Basal forebrain acetylcholine release during REM sleep is significantly greater than during waking

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Vazquez, Jacqueline, and Helen A. Baghdoyan. Basal forebrain acetylcholine release during REM sleep is significantly greater than during waking. Am J Physiol Regulatory Integrative Comp Physiol 280: R598–R601, 2001.—Cholinergic neurons of the basal forebrain supply the neocortex with ACh and play a major role in regulating behavioral arousal and cortical electroencephalographic activation. Cortical ACh release is greatest during waking and rapid eye movement (REM) sleep and reduced during non-REM (NREM) sleep. Loss of basal forebrain cholinergic neurons contributes to sleep disruption and to the cognitive deficits of many neurological disorders. ACh release within the basal forebrain previously has not been quantified during sleep. This study used in vivo microdialysis to test the hypothesis that basal forebrain ACh release varies as a function of sleep and waking. Cats were trained to sleep in a head-stable position, and dialysis samples were collected during polygraphically defined states of waking, NREM sleep, and REM sleep. Results from 22 experiments in four animals demonstrated that means ± SE ACh release (pmol/10 min) was greatest during REM sleep (0.77 ± 0.07), intermediate during waking (0.58 ± 0.03), and lowest during NREM sleep (0.34 ± 0.01). The finding that, during REM sleep, basal forebrain ACh release is significantly elevated over waking levels suggests a differential role for basal forebrain ACh during REM sleep and waking.

arousal state control; electroencephalogram activation; substantia innominata; nucleus basalis; in vivo microdialysis

BASAL FOREBRAIN NEURONS participate in motor control (19), thermoregulation (28), learning (16), memory (23), and arousal state control (8). The chemical neuroanatomy of basal forebrain projection neurons is heterogeneous and includes cholinergic and GABAergic cells (5). Basal forebrain cholinergic neurons project topographically to the entire cerebral cortex and play a crucial role in cortical electroencephalographic (EEG) activation, behavioral arousal, and selective attention (8, 23, 24). Loss of basal forebrain cholinergic neurons contributes to the cognitive deficits characterizing Alzheimer’s disease, Parkinson’s disease, and dementia with Lewy bodies (21). Electrical stimulation of the basal forebrain increases cortical ACh release and causes EEG activation, whereas lesions of basal forebrain cholinergic neurons decrease cortical ACh release and reduce cortical activation (reviewed in Ref. 23). Consistent with these findings are data showing that during behavioral states characterized by EEG activation, such as waking and rapid eye movement (REM) sleep, cortical ACh release is at its highest level (7, 14). During states characterized by reduced EEG activation, such as non-rapid eye movement (NREM) sleep and anesthesia, cortical ACh release is low (reviewed in Ref. 12). The foregoing data support a role for ACh in regulating EEG and behavioral arousal. Given that the source of cortical ACh is the basal forebrain, cholinergic neurotransmission within the basal forebrain also may vary as a function of arousal state. No previous studies, however, have characterized ACh release within the basal forebrain during sleep and wakefulness. Therefore, the present study used in vivo microdialysis to test the hypothesis that ACh release in the basal forebrain varies significantly as a function of waking, NREM sleep, and REM sleep. Portions of these data have been presented as an abstract (30).

METHODS

Surgical preparation. Adult male cats (n = 4) were anesthetized with isoflurane (1–3% in O2) and implanted with standard electrodes for polygraphic identification of sleep-wake states. A craniotomy was performed over the cerebral cortex, and an acrylic well was built to provide access to the

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basal forebrain during subsequent experiments (11). Plastic sleeves were embedded within the acrylic to permit head-stable dialysis. Animals were free to move their limbs and make shifts in body posture.

Polygraphic identification of sleep and waking. With standard polygraphic criteria (29), waking was identified by a low-voltage, high-frequency cortical EEG, an electrocorticogram (EOG) that showed frequent eye movements, the presence of muscle tone as indicated by neck muscle electromyogram (EMG), and the absence of pontogeniculocipital (PGO) waves recorded from depth electrodes placed bilaterally in the lateral geniculate bodies of the thalamus. Behaviorally, open eyes and occasional shifts of body position characterized waking. NREM sleep was indicated when the cortical EEG exhibited a high-voltage, low-frequency pattern typified by slow waves and/or spindles. During NREM sleep, eyes were closed and the EOG showed few eye movements, EMG tone was lower than observed during waking, and there was an absence of movement. Electrographic measures of REM sleep included a cortical EEG with a low-voltage, high-frequency pattern similar to waking. During REM sleep, eyes were closed and rapid eye movements were recorded from the EOG, the EMG showed skeletal muscle atonia with phasic twitches, and PGO waves were recorded from the thalamus.

Experimental procedure. All experiments strictly followed the Guide for the Care and Use of Laboratory Animals (7th ed., National Academy of Sciences Press, Washington, DC, 1996). Cats were trained to sleep in a head-stable position (Kopf Instruments) in the laboratory. During waking, a microdialysis probe was aimed stereotaxically for the substantia innominata (4) at sites ranging from anterior (A) 14.5 to L5.5, and vertical (V) tia innominata (4) at sites ranging from anterior (A) 14.5 to L5.5, and vertical (V)

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In vivo microdialysis and HPLC. Microdialysis procedures have been described in detail (11). Briefly, microdialysis probes (CMA/10) had a polycarbonate membrane with a 20-kDa cutoff, 2-mm membrane length, and 0.5-mm membrane diameter. The probe was perfused continuously with Ringer (147 mM NaCl, 2.4 mM CaCl2, 4.0 mM KCl, 10 μM neostigmine) at a rate of 3 μl/min using a CMA/100 pump. Each 30-μl sample was collected during unambiguously scored states of waking, NREM sleep, or REM sleep and injected into the HPLC system for quantification of ACh. All of the 30-μl dialysis samples for waking and NREM sleep were obtained from single, uninterrupted episodes. In cats, the mean duration of REM sleep epochs is ~5–6 min (2). Thus ACh levels during REM sleep were assessed by collecting dialysate during multiple REM sleep episodes. Dialysis samples were not collected during transitions from waking to NREM sleep, when cats appeared drowsy and showed a mixed-frequency EEG. A 50 mM Na2HPO4 mobile phase solution, pH 8.5, at 1.0 ml/min (pressure = 2,500–3,000 lb/in2), carried each sample to an analytic column, permitting separation of ACh and choline. Hydrogen peroxide then was produced in an immobilized enzyme reactor column in amounts proportional to ACh and measured at a 0.5-V applied potential on a platinum electrode referenced to an Ag+/AgCl electrode. Chromatograms were recorded directly on a flatbed recorder and simultaneously digitized using ChromGraph software and stored on disk. Area under the chromatographic peak for each dialysis sample was compared with the peak areas generated from known ACh concentrations to determine the picomole value of ACh in each sample.

Data analysis. Descriptive statistics and a repeated-measures, one-way ANOVA were used to determine the effect of sleep-wake state on ACh release. Post hoc comparisons were performed using Tukey’s multiple pairwise comparison tests. A probability (P) value < 0.05 was used to evaluate the significance of all statistical tests. Histological sections were compared with the coronal plates of a stereotaxic atlas (4) to confirm dialysis probe placement in the substantia innominata.

RESULTS

The results are based on 2,700 min of microdialysis sampling obtained during 22 experiments in four cats. Figure 1 shows a typical microdialysis probe site in the substantia innominata and underlying diagonal band region of the basal forebrain. Figure 2A shows representative chromatograms peaks of basal forebrain ACh release during waking, NREM sleep, and REM sleep. Figure 2B summarizes mean + SE ACh release during waking (103 dialysis samples), NREM sleep (139 dialysis samples), and REM sleep (28 dialysis samples). Per experiment, 3–10 dialysis samples were collected during waking and NREM sleep, and 1–3 samples

Fig. 1. Histological localization of a dialysis site. Digitized coronal section of cat basal forebrain at ~14.5 mm anterior to stereotaxic zero (4). The arrowhead marks the midpoint of a dialysis site, located in the substantia innominata (SI). AC, anterior commissure; DBH, nucleus of the diagonal band of Broca, horizontal division; IC, internal capsule; LV, lateral ventricle; OC, optic chiasm; V3, third ventricle.
were collected during REM sleep. ANOVA revealed a significant state main effect on basal forebrain ACh release. ACh release during REM sleep (0.77 ± 0.07 pmol/10 min) was significantly elevated (32%) compared with ACh release during waking (0.58 ± 0.03). ACh release during NREM sleep (0.34 ± 0.01 pmol/10 min) was significantly decreased below waking (−41%) and REM sleep (−55%) levels.

**DISCUSSION**

The present data show that ACh release in the substantia innominata region of the feline basal forebrain is greatest during REM sleep, intermediate during waking, and lowest during NREM sleep. To the best of our knowledge, this is the first study quantifying basal forebrain ACh release during polygraphically defined states of sleep and waking. The following discussion relates this state-dependent pattern of basal forebrain ACh release to current knowledge about the REM sleep-related roles of ACh in other brain regions.

Basal forebrain cholinergic neurons long have been known to participate in the regulation of behavioral arousal and cortical EEG activation via their widespread projections to the entire neocortex (reviewed in Refs. 8, 23, 24, 28). Whereas cortical ACh release is equally high during REM sleep and waking (7, 14), the present study shows that ACh release within the basal forebrain is significantly greater during REM sleep than during waking (Fig. 2). Other feline brain regions in which ACh release is greater during REM sleep than during waking include the hippocampus (14), dorsal (10) and medial (11) regions of the pontine reticular formation, and the nucleus paramedianus of the caudal medullary reticular formation (9). In the hippocampus, pontine reticular formation, and nucleus paramedianus, ACh is known to play a key role in generating theta rhythm during REM sleep (14), the state of REM sleep (1), and the motor atonia characteristic of REM sleep (9), respectively. In contrast, the functional roles of ACh within the substantia innominata region of the basal forebrain remain to be identified. Increased cholinergic neurotransmission in the basal forebrain has been suggested to contribute to the pathophysiology of canine narcolepsy (19). The current finding that during REM sleep ACh release in the basal forebrain is significantly elevated over waking levels, suggests a differential role for basal forebrain ACh during waking and REM sleep.

Direct administration of the cholinergic agonist carbachol into the substantia innominata region of the feline basal forebrain increased waking, decreased REM sleep, and inhibited the ability of pontine carbachol to enhance REM sleep (2). This finding demonstrated an interaction between cholinoceptive basal forebrain and pontine systems in the regulation of REM sleep. In normal dog, microinjection of carbachol into the substantia innominata also increased waking and decreased sleep, whereas in the narcoleptic dog, the same dose of carbachol triggered cataplexy when delivered to the substantia innominata (19). Cataplexy, the sudden loss of postural muscle tone during waking, is one of the primary symptoms of the neurological disorder narcolepsy (15). The similarity between cataplexy and the normal muscle atonia of REM sleep has led to the hypothesis that cataplexy results from a triggering of REM sleep atonia mechanisms during waking (26). Relatively high levels of basal forebrain ACh release during REM sleep (Fig. 2) and cholinergic triggering of cataplexy from basal forebrain (19) are consistent with this hypothesis. Most recently, it has been demonstrated that basal forebrain ACh release in control and narcoleptic dogs is increased selectively during the Food Elicited Cataplexy Test (22), a behavioral bioassay for cataplexy that involves learning, memory, emotional stimulation, and reward (18). This finding suggests a role for basal forebrain ACh during waking in emotional- and trained-reward-associated behaviors (22).

The present data (Fig. 2) stimulate questions about the sources of cholinergic input to the basal forebrain and the mechanisms modulating ACh release within the basal forebrain. Potential sources of basal forebrain ACh include the brain stem laterodorsal and pedunculopontine tegmental nuclei and recurrent collaterals of local cholinergic neurons (8, 25). The relative contribution to basal forebrain ACh release of...
extrinsic (brain stem) and intrinsic (basal forebrain) sources is not known. Factors modulating ACh release within the basal forebrain only recently have begun to be identified, and new data indicate a role for muscarinic autoreceptors (3), nitric oxide (32), GABA_A receptors (31), and excitatory amino acids (13). The present findings demonstrating an REM sleep-dependent increase in basal forebrain ACh release encourage future studies aiming to elucidate the neurochemical mechanisms by which basal forebrain ACh release is modulated, and the functional roles of cholinergic neurotransmission within the basal forebrain.

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