic neurons to direct thermal stimulation is strongly depressed during DS [4]. The possibility that there are specific cellular processes related to sleep in the hypothalamic-preoptic region is also suggested by recent biochemical results from this laboratory [5]. These findings have shown that the concentration of a second messenger (cAMP) changes in this region in accordance with the modification of the sleep cycle induced by a broad variation of ambient temperature (Ta). In particular, cAMP concentration decreases during sleep deprivation and increases during sleep recovery. Furthermore, both conditions are characterized by the disappearance of the nucleotide circadian rhythm (light-minimum and dark-maximum) observed in control conditions [6].

The present study was performed in order to assess whether the functional states corresponding to SS and DS, respectively, might also be characterized by changes in the concentration of cAMP in the preoptic area.

Materials and methods

45 male Sprague-Dawley rats (300 g) housed in normal laboratory conditions (Ta 22 ± 0.5 °C, food and water ad libitum, 12:12 h light-dark schedule (LD); L: 07.00-19.00 h) were used. Animals were implanted with surface electrodes for EEG recording under general anesthesia (50 mg/kg ketamine-HCl and 1 mg/kg flunitrazepam, i.p.). Recording sessions were started 3-5 days after surgery, and sacrifice was carried out between 13.00 and 18.00 h. Animals were acclimatized to the experimental condition during the first L hours of the recording day and during this period the sleep pattern was monitored. They were assigned to each experimental session according to a randomized block experimental design and sacrificed in liquid nitrogen (at least 30 s from the onset of SS and DS) by opening the cage floor by remote control. The removal of samples from the preoptic area and the cerebral cortex, taken as a control, was performed as previously described [7]. The samples were homogenized in 150 µl of 5% ice-cold trichloracetic acid (TCA) and centrifuged at 10,000 g for 10 min at 2 °C. 100 µl of 1 M HCl was added to 100 µl of supernatant which, following the extraction of TCA with aqueous diethyl ether, was lyophilized and stored at − 80 °C. The sediments were resuspended in 150 µl of 1 M NaOH and total protein concentration was determined on 40-µl aliquots using a modified Bradford's protein assay [8], with rabbit gamma-globulins as standard (Sigma, UK). cAMP was determined by means of a competitive radiobinding assay using a commercial kit (Amersham, UK). The statistical analysis of the results was carried out by means of covariance analysis (regression of pmol of cAMP on mg of protein).

Results and discussion

The results are summarized in tables 1 and 2. As indicated in table 1, the average sleep stage duration before animal sacrifice is longer in the case of SS than in that of DS. This is due to the fact that the sacrifice during SS was performed only when cortical activity was characterized by high amplitude and low frequency waves[9]. The lack of correlation between the distribution of concentration values and sleep stage duration (table 1) demonstrates that such a time period bears no relevance to cAMP concentration. Table 2 shows that cAMP concentration in the preoptic area was significantly higher during SS than during DS. In this area the transition from SS to DS was marked by a reduction in cAMP concentration of approximately three pmol/mg protein. In contrast, no significant changes in nucleotide concentration were detected in the cerebral cortex under the same experimental conditions.

A stable concentration of cAMP in the rat microwave-fixed cerebral cortex has also been found during sleep by Ogasahara et al.[10]. In spite of the different experimental and nucleotide determination procedures (radioimmunoassay) the cortical cAMP level reported in their study is very similar to ours. Furthermore, no change in cAMP concentration which could be associated with sleep stages was found in other brain regions; however, in their study measurements at the diencephalic level were not included.

The decrease in preoptic cAMP concentration from SS to DS is small but consistent. Since many neurotransmitters are known to influence cAMP accumulation[11], it is not possible at the moment to define the nature of the mechanism involved. However, our findings would suggest that a modification in cellular metabolic activity in the preoptic area is associated with different functional states.

Table 1. Duration (s, mean ± SD) of the sleep stage before animal sacrifice and linear correlation coefficient between standard scores of cAMP concentration vs duration.

	Stage duration	Correlation coefficient		
SS	144 ± 93 (23)	PO	0.074	NS
	(40–360)	CC	0.001	NS
DS	50 ± 22 (22)	PO	−0.141	NS
	(30–95)	CC	0.215	NS

SS, synchronized sleep; DS, desynchronized sleep; PO, preoptic area; CC, cerebral cortex; NS, not significant. Number of observations and range between brackets.

Table 2. cAMP concentration (pmol/mg protein: mean ± SEM) changes in the preoptic area and the cerebral cortex during synchronized and desynchronized sleep.

	SS	DS	F	df	P
PO	19.22 ± 1.31 (23)	16.07 ± 0.96 (22)	5.23	1/39	<0.05
CC	8.15 ± 0.60 (23)	8.93 ± 0.70 (22)	1.99	1/39	NS

PO, preoptic area; CC, cerebral cortex; SS, synchronized sleep; DS, desynchronized sleep; F, Fisher's F; df, degrees of freedom; NS, not significant. Number of observations between brackets.

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