ORIGINAL ARTICLE

REM Sleep Regulating Mechanisms in the Cholinergic Cell Compartment of the Brainstem

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Abstract

Rapid eye movement (REM) sleep is a highly evolved yet paradoxical behavioral state (highly activated brain in a paralyzed body) in mammalian species. Since the discovery of REM sleep and its physiological distinction from other sleep states[1], a vast number of studies in neurosciences have been dedicated toward understanding the mechanisms and functions of this behavioral state. Collectively, studies have shown that each of the physiological events that characterize the behavioral state of REM sleep is executed by distinct cell groups located in the brainstem. These cell groups are discrete components of a widely distributed network, rather than a single REM sleep center. The final activity within each of these executive cell groups is controlled by the ratio of cholinergic neurotransmission emanating from the pedunculopontine tegmentum (PPT) to aminergic neurotransmission emanating from the locus coeruleus (LC) and raphe nucleus (RN). In this review, we summarize the most recent findings on the cellular and molecular mechanisms in the PPT cholinergic cell compartment that underlie the regulation of REM sleep. This up-to-date review should allow clinicians and researchers to better understand the effects of drugs and neurologic disease on REM sleep.

Keywords: REM Sleep, Neurotransmitters, Receptors, Intracellular signal transduction, Cholinergic cell, Brainstem.

Introduction

The rest-activity cycle is one of the basic signs of all living organisms. Rest is a passive process during which living organisms "slow down" and become rejuvenated for subsequent activities. In homeothermic vertebrates, like mammals and birds, the rest part of this rest-activity cycle evolved as sleep. This sleep, in addition to providing rest and micromanagement of energy savings, provides a biological advantage in the formation of complex memory and in the physiological fight against infections. Sleep in mammals could be defined by:[1] characteristic changes in posture;[2] raised sensory threshold; and[3] distinctive electrographic signs. Sleep is usually associated with a marked diminution of motor activity and an assumption of recumbent postures. Typically the eyes close and the somatic musculature becomes hypotonic. The threshold to external stimulation increases and animals become progressively less responsive to external stimuli as sleep deepens. Beneath the calm exterior of sleeping mammals, dynamic forces are at play. There are mechanisms that regulate the two

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alternating sleep states that occur while mammals are "asleep"[2]. Normally, when we first enter the sleep state, it is via quiet (non-rapid eye movement; non-REM) sleep, which is a state that, behaviorally, is not very dramatic. We simply lie still, breathing slowly. Our eyes drift slowly back and forth and every once in a while we shift our sleep position. When first falling asleep, individuals may progressively lose awareness of the outside world and experience micro-hallucinations and illusions of movements of the body in space. During non-REM sleep there are decreases in body temperature, blood pressure, heart rate, and respiratory rate. However, the pulsatile release of growth and sex hormones from the pituitary gland and production of antibodies increases during non-REM sleep. The concomitance of these events gives further credence to the notion that non-REM sleep may be functionally associated with anabolic processes benefiting the somatic tissues[3]. Non-REM sleep is characterized by a progression of change in the electroencephalogram (EEG) from a low-amplitude, high-frequency to a high-amplitude, low-frequency pattern. Throughout this process, as the EEG frequency is decreasing and the amplitude is increasing, muscle tone progressively declines and may be lost altogether in most of the somatic musculature. Slow rolling eye movements first replace the rapid saccadic eye movements of waking and then subside, with the eyes finally assuming a divergent upward gaze. After varying amounts of time (depending upon the size of the animal and its brain), this progressive set of changes in the EEG reverses itself and the EEG eventually resumes the low-amplitude, fast character previously seen in waking. Instead of waking, however, behavioral sleep persists. Muscle tone, that at first passively decreased during non-REM sleep, is now actively inhibited. Stereotyped bursts of saccadic eye movements called rapid eye movements (REMs) appear in the electrooculogram (EOG) and give this state the name REM sleep. This phase of sleep has also been called activated sleep (or paradoxical sleep; due to increased EEG activation). Supplemental to these polysomnographic signs, other REM sleep-specific physiological signs are: myoclonic twitches in the facial, digital, and even major proximal skeletal muscles, pronounced fluctuations in cardio-respiratory rhythms and core body temperature, and penile erection in males and clitoral engorgement in females.

In the fifty years since pioneering neuroscientists began to identify specific centers for well-characterized states of non-REM and REM sleep[2,4,5], our understanding of the neurobiology of sleep in terms of brain structures, cell types, neurotransmitters and their receptors, intracellular molecular cascades, neuronal networks, and synaptic interactions has progressed dramatically. Similarly, considerable progress has also been made in identifying some of the positive functions of sleep. It is remarkable how far we have come in understanding the different aspects of sleep neurobiology — now we have an independent specialty in medicine, sleep medicine. Our growing understanding of the cellular and molecular aspects of sleep mechanisms will likely yield significant new insights into the basic underpinnings of sleep as well as the causes of sleep disorders and other disorders that are affected by sleep disorders. Since this article is dedicated only to REM sleep, this review summarizes the years of investigative work from our laboratories and that of others on the neurobiology of REM sleep. In this review, based on what we know at this time, we discuss neural mechanisms of REM sleep without detailing the results of individual studies. Although much remains to be learned, this review is intended to provide the most simple yet complete description of the neurobiological mechanisms of REM sleep. While, in this review, we do not discuss the pathophysiological aspects of sleep disorders and pharmacological treatments for any specific sleep disorder, this simplified knowledge of the neurobiological mechanisms of REM sleep will aid in the development of rational schematics of behavioral and pharmacological therapies both for REM sleep disorders and their comorbidities. Therefore, it is expected that this simplified knowledge will be valuable for sleep medicine physicians, who often encounter questions that require an understanding of the neurobiology of REM sleep, as well as scientists designing experiments to further our understanding of the molecular mechanisms of REM sleep.

Mechanisms of REM Sleep Sign Generation

REM sleep is characterized by a constellation of events including the following:[1] an activated (high frequency, low amplitude) cortical EEG activity pattern;[2] marked atonia of the postural muscles;[3] REM's [4] a theta rhythm within the hippocampus;[5] field potentials in the pons (P-wave), lateral geniculate nucleus and occipital cortex (ponto-geniculo-occipital [PGO]) spikes;[6] myoclonic twitches, most apparent in the facial and distal limb musculature;[7] pronounced fluctuations in cardio-

respiratory rhythms and core body temperature; and[8] penile erection and clitoral tumescence. Evidence from animal studies has suggested that each of these events of REM sleep is executed by distinct cell groups located in the brainstem (Fig. 1)[2,6,7]. These cell groups are discrete components of a widely distributed network, rather than a single REM sleep "center"[2]. More specifically: muscle atonia is executed by the activation of neurons in the locus coeruleus alpha; REMs result from the activation of neurons in the peri-abducens reticular formation; PGO waves emerge from activation of neurons in the caudo-lateral peribrachial area of predator mammals and in the dorsal part of the nucleus subcoeruleus of prey mammals; hippocampal theta rhythm is produced via the activation of neurons in the pontis oralis; muscle twitches appear with the activation of neurons in the nucleus gigantocellularis (especially the caudal part); and increased brain temperature and cardio-respiratory fluctuations result from the activation of neurons in the parabrachial nucleus. The cortical EEG activation sign of REM sleep, however, is collaboratively executed by activation of neurons in the mesencephalic reticular formation and rostrally-projecting bulbar reticular formation (also called the medullary magnocellular nucleus). It should be noted here that the mentioned cell groups simply represent the executive neurons for the individual signs. Final expression of each is executed by the relevant neuronal circuit unique to that REM sleep sign. In essence, each of these REM sleep signs has a separate, specialized network. Thus, each of these REM sleep signs could be modulated by multiple neurotransmitters at multiple sites of their circuit.

Turn-on and turn-off conditions of REM sleep generating executive neurons are regulated by the ratio

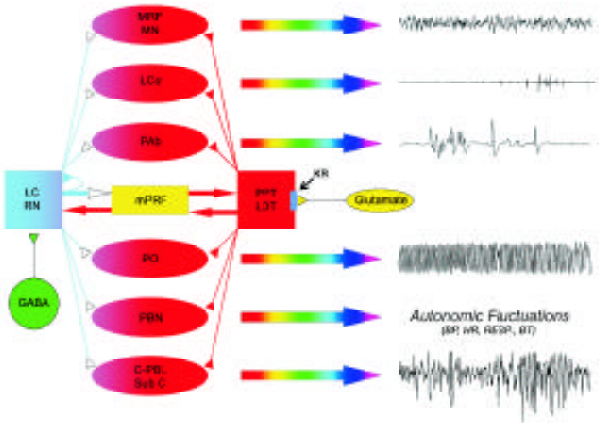

Figure 1: Cellular-Molecular-Network model of physiological mechanisms for the generation of REM sleep.

of available aminergic and cholinergic neurotransmitters within those cell groups (Fig. 1)[2,8]. Aminergic neurotransmitters are released from noradrenergic cells in the nucleus locus coeruleus (LC) and serotonergic cells in raphe nuclei (RN), while cholinergic neurotransmitters originate from the pedunculopontine tegmentum (PPT). The activity of both aminergic and cholinergic cells is approximately equal during wakefulness, and the onset of non-REM sleep results in an equal reduction in activity. Therefore, the ratio of aminergic to cholinergic neurotransmitters in REM sleep generators is proportionate during wakefulness and throughout non-REM sleep. During REM sleep, however, aminergic cell activities are markedly reduced or absent and cholinergic cell activities are comparatively high[9-15]. The level of cholinergic cell activity during REM sleep is roughly 35% less than that of wakefulness. Thus, when a hypothetical ratio of aminergic and cholinergic neurotransmitters is 1:1, the REM sleep sign-generator remains in the turned-off condition (during wakefulness and non-REM sleep); however, when this ratio is 0:0.65, the generator is turned-on to express REM sleep signs[2,11]. A detailed description of the physiological mechanisms underlying REM sleep can be found elsewhere[2].

Neuronal Activity Within the PPT Cholinergic Cell Compartment and Expression of REM sleep

The PPT is situated in the dorsolateral tegmentum and contains a prominent group of cholinergic neurons that project widely throughout the brainstem and forebrain[6,16-18]. To date, almost all studies have consistently demonstrated that the activation of cholinergic cells in the PPT is one of the most critical steps for the generation and maintenance of REM sleep[2,13]. Interestingly, studies have shown that the activation of cholinergic cells in the PPT is also involved in the termination of REM sleep episodes, by inducing wakefulness[2]. Single cell recordings from the PPT in behaving cats and rats have identified several different classes of cells whose firing rates correlate with both wakefulness and REM sleep[6,11,19-21]. Neuronal activity within the PPT cholinergic cell compartment is minimal (about 7.4% of its maximum capacity) when extracellular levels of GABA are at their highest and levels of glutamate are at their lowest[11,22]. In this condition non-REM sleep is expressed via suppression of both wakefulness and REM sleep. Gradually, this neuronal activity increases

to a level about 65% of its maximum capacity — a result of a reduction in GABA and corresponding increase in glutamate[11,23,24]. This condition ushers in REM sleep. Eventually, neuronal activity reaches its maximum output, when glutamate is at its maximum level and GABA is at its lowest level[11,22,24,25]. This condition terminates REM sleep to induce wakefulness.

Neurotransmitter Receptor-mediated Activation/Inactivation of PPT Cholinergic Cells and Expression of REM sleep

As discussed above, the regulation of REM sleep and wakefulness are dictated by the level of neuronal activation within the cholinergic cell compartment of the PPT, which in turn is regulated by neurotransmitter-specific receptor-mediated excitation and inhibition of PPT cells[26]. For example, localized neuropharmacological studies have demonstrated that glutamate activation of kainate receptors on PPT cholinergic cells ultimately induces REM sleep, whereas glutamate activation of N-methyl-D-aspartate (NMDA) receptors ultimately induces wakefulness[26-29]. On the other hand, the inhibitory GABA activates GABA-B receptors which inhibit PPT cholinergic cells and suppress REM sleep and wakefulness[22,30]. Although glutamate and GABA are the two most important neurotransmitter/receptor systems within the PPT cholinergic cell compartment for regulating REM sleep, there are other neurotransmitter/receptor systems that could also influence the expression of REM sleep. More recently, it has been shown that PPT cholinergic neurons exhibit REM sleep-associated immunoreactivity of cAMP-response-element-binding protein (pCREB), indicating that the PPT intracellular transcription process is involved in the neurotransmitter receptor activation/inactivation-mediated regulation of wakefulness and REM sleep[13].

Intracellular signaling mechanisms in the PPT cholinergic cell compartment and regulation of REM sleep

Coordinated regulation of intracellular signaling pathways is essential for the receptor activation mediated transcription and translation processes in neuronal function that appear to underlie complex behavioral responses, including the regulation of sleep/wake states (Fig. 2)[26]. Several signaling pathways are thought to play important roles in neuronal receptor activation/inhibition-mediated regulation of the sleep/wake cycle, including cAMP-protein kinase A (cAMP-PKA), Ca2+/calmodulin-dependent protein kinase II (CaMKII) and the mitogen-activated protein kinases (MAPK)[26]. It is well known that activation of kainate receptors increases cytoplasmic free calcium concentration[31-35]. In neurons, calcium ions can stimulate the production of cAMP and the activation of protein kinase A (PKA) via the activation of adenylyl cyclase (AC)[36-39]. Thus, it is possible that the activation of kainate receptors in the PPT could also activate the cAMP-PKA signal transduction pathway to induce REM sleep. Indeed, one recent study has demonstrated that the inhibition of AC in the cholinergic cell compartment of the PPT suppresses REM sleep in the freely moving rat[40]. Yet another recent study demonstrated that REM sleep increases with increased catalytic subunit of PKA and PKA enzymatic activity in the cholinergic cell compartment of the PPT[41]. This study also demonstrated that the pharmacological inhibition of PPT intracellular cAMP-PKA activity suppresses REM sleep. Accordingly, it appears that the cAMP-PKA intracellular signaling pathway is critically involved in the cholinergic cell compartment of the PPT for the regulation of cholinergic tone in the REM sleep sign-generators. Rapid desensitization (in seconds) is one of the important characteristics of the kainate receptors (GluR6), but increased cytosolic PKA activity can phosphorylate GluR6 subunits and modulate channel function for about 3-25 minutes[42]. In turn, this cytosolic PKA activity mediated phosphorylation increases the number of active receptors and effectively enhances the gating properties of the channels[42]. So the increased PKA activity may also be responsible for the sustained activity of the PPT cells and maintenance of normal REM sleep episodes. Indeed, a recent study has unequivocally demonstrated that the activation of intracellular cAMP-PKA activity in the cholinergic cells of the PPT is a causal intracellular mechanism for the induction as well as maintenance of REM sleep[43]. The importance of intracellular cAMP-PKA activity in the cholinergic cells of the PPT in the regulation of REM sleep is further confirmed by the fact that activation of GABA-B receptors activates Gi/Go G proteins, which are known to inhibit AC, which in turn prevents activation of the cAMP-PKA signal transduction pathway, which suppresses REM sleep[30,44-46]. Therefore, suppression of neuronal activity with the PPT cholinergic cell

compartment during NREM sleep may be due to localized activation of GABA-B receptors, which ultimately inhibits the cAMP-PKA signal transduction pathway (Fig. 2).

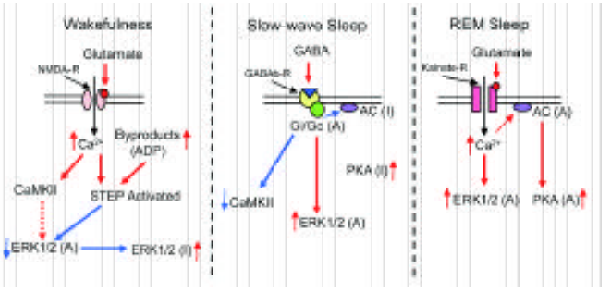

Figure 2: Model for intracellular signal transduction interactions in the pedunculopontine tegmental (PPT) cholinergic neurons involved in the regulation of wakefulness, slow-wave sleep, and REM sleep. Long red arrows with solid lines between molecules: direction of activating effects on the target molecule. Long blue arrows with solid lines between molecules: inactivating effects on the target molecule. Dotted red arrow: a momentary activation before the effect disappears. Short red and blue arrows: increased and decreased levels of molecules, respectively. Symbols: (A) and (I) next to molecules indicate its active and inactive forms. NMDA-R, N-methyl-D-aspartate receptor; Ca^{2+}, calcium; ADP, adenosine diphosphate; STEP, Striatal Enriched Tyrosine Phosphatase; CaMKII, calcium/calmodulin-dependent protein kinase II; ERK1/2, extracellular signal-regulated kinases 1/2; GABA, gamma-aminobutyric acid; GABAb-R, gamma-aminobutyric acid B receptor; AC, adenylyl cyclase; Gi/Go, G protein subunit; PKA, protein kinase A; Kainate-R, kainate receptor.

Given that activated NMDA receptors conduct calcium ions (Ca2+), it is reasonable to suggest that the PPT NMDA receptor activation-induced wakefulness may involve the Ca2+/calmodulin-dependent protein kinase II (CaMKII), a serine/threonine kinase constituting roughly 1-2% of total brain protein[44,47]. Importantly, activation of NMDA receptors potentiates CaMKII activity[48,49]. When active, CaMKII transduces membrane-mediated Ca2+ currents to affect transcriptional targets, such as the cAMP response element binding protein (CREB)[50]. Notably, CaMKII is capable of autophosphorylation, which unhinges the enzyme from direct Ca2+ -mediated activity[51]. Recently we have demonstrated that the levels of CaMKII and phosphorylated CaMKII expression in the PPT decreases with low wakefulness/high REM sleep and increases with high wakefulness/low REM sleep periods[52]. This study also demonstrated that with increased PPT CaMKII activity observed during high wakefulness/low REM sleep, there were marked shifts in the expression of genes that are involved in components of various signal transduction pathways. Collectively, the results of this study suggest that the increased CaMKII activity within the PPT neurons is associated with increased wakefulness at the expense of REM sleep, and this process is accomplished through the activation of a specific gene expression profile[52]. Moreover, a more recent study that utilized a combination of behavioral, pharmacological, and molecular techniques has provided direct evidence that CaMKII intracellular signaling within PPT neurons is indeed involved in the induction and maintenance of wakefulness that ultimately terminates REM sleep[53]. Since within the normal sleep-wake cycle, the end of REM sleep corresponds with the beginning of wakefulness — a transition that requires increased PPT neuronal activity — and also based on the results discussed above, it is therefore reasonable to suggest that increased CaMKII activity in the PPT neurons is the intracellular signaling mechanism for the behavioral transition from REM sleep to wakefulness (Fig. 2).

Another intracellular signaling system, MAPK, represents a highly conserved family of enzymes comprising the extracellular signal-regulated kinases that contain many isoforms (ERK1/2/3/4/5/7), the c-Jun N-terminal kinases/stress-activated protein kinases (JNK/SAPK) and p38 MAPK[54]. Among the MAPK family, ERK1/2, which contains the subtypes 1 and 2, represents the most studied and thoroughly characterized signaling pathway. ERK1/2 are serine-threonine kinases that are highly expressed in the central nervous system and have emerged as important elements in neuronal signal transduction[55,56]. The activation of ERK1/2 is a critical component of the neuronal response[57-61]. ERK1/2 participates in diverse important neuronal processes, for instance neuronal maturation and survival, synaptic plasticity and learning and memory[62-65]. A variety of neurotransmitters including glutamate, GABA, acetylcholine, dopamine, and nitric oxide regulate ERK1/2 activity[66-69]. The expression and activity of the ERK1/2 signaling pathway are also known to be modulated by the activation and inactivation of both the cAMP-PKA and the CaMKII signaling pathways[56,70]. It is also known that the activity of ERK1/2 signaling can be modulated by the activation and inactivation of NMDA, kainate, and GABA-B receptors[67,68]. Recently, we have demonstrated that the expression of ERK1/2, pERK1/2, and the activity of ERK1/2 increased proportionately with increased sleep[71]. Conversely, the expression of ERK1/2, pERK1/2,

and the activity of ERK1/2 decreased proportionately with increased wakefulness. These findings suggested that the up-regulation of ERK1/2 signaling pathway within the PPT is involved in the maintenance of sleep by suppressing wakefulness (Fig. 2).

Mammalian cells possess numerous signal transduction pathways and their interactions with each other are different in different cell types[56,72-77]. These differential interactions of numerous signaling pathways regulate the distribution, duration, intensity and specificity of the cellular response[65,69,78-82]. Recent studies have shown that the activation of the PKA signaling pathway in PPT cells promotes REM sleep[40,41,43], whereas activation of the CaMKII pathway in PPT cells promotes wakefulness by suppressing REM sleep[52,53]. Additionally, activation of the ERK1/2 signaling pathway in PPT cells promotes both SWS and REM sleep by suppressing wakefulness[71]. Thus, it appears that like in other cell types, in the PPT cells, PKA, CaMKII, and ERK1/2 signaling systems interact to regulate sleep-wake stages. Wakefulness is the behavioral and physiological manifestation of NMDA receptor activation-mediated up-regulation of CaMKII and ERK1/2 signaling and activation of Striatal Enriched Tyrosine Phosphatase (STEP) in the PPT[71]. This transient up-regulation of the ERK1/2 signal is then quickly down-regulated by the activated STEP. However, the up-regulation of CaMKII remains throughout periods of wakefulness[71]. SWS is the behavioral and physiological representation of GABA-B receptor activation-mediated up-regulation of ERK1/2 and down-regulation of CaMKII and PKA signaling in the PPT. REM sleep is the physiological and behavioral result of kainate-receptor activation-mediated up-regulation of PKA and ERK1/2 and down-regulation of CaMKII signaling in the PPT (Fig. 2). These complex interactions are described in further detail in a previous publication[71].

Conclusion and Future directions

As with all biomedical research, the ultimate goal of sleep research is to understand the basic mechanisms of sleep regulation and thereby find causes and cures for sleep disorders. The wealth of information presented here shows just how much progress neurobiological research has made in identifying the brain areas, cell types, neurotransmitters and their receptors, intracellular molecular cascades, neuronal networks, and synaptic interactions integral to the regulation of REM sleep. Based on this progress, a number of remedies for insomnia and other sleep disorders have been developed. At this time, the principal drugs used in sleep medicine are GABA-mimetic drugs, which are effective in restoring non-REM sleep. Unfortunately, all of these non-REM sleep-inducing drugs suppress REM sleep. Therefore, the immediate challenge to sleep research is to identify a novel pharmacological agent that restores non-REM as well as REM sleep. Ongoing molecular studies suggest that progressive research in intracellular signaling pathways holds perhaps the greatest potential to fully elucidate the mechanisms by which normal sleep architecture is generated and maintained. Thus, in the future, it is expected that more and more studies will seek to expand our knowledge of sleep regulation at the molecular level, which ultimately will facilitate the design of a new generation of drugs that will be more effective in the treatment of both sleep disorders and other ailments that are affected by sleep disorders.

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