[Frontiers in Bioscience 8, d520-550, May 1, 2003]

BIOCHEMICAL REGULATION OF NON-RAPID-EYE-MOVEMENT SLEEP

Ferenc Obal Jr. [1] and James M. Krueger [2]

[1] Department of Physiology, University of Szeged, A. Szent-Györgyi Medical Center, 6720 Szeged, Hungary, [2] Department of Veterinary and Comparative Anatomy, Pharmacology, and Physiology, Washington State University, Pullman, WA 99164-6520

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1. ABSTRACT

The concept, that sleep regulatory substances (sleep factors) exist, stems from classical endocrinology and is supported by positive transfer experiments in which tissue fluids obtained from sleepy or sleeping animals elicited sleep when injected into recipient animals. The transfer experiments concluded with the identification of four sleep factors: delta sleep-inducing peptide (DSIP), uridine, oxidized glutathione, and a muramyl peptide. A physiological sleep regulatory role, however, has not been determined for these substances. In contrast, transfer experiments did not play a part in the development of the strong experimental evidence that implicated the currently known sleep factors in sleep regulation. These substances include adenosine, prostaglandin D2 (PGD2), growth hormone-releasing hormone (GHRH), interleukin-1 (IL1) and tumor necrosis factor (TNF). They promote non-REMS in various species, inhibition of their action or endogenous production results in loss of spontaneous sleep, and their synthesis and/or release display variations correlating with sleep-wake activity. Although the source of these substances vary they all enhance sleep by acting in the basal forebrain / anterior hypothalamus – preoptic region. It is also characteristic of these substances that they interact in multiple ways often resulting in mutual stimulation or potentiation of each other. Finally, there is a third group of substances whose significance in sleep regulation is less clear but for which there are two or more lines of evidence suggesting that they may have a role in modulating non-REM sleep (NREMS). This group includes oleamide, cortistatin, cholecystokinin (CCK), insulin, and nitric oxide (NO). More sleep regulatory substances are likely to be discovered in the future although it is a long and difficult process requiring multiple laboratories to generate sufficient convincing data to implicate any one of them in sleep regulation.

2. INTRODUCTION

In 1898 Tigerstedt and Bergman (1) described renin, the first hormone discovered, but their paper elicited little interest at the time. Four years later, Bayliss and Starling (2) published a paper in which they described secretin- another hormone. Bayliss and Starling developed the concept of humoral regulation and coined the word "hormone". The publication of their paper in 1902 is generally regarded as the birth date of endocrinology. The discovery of secretin initiated a long-lasting race to identify specific hormones, and the number of known hormones increased exponentially over the subsequent decades. During this period, the major tool of classical endocrinology was the transfer experiment, in which extracts from the stimulated tissues of donor animals were transferred to recipient animals. An appropriate response to the extract indicated the presence of a humoral agent released into tissue fluids.

Bayliss and Starling's discovery also paved the road for the theory of humoral regulation of sleep: Theoretically, a sleep hormone, i.e. a sleep factor, regulates sleep. This concept received a major boost from the results of two groups performing transfer experiments in sleep-deprived dogs. Ishimori in Japan (reviewed in (3)) transferred dialysates of brain homogenates, whereas Legendre and Pieron (4) in France used the cerebrospinal fluid (CSF), serum, and emulsion of cerebral cortex. In both studies, the recipient animals exhibited somnolence

and sleep. During the subsequent 80-90 years, many laboratories repeatedly confirmed the existence of sleep promoting materials in various tissue fluids, although some negative findings were also reported (reviewed in (3)).

A hormone must fulfil many criteria, but perhaps the most fundamental is that it has to be chemically identified. At the beginning of the 20th century, biologically active substances could not be characterized because of the limitations of chemistry. The structural determination of many small molecular compounds (thyroid hormones, histamine, catecholamines, acetylcholine) was delayed for several decades. Steroid chemistry became a powerful tool in the 1940s and 1950s. The identification of most of the peptides, which comprise the vast majority of hormones, only occurred in the 60's and thereafter. Even today, extraction and chemical identification of substances found in minute amounts in tissue fluids is extremely tedious. We are aware of only three groups whose experiments in sleep research resulted in the chemical identification of factors putatively responsible for transferring somnolence. The difficulties of these experiments are clearly shown by the time elapsed from the publication of the successful transfer experiments to the final identification of the sleep factors: the shortest and the longest intervals were 5 and 17 years, respectively. The 3 research groups, led by Monnier, Uchizono, and Pappenheimer, used two fundamentally different approaches. Two out of the three experiments mentioned above were based upon the assumption that the sleep factor accumulates during wakefulness and diminishes during sleep. Consequently, the longer the wake period is, the higher the concentration of the sleep factor becomes. This experimental paradigm repeated the classical studies of Legendre and Pieron, and of Ishimori. In contrast, the third group anticipated high concentrations of the sleep factor during sleep, and reduced amounts during wakefulness. In each of these approaches, tissue fluids were collected for transfer when the sleep factor was theoretically highest.

Hess (5) reported that electrical stimulation in the thalamus and rostral hypothalamus (trophotropic area) elicits sleep. This finding provided evidence supporting the active sleep theory (sleep results from the increased activity of particular neuronal groups, i.e. sleep centers) and refuted the prevailing passive sleep theory (sleep results from a withdrawal of wake promoting stimuli). Monnier (a student of Hess) stimulated the thalamus in rabbits and subsequently dialyzed the venous blood leaving the brains of the sleeping animals. The dialysate elicited sleep (non-rapid eye movement sleep=NREMS) when transferred into recipient rabbits, implicating the presence of a sleep promoting material (6). Among the most exciting publications in sleep research are the papers describing the dialysis, purification, isolation, identification, and synthesis of the sleep promoting material. The substance, a nonapeptide, was identified by Schoenenberger *et al*. (7) and named delta sleep-inducing peptide (DSIP). As soon as synthetic DSIP became available, many laboratories tested it in a number of species, and in the 1980's published a vast number of papers describing the biological activities and structure-activity relationships of DSIP (reviewed in (8;9)). DSIP-like immunoreactivity was described in a range of tissue fluids from the CFS to milk. Although exogenous DSIP may modulate endocrine secretions (e.g. inhibition of adrenocorticotropic hormone (ACTH) secretion, stimulation of GH secretion) and reduce stress, consistent sleep-promoting activity could not be verified (reviewed in (10)). Some research suggests that DSIP acts indirectly on sleep by modulating circadian rhythms (11) or by reducing stress (12). Our own experiments suggest that DSIP analogs that are resistant to enzymatic breakdown may enhance a pre-existing sleep process, but DSIP per se does not promote sleep in the rat (13). Furthermore, the antibodies to DSIP may cross-react with proteins not implicated in sleep regulation (14). To our knowledge, the gene or mRNA determining DSIP has never been identified.

Uchizono and colleagues (15) induced sleep in rats by infusing brain stem extracts from sleep deprived donor rats into the third ventricle of recipient rats. Appropriately, the putative sleep factor was dubbed sleep-promoting substance (SPS); the chemical identification was completed by Komoda *et al*. (16;17) and the biological actions analyzed by Inoué *et al*. (18-20). In fact, the authors found signs of multiple SPSs and successfully identified two substances: a pyrimidine nucleoside, uridine, and a small peptide, oxidized glutathione. When continuously infused into the cerebral ventricles of rats, both uridine and oxidized glutathione promote NREMS and rapid eye movement sleep (REMS) in a narrow dose range. Similar findings were obtained after intracerebroventricular (*icv*) bolus injection of oxidized glutathione into rabbits (21). Proposed mechanisms of action are modulations of neuronal metabolism by uridine, and modulation of glutamatergic transmission by glutathione. Some observations suggest that uridine may act as a neurotransmitter: uridine release can be elicited by depolarization of hippocampal and thalamic neurons, and uridine can modulate calcium currents and inhibit neuronal activity (22;23). Neuronal depolarization may also elicit release of glutathione (24). The reduced-oxidized glutathione system is a redox system in the brain. The sleep promoting activity of glutathione is an important argument for the neuronal detoxication theory of sleep function proposed by Inoué (25). Glutathione concentration decreases in the hypothalamus and, to a lesser extent, in the thalamus of sleep deprived rats, and this is interpreted as a sign of oxidative stress associated with sleep deprivation (26).

Pappenheimer *et al*. (27;28) demonstrated the presence of a sleep promoting material, Factor S, in CSF by transferring CSF samples from sleep-deprived goats into cats, rats, and rabbits. In a Bayliss-Starling memorial lecture (29), Pappenheimer described his personal recollections of the first experiments and admitted that they did not expect Legendre and Pieron's findings to be replicated but, to their great surprise, the samples clearly enhanced sleep in the recipient animals. These experiments also produced a fundamental observation: sleep deprivation increased not only the duration of subsequent NREMS but also the amplitudes of EEG slow waves during recovery

NREMS (30). There was insufficient CSF for the chemical identification of Factor S, and the experiments continued with extracts from brains from sleep deprived goats, sheep, rabbits, and cows. A substance with chemical characteristics indistinguishable from Factor S was described in human urine (31). Factor S derived from urine was characterized as a muramyl peptide (32). Muramyl peptides, components of bacterial cell wall peptidoglycans, are not synthesized by mammalian cells; at the time, this limited the acceptance of muramyl peptides as an endogenous sleep factor. Nevertheless, muramyl peptides enter the body from the intestinal bacterial flora, and an mRNA for peptidoglycan binding protein (PGRP- a protein that binds the lower molecular weight muramyl peptides) was found recently in brain (33). PGRP mRNA increases in brain in response to sleep deprivation. Muramyl peptides strongly promote NREMS and generally suppress REMS, e.g., *icv* injection of 1 pmol of the muramyl peptides isolated from urine enhances NREMS for 6 hours in rabbits (34). Muramyl peptides elicit fever and other signs of the acute phase response by stimulating release of proinflammatory cytokines from macrophages. Endotoxin, a cell wall component of Gram-negative bacteria, also activates the cytokine cascade and elicits acute phase response including sleep. Thus, identification of Factor S as muramyl peptides eventually led to the discovery of the sleep-promoting activity of cytokines (35;36) and later, of other growth factors. These findings provided explanation for the mechanism of somnolence associated with infectious diseases, and initiated studies of links between sleep and immune regulation (see Toth and Opp, this volume).

Although they were not isolating an unknown sleep factor, Drucker-Colin *et al*. adapted the principles of the classical transfer experiment to study the role of vasoactive intestinal peptide (VIP) in REMS rebound after REMS deprivation. The authors established the accumulation of a REMS-promoting material in the CSF of REMS-deprived cats by injecting the fluid into recipient animals rendered insomniac by intraperitoneal (*ip*) injection of para-chlorophenylalanine (37). Next the CSF samples were pretreated with antibodies to VIP and then injected into recipient animals. This anti-VIP treatment eliminated the REMS-promoting activity of the samples, suggesting that VIP was the substance responsible for the effect (38).

Irrespective of the significance of the individual substances identified as sleep factors, the legacy of these heroic experiments includes some general conclusions that were not obvious 50 or 100 years ago. One major lesson is the absence of a single factor specific for sleep. In fact, multifactorial regulation is a fundamental requirement for the stability of all physiological processes, and sleep is homeostatically regulated to a high level of stability (39). Multifactorial regulation also implies variable significance of individual factors depending upon experimental conditions (40, 41). For example, the factors promoting sleep in a mildly warm environment, after sleep deprivation, after eating, and during infectious diseases may differ. Another lesson of these experiments is that sleep factors are not necessarily hormones in the classical sense. Within the past decade, the spectrum of humoral intercellular communication has become extremely large: a single substance may act as a growth factor, hormone, paracrine, and neurotransmitter. It is perhaps appropriate to regard as sleep factors all endogenous substances that promote sleep.

Many substances disturb sleep by altering some homeostatic functions and causing discomfort. In contrast, the number of substances capable of enhancing sleep is much smaller. Sleep responses are highly dependent on experimental conditions, and therefore several laboratories must successfully replicate experiments in various species to confirm a substance's role in sleep. If a substance consistently promotes sleep, it nevertheless cannot be assigned a role in sleep regulation without further study: Changes in sleep after inhibiting the action/production of the endogenous substance, relationships between sleep and the endogenous synthesis/release of the substance, its action sites and mechanisms of action, and the regulation of its endogenous production also must be described. To date, various laboratories have published large amounts of data that implicate adenosine, prostaglandin D2 (PGD2), cytokines/growth factors (e.g. interleukin-1 (IL1) and tumor necrosis factor (TNF)), and growth hormone-releasing hormone (GHRH) in the regulation of NREMS (Table 1). In addition, a number of candidates may become classified as sleep regulatory substances in the future; these are only briefly discussed in this review. Classical neurotransmitters such as serotonin, catecholamines, acetylcholine, GABA, histamine, glutamate, etc. are omitted (reviewed in 41).

3. ADENOSINE

When adenosine triphosphate (ATP) is hydrolyzed as a function of tissue metabolic activity, the purine nucleoside adenosine is released making it ideally suited to participate in autoregulatory processes. As such, it was implicated in the regulation of microcirculation long before it was considered as a sleep regulatory substance (e.g. (42). Arterioles express predominantly A2 receptors, which stimulate cyclic adenosine monophosphate (cAMP) production in vascular smooth muscle and elicit vasodilation (A1 receptors inhibit cyclic cAMP production and / or stimulate phospholipase C, and mediate vasoconstriction in some vascular beds such as those in the kidney). The short life (< 10 s) of adenosine in the blood ensures that it acts locally. Although the physiological significance of the adenosine-induced vasodilation remains to be verified, this elegant mechanism allows tissues to regulate their own blood flow according to need. Thus, the microcirculation of blood in heart and perhaps in skeletal muscle is regarded as a major action site for adenosine autoregulatory activity. Adenosine is also a strong vasodilator in cerebral vessels (43).

Neurons use ATP extensively to maintain the ion distribution necessary for excitability, metabolism, transmitter synthesis etc. Neurons are capable of synthesizing adenosine required for production of ATP, but they also have membrane transporters which take up

Table 1. Comparison of the characteristics of sleep factors

	ADENOSINE	PGD2	GHRH	IL1	TNF
Source	Wake-active neurons in basal forebrain and neocortex	Macrophages in leptomeninges, epithelial cells in choroids plexus, CSF	GHRHergic neurons in mediobasal hypothalamus	Glia, and neurons in hypothalamus and other brain areas	Glia and neurons in hypothalamus and other brain areas
Target structure	Basal forebrain, POAH	Arachnoidea on the surface of basal forebrain	POAH	Multiple (see actions)	POAH, locus ceruleus
Action	1. Inhibition of wake-active neurons (A1) 2. Disinhibition of sleep-active neurons (A1) 3. Stimulation of sleep-active neurons (A2)	Release of adenosine from arachnoid cells	Stimulation of GABAergic neurons	1. Stimulation of sleep-active neurons in POAH 2. Inhibition of 5Htergic neurons in raphe 3. Stimulation of adenosine (hippocampus) 4. Stimulation of PGD2 production	1. Stimulation of sleep-active neurons in POAH? 2. Inhibition of noradrenergic neurons in locus ceruleus 3. Stimulation of PGD2 production
Sleep-related variations (spontaneous sleep, sleep deprivation, diurnal rhythm.)	Basal forebrain, neocortex	CSF, neocortex	Hypothalamus	Hypothalamus, neocortex, hippocampus, CSF	Hypothalamus, blood
Effects of acute inhibition	Sleep suppression	Sleep suppression	Sleep suppression	Sleep suppression	Sleep suppression
Chronic deficiency	?	?	Sleep loss in the rest period	Sleep loss in the active period	Sleep loss in the rest period
Species responsive	Cat, rat, human, drosophila	Rat, monkey, mouse, human	Rat, rabbit, mouse, human	Rat, rabbit, mouse, cat, monkey, human	Rat, rabbit, mouse, sheep
Action often associated with the sleep effect	hypothermia	hyperthermia	GH secretion	hyperthermia	hyperthermia

See the text for references. POAH: preoptic region/anteriorhypothalamus; CSF: cerebrospinal fluid

adenosine from the extracellular fluid. The source of adenosine in the extracellular fluid is, in part, intracellular adenosine. After hydrolysis of ATP and cAMP, excess adenosine is transported out of the cell along its concentration gradient. ATP is also found in some neurotransmitter-containing vesicles and therefore it is co-released with neurotransmitters such as acetylcholine, noradrenaline, and dopamine. Extracellular ATP is metabolized to adenosine by means of ectoenzymes. Theoretically, the extracellular concentration of adenosine, therefore, parallels neuronal activity. Assuming that wakefulness and sleep are associated with intense and modest neuronal metabolic and electrical activity, respectively, adenosine may provide an autoregulatory mechanism for neurons through which sleep ensues as a function of previous wakefulness. Thus, sleep through adenosine is a tool of metabolism-dependent autoregulation for neurons in the same way vasomotion is a tool of metabolism-related autoregulation of the blood supply in tissues.

Intracerebral or systemic injection of adenosine or adenosine agonists induces increases in sleep duration and/or enhances EEG slow wave activity during NREMS (44-46). Caffeine and theophylline are adenosine antagonists widely used in various cultures to stimulate vigilance. An adenosine agonist increases whereas caffeine inhibits duration of rest in Drosophila though caffeine also kills the flies when the dose is high (47). Adenosine, however, has many systemic actions, e.g. in addition to circulatory effects, it also decreases metabolic rate (48). It seems, sleep responses to central administration of compounds acting on adenosine receptors occur without changes in body temperature or at lower threshold doses than the doses altering body temperature (44;45). Fundamental observations implicating adenosine in sleep

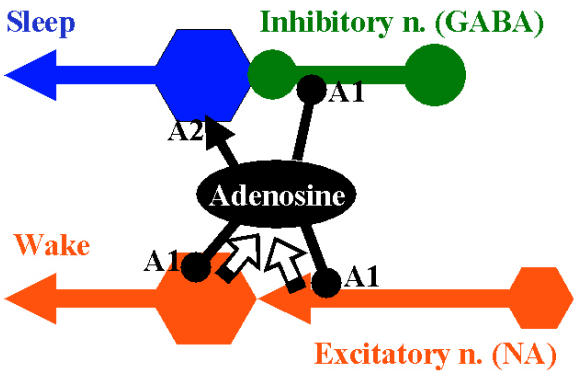

Figure 1. Putative mechanisms of sleep promotion by adenosine. Activity-dependent adenosine release occurs from wake-active neurons and terminals in the basal forebrain. Adenosine inhibits wake-active cholinergic and non-cholinergic neurons and terminals (presynaptic inhibition) via A1 receptors. A1 receptors also mediate presynaptic inhibition of the GABAergic neurons that inhibit sleep-active neurons resulting in a disinhibition of these neurons. The activity of sleep-active neurons might be further enhanced by adenosine acting on stimulatory A2 receptors. The disinhibited and directly stimulated sleep-active neurons are not necessarily the same neurons. Disinhibition is suggested to occur in sleep-active neurons in the POAH whereas the sleep-active neurons stimulated by A2 receptors are assumed to reside in the VLPO (see also Figure 2).

regulation were obtained in experiments with microdialysis which allow detection of sleep-related variations in extracellular adenosine in the brain, and the long-term delivery of adenosine, adenosine antagonists and agonists, and adenosine transport blockers into the brain tissue. These experiments demonstrate that extracellular concentrations of adenosine increase during wakefulness and decrease in sleep (both NREMS and REMS) in various structures of the brain (i.e. cholinergic area of the basal forebrain, preoptic region / anterior hypothalamus, thalamus, pedunculopontin tegmentum, dorsal raphe) (49-51). However, sustained increases in extracellular adenosine occur only in the basal forebrain and to some extent in the cerebral cortex during sleep deprivation. After 5 hours of wakefulness adenosine starts declining in the cerebral cortex. In contrast, adenosine stays high throughout sleep deprivation and decreases gradually during recovery sleep in the basal forebrain. These variations in exctracellular adenosine concentrations suggest that the neurons and terminals, whose wake-associated activity may determine subsequent sleep, reside in the basal forebrain. A1 receptor knockout mice sleep less than their normal siblings (-11 % in 24 h), and their ability to respond with enhanced EEG slow wave activity to sleep deprivation is compromised though recovery sleep time is longer in these mice than in controls (52). More recent experiments, however, suggest that A1 receptor knockout mice sleep normally and respond normally to sleep deprivation when they are fully adapted to the recording conditions (Porkka-Heiskanen, personal communication).

Cholinergic projection neurons represent a significant portion of the basal forebrain wake-active neurons (see Zaborszky in this volume). The site in the basal forebrain where adenosine accumulates during sleep deprivation, corresponds to the area of the cell bodies of the cholinergic neurons. The cholinergic, and perhaps also the non-cholinergic wake-active neurons are regarded as the major target for the sleep-promoting action of adenosine (51). These neurons and the terminals ending on the wake-active neurons (e.g. ascending noradrenergic innervation) might also be the source of wake-dependent adenosine release (Figure 1). Adenosine acting on A1 receptors elicits hyperpolarization through opening of potassium channels, and thus causes inhibition. Administration of A1 receptor antagonists into the basal forebrain cholinergic area decreases both NREMS and REMS in the cat (51). Both NREMS and REMS increase when the extracellular adenosine concentration is raised by means of an adenosine transport blocker infused locally into the basal forebrain in cats and rats (49). The effect can be large: 40 % increases in NREMS were observed during the 3-hour infusion period. Local application of adenosine into the basal forebrain enhances NREMS and REMS in the rat (51) but, interestingly, increases only REMS the cat (53). A1 receptors may also mediate presynaptic inhibition via reducing N-type calcium currents (54). Thereby adenosine may reduce stimulatory input to the wake-active neurons.

The mechanism of sleep promotion by adenosine is based upon a withdrawal of the stimulatory activity of the cholinergic and non-cholinergic basal forebrain neurons. Some observations, however, indicate that adenosine also may stimulate sleep-active neurons in the preoptic region / anterior hypothalamus. Presumably the sleep-active neurons are under GABAergic inhibition, and adenosine may cause disinhibition of these neurons by inhibiting the GABAergic neurons presynaptically (51). Alternatively, adenosine may directly stimulate sleep-active neurons via A2 excitatory receptors. Although the majority of the data indicates that the promotion of sleep is mediated almost exclusively by A1 receptors, others suggest that adenosine may act on A2a receptors expressed in neurons located in the shell of the nucleus accumbens (55) or in the ventrolateral preoptic region (VLPO) (56). While local application of adenosine consistently inhibits wake-active neurons some sleep-active neurons are inhibited by adenosine and some might be stimulated by A1 antagonists (57). These variations might be related to differences in the presynaptic and postsynaptic actions of adenosine or to differences in the A1/A2 receptor ratio among neurons. NREMS and REMS increase after elevations of adenosine concentrations in the laterodorsal tegmentum, and REMS is enhanced in response to infusion of an adenosine transport blocker into the dorsal raphe nucleus (51). However, since sleep deprivation-related variations in adenosine concentrations are not detected in these structures the significance of these observations remains to be determined.

Because A1 receptors inhibit production of cAMP, it is interesting to note that the highest cAMP concentrations in the preoptic region occur during wakefulness, and cAMP

decreases in NREMS and REMS (58). Adenosine elicits expression of c-fos protein in non-cholinergic neurons in the basal forebrain of rats, a response similar to the effect of sleep deprivation (59). In addition, sleep deprivation stimulates translocation of another transcription factor, nuclear factor kappa B (NFkB) into the cell nucleus in the basal forebrain. Adenosine elicits the same response in basal forebrain slices *in vitro* (60). This action is mediated by A1 receptors and the phospholipase C – inositol triphosphate second messenger pathway. NFkB can induce production of other substances implicated in sleep regulation or may up-regulate A1 receptors and thereby it can prolong and enhance promotion of sleep by adenosine. Interestingly, other putative sleep factors such as IL1 also stimulate NFkB (61).

The sleep promoting activity of adenosine has been linked to the restorative function of sleep proposed by Karnovsky (62) and Benington and Heller (63). Glycogen is the largest energy store for brain, and NREMS promotes accumulation of glycogen in glial cells (62). Adenosine stimulates glycogen synthesis in glial cells *in vitro* (64). Theoretically, therefore, glycogen depletion during wakefulness may provide the homeostatic sleep drive which determines subsequent sleep. Large decreases in glycogen levels of rat brain were observed after 12-24-hours of sleep deprivation in one study and glycogen levels were increased during recovery sleep (65). However, glycogen decreased only in the cerebellum of young rats after 6-12-hours sleep deprivation, and it did not change or increased (in older rats) in the cerebral cortex in another study (66). Sleep deprivation (6 h) failed to alter cortical glycogen contents (but glycogen decreased in the brain stem) in one strain of mice whereas this treatment increased cortical glycogen contents in another strain (67). Signs of enhanced glial glycogen synthesis were also found after sleep deprivation (64). It is possible that the deprivation-induced increased adenosine release helps to replenish glycogen stores already during wakefulness (66) though VIP, and in particular noradrenaline, which is likely to be released in great quantities during deprivation, are also powerful stimulators of glycogen synthesis (68). Whether depletion of glycogen is in fact the driving force of sleepiness remains controversial.

4. PROSTAGLANDINS (PGs)

Although sleep responses to prostaglandins were reported previously (e.g. 69;70) systematic studies of this issue has been performed almost exclusively in Hayaishi's laboratory. These experiments resulted in discoveries whose importance may go beyond sleep regulation including the isolation and cloning of the enzyme synthesizing PGD, identification of β-trace protein with human PGD synthase, development of pharmacological inhibitors of PGD synthase, and determination of the distribution of PGD synthase and the prostanoid receptor mediating PGD actions in the brain (reviewed in (71).

Prostaglandins are unsaturated fatty acids containing 20 carbon atoms and a cyclopentane ring. They are eicosanoids produced from arachidonic acid through the cyclooxygenase (COX: COX1 (or constitutive COX) and COX2 (or inducible COX)) pathway. COX's activity results in production of PGH2 that is further converted by specific enzymes into prostacyclin (PGI2), thromboxane (TXA2), or various PGs (PGD2, PGE2, and PGF2α, though PGF2α is also a metabolite of PGE2). PGD2 seems to be a major PG in the central nervous system in various mammals. Lipocalin-type glutathione-independent PGD synthase is expressed in the brain whereas hematopoetic glutathione-dependent PGD synthase is found predominantly in peripheral tissues. Unlike the hematopoietic PGD synthase, the brain-type enzyme is reversibly inhibited by quadrivalent selenium compounds (reviewed in 72).

PGD2 is the major sleep-promoting eicosanoid. In rats, it stimulates both NREMS and REMS after microinjection into the preoptic area or during continuous infusion into the third ventricle (18; 73). In rhesus monkeys, NREMS and REMS are also increased in response to continuous infusion of PGD2 into the lateral or third ventricle (74). However, single bolus injection of PGD2 into the lateral ventricle does not alter sleep in rabbits (75). Amounts of PGD2 are slightly (1.2-1.5-fold) higher in the brain of transgenic mice expressing excess human PGD synthase than in wild type mice (76). Spontaneous sleep is not altered in these transgenic mice, although their spontaneous locomotor activity is significantly enhanced. A tail clip (to obtain samples for determining the transgene), however, elicits robust rises in brain PGD2 contents in the transgenic mice, and this response is associated with significant and selective increases in NREMS. It is assumed that pain from the tail clip activates PGD synthase. Inhibition of PGD2 synthesis by means of inhibitors of COX is followed by decreases in NREMS and REMS in rats (77). Sleep also decreases after blockage of PGD synthase by means of selenium compounds (78). Inhibition of PGD synthase increases the incidence of arousal-like behavior in fetal sheep which can be reversed by means of *icv* infusion of PGD2 suggesting that PGD2 has role in the maintenance of prenatal sleep (79).

PGD2 concentrations in the CSF are high during the rest (light) period and low during the active (dark) period in the rat (80). More specifically, PGD2 concentrations tend to be higher in the CSF (81) and the cerebral cortex (82) during NREMS than in wakefulness. Further, sleep deprivation enhances PGD2 contents of the CSF in rats (81). However, the source of PGD2 is neither brain tissue nor neurons. PGD synthase is expressed predominantly in the leptomeninges, and the epithelial cells of the choroid plexus, and oligodendrocytes (83). The enzyme is secreted as beta-trace protein by the choroid plexus into the CSF in humans (84). Thus, PGD2 and PGD synthase come from the membranes encapsulating the brain (Figure 2). The prostanoid receptor mediating PGD2 action is also predominantly expressed in the leptomeninges (85). The receptors responsible for the NREMS-promoting activity of PGD2 reside in a circumscribed area near VLPO on the surface of the basal forebrain. It is suggested that the arachnoid cells function as chemoreceptors responsive to PGD2, they release adenosine that stimulates the sleep-active neurons in the VLPO via A2a adenosine receptors

Figure 2. Proposed mechanism of sleep induction by PGD2. PGD2 is produced in, and is secreted into the CSF by the leptomeninges and the choroids plexus. PGD2 acts on arachnoid trabecular cells in a circumscribed area on the surface of the basal forebrain, and elicits adenosine release. Adenosine diffuses to the VLPO situated nearby and stimulates sleep-active neurons via A2 receptors. CSF: cerebrospinal fluid; VLPO: ventrolateral preoptic nucleus.

(86). In the preoptic area / anterior hypothalamus of rats, local administration of PGD2 stimulates and inhibits 30 % of the sleep- and wake-active neurons, respectively (87). It is not clear if these effects are also mediated by adenosine particularly because these types of actions are reported for A1 receptor agonists (see above), but A1 receptors are not implicated in PGD2 effects.

Another PG, PGE2 was also implicated in sleep regulation. Originally, PGE2 was proposed to inhibit sleep and possibly act as a wake promoting substance (88). In fact, PGE2 behaved in a way opposite to PGD2 in many tests. For instance, PGE2 decreased sleep (89), stimulated wake-active neurons in the basal forebrain (87), PGE2 concentration increased in wakefulness and decreased in sleep in the hypothalamus (90), and infusion of a PGE2 antagonist into the third ventricle decreased wakefulness (91). In a more recent study, however, concentrations of PGE2 (and those of PGF2 alpha) displayed the same diurnal and sleep-related variations as PGD2 in the CSF, and application of PGE2 into the PGD2-responsive subarachnoid space promoted sleep (81). The effects of PGE2 on sleep may vary with the site of application, for example PGE2 may act on PGD2 receptors in the basal forebrain subarachnoid area. Regardless, current findings do not support the idea that sleep-wake activity is regulated by the ratio of PGD2/PGE2. Finally, the effects on sleep were also tested of eicosanoids generated from arachidonic acid via the lipoxygenase pathway (92). When infused into the third ventricle, lipoxins (LXA4 and LXB4) and the leukotriene D4 (LTD4) increased duration of NREMS but they were less potent than PGD2.

Many eicosanoids alter body temperature; PGE2 is regarded as a major mediator of fever. Increases in body temperature are also observed after PGD2 in some sleep experiments but this effect was not consistent in all studies, perhaps because the sleep-promoting dose is lower than the pyrogenic dose. Thus, the sleep-promoting activity of PGD2 is not attributed to thermoregulatory actions.

The concepts of volume conduction of PGD2 in the CSF and its acting at a specific brain site, are attractive since sleep need could be conveyed from very distant parts of the brain to sleep generating structures. On the other hand, it is not pleasing to envision sleep as the function of the membranes surrounding the brain. The stimuli, however, that result in PGD2 and PGD synthase secretion, might derive from the brain. For example, some cytokines, e.g. IL1 and TNF alpha, can enhance production PGD2, and PGD2 may in fact be involved in mediation of sleep in response to these cytokines (93;94). From this viewpoint, PGD2 synthesis by the leptomeninges and choroid plexus could be considered an amplification mechanism in the sleep process.

5. HORMONES OF THE SOMATOTROPIC AXIS

Growth hormone (GH), a major anabolic hormone of the body, is secreted by the anterior pituitary. Synthesis and release of GH is controlled by the hypothalamus via two neurohormones, GHRH and somatostatin, which are secreted into the pituitary portal vessels at the median eminence. GHRH stimulates and somatostatin inhibits GH production and release. Secretion and synthesis of GHRH and somatostatin vary inversely with a period of approximately 3 hours in rats (95;96). Bursts of GHRH release are followed by GH secretion at 3-4 hour intervals in male rats (97). GH actions are in part mediated by insulin-like growth factor-1 (IGF-1). IGF-1 is a paracrine substance produced in various tissues including the brain, and it is also a hormone which is secreted by the liver into the circulation. There are many feed back loops within the somatotropic system. It is important for the present discussion that acute rises in IGF-1, GH and somatostatin inhibit release and / or production of GHRH (somatostatin and IGF-1 may mediate the effects of GH on GHRH). Ghrelin is a new member of the somatotropic system (98). Ghrelin is a hormone produced by the stomach, and it is also a neurotransmitter in the hypothalamus. Ghrelin stimulates the GH-secretagogue (GHS) receptors which are distinct from GHRH receptors. Although ghrelin is capable of stimulating GH secretion by the pituitary *in vitro*, it stimulates hypothalamic GHRHergic neurons *in vivo*, and GHRH may mediate the GH secreatgogue action of ghrelin *in vivo*. Thus, while GHRH is recognized as an important sleep regulatory factor, other members of the somatotropic system may also modulate sleep either through acting on GHRH or through metabolic actions independent of GHRH.

5.1. GHRH

GHRH is a peptide (reviewed in (99), a member of the secretin-glucagon peptide family, and it has significant structural homology to VIP. VIP and GHRH may mutually cross-react to some extent with each other's receptor. The homology to VIP was the feature of GHRH which prompted us to test the sleep effects of GHRH (100). VIP is implicated in the regulation of REMS but it also stimulates NREMS in rats (101-103) but not in cats (104) or rabbits (105). Yet antibodies or antagonists to VIP do not alter NREMS in rats (101;106), therefore, the effects on NREMS might be mediated through a related receptor perhaps that of GHRH. Links between NREMS and activation of GHRH is suggested by the well-documented NREMS-associated GH secretion which also occurs in rats (e.g. (107). Subsequent experiments supported the NREMS promoting activity of GHRH but it has never been determined whether exogenous VIP in fact stimulates NREMS via GHRH receptors in the rat.

GHRH promotes NREMS in rats, rabbits, mice, and humans. The NREMS-promoting activity was demonstrated in a variety of experimental models after intraventricular (*icv*): (rats (108-110) and rabbits (110)), intravenous (humans (e.g. (111-113), and rats (114)), intraperitoneal (mice (115)), intranasal (spray in humans (116)), and intrapreoptic (rats, (117)) administrations. GHRH enhances NREMS duration and the intensity (as measured by slow wave activity in the EEG) although brain (body) temperature is not altered. In animals, increases in NREMS occur within 1 hour of GHRH injection. The duration and the magnitude of the effect vary with the dose, the site of injection, the species etc. For example, in rabbits 1 nmol/kg *icv* injected GHRH induces 53 min excess NREMS within the first 6 postinjection hours, but it is only half as effective in rats (injected at the onset of the dark period). The NREMS promoting activity may disappear when the dose of GHRH is high perhaps due to an inhibition of endogenous GHRH by GH (114). Other factors that may influence the sleep response include time of the day, single versus repeated administration, and the slope of the rise in plasma GH concentrations, and gender (see Steiger in this volume). For instance, in humans, continuous infusion of GHRH fails to increase sleep (118). GHRH stimulates NREMS in hypophysectomized rats (114), thus indicating that GH is not involved in the mediation of GHRH-induced NREMS. GHRH may also enhance REMS but the REMS promoting activity is not consistent and hypophysectomy blocks the REMS response to GHRH suggesting that it requires GH (114).

Suppression of endogenous GHRH actions by means of immunoneutralization of GHRH or by means of a competitive antagonist is followed by decreases in NREMS (119;120). Inhibition of GHRH through negative feedbacks in the somatotropic axis also inhibits NREMS (115). NREMS decreases in mutant dwarf rats (*dw/dw* rats) (121) and mice (*lit/lit* mice) (115;122) with GHRH receptor deficiencies, and in transgenic mice with decreased GHRH production (Th-hGH transgenic mice; they express human GH in typrosine hydroxilase positive neurons, and the GH secreted in the brain inhibits endogenous GHRH production) (123). The reduction of NREMS is approximately 2 hours per day, it occurs predominantly during the diurnal rest period, and it is comparable to those observed after immunoneutralization of GHRH. Immunoneutralization of GHRH blocks the NREMS response to a 3-hour sleep deprivation in rats (120). Further, sleep deprivation (4 hours)-induced enhancements in EEG slow wave activity are greatly attenuated in the *dw/dw* rats with a defect in GHRH receptor signaling (121). However, the sleep responses to sleep deprivation are essentially normal in the *lit/lit* mouse (4-hours sleep deprivation) with non-functional GHRH receptors (unpublished). Recovery sleep after 12 hours of sleep deprivation is also normal in the transgenic Mt-rGH mice, which express rat GH stimulated by the promoter region of the metallothionein gene, though these mice have less GHRH (124). The cause of these differences is currently not known.

GHRH mRNA levels (125) and GHRH contents (126) in the hypothalamus display diurnal variations. These variations are such that they suggest significant GHRH synthesis and release during the first portion of the diurnal rest (light) period when the highest amount and most intense NREMS occurs in the rat. Eight hours of sleep deprivation results in a depletion of GHRH from the hypothalamus indicating its release (126); this process continues during recovery sleep for another 2 hours. Depletion-induced synthesis of GHRH may explain the significant rise in GHRH mRNA level that occurs at the termination of sleep deprivation (127). In situ hybridization studies suggest that the diurnal variations in GHRH mRNA occur in the periventromedial area in the vicinity of the arcuate nucleus whereas sleep deprivation stimulates GHRH mRNA in the paraventricular nucleus (128). A recent observation provides indirect evidence for the sleep deprivation-induced GHRH release. Thus, massive GHRH exposure causes a significant down-regulation of GHRH receptors in the pituitary (129). Determination of GHRH binding and GHRH receptor mRNA in the rat hypothalamus shows a 50% down-regulation in GHRH receptors after 8 hours of sleep deprivation whereas GHRH receptors in the pituitary are unaltered (130). It seems that GHRH release occurs only in the hypothalamus and not in the pituitary during sleep deprivation. Indeed, GH secretion tends to be inhibited during sleep deprivation (131). The progressive release of GHRH in the hypothalamus may contribute to the development of sleepiness during sleep deprivation.

Unlike many other neuropeptides, GHRH is confined to a small area in the brain (132). GHRH-containing neurons in the arcuate nucleus are the major source of the hypophyseotropic projections terminating in the median eminence (Figure 3). A much smaller number of GHRHergic neurons is found around the ventromedial nucleus, close to the arcuate nucleus, and in the parvicellular portion of the paraventricular nucleus. These extra-arcuate GHRHergic neurons seem to innervate the periventricular nucleus and the anterior hypothalamus / preoptic area. Promotion of sleep is the function of intrahypothalamic GHRH action: microinjection of GHRH into the anterior hypothalamus / preoptic area enhances NREMS whereas administration of a GHRH antagonist into this region inhibits spontaneous NREMS and recovery after sleep deprivation (117). That diurnal and sleep deprivation-induced changes in GHRH mRNA are detected in the periventromedial and paraventricular neurons suggests that the extra-arcuate GHRHergic neurons are important for sleep regulation (128). Intra-arcuate neurons, however, also innervate various parts of the hypothalamus (133;134) and thus, may also modulate sleep. Monosodium glutamate treatment in neonatal rats destroys the arcuate nucleus but the extraarcuate GHRHergic neurons survive (135). Recorded from as adults, the monosodium glutamate treated rats display decreases in NREMS similar to those observed in rats with GHRH receptor deficiency (136). That communications between the mediobasal hypothalamus (arcuate nucleus and ventromedial nucleus) and the anterior hypotalamus / preoptic area are significant for sleep is supported by experiments with transections at the rostral pole of the arcuate nucleus in rats (137). NREMS and REMS decrease markedly during the light period after a cut between the arcuate nucleus and the anterior hypothalamus. Finally, GHRH elicits increases in

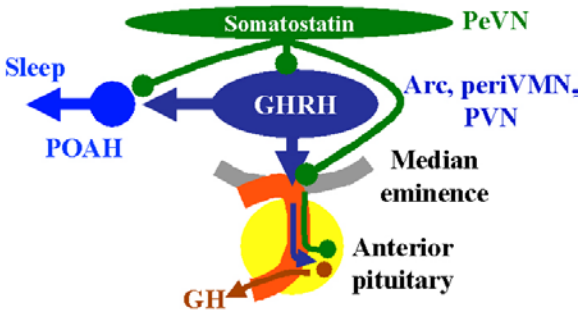

Figure 3. Stimulation of growth hormone (GH) secretion and sleep by hypothalamic GHRH. GHRH-containing neurons reside in the arcuate nucleus (Arc), around the ventral surface of the ventromedial nucleus (periVMN), and in the parvicellular portion of the paraventricular nucleus (PVN). The GHRH released from terminals in the median eminence is taken up by the blood and is carried into the anterior pituitary where it stimulates GH secretion. Sleep promoting activity of GHRH is mediated by GABAergic neurons in the proptic region – anterior hypothalamus (POAH). Somatostatin-containing neurons in the periventricular nucleus (PeVN) project to the median eminence. Somatostatin delivered to the pituitary somatotroph cells inhibits GH secretion. Somatostatin also inhibits GHRHergic neurons. It is assumed that somatostatin acts both on the cell bodies and the terminals of GHRHergic neurons. There might be more than one somatostatin-containing neuronal pool involved in the control of GHRHergic activity.

cytosolic calcium in cultured hypothalamic neurons obtained from fetal rats (138). The GHRH responsive neurons are GABAergic. There is a strong possibility, therefore, that GABAergic neurons mediate the sleep-promoting activity of hypothalamic GHRHergic neurons.

IL1 stimulates the GHRHergic system (139), and this action contributes to the enhancements of NREMS elicited by IL1 (140). IL1 up-regulates expressions of both the GHRH gene and GHRH receptor gene in fetal hypothalamic neuronal cultures (141). *Icv* injection of IL1 also increases GHRH and GHRH receptor mRNA levels in the hypothalamus *in vivo*. In addition, a significant percentage of the cultured neurons that are responsive to GHRH also produce calcium signals after IL1 treatment (138). It is hypothesized that GHRH may act by amplifying and prolonging IL1 actions: IL1 elicits acute and direct stimulation of sleep-active neurons (142), simultaneously, IL1 enhances GHRH release and synthesis in GHRHergic neurons and up-regulates GHRH receptors which also increase sleep.

GHRH stimulates GH secretion and NREMS through two independent outputs. Perhaps the major significance of this mechanism is that it is capable of synchronizing the anabolic processes of the body to a state when rest occurs. Theoretically, the GHRHergic system may convey the need for sleep arising in the brain, and the need for anabolism in the body to structures in the anterior hypothalamus / preoptic region that mediate sleep. Unfortunately, the regulation of GHRH is currently unclear.

5.2. Somatostatin

Somatostatin (somatostatin-14 and somatostatin 28) is a cyclic peptide. It is an ubiquitous inhibitory neurotransmitter in the brain, an inhibitory neurohormone released from the hypothalamus to act in the pituitary, and an inhibitory paracrine in the gastrointestinal system and the pancreas. Somatostatin modulates the activity of the somatotropic axis in multiple ways. The hypophyseotropic somatostatinergic nerurons reside in the periventricular nucleus and project to the median eminence. Somatostatin is delivered to the pituitary somatotrophs by the blood to inhibit GH secretion. Periventricular somatostatinergic neurons inhibit GHRH-containing neurons in both the arcuate nucleus and the median eminence (Figure 3) (143;144). Finally, GHRHergic neurons are also inhibited by somatostatin-containing interneurons residing in the arcuate nucleus (145).

The distribution of the five somatostatin receptors (sst1-5) in brain have been determined (146). Inhibition of GH and GHRH results from sst2 receptor activity. There are somatostatin analogs, such as octreotide, which are more resistant to breakdown than somatostatin which is eliminated within a few minutes in tissues. Octreotide is a potent agonist on human sst2 and sst5 receptors (but sst5 receptors in the rat differ from human sst5 receptors), it displays modest affinity for sst3 receptors, and does not bind sst1 and sst4 receptors (147).

One may anticipate that a peptide, which inhibits GHRH, would inhibit sleep. Somatostatin is one of the first hypothalamic neurohormones tested in a sleep assay and indeed, inhibition of sleep in rats was reported (148;149). Systemic or *icv* injection of octreotide also elicits prompt and dose-dependent suppression of NREMS in rats (150;151). This suppression is followed by a tendency for increased sleep duration and prominent enhancements in EEG slow wave activity during NREMS 2 to 3 hours postinjection. In human studies, systemic injection of somatostatin failed to alter sleep in young subjects (111) but impaired sleep in the elderly (152). The more potent octreotide, however, decreased sleep in young subjects (153).

Since somatostatin and its receptors are expressed in various structures in the brain the sleep response to exogenous somatostatin or octreotide may have little to do with the somatotropic axis. For instance, *icv* octreotide elicits angiotensin-like effects (drinking, vasopressin secretion, increases in blood pressure) which themselves disturb sleep. However, the angiotensinergic actions of octreotide are blocked by angiotensin antagonists or angiotensin convertase inhibitors (151;154) while the octreotide-induced sleep suppression remains unaltered (150). Determination of hypothalamic GHRH contents verifies that octreotide causes an immediate angiotensin release and an inhibition of GHRH release resulting in GHRH accumulation (155). The accumulated GHRH is

released in hour 2 postinjection and thereafter when intense NREMS and GH secretion recurs. After *icv* octreotide, different brain structures mediate the angiotensin-like actions and the sleep suppression (156). Octreotide elicits drinking when injected into the subfornical organ and the dorsal periventricular area including the paraventricular nucleus. In contrast, sleep is suppressed after microinjection of octreotide into the arcuate nucleus, anterior hypothalamus and medial preoptic region. The sleep suppressive sites correspond to the location of the GHRHergic cell bodies and terminals. Indeed, the sleep response to octreotide is attenuated in transgenic mice with decreased GHRH production (Mt-rGH mice) (124) and it is abolished in mutant mice with deficient GHRH receptors (*lit/lit* mice) (122). These findings suggest that somatostatin can suppress sleep by inhibiting GHRHergic neurons, an effect attributed to stimulation of sst2 receptors.

A series of experiments demonstrates that somatostatin has a REMS-promoting activity (e.g. (157)). Unfortunately, NREMS is often not reported in these papers or the time blocks presented are too long to detect an immediate and short lasting inhibition of NREMS (157;158). The action site of somatostatin-induced REMS is localized in the brain stem (159;160), and thus, it might be independent of the somatotropic axis.

5.3. Growth hormone (GH)

Many observations demonstrate that GH may promote REMS (e.g. (161), however, these are not reviewed in this chapter. Some findings suggest that an acute rise in GH concentration may inhibit NREMS (162), in a manner consistent with negative feed back inhibition of GHRH. In fact, in an experiment studying sleep in humans whose bedtime was delayed, decreases in slow wave sleep were attributed to GH secretion prior to sleep onset (163). Chronically high and low GH secretions, however, may alter NREMS in humans (reviewed in (164)). In particular, chronic excess of GH may be associated with enhancements in EEG power in all frequency bands. The predominant response to immunoneutralization of GH is a suppression of slow wave activity during NREMS in rats (165). Mutant and transgenic dwarf rats and mice, however, do not display alterations in EEG slow wave activity during spontaneous NREMS (121). In addition, GH replacement in the *lit/lit* mice with a defect in GHRH receptors does not alter NREMS though it normalizes REMS (122). Finally, transgenic mice producing huge amounts of excess GH (Mt-rGH mice) display large increases in REMS with a modest increase in NREMS time (124). This change in NREMS is at variance with the suggested role of GHRH in sleep regulation because production of GHRH is decreased in these mice. It is speculated that excess GH stimulates NREMS through mechanisms such as production of free radicals and enhanced synthesis of cytokines, which do not occur at physiological GH concentrations.

5.4. Insulin-like growth factor-1 (IGF-1)

Icv injection of a high dose of IGF-1 promptly inhibits sleep in rats and rabbits (166). Inhibition of sleep occurs simultaneously with inhibition of GH secretion and it is attributed to an inhibition of GHRH. Low doses of IGF-1, however, increase NREMS (167). This may result from some metabolic action. It is possible that the effects of chronically high GH concentrations are mediated via IGF-1.

6. CYTOKINES

During the past 20 years a wealth of information has accumulated concerning a new, now relatively large, class of low molecular weight proteins collectively termed cytokines. Since the work has been conducted primarily by immunologists, the names often reflect immunological cells, actions, or processes, e.g., IL or TNF. Cytokines act in autocrine, paracrine, and endocrine fashions to regulate important facets of the immune response such as the acute phase response. In this essential host defense response, cytokines trigger constitutional symptoms of acute illness such as malaise as well as behavioral manifestations such as somnolence, anorexia, fever and social withdrawal (see Toth and Opp this volume for a more detailed review of sleep-host defense mechanisms). A body of evidence now indicates that most aspects of the acute phase response are driven by a complex array of cytokines in association with classical stress hormones.

Cytokines are also hypothesized to be involved in physiological processes such as sleep, food intake, development and gastrointestinal function (e.g., 168-170). The central idea involved in this hypothesis is that under physiological conditions, basal levels of cytokines or their receptors or modifier proteins vary in subtle ways with one or more physiological processes (see 171). Response specificity for any one physiological function such as sleep would arise from multiple interactions of several cytokines and hormones. After pathological disturbance, the production of one or more cytokines would be greatly amplified by the pathological stimulus, e.g., microbial products, in a site-specific manner. Such amplified cytokine production would induce pathology in a manner analogous to the pathologies produced by excessive hormone production. Although regulation of physiological processes likely gains stability by the involvement of multiple pleiotropic cytokines, the involvement of individual cytokines in sleep regulation can be studied since their activity on sleep are often large. In this essay, we focus on IL1 beta and TNF alpha since their sleep regulatory roles are extensively studied. We also briefly mention how other cytokines, such as nerve growth factor and brain-derived neurotrophic factor, IL4, IL10, IL13, and IL18, all affect sleep and are affected by sleep.

6.1. Interleukin-1 beta

IL1 beta has a molecular weight of about 17 kD. Its role in inflammation and immunity are well characterized (172) and it was the first cytokine for which sleep-promoting activity was described (35). A relatively large number of separate gene products compose the IL1 family of molecules. There are three IL1 ligands, IL1 alpha, IL1 beta and an IL1 receptor antagonist (IL1 RA); these members show limited amino acid homology although they share a higher homology in their predicted 3-dementional topologies (173).

Two IL1 receptors have been identified, Type I and Type II (174-176) and all three ligands bind to both receptors. The IL1 Type I and II receptors possess three extra cellular immunoglobulin-like domains, limited homology and different binding characteristics. In addition, the receptors are part of the Toll-like receptor family (177-179) with other members of this family also contributing to host defense and probably to sleep as well. For instance, Toll-like receptors 2 and 4 bind bacterial products then trigger intracellular events, which involve proteins also used by the IL1 Type I receptor, e.g., MyD88 and the IL1 receptor associated kinase (IRAK). Both of these substances are also used by another Toll-like receptor, the IL18 receptor.

The two receptors have different functions. The Type I receptor is the signal transduction receptor, while the Type II receptor is a decoy receptor having a truncated non-signaling intracellular domain (180). Only 10 Type I receptors per cell need to be occupied to activate cells. The IL1 RA competes with IL1 alpha and IL1 beta and, as its name implies, it antagonizes the actions of IL1 alpha and beta. An IL1 accessory protein (IL1 AP) shares limited homology with Type I and II receptors (181;182) and forms a complex with the Type I receptor and either IL1 alpha or beta, but not with the IL1 RA. The IL1 AP increases the binding affinity of IL1 beta for the Type I receptor (182). Ultimately, the Type I receptor-ligand complex triggers a complex signaling cascade, sharing several intracellular proteins with other signaling cascades leading to NFkB activation. Other IL1-activated signaling pathways have also been described; they include activation of mitogen-activated protein kinases and protein kinase C and induction of other second messengers including intracellular calcium (see below), cAMP and ceramide (reviewed 172;178).

IL1 alpha and beta are made as precursor molecules and mature forms are released from cells after processing. However, the IL1 alpha precursor is biologically active while pre-IL1 beta requires processing. IL1 beta converting enzyme (ICE) cleaves pre-IL1 beta at two sites, thereby producing the mature 17 kD IL1 beta fragment. Soluble IL1 receptors result from shedding of the extracellular domain of both IL1 receptors. Finally, an alternatively processed cDNA for the Type II receptor which only encodes the extracellular domain of the receptor was described (183). The soluble IL1 receptors bind IL1 and thereby lower IL1 concentrations; their physiological role remains incompletely understood (reviewed 178).

The IL1 family of molecules are expressed in the brain (reviewed 169;184). IL1 beta is produced by glia, endothelial cells and neurons (e.g., 185) and the receptors are also found on a variety of cell types, including neurons (e.g., 138). IL1 is one of the several cytokines, including TNF alpha and NGF, whose production is enhanced by activation of NFkB. In addition, these cytokines also activate NFkB. As a consequence, they form positive feedback loops capable of rapid self amplification as well as NFkB activation. Furthermore, there are many negative feedback signals that either reduce IL1 production or limit NFkB activation. Molecules involved in these negative feedback mechanisms include the soluble IL1 receptors, the IL1 RA, the corticotropin releasing hormone (CRH)-glucocorticoid axis, several cytokines termed "anti-inflammatory" cytokines such as IL4, 10, and 13, and transforming growth factor beta. All of these substances inhibit sleep (e.g, 186;187) (see below).

Bolus injection of IL1 beta either directly into brain areas, *icv*, intravenously (*iv*) or intraperitoneally (*ip*) enhances NREMS (reviewed 188). These effects can be large, for example, *icv* injection of about 600 femtomoles of IL1 beta into rabbits induces about 2 hours of extra NREMS during the first 12 hours post injection (189). IL1 beta has been found to induce excess NREMS in every species thus far tested, mice, rats, rabbits, monkeys, and cats (35;190-194). Humans receiving IL1 for therapy report excessive sleepiness (195). In rats (196) and cats (191) low doses of IL1 promote sleep while higher doses inhibit sleep. Furthermore, these effects depend upon the time of day IL1 is given (196). Thus, some doses of IL1 can promote NREMS after nighttime injections while the same dose given during the day inhibits NREMS. In addition to enhancing duration of NREMS, IL1 beta also induces enhanced EEG slow wave activity during NREMS (35). This effect is dependent upon the route of administration; thus, after *icv* or *iv* IL1 injection EEG delta waves are enhanced. In contrast, after *ip* injection in rats and mice IL1 beta induces decreased EEG delta waves, although IP-IL1 still induces enhanced duration of NREMS (197).

Low somnogenic doses of IL1 have little effect on duration of REMS in rats and rabbits. However, somnogenic doses of IL1 beta that induce large increases in NREMS inhibit duration of REMS and doses that inhibit NREMS also inhibit REMS. The sleep occurring after somnogenic doses of IL1 appears to be relatively normal in that animals continue to cycle through wakefulness, NREMS and REMS stages and the animals are easily aroused by various stimuli. However, after the high sleep inhibiting doses of IL1, sleep becomes fragmented, sleep postures are abnormal and animals are less responsive to handling.

Inhibition of IL1 beta inhibits spontaneous NREMS. Thus, anti-rabbit IL1 beta (190) and anti-rat IL1 beta (198) inhibit spontaneous NREMS in rabbits and rats respectively. Similarly, the IL1 RA, or a fragment of the soluble IL1 receptor, inhibit spontaneous sleep (199). Substances, which inhibit IL1 production, also inhibit spontaneous NREMS, e.g. CRF, IL4, IL10, IL13, transforming growth factor beta and alpha melanocyte stimulating hormone (MSH) (e.g., 200; reviewed 188). Further, inhibition of IL1 using either the soluble IL1 receptor fragment or an anti-IL1 antibody attenuates sleep rebound after sleep deprivation (190;198;201). Finally, substances such as muramyl dipeptide induce IL1 production and NREMS and inhibitors of IL1 also attenuate muramyl dipeptide-induced sleep responses (202).

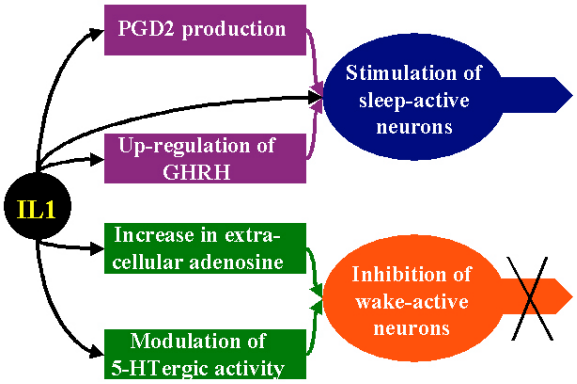

Figure 4. Mechanisms implicated in sleep promotion by IL1. It is assumed that use-dependent release of IL1 may occur from neurons and glial cells in various structures in the brain. IL1 acts in part via alterations in neuronal activity locally, and in part via projection systems which mediate sleep-like activity throughout the brain. IL1 directly stimulates sleep-active neurons in the POAH, and up-regulates the GHRHergic system. IL1 stimulates PGD2 production, and PGD2 also stimulates sleep mechanisms in the basal forebrain. In addition, IL1 may inhibit mechanisms promoting wakefulness. This may result from a modulation of serotonergic activity in the dorsal raphe nucleus, and from enhancements in adenosine release. Indirect evidence from IL1-induced adenosine release was found in the hippocampus where IL1 inhibited glutamatergic excitation of hippocampal neurons without altering GABAergic inhibition (217).

Mutant mice lacking the IL1 type I receptor do not exhibit sleep responses if given IL1 thereby implicating the type I receptor in IL1-induced sleep responses (193). These mutant mice also have less spontaneous NREMS than their control strain and this effect is greatest during dark hours. However, these mutant mice are capable of sleep responses if given TNF alpha suggesting their functional sleep impairment involves only IL1 signaling.

Brain levels of IL1 or IL1 beta mRNA seem to vary with sleep propensity. CSF levels of IL1-like activity in cats vary with the sleep-wake cycle (203). In rats, highest hypothalamic levels of IL1 beta (204) and IL1 beta mRNA (184) occur at the beginning of daylight hours, a time when rat NREMS is maximal. Further, sleep deprivation results in enhanced hypothalamic IL1 mRNA levels (184;205). In addition, changes in cortical and hippocampal IL1 beta mRNA levels also vary with sleep propensity.

IL1 blood levels may also vary with sleep. In humans, peak levels of IL1 in humans occur at sleep onset (206). IL1 blood levels also increase during sleep deprivation (207;208). An issue related to changing systemic cytokines and their effects on the central nervous system function is the question of how they, as proteins, pass the blood brain barrier. Apparently, several methods are used including, passage through permeable areas of the blood brain barrier such as the median eminence, transport mechanisms specific for cytokines, induction of secondary small relatively lipid-soluble biologically active molecules such as PGs and NO, and signaling the brain via neural afferents. For instance, vagotomy blocks the sleep promoting actions of low doses of *ip* IL1 (197). Interestingly, IL1 given *ip* enhances hypothalamic IL1 beta mRNA and this action is also blocked by vagotomy (209). Finally, excessive food intake enhances NREMS and hypothalamic levels of IL1 beta mRNA and vagotomy blocks these effects as well (210;211). However, it remains to be determined what role, if any, circulating IL1 beta has in physiological sleep regulation.

The site(s) of action of IL1 responsible for sleep remains to be determined. Results from microinjection studies suggest that IL1 may act at multiple action sites (Figure 4). Thus, injection of IL1 into the locus coeruleus (212) or dorsal raphe (213) induces enhanced NREMS. In contrast application of IL1 into the hypothalamic paraventricular nucleus induces wakefulness (214) perhaps through activation of the corticotropic system. In a more extensive study, IL1 injection into several ventricular sites and subarachnoid sites resulted in enhanced sleep (94). The most active sites were those in close proximity to the anterior hypothalamus. Within the anterior hypothalamus/preoptic area IL1 beta excites sleep-active neurons and inhibits wake-active neurons (142). There is a more extensive literature implicating the anterior hypothalamus for the site of IL1-induced fevers (215). However, IL1-induced fevers are separable, in part, from IL1-induced sleep responses (35).

There are several likely downstream events involved in IL1-enhanced NREMS. If rats are pretreated with anti-GHRH antibodies, then given IL1, the expected sleep responses do not occur thereby suggesting that GHRH is involved in IL1-induced sleep (140). A subpopulation of GABAergic hypothalamic neurons contains receptors for both GHRH and IL1 and the stimulation of either receptor enhances cytosolic calcium (138). This latter experiment implies that the somnogenic actions of GHRH and IL1 result from stimulation of the same cells although this relationship has yet to be determined.

In addition, IL1 induces increases in PGs (195), NO (216) and adenosine (217); thus, all of these could be thus involved in the somnogenic actions of IL1. Finally, IL1 interacts with many neurotransmitter systems, e.g. GABA (218) and serotonin (219) and acetylcholine (220) and these actions could also be related to IL1-induced sleep (reviewed 221).

6.2. Tumor necrosis factor alpha

TNF alpha is synthesized as a 26 kD membrane associated protein (222). Soluble TNF alpha, a 17 kD protein, is cleaved from the 26 kD membrane associated protein by TNF alpha converting enzyme (TACE). TNF alpha production is tightly regulated in a tissue specific manner (reviewed 223) with transcription, translation and secretion, all controlled at multiple points. The 5' flanking region of the TNF alpha gene contains several regulatory sites. TNF alpha induces its own expression, in part, via an NFkB regulatory site.

There are two TNF cell-surface receptors (R), the 55 kD TNFR and the 75 kD TNFR. 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 helps explain the pleiotropic actions of TNF. The TNF receptor family is divided into 2 large groups based on the adaptor proteins recruited in response to ligand binding. One such class includes the 55 kD TNFR and several additional proteins, e.g., FADD, TRADD, RIP, TRAF-2 (reviewed 224-228). TNF receptor trimerization is mediated by the TNFR-gamma 1 subunit. This association causes the displacement of a protein, SODD (silencer of death domains), from the intracellular domains of the gamma 1 monomers and the formation of a binding site for another protein, TRADD (TNF receptor associated death domain). TRADD then recruits FADD, RIP and TRAF2. TRAF2 is associated with CIAP1 and CIAP2, (cellular inhibitors of apoptosis) which inhibit caspases associated with FADD. These resulting complexes signal via several pathways; including NFkB activation, AP-1 and caspases 8 and 3.

One major TNF activated signaling pathway activates new gene transcription, while another leads to cell death. 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 (reviewed 224). For instance, the cellular inhibitors of apoptosis (CIAP1 and CIAP2) recruited by TRAF2 and TRAF1 and associated with TRADD, inhibit caspase activation (229). Also both the NFkB and AP-1 pathways contribute to the antiapoptotic action of TNF alpha.

Both TNF receptors are cell-surface receptors; they form trimeric complexes with TNF (reviewed 228). 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 majority of the 75 kD TNFRs are located on the cell surface, while the steady-state location of most of the 55 kD TNFRs are internal. The ectodomains of both receptors are shed via the actions of TACE to form soluble (S) receptors. The 55 kD sTNFR is a constituent of normal CSF (230) although its physiological role remains unknown. However, given the clinical usefulness of the sTNFR and its effects on sleep and fatigue, it likely plays a role in sleep regulation.

TNF alpha is expressed by microglia, astrocytes and neurons (231-234) and has a variety of biological actions in the central nervous system including a role in mediating brain damage and in neuroprotection. Whether TNF alpha is protective or damaging may depend upon the receptor type present, TNF-55 kD or the TNF 75 kD receptor (235-237) and the stimulus context, the presence and absence of substances that modify TNF alpha activity (238;239). TNF alpha participates in mediating whole organism processes including fever (215;240) and food intake (241). TNF alpha also plays a role in brain development (reviewed 170). TNF alpha seems to participate in neuronal connectivity, (also called plasticity). For instance, TNF alpha potentiates AMPA-induced postsynaptic potentials (242), AMPA-induced cytosolic Ca^{++} increases (138) as well as several voltage-dependent calcium channels (De *et al* submitted) (243;244)). AMPA receptors and intracellular calcium modulate neural plasticity.

The ability of TNF alpha to promote NREMS was first described in 1987 by Shoham *et al* (36). If TNF alpha is given *icv*, *iv* or *ip*, it enhances duration of NREMS. For instance, mice receiving TNF *ip* (3 micrograms) spend about 90 minutes extra in NREMS during the first 9 hours postinjection (245). In addition, NREMS after TNF alpha treatment is associated with supranormal EEG slow waves (36). TNF alpha is somnogenic in all species thus far tested: rabbits (36), mice (245), rats (246), and sheep (247). TNF alpha has little effect on REMS if low NREMS-promoting doses are used, however, higher doses inhibit REMS. Sleep following TNF alpha treatment appears to be normal in the sense 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 treatment.

Inhibition of TNF alpha inhibits spontaneous NREMS. Thus, treatment with anti-TNF alpha antibodies (248), full-length soluble TNF receptors (249) or TNF soluble receptor fragments containing the TNF recognition site (250), all inhibit spontaneous NREMS in rabbits and rats. Further, pretreatment of animals with TNF inhibitors prior to sleep deprivation reduces the expected sleep rebound that normally occurs after sleep deprivation (250). Substances that inhibit TNF alpha action or production also inhibit spontaneous sleep, e.g., IL4, 10 and 13. In addition, these substances also inhibit the production of certain other cytokines, e.g., IL1 beta, 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 NFkB-sensitive cytokines. Furthermore, inhibition of TNF also blocks the increases in NREMS observed in response to an acute mild increase in ambient temperature (251).

Mice lacking the TNF 55 kD receptor fail to exhibit NREMS responses if given TNF alpha, thereby implicating this receptor in TNF alpha-enhanced sleep (245). These mice also sleep less than corresponding control strains; the reduced NREMS occurs mostly during daylight hours. In contrast, mice lacking the IL1 Type I receptor sleep less than controls during dark hours (193). If animals are given both the soluble IL1 receptor plus the soluble TNF receptor, spontaneous NREMS is reduced across day and night hours (189).

Hypothalamic levels of TNF alpha (252) and the TNF alpha mRNA (253) vary diurnally and are influenced by sleep deprivation. The highest levels in rats occur at

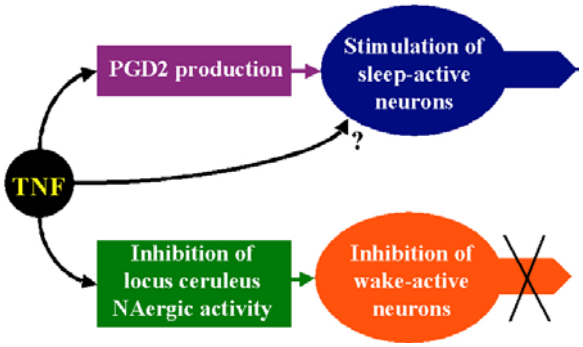

Figure 5. Promotion of sleep by TNF. Similarly to IL1, TNF might be produced in various structures and may have multiple action sites. TNF elicits sleep when injected into the POAH but direct action of TNF on sleep-active neurons has not been reported yet. Like IL1, TNF also stimulates PGD2 production and may used the PGD2-mechanism of sleep induction. TNF seems to inhibit the noradrenergic (NAergic) wake-mechanism in the locus ceruleus.

daybreak. The amplitude of the day-night changes in TNF protein is about 10 fold and mRNA about 2 fold, this reflects the predominate post-transcriptional regulation of TNF. After sleep deprivation, hypothalamic TNF mRNA also increases (254;255). Sleep deprivation also increases the expression in brain of the 55 kD TNF receptor mRNA (255). In normal humans blood levels of TNF alpha correlate with EEG slow wave activity (256). After sleep deprivation, circulating levels of TNF (257) and the 55 kD TNF soluble receptor, but not the 75 kD soluble receptor, increase (258). As mentioned above, the 55 kD soluble receptor is a component of normal CSF.

Clinical conditions associated with sleepiness also seem to correlate with higher blood levels of TNF. Thus, sleep apneic patients have elevated TNF plasma levels (259-261). AIDS patients have disrupted TNF and sleep rhythms (256). TNF alpha is elevated in chronic fatigue patients (262) chronic insomnia patients (263) and in pre-eclampsia patients (264). Postdialysis fatigue is associated with higher TNF levels (265;266) and cancer patients receiving TNF report fatigue (267). Rheumatoid arthritis patients receiving the soluble TNF 75 kD receptor report reduced fatigue (268). Consequently, systemic TNF, like IL1, likely signals the brain via different mechanisms; one involves vagal afferents since vagotomy attenuates *ip*-TNF alpha-induced NREMS responses (269). The effects of systemic bacterial products such as endotoxin may also involve TNF. For instance, in humans, endotoxin doses that induce transient increases in sleep also induce concomitant increases in circulating TNF alpha (270). In addition, the soluble TNF receptor fragment attenuates muramyl dipeptide-enhanced NREMS as well in rabbits (271).

The site(s) of action of TNF-alpha-induced NREMS include the preoptic area of the anterior hypothalamus and the locus ceruleus (Figure 5). Thus, microinjection of TNF into the preoptic area enhances NREMS in rats (272). In contrast, injection of a soluble TNF receptor fragment into this site inhibited spontaneous NREMS (272). De Sarro *et al* (212), in the same study in which they injected IL1, also microinjected TNF alpha into the locus ceruleus. After a brief period of excitation, prolonged increases in sleep and EEG synchronization were observed. These effects were antagonized by anti-TNF alpha antibodies. Finally, Terao *et al* (93) microinfused TNF alpha into the subarachnoid space just beneath the basal forebrain in rats and observed enhanced NREMS and reduced REMS.

The downstream events involved in TNF alpha-enhanced NREMS likely involve many of the same substances responsible for IL1 beta-enhanced NREMS. For example, Terao *et al* (93) showed that TNF alpha-induced NREMS was blocked by an inhibition of COX-2 thereby implicating PGs in TNF somnogenic action. We recently confirmed this finding in another species (Kubota *et al* unpublished). TNF alpha also enhances NO production.

6.3. Other Cytokines / Growth factors

Several additional cytokines have been tested for their effects on sleep (Table 2). However, none have an extensive body of evidence firmly demonstrating their involvement in sleep regulation.

Nerve growth factor (NGF) induces both NREMS (273) and REMS (273;274) if injected either into the pontine reticular formation or *icv*. Further, if NGF receptive basal forebrain cholinergic neurons are selectively removed using an immunotoxin conjugated to an anti-p75 NGF receptor, there is a transient loss of NREMS and a more permanent loss of REMS (275). Giant reticular cells and neurons in the mesencephalic trigeminal nucleus are immunoreactive for the p75 and trk A receptor suggesting that these neurons may modify NGF-induced REMS (276). After sleep loss NGF1-A (an immediate early gene also called Erg-1 and Zif 268) upregulates in the locus ceruleus (277). NGF also upregulates in the somatosensory cortex after sleep loss (278). Notably, after *icv* injection, NGF induces a reduction in EEG slow wave activity during NREMS. In contrast, most other somnogenic cytokines, e.g., IL1 and TNF, induce enhanced EEG slow wave activity.

Brain-derived neurotrophic factor (BDNF) may also play a role in sleep regulation. BDNF promotes both NREMS and REMS in rabbits although in rats, only NREMS was increased after *icv* injection (279). Like NGF, BDNF reduces EEG slow wave activity during NREMS in rabbits. BDNF mRNA upregulates during sleep deprivaton (280;281) and down regulates during sleep (282). A related neurotrophic factor, glial cell-line-derived neurotrophic factor (GDNF), enhances NREMS in rats and rabbits, but, of the doses tested, does not affect EEG slow wave activity during sleep. GDNF did not affect REMS after low somnogenic doses, but inhibited REMS after high doses (283).

Table 2. NREMS response to cytokines and growth factors

Cytokine/Growth Factor[1]	Effect on NREMS[2]	Reference
Interleukin-1alpha	↑	362
Interleukin-1 receptor antagonist	↓	363
Interleukin-2	↑	364; 365
Interleukin-4	↓	366
Interleukin-6	↑→↓	367; 368
Interleukin-10	↓	200; 369
Interleukin 13	↓	370
Interleukin-15	↑	365
Interleukin-18	↑	371
Epidermal growth factor	↑	372;
Acidic fibroblast growth factor	↑	373; 374; 375
Nerve growth factor	↑	273
Brain derived neurotrophic factor	↑	279
Glia-derived neurotrophic factor	↑	283
Interferon alpha	↑→↓	364; 376; 377; 378; 379; 380
Interferon gamma	↑	381
Tumor necrosis factor beta	↑	382
Transforming growth factor beta	↓	370
Granulocyte-macrophage colony-stimulating factor	↑	383
Granulocyte colony-stimulating factor	↓	384
Insulin-like growth factor	Small dose: ↑; high dose: ↓	166;167
Soluble TNF receptor	↓	249
Soluble IL1 receptor	↓	201

[1]IL1 beta and TNF alpha are omitted from this list, they are reviewed extensively in the text. [2]↑indicates increase; ↓decrease; and → no change in duration of NREMS.

Several other cytokines also promote sleep (Table 2); the list includes IL1 alpha, IL6, IL15, IL18, epidermal growth factor, acidic fibroblast growth factor, colony stimulating factors, interferon alpha, interferon gamma, and TNF beta. In contrast, several cytokines referred to as anti-inflammatory cytokines, inhibit NREMS including the IL1 RA, IL4, IL10, IL13, insulin-like growth factor, transforming growth factor beta and as mentioned above, the soluble IL1 and TNF receptors. Although collectively these data strongly suggest that the brain cytokine network participates in sleep regulation, it is premature to conclude that any of these substances other than IL1 beta and TNF alpha is a sleep regulatory substance.

7. OTHER PUTATIVE SLEEP REGULATORY FACTORS

7.1. Oleamide

Oleamide is an endogenous unsaturated fatty acid amide containing 18 carbon atoms (cis-9,10-octadecenamide) (284;285). It is structurally related to sphingosine and sphinganine, and to the endogenous cannabinoid, anandamide. Microsome preparations from brain contain the enzymes synthesizing oleamide (286). The enzyme that inactivates oleamide, fatty acid amide hydrolase (FAAH), also metabolizes anandamide (287). This membrane-associated enzyme is expressed by the soma of various neurons and by the epithelial cells of the choroid plexus (288). Anandamide is converted by FAAH into arachidonic acid, the precursor of PGD2 (287).

Oleamide was isolated from CSF of sleep deprived cats (284;285). Oleamide accumulation was reported after 6 hours of sleep deprivation in rat CSF (289). However, the source of oleamide in the CSF is currently not known. *ip* injection of oleamide decreased motor activity and caused sedation in rats in one experiment (285), increased sleep in another study (290), and it failed to alter sleep in a third experiment (291). *ip* administration of oleamide increased NREMS time (19 minutes of extra sleep in 2 hours) in mice (292). When injected into the cerebral ventricles, oleamide shortened sleep latency without alterations in total sleep time in rats (289). Oleamide causes hypothermia that seems to be independent of the effects on sleep (290;293). The behavioral responses to oleamide are very similar as those elicited by anandamide in various tests (293). Increases in NREMS time were also reported after *icv* injection of anandamide (294).

The mechanism of oleamide's actions is not clear. Oleamide is capable of interacting with multiple neurotransmitter receptors and membrane processes. For example, oleamide inactivates gap junction channels between glial cells (295), and suppresses function of voltage gated sodium channels in neurons (296). Oleamide modulates the activity of 5HT2a, 5HT2c, and 5HT7 receptors. In addition, oleamide enhances current through the GABA-A receptor (reviewed in 292) an action implicated as a possible mechanism affecting sleep (297). The obvious structural homology between oleamide and anandamide and that exogenous (delta9-tetrahydrocannabinol in marijuana) and endogenous cannabinoids can also elicit sleepiness suggests the possibility that oleamide acts somehow through the central cannabinoid receptor, CB1. Indeed, an antagonist to CB1

receptor blocks the sleep response to oleamide (298). The endogenous cannabinoid system has been implicated in oleamide effects on sleep by two different ways. First, oleamide may act on CB1 receptors. However, binding study results are inconsistent (e.g. (299), (300). Second, high oleamide concentrations may inhibit inactivation of anandamide by occupying the common hydrolysing enzyme, FAAH, and in that case, the sleep modulation by oleamide is exclusively due to anandamide (299). However, other findings suggest that anadamide and oleamide act through distinct receptors (301). It is interesting to note that FAAH inhibition might be involved in the sleep promoting activity of a bromine compound, 1-methylheptyl-gamma-bromoacetate (288). This compound was isolated from human CSF but it enhances REMS and not NREMS (reviewed in (3)).

7.2. Cortistatin

Cortistatin is a brain-specific peptide with high homology to somatostatin (reviewed in (302). Although several cleavage sites have been identified in preprocortistatin, it is produced predominantly as cortistatin composed of 14 or 29 amino acid residues, and the cortistatin-14 is the major peptide secreted. Eleven of the 14 amino acid residues are the same as those in somatostatin 14 including the residues that render somatostatin cyclic. Cortistatin is, however, the product of a gene different from the somatostatin gene (303). Rat, mouse and human cortistatins have been cloned. Unlike somatostatin, which is ubiquitous in the central nervous system, cortistatin is expressed almost exclusively in the cerebral cortex and the hippocampus where it is colocalized with GABA in interneurons (304). There is a partial overlap in somatostatin and cortistatin expression.

Icv administration of cortistatin in rats increased duration of NREMS by 35 % during the first 4 hours postinjection (305). Apart from this first publication, published sleep data are scarce on cortistatin. It is suggested that preprocortistatin mRNA levels in the brain peak during the active period of the day and are enhanced by sleep deprivation in rats (302). Cortistatin causes hyperpolarization of neocortical and hippocampal neurons making them less responsive to cholinergic (305) and glutamatergic activation (306). In addition, cortistatin may enhance hyperpolarization-activated cation currents promoting development of oscillatory activity in neuronal groups (302).

If cortistatin-induced enhancements in NREMS are not preceded by sleep suppressions then the sleep responses to cortistatin are clearly different from those elicited by somatostatin or the somatostatin analog, octreotide (see somatostatin in this chapter). Yet, cortistatin receptors have not been identified. Cortistatin and somatostatin bind with equal affinity to somatostatin receptors, and cortistatin behaves like a somatostatin analog (302). Consequently, the effects of cortistatin and somatostatin on sleep might result from differences in the somatostatin receptors they act on. Structure-activity studies suggest that sst3 receptors, prevalent in the cortex and hippocampus, may have a prominent role in the somnogenic action of cortistatin (307). Cortistatin, however, is also capable of stimulating sst2 receptors which are implicated in the sleep suppreessive action of octreotide. For example, cortistatin can inhibit GH secretion (308) and can suppress the activity of neurons in the locus coeruleus (309); these are predominantly sst2 actions. Depression of glutamate sensitivity is also mediated by sst2 receptors (306). Nevertheless, some features of cortistatin actions are not shared by somatostatin (e.g. stimulation of hyperpolarization-activated current in hippocampal neurons, binding to GHS receptors (310)) and therefore, the existence of specific cortistatin receptors cannot be excluded (302).

7.3. Cholecystokinin (CCK)

In various species, including humans, feeding is often followed by sleepiness or sleep (311), and meal size and the duration of subsequent sleep are positively correlated (312). In rats, refeeding after food deprivation induces prompt increases in NREMS (313). Postprandial sleep is part of the satiety syndrome together with decreases in motor activity and social withdrawal (314). In cats and rats, intragastric or intraduodenal administration of nutrients, particularly fat, elicits signs of sleep suggesting that the sleep promoting stimuli originate, at least in part, from the gastrointestinal system (315;316). The gastrointestinal tract produces a number of hormones including CCK (CCK sulfate ester).

CCK is a peptide hormone released from the upper intestine, predominantly from the duodenum in response to fat and protein in the lumen. CCK exhibits strong homology to gastrin. It is produced in several forms but the CCK octapeptide is the major secreted species. CCK elicits bile ejection into the dudodenum, stimulates enzyme secretion in the exocrine pancreas, and inhibits gastric emptying. These effects are mediated by CCK-A receptors (reviewed in 317). Sulfation of the tyrosine residue in position 7 greatly enhances binding of CCK to A receptors. There are also CCK-B receptors in the gastrointestinal system and gastrin is the major ligand for these receptors at the periphery. CCK is a very common neurotransmitter in the central nervous system. CCK-B receptors are the predominant receptors in the brain and these receptors mediate the central actions of CCK.

Systemic administration of CCK elicits symptoms of satiety (314): it decreases feeding and motor activity, and rapidly elicits NREMS in rats (318-320), cats (315), and rabbits (321). In addition to promoting NREMS, CCK may induce slow waves and spikes in the waking EEG of rats (322). In young rats, an intraperitoneal injection of a high dose of albumin elicits CCK/gastrin releases and promotes NREMS though, the role of CCK remains to be verified in the albumin-induced sleep response (323). Human subjects tend to feel more sleepy after high fat/low carbohydrate meal which stimulates CCK than after low fat/high carbohydrate meal which induces high insulin secretion (311). CCK injection also elicits peripheral vasodilation and decreases in body temperature (324).

Although CCK may act in those brain areas where the blood-brain barrier is penetrable, systemic CCK

does not enter the central nervous system suggesting that peripheral CCK-A receptors mediate the behavioral effects of systemic CCK. In fact, intracerebral administration of CCK (321), or systemic injection of B receptor agonists do not promote NREMS (325), and CCK-B receptor antagonists do not suppress sleep (326). The role of peripheral CCK-A receptors has been verified in the CCK-induced reductions in food intake (327). Enhancements in sleep after refeeding in food deprived rats is inhibited by A receptor antagonists supporting the hypothesis that postprandial sleep depends on CCK acting on A receptors (313). At variance to this, a CCK-A receptor antagonist failed to inhibit postprandial fatigue and sleepiness in humans; in fact, the antagonist increased somnolence (328).

In order to elicit sleep, peripheral actions of CCK have to be conveyed into the brain. The vagus nerve is the candidate for signaling CCK actions to the brain because the sensory fibers of the vagus express CCK receptors (both A and B receptors) (329), and because the vagus' role has been demonstrated in CCK-induced reduction of food intake (330). The sensory neurons of the vagus are sites for interactions between CCK and IL1: IL1 enhances vagal responsiveness to CCK (331), and CCK-A receptors are implicated in the mediation of IL1 effects on vagal afferents (332). CCK-A receptors might not modulate sleep tonically for sleep is normal in mutant rats with a CCK-A receptor gene deficiency (333). Finally, CCK-B receptors have been implicated in anxiety, and B receptor antagonist display anxiolytic acitivity (334). B receptor antagonists were reported to improve sleep in aged rats (335).

7.4. Insulin

Insulin is best known for stimulating glucose uptake in skeletal muscle and adipocytes though the hormone also plays a fundamental role in regulating many metabolic processes by controlling enzyme expression. The endocrine pancreas produces insulin, a peptide composed of two chains. The rise in blood glucose concentration is the major stimulus for release. Insulin secretion is stimulated soon after a meal in response to glucose absorption, and to hormones with insulin-secretagogue activity produced by the gastrointestinal tract, e.g. gastric insulinotropic peptide (GIP). Thus, if insulin has sleep promoting activity then, theoretically, it may contribute to the mediation of postprandial sleep.

Robust increases in NREMS were reported in rats in response to either systemic insulin injection (336) or long term (3 days) *icv* insulin infusion (337). Conversely, *icv* or systemic infusion of antibodies to insulin decreases NREMS. Large decreases in NREMS and REMS were reported in rats with experimental diabetes mellitus, and sleep could be normalized by means of systemic insulin infusion (338). In another experiment, much smaller decreases in NREMS were found in diabetic rats and these changes occurred strictly in the second portion of the light period (339). In addition, mice expressing human insulin transgene sleep more than control mice. However, mice bearing, but not expressing the transgene, also display increased NREMS duration, and, therefore, the role of insulin could not be determined (340). Compared to human insulin, porcine insulin increased EEG spectral power in the spindle frequency range in humans with insulin-dependent diabetes mellitus (341).

Collectively, these findings suggest that sleep modulation by insulin occurs through a central action site and not via blood glucose concentration. Because insulin also stimulates IGF receptors, sleep responses to *icv* injected IGF-1 and insulin were compared. Low doses of IGF-1 enhance NREMS (see IGF-1 in this chapter), and the molar dose of IGF-1 promoting sleep is much smaller than the molar dose of insulin required for the same effect (167). It is possible, therefore, that IGF receptors mediate the insulin effects on sleep. Data do not support involvement of insulin in the mediation of postprandial sleep.

7.5. Nitric oxide (NO)

Nitric oxide, a highly lipid soluble gas, is produced from arginine by the enzyme, NO synthase (NOS). There are three main NOS types: neural (Type I, nNOS), inducible (Type II, iNOS in macrophages, astrocytes etc.), and constitutive (Type III, eNOS in endothelial cells). Neural and constitutive NOSs are stimulated by intracellular calcium increases, whereas iNOS requires stimulation of gene expression. For example, cytokines elicit expression of inducible NOS. NO is highly diffusible and has a very short life measured in seconds, thus, it acts in the vicinity of production site by stimulating cytoplasmic guanylyl cyclase. The NO released by endothelial cells relaxes vascular smooth muscle and dilates vessels. There are constant NO releases, therefore, inhibition of NO synthesis results in vasoconstriction and hypertension if the inhibition is systemic. NO plays an important role in changes in cerebral vasomotion across the various states of vigilance (342). Phagocytes use NO, a highly reactive molecule, for bacterial destruction. NO produced by iNOS in phagocytes also contributes to local blood flow regulation when phagocytes are activated, e.g. in inflammation. Neuronal NO modulates neurotransmitter release. Soluble NOS activity in brain samples has a diurnal rhythm with a peak in the active period in the rat (343). NO determination by means of voltametry indicates enhanced NO production in wakefulness and decreased NO releases in both NREMS and REMS (344).

There is perhaps no function of the body in which NO has not been implicated, and sleep regulation is no exception. In the case of sleep, however, the findings linking NO to NREMS are controversial whereas the results suggesting a role for NO in REMS regulation are fairly consistent. Both decreases (345-348) and increases (349;350) in NREMS were reported after systemic administration of NOS inhibitors acting on either predominantly on nNOS or both on nNOS and eNOS. A NOS inhibitor also suppressed sleep enhancement after sleep deprivation (351). A nNOS inhibitor decreased both sleep and locomotor activity with a general suppression of EEG power (352). *Icv* injection of a NO donor increased sleep in rats (353) whereas NOS inhibitors decreased sleep in rats and rabbits (345;346). The biochemical activity of the systemically administered non-selective NOS inhibitor was verified in brain samples (354). However,

voltammetric detection of the NO signal in the cerebral cortex suggested that these compounds either did not cross the blood brain barrier or were only partially effective in the brain tissue, acting exclusively or predominantly on endothelial NOS (350). In this case, the sleep effects are due to changes in blood pressure or in cerebral circulation.

In contrast, voltametry measurements supported the effectiveness of systemically-injected selective neural NOS inhibitors but the sleep findings still remained inconsistent. Microinjection of NOS inhibitors into the brain stem, (dorsal raphe and pedunculopontine tegmentum), consistently reduced REMS in various laboratories, and in some experiments, inhibition of NREMS was also noted (345;347;350;355-357). In agreement with this, local NO donor application into the pedunculopontine tegmentum increased REMS and NREMS (356). Spontaneous sleep-wake activity was recently recorded in mutant mice lacking either nNOS or iNOS (358). NREMS was not altered in the nNOS knockout mice whereas the iNOS knockout mice had slightly less NREMS than their controls during the dark period. Major changes occurred in REMS. REMS decreased in the nNOS knockout and increased significantly in the iNOS knockout mice. The results verify the significance of NO generated by nNOS in REMS regulation. It seems that iNOS may also modulate sleep through decreasing REMS and slightly increasing NREMS. It is not clear where in the brain the NO produced by iNOS acts but this mechanism may contribute to the infection associated changes in sleep.

NO effects on sleep may vary with brain structures since NO is produced in various brain regions, but its specific actions are always local. NO seems to stimulate acetylcholine release in the pontine reticular formation (355), it may modulate serotonergic activity in the raphe (350), and it may inhibit noradrenaline release in the pedunculopontine area (359). These effects may explain the sleep responses, particularly the REMS promoting activity of NO in the brain stem. NO did not seem to alter acetylcholine release in the basal forebrain, and local injection of a NOS inhibitor into the basal forebrain failed to alter sleep (360). Other observations suggest that NO may stimulate accumulation of adenosine (361). Instead of systemic approaches, studies of local NO actions may promote the understanding of the role of this molecule in sleep regulation.

8. PERSPECTIVES

We make the following general conclusions based on our review of the putative sleep regulatory substances. 1. Although the importance of an individual substance to spontaneous sleep may vary, definitely more than one sleep factor exists and any one factor might have a particular significance in special conditions. 2. The basal forebrain (including the anterior hypothalamus / preoptic area) emerges as a major action site for many NREMS-promoting substances. The basal forebrain may provide the neural network that can synchronize sleeping or waking activity in various brain regions (see sleep function in this volume). This network, which is not discussed herein, may include GHRH, GABA and galanine as sleep-promoting neurotransmitters and acetylcholine, histamine, hypocretin/orexin (and GABA in some neurons) as wake-promoting neurotransmitters (see Szymusiak and McGinty in this volume). The sleep-wake network also possesses an autoregulatory mechanism through adenosine. 3. The idea of use-dependent sleep regulation implies that the need for sleep develops in areas which are in significant use during wakefulness (see sleep function in this volume). This can be anywhere in the brain but the cerebral cortex might be particularly important. In addition to neural projections, the CSF may be an effective medium conveying the sleep need, through secreted sleep factors, to the basal forebrain. PGD2 can reach the basal forebrain through this route. 4. Use dependent activation of factors in the cerebral cortex and elsewhere may promote localized sleep mechanisms. Growth factors, adenosine, interneurons containing cortistatin and GABA might be involved in this mechanism. 5. Several observations suggest that there are interactions among the identified sleep factors: they often stimulate each other. The complexity through these interactions may provide the exceptional homeostatic stability of sleep regulation.

9. ACKNOWLEDGMENTS

This work was supported in part by the National Institutes of Health (USA) grant number NS25378, NS27250, NS31453 and HD36520 to J.K. and by the Hungarian National Science Foundation (OTKA 30456) and Ministry of Health (ETT P-04/033/2000) to F.O. We thank Danielle Nelson and Deborah Duricka for their help with this manuscript.

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Key Words: EEG, Sleep, Sleep Regulation, Cytokine, TNF, Hormones, Review

Send correspondence to: James M. Krueger, Ph.D., Department of VCAPP, PO Box 646520, Pullman, WA 99164-6520, Tel:509-335-8212, Fax: 509-335-4650, E-mail: Krueger@vetmed.wsu.edu