Somnogenic relationships between tumor necrosis factor and interleukin-1

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Takahashi, Satoshi, Levente Kapás, Jidong Fang, and James M. Krueger. Somnogenic relationships between tumor necrosis factor and interleukin-1. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1132–R1140, 1999.—Both tumor necrosis factor (TNF) and interleukin (IL)-1 are somnogenic cytokines. They also induce each other’s production and both induce nuclear factor kappa B activation, which in turn enhances IL-1 and TNF transcription. We hypothesized that TNF and IL-1 could influence each other’s somnogenic actions. To test this hypothesis, we determined the effects of blocking both endogenous TNF and IL-1 on spontaneous sleep and on sleep rebound after sleep deprivation in rabbits. Furthermore, the effects of inhibition of TNF on IL-1-induced sleep and the effects of blocking IL-1 on TNF-induced sleep were determined. A TNF receptor fragment (TNFRF), as a TNF inhibitor, and an IL-1 receptor fragment (IL-1RF), as an IL-1 inhibitor, were used. Intracerebroventricular injection of a combination of the TNFRF plus the IL-1RF significantly reduced spontaneous non-rapid eye movement sleep by 87 min over a 22-h recording period. Pretreatment of rabbits with the combination of TNFRF and IL-1RF also significantly attenuated sleep rebound after sleep deprivation. Furthermore, the TNFRF significantly attenuated IL-1-induced sleep but not fever. Finally, the IL-1RF blocked TNF-induced sleep responses but not fever. Results indicate that TNF and IL-1 cooperate to regulate physiological sleep.

cytokine; fever; electroencephalogram; rabbit

TUMOR NECROSIS FACTOR (TNF)-α and interleukin (IL)-1β are involved in sleep regulation (reviewed in Ref. 24). TNF-α mRNA, IL-1β mRNA, and their receptor mRNAs are constitutively expressed in brain (3, 4; reviewed in Ref. 25). TNF-α mRNA and IL-1β mRNA and TNF bioactivity (10) levels in rat brain vary with the sleep-wake cycle (5, 46); highest levels occur during light hours (the sleep period). In cats, IL-1 cerebrospinal fluid levels (33) vary with the sleep-wake cycle, and in rats, IL-1β mRNA levels in brain increase during sleep deprivation (34, 47). Furthermore, human plasma levels of IL-1 and TNF and the ability of circulating white blood cells to produce these cytokines increase after sleep deprivation and vary with the normal sleep-wake cycle (reviewed in Ref. 24). Administration of exogenous TNF or IL-1 induces increases in non-rapid eye movement sleep (NREMS) and electroencephalographic (EEG) slow-wave activity (SWA) in species such as rats (31, 35, 38, 55), rabbits (18, 28, 44), mice (8, 9), cats (45), and monkeys (11). Furthermore, inhibition of endogenous TNF or IL-1 attenuates various sleep responses. TNF inhibitors, e.g., TNF antibodies (49), the TNF soluble receptor I (53), or a fragment of the TNF soluble receptor (50–52), attenuate physiological sleep, sleep responses after sleep deprivation, muramyl dipeptide (MDP)-induced sleep, and warm environmental temperature-induced sleep. Blocking IL-1 using IL-1 antibodies (36), the IL-1-receptor antagonist (37), or an IL-1 soluble receptor fragment (48, 50) also reduces spontaneous sleep, sleep rebound after sleep deprivation, and MDP-induced sleep. Finally, TNF 55-kDa receptor knockout mice (8) or IL-1 type 1 receptor knockout mice (9) sleep less than control strains. Collectively, these data suggest that both TNF and IL-1 are involved in sleep regulation.

Although IL-1 and TNF induce each other’s production (1, 7, 41) and possess similar biological activities, the relationships between the somnogenic actions of TNF and IL-1 remain unknown. In IL-1 type 1 receptor knockout mice, TNF-α is somnogenic (9); conversely, in TNF 55-kDa receptor knockout mice, IL-1 is somnogenic (8). Nevertheless, we hypothesized that under normal circumstances TNF and IL-1 work together to regulate sleep. If this hypothesis is correct, inhibition of both IL-1 and TNF should reduce physiological sleep to a greater extent than inhibition of either alone. Furthermore, blocking endogenous IL-1 should attenuate TNF-induced sleep and inhibition of endogenous TNF should alter IL-1-induced sleep. We report herein that TNF and IL-1 are closely linked to regulate sleep.

MATERIALS AND METHODS

Materials

A TNF receptor fragment (TNFRF), which corresponds to amino acid residues 119–138 of the human recombinant TNF soluble receptor I (32), was synthesized by Dr. J. M. Seyer (Department of Biochemistry, University of Tennessee, Memphis, TN). Its amino acid sequence is QEKQNTV FLRENE(G); the underlined alanines were used as substitutes for cysteine. The terminal glycine was used to simplify synthesis (32). Inhibitory activity of the peptide against TNF cytotoxicity in mouse L-M cells was previously used to characterize this peptide (32). An IL-1 receptor fragment (IL-1RF), which is a synthetic peptide corresponding to the amino acid residues 86–95 of the human type I IL-1 receptor, was also synthesized by J. M. Seyer. Its amino acid sequence is YCLRIKISAK (54). It has binding sites of the parent molecule and inhibits IL-1 actions (54). A dose of 25 µg for both the TNFRF and the IL-1RF was used because previously we showed that this dose significantly inhibited sponta-
neous sleep and the sleep induced by the respective cytokines (50, 53). Human recombinant TNF-α and human recombinant IL-1β were obtained from R&D Systems (Minneapolis, MN). All substances were dissolved in pyrogen-free isotonic saline (PFS) from Abbott Laboratories (North Chicago, IL). Intracerebroventricular injections were done using a volume of 25 µl between 0830 and 0930.

Intracerebroventricular injections were done using a volume of 25 µl of PFS 10 min apart on the control day (PFS). On the first experimental day, they were injected intracerebroventricularly with 25 µl PFS and IL-1β (10 ng) 10 min apart (IL-1β). One week later, they received two injections: first 25 µg of the TNFRF followed 10 min later by 10 ng IL-1β (pretreatment with TNFRF). After the second injection, EEG, Tbr, and motor activity were recorded for 23 h.

Experiment 4: Effects of the IL-1RF on TNF-α-induced sleep and fever. The rabbits (n = 6) received two intracerebroventricular injections of PFS 10 min apart on the control day (PFS). On the first experimental day, they were injected intracerebroventricularly with PFS and TNF-α (250 ng) 10 min apart (TNF-α). One week later, they received two injections: first, 25 µg of the IL-1RF followed 10 min later by 250 ng TNF-α (pretreatment with IL-1RF). After the second injection, animals’ EEG, Tbr, and motor activity were recorded for 23 h.

Recording and Analysis

The rabbits were allowed relatively unrestricted movement inside the recording cages. A flexible tether connected the EEG electrodes and thermostim to an electronic swivel. Body movements were detected by ultrasonic detectors (Biomedical Instrumentation, University of Tennessee). The leads from the electronic swivel and movement detectors were routed to Grass 7D polygraphs in an adjacent room. EEG was filtered below 0.1 Hz and above 35 Hz. The amplified signals were digitized at a frequency of 128 Hz for EEG and at 2 Hz for Tbr and motor activity. Tbr values were saved on the computer in 10-s intervals. Tbr values sampled in 10-min intervals were used for statistical analyses. Online Fourier analysis of the EEG was performed. Vigilance states were determined offline in 1-s epochs by individuals unaware of the treatment. The vigilance states wakefulness (W), NREMS, and rapid eye movement sleep (REMS) were visually identified using criteria previously reported (18, 44, 49). Briefly, W was characterized by fast, low-amplitude EEG waves, gradually increasing Tbr, and high incidence of gross body movements. NREMS was associated with slow, high-amplitude EEG waves, slowly decreasing Tbr, and lack of body movements. In contrast, REMS was characterized by fast, low-amplitude EEG waves, appearance of rhythmic theta-EEG, rapidly increasing Tbr at REMS onset, and lack of motor activity. Time spent in each vigilance state was calculated for 1- and 2-h intervals and for the entire recording periods. The EEG power density values were summed in four frequency bands for each 10-s epoch; delta (0.5–4.0 Hz), theta (4.5–8.0 Hz), alpha (8.5–12.0 Hz), and beta (12.5–30 Hz)-wave activities were calculated. Hourly average of the EEG delta-wave activity during NREMS (EEG SWA) was determined. Percent changes in EEG SWA from time-matched values during the baseline period were calculated.

Statistical Analysis

In experiment 1, the differences between the effects of non-SD with PFS and TNFRF and IL-1RF first Tbr, were lost from one animal due to mechanical failure.

In experiment 3, the differences between the effects of non-SD with PFS and TNFRF and IL-1RF were evaluated. In experiment 4, the differences among the effects of non-SD with PFS and IL-1RF and pretreatment with TNFRF were evaluated.
evaluated. In experiment 4, the differences between PFS, TNF-\(\alpha\), and TNF-\(\alpha\) plus pretreatment with IL-1RF were evaluated. All analyses were performed with two-way ANOVA for repeated measures across the entire recording period. The first independent variable is treatment and the second independent variable is time. These were followed by the Student-Newman-Keuls (SNK) test. A significant level of \(P < 0.05\) was accepted.

RESULTS

Experiment 1: Effects of the TNFRF and the IL-1RF on Spontaneous Sleep and \(T_{br}\)

Rabbits receiving control injections displayed the typical daily variations of sleep; e.g., NREMS and REMS were greater during daylight hours than during dark hours, and at the transition between daylight and dark hours there was a decrease in NREMS. If animals were pretreated with both the TNFRF and the IL-1RF, NREMS was suppressed across the 22-h recording period (Fig. 1; Table 1). Control animals spent 535 min in NREMS; after pretreatment with the receptor fragments, the animals only spent 448 min in NREMS. Although daytime values of REMS were lower after pretreatment with the receptor fragments, this decrease did not reach statistical significance. Neither EEG SWA nor \(T_{br}\) was affected by the receptor fragments.

Experiment 2: Effects of the TNFRF and the IL-1RF on Sleep and \(T_{br}\) After 6 h of Sleep Deprivation

The 6-h SD with PFS induced a significant NREMS rebound that occurred across the 17-h recording period after SD (Fig. 2). EEG SWA during NREMS was also significantly enhanced after SD. However, the amount of time spent in REMS was not affected by SD in PFS-treated rabbits. Pretreatment with the TNFRF plus the IL-1RF significantly suppressed NREMS rebound after SD (Fig. 2; Table 2). Furthermore, in the SD receptor fragment-treated rabbits, the time spent in NREMS did not differ from that observed in non-SD controls. SD-enhanced EEG SWAs also were significantly inhibited by the receptor fragments; however, EEG SWAs were still enhanced compared with non-SD controls. In contrast, pretreatment with the receptor fragments induced significant increases in REMS after SD. \(T_{br}\) was significantly elevated during SD, from a baseline of 38.3 ± 0.06°C to 39.7 ± 0.10°C [ANOVA during the 6-h SD: treatment effects \(F(2,8) = 38.27, P < 0.0001\); SNK test: non-SD vs. SD with PFS q(3,8) = 11.12, \(P < 0.05\)]. Pretreatment with the receptor fragments did not affect the increases in \(T_{br}\) during SD (39.6 ± 0.07°C) [SNK test: non-SD vs. SD with TNFRF and IL-1RF q(2,8) = 10.26, \(P < 0.05\); SD with PFS vs. SD with TNFRF and IL-1RF q(2,8) = 0.09, NS]. There were no significant differences in \(T_{br}\) among the three treatment groups after SD.

Experiment 3: Effects of the TNFRF on IL-1\(\beta\)-Induced Sleep and Fever

IL-1\(\beta\) itself significantly increased the amount of NREMS, decreased REMS, enhanced EEG SWA, and induced fever during the 23-h postinjection period (Fig. 3; Table 3). Pretreatment with the TNFRF significantly attenuated the IL-1-induced increases in NREMS and EEG SWA. The onsets of TNFRF-induced attenuation of IL-1-induced NREMS and EEG SWAs were delayed by ~4 h after IL-1 injection but then persisted throughout most of the remaining recording period. IL-1\(\beta\)-suppressed REMS was slightly reversed by pretreatment with the TNFRF, but not significantly, across the
 Effects of intracerebroventricular injection of 25 µg TNFRF and 25 µg IL-1RF on spontaneous sleep and Tbr in rabbits

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NREMS, % recording time</th>
<th>REMS, % recording time</th>
<th>EEG SWA, change from control</th>
<th>Tbr, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline + saline)</td>
<td>40.5 ± 1.67</td>
<td>3.5 ± 0.11</td>
<td>0</td>
<td>38.09 ± 0.13</td>
</tr>
<tr>
<td>TNFRF + IL-1RF</td>
<td>33.9 ± 1.62*</td>
<td>3.4 ± 0.23</td>
<td>-4.50 ± 1.4</td>
<td>37.99 ± 0.12</td>
</tr>
</tbody>
</table>

Sleep values are means ± SE of percent of recording time spent in non-rapid eye movement sleep (NREMS) or rapid eye movement sleep (REMS) during 22-h recording periods; n = 7. Electroencephalogram (EEG) slow-wave activity (SWA) during NREMS is expressed as mean ± SE of hourly values of percent deviation from baseline [100 x (experimental − control)/control]. Brain temperature (Tbr) values are means ± SE of values collected in 10-min intervals across entire recording period. *P < 0.05; significant treatment effect vs. control (within-group comparisons by means of ANOVA for repeated measures followed by Student-Newman-Keuls test). NREMS: 2-way repeated ANOVA for 22-h recording period, treatment effects F(1,6) = 19.04, P < 0.005; Student-Newman-Keuls test, control vs. tumor necrosis factor (TNF) receptor fragment (RF) + interleukin (IL)-1RF q(2,6) = 6.17; P < 0.05. REMS: 2-way repeated ANOVA for 22-h recording period, treatment effects F(1,6) = 0.51, NS. SWA: 2-way repeated ANOVA for 22-h recording period, treatment effects F(1,6) = 3.00, NS. Tbr: 2-way repeated ANOVA for 22-h recording period, treatment effects F(1,6) = 0.34, NS.

Experiment 4: Effects of the IL-1RF on TNF-α-Induced Sleep and Fever

TNF-α itself significantly increased the amount of NREMS, decreased REMS, enhanced EEG SWA, and induced fever during the 23-h postinjection period (Fig. 4; Table 4). The magnitudes of these effects were similar to those induced by IL-1β in experiment 3. The IL-1RF almost completely blocked the effects of TNF-α on NREMS; these effects were evident in the first postinjection hour, then persisted throughout the recording period. TNF-α-enhanced EEG SWAs were also suppressed by pretreatment with the IL-1RF (Fig. 4; Table 4); these effects were also evident in the first postinjection hour. Furthermore, TNF-α-induced suppression of REMS duration was significantly attenuated by pretreatment with the IL-1RF. However, TNF-α-induced fever was not attenuated by pretreatment of IL-1RF.

DISCUSSION

Results presented here are consistent with earlier findings. Previously, the somnogenic actions of IL-1β and TNF-α have been described (reviewed in Ref. 25). Furthermore, inhibition of either IL-1 or TNF reduces spontaneous sleep and attenuates sleep rebound after SD (24). Current data extend those findings by showing that the inhibition of both IL-1 and TNF results in a slightly greater sleep loss than inhibition of either one alone [NREMS loss of 87 min over 22 h after inhibition of both IL-1 and TNF vs. 68 min over 21 h after TNFRF (51) and 57 min over 23 h after IL-1RF (50)]. Although these differences are small, they suggest some degree of interaction between IL-1 and TNF because the individual inhibitory effects were not additive and simultaneously that they act independently because, after inhibition of both cytokines, sleep loss was greater than after inhibition of either alone. Regardless, the inhibition of both IL-1 and TNF did not completely eliminate sleep; that result is consistent with the previous hypoth-
Table 2. Effects of intracerebroventricular injection of 25 µg TNFRF and 25 µg IL-1RF on sleep and T br after sleep deprivation in rabbits

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NREMS, % recording time</th>
<th>REMS, % recording time</th>
<th>EEG SWA, change from control</th>
<th>T br, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline + saline)</td>
<td>38.8 ± 2.02</td>
<td>2.8 ± 0.23</td>
<td>0</td>
<td>38.34 ± 0.14</td>
</tr>
<tr>
<td>Saline + saline with SD</td>
<td>47.6 ± 2.41†</td>
<td>3.1 ± 1.35</td>
<td>32.52 ± 5.14*</td>
<td>38.46 ± 0.14</td>
</tr>
<tr>
<td>TNFRF + IL-1RF with SD</td>
<td>41.5 ± 1.91†</td>
<td>4.6 ± 0.68†</td>
<td>15.24 ± 4.86†</td>
<td>38.21 ± 0.18</td>
</tr>
</tbody>
</table>

Sleep values are means ± SE of percent of recording time spent in NREMS or REMS during 17-h post-sleep deprivation (SD) recording period; n = 6. EEG SWA during NREMS is expressed as means ± SE of hourly values of percent deviation from baseline [100 x (experimental − control)/control] in each hour. T br values are means ± SE of values collected in 10-min intervals across entire recording period. In T br samples, data were lost from 1 animal due to mechanical failure. *P < 0.05. significant treatment effects vs. control (within-group comparisons by means of ANOVA for repeated measures followed by Student-Newman-Keuls test). † P < 0.05, significant treatment effects vs. saline + saline with SD (within-group comparisons by means of ANOVA for repeated measures followed by Student-Newman-Keuls test). NREMS: 2-way repeated ANOVA for 17-h post-SD period, treatment effects F (2,10) = 24.35, P < 0.005; Student-Newman-Keuls test, control vs. saline + saline with SD q(3,10) = 9.64, P < 0.05, saline + saline with SD vs. TNFRF + IL-1RF with SD q(2,10) = 6.67, P < 0.05, control vs. TNFRF + IL-1RF with SD q(2,10) = 2.96, NS. REMS: 2-way repeated ANOVA for 17-h post-SD period, treatment effects F (2,10) = 13.80, P < 0.005; Student-Newman-Keuls test, control vs. saline + saline with SD q(2,10) = 1.23, NS, saline + saline with SD vs. TNFRF + IL-1RF with SD q(2,10) = 5.73, P < 0.05, control vs. TNFRF + IL-1RF with SD q(3,10) = 5.73, P < 0.05. SWA: 2-way repeated ANOVA for 17-h post-SD period, treatment effects F (2,10) = 14.56, P < 0.005; Student-Newman-Keuls test, control vs. saline + saline with SD q(3,10) = 7.63, P < 0.05, saline + saline with SD vs. TNFRF + IL-1RF with SD q(2,10) = 4.05, P < 0.05, control vs. TNFRF + IL-1RF with SD q(2,10) = 3.57, P < 0.05. T br: 2-way repeated ANOVA for 17-h post-SD period, treatment effects F (2,8) = 0.73, NS.

The intracerebral administration of the IL-1RF plus the TNFRF blocked sleep rebound after SD, thereby suggesting that central pools of cytokines play an important role in sleep responses to sleep loss. Previously, we had shown that central injection of the IL-1RF, but not intravenous injection of the IL-1RF, attenuated sleep rebound after SD (48). Furthermore, the TNF soluble receptor is a normal component of cerebrospinal fluid (42); comparative data for the IL-1 soluble receptor have not been published. It is thus possible that the physiological role of these soluble receptors is to act as endogenous inhibitors of these proinflammatory cytokines in brain. A different line of evidence also suggests that central cytokines are important to sleep physiology. Rats fed a palatable diet also induce increases in NREMS responses (12). The palatable diet also induces increases in IL-1β mRNA levels in the liver and in the brain (13). Intraperitoneal injection of IL-1β also induces increases in NREMS and increases in liver and brain production of IL-1β mRNA; these responses are blocked or attenuated (depending on dose) after vagotomy (14). These data suggest that peripheral somnogenic signals, including IL-1β, elicit sleep via central changes in cytokine levels.

The inhibition of spontaneous NREMS by the IL-1RF and TNFRF was not associated with an inhibition of EEG SWAs. In contrast, after SD, the enhanced EEG...
Table 3. Effects of intracerebroventricular injection of 25 µg TNF-RF on 10 ng IL-1β-induced sleep and febrile responses in rabbits

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NREMS, % recording time</th>
<th>REMS, % recording time</th>
<th>EEG SWA, % change from control</th>
<th>Tbr, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>43.4 ± 1.94</td>
<td>4.3 ± 0.25</td>
<td>0</td>
<td>38.30 ± 0.40</td>
</tr>
<tr>
<td>Saline + IL-1β</td>
<td>51.5 ± 3.30</td>
<td>2.5 ± 0.34*</td>
<td>11.33 ± 3.45*</td>
<td>38.97 ± 0.53*</td>
</tr>
<tr>
<td>TNFRF + IL-1β</td>
<td>44.0 ± 2.11†</td>
<td>3.2 ± 0.53*</td>
<td>0.83 ± 3.03†</td>
<td>38.82 ± 0.50</td>
</tr>
</tbody>
</table>

Sleep values are means ± SE of percent of recording time spent in NREMS or REMS during 23-h recording period; n = 6. EEG SWA during NREMS is expressed as means ± SE of values collected in 10-min intervals across entire recording period. In Tbr samples, data were lost from 1 animal due to mechanical failure. *P < 0.05, significant treatment effect vs. control (within-group comparisons by means of ANOVA for repeated measures followed by Student-Newman-Keuls test). †P < 0.05, significant treatment effects vs. saline + IL-1β (within-group comparisons by means of ANOVA for repeated measures followed by Student-Newman-Keuls test). NREMS: 2-way repeated ANOVA for 23-h postinjection period, treatment effects F(2,10) = 8.31, P < 0.01; Student-Newman-Keuls test, control vs. saline + IL-1β q(3,10) = 5.17, P < 0.05, saline + IL-1β vs. TNFRF + IL-1β q(2,10) = 4.79, P < 0.05, control vs. TNFRF + IL-1β q(2,10) = 0.30, NS. REMS: 2-way repeated ANOVA for 23-h postinjection period, treatment effects F(2,10) = 11.32, P < 0.005; Student-Newman-Keuls test, control vs. saline + IL-1β q(3,10) = 6.68, P < 0.05, saline + IL-1β vs. TNFRF + IL-1β q(2,10) = 2.61, NS, control vs. TNFRF + IL-1β q(2,10) = 4.07, P < 0.05. SWA: 2-way repeated ANOVA for 23-h postinjection period, treatment effects F(2,10) = 5.41, P < 0.05, saline + IL-1β vs. TNFRF + IL-1β q(2,10) = 4.80, P < 0.05, control vs. TNFRF + IL-1β q(2,10) = 0.60, NS. Tbr: 2-way repeated ANOVA for 23-h postinjection period, treatment effects F(2,10) = 9.91, P < 0.005; Student-Newman-Keuls test, control vs. saline + IL-1β q(3,10) = 5.99, P < 0.05, saline + IL-1β vs. TNFRF + IL-1β q(2,10) = 1.30, NS, control vs. TNFRF + IL-1β q(2,10) = 4.68, P < 0.05.

Slow waves were attenuated if rabbits were pretreated with the receptor fragments. These results are consistent with those previously published in which either IL-1RF or TNFRF were used alone (51, 52). EEG SWAs are thought to be indicative of the intensity of sleep (2) because “supranormal” E EG slow waves characterize NREM S after SD (40). Several lines of evidence indicate that the regulation of NREM S duration is independent, in part, from the regulation of EEG SWAs. For example, after immunotoxin lesion of basal forebrain cholinergic neurons, NREM S duration remains relatively normal whereas EEG SWAs decrease (21). In contrast, some drugs (e.g., atropine) or manipulations (e.g., hyperventilation) induce high-amplitude EEG slow waves dissociated from sleep. Regardless, current data are consistent with the notion that after SD the increased EEG slow-wave power reflects the intensity of NREM S (2) and that IL-1 and TNF play a role in this association.

In the sleep-deprived control rabbits, there was not an increase in REMS after the 6-h SD period. This result is consistent with what we have previously published (48, 51). In contrast, after pretreatment with the receptor fragments, REMS increased after SD; this also is consistent with our previous studies in which either the IL-1RF or the TNFRF was used. These results suggest that the NREM S pressure, occurring after SD, suppresses REMS. Finally, these data clearly indicate that the IL-1RF and TNFRF effects on sleep are not the result of nonspecific activation because, after their injection, increases in REMS were observed.

Current results suggest that the somnogenic actions of TNF and IL-1 are independent of their effects on Tbr. Thus inhibition of both cytokines inhibited sleep but failed to affect Tbr. Furthermore, the pyrogenic actions...
of IL-1 were not blocked by the TNFRF nor were those of TNF blocked by the IL-1RF. These results are consistent with previous data (reviewed in Ref. 27). For example, low doses of IL-1 enhance sleep without affecting T_{br} (38). Furthermore, these sleep-promoting actions of IL-1 are not affected by anti-pyretics (22, 28). Finally, nitric oxide synthase (NOS) inhibitors, if given centrally, block IL-1-induced sleep responses, but not fevers (23). Collectively, these data clearly indicate that sleep and fever mechanisms are separate. Regardless, other aspects of the relationships between sleep, cytokines, and T_{br} regulation are not so clearly separate. Thus the increases in NREMS induced by acute mild increases in ambient temperature are blocked if animals are pretreated with the TNFRF, although the ambient temperature-induced changes in T_{br} are not (52). In contrast, the IL-1RF does not block ambient temperature-induced sleep responses but does block T_{br} responses to ambient temperature (29).

In addition to the apparent independent IL-1 and TNF mechanisms involved in sleep responses to ambient temperature, other data suggest that these cytokines can independently affect sleep. Thus, as already mentioned, in IL-1 type I receptor knockout mice TNF, but not IL-1, is somnogenic (9). Similarly, in TNF 55-kDa receptor knockout mice, IL-1, but not TNF, is somnogenic (8). Furthermore, the current results indicate that there are differences in the time courses of inhibition after giving IL-1 plus TNFRF versus TNF plus IL-1RF. The IL-1RF inhibition of TNF-α-induced sleep occurred rapidly, whereas the TNFRF inhibition of IL-1β-induced sleep was delayed by ~4 h before it became manifest. Because IL-1 and TNF not only induce each other’s production but also induce their own production and may do so via neuronal signals (1, 7, 14, 41), the potential for nonlinear amplifications and asymmetries of inhibition abound.

Inherent within the concept of sleep homeostasis is the idea that sleep-regulatory substance(s) increase as a result of the neuronal activity of W and act on populations of neurons to alter input-output relationships (26). Thus the propensity for sleep and its intensity and duration are dependent on prior neuronal activity. There is much evidence suggesting that humoral factors are involved in the induction of state shifts. Many studies have demonstrated that the transfer of cerebrospinal fluid from sleep-deprived animals to control animals enhances sleep in the recipient (reviewed in Ref. 25). It is likely that IL-1β and TNF-α are two endogenous substances involved in physiological sleep regulation (see introduction). Results from this study suggest they act in concert to affect sleep, although under certain experimental conditions they can act independently to promote sleep. Both IL-1 and TNF must interact with other molecules to elicit sleep, and several mechanisms have been identified via which one or both of these cytokines could affect sleep. For example, both IL-1β and TNF-α enhance nitric oxide (NO) production. Inhibition of NOS inhibits sleep (17, 23), whereas NO donor substances enhance sleep (6, 20). Other putative sleep-regulatory substances also affect sleep either by directly affecting IL-1 or TNF or by affecting downstream events. For example, adenosine augments IL-1-enhanced NO production (43), and in astrocytes and other tissues adenosine enhances NO production (15, 16). IL-4 and IL-10 inhibit sleep and inhibit production of IL-1 and TNF (29, 39). It is likely that the biochemical orchestration of sleep is complex, involving many substances. Although none may be necessary for sleep, several, such as IL-1 and TNF, are important components of normal sleep regulation as evidenced by findings such as those described in this report.

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