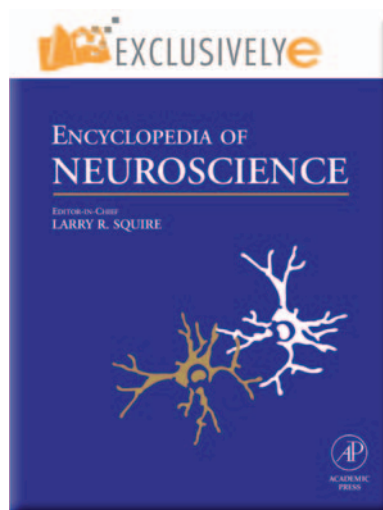


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Sleep and Sleep States: Cytokines and Neuromodulation

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Introduction

Sleep is of central importance to neurobiology, yet our understanding of the biochemical and neurophysiological mechanisms of sleep is rudimentary and sleep function has not been experimentally demonstrated. Our quality of life, performance, and mental well-being are all adversely affected by even a single night's loss of sleep. Chronic sleep loss is associated with pathologies such as the metabolic syndrome. Primary sleep disorders increase morbidity and mortality. Sleep plays a central role in many mental and physiological functions – for example, memory improves during sleep. It is thus important that we understand sleep regulatory mechanisms. This article focuses on the biochemical regulation of sleep and discusses how recent knowledge has led to new insights into how the brain is organized to produce sleep.

There are two somewhat independent literatures concerning the fundamental mechanisms of sleep regulation. One is based on neurophysiological methods, and this literature has led to the identification of circuits involved in non-rapid eye movement sleep (NREMS) regulation, such as corticothalamic projections and the hypothalamic ventrolateral preoptic and median preoptic circuits, and those involved in rapid eye movement sleep (REMS) regulation, such as the laterodorsal tegmental nucleus. Satisfactory explanations of how these circuits impose sleep on the brain and how they keep track of past sleep–wake activity likely will involve the biochemical mechanisms that interact with these circuits. Such interactions are particularly important for sleep homeostasis, the phenomenon exemplified by sleep rebound after prolonged wakefulness. The second sleep regulatory literature is based on biochemical methods. This work has its basis in the homeostatic nature of sleep and the nearly 100-year-old finding that has been replicated many times showing that the transfer of cerebrospinal fluid from sleep-deprived, but not control, animals enhances sleep in the recipients.

During the past 25 years, several sleep regulatory substances have been identified and extensively tested. However, only a handful are strongly implicated in sleep regulation. The list includes tumor necrosis

factor- α (TNF- α), interleukin-1 β , growth hormone releasing hormone (GHRH), prostaglandin D₂, and adenosine for NREMS regulation and vasoactive intestinal peptide, prolactin (PRL), and nitric oxide for REMS. When injected into animals, each of these substances promotes sleep; if they are inhibited, spontaneous sleep is reduced, their levels in brain vary with sleep propensity, and they act on sleep regulatory circuits to promote sleep. Here, we focus on TNF- α in NREMS regulation because it is the only substance identified whose circulating levels in humans correlate with sleepiness and sleep. We also discuss PRL because it has been implicated in REMS regulation by several laboratories and is a cytokine. In addition to these humoral agents, all neurotransmitters have been implicated in sleep–wake regulation; this literature is not discussed here. There is yet another literature demonstrating changes in gene expression, usually determined by changes in mRNA levels using gene chips, with sleep or sleep loss. That literature is distinguished from the sleep regulatory literature because every physiological function changes during sleep; thus, it is not possible from gene expression data alone to know if sleep *per se* is causative of the change. It is important to recognize that those humoral agents implicated in NREMS and REMS regulation affect each other's production and seem to act in concert with each other to affect sleep (Figure 1).

TNF- α in Sleep Regulation

The ability of TNF- α to promote NREMS was first described in 1987. TNF- α given directly into the brain or systemically enhances the duration of NREMS. For instance, mice receiving 3 μ g TNF- α intraperitoneally spend approximately 90 min extra in NREMS during the first 9 h postinjection. In addition, NREMS after TNF- α treatment is associated with supranormal electroencephalogram (EEG) delta (0.5–4 Hz) waves, which are indicative of a greater intensity of NREMS. TNF- α is somnogenic in all species thus far tested: rabbits, mice, rats, and sheep. TNF- α has little effect on REMS if low NREMS-promoting doses are used; however, higher doses inhibit REMS. Sleep following TNF- α treatment appears to be normal in that sleep architecture remains normal, sleep remains easily reversible, postures remain normal, and animals remain responsive to handling. Changes in sleep-coupled autonomic functions, such as the decreases in brain temperature upon entry into NREMS, also persist after TNF- α treatment.

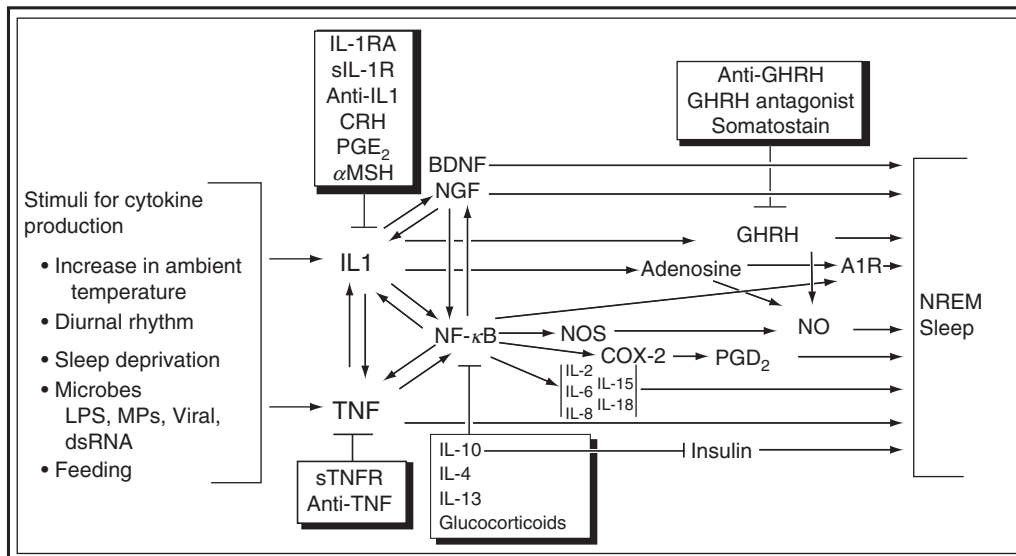


Figure 1 Molecular networks are involved in sleep regulation. Substances in boxes inhibit sleep and inhibit the production or actions of sleep-promoting substances illustrated via feedback mechanisms. Inhibition of one step does not completely block sleep since parallel sleep-promoting pathways exist. These redundant pathways provide stability to sleep regulation. Our knowledge of the biochemical events involved in sleep regulation is more extensive than that illustrated. The molecular network shown here possesses many of the characteristics of biological networks and engineered systems. Thus, the network is modular in that several proteins (cytokines) are working in 'overlapping coregulated groups' in this pathway. Second, the molecular network is robust in that removal of one of the components does not result in complete sleep loss. Third, the network operates as a recurring circuit element with multiple feedback loops affecting other pathways to the extent that similar networks involving many of the same substances and component network parts are used to regulate body temperature, inflammatory responses, microcirculation, memory, food intake, etc., and these systems, to a limited degree, coregulate. Specificity for any one physiological process, such as sleep, results from multiple interacting molecular and cellular circuits, each possessing different, but similar, reactivity. A1R, adenosine A1 receptor; α MSH, α -melanocyte-stimulating hormone; anti-IL1, anti-IL-1 antibodies; anti-TNF, anti-TNF antibodies; BDNF, brain-derived neurotrophic factor; CRH, corticotrophin-releasing hormone; GHRH, growth hormone releasing hormone; IGF1, insulin-like growth factor 1; IL-1RA, IL-1 receptor antagonist; LPS, lipopolysaccharide; MPs, muramylpeptides; NF- κ B, nuclear factor-kappa B; NGF, nerve growth factor; NOS, nitric oxide synthase; PGD₂, prostaglandin D₂; sIL-1R, soluble IL-1 receptor; sTNFR, soluble TNF receptor; TGF β , transforming growth factor- β .

Inhibition of TNF- α inhibits spontaneous NREMS. Thus, treatment with anti-TNF- α antibodies, the full-length soluble TNF receptor, or TNF soluble receptor fragments containing the TNF recognition site all inhibit spontaneous NREMS in rabbits and rats. Furthermore, pretreatment of animals with TNF inhibitors prior to sleep deprivation reduces the expected sleep rebound that normally occurs after sleep loss. Substances that inhibit TNF- α action or production also inhibit spontaneous sleep (e.g., interleukin-4, -10, and -13) (Figure 1). In addition, these substances also inhibit the production of certain other cytokines, such as interleukin-1 β ; therefore, their action on sleep may not be specific to TNF. However, they do form part of the negative feedback loops that help to regulate these nuclear factor-kappa B (NF- κ B)-sensitive cytokines. Furthermore, inhibition of TNF also blocks the increases in NREMS observed in response to an acute mild increase in ambient temperature. Mice lacking the TNF 55-kDa receptor fail to exhibit NREMS responses if given TNF- α , thereby implicating this receptor in

TNF- α -enhanced sleep. These mice also sleep less than corresponding control strains; the reduced NREMS occurs mostly during daylight hours.

Brain levels of TNF- α and the TNF- α mRNA vary diurnally and are influenced by sleep deprivation. The highest levels in rats occur at daybreak. The amplitude of the day-night changes in TNF protein is approximately tenfold and that in mRNA approximately twofold; this reflects the predominate posttranscriptional regulation of TNF. After sleep loss, hypothalamic TNF- α mRNA also increases. Sleep deprivation also increases the expression in brain of the 55 kDa TNF receptor mRNA. TNF- α serum levels increase in mice after sleep deprivation but not after stress. In normal humans, blood levels of TNF- α correlate with EEG delta wave activity. After sleep loss, circulating levels of TNF- α and the 55 kDa soluble TNF receptor, but not the 75 kDa TNF soluble receptor, increase.

Clinical conditions associated with sleepiness correlate with higher blood levels of TNF. Thus, patients with sleep apnea, chronic fatigue, chronic insomnia,

myocardial infarct, excessive daytime sleepiness, and preeclampsia all have elevated TNF plasma levels. AIDS patients have disrupted TNF and sleep rhythms. Postdialysis fatigue is associated with higher TNF levels, and cancer patients receiving TNF report fatigue. Furthermore, the 308A TNF- α polymorphic variant is associated with the metabolic syndrome and sleep apnea. Rheumatoid arthritis patients receiving the soluble TNF 75 kDa receptor report reduced fatigue. Sleep apnea patients treated with the soluble TNF receptor have reduced sleepiness. If obstructive sleep apnea patients are surgically treated, their elevated TNF- α plasma levels return to normal.

Systemic TNF, like several other cytokines, signals the brain via multiple mechanisms; one involves vagal afferents since vagotomy attenuates intraperitoneal TNF- α -induced NREMS responses. The effects of systemic bacterial products such as endotoxin, a component of gram-negative bacterial cell walls, may also involve TNF. For instance, in humans, endotoxin doses that induce transient increases in sleep also induce concomitant increases in circulating TNF- α . In addition, the soluble TNF receptor fragment attenuates bacterial cell wall peptidoglycan-enhanced NREMS in rabbits.

The site(s) of action of TNF- α -induced NREMS includes the preoptic area of the anterior hypothalamus, the locus coeruleus, and the somatosensory cortex. Thus, microinjection of TNF- α into the preoptic area enhances NREMS in rats. In contrast, injection of a soluble TNF receptor fragment into this site inhibits spontaneous NREMS. Microinjection of TNF- α into the locus coeruleus, after a brief period of excitation, induces prolonged increases in sleep and EEG synchronization. These effects are antagonized by anti-TNF- α antibodies. Microinfusion of TNF- α into the subarachnoid space just beneath the basal forebrain in rats enhances NREMS and reduces REMS. Finally, unilateral application of TNF- α onto the surface of the somatosensory cortex induces unilateral state-dependent increases in EEG delta wave power. Conversely, the soluble TNF receptor unilaterally reduces EEG power during the NREMS occurring after sleep deprivation. These latter two findings are germane to the brain organization of sleep and are discussed further later.

TNF Cell Biology

TNF signaling is an intense area of research due to its clinical importance and its inherent interest. TNF- α is synthesized as a 26 kDa membrane-associated protein. Soluble TNF- α , a 17 kDa protein, is cleaved from the 26 kDa membrane-associated protein by

TNF- α converting enzyme. TNF- α production is tightly regulated in a tissue-specific manner, with transcription, translation, and secretion all controlled at multiple points. For instance, the 3' UTR of TNF contains 24 miRNA complementary sites, suggesting translational regulatory sites. TNF- α induces its own expression, in part, via an NF- κ B regulatory site (Figure 1). TNF- α can exist as a transmembrane protein that has biological activity. This membrane integrated form of TNF- α also receives signals and thus acts as a receptor, as well as a ligand.

There are two TNF cell surface receptors, the 55 kDa TNF receptor and the 75 kDa TNF receptor. The intracellular domains of these receptors lack intrinsic enzymatic activity. Rather, both receptors signal by recruitment of cytosolic proteins via protein-protein interaction domains. The diversity of these adaptor proteins and their ability to interact with other members of the TNF receptor superfamily help explain the pleiotropic actions of TNF- α . The TNF receptor family is divided into two large groups based on the adaptor proteins recruited in response to ligand binding. One major TNF-activated signaling pathway activates new gene transcription, whereas the other leads to cell death. Activation of the cell death pathway via the caspases 8 and 3 appears to be a rare physiological event because TNF-responsive gene products function to prevent cell death.

Both TNF receptors are cell surface receptors; they form trimeric complexes with TNF. The spatial distribution of the receptor and associated intracellular adaptor molecules is likely an important determinant of cellular specificity of action. Consequently, TNF activates signaling at multiple subcellular compartments, including the plasma membrane, mitochondria, and the nucleus. The ectodomains of both receptors are shed to form soluble receptors. The 55 kDa soluble TNF receptor is a constituent of normal cerebrospinal fluid, although its physiological role is unknown. However, given its effects on sleep and fatigue, it likely plays a role in sleep regulation.

TNF- α is expressed by microglia, astrocytes, and neurons and has a variety of biological actions in the brain, including a role in mediating both brain damage and neuroprotection. Whether TNF- α is protective or damaging may depend on the receptor type present – either the TNF 55 kDa or the TNF 75 kDa receptor – as well as the stimulus context and the presence or absence of substances that modify TNF- α activity. In addition to sleep, TNF- α has a role in thermoregulation, food intake, brain development, and neuronal connectivity. For instance, TNF- α potentiates AMPA-induced postsynaptic potentials,

AMPA-induced cytosolic Ca^{2+} increases, as well as several voltage-dependent calcium channels. TNF has a role in synaptic scaling, a process involved in maintaining synaptic diversity and plasticity.

Other NREMS Cytokine and Peptide Regulatory Substances

There is extensive evidence implicating both interleukin- 1β (IL- 1β) and GHRH in NREMS regulation that parallels that described previously for TNF- α . Thus, for example, inhibition of either GHRH or IL- 1β inhibits spontaneous sleep. Mutant mice lacking functional GHRH receptors or mice lacking the IL- 1β type I receptor have substantially less NREMS than respective strain controls. IL- 1β induces growth hormone release via a hypothalamic mechanism that includes GHRH; anti-GHRH antibodies block IL- 1β -induced growth hormone release. These antibodies also block IL- 1β -induced NREMS. In fact, hypothalamic GABAergic neurons are receptive to both IL- 1β and GHRH; both substances enhance intracellular calcium levels in these neurons. It is speculated that these neurons are the sleep-active hypothalamic neurons that increase their firing rates in response to IL- 1β . In contrast, wake-active hypothalamic neurons decrease their firing rates in response to IL- 1β . IL- 1β and GHRH are related to TNF via the pathways outlined in [Figure 1](#); collectively, it seems likely that these three substances form part of an NREMS regulatory network. Many other cytokines and members of the somatotrophic axis can also affect sleep, but extensive knowledge concerning their roles in sleep regulation is lacking. They could in their own right be sleep regulatory substances or they may affect sleep via TNF- α , IL- 1β , or GHRH.

Brain Organization of Sleep and TNF- α

Many investigations support the idea that sleep is regulated by specific brain circuits, such as the anterior hypothalamus. Perhaps the best evidence for this paradigm with regard to NREMS is that electrical, chemical, or thermal stimulation of the anterior hypothalamus induces NREMS. However, this paradigm inadequately addresses the issue of how the brain keeps track of its prior sleep-wake history over long periods of time. The transfer of cerebrospinal fluid from a sleep-deprived animal, but not a control animal, into a fully rested recipient enhances sleep in the recipient. This finding suggests that the homeostatic processes for sleep involve sleep regulatory substances.

There are also numerous results suggesting that sleep can be targeted to brain areas disproportionately activated during prior wakefulness. For instance, unilateral stimulation of the somatosensory cortex in awake humans produces asymmetry in EEG delta wave activity during subsequent NREMS. Similar results were obtained from rats and mice. Furthermore, dolphins only show EEG slow wave sleep on one side of the brain at a time. After a specific learning task, the subsequent EEG delta wave activity during sleep is enhanced in the area activated by the learning task. This finding reinforced previous work showing that either EEG activity or brain metabolism during sleep is regionally localized depending on prior use during wakefulness.

Our view of brain organization of sleep posits that sleep is initiated within neural assemblies as a function of prior cellular activity and this provides the mechanism for the localization and targeting of sleep, as well as for sleep homeostasis. In this view, for example, TNF- α is produced and released in response to neural and glial activity. These substances in turn are posited to be made and act locally in autocrine, paracrine, and juxtacrine ways to alter the input-output relationships of neural assemblies (e.g., cortical columns, barrels, or a cortical-thalamic-cortical loop) and thereby induce a functionally different state in these assemblies. Indeed, cortical TNF- α levels increase during prolonged wakefulness and during seizures – two conditions thought to be associated with enhanced cellular activity – and activity-dependent increases in TNF- α appear to be important for the developing nervous system. Furthermore, whisker stimulation upregulates TNF- α expression in neurons in the stimulated whisker's corresponding somatosensory barrel, but not in adjacent barrels, thereby directly demonstrating activity-dependent production of TNF- α .

Individual auditory and somatosensory cortical columns oscillate between functional states as defined by the amplitude of auditory- or whisker stimulation-induced surface evoked potentials. One of the functional states usually occurs simultaneously with whole animal sleep. This sleeplike state is dependent on its prior history in the sense that the probability of its occurrence is higher if, for the 15 min period prior to the measurement, it had been in the other functionally defined state. Furthermore, if two whiskers are stimulated, one at twice the rate of the other, the probability of the cortical column being in the sleep state is greater in the column receiving afferent input from the more rapidly stimulated whisker. If such changes in the functional state are dependent

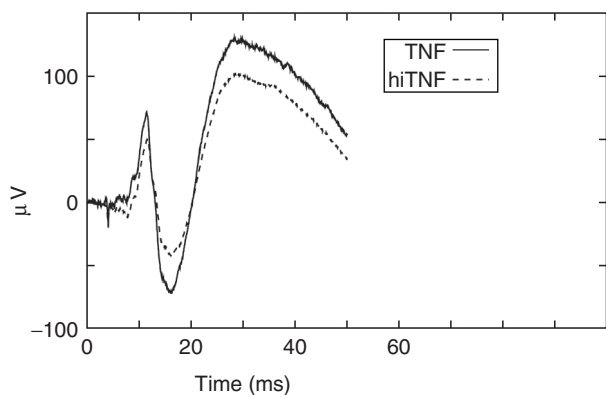


Figure 2 TNF- α enhances the amplitude of surface evoked potentials induced by whisker stimulation. A rat with two-bilateral 12 electrode arrays plus a guide cannula was trained to accept the restraint associated with the recording apparatus and slept during recordings. If the rat was pretreated with TNF- α applied to the surface of the somatosensory cortex, surface evoked potentials were enhanced during quiet sleep compared to those occurring after heat-inactivated TNF- α (hiTNF). We propose that cytokines are driving neurons within cortical columns into a down state and thereby amplify surface evoked potentials. We also propose that the sleep functional state of the column is promoted by cytokines produced in response to cellular activity and is a function of the number of neurons in the down state.

on activity-dependent sleep regulatory substances such as TNF- α , then one would predict that direct application of TNF- α onto the surface of the cortex would drive the affected neural assemblies to simultaneously express the same functional state. Indeed, when TNF- α is applied in this manner, EEG delta power is enhanced unilaterally. Furthermore, if TNF- α is applied to the surface of the cortex and individual cortical columns are probed by whisker twitches, the probability of the column being in the sleeplike state increases (Figure 2). Such experiments strongly support the notion that sleep begins as a local process, being induced by the production of activity-dependent sleep regulatory substances such as TNF- α . Such a view has profound implications for our understanding of sleep regulation, sleep function, and unconsciousness.

Regulation of REMS by Prolactin and Related Molecules

The first anecdotal account implicating PRL in REMS regulation was from an experiment in which REMS was enhanced after systemic administration of PRL in hypophysectomized pontine cats. Subsequently it was shown that systemic PRL causes dose-dependent enhancements of REMS in rabbits. Thereafter, several

lines of evidence implicated PRL in the regulation of REMS. The following were the major observations:

1. Injection of exogenous PRL stimulates REMS in cats, rabbits, and rats.
2. The increases in REMS develop slowly, over 2 or 3 h postinjection, and are maintained for several additional hours.
3. PRL-induced changes in sleep are selective for REMS.
4. PRL enhances REMS only during the light period in the rat.
5. In contrast, REMS is suppressed during the light period and is enhanced at night in mutant, PRL-deficient rats and PRL knockout mice.
6. Rats rendered chronically hyperprolactinemic by means of grafting pituitaries under the kidney capsule exhibit increases in REMS.
7. Both REMS and NREMS are enhanced in hyperprolactinemic pseudopregnant rats.
8. Restraint stress and ether stress are associated with enhanced circulating PRL and enhanced REMS. Restraint stress also enhances hypothalamic release of PRL, although stressor-induced release of pituitary PRL is thought to be responsible for the increases in REMS after stress.

Intracerebral administration of PRL or antibodies to PRL stimulates or inhibits REMS, respectively. The modulation of REMS is therefore a central action of PRL. PRL may come from two sources: it is produced by neurons in the hypothalamus and brain stem, and it is also released into the blood from the anterior pituitary. Circulating PRL is transported into the brain via specific receptor-mediated transport mechanisms residing in the choroid plexus. The REMS-promoting activity of blood-borne PRL is supported by the observation that REMS is enhanced in response to systemically injected PRL, excess PRL released from pituitary grafts, or stimulation of endogenous PRL secretion from the pituitary. Nevertheless, only slight decreases in REMS are observed when basal, nonstimulated blood PRL is suppressed by means of immunoneutralization. Although plasma PRL levels in humans are sleep related, being highest in the early morning hours, patients with PRL-producing adenoma fail to display changes in REMS; instead, their NREMS is increased. In summary, increases in blood PRL stimulate REMS in animal models, but circulating PRL at normal concentrations in the blood has only a minor effect on the regulation of REMS. The impact of the increased blood PRL concentrations on REMS is, in part, attributed to stimulation of the expression of PRL

receptors in the choroid plexus, whereby PRL enhances its own transport into the brain. It is thus posited that intracerebral PRL modulates REMS under normal conditions, whereas pituitary PRL provides additional stimulating influence when PRL secretion is high (e.g., during stress). PRL entering the brain from the systemic circulation acts on the same structures normally under the influence of intracerebral prolactinergic neurons.

Intracerebroventricular injections of hypothalamic peptides that cause pituitary release of PRL – vasoactive intestinal peptide (VIP) and pituitary adenylyl cyclase activating peptide, both of which promote REMS – elicit expression of PRL mRNA in the hypothalamus and anti-PRL antibodies can block VIP-enhanced REMS responses. Another hypothalamic releasing factor for pituitary PRL, prolactin releasing peptide, also enhances REMS in rats if injected centrally.

The mechanism by which PRL increases REMS is unclear. PRL may modulate the circadian regulation of REMS at the level of the hypothalamus, or it may act in the brain stem structures generating REMS. The long (2 or 3 h) latency of the REMS response to exogenous PRL suggests that some metabolic actions mediate the sleep effects. Finally, growth hormone, which is structurally related to PRL, also stimulates REMS. Human growth hormone has a high affinity to PRL receptors and promotes REMS in both humans and rats.

The neuronal circuits responsible for the generation of REMS reside in the brain stem. The brain stem control of REMS involves the interactions between REMS-on neurons, which promote REMS, and REMS-off neurons, which inhibit REMS. Acetylcholine (ACh) is the best characterized neurotransmitter that promotes REMS. Cholinergic neurons localized in the laterodorsal tegmental nucleus (LDT) and the pedunculopontine tegmental nucleus (PPT) are involved in the generation of REMS signs such as EEG desynchronization, ponto-geniculo-occipital waves, and muscle atonia. Cholinergic neurons increase their firing rates approximately 20 s before the EEG signs of REMS and maintain higher activity levels during REMS than during NREMS, suggesting that these neurons participate in the initiation and maintenance of REMS. Numerous microinjection and microinfusion studies show that REMS is enhanced by cholinergic agonists and inhibited by cholinergic antagonists. Furthermore, kainate lesions of the PPT result in prolonged decreases in REMS. Anatomical data indicate that single cholinergic neurons in the LDT or PPT project to

multiple brain regions that are responsible for the generation of individual signs of REMS. Therefore, these cholinergic neurons might be involved in the orchestration of different REMS signs to generate REMS.

A Proposed Mechanism for PRL-Enhanced REMS

REMS is influenced by metabolism. The length of the REMS cycles correlates with brain size and general metabolic activity. The brain primarily utilizes glucose as an energy source. Glucose is transported from the blood and into astrocytes through an active transport system and metabolized into pyruvate, the main fuel for neurons. In neurons, pyruvate may be metabolized into lactate by the enzyme lactate dehydrogenase in the absence of oxygen, or it may be metabolized into CO₂ and H₂O through the oxidative pathway which involves many enzyme actions, including pyruvate dehydrogenase (PDH). REMS is selectively suppressed during hypoxic hypoxia, whereas NREMS or waking may be enhanced. In contrast, an increase in oxygen availability induces a significant increase in REMS in chronic pontine cats kept at a fixed temperature. The high glucose and oxygen consumption levels during REMS compared to those during NREMS suggest that PDH activity is enhanced during REMS. It is suggested that glucose may be metabolized through the anaerobic pathways during wakefulness, converted to glycogen during NREMS, and metabolized through the aerobic pathways during REMS.

Stimulation of PDH in the mesencephalic/pontine cholinergic cells may serve as a final common pathway for various experimental interventions that promote REMS, such as REMS deprivation, immobilization stress, or administration of PRL. The connection between PRL and PDH was suggested by observations that PRL stimulated PDH activity in the prostate and the mammary gland. Increases in brain stem PDH activity occur in response to systemic injection of PRL.

The synthesis of ACh is critically dependent on the supply of acetyl-CoA, which in turn relies on the oxidation of pyruvate by the PDH complex during high glucose and oxygen consumption. PDH is particularly enriched in brain cholinergic neurons. Enhanced PDH activity results in increases in the concentration of acetyl-CoA and thereby stimulates the synthesis of ACh. Furthermore, brain cholinergic neurons are vulnerable to the deficiency of PDH. ACh levels are decreased by the PDH inhibitor 3-bromopyruvate

in brain homogenates or in the brain after local injection of this compound into the basal forebrain. Decreases in PDH activity elicited by experimental thiamine deficiency are also associated with the suppression of REMS.

Based on these observations, the proposed mechanism of the effects of PRL on sleep is as follows: PRL in the brain stimulates PDH and thereby energy production in neurons. This stimulation of PDH results in enhancement of ACh synthesis in cholinergic neurons because of an increased supply of acetyl-CoA. In our opinion, increased ACh production in itself does not trigger REMS. Due to the increased ACh pool, however, the quantity of ACh released increases whenever REMS spontaneously occurs. Therefore, PRL does not trigger REMS; instead, it facilitates spontaneous REMS. Furthermore, there is no evidence to suppose that PRL is specific to REMS-promoting cholinergic neurons. Cholinergic neurons also exist in the basal forebrain. These neurons are implicated in the mechanism of arousal, and their activities are associated with wakefulness. The importance of normal PDH activity has been demonstrated in the function of cholinergic neurons of the basal forebrain in experiments modeling the pathology of Alzheimer's disease. Stimulation of PDH in these neurons may explain the promotion of wakefulness by PRL during the dark period in rats. If further experiments substantiate that PRL acts via an increased synthesis of ACh due to enhanced activity of PDH, then PRL might be the first metabolic hormone whose effects on sleep can be linked to a distinct biochemical mechanism.

Conclusion

Our understanding of the biochemical regulation of sleep has progressed rapidly during the past 20 years. We now have a firm understanding of at least some of the humoral substances involved in sleep regulation. No doubt, other sleep regulatory substances will be described. Perhaps more important, these substances have already given insight into how the brain is organized to produce sleep. These mechanisms include a dynamic oscillation of states within neural assemblies coordinated into coherent

whole animal states by sleep regulatory circuits and homeostatically driven by the accumulation of sleep regulatory substances.

See also: Dopamine Control of Arousal; Sleep and Sleep States: Thalamic Regulation; Sleep and Sleep States: Hypothalamic Regulation; Sleep and Sleep States: Histamine Role; Sleep–Wake State Regulation by Noradrenaline and Serotonin; Sleep–Wake State Regulation by Acetylcholine.

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