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ACETYLCHOLINE AND THE REGULATION OF REM SLEEP: BASIC MECHANISMS AND CLINICAL IMPLICATIONS FOR AFFECTIVE ILLNESS AND NARCOLEPSY[1]

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INTRODUCTION

Despite over half a century of scientific inquiry into the basic brain processes underlying the alternation of mammalian states of arousal and sleep, we have little understanding of the mechanisms subserving this basic behavior. More recent experimental and theoretical reviews have proposed multicenter, interdigitated anatomical systems, with multiple neurochemical signatures, involved in the elaboration of the sleep state (1–3). However, no construct has yet successfully described the natural processes underlying sleep initiation, sleep maintenance, sleep stage alternation, or the subsequent relationship of sleep stage alternation to the processes underlying wakefulness and its maintenance or sleep-wake pathologies. Recent evidence suggests that particular subsets of brain stem cholinoceptive neurons are involved in the elaboration

of major behavior and are physiological constituents of rapid-eye-movement (REM) sleep (1, 2). To date these collective studies provide the most complete description of the potential anatomical and neurochemical substrate for the initiation and maintenance of mammalian states of arousal and sleep.

In this review we focus on and summarize the evidence for the role of cholinergic mechanisms in REM sleep. Interest in this neurochemical system has increased during the past ten years, and important advances have been made in histochemically identifying the cholinergic neuron. New anatomical techniques have been useful in elucidating the role of the cholinergic system in REM sleep generation. We review the evidence that a diffuse network of cholinoceptive neurons in the medial pontine reticular formation (PRF) primes, initiates, and maintains the consolidated state of REM sleep. Neither the origin of the cholinergic input to cholinoreceptive neurons nor the chemical identity of these neurons is fully established at this time.

The realization that cholinergic mechanisms play an important role in the initiation and maintenance of REM sleep coincides with increased interest in states or conditions in which REM sleep occurs earlier, or more, than usual (4). These conditions include clinical disorders such as major depressive disorder, narcolepsy, and, perhaps, other disorders such as obsessive-compulsive disorder and some schizophrenias in which short REM latency (elapsed time from onset of sleep to onset of the first REM period) occurs. These observations raise the possibility that abnormal activation of central cholinergic mechanisms may be part of the pathophysiology of these clinical conditions.

THE NEUROANATOMICAL ORGANIZATION OF THE CHOLINERGIC SYSTEM

Acetylcholine is a neurotransmitter in the central nervous system, as was experimentally determined over a decade ago (5). However, lack of accurate mapping procedures comparable to those used to localize serotonin and the catecholamines has hindered progress in determining the organization of acetylcholine. New procedures, such as autoradiography to locate cholinergic receptors (6), immunohistochemical labeling of the acetylcholine-synthesizing enzyme, choline-acetyltransferase (ChAT) (7), and identification of acetylcholine in neurons (8), are only now providing information on the organization of the acetylcholine system and its potential role in sleep and other behaviors such as information processing (9).

Earlier methods for identifying cholinergic neurons involved detection of acetylcholinesterase (AchE), the enzyme that metabolizes acetylcholine. This procedure, developed by Koelle & Friedenwald (10), was used by Lewis & Shute (11) to provide early maps of the cholinergic system. Conclusions from

this procedure have been questioned, as this approach provides a limited, indirect method of localizing cholinergic pathways. Moreover, AchE is found in places devoid of acetylcholine, such as the zona incerta, hypothalamic arcuate and dorsomedial nuclei, lateral posterior hypothalamus, and substantia nigra (12, 13). This procedure, therefore, falsely indicates many positive sites.

A more direct histochemical marker of cholinergic pathways has been developed. Recently, immunohistochemical procedures have been used to label ChAT. Using this immunohistochemical technique, Mesulam and coworkers (14) have identified six major cholinergic groups. Cholinergic groups 1 and 2 (Ch 1 and 2) lie within the medial septal nucleus and the vertical limb nucleus of the diagonal band, respectively. Neurons from these two groups innervate the hippocampus. Group Ch 3 is partly contained in the horizontal limb nucleus of the diagonal band and innervates the olfactory bulb. The largest collection of cholinergic cells is located within the nucleus basalis of Meynert and in the nucleus of the ansa lenticularis, in the nucleus of the ansa peduncularis, and in the medullary laminae of the globus pallidus and substantia innominata. Collectively, these cholinergic neurons are referred to as the Ch 4 group, and they are the principal cause of the cholinergic innervation of the amygdala and neocortex. Loss of these cholinergic neurons has been hypothesized to cause the cognitive deterioration observed in Alzheimer's disease (15).

In the brain stem the largest collection of cholinergic cells occurs in the pedunculo-pontine nucleus (Ch 5), which borders the brachium conjunctivum, and the lateral dorsal tegmental nucleus (Ch 6), which is medial to the locus coeruleus. These two groups, which form the ascending cholinergic pathway of Lewis & Shute (11, see also 16), innervate the thalamus, hippocampus, hypothalamus, and cingulate cortex (14, 16–18). In the brain stem, cholinergic neurons are also found in the cranial motor nerve nuclei and the solitary nucleus.

ACETYLCHOLINE AND THE TONIC AND PHASIC COMPONENTS OF REM SLEEP

REM sleep was first discovered with the observation of a wakelike EEG associated with bursts of rapid eye movements during behavioral sleep in man (19, 20). When subjects were awakened from this state, they frequently reported dreaming (20). Since then sleep and REM sleep have been identified in virtually all mammals, some birds, and, perhaps, in reptiles (21).

REM sleep is composed of both tonic (occurring throughout the REM sleep episode) and phasic (occurring only sporadically during REM sleep) events. The major tonic events include cortical desynchronization, loss of muscle

tone in antigravity musculature, and theta activity in the dorsal hippocampus. The phasic events include monophasic waves in pons, lateral geniculate nucleus, and occipital cortex (these waves are called ponto-geniculo-occipital waves, i.e. PGO waves), and rapid eye movements. As we review below, data from lesion, transection, pharmacological, and single-unit studies indicate that the various tonic and phasic components of REM sleep are controlled by discrete reticular formation (RF) nuclei. Moreover, a collection of neurons in the medial pontine reticular formation (PRF) may trigger the discrete reticular nuclei individually responsible for the various tonic and phasic components of REM sleep.

Acetylcholine and Cortical Desynchronization

Cortical EEG desynchronization is an important, distinguishing tonic feature of REM sleep. During both REM sleep and waking the cortical EEG typically shows low-voltage, fast activity, whereas orthodox (NREM) sleep is characterized by the presence of slow (1–3 Hz), high-amplitude waves, sleep spindles (12–14 Hz), and K-complexes.

Moruzzi & Magoun (22) showed that the mesencephalic reticular formation was important for desynchronizing the cortex. Over the years various RF nuclei, particularly the norepinephrine-containing locus coeruleus (LC), have been implicated in cortical EEG desynchronization (23). Data from lesion and single unit studies, however, show that the LC is not necessary for cortical desynchronization during waking and REM sleep (3, 24–26).

Cholinergic mechanisms, on the other hand, do appear to play an important role in cortical desynchronization. Systemically administered atropine, a cholinergic antagonist, readily produces slow, high-amplitude waves, even during behavioral waking (27). Local infusion of cholinergic agonists, such as carbachol, bethanechol, or oxotremorine, into the RF increases cortical desynchronization (28–32). Intense behavioral and EEG arousal is noted with carbachol injections into the mesencephalic RF (28, 33, 34).

Acetylcholine and Rapid Eye Movements

Evidence from lesion studies (35) indicates that the vestibular nuclei may be involved in rapid eye movements during REM sleep. Evidence supporting this hypothesis comes from studies that show that phasic changes in firing rates of vestibular neurons occur during REM sleep in intact cats (36), in the decerebrate preparation (37, 38), and in acute decerebrate animals treated with acetylcholine-potentiating agents such as physostigmine (37, 38). Furthermore, Mergner & Pompeiano (39) showed that increased discharge rates of medial vestibular neurons and abducens motoneurons, which innervate the lateral rectus muscles of the eye, occur 11–15 msec prior to activity in the

lateral rectus muscle. Vestibular lesions, however, abolish bursts of rapid eye movements but do not interfere with isolated, slow eye movements or with REM sleep per se.

The excitation in vestibular and abducens motoneurons may be generated by nuclei located within the paramedian RF (40, 41). Indeed, reticular units do show bursts preceding saccadic eye movements (40–44). Moreover, cells in the giganto-cellular tegmental field show cyclic changes in discharge rates before rapid eye movements, not only in intact animals (45) but also in decerebrate animals treated with acetylcholine-potentiating agents (46, 47).

Acetylcholine and PGO Waves

Ponto-geniculo-occipital (PGO) waves are slow, monophasic potentials that occur either singly or in clusters of three to four just prior to and during REM sleep. These waves are recorded in cats, from the pons, lateral geniculate nucleus (LGN), and occipital cortex, areas directly related to the visual system.

The neurons responsible for PGO waves are hypothesized (for review see 48) to be located in and around the brachium conjunctivum (which Sakai (48) calls the "X" area), the rostral part of the lateral parabrachial nucleus, which is just caudal to the "X" area, and the rostral part of the locus coeruleus. Some neurons in these areas fire in phasic bursts (3–5 spikes) as much as 5–25 msec before the onset of a PGO wave (48, 49). During wakefulness some of these units also fire in conjunction with eye movements. Electrical stimulation of this area induces PGO waves, whereas electrical ablation abolishes the PGO waves (48).

The neurons responsible for PGO potentials appear to be cholinergic or at least cholinoceptive in nature. Atropine significantly reduces PGO bursts (50), whereas physostigmine triggers PGO bursts in collicular or pontine-transected cats (51). Significantly, microinfusions of carbachol in the dorso-lateral pontine tegmentum induce PGO activity selectively (28, 31). A vestibular component also appears to be involved because carbachol microinfusion into the vestibular region can evoke PGO waves tightly coupled to stereotyped eye movements (P. J. Shiromani, personal observations).

The elaboration and discharge of PGO waves are not exclusively under cholinergic control; noradrenergic and serotonergic inputs from the locus coeruleus and dorsal raphe nucleus (DRN) are hypothesized to inhibit the cholinergic PGO executive neurons (48). Evidence in support of the inhibitory influence is provided by studies showing that electrical stimulation of DRN inhibits PGO waves (52), whereas lesions or cooling of DRN immediately releases PGO activity (53). Treatments that decrease serotonin or norepinephrine also release PGO activity immediately (53). Moreover, DRN

and LC neurons stop activity just prior to, and in temporal contiguity with, PGO activity (54–57).

The anatomical profile of the PGO neuronal network confirms the electrophysiological and pharmacological evidence. Using retrograde tracer and immunohistochemical studies, Sakai (for review see 53) demonstrated that neurons in the "X" area project directly to the LGN (where PGO waves are most easily recorded) and stain positively for ChAT, a specific marker of cholinergic neurons. In turn, the neurons in the "X" area receive norepinephrine and serotonin afferents from the DRN and LC.

Acetylcholine and Atonia

Much evidence supports the hypothesis that the cataplectic episodes of narcoleptic humans and dogs are related to the muscle atonia of normal REM sleep. The inhibition of antigravity musculature is hypothesized (48) to result from activation of a discrete group of nonmonoaminergic cells located ventrally in the LC complex. These peri-LC neurons (48) may exert an excitatory influence on magnocellular neurons located in the medullary RF. The magnocellular neurons correspond to the medullary inhibitory center previously described by Magoun & Rhines (58) and are postulated to induce a nonreciprocal inhibition of spinal motoneurons by exciting spinal inhibitory interneurons (47, 59–62).

Electrical stimulation of the medullary RF, especially the magnocellular, elicits generalized inhibition of spinal motoneurons (48), whereas bilateral electrical ablation of the peri-LC and medial LC abolishes the atonia during REM sleep (63). Moreover, neuronal activity within the peri-LC and the magnocellular is high during periods of atonia in REM sleep (48, 64). The dorso-lateral pontine tegmentum, which contains the peri-LC, exhibits intense metabolic activity, as determined by the 2-deoxyglucose (2-DG) method, during concussion-induced behavioral suppression (65). Investigators suggested (65, 66) that a common mechanism underlies the atonia of REM sleep and the behavioral suppression that follows concussion. Horseradish peroxidase technique (HRP) studies (67, 68) show connections between the peri-LC, the magnocellular, nucleus, and the spinal cord.

The cholinergic mechanism is also implicated in the phenomena of atonia. Infusion of carbachol into the pontine tegmentum readily induces cataplexy in cats (28, 29, 31, 34, 66, 69–71). In acute decerebrate cats, systemic infusion of physostigmine induces cataplexy; such a loss of decerebrate rigidity occurs at regular intervals only with a chronic preparation (72). In narcoleptic dogs, systemically administered cholinomimetics precipitate cataplectic episodes, whereas muscarinic receptor blockers delay these episodes, and nicotinic agents have no effect (73, 74). Moreover, in narcoleptic dogs, increased

muscarinic receptor binding is found in several pontine sites (73). In addition, ChAT is found in these areas (7, 75, 76).

ACETYLCHOLINE AND REM SLEEP GENERATION

Although discrete RF nuclei may be responsible for generating the major tonic and phasic components of REM sleep, we suggest that a PRF cholinoceptive mechanism primes the various RF nuclei. Historically, the cholinergic system was the first neurotransmitter system implicated in REM sleep generation. Indeed, Jouvet (77) initially hypothesized that cholinergic mechanisms served an executive function in REM sleep generation. However, he subsequently formulated the "mono-aminergic theory of sleep" (23) when the development of the Falck–Hillarp histofluorescence technique (78) revealed that monoamine pathways originating from the brainstem regions were implicated in arousal, sleep, and REM sleep. The finding that the monoamine system innervated almost the entire brain and spinal cord suggested that this system was involved in orchestrating widespread electrophysiological changes associated with the sleep-wake cycle. Considerable research shows that dopamine, norepinephrine, and serotonin play important roles in sleep and arousal (23, 79). These neurotransmitters are also implicated in the regulation of the REM sleep process but may not be directly involved in REM sleep generation. Instead, these neurotransmitters are currently hypothesized to exert an inhibitory control on a diffusely represented neuronal system responsible for the initiation and maintenance of REM sleep (1). Considerable work has been done on establishing the inhibitory role of the catecholamines (principally norepinephrine) and serotonin in REM sleep, however very little is known about the diffuse REM sleep generating system.

Pharmacological studies show that cholinergic mechanisms in the medial PRF play an important role in the generation of REM sleep. For example, in cats and rats administration of cholinergic agonists, e.g. carbachol, directly into the PRF evokes elements of REM sleep (atonia, PGO waves, rapid eye movements) or complete REM sleep, which may be unusually long (28–32, 34, 69, 70, 80–82). Infusions into midbrain or medullary sites fail to induce REM sleep (28, 34, 70), whereas local infusion of scopolamine blocks the cholinomimetic-induced and physiologic REM sleep (31, 82). Recently, Shiromani & McGinty (70) found that some medial PRF neurons increase discharge in conjunction with the carbachol-induced REM sleep. The medial PRF appears to be unique because blood pressure changes during the carbachol-induced REM sleep (when the cabachol infusions are made in the medial PRF) are similar to those during physiological REM sleep (34). During REM sleep increased release of acetylcholine occurs in cortex (83, 84) and striatum (85) of normal cats and in ventricular perfusates of conscious

dogs (86). In human subjects, intravenous infusions of physostigmine or arecoline during non-REM sleep decrease the latency to REM sleep, although infusions during or immediately after REM sleep produce arousal (87–91). In addition, an orally active muscarinic agonist (RS-86) shortens REM latency in normal volunteers (92).

The cholinomimetic-induced and normal REM sleep may be mediated by muscarinic receptor activation because scopolamine and atropine block normal and cholinomimetic-induced REM sleep (28, 31, 82, 91). In narcoleptic dogs, as mentioned, muscarinic receptor agonists readily trigger cataplexy, whereas nicotinic agonists have no effect (74). In narcoleptic dogs, increased muscarinic receptors are located in the medial PRF regions implicated in REM sleep generation (73, 93, 94). In normal human subjects, a three-consecutive-morning treatment with scopolamine decreases the latency to REM sleep at night (95). In rats, REM sleep augmentation occurs in conjunction with muscarinic receptor up-regulation during withdrawal from a seven-day chronic scopolamine treatment (96). In this study increased muscarinic density was found in the caudate and hippocampus but unchanged density was observed in the cortex, brainstem, and cerebellum. Finally, increased REM sleep is found in a strain of rats genetically inbred for increased numbers of central cholinergic receptors (P. J. Shiromani, D. Overstreet & J. C. Gillin, unpublished observations).

Numerous pharmacological studies implicate cholinergic mechanisms in REM sleep generation, however, they only indicate that neurons sensitive to acetylcholine can generate REM sleep. Indeed, we find no cholinergic cell bodies in the medial PRF, an area traditionally implicated in REM sleep generation (97). Also, we show that some neurons in the medial PRF show a progressive increase in discharge from waking to REM sleep (97). Previously, Sakai (48) had identified some "REM-on" neurons and suggested that they are important for REM sleep generation, since they show a unique firing increase related to REM sleep.

Few, if any, cholinergic cell bodies are located in the medial PRF. Therefore, the control system in the medial PRF responsible for REM sleep generation is apparently not mediated by intrinsic PRF cholinergic neurons. As an alternate hypothesis, we suggest that an extrinsic cholinergic input "primes" the cholinoceptive REM sleep neurons in the medial PRF. Thus, the medial PRF may actually represent a final common path in a sequence of events originating elsewhere. Now, we must determine the source of the cholinergic input to the medial PRF.

Two cholinergic groups are the possible source of cholinergic afferents to the medial PRF. The first choice of a group is the cholinergic cells in the pedunculo-pontine (PPG) and lateral dorsal tegmental (LDT) groups. These cells form the largest collection of cholinergic cells in the PRF (7, 14, 75, 98),

and considering that REM sleep originates from the PRF, this collection of cells is a logical choice of a possible source. These two groups form the ascending cholinergic pathway of Shute and Lewis (11, 16), which innervates the thalamus, hippocampus, hypothalamus, and cingulate cortex (14–18). The PPG and LDT may also be a component of the ascending reticular-activating system described by Moruzzi & Magoun (22).

Much evidence indicates that cholinergic cells in the PPG and LDT play a very important role in some tonic and phasic components of REM sleep. For example, Steriade et al (99) suggest that EEG activation during waking and REM sleep may be result from a tonic activation of an ascending cholinergic system in the rostral reticular core. Steriade et al (99) suggest that midbrain-subthalamic-thalamic-cortical loops underlie the ascending activating reticular influences. The PPG and LDT innervate the thalamus (for review see 16), and microinfusion of carbachol into the rostral portions of the PPG induces a sustained behavioral arousal characterized by EEG desynchronization (28, 34). Indeed, acetylcholine is released from the cerebral cortex during REM sleep (84). PGO activity is another component of REM sleep hypothesized to be under the control of cholinergic cells in the PPG and LDT (48, 53). PGO waves are a phasic component of REM sleep, and they occur just before and during REM sleep. The PPG and LDT project to the LGN (where PGO waves are recorded easily) (48, 53, 100–102). Some neurons in the PPG and LDT fire before and with PGO, leading some investigators to suggest that these are PGO executive neurons (48, 53, 103). Finally, the muscle atonia that accompanies REM sleep may be a result of descending cholinergic neurons in the more medial-caudal portions of the PPG located in the nucleus subcoeuruleus and locus coeruleus-alpha (48, 53, 68).

The other possible source of cholinergic afferents to the medial PRF is the cholinergic cells in the basal forebrain. Since rostral transection does not eliminate REM-like states in the isolated pons, we postulate that cholinergic inputs from the basal forebrain or other rostral sites might exert modulation control over REM sleep rather than be the sole regulation of REM sleep. This region along with the raphe is considered to be one of the somnogenic sites (104). Electrolytic (104, 105) and kainic acid (106) lesions of the basal forebrain produce long-lasting insomnia, and electric stimulation produces sleep (107). Diathermic warming in this region also produces sleep (108). More importantly, Szymusiak & McGinty (109) found that some basal forebrain neurons discharge only during sleep. The chemical identity of this type of basal forebrain neuron is unknown, but since these cells are localized in areas found to contain cholinergic cells (7), the cells causing sleep may be cholinergic.

Most studies have examined the ascending projections of the cholinergic neurons. The descending projections of the basal forebrain are not thoroughly

examined. Recently, retrograde studies demonstrated that there is a descending projection from the basal forebrain to the PRF in rats (110). However, these studies did not determine whether cholinergic neurons innervated the PRF. This determination is vitally important considering that the basal forebrain represents a somnogenic center whereas the PRF is important for arousal and REM sleep. The interplay between cholinergic basal forebrain cells and cholinoceptive medial PRF cells may be responsible for the regular transition from waking to sleep to REM sleep. For example, we noted earlier that some basal forebrain neurons discharge selectively during non-REM sleep. If these neurons are cholinergic, then increased discharge during non-REM sleep could release acetylcholine in the medial PRF and prime the medial PRF cholinoceptive neurons responsible for REM sleep. Subsequent interplay between acetylcholine, catecholamine, and indoleamine neuronal systems, and other chemically unidentified neurons in the pons, may then be responsible for the initiation and maintenance of REM sleep (1, 111).

Even though we suggest that a cholinergic input primes the cholinoceptive medial PRF, the medial PRF neurons may have an intrinsic property to exhibit a tonic or bursting firing pattern similar to that seen in thalamic neurons (112–114). Indeed, Greene & McCarley (115) have begun to examine, in pontine slices, the firing pattern of medial PRF neurons, and they find similarities with thalamic neurons.

SUMMARY OF BASIC MECHANISMS

Considerable evidence indicates that discrete brain stem nuclei are responsible for the various tonic and phasic components of REM sleep. The preponderance of evidence suggests that these discrete nuclei are activated and REM sleep ensues when a group of medial PRF neurons begin to fire. The evidence derived from pharmacological and anatomical studies indicates that the medial PRF neurons instrumental to REM sleep generation are cholinoceptive and are dependent on a cholinergic input for activation. Although we focus on the cholinergic system in this review, we stress that the catecholamine and serotonin systems also play very important roles and that the interplay between these transmitter systems may be responsible for the orderly transition between waking, sleep, and REM sleep. In addition, the chemical identity of the apparent cholinoreceptive cells in medial PRF remains unknown; they are not cholinergic, serotonergic, or catecholaminergic.

CHOLINERGIC REGULATION OF REM SLEEP: CLINICAL IMPLICATIONS

The evidence for cholinergic involvement in REM sleep, reviewed above, has implications for conditions in which short REM latency occurs. The two

best-documented clinical disorders with short REM latency are depression and narcolepsy, which we briefly review.

Short REM latency has been reported in some patients with severe obsessive-compulsive disorder, schizophrenia, alcoholism, anorexia nervosa, and attention-deficit disorder. Controversy exists regarding some of these later conditions, and the significance of the reports is not yet clear. Further research is needed to determine the clinical specificity of short REM latency and other sleep disorders in depression.

In addition, short REM latency may occur in normal subjects under certain conditions, such as withdrawal from drugs that suppress REM sleep, following deprivation of REM sleep, and under altered sleep-wake patterns, such as naps, free-running conditions in which the subject lives in an environment free of time cues, and on experimental "short days" (i.e. 30 minutes sleep and 60 minutes wake). A circadian rhythm of REM latency and REM sleep exists, which is roughly in phase with the core body-temperature rhythm. REM latency tends to be longest and REM percentage lowest when body temperature is highest, for example, in the early evening. In contrast, REM latency is shortest and REM percentage highest when temperature is lowest, for example, at the end of the normal rest period. REM latency also varies with age; it is highest in early adolescence and tends to decline moderately after midlife.

Many studies from around the world demonstrate that short REM latency is a state-dependent characteristic of patients with moderate-to-severe depression, particularly those with endogenous, melancholic, or primary subtypes (for reviews see 116, 117). In addition, some, but not all, studies report increased duration of the first REM period and increased ocular activity during REM periods (increased REM density). Loss of total sleep time, poor sleep efficiency (percentage time asleep in bed), and low amounts of delta (Stages 3 & 4) sleep are also commonly reported. The sleep alterations in depression tend to be worse in older than in younger patients compared with age-matched controls.

Short REM latency in depression appears to be associated with hypercortisolemia in depression, as manifested either by an elevated concentration of plasma cortisol (118) or with "escape" on the dexamethasone-suppression test (DST) (119–122).

Many biochemical theories of affective disorder have been proposed. Included is the cholinergic-aminergic imbalance hypothesis, originally proposed by Janowsky et al (123). Based on inferences from pharmacological studies, they suggest that depression results from an increased ratio of central cholinergic to aminergic activity, while mania results from the opposite. For example, physostigmine and other cholinomimetics drugs have antimanic effects in manic patients and depressogenic effects in normal controls and depressed patients, especially in depressives.

The cholinergic-aminergic balance hypothesis of affective disorders provides an explanatory mechanism for both short REM latency and hypercortisolemia in depression. As reviewed already, considerable evidence suggests that cholinergic mechanisms help regulate REM latency and REM density in normal sleep. In addition, cholinergic mechanisms facilitate release of plasma ACTH and cortisol. Physostigmine has been reported to elevate plasma ACTH levels to a greater extent in depressives than in normals (124) and to reverse dexamethasone suppression of cortisol in normal subjects (125).

It has not been possible to test the cholinergic-aminergic hypothesis directly. Nevertheless, depressive sleep patterns, short REM latency, elevated REM density, and poor sleep efficiency, can be induced in normal volunteers by the administration of scopolamine for three consecutive mornings (126). In addition, following this treatment, sleep records of normal volunteers were chosen as "depressed" by a discriminant function analysis that had previously separated depressed, insomniac, and normal sleep records successfully (127). As stated, administration of scopolamine in this fashion may induce muscarinic receptor supersensitivity (95). These observations suggest that a central, functional muscarinic supersensitivity is present in patients with affective disorders.

To test this hypothesis, we developed the Cholinergic REM Induction Test (CRIT), which was originally used to demonstrate muscarinic supersensitivity in normal volunteers following administration of scopolamine (91, 128). In the CRIT, arecoline (0.5 or 1.0 mg) is administered during the second NREM period of the night, and the latency to the second REM period is measured. Our original studies indicated that patients with bipolar depression (both in a state of clinical remission and ill) responded more rapidly than normal controls (91, 129, 130). Further studies in identical twins suggested that the response on the CRIT might be partially under genetic control (131). Since the original study, Sitaram and his colleagues have confirmed the general cholinergic muscarinic supersensitivity in patients with endogenous depression using the CRIT (132–134). Our own recent preliminary results confirm a faster REM induction in patients with major depressive disorder and bipolar depression with an arecoline dose of 1.0 mg than with a 0.5-mg dose.

Using another sleep measure to assess cholinergic supersensitivity in depression, Berger et al (135) found that depressed patients were more likely than normal controls to awaken following an infusion of physostigmine (0.5 mg), administered shortly after sleep onset. Since we had previously shown time- and dose-dependent arousal to intravenously administered physostigmine in sleep, this result may be consistent with increased responsiveness to cholinomimetics in depressives compared to controls.

More recently, Berger et al (136) reported that the experimental muscarinic agonist, RS-86, shortens REM latency to a greater extent in depressives than

in normal controls. All of these sleep studies, employing a variety of cholinergic agonists (arecoline, physostigmine, and RS-86), support the hypothesis that REM sleep can be induced more rapidly with cholinomimetics in patients with depression than in normal volunteers. Although no studies provide direct evidence of increased muscarinic receptor density in patients with depression, these data from sleep studies suggest that functional muscarinic supersensitivity may be a feature of depression.

The other disorder characterized by short REM latency is narcolepsy, a chronic disease in man, characterized by excessive daytime sleepiness and attacks of cataplexy, often precipitated by emotional arousal (137–139). The clinical symptoms of narcolepsy probably represent dissociated features of normal REM sleep. For example, cataplexy may be the normal muscle atonia accompanying REM sleep. Genetics play an important role in narcolepsy and virtually all patients with narcolepsy have the HLA antigen DR2 (140). Narcolepsy also occurs in certain animals. Recent evidence suggests that muscarinic receptor density is increased in brainstem of narcoleptic dogs (73, 93, 94). Furthermore, dopamine turnover appears to be reduced in narcoleptic dogs (141).

As mentioned above, short REM latency also occurs when normal subjects sleep near the low point of their circadian temperature rhythm. Studies of autopsied brain tissue in man (142) and rat (143) suggest that various neurotransmitter receptors, including muscarinic receptor density, have a circadian rhythm. Thus, the circadian rhythm of muscarinic receptor density in selected brain regions may underlie the circadian rhythm of REM latency and REM sleep.

Finally, in the context of circadian rhythms, cholinergic nicotinic mechanisms may be involved in mediating the phase position of the suprachiasmatic nucleus (SCN), the best-established circadian clock in mammalian brain. Nicotinic agonists, like light, can advance or delay the phases of circadian rhythms, depending upon the time of administration of the stimulus (creating the classical phase-response curve) (144).

CONCLUSION

A role for cholinergic neurons in the control of REM sleep and its physiological components seems firmly established. The anatomical basis for this cholinergic involvement needs further investigation. The role of other neurotransmitter systems in the general control of sleep and circadian rhythms also needs investigation. In terms of clinical implications, the involvement of cholinergic mechanism in normal sleep may provide clues to pathophysiological mechanisms in certain disorders.

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