Vagotony attenuates tumor necrosis factor-α-induced sleep and EEG δ-activity in rats

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Kubota, Takeshi, Jidong Fang, Zhiwei Guan, Richard A. Brown, and James M. Krueger. Vagotomy attenuates tumor necrosis factor-α-induced sleep and EEG δ-activity in rats. Am J Physiol Regulatory Integrative Comp Physiol 280: R1213–R1220, 2001.—Much evidence suggests that tumor necrosis factor-α (TNF-α) is involved in the regulation of physiological sleep. However, it remains unclear whether peripheral administration of TNF-α induces sleep in rats. Furthermore, the role of the vagus nerve in the somnogenic actions of TNF-α had not heretofore been studied. Four doses of TNF-α were administered intraperitoneally just before the onset of the dark period. The three higher doses of TNF-α (50, 100, and 200 μg/kg) dose dependently increased nonrapid eye movement sleep (NREMS), accompanied by increases in electroencephalogram (EEG) slow-wave activity. TNF-α increased EEG δ-power and decreased EEG α- and β-power during the initial 3 h after injection. In vagotomized rats, the NREMS responses to 50 or 100 μg/kg of TNF-α were attenuated, while significant TNF-α-induced increases in NREMS were observed in a sham-operated group. Moreover, the vagotomized rats failed to exhibit the increase in EEG δ-power induced by TNF-α intraperitoneally. These results suggest that peripheral TNF-α can induce NREMS and vagal afferents play an important role in the effects of peripheral TNF-α and EEG synchronization on sleep. Intraperitoneal TNF-α failed to affect brain temperature at the doses tested, thereby demonstrating that TNF-α-induced sleep effects are, in part, independent from its effects on brain temperature. Results are consistent with the hypothesis that a cytokine network is involved in sleep regulation.

electroencephalogram; brain; vagus nerve

TUMOR NECROSIS FACTOR (TNF)-α is involved in physiological sleep regulation (reviewed in Ref. 20). Administration of exogenous TNF-α induces physiological nonrapid eye movement sleep (NREMS) in various species (8, 18, 27, 35). TNF-enhanced sleep appears to be similar to physiological sleep; for instance, it is readily reversible, and sleep-coupled autonomic changes [e.g., brain temperature (T_br)] remain intact (reviewed in Ref. 19). Inhibition of endogenous TNF-α activity during the light period using an anti-TNF-α antibody (30), the soluble TNF receptor (34), or fragments of soluble TNF receptors (31–33) reduces spontaneous sleep. TNF-α is constitutively expressed in the normal brain (2). Brain TNF-α and its mRNA levels correlate with sleep propensity; they are highest at light onset, which is the period of maximal sleep in rats (3, 9). TNF receptor mRNA is also expressed in the normal brain (16). There are two cell surface receptors for TNF (55 and 75 kDa), and the TNF 55-kDa receptor is thought to be involved in sleep regulation (8). Mice lacking the 55-kDa TNF receptor do not exhibit enhanced NREMS responses if given exogenous TNF; these mice also have less spontaneous sleep than do control strains of mice (8). TNF-α activates nuclear factor-κB (NF-κB), a transcriptional factor that can promote transcription of several substances also implicated in sleep regulation, e.g., nitric oxide synthase, cyclooxygenase-2 (Cox-2), the adenosine A_1 receptor, interleukin (IL)-1β, nerve growth factor, and TNF-α (reviewed in Ref. 20).

Many TNF-α actions on the central nervous system (CNS), including anorexia and changes in body temperature and sleep, occur after peripheral administration of TNF-α (8, 23, 27, 29). In humans, blood levels of TNF-α are related to electroencephalogram (EEG) δ-activity during sleep (5). Also, higher plasma levels of TNF-α are thought to contribute to daytime somnolence in patients with obstructive sleep apnea (7). Sleep loss is also associated with increases in TNF plasma levels (15), and the ability of circulating white blood cells to produce TNF-α is enhanced after sleep deprivation (36, 38). Although these findings suggest that peripheral TNF-α is associated with, and may affect, the sleep/wake cycle, the effects of peripheral administration of TNF-α on sleep have not been elucidated in rats. Moreover, how peripheral TNF-α affects the CNS remains obscure. TNF-α could penetrate the blood-brain barrier and thereby affect the CNS (11). Another possible mechanism is that cytokines may act on brain capillary endothelium and circumventricular organs (22). TNF might also act on vagal afferents to transmit information to the brain in a manner similar to that demonstrated for IL-1β (13, 14). For instance,

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Hansen and Krueger (13) reported that subdiaphragmatic vagotomy attenuated IL-1β-induced sleep and fever responses. They also showed that vagotomy blocked IL-1β mRNA expression in the brain that was induced by intraperitoneal administration of IL-1β (14). Furthermore, considerable evidence suggests that the activity of vagal afferents can affect sleep. For instance, EEG synchronization is affected by stimulation of vagal afferents (reviewed in Ref. 25). Repetitive intestinal stimulation or carotid sinus stimulation can induce sleep (reviewed in Ref. 25). Thus we hypothesized that the sleep-promoting effects of systemic TNF-α may be mediated in part by intact vagal afferents. We report here that vagotomy attenuated intraperitoneal TNF-α-induced sleep responses.

**MATERIALS AND METHODS**

**Agents**

Rat recombinant TNF-α was purchased from Peprotech (Rocky Hill, NJ). In a preliminary study, rats were given recombinant rat TNF-α (50 μg/kg) purchased from R&D Systems (Minneapolis, MN); results were indistinguishable from those reported here (data not shown). TNF-α was dissolved in pyrogen-free saline (PFS; Abbott Laboratories, North Chicago, IL) and stored at −20°C until the experiment.

**Animals and Surgery**

Male Sprague-Dawley rats (270–350 g for experiment I and 320–400 g for experiment II) were purchased from Taconic Farms (Germantown, NY). The rats were kept on a 12:12-h light-dark cycle (lights on at 0900) at 23 ± 2°C ambient temperature. They had free access to water and food during the experiment. The implantation surgery for EEG and electromyogram (EMG) electrodes and thermistors was performed under ketamine and xylazine (87 and 13 mg/kg, respectively) anesthesia. Stainless steel jewelry screws for EEG recording were placed over the frontal and parietal cortices. An EMG electrode was implanted in the dorsal neck muscles. To measure Tθ, a calibrated 30-kΩ thermistor (model 44008; Omega Engineering, Stanford, CT) was placed on the dura mater over the parietal cortex. The leads from the EEG and EMG electrodes and the thermistor were routed to a Teflon pedestal. They were attached to the skull with dental acrylic (Duz-All; Coralite Dental Products, Skokie, IL).

**Recording and Analysis**

After the recovery period (at least 1 wk), rats were moved to a sleep-recording chamber (model 352600; Hot Pack, Philadelphia, PA), and they were acclimated to the experimental chamber for at least 7 days. The rats were allowed relatively unrestricted movement inside the recording cages. A flexible tether connected the electrodes and thermistor leads to an electronic swivel (SL6C; Plastics One, Roanoke, VA). The leads from the swivel were routed to Grass model 7D polygraphs in an adjacent room. The EEG was filtered below 0.1 Hz and above 35 Hz. The amplified EEG and EMG signals were digitized at a frequency of 128 Hz, and on-line Fourier analysis of the EEG was performed. Tθ signals were digitized at 2 Hz. The EEG, EMG, and Tθ data were saved on the computer in 10-s intervals. The vigilance states of wakefulness, NREMS, and rapid eye movement sleep (REMS) were determined off-line in 10-s epochs according to criteria previously reported (21). Briefly, wakefulness was characterized by fast low-amplitude EEG waves, gradually increasing Tθ, and a high incidence of gross body movements. NREMS was associated with slow high-amplitude EEG waves, slowly decreasing Tθ, and lack of body movement. In contrast, REMS was characterized by fast low-amplitude EEG waves, appearance of rhythmic 8-EEG, rapidly increasing Tθ at REMS onset, and lack of body movement. The amount of time spent in each vigilance state was calculated hourly. In addition, the number and duration of NREMS and REMS episodes were determined using a computer program. EEG 5-wave activity during NREMS, also called EEG slow-wave activity (SWA), was determined by using 2-h time blocks because within 1-h time blocks there were some missing data. EEG power spectrum analysis during the initial 3-h post-TNF treatment during NREMS was also performed for the 0.5- to 25-Hz frequency range.

**Experimental Protocols**

**Experiment I: Effects of intraperitoneal administration of TNF-α on spontaneous sleep in rats**. Twenty-eight rats were used for this experiment. All rats received PFS intraperitoneally on the control day. The next day, rats were injected with one of the following four doses of TNF-α: 10 μg/kg (n = 8), 50 μg/kg (n = 7), 100 μg/kg (n = 7), and 200 μg/kg (n = 6). The injection volume for each rat was 1 ml/kg, and a corresponding volume of PFS was given as control. All injections took place between 2030 and 2100. After the injection, EEG, EMG, and Tθ were recorded for the next 12 h.

**Experiment II: Effects of subdiaphragmatic vagotomy on TNF-α-induced sleep**. Fourteen rats were randomly assigned to one of two groups. The rats in the first group (sham group; n = 7) received only pyloroplasty surgery. The rats of the second group (vagotomy group; n = 7) received bilateral subdiaphragmatic vagotomy and pyloroplasty under ketamine and xylazine (87 and 13 mg/kg, respectively) anesthesia according to the method of Hansen et al. (12–14). Four weeks later, to verify the effectiveness of the vagotomy, rats received intraperitoneal injections of saline and 4 μg/kg CCK (Sigma, St. Louis, MO) after 22 h of food deprivation, as previously described (12–14). CCK inhibits food intake in normal or sham-operated animals but not in vagotomized rats; this test was originally described by Smith et al. (28) and is now widely used to verify the effectiveness of subdiaphragmatic vagotomies (reviewed in Ref. 26). Three days were allowed between the saline and CCK injections. Food intake (in g) was measured during the first hour after injection. Rats with verified vagotomies and sham rats then received EEG and EMG electrodes and thermistor implantation surgery as described above in experiment I. After the second surgery, an additional 2 wk were allowed for recovery and adaptation before the sleep experiment was started. After the first surgery, rats went through an initial 1- to 2-day period of weight loss, then after that they gained weight at the same rate as sham-operated controls (13). These rats received two doses of TNF-α 10 days apart. All rats received an intraperitoneal injection of PFS on the control day. On the next day, they received intraperitoneal TNF-α (50 μg/kg (n = 6 in both groups) or 100 μg/kg (n = 7 in both groups)). The injection volume was 1 ml/kg. After the injection, EEG, EMG, and Tθ were recorded for 12 h. All injections took place between 2030 and 2100.

**Statistical Analysis**

Two-way ANOVA for repeated measures across the 12-h recording period was used. The first independent variable
was the treatment (saline vs. TNF-α), and the second independent variable was time. When ANOVA indicated significant effects, it was followed by the Student-Newman-Keuls (SNK) test to reveal where the significant effect occurred. Four 3-h time blocks were used for the analyses of the time spent in each vigilance state, EEG SWA, and T_br. For the sleep episode data, one-way ANOVA for repeated measures was used for the entire 12-h time period. Paired or unpaired t-tests were used for the comparison of the body weight and CCK test data. For power spectrum analysis data, the EEG power density values were summed in four frequency bands—slow-wave (0.5–4.0 Hz), slow (4.5–8.0 Hz), α (8.5–12.0 Hz), and β (12.5–25.0 Hz)-wave activities; separate ANOVAs for each bandwidth were performed for the initial 3-h time block. A significance level of P < 0.05 was accepted.

RESULTS

Experiment I: Effects of Intraperitoneal Administration of TNF-α on Spontaneous Sleep in Rats

NREMS dose dependently increased after TNF-α intraperitoneal injections [ANOVA for the 12-h period, 50 μg/kg: treatment effect, F(1,6) = 6.00, P < 0.05; 100 μg/kg: treatment effect, F(1,6) = 30.38, P < 0.005 with time-treatment interaction, F(3,18) = 3.38, P < 0.05; 200 μg/kg: treatment effect, F(1,5) = 8.00, P < 0.05 with time-treatment interaction, F(3,15) = 9.38, P < 0.005; Table 1]. These effects were most evident during the initial 6 h after the injections (Fig. 1). The increase in time spent in NREMS resulted from an increase in the number and duration of NREMS episodes, although neither of these parameters was significantly increased after intraperitoneal TNF-α (Table 1). The lower three doses of TNF-α did not affect REMS. The highest dose of TNF-α (200 μg/kg) significantly decreased REMS [ANOVA, treatment effect; F(1,5) = 11.60, P < 0.05], and this effect was due to a decrease in the number of REMS episodes [ANOVA, F(1,5) = 7.97, P < 0.05].

Although SNK tests in each of the time blocks did not reach significance, EEG SWA during NREMS increased after the two higher doses of TNF-α during the initial 4 to 6 h postinjection [ANOVA, 100 μg/kg: time-treatment interaction, F(3,18) = 7.66, P < 0.005; 200 μg/kg: time-treatment interaction, F(3,15) = 3.48, P < 0.05; Fig. 1]. EEG power spectrum analysis during the initial 3 h also showed that TNF-α dose dependently increased EEG δ-power and decreased EEG α- and β-activities [ANOVA for δ-band; 200 μg/kg; F(1,5) = 10.80, P < 0.05; α-band; 100 μg/kg; F(1,6) = 9.54, P < 0.05; 200 μg/kg; F(1,6) = 16.70, P < 0.01; β-band; 100 μg/kg; F(1,5) = 11.30, P < 0.05; 200 μg/kg; F(1,5) = 17.40, P < 0.01; Table 2 and Fig. 2A]. TNF-α failed to affect T_br after any dose.

Experiment II: Effects of Subdiaphragmatic Vagotomy on TNF-α-Induced Sleep

The mean body weights in the sham and vagotomy group just before the CCK test were 382.9 ± 7.3 and 364.7 ± 6.7 g, respectively (unpaired t-test, not significant (NS)). CCK significantly inhibited food intake in the sham group (saline 8.2 ± 1.9 g vs. CCK 4.7 ± 0.8 g, paired t-test, P < 0.05) but not in the vagotomy group (saline 5.4 ± 0.5 g vs. CCK 5.9 ± 0.8 g, paired t-test; NS).

NREMS was significantly increased after 50 or 100 μg/kg of TNF-α intraperitoneally in the sham group; these effects were similar in magnitude to those described in experiment I [ANOVA, treatment effect; 50 μg/kg: F(1,5) = 6.76, P < 0.05; 100 μg/kg: F(1,6) = 115.86, P < 0.0001; Table 3 and Fig. 3]. The enhanced NREMS in the sham group was due to an increase in the mean duration of NREMS episodes [ANOVA, 100 μg/kg: F(1,6) = 11.00, P < 0.05]. In the vagotomy group, TNF-α given intraperitoneally failed to significantly enhance NREMS, although small nonsignificant increases in NREMS were observed [ANOVA, treatment effect; 50 μg/kg: F(1,5) = 1.42 (NS); 100 μg/kg: F(1,6) = 3.39 (NS)]. In the vagotomy group, the mean duration of NREMS episodes was suppressed after intraperitoneal TNF-α [ANOVA, 100 μg/kg: F(1,6) = 7.30, P < 0.05]. Although numbers of NREMS episodes tended to increase after intraperitoneal TNF-α in both groups, these changes did not reach significance. In the sham group, REMS significantly decreased after 100

<table>
<thead>
<tr>
<th>Conditions</th>
<th>During the recording period, min</th>
<th>Duration of episodes, min</th>
<th>No. of episodes</th>
<th>During the recording period, min</th>
<th>Duration of episodes, min</th>
<th>No. of episodes</th>
<th>T_br During the recording period, min</th>
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</thead>
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<tr>
<td>Substance</td>
<td>Dose, μg/kg</td>
<td>n</td>
<td></td>
<td></td>
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<tr>
<td>TNF-α</td>
<td>0</td>
<td>8</td>
<td>185.4 ± 8.0</td>
<td>2.00 ± 0.10</td>
<td>93.3 ± 3.8</td>
<td>35.8 ± 7.4</td>
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<td></td>
<td>10</td>
<td>6</td>
<td>185.1 ± 11.6</td>
<td>2.01 ± 0.12</td>
<td>92.9 ± 4.7</td>
<td>31.8 ± 6.0</td>
<td>1.46 ± 0.11</td>
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<td>TNF-α</td>
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<td>186.2 ± 11.2</td>
<td>1.95 ± 0.09</td>
<td>95.9 ± 5.2</td>
<td>26.2 ± 5.9</td>
<td>1.38 ± 0.12</td>
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<td>50</td>
<td>6</td>
<td>216.3 ± 17.5</td>
<td>2.13 ± 0.19</td>
<td>105.2 ± 10.9</td>
<td>25.3 ± 4.2</td>
<td>1.32 ± 0.07</td>
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<tr>
<td></td>
<td>0</td>
<td>7</td>
<td>169.9 ± 7.2</td>
<td>1.92 ± 0.11</td>
<td>89.3 ± 2.7</td>
<td>24.9 ± 2.8</td>
<td>1.29 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>6</td>
<td>232.9 ± 12.2**</td>
<td>2.15 ± 0.12</td>
<td>110.6 ± 8.8</td>
<td>29.0 ± 4.5</td>
<td>1.34 ± 0.09</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0</td>
<td>6</td>
<td>188.2 ± 12.9</td>
<td>2.26 ± 0.15</td>
<td>85.2 ± 8.8</td>
<td>34.9 ± 5.6</td>
<td>1.30 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>6</td>
<td>247.2 ± 14.2</td>
<td>2.39 ± 0.09</td>
<td>104.3 ± 8.3</td>
<td>25.6 ± 5.1**</td>
<td>1.23 ± 0.11</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. TNF-α, tumor necrosis factor-α. Sleep times are expressed as the number of minutes spent in nonrapid eye movement sleep (NREMS) or rapid eye movement sleep (REMS) for the 12-h postinjection period. Brain temperatures (T_br) were collected in 10-s intervals. The number and length of the episodes were determined using a computer program with the criterion that each episode lasted at least 30 s. *P < 0.05 vs. vehicle treatment.
μg/kg of intraperitoneal TNF-α [ANOVA, treatment effect; \( F(1,6) = 13.49, P < 0.05 \)], and this was due to a decrease in the number of REMS episodes [ANOVA, 100 μg/kg: \( F(1,6) = 16.70, P < 0.01 \)]. The REMS inhibitory effect was not observed in the vagotomy group (Table 3 and Fig. 3).

Although the changes in EEG SWA after 50 and 100 μg/kg of TNF-α injections did not reach significance in either group, EEG power spectrum analysis during the initial 3 h showed that EEG δ-power tended to be enhanced after 50 or 100 μg/kg of intraperitoneal TNF-α in the sham group but not in the vagotomy group. The increase in EEG δ-power after 100 μg/kg of intraperitoneal TNF-α in the sham group was significantly higher than that in the vagotomy group [\( F(1,12) = 4.85, P < 0.05 \); Table 2 and Fig. 2B]. EEG power spectrum analysis also showed that 100 μg/kg of TNF-α significantly decreased θ-, α-, and β-power in the sham group [ANOVA for θ-band; \( F(1,6) = 10.50, P < 0.05 \); α-band: \( F(1,6) = 14.50, P < 0.01 \); β-band: \( F(1,6) = 7.92, P < 0.05 \)] and δ-power in the vagotomy group [ANOVA; \( F(1,6) = 8.50, P < 0.05 \); Table 2 and Fig. 2B]. As in experiment I, TNF-α did not affect the Tbr.

Table 2. EEG power spectrum analysis during initial 3 h after TNF treatment

<table>
<thead>
<tr>
<th>Rat</th>
<th>TNF-α Dose, μg/kg</th>
<th>n</th>
<th>Δ (0.5–4.0 Hz)</th>
<th>θ (4.5–8.0 Hz)</th>
<th>α (8.5–12.0 Hz)</th>
<th>β (12.5–25.0 Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>8</td>
<td>100.6 ± 4.3</td>
<td>96.0 ± 3.4</td>
<td>97.0 ± 3.3</td>
<td>97.9 ± 3.6</td>
</tr>
<tr>
<td>Normal</td>
<td>50</td>
<td>7</td>
<td>108.3 ± 5.5</td>
<td>97.1 ± 1.9</td>
<td>94.3 ± 3.0</td>
<td>96.4 ± 3.2</td>
</tr>
<tr>
<td>Normal</td>
<td>100</td>
<td>7</td>
<td>115.9 ± 7.4</td>
<td>91.9 ± 5.0</td>
<td>78.5 ± 5.8</td>
<td>76.7 ± 4.2</td>
</tr>
<tr>
<td>Normal</td>
<td>200</td>
<td>6</td>
<td>118.7 ± 4.8</td>
<td>92.3 ± 5.1</td>
<td>82.3 ± 4.3</td>
<td>80.8 ± 4.2</td>
</tr>
<tr>
<td>Sham</td>
<td>50</td>
<td>6</td>
<td>105.2 ± 5.6</td>
<td>97.8 ± 3.3</td>
<td>93.7 ± 2.3</td>
<td>96.0 ± 3.4</td>
</tr>
<tr>
<td>Sham</td>
<td>100</td>
<td>7</td>
<td>99.1 ± 3.2</td>
<td>96.5 ± 1.4</td>
<td>93.9 ± 2.9</td>
<td>95.6 ± 3.7</td>
</tr>
<tr>
<td>Vagotomy</td>
<td>50</td>
<td>6</td>
<td>114.5 ± 7.3</td>
<td>90.1 ± 3.6</td>
<td>83.2 ± 4.0</td>
<td>81.8 ± 6.5</td>
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<tr>
<td>Vagotomy</td>
<td>100</td>
<td>7</td>
<td>94.6 ± 5.3</td>
<td>89.8 ± 3.7</td>
<td>88.1 ± 4.9</td>
<td>91.8 ± 5.1</td>
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</table>

Values are means ± SE; n, no. of rats. Electroencephalogram power density values were summed in the following four frequency bands: Δ (0.5–4.0 Hz), θ (4.5–8.0 Hz), α (8.5–12.0 Hz), and β (12.5–25.0 Hz). Values obtained during NREMS during the first 3 h postinjection in each rat were averaged. Next, rats were normalized to the percent values of the control recording. *P < 0.05 vs. control. †P < 0.05 vs. sham group.
DISCUSSION

One of the major findings of this study is that intraperitoneal administration of TNF-α increased NREMS in rats. Previously, we showed that intravenous injections of TNF-α enhanced NREMS in rabbits (18) and that intraperitoneal injections of TNF-α increased NREMS in mice (8). The current results extend those findings to rats and expand on them by providing EEG spectral analysis after TNF-α treatment.

An important finding in the current study is that vagotomy attenuates TNF-α-induced NREMS responses. This finding is similar to that reported by Hansen and Krueger (13) after intraperitoneal IL-1β injection in vagotomized animals. In that study, the NREMS-promoting actions of intraperitoneal IL-1β in vagotomized rats were blocked after a low dose of IL-1β, attenuated after a midlevel dose, and not affected after a high IL-1β dose. As a consequence, those authors concluded

![Fig. 2. EEG power spectrum analysis for the initial 3 h postinjection during NREMS. Average power values for each animal and for each frequency band during control recording were normalized to 100%, and then all frequency band densities in the TNF-α treatment groups were converted to relative power data. A: 10 μg/kg (●), 50 μg/kg (○), 100 μg/kg (▲), and 200 μg/kg (▲) of TNF-α treatment. TNF-α dose dependently increased EEG δ-power and decreased EEG α- and β-powers. B: 50 μg/kg of TNF-α treatment in the vagotomy group (●), 50 μg/kg of TNF-α treatment in the sham group (○), 100 μg/kg of TNF-α treatment in the vagotomy group (▲), and 100 μg/kg of TNF-α treatment in the sham group (▲). In the vagotomy group, there was no significant increase in EEG δ-power after the TNF-α injections.](image-url)
It is likely that similar mechanisms exist for the systemic actions of TNF-α on the brain. Thus there is an active transport system for TNF-α from blood to brain (11). TNF-α also can enter the brain via circumventricular organs; lesion of the area postrema attenuates TNF-induced anorexia (1). TNF could also act directly on organs; lesion of the area postrema attenuates TNF-vagotomy groups. Vehicle control (100 μg/kg) in the sham and vagotomy groups. Vagus nerve stimulation blocks conditioned taste aversion (10) and hyperalgesia (37) induced by systemic TNF-α.

In this study, we also showed that systemic TNF-α administration increases EEG δ-wave activity. This response was not observed in the vagotomized rats. EEG δ-wave amplitudes are thought to reflect the intensity of NREMS. For example, EEG SWA increases to supranormal levels during the deep sleep after sleep deprivation in rabbits (24). The effects of TNF-α on EEG SWA were different from those of IL-1β. In rats, intraperitoneal injection of IL-1β induces biphasic EEG SWA responses. Thus EEG SWA is significantly inhibited in the first 2-h post-IL-1β treatment and is then followed by an increase in EEG SWA for 4 h (13). Furthermore, that report showed that those changes in EEG SWA induced by IL-1β were not affected by subdiaphragmatic vagotomy. Our previous study showed that systemic TNF-α decreased EEG SWA in mice (8). The reason for this discrepancy is unknown; probably, species differences are involved. The current study is consistent with the previous finding that blood levels of TNF-α are related to the EEG δ-activity during sleep in humans (5). Furthermore, Chase et al. (4) showed that electrical stimulation of vagal afferents induced EEG synchronization in cats. Thus it is likely that systemic TNF-α stimulates vagal afferents and thereby increases EEG SWA.

In the current study, we did not show any changes in Tbr in response to TNF-α. In contrast, several reports suggest that TNF-α is involved in fever responses. In rabbits, intracerebroventricular injection or intravenous injection of TNF-α induces fevers (18, 23). Furthermore, inhibition of TNF-α using a TNF receptor fragment inhibits muramyl dipeptide-induced fever (32). TNF-α induces Cox-2 via the activation of NF-κB and thereby promotes the production of prostaglandins (17). Terao and co-workers (35) reported that continuous infusion of TNF-α into the subarachnoid space of the rostral basal forebrain induces fever, and these reactions are blocked by a Cox-2 inhibitor in rats. Although TNF-α is a pyrogenic substance when it is directly administered in the CNS, the effects of peripheral TNF-α on body temperature are complicated. De-riijk and Berkenbosch (6) reported that intravenous
administration of lipopolysaccharide induced hypothermia, and this effect was due to the release of TNF from peripheral macrophages. It was hypothesized that peripheral TNF activates the arginine vasopressin system, which is thought to act as an antipyretic in the ventral septal area. Thus TNF-α can simultaneously activate central pyretic and antipyretic mechanisms in rats, and this might explain why we did not observe TNF-induced effects on body temperature in this study. Regardless of such possibilities, the current results clearly indicate that TNF-α-induced fevers are not responsible for TNF-α-induced NREMS responses.

In summary, we reported herein that intraperitoneal administration of TNF-α induces NREMS and EEG synchronization in rats without a concomitant increase in Tpr. The sleep responses were attenuated by subdiaphragmatic vagotomy. These results suggest that TNF-α stimulates vagal afferents, and this action is involved in the modulation of sleep by systemic TNF-α.

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