Humoral Regulation of Sleep

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Cytokines and hormones, including interleukin-1, tumor necrosis factor, growth hormone-releasing hormone, vasoactive intestinal polypeptide and prolactin, are involved in sleep regulation. These substances enhance sleep, inhibition of them inhibits sleep, and their brain levels vary with sleep. This knowledge helps our understanding of the humoral regulation of sleep.

To understand how the brain produces thought, memory, emotion or regulates bodily functions, it is very likely that we will first have to understand what sleep does to the brain. Sleep function remains a major physiological riddle. A necessary step in deciphering sleep function is to determine the mechanism by which the brain produces sleep. Two fundamental processes have been implicated in sleep regulation (see Ref. 2 for review). One of them is determined by the circadian pacemaker(s) and is proposed to act as a "sleep threshold." This process describes the common experience that the easiness of falling asleep varies with the circadian rhythm. The other process, called "homeostatic sleep regulation," encompasses the fact that the daily duration of sleep is fairly constant in a given individual. The concept of homeostatic sleep regulation implies that sleep is wake dependent; unusually long wake periods are followed by enhanced sleep. These circadian and homeostatic processes result from the interaction of humoral and neural mechanisms. Neural mechanisms have been extensively reviewed elsewhere (e.g., Ref. 15); this review focuses on humoral mechanisms.

There are no direct measures of sleep; it is inferred from variations in physiological parameters such as the electroencephalogram (EEG), the electromyogram, changes in brain temperature, and eye movements. Sleep is divided into two main states, non-rapid eye movement sleep (NREMS) and rapid eye movement sleep (REMS). The EEG during NREMS exhibits sleep spindles and large slow waves, whereas the EEG during REMS is similar to that in wakefulness. There is a continuous structured cycling between wakefulness, NREMS, and REMS. In many laboratory animals, individual bouts of each of these vigilance states typically last less than 5 min. Collectively, over a day, rats and rabbits will spend about 40–50% of the time in NREMS and 5–15% of the time in REMS. There is also a strong influence of circadian rhythms on sleep/wake cycle organization. For example, rats sleep more during the day than during the night, whereas rabbits are active at dawn and dusk.

The hypothesis that sleep is regulated, in part, by humoral mechanisms is ancient, being first proposed by Aristotle. Within the context of the humoralism movement, Aristotle suggested that vapors emanating from the stomach during food digestion were transported throughout the body thereby causing sleepiness. Although the details of this hypothesis have changed, such as the chemical specification of the "vapors" and where they originate and act, the fundamental idea of the existence of a sleep regulatory substance is implicit in Aristotle's hypothesis. The modern pursuit of sleep regulatory substances began at the turn of the century with Legendre and Pieron (1913) and Ishimori (1909), who took cerebrospinal fluid from sleep-deprived dogs and transferred it to normal dogs. The recipient animals were then observed to sleep longer than...
usual. Over the next 80 years, many investigators successfully repeated this type of experiment, showing the accumulation of sleep-promoting substances in cerebrospinal fluid and/or the brain although failures were also encountered (reviewed in Refs. 10, 11).

It is now known that many substances can affect sleep, although convincing evidence for the involvement in physiological sleep regulation exists only for a small number of these substances. This list includes the cytokines, interleukin-1 (IL-1) (9,10) and tumor necrosis factor (TNF) (4), and growth hormone (GH)-releasing hormone (GHRH) (11,12), prostaglandin D2 (5), and adenosine (6) for NREMS and prolactin (PRL) (14), vasoactive intestinal peptide (VIP) (3,7), and corticotropin-like intermediate lobe peptide (8) for REMS. This article briefly reviews the evidence linking IL-1, TNF, GHRH, PRL, and VIP to sleep regulation.

Cytokines in sleep regulation

Cytokines are best known for their roles in inflammation and triggering the acute phase response; one facet of the acute phase response is somnolence. The hypothesis that cytokines, such as IL-1 and TNF, are involved in physiological processes is less well studied. The central idea of this hypothesis is that, under normal conditions, low basal levels of cytokines vary in subtle ways with one or more physiological processes. Removal of any one somnogenic cytokine inhibits normal sleep but does not completely disrupt sleep due to the redundant nature of the cytokine network. After pathological disturbances, the production of one or more cytokines would be greatly amplified via microbial (or other pathological) stimuli. Such amplified cytokine production would then induce pathology in a manner analogous to the pathologies produced by excessive production of hormones.

Interleukin-1. IL-1 is a 17-kDa polypeptide with autocrine, paracrine, and endocrine roles. IL-1 seems to play physiological roles in the regulation of sleep, appetite, brain development, gastrointestinal function, and several endocrine systems, such as the GH-GHRH-insulin-like growth factor axis and the corticotropin-releasing hormone-adrenocorticotropic hormone-glucocorticoid axis. There are now numerous reports indicating that IL-1, IL-1 receptors, and other members of the IL-1 family of molecules are constitutively expressed in normal brain. IL-1 cerebrospinal levels vary in phase with the sleep-wake cycle, with highest levels occurring at sleep onset. Furthermore, there is a diurnal rhythm of IL-1 mRNA in the hypothalamus, hippocampus, and cortex of rats, with the highest levels corresponding to peak sleep periods. In humans, plasma levels of IL-1 peak at the onset of sleep. Finally, after sleep deprivation, brain stem and hypothalamic levels of IL-1 mRNA increase as well as circulating levels of IL-1.

Administration of exogenous IL-1 via intraperitoneal, intravenous, or intracerebroventricular routes induces relatively large increases in NREMS in rats, rabbits, mice, cats, and monkeys (reviewed in Ref. 9). For example, mice given 0.4 g IL-1 intraperitoneally doubled their time spent in NREMS during the first 6 h after injection from 118 min to 246 min (4). Similar results were obtained in a different strain of mice (Fig. 1). These large increases in NREMS, although complex (e.g., vary with species, dose, route of administration, and time of day), are usually accompanied by increases in EEG slow-wave amplitudes; supranormal EEG waves are thought to reflect the intensity of NREMS. Similarly, substances that induce IL-1 production (e.g.,

FIGURE 1. Effects of tumor necrosis factor (TNF; left) and interleukin-1 (IL-1; right) on mouse non-rapid eye movement sleep (NREMS). NREMS is expressed as % of time spent in that state; individual values are means ± SE for 2-h periods. Both TNF (3 µg) and IL-1 (0.4 µg) were injected intraperitoneally into B6 x 129 mice at the onset of dark hours (time = 0); 6 mice were used for each experiment. After injection, the electroencephalogram (EEG) and electromyogram were recorded for next 23 h. Both IL-1 and TNF induced large increase in NREMS, which lasted ~9 h.
lipopolysaccharide) enhance NREMS and EEG slow-wave amplitudes. Conversely, inhibition of endogenous IL-1, using the IL-1 receptor antagonist, anti-IL-1 antibodies, or the soluble IL-1 receptor, reduces spontaneous sleep, inhibits sleep rebound after deprivation, and attenuates the NREMS responses induced by microbial products or mild increases in ambient temperature.

Tumor necrosis factor. TNF is a proinflammatory cytokine of about 17 kDa and, like IL-1, most of the research on TNF has focused on its role in host defense systems. Although TNF is less well studied than IL-1, a growing body of evidence suggests that TNF is also involved in the regulation of several physiological processes (e.g., sleep regulation, brain development, and hormonal and appetite regulation). TNF, TNF receptors, and other members of the TNF-family of molecules are constitutively expressed in the brain. TNF mRNA is induced in brain by microbial products, and there is a diurnal rhythm of TNF mRNA in the hypothalamus and hippocampus. Consistent with the diurnal rhythm of TNF mRNA, there is a diurnal rhythm of TNF protein in the brain. Finally, there is a diurnal rhythm of TNF in plasma, and the ability of circulating monocytes to produce TNF is coupled to the sleep-wake cycle and increases during sleep deprivation.

Many data support the notion that TNF is involved in physiological NREMS regulation; these data parallel those demonstrating the involvement of IL-1 in sleep regulation. Administration of exogenous TNF to rabbits, rats, or mice induces increases in the duration of NREMS (Fig. 1) (reviewed in Ref. 10). For example, if mice are given 3 μg TNF intraperitoneally, they get 81 min extra of NREMS during the first 9 h postinjection (4). Furthermore, TNF induces increases in EEG slow-wave amplitudes that are similar to those observed after sleep deprivation, during infection, or after IL-1 treatment. Inhibition of TNF inhibits spontaneous sleep, sleep rebound after sleep deprivation, as well as microbial product-induced increases in NREMS and mild increases in ambient temperature-induced increases in NREMS. Finally, mice that lack the TNF 55-kDa receptor sleep less than control strains or other strains of mice recorded under the same conditions; these knockout mice sleep about 90 min less during daylight hours than strain controls (4).

Interactions between IL-1 and TNF. The relationship between TNF and IL-1 in sleep regulation is only beginning to be understood. Many stimuli, e.g., muramyl dipeptide, induce both IL-1 and TNF production. Furthermore, IL-1 and TNF also induce each other's production. It is likely that IL-1 and TNF belong to a functional gene group involved in sleep regulation and act in concert with each other. For example, inhibition of TNF attenuates IL-1-induced NREMS. Similarly, inhibition of IL-1 attenuates TNF-induced NREMS. In contrast to these findings, recent data in knockout mice show that IL-1 and TNF can also affect sleep independently. Thus, in IL-1 type 1 receptor knockout mice, TNF, but not IL-1, induces robust increases in NREMS. Conversely, in TNF 55-kDa receptor knockout mice, IL-1, but not TNF, induces large increases in NREMS (4).

Systemic cytokines and the brain

Whether blood levels of cytokines are relevant to central nervous system (CNS) function remains unknown, although this seems to be the case. Systemic injections of IL-1 or TNF, or substances that induce cytokine production (e.g., lipopolysaccharide), result in altered CNS function (e.g., fever and sleep). Furthermore, cytokine levels in blood change during infection when there are changes in CNS function. It is still unclear, however, how such molecules, whether systemically released or experimentally injected, gain access to the brain, because most are relatively large and are hydrophilic peptides, which aren't expected to readily cross the blood-brain barrier. Although various transport hypotheses have addressed this issue, recent evidence has suggested that cytokines may signal the CNS via vagal afferents (reviewed in Ref. 1). Indeed, IL-1- and LPS-induced sleep, fever, and various other facets of the acute phase response are blocked or attenuated by subdiaphragmatic vagotomy.

Additional evidence also suggests a possible involvement of systemic cytokines in sleep regulation. For example, as previously mentioned, plasma levels of IL-1 peak at the onset of sleep and increase after sleep deprivation. Similarly, there is a diurnal rhythm of TNF in plasma and TNF production by increases in the number of monocytes after sleep deprivation. Furthermore, some evidence suggests that gastrointestinal bacteria are a source of sleep-inducing agents. In rats placed on an antibiotic regimen (neomycin and metronidazole in drinking water), there is a significant reduction in NREMS as well as an increase in sleep latency. Furthermore, stressors, such as sleep deprivation, starvation, or protein malnutrition, can result in gastrointestinal barrier dysfunction and increased bacterial translocation across the gut, potentially leading to lethal systemic infections. One interpretation of these data is that bacterial products could influence everyday sleep regulation (reviewed in Ref. 10); the relatively rapid appearance of viable bacteria in lymph nodes of sleep-deprived rats is consistent with such a hypothesis.
Hormones in sleep regulation

The GHRH-somatostatin-GH axis and sleep. Two hypothalamic hormones, GHRH, which stimulates GH release, and somatostatin (SRIF), which inhibits GH, play a major role in the regulation of pituitary GH secretion. GHRH is also involved in the regulation of sleep (reviewed in Ref. 11). Intracerebroventricular administration of GHRH promotes NREMS in rats, rabbits, and humans. Rabbits given 3 nmol GHRH centrally have about 1 h extra of NREMS during the first 6 h after injection. Increases in NREMS and EEG slow-wave activity occur relatively rapidly in postinjection hour 1. GHRH also increases REMS; however, increases in REMS are delayed 2 h or more. The increases in NREMS likely result from the direct action of GHRH, whereas the REMS response may occur in response to enhanced GH and SRIF release stimulated by GHRH (see below and Fig. 2). Inhibition of GHRH using either a GHRH antagonist or anti-GHRH antibodies selectively reduces spontaneous NREMS and GH secretion. Anti-GHRH antibodies also suppress the expected sleep rebound after sleep deprivation in rats. Furthermore, blockade of endogenous GHRH significantly attenuates increases in NREMS and GH secretion elicited by IL-1. Finally, there is a significant reduction of NREMS in a strain of transgenic mice with a deficiency in the somatotropic system. All these findings indicate that endogenous GHRH is involved in physiological sleep regulation and in the mechanism of enhanced sleep after sleep deprivation.

In the hypothalamus, two GHRH-containing neuronal pools can be clearly distinguished: the GHRH-containing neurons in the arcuate nucleus and the extra-arcuate GHRH-containing neurons found around the ventromedial nucleus and in the perifornical area. The GHRH-containing neurons in the arcuate nucleus project to the median eminence. The extra-arcuate GHRH-containing neurons may also contribute to the regulation of GH secretion. In addition, these neurons innervate various parts of the basal forebrain, particularly the preoptic area, a region known to be involved in sleep regulation.

It is likely that promotion of NREMS is mediated by GHRH released in the basal forebrain, since microinjection of GHRH into this area induces increases in NREMS. Within the hypothalamus, GHRH mRNA has a diurnal rhythm with highest levels corresponding to the onset of the diurnal sleep period (Fig. 3). Furthermore, GHRH mRNA is reported to increase during short-term sleep deprivation.

GH was the first hormone for which a sleep-related secretory pattern was discovered. In humans, the major GH release is associated with deep NREMS onset, generally occurring soon after sleep onset (reviewed in Ref. 11). Correlation between NREMS and GH secretion has also been documented in other species. Many observations indicate that sleep disturbances are accompanied by reduced GH secretion or a greatly altered GH secretory pattern. Experiments involving sleep deprivation provided further support for the coupling of NREMS and GH secretion; large surges of GH release occur during NREMS after sleep deprivation. However, dissociations between NREMS and GH secretion may be observed under certain conditions. GHRH is also a likely candidate for being the hypothalamic factor linking sleep and GH secretion.

Pituitary GH secretion increases in response to GHRH, GH elicits SRIF release, and some evidence suggests that GHRH itself might also stimulate hypothalamic SRIF secretion (Fig. 2). GH, SRIF, and a long-lasting SRIF agonist, octreotide, promote REMS, whereas immunoneutralization of endogenous SRIF decreases REMS and abolishes the increases in REMS elicited by cholinomimetics in rats. Selective increases in REMS were reported in response to systemic injection of GH in humans, cats, and rats. High doses of GH tend to reduce NREMS, which can be

FIGURE 2. Role of growth hormone-release hormone (GHRH) in sleep regulation. Stimulation of growth hormone (GH) secretion and promotion of non-rapid eye movement sleep (NREMS) are shown as 2 independent outputs of hypothalamic GHRH. Normally, these events are synchronized with a major GH surge occurring in association with sleep onset or deep NREMS soon after sleep onset. Selective inhibition of either output is also possible: hypophysectomy blocks the GHRH-induced GH release but not sleep, and disruption of sleep by environmental stimuli may not interfere with GH secretion. Small doses of interleukin-1 (IL-1) elicit GH secretion and promote sleep in part via GHRH. Higher IL-1 concentrations, however, inhibit both sleep and GH secretion. This is attributed to an IL-1-induced release of corticotropin-releasing hormone (CRH), which, at least in the rat, stimulates somatostatin (SRIF). SRIF inhibits GHRH and GH (the latter is not shown).
explained by assuming a negative feedback inhibition of endogenous GHRH. SRIF and GH, therefore, may mediate the increases in REMS following the administration of GHRH and GH is likely to act by stimulating SRIF release. This notion is reinforced by the finding that systemic GHRH promotes NREMS but not REMS in hypophysectomized rats (12).

VIP and prolactin

Central injection of VIP elicits selective increases in REMS in cats and rabbits without significant changes in NREMS. In rats VIP enhances both NREMS and REMS. The possible physiological role of endogenous VIP in sleep regulation is indicated by the finding that the activity of a REMS-promoting substance accumulating in cerebrospinal fluid of REMS-deprived cats is immunoneutralized by treating the cerebrospinal fluid sample with anti-VIP antibodies. Furthermore, central administration of anti-VIP serum or a competitive VIP antagonist induces selective suppression of REMS in rats. This latter observation also indicates that the increase in NREMS found after central administration of VIP in rats is probably a nonspecific effect that may be attributed to a cross reaction of exogenous VIP with GHRH receptors.

The origin of the VIP found in cerebrospinal fluid after sleep deprivation and the exact mechanism by which VIP elicits REMS remain unknown. VIP may elicit REMS via direct actions on neurons; microinjection of VIP into the pontine tegmentum, an area involved in REMS regulation, enhances REMS (3). VIP may also influence REMS via PRL. Hypothalamic VIP-containing neurons project to the median eminence. VIP released into the portal blood acts as a hypothalamic releasing factors for pituitary PRL secretion. PRL is transported from the blood to the cerebrospinal fluid by a specific transport mechanism residing in the choroid plexus.

Systemic administration of PRL enhances REMS in rats, rabbits and cats. Immunoneutralization of systemic PRL blocks systemically administered VIP-enhanced REMS. Hyperprolactemic rats bearing transplanted pituitary grafts have pronounced increases in REMS (Fig. 4) (13). Nevertheless, immunoneutralization of circulating PRL in normal rats only slightly inhibits spontaneous REMS, thereby suggesting that central PRL may be more important in REMS regulation than circulating PRL. PRL is found in the brain, and PRL-containing neu-
rons in the hypothalamus project to various areas in the brain. Indeed, central administration of PRL also enhances REMS in rats and VIP induces increases in hypothalamic PRL mRNA levels.

In conclusion, within the past decade, great strides have been made in our understanding of the humoral regulation of sleep. This knowledge may promote insights into sleep function.

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References