Interleukin-4 inhibits spontaneous sleep in rabbits

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Kushikata, Tetsuya, J idong Fang, Ying Wang, and J ames M. Krueger. Interleukin-4 inhibits spontaneous sleep in rabbits. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1185–R1191, 1998.—Proinflammatory cytokines, including interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), are involved in sleep regulation. IL-4 is an antiinflammatory cytokine that inhibits proinflammatory cytokine production. The hypothesis that IL-4 should attenuate sleep was studied by determining the effects of IL-4 on rabbit spontaneous sleep. Thirty-six rabbits were used. Four doses of IL-4 (0.25, 2.5, 25, and 250 ng) were injected intracerebroventricularly during the rest (light) period. One dose of IL-4 (25 ng) was injected during the active (dark) cycle. Appropriate time-matched control injections of saline were done in the same rabbits on different days. The three highest doses of IL-4 significantly inhibited spontaneous non-rapid eye movement sleep if IL-4 was given during the light cycle. The highest dose of IL-4 (250 ng) also significantly decreased rapid eye movement sleep. On the other hand, IL-4 administered at dark onset had no effect on sleep. The sleep inhibitory properties of IL-4 provide additional evidence for the hypothesis that a brain cytokine network is involved in the regulation of physiological sleep.

non-rapid eye movement sleep; rapid eye movement sleep; electroencephalogram; cytokines; brain temperature

The proinflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) are involved in physiological sleep regulation (reviewed in Ref. 20). Administration of exogenous TNF-α (5, 16, 29) or IL-1β (6, 8, 22, 23, 33, 43, 51) enhances non-rapid eye movement sleep (NREMS) in a variety of species. Conversely, inhibition of TNF or IL-1 using antibodies (31, 49), soluble receptors (46–48) or, in the case of IL-1, the IL-1 receptor antagonist (IL-1RA) (34) inhibits spontaneous sleep. These inhibitors also inhibit sleep rebound after sleep deprivation (31, 45), the excess NREMS associated with acute mild increases in ambient temperature (49), and the excess NREMS associated with administration of bacterial products (46). In rats, IL-1β mRNA levels in the hypothalamus, cerebral cortex, brain stem, and hippocampus are highest during peak sleep periods (daytime) (44) and increase in the brain after sleep deprivation (26). In cats, IL-1 cerebrospinal fluid levels vary in phase with the sleep-wake cycle (25). TNF bioactivity levels in rat hypothalamus and cortex are about 10-fold greater during peak sleep periods than during waking hours (7). Mutant mice lacking the TNF 55-kDa receptor or mice lacking the IL-1 type 1 receptor sleep less than their respective strain controls (5, 6). Circulating levels of IL-1 and TNF are also affected by the sleep-wake cycle and sleep deprivation (4, 9, 12, 27, 52); highest levels are associated with enhanced sleep or sleepiness.

The regulation of proinflammatory cytokines is complex and not very well understood in the brain. Nevertheless, some substances associated with specific cytokines such as the IL-1RA or the TNF and IL-1 soluble receptors seem to act as endogenous antagonists, and indeed these substances inhibit spontaneous sleep (34, 46, 47). Furthermore, there is a class of antiinflammatory cytokines that includes IL-4 and IL-10. Each of these cytokines has a unique set of biological activities, although both, in one manner or another, inhibit proinflammatory cytokines. Previously, Opp, et al. (35) showed that IL-10 inhibits spontaneous NREMS, thereby providing further evidence that proinflammatory cytokines are involved in physiologic sleep regulation. IL-4 inhibits IL-1β (13, 28) and TNF-α (3, 24) production and it increases the production of the IL-1RA (11, 42) and release of the soluble TNF receptor (14). Furthermore, IL-4 inhibits production or release of other substances implicated in sleep regulation, e.g., nitric oxide (NO) (19) and insulin (39). We predicted therefore that IL-4 should inhibit NREMS. We report herein that IL-4 inhibits sleep after about an 8- to 10-h delay, without affecting body temperature or amplitudes of electroencephalographic (EEG) slow waves.

MATERIALS AND METHODS

Recombinant human IL-4 was purchased from Genzyme (Cambridge, MA). Substances were dissolved in pyrogen-free isotonic NaCl (PFS; Abbott Laboratories, North Chicago, IL). The intracerebroventricular injection was done using a volume of 50 µl PFS.

Animals. Male New Zealand White Pasteurella-free rabbits (4.5–5.5 kg) were provided with a lateral cerebral ventricular guide cannula, stainless steel EEG electrodes, and a brain thermistor, using ketamine-xylazine (35 and 5 mg/kg) anesthesia as previously described (46). In brief, the guide cannula was placed in the left lateral ventricle for intracerebroventricular injection. The EEG electrodes were placed over the frontal and parietal cortices. A calibrated 30-kΩ thermistor (model 44008; Omega Engineering, Stamford, CT) was implanted on the dura mater over the parietal cortex to measure brain temperature (Tbr). The leads from the EEG electrodes and the thermistor were routed to a Teflon pedestal (Plastics One, Roanoke, VA). The pedestal and the guide cannula were attached to the skull with dental acrylic (Duz-All, Coradite Dental Products, Skokie, IL). After a 2-wk recovery period, the animals were placed in sleep-recording chambers (Hot Pack 352600, Philadelphia, PA) for at least one 24-h habituation period. The rabbits were kept on a 12:12-h light-dark cycle (lights on at 0600) at 21 ± 1°C.
ambient temperature. Water and food were available ad libitum throughout the experiment.

Experimental protocols. A total of 36 rabbits were used. The rabbits received 50 µl PFS intracerebroventricularly on a separate control day. The same animals were given 50 µl IL-4 intracerebroventricularly 1 day before or after the PFS injection. Four doses of IL-4 were used during the light cycle: 0.25 ng (n = 6), 2.5 ng (n = 7), 25 ng (n = 8), and 250 ng (n = 7). Injections took place between 0825 and 0915. Rabbits are crepuscular and have distinct circadian rhythms in their sleep. During daylight hours rabbits spend more time in NREMS and in REMS than they do during dark hours (46). It was of interest therefore to determine whether injection of IL-4 had the same effects at night as during the day. Eight rabbits received IL-4 (25 ng icv) at dark onset (1800); the IL-4 had the same effects at night as during the day. Eight was of interest therefore to determine whether injection of NREMS and in REMS than they do during dark hours (46). It was of interest therefore to determine whether injection of IL-4 had the same effects at night as during the day. Eight rabbits received IL-4 (25 ng icv) at dark onset (1800); the same rabbits received timedate-controlled injection of PFS on a separate day. After injections, EEG, Tbr, and motor activity were recorded for the next 23 h as described previously (46).

Recording and analysis. The rabbits were allowed relatively unrestricted movement inside the recording cages. A flexible tether connecting the electrodes and thermistor led to an electronic swivel (SL6C, Plastics One). Body movements were detected by ultrasonic detectors (Biomedical Instrumentation, The University of Tennessee, Memphis, TN). The leads from the electronic swivels and movement detectors were routed to Grass 7D polygraphs in an adjacent room. The EEG was filtered below 0.1 Hz and above 35 Hz. The amplified signals were digitized at frequency of 128 Hz for the EEG and 2 Hz for Tbr and motor activity. Tbr and EEG data were saved on the computer in 10-s intervals. Tbr values sampled in 3-h intervals were used for statistical analysis. Some of the Tbr values were lost because of technical problems and therefore the sample sizes for Tbr were lower than for sleep data. Online Fourier analyses of the EEG were performed. The vigilance states were determined offline in 10-s epochs. The vigilance states of wakefulness (W), NREMS, and REMS were visually identified in 10-s epochs using criteria previously reported (33). 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, rapidly increasing Tbr at REMS onset, and lack of motor activity interrupted by phasic motor activity. The amount of time spent in each vigilance state was calculated for 2-h intervals for graphical display. Three-hour time blocks were used for statistical analyses. In addition, the number of NREMS and REMS episodes, the mean episode length, and mean length of sleep cycles (REMS-REMS interval) were determined using a computer program with the criterion that each episode lasted at least 30 s. 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 (12.0–30.0 Hz)-wave activities. Hourly averages of the EEG power density values in the four frequency bands were determined for W, NREMS, and REMS separately.

Statistical analysis. All analyses were performed with two-way ANOVA for repeated measures across the entire 23-h recording period and the 12-h dark period and for the 3-h time blocks followed by Student-Newman-Keuls test (Tables 1 and 2). A significant level of P < 0.05 was accepted.

RESULTS

The lowest dose of IL-4 (0.25 ng) had no effect on the sleep parameters measured (Tables 1 and 2). The three highest doses of IL-4 used during the light phase significantly inhibited duration of NREMS (Table 1) [ANOVA values were 2.5 ng: F(1,6) = 10.127, P = 0.019; 25 ng: F(1,7) = 9.760, P = 0.017; 250 ng: F(1,6) = 9.138, P = 0.023]. These NREMS inhibitory effects began 8–10 h postinjection and continued throughout the remainder of the recording period (results obtained after the 25-µg dose are shown in Fig. 1); the largest effects were observed during the 12-h dark period (Table 1). In contrast, the 25-ng dose given at dark onset failed to affect any sleep parameters tested (Fig. 1 and Tables 1 and 2). The two intermediate doses of IL-4 tested, 2.5 and 25 ng, failed to affect duration of REMS across the 23-h recording period. After these two doses of IL-4, the distribution of REMS across the 23-h recording period remained similar to that observed after injections of PFS, with higher amounts of REMS occurring during daylight hours (e.g., Fig. 1 and Table 1). After the highest dose of IL-4, 250 ng, REMS was inhibited [ANOVA; treatment effect F(1,6) = 7.810, P = 0.031]; this inhibition began within 2–4 h postinjection and continued throughout the remaining portion of the recording period. Although NREMS was inhibited after the highest dose across the 23-h recording period, during the dark hours this inhibition did not reach

Table 1. IL-4 inhibits duration of NREMS

<table>
<thead>
<tr>
<th>Substance</th>
<th>Dose, ng</th>
<th>Time of Injection</th>
<th>n</th>
<th>NREMS during 23-h period, min</th>
<th>NREMS during 12-h dark period, min</th>
<th>REMS, min</th>
<th>REMS during 12-h dark period, min</th>
<th>Tbr (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>0</td>
<td>0900</td>
<td>6</td>
<td>534.8 ± 18.0</td>
<td>260.3 ± 4.9</td>
<td>35.6 ± 4.6</td>
<td>16.6 ± 3.8</td>
<td>39.4 ± 0.03</td>
</tr>
<tr>
<td>IL-4</td>
<td>0.25</td>
<td>0900</td>
<td>7</td>
<td>524.5 ± 29.3</td>
<td>246.8 ± 10.9</td>
<td>36.6 ± 8.5</td>
<td>16.9 ± 6.2</td>
<td>39.4 ± 0.03</td>
</tr>
<tr>
<td>IL-4</td>
<td>2.5</td>
<td>0900</td>
<td>8</td>
<td>561.4 ± 12.5</td>
<td>277.0 ± 7.1</td>
<td>46.5 ± 3.5</td>
<td>14.8 ± 1.7</td>
<td>38.0 ± 0.02</td>
</tr>
<tr>
<td>IL-4</td>
<td>25</td>
<td>0900</td>
<td>6</td>
<td>520.1 ± 9.3*</td>
<td>251.4 ± 6.7*</td>
<td>45.4 ± 4.5</td>
<td>15.0 ± 1.9</td>
<td>39.0 ± 0.03</td>
</tr>
<tr>
<td>IL-4</td>
<td>250</td>
<td>0900</td>
<td>7</td>
<td>553.9 ± 12.9</td>
<td>280.7 ± 8.0</td>
<td>54.4 ± 4.0</td>
<td>23.0 ± 3.1</td>
<td>38.7 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. rabbits studied. NREMS, non-rapid eye movement sleep; Tbr, brain temperature; IL-4, interleukin-4. *Significant difference, P < 0.05.
The duration and number of NREMS episodes and number of REMS episodes tended to decrease after the three highest doses of IL-4 used during the light phase. However, these effects were not significant. Similarly the sleep cycle length was not significantly affected by any dose of IL-4 tested (Table 2). All doses of IL-4 tested failed to affect Tbr (Table 1) or power density in the four frequency bands of the EEG during any of the vigilance states; by way of example, EEG results obtained after the 25-ng dose during the light phase are shown in Fig. 2. This 25-ng dose also failed to affect EEG activity if given at dark onset (data not shown).

Although not systematically quantified, IL-4 did not induce abnormal behavior in the sense that animals continued to cycle through sleep and wakefulness episodically.

Table 2. Effect of IL-4 on sleep episodes and sleep cycles

<table>
<thead>
<tr>
<th>Substance</th>
<th>Dose, ng</th>
<th>Diel Phase</th>
<th>n</th>
<th>Duration of NREMS Episodes, min</th>
<th>No. of NREMS Episodes</th>
<th>Duration of REMS Episodes, min</th>
<th>No. of REMS Episodes</th>
<th>Sleep Cycle Length REMS-REMS Intervals, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>0</td>
<td>Light</td>
<td>6</td>
<td>1.75 ± 0.14</td>
<td>6</td>
<td>0.14</td>
<td>1.73 ± 0.14</td>
<td>308±22.4</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>Light</td>
<td>6</td>
<td>1.77 ± 0.12</td>
<td>6</td>
<td>0.14</td>
<td>1.67 ± 0.12</td>
<td>296±32.6</td>
</tr>
<tr>
<td>IL-4</td>
<td>0</td>
<td>Dark</td>
<td>6</td>
<td>2.57 ± 0.13</td>
<td>6</td>
<td>0.14</td>
<td>2.50 ± 0.20</td>
<td>241±13.5</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>Dark</td>
<td>6</td>
<td>2.43 ± 0.12</td>
<td>6</td>
<td>0.14</td>
<td>2.27 ± 0.16</td>
<td>224±9.7</td>
</tr>
<tr>
<td>IL-4</td>
<td>0</td>
<td>Dark</td>
<td>6</td>
<td>2.44 ± 0.14</td>
<td>6</td>
<td>0.14</td>
<td>2.35 ± 0.16</td>
<td>242±19.0</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>Dark</td>
<td>6</td>
<td>2.37 ± 0.14</td>
<td>6</td>
<td>0.14</td>
<td>2.26 ± 0.23</td>
<td>218±13.3</td>
</tr>
<tr>
<td>IL-4</td>
<td>0</td>
<td>Dark</td>
<td>6</td>
<td>2.05 ± 0.14</td>
<td>6</td>
<td>0.14</td>
<td>2.25 ± 0.14</td>
<td>264±16.6</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>Dark</td>
<td>6</td>
<td>2.09 ± 0.13</td>
<td>6</td>
<td>0.14</td>
<td>2.22 ± 0.11</td>
<td>252±10.2</td>
</tr>
<tr>
<td>IL-4</td>
<td>0</td>
<td>Light</td>
<td>6</td>
<td>2.65 ± 0.24</td>
<td>6</td>
<td>0.14</td>
<td>2.57 ± 0.20</td>
<td>228±14.5</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>Light</td>
<td>6</td>
<td>2.52 ± 0.17</td>
<td>6</td>
<td>0.14</td>
<td>2.41 ± 0.23</td>
<td>216±13.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. rabbits studied.
DISCUSSION

The major finding reported here is that IL-4 inhibits NREMS. This action of IL-4 is likely due to IL-4 inhibition of production of endogenous somnogens such as IL-1, TNF, NO, and insulin, as well as its stimulatory actions on endogenous sleep-inhibitory substances such as the IL-1RA and the soluble TNF receptor, although it is emphasized that the time courses of these effects in brain remain unknown. The IL-4-induced inhibition of NREMS did not manifest until 8–10 h after injection. There are several possibilities for this delay. For example, the time for the diffusion of IL-4 to effective sites could be long. The location of these sites remains unknown. Alternatively, the NREMS inhibitory properties of IL-4 could be due to its ability to inhibit new production of IL-1β and TNF-α. During the first few daylight hours, brain levels of IL-1β mRNA (44) and TNF-α mRNA (1) and TNF protein (7) are highest. IL-4 was injected 3 h after lights were turned on and thus IL-1 and TNF levels were likely already high. These high levels may be sufficient to maintain sleep for several hours. Consistent with this interpretation are the findings that bolus injection of either IL-1β or TNF-α induces excess NREMS for hours (5, 6, 22). The effects of IL-4 inhibition of IL-1 and TNF production thus could probably not manifest themselves for several hours. In contrast, if endogenous levels of IL-1 or TNF are rapidly reduced, using antibodies or soluble receptors, NREMS is quickly inhibited (30, 45, 46, 48). Furthermore, the current finding that injection of IL-4 at dark onset, when brain IL-1β mRNA and TNF-α mRNA levels are lowest and hence production likely lowest, failed to alter NREMS, is consistent with the interpretation that IL-4 inhibitory effects are due to inhibition of production of these substances. Neverthe-
less, because IL-4 affects several additional substances that alter sleep, it is likely that IL-4 inhibitory actions result from its net effects on all of these substances.

The delay in IL-4 inhibition of NREMS is in part different from the published effects of IL-10 on NREMS. In rats the most effective dose of IL-10 used (50 ng icv) inhibited NREMS during the first 10 postinjection hours (35), whereas after a higher dose (100 ng icv) there was a prolonged delay before NREMS was inhibited. IL-10 and IL-4 share the ability to inhibit production of IL-1 and TNF; the difference in their time courses of effects on NREMS is thus unexpected. Nevertheless, different species were used in these two studies, and in neither study was species-specific IL-4 (this study) or IL-10 (35) used. In a preliminary study, we recently found that in rabbits the inhibitory effects of IL-10 were similar to those of IL-4 reported here; after an 8- to 10-h delay NREMS was inhibited. Furthermore, in that study IL-10 did not affect NREMS if it was given at dark onset (Kushikata, unpublished data).

The doses of IL-10 that Opp and colleagues (35) used did not affect REMS, and the three lower doses of IL-4 used in this study also failed to affect REMS. These results suggest that the regulatory mechanisms for NREMS and REMS are in part distinct. Similar conclusions have been reached by others (e.g., Ref. 51). However, the highest dose of IL-4 tested did inhibit REMS. This REMS inhibition effect was evident within 2–4 h postinjection and then persisted throughout the recording period. The mechanisms of IL-4-induced REMS inhibition are unknown, although IL-4 does inhibit NO synthesis, and brain stem NOergic mechanisms are implicated in REMS regulation (2, 15, 18). However, IL-4 inhibits production of inducible NO synthase, and it is assumed that neural NO synthase is involved in REMS regulation because it colocalizes with choline acetyltransferase in the brain stem, although direct evidence for the involvement of neural NO synthase in REMS regulation is weak.

Although IL-4 inhibits duration of NREMS, it does not affect EEG slow-wave activities (SWA) during NREMS. EEG SWA are posited to be indicative of the intensity of NREMS; the greater the magnitude of EEG SWA, the more intense NREMS (see Refs. 36 and 51 for reviews). During the deep sleep that follows sleep deprivation EEG SWA are “supranormal” (37). Nevertheless, several studies have shown that changes in duration of NREMS can be distinct from changes in EEG SWA. Thus the effects of IL-1β on duration of NREMS and EEG SWA are independent of each other and differentially depend on the time of day IL-1 is given. In rats high doses of IL-1 intracerebroventricularly inhibit NREMS yet enhance EEG SWA (low doses enhance both) (23, 33). Lesions of the anterior preoptic hypothalamus induce transient decreases in NREMS and long-term decreases in EEG SWA (40). Immunoregulations of nerve growth factor-receptive neurons induce differential effects on duration of NREMS and EEG SWA (17). Some hypnotics such as benzodiazepine derivatives decrease low-frequency activity (0.25–10 Hz) while increasing high-frequency (17.0–25.0 Hz) activity. Furthermore, a cafeteria diet induces increases in NREMS but simultaneously decreases EEG SWA (10). Collectively, these data suggest independent regulatory mechanisms for NREMS and EEG SWA; current data are in agreement with this conclusion.

IL-4 also failed to affect Tbr. The changes in Tbr associated with changes in vigilance states remained clear in the IL-4-treated animals, although the magnitudes of these changes were not quantified in this study. These state-coupled changes in Tbr are robust; e.g., they persist in fer bile animals (53) and they persist in animals given cryogenic substances such as α-melanocty-stimulatory hormone (32). IL-4 also failed to induce fevers or reduce Tbr. This suggests that proinflammatory cytokines may have little to do with the regulation of normal body temperature. Similar conclusions were reached earlier (21).

In conclusion, current data are consistent with the hypothesis that the cytokine network in brain is involved with physiological NREMS regulation. IL-4 and other antiinflammatory cytokines may play a role in this process.

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