MINIREVIEW

Cytokine Involvement in the Regulation of Sleep (43474)

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Cytokines. The term cytokine refers to a group of regulatory proteins that are produced by a large number of cell types in response to a variety of stimuli, and include substances such as the interleukins (IL), tumor necrosis factor (TNF), and interferons (IFN). Cytokines are key mediators of many of the physiological responses to infection or trauma, and these responses are collectively referred to as the acute phase response (APR). The role of cytokines in the complex physiological changes of the APR has been extensively reviewed elsewhere (1, 2). The behavioral changes associated with the APR include altered vigilance (3, 4). This Minireview examines cytokine-induced changes in vigilance and the hypothesized mechanisms whereby these cytokines are likely to contribute to altered sleep during microbial disease.

Sleep. Virtually every physiological system is affected in some way during sleep. Sleep is a complex phenomenon resulting from cellular interactions that are modulated by biochemically and anatomically diffuse regulatory mechanisms. The occurrence of sleep is inferred primarily from the correlation of electrophysiological and physiological parameters with behavioral characteristics. Parameters frequently used to identify sleep include the electroencephalogram (EEG), electrooculogram, electromyogram, brain temperature (Tbr), gross body movements, respiration rate, and heart rate. The most critical parameter is the EEG. In addition to providing basic information about vigilance states, the EEG can also be analyzed using band-pass filters and mathematical transformations to derive additional information. For example, the amplitudes of the EEG waves in the slow frequency bands are thought to represent an index of the depth or intensity of sleep (5).

The identification of vigilance states based on polygraphic recordings is possible because of the correlation between behaviorally identified sleep (i.e., sleep determination based on body posture and eye state) and changes in the electrophysiological parameters discussed above. These parameters change in a predictable manner with transitions from one state of vigilance to another. States of vigilance are generally divided into three stages: wakefulness, characterized by locomotor activity, gradually increasing Tbr, and low-voltage high-frequency activity in the EEG; non-rapid-eye-movement sleep (NREMS; also called slow-wave sleep or quiet sleep), during which the EEG exhibits a high-voltage slow-frequency pattern, body movements are restricted to occasional twitches, and Tbr initially declines and subsequently stabilizes; and rapid eye movement sleep (REMS; also called active sleep, paradoxical sleep, or dream sleep), which is defined by a high-frequency low-voltage EEG, a rapid increase in Tbr at REMS onset, and phasic body twitches. Changes in these parameters with vigilance state transitions are illustrated graphically in Figure 1.

The architecture of sleep is species specific. The species most commonly used for laboratory sleep research, the rat, rabbit, and cat, demonstrate different patterns of sleep-wake activity. The rabbit is primarily crepuscular, with periods of activity centered around the transitions between the dark period and the light period. On a 12:12-hr light:dark cycle, rabbits spend about 40–50% of the light period asleep compared with about 25–35% of the dark period. In contrast, the rat is a highly circadian animal, spending approximately 70% of the light period and about 30% of the dark

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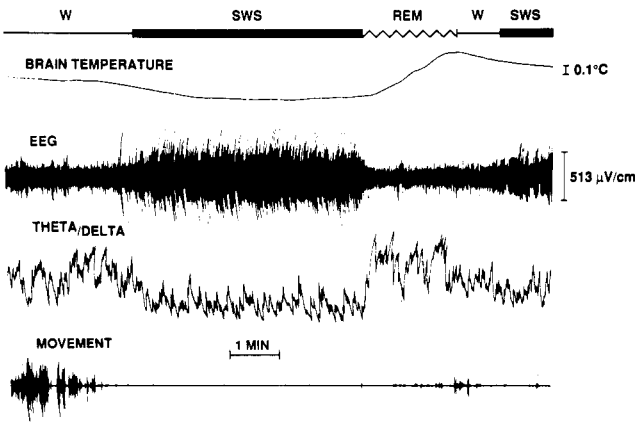

Figure 1. Representative polygraphic recording from a rabbit. Designation of vigilance state as either wakefulness (W), slow wave sleep (SWS; the human equivalent of non-rapid-eye-movement sleep), or rapid eye movement sleep is based on the direction and rate of change of brain temperature, the amplitude of the EEG waves, the ratio of θ- to δ- frequency components of the EEG, and the presence or absence of body movement. Figure reprinted with permission from American Physiological Society (copyright 1988), American Journal of Physiology (Ref. 80).

period asleep. Cats show tremendous variability in sleep architecture depending upon the laboratory environment. However, cats generally sleep more during the light period, with major sleep episodes occurring in the late morning and in the late afternoon or early evening.

Cytokines and Sleep

Although the idea of humoral regulation of sleep is an ancient one, modern experiments addressing this issue did not begin until the turn of the 20th century. Legendre and Piéron (6) and Ishimori (7) independently postulated that a "hypnotoxin" accumulated in the cerebrospinal fluid (CSF) of sleep-deprived dogs based on the observation that CSF from sleep-deprived animals induced a long-lasting, deep sleep when it was transferred into normal, recipient animals. The specific substance(s) was never identified, but these experiments have had an indelible impact on our current thinking of the humoral regulation of sleep and of altered sleep in response to a variety of stressors.

In the late 1960s, investigations into the existence/identificaton of a hypnotoxin were renewed. Pappenheimer and colleagues (5) isolated a sleep-promoting substance from the CSF of sleep-deprived animals, and later a similar (if not identical) substance derived from brain and urine was identified as a muramyl peptide (MP; 8). MP had already been described as immunoadjuvants that induced the synthesis and release of IL-1 (known at that time by several names, including endogenous pyrogen and lymphocyte-activating factor). The hypothesized mediation of MP actions via IL-1 led to the first report of the somnogenic actions of IL-1 (9, 10). The remainder of this Minireview will focus on the effects of three groups of cytokines, IL, TNF, and IFN, on states of vigilance, and the interactions that we hypothesize are involved in the altered sleep that occurs in response to infectious challenge. Published reports of the effects of these cytokines on sleep parameters and on temperature regulation are summarized in Table I.

Interleukins

IL-1. IL-1 is the cytokine that has been most extensively characterized with regard to its effects on sleep and its possible involvement in the regulation of sleep. The two forms of IL-1, IL-1α and IL-1β, are derived from two distinct gene products by posttranslational processing of large precursor molecules (1) and share many biological properties, although they have limited structural homology (approximately 26%; 1). Most studies to date on somnogenic properties of IL-1 have used IL-1β, which is the predominant form found in tissue fluids and plasma (2). To date, the effects of IL-1 on sleep have been reported in rats, rabbits, and cats.

The effects of IL on sleep were initially described in rabbits injected intracerebroventricularly or intravenously with IL-1 purified either from human mononuclear cells or from stimulated rabbit peritoneal exudate cells (10). The central administration of IL-1 induced dose-related increases in the amount of time spent in NREMS for 6 hr or more after injection. These increases in NREMS temporally coincided with the development of fever. Systemic administration of IL-1 also induced a febrile response and enhanced sleep, but the somnogenic effect was of shorter duration (1–2 hr) than that occurring after intracerebroventricular injection.

The time courses for febrile and somnogenic responses to IL-1 generally parallel each other. However, the febrile responses to IL-1, but not the somnogenic responses, can be prevented by pretreatment with either antipyretic protein synthesis inhibitors (e.g., anisomycin) or by cyclo-oxygenase inhibitors (e.g., indomethacin, aspirin) (10, 11). These observations imply that altered sleep in response to IL-1 is not likely to be a direct consequence of fever. The literature indicating that somnogenic and pyrogenic responses to IL-1 and other cytokines can be dissociated has been reviewed elsewhere (12).

The early reports of somnogenic and pyrogenic actions of IL-1 in rabbits have been replicated numerous times (13–18). General characteristics of responses of rabbits to IL-1 include dose-related increases in the amount of NREMS, with a concomitant increase in the amplitudes of EEG slow waves, suppression of REMS, and fever. Long-term recordings (18) indicate that the somnogenic responses of rabbits to intracerebroventricularly administered IL-1 are biphasic, i.e., there is an initial increase in the amount of NREMS that lasts 10–12 hr, followed by a significant reduction in the amount of NREMS 24 hr after injection (Fig. 2).

Table I. Effects of IL, TNF, and IFN on EEG Slow Waves in the δ-Frequency Band, Duration of NREMS, Duration of REMS, and on Brain or Body Temperature

Cytokine	Species	Light:dark cycle[a]	Delivered[b]	EEG[c]	NREMS	REMS	Fever	Ref.
IL-1β	Rabbit	L	icv, iv	↑	↑	NR	+	10
IL-1β	Rabbit	L	icv	↑	↑	↓	+	13
IL-1β	Rabbit	L	icv, iv	↑	↑	↓	+	14
IL-1β	Rabbit	L	icv	↑	↑	↓	+	15
IL-1β	Rabbit	L	icv	↑	↑	↓	+	16
IL-1β	Rabbit	L	μi[d]	NR	↑	→	+	101
IL-1β	Rabbit	L	μi[e]	NR	→	→	+→	101
IL-1β	Rabbit	L	icv	↑	↑	↓	+	17
IL-1β	Rabbit	L	icv	↑	↑	↓	+	18
IL-1β[f]	Rat	L	icv	↑	→	↓	+	19
IL-1β	Rat	L	icv	↑	↑↓	↓	+	102
IL-1β	Rat	D	icv	NR	↑	↓	→	103
IL-1β	Rat	L	icv	↑	↑↓	→↓	+	20
IL-1β	Rat	D	icv	↑↓	↑	→↓	+	20
IL-1β	Cat	L	icv	NR	↑↓	↑↓	+	21
IL-1α	Rabbit	L	icv	↑	↑	↓	+	17
hrIL-2	Rat	L	icv, μi[g]	↑[h]	↑	NR	NR	33
hrIL-2	Rat	L	μi[f]	→[h]	→	NR	NR	33
Rat IL-2	Rat	L	icv, μi[g]	↑[h]	↑	NR	NR	33
Rat IL-2	Rat	L	μi[f]	→[h]	→	NR	NR	33
IL-6	Rabbit	L	icv	→	→	→	+	35, 36
TNF-α	Rabbit	L	icv, iv	↑	↑	↓	↑	14
TNF-α	Cat	L	iv[j]	↑[k]	NR	NR	NR	104
TNF-α	Rabbit	NR	sc	↑[h]	NR	NR	NR	104
TNF-β	Rabbit	L	icv	NR	↑	NR	↑	39
hIFN-α	Rabbit	L	iv, icv	↑	↑	→	↑	47
hIFN-α	Monkey	L	iv	NR	↑[l]	↑[l]	NR	46
hIFN-α	Rat	L	icv	↑[k]	↑	↓	NR	49
hIFN-α	Rat	L	icv, μi[g]	↑[h]	↑	NR	NR	33
hIFN-α	Rat	L	iv	→[h]	↑	NR	NR	33
hIFN-α	Rat	L	μi[f]	→[h]	→	NR	NR	33
hIFN-β	Rat	L	icv, iv, μi[g]	↑[h]	↑	NR	NR	33
hIFN-β	Rat	L	μi[f]	→[h]	→	NR	NR	33
Rat IFN	Rat	L	icv, μi[g]	↑[h]	↑	NR	NR	33
R IFN-A[m]	Rat	NR	ip	↑[n]	NR	NR	NR	48

[a] Period of the light:dark cycle when substance was administered.
[b] Route substance was delivered: icv, intracerebroventricular; μi, microinjection.
[c] Amplitudes of EEG slow waves in the δ-frequency band (0.5–3.5 Hz). NR, not reported; ↑, increase; ↓, decrease; →, no change; +, brain or body temperature change of >0.5°C relative to initial temperature.
[d] Microinjection into the aqueduct of Sylvius.
[e] Microinjection into 41 sites in basal forebrain and brain stem.
[f] Astrocyte-derived IL-1-like substance.
[g] Microinjection into the locus ceruleus.
[h] Total power in the δ-frequency band (0.5–3.5 Hz).
[i] Microinjection into the dorsal hippocampus, caudate nucleus, substantia nigra, and ventromedial hypothalamus.
[j] Immobilized animal, acute study.
[k] Synchronization of EEG.
[l] Reduced latency.
[m] Recombinant leukocyte IFN-A.
[n] Amplitudes of the dominant frequency in the EEG.

This finding is of importance in terms of altered sleep induced by infectious challenge (see below).

Responses to exogenous cytokines are influenced by interactions with circadian regulatory mechanisms for sleep. The first report of IL-1 effects on the sleep of rats (19) did not directly address this issue, but it did provide an early indication that complex circadian interactions would be a complicating factor in this species. In this study (19), a single dose of an astrocyte-derived IL-1-like substance was injected intracerebroventricularly into rats during the light period. A febrile response developed, but the amount of NREMS was not altered, despite increased amplitudes of EEG slow waves and a reduction in the amount of REMS. The increase in EEG slow wave amplitudes was evidence that IL-1 might influence sleep mechanisms of the rat. However, these responses of rats to this IL-1-like substance were not as robust as those reported for rabbits.

The interactions between circadian phase and responsiveness of rats to IL-1 were later addressed by

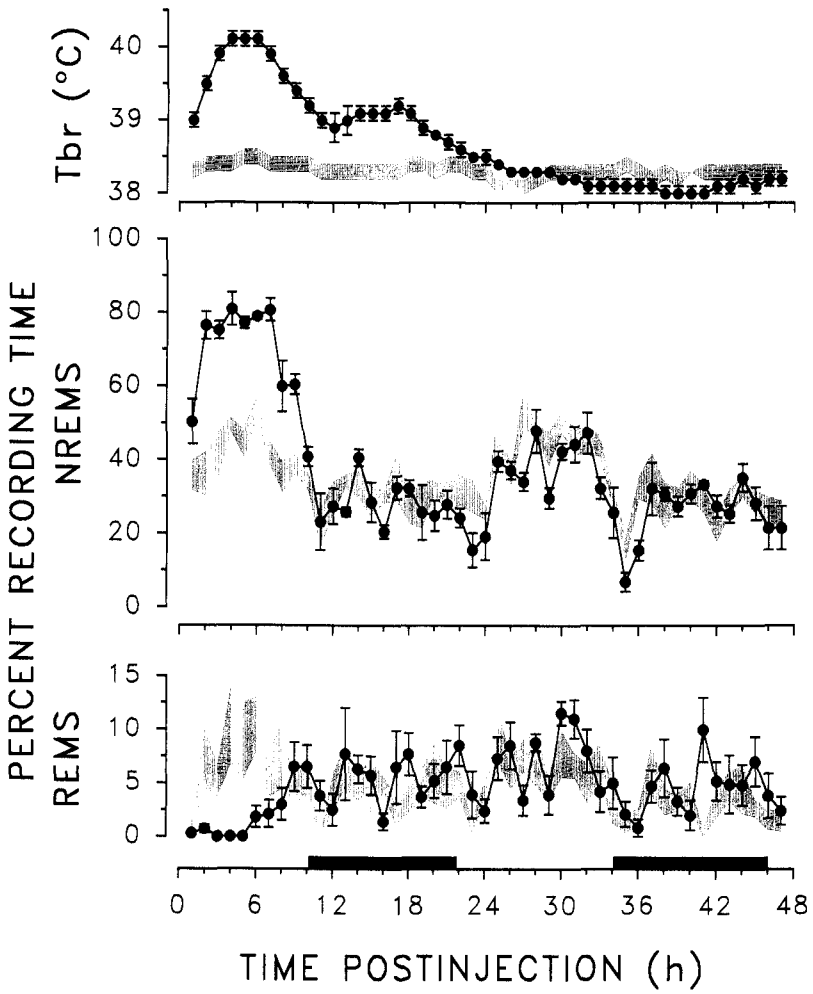

Figure 2. Changes in percentage of recording time spent in NREMS, in REMS, and Tbr induced by intracerebroventricular injection of 10 ng of IL-1β into rabbits. Closed circles represent the mean ± SE of values recorded from seven animals, and the shaded areas represent the mean ± SE of values recorded from the same animals after intracerebroventricular injections of vehicle. The dark bars on the abscissa indicate the dark portion of the light:dark cycle.

injecting intracerebroventricularly various doses of IL-1 at both light onset and dark onset (20). Quantification of vigilance states, amplitudes of EEG slow waves, and brain temperature revealed that rats respond differently to IL-1 depending upon the time of administration. Low doses of IL-1 increase the amount of NREMS during the light period (the time when rats naturally sleep the most), but with increasing dose, rats demonstrate progressively more wakefulness. However, concurrent with increasing wakefulness is an increase in amplitudes of the EEG slow waves during NREMS. Thus, during the light period, IL-1-treated rats seem to compensate for sleeping less by sleeping more deeply. When IL-1 is injected as dark onset (the time when rats are most active), the amount of NREMS increases, but high doses result in reduced amplitudes of EEG slow waves during NREMS. In addition, these data indicate that in rats, somnogenic doses of IL-1 (i.e., doses inducing increased NREMS), do not invariably suppress REMS.

The only study to date concerning the effects of IL-1 on the sleep of cats (21) indicates responses that are similar in some ways to those of the rat. The amount of time spent in NREMS was increased by the lowest doses tested (10 and 20 nmol) and was suppressed at the highest dose (40 nmol). Febrile responses were recorded after all doses of IL-1 tested.

Additional physiological and anatomical evidence supports the hypothesis of IL-1 involvement in the regulation of sleep and is reviewed elsewhere (22). Substances that induce IL-1 production (e.g., endotoxin, MP, etc.) enhance NREMS, whereas substances that inhibit the synthesis and/or actions of IL-1 (e.g., corticotropin-releasing hormone [CRH], α-melanocyte-stimulating hormone [α-MSH], and glucocorticoids) inhibit sleep. Human plasma IL-1 concentrations increase during the course of sleep deprivation (23), and preliminary data indicate that this may also be true in rabbits (M. R. Opp, unpublished). IL-1 concentrations in plasma peak at NREMS onset in humans (24), IL-1 like activity in the CSF of cats varies with sleep cycle (25), and patients undergoing IL-1 therapy report ex-

cessive sleepiness (1). In addition, a specific IL-1 receptor antagonist and anti-IL-1β antibodies reduce sleep in normal rabbits (18, 26). IL-1β mRNA is present in rat brain (3, 27), receptors for IL-1 are present in the rat (28) and mouse (29) brain, neurons immunoreactive for IL-1 are found in the central nervous system (CNS) (30, 31), and cells of CNS origin can produce IL-1 (32).

IL-2. IL-1 induces the release of IL-2 (2), which suggests the possibility that the somnogenic actions of IL-1 could, in part, be mediated by IL-2. Administration of rat IL-2 or human recombinant (hr) IL-2 into the third cerebral ventricle of rats is reported to induce synchronization of the EEG and behavioral sleep (33). These effects were observed within 5–10 min of administration and lasted from 25 to 140 min, depending upon the dose. Similar observations were made when IL-2 was microinjected into the locus coeruleus, but not when it was microinjected into the dorsal hippocampus, caudate nucleus, substantia nigra, or ventromedial hypothalamus (33). However, injections of hrIL-2 into the lateral cerebral ventricle (67–250 ng) or intravenously (1000–2000 ng) into rabbits did not alter sleep or brain/body temperature (M. R. Opp, unpublished). These data suggest that the somnogenic actions of IL-1 are not likely to be mediated via IL-2 in the rabbit, although IL-2 may influence sleep in the rat.

IL-6. The cytokine IL-6 induces the full hepatic acute phase protein response both *in vivo* and *in vitro*, whereas IL-1 elicits the entire spectrum of acute phase protein responses *in vivo* (2), but affects only a subset of these proteins *in vitro* (34). These observations suggest that the *in vivo* effects of IL-1 may be mediated, in part, by IL-6 (34), and prompted a study to determine whether IL-6 was somnogenic (35, 36).

Intracerebroventricular injections of IL-6 (20–200 ng) into rabbits resulted in a dose-related febrile response. The febrile responses were evident by the second postinjection hour and persisted for up to 6 hr. A heat-inactivated 200-ng dose of IL-6 had no effect on Tbr. Time spent in NREMS or in REMS was not affected by any of these doses of IL-6. In addition, latencies to the first NREMS episode of duration >1 min and the amplitudes of EEG slow waves were not consistently changed after these treatments (36). Thus, hrIL-6 does not appear to induce somnogenic responses across the dose range tested.

Tumor Necrosis Factors

Two related tumor necrosis factors have been described. TNF-α (also called cachectin) is produced primarily by macrophages, whereas TNF-β (also called lymphotoxin) is a product of lymphocytes (37). These two forms of TNF exhibit about 30% amino acid sequence homology and have similar, though not identical, biological activities (reviewed in Ref. 37). TNF, in concert with other cytokines, contribute to the development of fever and anorexia in response to a variety of stressors. Central administration of TNF-α and TNF-β modifies neural activity in the hypothalamus (38), which indicates possible direct actions in the CNS.

The first data indicating that TNF might be involved in sleep regulation came from the experiments of Shoham *et al.* (14). They demonstrated that intracerebroventricular or intravenous injections of hrTNF-α promote NREMS, suppress REMS, and induce fevers in rabbits in a dose-dependent manner. After the intracerebroventricular injection of 0.5 μg of TNF-α, the amount of time spent in NREMS increased during the first postinjection hour, whereas a dose 10 times greater increased NREMS throughout the entire 6-hr recording period. The amount of time spent in NREMS increased significantly for 6 hr or more after systemic injection of 10 μg/kg of TNF-α. Increases in the average amplitudes of EEG slow waves paralleled the somnogenic effects of TNF-α. The somnogenic effects of TNF-α are, thus, similar, although not identical, to those of IL-1 (14) and IFN-α_2 (see below). A recent study using a wide dose range of both TNF-α and TNF-β (0.5–5000 ng) indicated that both TNF induce similar somnogenic and pyrogenic responses in rabbits (Fig. 3) (39). Furthermore, TNF-β, like TNF-α, also induces anorexia (L. Kapás, unpublished). The magnitude and time course of these responses are nearly identical, which indicates that, although the peripheral effects of TNF-α and TNF-β differ, the central effects of these two cytokines are similar, and are possibly mediated by the same receptors (40).

The mechanisms responsible for the somnogenic effects of TNF are not clearly understood. Because TNF have direct modulatory effects on the neurons of the hypothalamus (38, 41), a region involved in the regulation of sleep-wake activity, they could also have direct effects on sleep. However, the interval of about 1 hr between the administration of TNF and the onset of the somnogenic effects indicates that other factor(s) and/or mechanisms may contribute to the somnogenic actions of TNF. Autocrine mechanisms have been described by which TNF-α stimulates its own production or the production of other somnogenic substances, such as IL-1 (reviewed in Ref. 37). Thus, TNF, or IL-1 synthesized in response to TNF-α administration, could mediate the somnogenic effects of exogenously administered TNF.

Interferons

Like IL-1 and TNF, interferons have been implicated as endogenous somnogens in several species. Humans undergoing IFN therapy report excessive sleepiness or fatigue (42–45), and, in monkeys, hrIFN-α_2 reduces the latency to sleep (46). High doses of hrIFN-α_2 (47) or hrIFN-β (M. Kimura-Takeuchi and L. A. Toth, unpublished) are somnogenic when administered intravenously or intracerebroventricularly to rabbits. A rabbit IFN-α/β preparation was also somnogenic in this

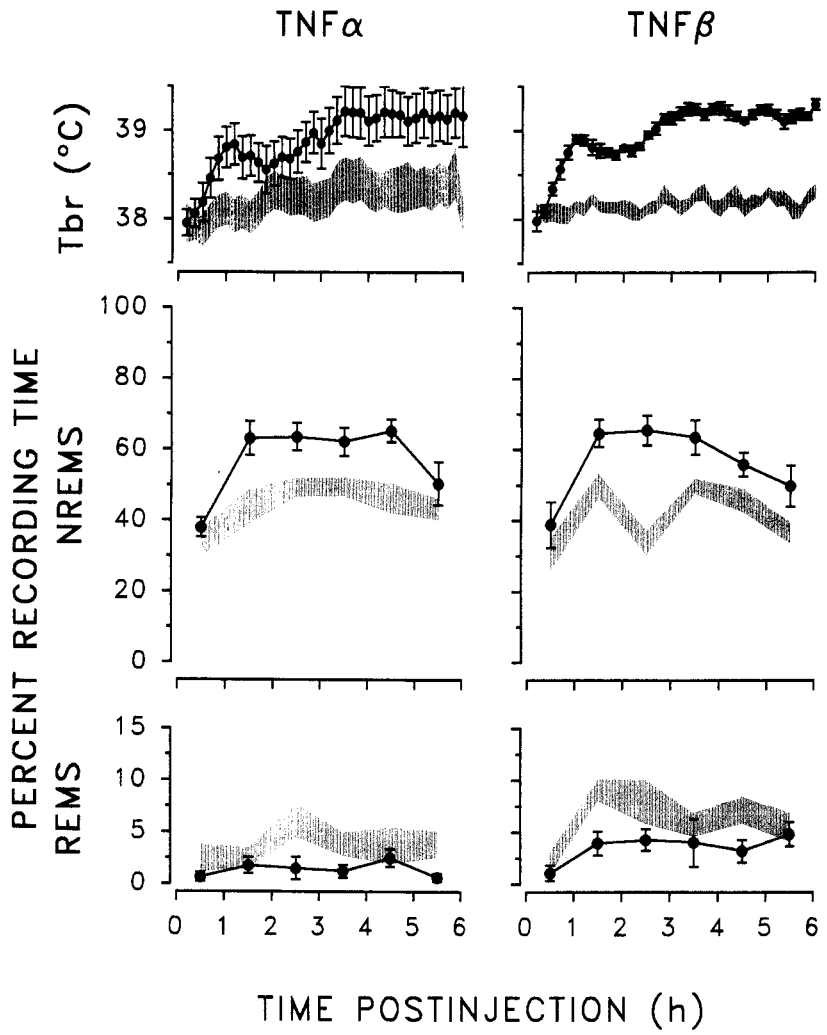

Figure 3. The effects of intracerebroventricular injection of 500 ng of TNF-α or of 200 ng of TNF-β on Tbr, and the percentage of recording time spent in NREMS and REMS in rabbits. The intracerebroventricular injections were given 3 hr after light onset. Each data point represents the mean ± SE for six animals. The shaded areas are the means ± SE for the same animals when injected intracerebroventricularly with vehicle.

species (M. R. Opp, unpublished). IFN-α_2 increases the amplitude of EEG slow waves, but does not reduce REMS (47). Increased EEG amplitudes were also observed in rats treated with intraperitoneal IFN (48). Similarly, a transient synchronization of the EEG and increases in EEG slow wave activity occur after the microinjection of IFN-α, IFN-β, or rat IFN into rat locus ceruleus, but not other brain regions (33). The administration of hrIFN-α into the lateral ventricles of rats also induced synchronization in the EEG in a dose-dependent manner (49).

Because IFN have historically been viewed as endogenous antiviral agents, the potential role of IFN as mediators of excessive sleep during viral disease is particularly relevant (see below). IFN-α and IFN-β are produced by most, if not all, nucleated cells (50), and the ability of leukocytes to produce IFN *in vitro* is enhanced during and after sleep deprivation (51). The identification of IFN as products of glial cells (52) suggests a CNS source for IFN involved in the mediation of sleep. However, the somnogenic and febrile responses to IFN do not develop until the second postinjection hour after intravenous or intracerebroventricular administration (47), which suggests a minimal time necessary to reach the site of action (e.g., crossing the blood-brain barrier) or to stimulate the synthesis and release of endogenous intermediates. For example, IFN induce human monocytes to secrete IL-1 (53), which could subsequently mediate the central effects of IFN. However, the observation that stimulation of glucose-sensitive and thermosensitive neurons and induction of fever by hrIFN-α_2 are not blocked by the endogenous IL-1 antagonist α-MSH (54) suggests that the effects of IFN are not mediated via IL-1.

Cytokines and Sleep during Infectious Disease

The study of CNS lesions produced during viral infections led von Economo (55) to describe sleep as an active process mediated by specific brain regions. Until recently, however, the relationship between sleep

and infection received little systematic investigation, despite common experiences of sleepiness during infectious disease, and the age-old practice of recommendation by physicians of bed rest as an aid in recuperation (e.g., see Ref. 56). Microbial infections are accompanied by constitutional symptoms that have given rise to common use of the generic term the flu to describe the fever and malaise that accompany acute infections of many etiologies. Current evidence suggests that sleep, like fever, is a common manifestation of infectious disease, and that physiological systems regulating the immune response, sleep, and body temperature are closely interrelated.

Sleep has not yet been completely characterized over the course of an infectious condition in humans, although abundant indirect evidence suggests that sleep is altered during such disease states. For example, viral infections have been linked to a variety of sleep disorders, such as chronic fatigue syndrome (57), and have been implicated as an epidemiological factor in the occurrence of sudden infant death syndrome (crib death) (58–60). Recent observations have indicated that HIV-infected patients who are asymptomatic demonstrate excess slow wave sleep (the human equivalent of NREMS) (61), in contrast to patients suffering from acquired immune deficiency syndrome, who commonly develop disturbed sleep (62).

Sleep patterns after infectious challenge have been most thoroughly characterized in the rabbit (63–65). Intravenous inoculation of rabbits with bacterial or fungal organisms induces sleep alterations that are characterized by initial increases in both the amount of NREMS and in the amplitudes of EEG slow waves during sleep (Fig. 4). These initial increases are followed by a decrease in these parameters. In contrast, REMS is suppressed for up to 48 hr after inoculation. Rabbits subjected to an abortive infection with influenza virus also develop enhanced sleep (66) and fever (66, 67). Like IFN-α_2 (47), and in contrast to bacterial and fungal challenge (62, 65), influenza inoculation does not significantly inhibit REMS in rabbits (66).

The precise temporal pattern of somnogenic responses to microbial challenge varies with the specific infectious agent used, and with the route of inoculation (62, 65). For example, inoculation with gram-negative bacteria induces a more rapid onset of enhanced sleep than does inoculation with gram-positive bacteria (Fig. 4) (62). Moreover, a natural pathogen of rabbits, the gram-negative bacterium *Pasteurella multocida*, administered intranasally to mimic spontaneously developing infections also alters sleep patterns in rabbits (65). These sleep changes develop after a longer latency and show a longer duration than do responses elicited by intravenous administration of the same organism. These differences in time course based on the route of inoculation could reflect a more slowly developing inflammatory response to organisms in the air spaces relative to the rapid and robust responses elicited by organisms in the circulation.

The severity of the disease process also influences the type of sleep changes that develop after microbial challenge; rabbits that died in response to the infection, or that were euthanized due to severely moribund conditions, demonstrated only a short period of sleep enhancement relative to that observed in rabbits that survived (64). The immune competence of the host also influences the type of sleep changes that develop after microbial challenge; rabbits treated with immunosuppressive and anti-inflammatory doses of glucocorticoids demonstrate a marked attenuation of the altered sleep induced by microbial challenge, although they continue to demonstrate other signs of disease (68). This observation, coupled with the fact that glucocorticoids antagonize the actions of many endogenous immune modulators, suggests a role for cytokines and other agents of inflammation in the mediation of altered sleep during infectious disease.

Rabbits inoculated with trypanosomes, the causative agent in humans of sleeping sickness, exhibit altered sleep that develops in temporal correlation with periods of parasitemia (69). Such observations indicate that the *in vivo* proliferation of the micro-organism is an important determinant in the development of the observed somnogenic effects. However, replication of the pathogen per se does not seem to be essential for manifestation of altered sleep. Inoculation of rabbits with heat-killed bacteria (63, 64) or with isolated bacterial cell walls (70) also induces sleep alterations that are qualitatively similar to those of viable bacteria, although the duration of these effects is often substantially reduced. In addition, mammalian macrophages produce somnogenically active substances of low molecular weight from phagocytized bacterial cell walls (71). Thus, viable bacteria are not essential for the induction of somnogenic responses after microbial challenge.

Several bacterial components are known to possess sleep-promoting properties. MP, the first microbial components to be characterized as somnogens (8), are the monomeric components of bacterial cell wall peptidoglycan (72). Whether administered intravenously, intraperitoneally, orally, or intracerebroventricularly, MP increase the amount of NREMS and enhance the amplitude of EEG slow waves during NREMS (8, 73). In addition to MP, bacteria contain other sleep-promoting substances. Endotoxin and its lipid A moiety, both components of the cell wall of gram-negative bacteria, promote NREMS in rabbits (74, 75). Interestingly, the somnogenic effects of lipid A and MP differ substantially in terms of latency and duration, as do the sleep patterns that develop after microbial challenge with gram-negative or gram-positive bacteria (76). Gram-negative and gram-positive bacteria differ with regard to the amount and molecular arrangement of

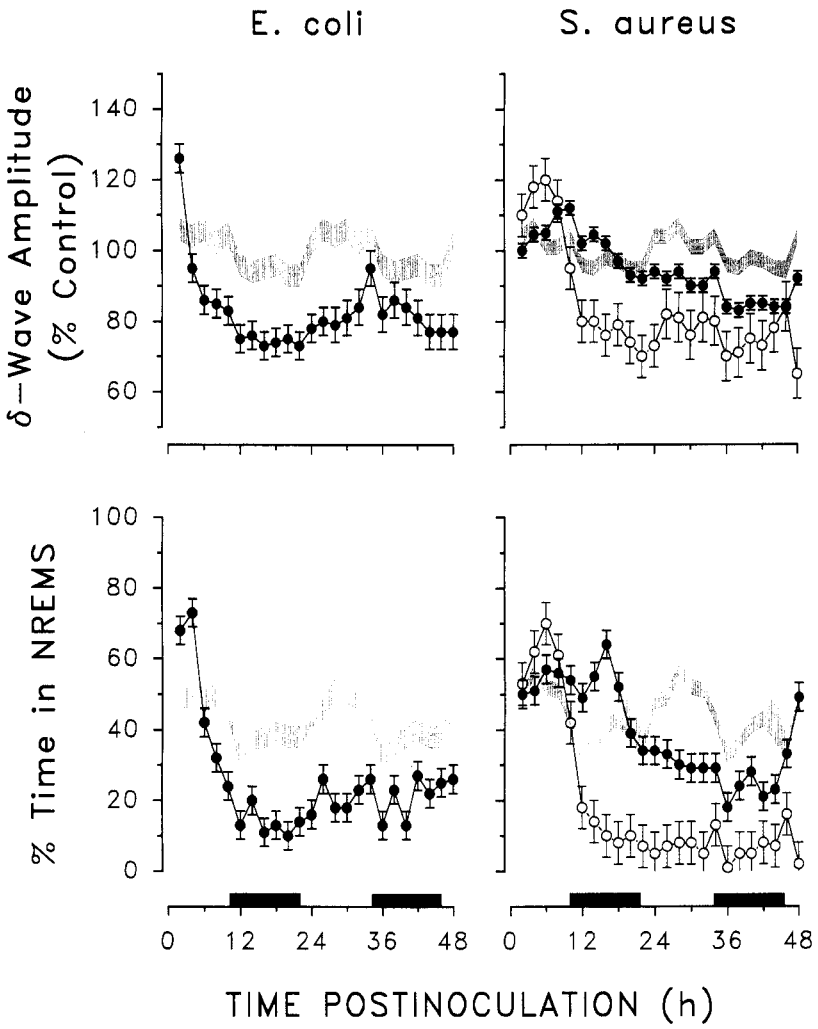

Figure 4. Effects of *Escherischia coli* (gram-negative bacteria) and *Staphylococcus aureus* (gram-positive bacteria) inoculation on sleep in rabbits. Rabbits were inoculated intravenously with $9.3 \pm 1.4 \times 10^7$ colony forming units (CFU) of *E. coli* ($n = 7$) or $5.9 \pm 1.4 \times 10^7$ CFU of *S. aureus* ($n = 12$). Sleep and δ-wave amplitudes during sleep were monitored for 48 hr after inoculation. The shaded areas represent the baseline values (mean ± SE) for a 24-hr period prior to inoculation; these values are replicated and graphically overlay data from the postinoculation period. Dark bars on the abscissa indicate the dark portion of the light:dark cycle. Rabbits inoculated with *S. aureus* exhibited two patterns of sleep alteration, early sleep (open circles) and late sleep (closed circles). Rabbits exhibiting late sleep patterns were more likely to survive the infectious challenge than were those animals that exhibited the early sleep pattern (data from Ref. 63).

MP in the cell wall, and in the presence or absence of endotoxin (77). Such factors could influence the time course of sleep alterations that develop after infectious challenge. Indeed, characteristic temporal patterns of fever, which in some cases have diagnostic value, accompany specific infectious conditions in humans (78).

Certain components of viruses are also somnogenic. Viral double-stranded RNA extracted from the lungs of mice infected with influenza virus (79) and a synthetic double-stranded RNA, poly I:C (80), both enhance NREMS in rabbits. Viral double-stranded RNA is postulated to be the microbial mediator that triggers at least some of the pathophysiological sequelae of viral infections (81).

The potential role of IFN in mediating sleep altered during viral disease has been examined in rabbits with an abortive influenza infection. In this model, serum antiviral activity (probably IFN) increased in temporal correlation with increases in NREMS and the febrile response (82), thereby supporting a potential role for IFN in sleep induction after viral inoculation. As additional support, rabbits rendered tolerant to the virus by repeated administration fail to develop fever, enhanced sleep, or increased serum IFN activity (66). Preliminary data indicate that central administration of IFN can induce excess sleep and fever, even in the virus-tolerant rabbit (M. Kimura-Takeuchi and L. A. Toth, unpublished). In addition, tolerance to the somnogenic and pyrogenic effects of influenza virus can be induced by pretreatment of rabbits with poly I:C (83), a potent stimulator of IFN production and release (81).

Although the chemical structures of microbial somnogens vary substantially, they are all potent modulators of host defense responses. The increased lassitude or sleepiness that commonly accompanies infectious disease, coupled with recent experimental demonstra-

tions that sleep actually is altered after microbial challenge, suggests that these behavioral manifestations of disease may also be regarded as part of the APR (3). Cytokines are of fundamental importance in the regulation of the APR, and probably mediate many of the immunological and behavioral sequelae of bacterial or viral disease. Indeed, the bacterial and viral products discussed above all alter cytokine production (reviewed in Ref. 84), and cytokine levels are altered *in vivo* during infections (85–90). As discussed above, several cytokines, including IL-1α, IL-1β, TNF-α, and IFN-α, have demonstrated somnogenic activity. The interactions between cytokines are complex and poorly understood, and parallel, sequential, and/or inhibitory effects on biological processes have been proposed for various cytokines. TNF, for example, induces a biphasic enhancement of sleep (14), promotes IL-1 production, and is implicated in many of the biological actions of IL-1 (91). IL-1 induces its own synthesis (92). IFN also stimulate IL-1 production, as well as promote sleep (47, 50, 53). IL-6 is postulated to mediate some of the systemic and central effects of IL-1 (93) and is pyrogenic, yet does not alter sleep (35). These interactions suggest that numerous cytokines could participate in the regulation of sleep alterations during infectious disease.

In summary, the host's response to infectious microbes (84) clearly includes alterations in normal sleep patterns (Fig. 4). Somnogenic factors are released secondary to macrophage processing of pathogenic microbes, and somnogenic cytokines are elaborated by the host as a result of immune stimulation. These two processes are hypothesized to induce altered sleep during infectious disease (Fig. 5). We further postulate that sleep could serve an adaptive function in combating infectious disease. For example, sleep, which is associated with a decreased metabolic rate and reduced muscular activity, could permit the animal to channel energy reserves into the production of fever at a time when food intake is likely to be reduced (94). Moreover, abundant evidence suggests that changes in sleep during infectious disease result from the activation and amplification of normal sleep regulatory mechanisms (76).

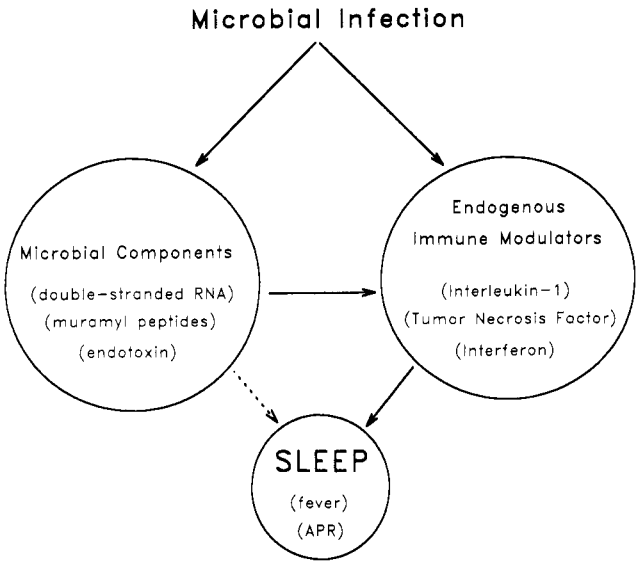

Figure 5. Schematic representation of hypothesized mechanisms for altered sleep during the course of an infectious disease. Somnogenic cytokines are elaborated by the host as a result of immune stimulation, and somnogenic microbial components are likely to be in the circulation during the infectious condition. These two processes are hypothesized to modulate normal sleep regulatory mechanisms, resulting in altered sleep.

Cytokines and the Regulation of Sleep

An important issue currently being addressed in the study of cytokine involvement in sleep regulation focuses on the interactions of cytokines with neural and endocrine systems. Cytokines could, for example, act directly on "somnogenic" neural networks to alter sleep or, alternatively, cytokine effects on sleep could be mediated via other humoral factors. The ability of IL-1 to alter neuronal activity in brain regions that are thought to be involved in sleep regulation (e.g., the hypothalamus) (41) provides supporting evidence for the hypothesis of direct action on neural networks. However, a large literature indicates that cytokines are only one component of a complex humoral regulatory network that modulates sleep. Other hypothalamic, pituitary, and gastrointestinal hormones are also constituents of this regulatory network (reviewed in Ref. 4), and many of these substances are secreted in response to cytokines. For example, growth hormone-releasing hormone may contribute in part to the somnogenic effects of IL-1 in that IL-1 stimulates the release of growth hormone (95, 96) probably via growth hormone-releasing hormone, and growth hormone-releasing hormone is somnogenic (96, 97). Another hypothalamic hormone, CRH, might be involved in the biphasic sleep response induced by IL-1 treatment and/or the sleep suppressive effects in rats of high doses of IL-1. Exogenously administered CRH reduces sleep (16) and blocks the somnogenic effects of IL-1 (16). Preliminary data indicate that the CRH antagonist, α-helical $CRH_{(9-41)}$, potentiates IL-1-induced sleep (M. R. Opp, unpublished), which suggests that CRH might provide a negative feedback signal for IL-1-induced sleep in addition to its well-documented involvement in the regulation of other IL-1 actions (98). These regulatory actions of CRH may be mediated through pituitary hormones such as α-MSH. CRH induces α-MSH production, α-MSH reduces sleep, and α-MSH inhibits IL-1-induced sleep (15). The contribution of other pro-opiomelanocortin peptides (e.g., adrenocorticotrophic hormone), is also possible, although a direct role for opioids in mediating the somnogenic effects of IL-1 is unlikely, given that systemic injection of naloxone does not alter IL-1-induced sleep (L. Kapás, unpublished).

Many of the biological actions of cytokines are mediated via the prostaglandins (PG), and experiments in rats and monkeys indicate that PG might also be involved in the regulation of sleep. In these species, PGD_2 enhances sleep and PGE_2 reduces sleep (99). In the rabbit, however, sleep-wake activity is not greatly altered by PGD_2, although PGE_2 does transiently reduce NREMS (100). Furthermore, central (L. Kapás, unpublished) or systemic (11) injection of the cyclooxygenase inhibitor indomethacin does not affect IL-1-induced sleep, but does prevent the febrile responses to this peptide.

Conclusions

Sleep can clearly be modulated by exogenous administration of cytokines, and is also likely to be regulated, in part, by endogenous cytokines. The synthesis of small peptide fragments from cytokines and the development of specific receptor antagonists and antibodies provide promising new tools with which to further examine the mechanisms responsible for cytokine-induced changes in vigilance. Although the mechanisms and sites of action mediating these somnogenic properties are not yet fully elucidated, the somnogenic responses to microbial challenge may represent amplifications of normal, physiological processes.

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