

# Vagotomy attenuates but does not prevent the somnogenic and febrile effects of lipopolysaccharide in rats

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**Kapás, Levente, Michael K. Hansen, Hee-Yoon Chang, and James M. Krueger.** Vagotomy attenuates but does not prevent the somnogenic and febrile effects of lipopolysaccharide in rats. *Am. J. Physiol.* 274 (*Regulatory Integrative Comp. Physiol.* 43): R406–R411, 1998.—The role of the vagus nerve in the somnogenic and pyrogenic effects of lipopolysaccharide (LPS) was studied in rats. Control rats ( $n = 8$ ) and rats subjected to bilateral subdiaphragmatic vagotomy (VX;  $n = 9$ ) were injected with 100  $\mu\text{g}/\text{kg}$  ip LPS at the beginning of the dark period. Sleep and brain temperature ( $T_{\text{br}}$ ) were recorded for 23 h after the injections. LPS caused increases in non-rapid eye movement sleep (NREMS) for 12 h after the injection in control rats. Sleep intensity, as indicated by the slow-wave activity (SWA) of the electroencephalogram during NREMS, was suppressed. LPS elicited biphasic  $T_{\text{br}}$  responses: an initial hypothermia was followed by increases in  $T_{\text{br}}$  that lasted for  $\sim 20$  h. In vagotomized rats, the NREMS responses to LPS were blunted. The magnitude of the LPS-induced NREMS increases was about one-half of that seen in control rats, and these sleep responses lasted only for 6 h. LPS did not affect SWA in VX animals. VX completely abolished the hypothermic responses to LPS and shortened the duration of the hyperthermia. The results suggest that the subdiaphragmatic vagi play an important, but not exclusive, role in the somnogenic and pyrogenic actions of intraperitoneally injected LPS.

thermoregulation; electroencephalogram slow-wave activity; vagus nerve

SYSTEMIC INFECTIONS, peripheral lipopolysaccharide (LPS) treatments, and several cytokines all induce central nervous system-related symptoms of the acute phase response, such as increased non-rapid eye movement sleep (NREMS) and fever (21). Although LPS itself does not enter the brain after systemic administration (6, 29), there are several possible mechanisms by which microbial products and the immune system may affect brain function. Numerous central effects induced by peripheral injection of LPS or cytokines are inhibited by vagus nerve transection, suggesting a neural communication pathway for systemic cytokines between the periphery and the brain. For example, interleukin-1 (IL-1)-induced fever (34), taste aversion (12), and depression in food-motivated behavior (2), as well as LPS-induced gene expression (11, 23, 33), behavioral changes (23), adrenocorticotropin hormone responses (11), hyperalgesia (35), and fever (30) are blocked or attenuated by vagotomy.

Sensory signals arriving via vagus afferents affect cerebrocortical activity and sleep stages. Electrical vagoaortic stimulation causes "vagoaortic sleep" in encephale isolé preparations (reviewed in Ref. 28). Depending on the stimulus parameters, vagal stimulation may cause electroencephalogram (EEG) synchroni-

zation or desynchronization and increases or decreases in the amounts of NREMS and REMS (reviewed in Ref. 28) and may suppress epileptic activities (36). Stimulation of the cervical vagus with electrical pulses that predominantly activate slow-conducting vagal afferents causes cortical synchronization, whereas stimuli that predominantly activate fast-conducting afferents elicit cortical desynchronization (4). The vagus may also mediate the somnogenic effects of physiological stimuli. For example, the sleep-inducing effects of increased eating are inhibited by subdiaphragmatic vagotomy (14). We hypothesized that the NREMS-promoting effects of systemic LPS treatment require intact vagus nerves in rats. To test this hypothesis, we studied the effects of systemic LPS injection on sleep and brain temperature ( $T_{\text{br}}$ ) in vagotomized and sham-operated rats.

## METHODS

**Materials.** LPS from *Escherichia coli* serotype 055:B5 (Sigma, St. Louis, MO) was dissolved in isotonic NaCl and was injected in a volume of 2 ml/kg ip.

**Animals.** Male Sprague-Dawley rats (250–350 g) were used. With the use of combined ketamine (87 mg/kg) and xylazine (13 mg/kg) anesthesia, nine rats were subjected to bilateral subdiaphragmatic vagotomy (VX group), supplemented with pyloroplasty. Eight sham-operated animals received only pyloroplasty (control group). Two weeks after the abdominal surgeries, all rats were implanted with cortical EEG and nuchal electromyographic (EMG) electrodes and a brain thermistor. The EEG electrodes were placed over the frontal, parietal, and occipital cortices, and the thermistor was placed on the dura over the parietal cortex. After a 1-wk recovery period, the animals were placed into sound-attenuated individual sleep recording cages for adaptation to the experimental conditions. During the 5- to 7-day habituation period, the animals were connected to recording cables and injected daily with isotonic NaCl solution intraperitoneally at dark onset. The animals were kept on a light-dark cycle of 12:12 h (light onset at 0800) and at  $26 \pm 1^\circ\text{C}$  ambient temperature for at least 2 wk before surgeries, during the recovery, habituation, and experimental periods. Water and food were available ad libitum throughout the experiment. After the experiments, the completeness of the vagotomies was verified using the 2-deoxy-D-glucose/neutral red test (5).

**Recordings.** EEG, EMG, and  $T_{\text{br}}$  were recorded by computer. EMG activity served as an aid for determining the vigilance states and was not further quantified. EEG was filtered below 0.1 Hz and above 40 Hz. The amplified signals were digitized at the frequency of 128 Hz for EEG and EMG and at 2 Hz for  $T_{\text{br}}$ . Single  $T_{\text{br}}$  samples were saved on the hard disk in 10-s intervals. Average  $T_{\text{br}}$  was calculated in 1-h time blocks. The vigilance states were determined off-line in 10-s epochs. EEG, EMG, and  $T_{\text{br}}$  were displayed on the computer monitor in 10-s epochs and also simultaneously in a more

condensed form in 12-min epochs. Wakefulness, NREMS, and REMS were distinguished as described before in detail (19). Time spent in NREMS and REMS was calculated in 1-h time blocks and for the whole 23-h period; in addition, the 23-h recording period was divided into, and sleep amounts were also calculated in, four (6, 6, 6, and 5 h) and two (12-h nighttime and 11-h daytime) time blocks after the injections. Numbers and average durations of the sleep episodes, which lasted for at least 20 s, were calculated separately for the entire dark and light periods.

On-line fast Fourier analysis (FFT) of the EEG was also performed in 10-s intervals on 2-s segments of the EEG in 0.5-Hz bands of the 0.5- to 4-Hz frequency range. The EEG power density values in the delta frequency range were summed for each 10-s epoch. Hourly average activities ( $\mu\text{V}^2$ ) were then calculated for the NREMS epochs. The delta activity during NREMS [also called slow-wave activity (SWA)] is often regarded as a measurement of sleep intensity. On the baseline day, the average SWA was computed across the entire 23 h for each rat to obtain a reference value for each animal. SWA for each hour on the baseline and test days was then expressed as a percent of that reference value. Group averages were calculated hourly and also for the entire dark and light periods.

**Experimental protocol.** On the baseline day, the animals were injected with isotonic NaCl intraperitoneally. On the test day, the rats received 100  $\mu\text{g}/\text{kg}$  ip LPS. The baseline and test days were separated by 3 recovery days when saline was injected intraperitoneally at dark onset, but sleep was not recorded. The order of the baseline and test days was counterbalanced. EEG, EMG, and  $T_{\text{br}}$  were recorded for 23 h after injections. Because of the malfunction of one thermistor, the animal number for  $T_{\text{br}}$  in the VX group is eight. Two rats from each group were excluded from the FFT analysis because their EEGs were contaminated with artifacts; the vigilance states of these animals, however, could be determined.

**Statistical analysis.** To analyze the effects of the LPS treatment within a group of animals, two-way analysis of variance (ANOVA) for repeated measures was performed. We compared values obtained on the baseline day to those obtained on the LPS day for sleep,  $T_{\text{br}}$ , or SWA values averaged in 1-h time blocks. Separate ANOVAs were performed for the first 6-h, the second 6-h, and the last 11-h period. When ANOVA indicated significant treatment effects, the Student-Newman-Keuls (SNK) test was performed a posteriori (Table 2).

The average nighttime and daytime sleep amounts, episode numbers and durations, and SWA after saline and LPS treatments within the same groups were compared using paired  $t$ -test; the comparisons between VX and control groups were done using SNK test (Table 1).

Differences in baseline sleep or  $T_{\text{br}}$  between the control and VX animals as well as the differences in the effects of LPS between two groups were determined using two-way ANOVA across the 23-h recording period on values averaged in 1-h time blocks.

## RESULTS

**Effects of LPS in control rats.** LPS induced excess NREMS in the dark period after a latency of 1 h (Fig. 1 and Tables 1 and 2). NREMS was significantly increased both in the first and second 6-h postinjection periods by  $55.2 \pm 10.6$  and  $14.0 \pm 2.8\%$ , respectively. This effect was mainly due to a significant increase in the numbers of NREMS epochs in the night. In the light

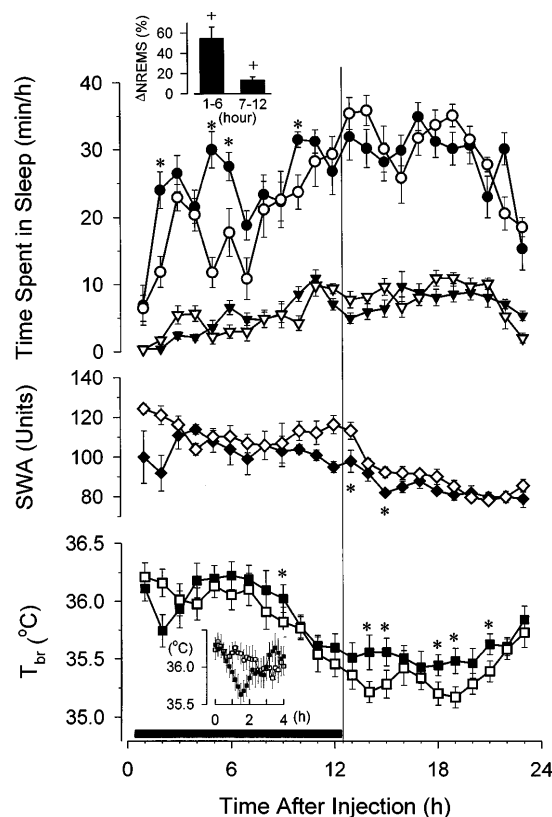


Fig. 1. Effects of lipopolysaccharide (LPS) on non-rapid eye movement sleep (NREMS; circles), rapid eye movement sleep (REMS; triangles), slow-wave activity of the electroencephalogram during NREMS (SWA; diamonds), and brain temperature ( $T_{\text{br}}$ ; squares) in control rats. Data points represent values averaged in 1-h intervals ( $\pm$ SE). Baseline day: open symbols; LPS treatment: solid symbols. *Top inset:* %change in NREMS from baseline in the 1st and 2nd 6-h time period after LPS injection. *Bottom inset:*  $T_{\text{br}}$  values for the 1st 4-h period after injections sampled in 10-min intervals. Horizontal dark bar: dark period of the day. \* Significant difference from baseline ( $P < 0.05$ , Student-Newman-Keuls test); † significant change from baseline ( $P < 0.05$ , paired  $t$ -test). LPS induced increases in NREMS, decreases in SWA, and biphasic  $T_{\text{br}}$  responses in control rats.

period, NREMS returned to the baseline levels. The amount of REMS, the number of REMS epochs, and the average duration of REMS episodes were not affected by LPS. During the first 12 h after LPS injection, there was a tendency toward decreased SWA. In the subsequent light period, the suppression reached a statistically significant level as indicated by ANOVA. There was a biphasic  $T_{\text{br}}$  response to LPS. After a transient suppression in the second hour after the LPS injection,  $T_{\text{br}}$  was significantly elevated by an average of 0.1–0.35°C for the rest of the dark period and throughout the light period.

**The effects of LPS in VX rats.** Vagotomy itself did not cause any gross behavioral changes in the animals. The rats appeared healthy and gained weight at a rate similar to the controls. In the light period of the baseline day, the amounts of NREMS in VX animals were  $\sim 8.2\%$  higher, whereas the amounts of REMS were  $\sim 15.1\%$  lower than those in controls ( $P < 0.05$  for both, SNK test) (Fig. 2 and Tables 1 and 2). There was no difference in the amount of sleep at night, and the

Table 1. Effects of saline and LPS on the amounts of REMS, NREMS, the number and average durations of NREMS and REMS epochs, and on SWA in the dark and the light period of the day

	Control				Vagotomized			
	Dark		Light		Dark		Light	
	Saline	LPS	Saline	LPS	Saline	LPS	Saline	LPS
NREMS, min	226.9 ± 11.3	290.1 ± 7.9*	324.9 ± 7.7	314.4 ± 11.7	253.9 ± 13.1	260.5 ± 16.7	351.6 ± 5.5†	326.4 ± 7.4*
REMS, min	54.9 ± 5.6	57.2 ± 3.5	88.7 ± 2.7	81.3 ± 3.2	53.0 ± 3.7	59.5 ± 6.1	75.3 ± 3.0†	79.3 ± 3.1
NREMS number of epochs	141.2 ± 2.5	191.5 ± 6.4*	191.3 ± 5.7	197.2 ± 6.2	146.3 ± 6.8	160.6 ± 11.3	191.0 ± 5.5	206.0 ± 3.6
REMS number of epochs	29.4 ± 2.5	31.2 ± 2.5	44.0 ± 1.9	42.8 ± 1.4	31.7 ± 2.8	33.1 ± 4.0	41.8 ± 2.6	41.8 ± 2.5
NREMS epoch length, min	1.63 ± 0.09	1.52 ± 0.06	1.71 ± 0.07	1.60 ± 0.06	1.75 ± 0.08	1.58 ± 0.05	1.86 ± 0.07	1.59 ± 0.04*
REMS epoch length, min	1.84 ± 0.09	1.86 ± 0.07	2.03 ± 0.05	1.91 ± 0.07	1.72 ± 0.11	1.84 ± 0.07	1.84 ± 0.09	1.93 ± 0.08
SWA	100.0 ± 0.0	93.2 ± 1.7*	100.0 ± 0.0	93.8 ± 0.9*	100.0 ± 0.0	103.6 ± 4.7	100.0 ± 0.0	95.6 ± 2.3

Values are means ± SE. LPS, lipopolysaccharide; NREMS, non-rapid eye movement sleep; SWA, slow-wave activity. \* Significant difference from saline treatment ( $P < 0.05$ , Student-Newman-Keuls test). † Significant difference from corresponding saline treatment in the control group ( $P < 0.05$ , Student-Newman-Keuls test).

average number and duration of NREMS and REMS episodes did not differ significantly between control and VX rats in either photoperiod. The  $T_{br}$  of the vagotomized rats on the baseline day was significantly lower by  $\sim 0.6$ – $1.2^\circ\text{C}$  than observed in the controls [ANOVA between the 23-h baseline days of VX and control rats on  $T_{br}$  values averaged in 1-h intervals, treatment effect:  $F(1,154) = 108.48$ ,  $P < 0.05$ ].

The effects of LPS on NREMS and SWA were significantly different in VX rats from those in control animals [ANOVA between the effects of LPS in control vs. VX rats across 23 h, treatment effects, NREMS:  $F(1,345) = 8.24$ ,  $P < 0.05$ ; SWA:  $F(1,234) = 9.03$ ,  $P < 0.05$ ]. NREMS increased in the first 6-h period only by  $25.8 \pm 9.9\%$  in VX animals, less than one-half of that observed in control rats. The NREMS-promoting effects of LPS were also shorter lasting; in the second 6-h period after LPS injection NREMS was below the baseline levels. In the light period, NREMS was significantly suppressed after LPS treatment. This suppression in NREMS was accompanied by a significant decrease in average NREMS epoch length. LPS did not affect the number of NREMS epochs or any measurements of REMS. In contrast to the SWA-suppressive effects in control rats, LPS did not affect SWA in VX animals.

Similar to that observed in control rats,  $T_{br}$  of VX rats was significantly elevated in the latter half of the dark

period after LPS injection [ANOVA for  $T_{br}$ , the effects of LPS in control vs. VX rats, treatment effects across hours 1–12:  $F(1,168) = 4.24$ ,  $P < 0.05$ ; across hours 13–23:  $F(1,154) = 12.16$ ,  $P < 0.05$ ]. There were two major differences, however, between the effects of LPS on  $T_{br}$  in control and VX animals. First,  $T_{br}$  did not decrease in the first 1–2 h after LPS injection and, second,  $T_{br}$  did not remain elevated throughout the 23-h recording period, it returned to the baseline level by the beginning of the light phase of the day in VX rats.

## DISCUSSION

*Effects of LPS in normal rats.* Our results are consistent with the previous findings that systemic administration of LPS increases the time spent in NREMS (3, 20, 22, 27). This increase is due to increased number of NREMS episodes (22; and present results). Previously, a suppression of REMS was also reported in the first 5–12 h after LPS injection (20, 22, 27). In our experiments, there was a tendency toward decreased REMS 2–3 h after injection and throughout the light period but these changes were not statistically significant. It is likely that the effects of LPS on sleep depend on the activity of sleep mechanisms at the time of the LPS treatment. We injected LPS at the beginning of the dark period when the amounts of REMS are the lowest in rats. In the previous studies, LPS was injected in the

Table 2. Effects of LPS on the amount of sleep, SWA, and  $T_{br}$  in control and vagotomized rats: statistical results

	Control			Vagotomized		
	Hours 1–6*	Hours 7–12†	Hours 13–23‡	Hours 1–6*	Hours 7–12†	Hours 13–23‡
NREMS	$F(1,7) = 43.16$ $P < 0.05$	$F(1,7) = 26.63$ $P < 0.05$	$F(1,7) = 1.35$ NS	$F(1,8) = 11.29$ $P < 0.05$	$F(1,8) = 3.34$ NS	$F(1,8) = 22.66$ $P < 0.05$
REMS	$F(1,7) = 0.31$ NS	$F(1,7) = 2.28$ NS	$F(1,7) = 3.14$ NS	$F(1,8) = 1.56$ NS	$F(1,8) = 0.43$ NS	$F(1,8) = 0.62$ NS
SWA	$F(1,5) = 0.86$ NS	$F(1,5) = 4.17$ NS	$F(1,5) = 10.53$ $P < 0.05$	$F(1,6) = 1.84$ NS	$F(1,6) = 0.03$ NS	$F(1,6) = 3.26$ NS
$T_{br}$	$F(1,7) = 0.07$ NS	$F(1,7) = 7.48$ $P < 0.05$	$F(1,7) = 66.07$ $P < 0.05$	$F(1,7) = 1.52$ NS	$F(1,7) = 13.79$ $P < 0.05$	$F(1,7) = 4.05$ NS

Two-way ANOVA for repeated measures was performed between the test (LPS) and corresponding baseline (saline) days. \* ANOVA across the 1st 6-h period after injections on values averaged in 1-h intervals. † ANOVA across the 2nd 6-h period after injections on values averaged in 1-h intervals. ‡ ANOVA across the 13- to 23-h period after injections on values averaged in 1-h intervals.  $P < 0.05$ : significant LPS effects. NS, nonsignificant difference between the effects of LPS and saline.

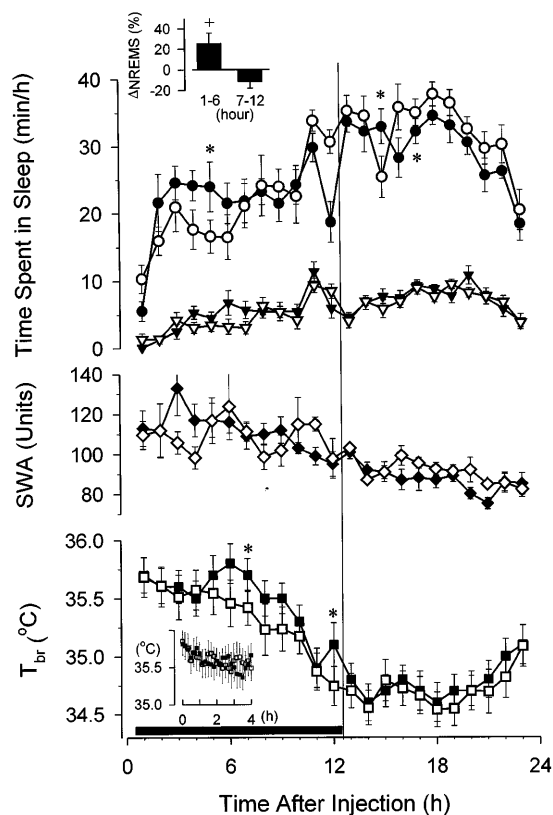


Fig. 2. Effects of LPS on NREMS, REMS, SWA, and  $T_{br}$  in vagotomized rats. See legend to Fig. 1 for details. The NREMS-inducing and febrile effects were attenuated and the hypothermic and SWA-suppressive actions of LPS were completely blocked in vagotomized rats. \*Significant difference from baseline ( $P < 0.05$ ).

light phase of the day when the amount of spontaneously occurring REMS is high. It is likely that the REMS mechanisms are more sensitive to the suppressive effects of LPS when they are highly activated in the light period in rats. Conversely, the NREMS-generating mechanisms may be more sensitive to the stimulatory effects of LPS at night when their spontaneous activity is low. Consistent with this notion, when LPS was injected in rats in the light period, it did not (16) or only slightly stimulated NREMS (3) or its NREMS-inducing effects appeared only after a latency of  $\sim 12$  h, i.e., in the subsequent dark period (22).

We observed suppressed sleep intensity, as indicated by the SWA of the EEG, during NREMS in response to LPS. In humans, SWA did not change after LPS injection (27). In rats, a biphasic SWA response to LPS was previously found: an initial increase was followed by suppressed SWA (22). It is likely that the timing of the LPS treatment is an important factor also in the SWA responses. IL-1 is a somnogenic cytokine that is assumed to play a key role in mediating the sleep-promoting effects of LPS (reviewed in Ref. 21). IL-1 primarily induces NREMS without stimulating SWA when injected at dark onset in rats (26); we injected LPS also at this time point. However, if IL-1 is given at the beginning of the light period, it stimulates SWA without promoting sleep or it even suppresses NREMS in higher doses (26). In the study where a transient

increase in SWA was found after LPS injection (22), the treatments were given at light onset and, similar to the effects of IL-1, not only sleep intensity increased but also the time spent in NREMS decreased in the light period in response to LPS injection. These findings indirectly support the hypothesis that IL-1 may be a key mediator of LPS-induced sleep and SWA responses.

Rats respond to systemic LPS injection with hypothermia (10, 32) and/or fever depending on the dose, ambient temperature, initial body temperature, and rat strain (9, 31). These complex thermoregulatory effects suggest that LPS can activate both pyretic and antipyretic mechanisms in rats; cytokines are thought to play a central role in both of these mechanisms (18). LPS elicits a biphasic thermoregulatory response in the dose range of 20–500  $\mu\text{g}/\text{kg}$  LPS if the animals are kept at subthermoneutral ambient temperature (below 28–30°C). The first phase is a dose-dependent decrease in body temperature, which is followed by fever (9). Our results are fully consistent with these findings. The time course of the hypothermic response in our control animals was very similar to that reported previously after systemic administration of 100  $\mu\text{g}/\text{kg}$  LPS; the decrease reached its maximum  $\sim 90$  min after the injection and the temperature returned to control level  $\sim 120$  min after the LPS treatment (9).

*The effects of VX on the sleep and  $T_{br}$  responses to LPS.* VX attenuated the sleep and febrile responses to LPS, suggesting that the subdiaphragmatic vagi play a significant role in the somnogenic and thermoregulatory effects of intraperitoneally administered LPS. Similarly, VX attenuated the sleep responses to systemic injections of IL-1 $\beta$  (15). It is possible that somnogenic cytokines, such as IL-1 and tumor necrosis factor (TNF) are released in the periphery in response to LPS and act directly on specific receptors on the vagus to promote NREMS. The presence of IL-1 binding sites in the vagal paraganglia (13) and the finding that intraportal injection of IL-1 $\beta$  stimulates the afferent activity of the vagus (25) support this possibility. It cannot be ruled out, however, that the vagus may serve a permissive role rather than be a direct target for cytokines in the somnogenic effects of LPS.

The finding that VX did not block the first phase of febrile or somnogenic effects of LPS indicates that the role of subdiaphragmatic vagi is not exclusive in the somnogenic and pyrogenic actions of LPS. It is likely that LPS also triggers sleep and thermoregulatory mechanisms acting at sites different from the subdiaphragmatic vagi. For example, significant amounts of intraperitoneally injected LPS and/or the cytokines produced in the periphery in response to LPS enter the systemic circulation; e.g., blood levels of LPS and cytokines are higher after LPS treatment (27, 29). When plasma levels of cytokines and LPS are relatively high, they may enter the brain in amounts sufficient to directly activate central somnogenic and pyrogenic structures in addition to the vagus nerve. This may take place at sites where the blood-brain barrier is missing, e.g., the organum vasculosum laminae terminalis or area postrema or via cytokine transporters (1).

Alternatively, circulating LPS and cytokines may stimulate the production of one or more low-molecular-weight messengers outside the blood-brain barrier. These messengers, in turn, cross the blood-brain barrier and act on neurons in the brain. One possible candidate for such a diffusible messenger is NO. LPS induces the excess production of NO by activating inducible NO synthase in endothelial cells (reviewed in Ref. 24). NO, when injected in the cerebral ventricle in the form of NO donor substances, induces increases in NREMS (17). It is possible, therefore, that LPS and/or cytokines produced in response to LPS trigger the production of NO in brain endothelia, and increased brain NO levels will ultimately induce NREMS.

A large fraction of systemically injected LPS is still present in the liver 6–24 h after the injection (29). Liver Kupffer cells produce significant amounts of cytokines on stimulation with LPS (reviewed in Ref. 7). It is possible that the levels of circulating cytokines 6–12 h after the LPS injection are not high enough to activate central mechanisms directly. However, the elevated levels of cytokines produced in the liver in response to residual levels of LPS could be sufficient to activate vagal afferents locally, therefore eliciting delayed sleep and febrile responses.

The hypothermic response to LPS was completely absent in the VX animals. Peripheral macrophage product(s) (9), possibly TNF- $\alpha$  (8), play a key role in LPS-induced hypothermia. It is hypothesized that TNF activates central antipyretic mechanisms, such as the septal arginine vasopressin system (8). Several effects of TNF- $\alpha$  are mediated by the vagus nerve (12), it is possible therefore that its activating effects on central antipyretic mechanisms also require intact vagus nerves. Alternatively, since LPS hypothermia involves reduced thermogenesis (9) and VX rats already have reduced  $T_{br}$ , it is possible that the mechanism that LPS acts on to suppress thermogenesis is already activated in VX animals.

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