Subdiaphragmatic vagotomy blocks the sleep- and fever-promoting effects of interleukin-1β

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Subdiaphragmatic vagotomy blocks the sleep- and fever-promoting effects of interleukin-1β. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1246–R1253, 1997.—The mechanism by which peripheral cytokines signal the central nervous system to elicit central manifestations of the acute phase response remains unknown. Recent evidence suggests that cytokines may signal the brain via the vagus nerve. To test this possibility, we examined sleep-wake activity and brain temperature (T_br) after the intraperitoneal administration of saline or three doses (0.1, 0.5, and 2.5 µg/kg) of interleukin-1β (IL-1β) in subdiaphragmatically vagotomized (Vx) and sham-operated (Sham) rats. The lowest dose of IL-1β (0.1 µg/kg) increased non-rapid eye movement sleep (NREMS) and slightly elevated T_br in Sham rats; both responses were blocked in Vx animals. The middle dose tested (0.5 µg/kg) increased NREMS and T_br in Sham animals; however, in Vx rats, the increase in NREMS was attenuated and the increase in T_br was blocked. The highest dose of IL-1β (2.5 µg/kg) induced increases in NREMS, decreases in rapid eye movement sleep, and a hypothermic response followed by a biphasic fever; these responses were similar in both Sham and Vx rats. These data provide strong evidence that the subdiaphragmatic vagus plays an important role in communicating both sleep and fever signals to the brain. However, there is clearly an alternative pathway by which IL-1 can signal the brain; whether it occurs through activation of other vagal afferents or through direct or indirect actions on the brain remains unknown.

cytokine; vagus nerve; non-rapid eye movement sleep; rapid eye movement sleep; brain temperature; electroencephalogram slow-wave activity

There is considerable evidence linking interleukin-1β (IL-1β) to physiological sleep regulation. Administration of exogenous IL-1β via intraperitoneal, intravenous, or intracerebroventricular routes results in relatively large increases in non-rapid eye movement sleep (NREMS) in rats, rabbits, mice, cats, and monkeys (reviewed in Ref. 19). Inhibition of endogenous IL-1, using the IL-1 receptor antagonist, anti-IL-1β antibodies, or the soluble IL-1 receptor, reduces spontaneous sleep (19) and inhibits sleep rebound after sleep deprivation (35). IL-1β and other members of the IL-1 family of molecules are constitutively expressed in the brain (19). Cat cerebrospinal fluid levels of IL-1 are reported to vary in phase with the sleep-wake cycle (24). Furthermore, there is a diurnal rhythm of IL-1β mRNA in the hypothalamus, hippocampus, and cortex of rats with the highest levels corresponding to peak sleep periods (34). Finally, after sleep deprivation, brain stem and hypothalamic levels of IL-1β mRNA increase (25).

In addition to its role in physiological sleep regulation, IL-1β also appears to play a key role in mediating the various facets of the acute phase response, such as loss of appetite, social withdrawal, fever, and excess sleep, which occur during infectious disease. Indeed, administration of exogenous IL-1β elicits all of these illness responses (17). In contrast, the administration of antibodies or antagonists to IL-1 blocks illness responses induced by agents such as lipopolysaccharide (LPS) (17, 18) or muramyl dipeptide (36). Although it is clear that IL-1β and other products of the immune system, such as tumor necrosis factor-α, have far-ranging effects on central nervous system functions, it is still unclear how such molecules, whether systemically released or experimentally injected, gain access to the brain because most are relatively large and hydrophilic peptides that are not expected to readily cross the blood-brain barrier.

Various hypotheses have been proposed to address this issue, including entry into the brain at sites where the blood-brain barrier is deficient, e.g., through circumventricular organs, and active transport into the brain (1, 3). Although each hypothesis has its supporting data, it is uncertain whether the amounts shown to enter the brain are sufficient to induce sleep and/or fever. Furthermore, peripherally released cytokines induce the synthesis and release of cytokines in the brain, and these locally produced cytokines are likely critical for brain-cytokine actions. For example, an intravenous injection of muramyl dipeptide increases sleep in rabbits; this sleep response is blocked with an intracerebroventricular injection of the IL-1 receptor fragment, an inhibitor of IL-1β (36). Hence, an alternative pathway by which peripheral cytokines can affect central nervous system functions has recently been suggested (reviewed in Refs. 3 and 39): that is, that neural afferents, such as the vagus nerve, are posited to transmit peripheral immune messages to the brain. Indeed, IL-1-induced fever (28, 38), taste aversion (11), and behavioral changes (5, 6), as well as LPS-induced fever (13, 32), gene expression (23, 37), and depression of food-motivated behavior (6) are blocked or attenuated by vagotomy.

In addition to this putative role of the vagal nerve in communicating immune signals to the brain, the vagus nerve is known to play a role in the regulation of vigilance. Vagal nerve stimulation induces electroencephalographic (EEG) synchronization (7) and excess sleep (30). The NREMS-promoting effects of increased food intake are inhibited by vagotomy (M. K. Hansen, L. Kapas, J. Fang, and J. M. Krueger, unpublished data). In addition, viscerosensory activity influences the sleep-wakefulness rhythm. For example, low-frequency stimulation of the small intestine and splanchnic nerve induces EEG activity characteristic of sleep that outlasts the period of stimulation (22), and repetitive intestinal stimulation increases sleep dura-
tion in both starved and satiated cats (21). We hypothe-
sized that the NREMS-promoting effects of intraperi-
toneal IL-1β are dependent on an intact vagal nerve and
that the role of the vagal nerve may vary with
 differing doses of IL-1. To test this hypothesis, subdia-
phragmatically vagotomized (Vx) and sham-operated
(Sham) rats were injected with three doses of IL-1β,
and the resultant effects on sleep-wake activity and
brain temperature (T_br) were evaluated.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (250–275 g at purchase; Harlan
Sprague Dawley, Indianapolis, IN) were used in this study.

Surgical Techniques

Vagotomy and pyloroplasty. Subdiaphragmatic vagotomy
and pyloroplasty were performed on rats as previously de-
scribed (M. K. Hansen, L. Kapás, J. Fang, and J. M. Krueger,
unpublished data). Briefly, after an overnight fast, rats were
anesthetized using ketamine-xylazine (87 and 13 mg/kg ip,
respectively). The stomach and lower esophagus were visual-
ized from an upper midline laparotomy. The stomach was
gently retracted down beneath the diaphragm to clearly
expose both vagal trunks. Each vagal trunk was ligated, and
at least 1 cm of the visible vagal nerve was dissected. In
addition, all neural and connective tissue surrounding the
esophagus immediately below the diaphragm was removed
to transect all small vagal branches. The vagotomy was supple-
mented with pyloroplasty to prevent gastric stasis. An inci-
sion was made parallel to the axis of the pylorus, through the
pyloric sphincter, and then the pylorus wall was recon-
structed by sutures perpendicular to the pylorus axis. The
stomach was returned to its normal position, and the inci-
sions were closed. Sham animals were also prepared, sub-
jected only to pyloroplasty.

It has been suggested that the lack of responsiveness in Vx
rats may be due to their poor health and malnutrition (31).

Sleep surgery. Three weeks after either Vx or Sham sur-
gery, the animals were implanted with cortical EEG and
nuchal electromyographic (EMG) electrodes and a brain
thermistor to measure T_br as previously described (20).

EEG, EMG, and T_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
and above 40 Hz. The amplified signals were digitized at a
frequency of 128 Hz for EEG and EMG and 2 Hz for T_br.

Recordings

Fig. 1. Effects of sham (Sham) or vagotomized (Vx) surgery on body
weights of rats for 2 wk after surgery. Values are means ± SE.

Experimental Protocol

Pyrogen-free saline (0.9% NaCl) was obtained from Abbott
Laboratories (North Chicago, IL), and recombinant human
human IL-1β was obtained from R&D Systems (Minneapolis, MN).
IL-1β was dissolved in pyrogen-free saline and delivered in an
injection volume of 2 ml/kg. All injections were given intraperi-
toneally.

One week after EEG-implant surgery, thus approximately
4 wk after either Vx or Sham surgery, the rats (n = 8 for each
group) were attached to recording cables for habituation.
During this 7- to 10-day period the animals received daily
intraperitoneal injections of saline at the time when the
experimental treatments were to be done. Three doses of
IL-1β were tested (0.1, 0.5, and 2.5 µg/kg). These doses were chosen on the basis of a dose-response curve determined in a pilot study of IL-1β on sleep-wake activity in rats (M. K. Hansen, L. Kapás, and J. M. Krueger, unpublished data). A within-subject experimental design was used; thus the animals were injected with saline on one day (control day) and with one dose of IL-1β on the following day (test day). At least a 1-wk period was allowed between test days. Furthermore, for each rat, recordings were taken on three separate control days. When rats received IL-1β, it was in the order of the lowest dose to highest dose. There were no signs of tolerance to IL-1β in either the pilot study or in this experimental study. All injections were given promptly at dark onset (1800). EEG, EMG, and Tbr were recorded for 23 h beginning at dark onset for each control day (n = 3 for each rat) and each test day.

Verification Procedures

On completion of the experiments the completeness of vagotomy was assessed using two independent approaches. The first test is based on the satiety effect of cholecystokinin (CCK, Ref. 33). Saline or CCK (4 µg/kg; Peninsula Laboratories) was injected intraperitoneally after 20 h of food deprivation, and food intake was measured after 1 h. The second test is based on the stimulation of gastric acid secretion via the vagus nerve by 2-deoxy-D-glucose (2-DG, Ref. 8) and was performed as previously described (M. K. Hansen, L. Kapás, J. Fang, and J. M. Krueger, unpublished data). Briefly, gastrotomy was performed along the greater curvature in anesthetized rats, the mucosa was exposed, and the bleeding points were ligated. A moistened gauze sponge was placed over the gastric mucosa, and 2 ml of 5% 2-DG were injected intravenously via the femoral vein. This was followed, after a period of 10 min, by 1 ml of a 1% solution of neutral red. The moistened sponge was periodically examined for the presence of a purple color. The neutral red, which is secreted in conjunction with gastric acid, appears purple on the sponge in those rats with an intact vagus; all Vx rats failed this test.

Statistical Analysis

The effects of IL-1β on sleep, SWA, and Tbr were determined by two-way analysis of variance (ANOVA) for repeated measures across the 23-h recording period. The first independent variable was the treatment (saline vs. IL-1β) and the second independent variable was time. When ANOVA indicated significant effects, the Student-Newman-Keuls (SNK) test was used to reveal where the significant effect had occurred. In all tests an α-level of P < 0.05 was taken as an indication of statistical significance.

RESULTS

Vagotomy Blocks Satiety Effect of CCK

CCK significantly inhibited food intake in Sham [F(1,14) = 32.748, P < 0.0001; SNK q(4,14) = 14.35, P < 0.01], but not Vx rats, thereby confirming the role of the vagus nerve in CCK-induced satiety (33). Food intake was decreased by about 50% in CCK-injected Sham rats compared with saline-injected Sham rats (3.25 ± 0.40 vs. 6.46 ± 0.4 g, respectively). In contrast, CCK did not significantly decrease food intake in Vx rats compared with saline injection (4.90 ± 0.7 vs. 5.55 ± 0.78 g, respectively).

Vagotomy Blocks Effects of 0.1 µg/kg IL-1β

In Sham rats 0.1 µg/kg IL-1β increased NREMS (Fig. 2, Table 1). On the control day Sham rats spent 61 ± 3 min in NREMS during the first 4 h compared with 90 ±
Table 1. Effects of intraperitoneal injections of IL-1β on sleep, slow-wave activity, and brain temperature in sham-operated and vagotomized rats: statistical results

<table>
<thead>
<tr>
<th>Treatment effect</th>
<th>0.1 µg/kg</th>
<th>0.5 µg/kg</th>
<th>2.5 µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>NREMS Treatment effect</td>
<td>F (1, 7) = 9.32*</td>
<td>F (1, 7) = 0.129</td>
<td>F (1, 7) = 0.129</td>
</tr>
<tr>
<td>Time-treatment interaction</td>
<td>F (5, 35) = 7.76†</td>
<td>F (5, 35) = 0.693</td>
<td>F (5, 35) = 0.693</td>
</tr>
<tr>
<td>REMS Treatment effect</td>
<td>F (1, 7) = 0.0826</td>
<td>F (1, 7) = 0.690</td>
<td>F (1, 7) = 0.690</td>
</tr>
<tr>
<td>Time-treatment interaction</td>
<td>F (11, 77) = 2.0127*</td>
<td>F (11, 77) = 0.645</td>
<td>F (11, 77) = 0.645</td>
</tr>
<tr>
<td>SWA Treatment effect</td>
<td>F (1, 7) = 0.202</td>
<td>F (1, 7) = 0.206</td>
<td>F (1, 7) = 0.206</td>
</tr>
<tr>
<td>Time-treatment interaction</td>
<td>F (11, 77) = 0.949</td>
<td>F (11, 77) = 0.212</td>
<td>F (11, 77) = 0.212</td>
</tr>
<tr>
<td>Tbr Treatment effect</td>
<td>F (1, 7) = 0.145</td>
<td>F (1, 7) = 0.0462</td>
<td>F (1, 7) = 0.0462</td>
</tr>
<tr>
<td>Time-treatment interaction</td>
<td>F (22, 154) = 2.374†</td>
<td>F (22, 154) = 0.4515</td>
<td>F (22, 154) = 0.4515</td>
</tr>
</tbody>
</table>

Each animal in sham-operated (Sham) and vagotomized (Vx) groups received 3 different doses of interleukin-1β (IL-1β) and 3 separate saline injections. Effects of IL-1β on non-rapid eye movement sleep (NREMS), rapid eye movement sleep (REMS), electroencephalographic (EEG) slow-wave activity (SWA), and brain temperature (Tbr) were analyzed by 2-way ANOVA for repeated measures across 23-h recording period. F values are given for treatment effect (saline vs. IL-1β) and for time-treatment interaction. When ANOVA indicated significant effects Student-Newman-Keuls multiple comparison test was used to determine where significant effects had occurred. *P < 0.05; †P < 0.005; and ‡P < 0.0005.

Vagotomy Attenuates NREMS-Inducing Effects of 0.5 µg/kg IL-1β

The intraperitoneal injection of 0.5 µg/kg IL-1β increased NREMS and Tbr in Sham rats (Fig. 3, Table 1). NREMS was increased in the first 6 h [SNK q(4, 21) = 9.307, P < 0.01]. In addition, this dose of IL-1β significantly increased Tbr in hour 2 [SNK q(4, 154) = 4.40, P < 0.01]. In contrast, this dose of IL-1β failed to induce significant changes in NREMS or Tbr in Vx rats. REMS and EEG SWA were not altered in either group.

![Graphs showing time spent in sleep, EEG SWA, and Tbr over time for Sham and Vx groups with different doses of IL-1β.](http://ajpregu.physiology.org/)
9.596, P < 0.01], and T_{br} was increased in hours 1 and 2 [SNK (hour 1) q(3,154) = 3.75, P < 0.05; (hour 2) q(10,154) = 5.56, P < 0.01]. In Vx rats NREMS was also significantly increased [SNK q(3,21) = 4.829, P < 0.01]; however, this response was significantly attenuated in Vx rats compared with Sham rats (t_{14} = 4.09, P < 0.002). NREMS was increased by about 58 min in Sham rats compared with about 22 min in Vx rats during this time period. The increase in T_{br} was completely blocked in Vx animals. REMS was not affected in either group, and there was a slight, but not significant, decrease in EEG SWA in both Sham and Vx rats.

Vagotomy Does Not Block Effects of 2.5 µg/kg IL-1β

The highest dose of IL-1 tested, 2.5 µg/kg, resulted in similar effects in both Sham and Vx rats (Fig. 4, Table 1). IL-1 significantly increased NREMS in the first 12-h time period [SNK (Sham) q(2,7) = 13.39, P < 0.01; (Vx) q(2,7) = 10.53, P < 0.01]. In Sham rats IL-1 increased NREMS by about 91 min (42%); in Vx rats IL-1 increased NREMS by about 74 min (35%). There was a decrease in REMS in both Sham and Vx rats. A significant suppression occurred in the first 4 h postinjection [SNK (Sham) q(5,35) = 7.766, P < 0.01; (Vx) q(2,35) = 7.952, P < 0.01]. EEG SWA was significantly inhibited in the first 2-h time period [SNK (Sham) q(14,77) = 9.24375, P < 0.01; (Vx) q(16,77) = 9.5792, P < 0.01]. This was followed by an increase in SWA for 4 h and then a suppression for most of the remaining recording time. IL-1 significantly increased T_{br}. In both Sham and Vx rats, an initial hypothermic response was followed by a biphasic fever.

Vagotomy Does Not Affect Normal Sleep-Wake Activity or T_{br}

To compare sleep-wake activity and T_{br} in Sham and Vx rats, the data on the 3 control days for each group were averaged. Confirming a previous report (M. K. Hansen, L. Kapás, J. Fang, and J. M. Krueger, unpublished data), vagotomy does not significantly alter sleep-wake activity or T_{br} (Fig. 5). In both Sham and Vx rats the distribution of the states of vigilance followed a normal diurnal pattern with high percentages of sleep during the day. Maximum durations of NREMS and REMS occurred during the first and second portions of the light period, respectively. Furthermore, T_{br} varied with the diurnal cycle, being higher during the dark period (the behaviorally active period of rats).

DISCUSSION

The present results demonstrate that the vagus is indeed a crucial component in the pathway by which peripheral IL-1β signals the central nervous system to elicit various components of the acute phase response. However, the importance of the subdiaphragmatic vagus varies greatly depending on the dose of IL-1β used. Thus at low doses both IL-1β-induced NREMS and fever were completely abolished by subdiaphragmatic vagotomy. These results are consistent with the find-
Fig. 5. Effect of subdiaphragmatic vagotomy on normal NREMS, REMS, SWA, and $T_{hr}$. Data points represent averages for 3 control (saline treatment) days in Sham (open symbols) and Vx rats (solid symbols). Data are means $\pm$ SE.

ings that IL-1$\beta$ increases the afferent activity of the vagal nerve (26), and IL-1 receptors are found on paraganglia in the hepatic vagus (12). Furthermore, they are consistent with a previous study that found that the somnogenic and pyrogenic effects of LPS (100 $\mu$g/kg ip) are attenuated in Vx rats (15). Finally, they are consistent with the finding that vagotomy blocked the increase in sleep, which accompanies increased feeding (M. K. Hansen, L. Kapás, J. Fang, and J. M. Krueger, unpublished data).

The finding that vagotomy did not block the sleep and fever responses to a high dose of IL-1$\beta$ indicates that IL-1$\beta$ also triggers sleep and thermoregulatory mechanisms that are not dependent on intact subdiaphragmatic vagi. It is expected that after the intraperitoneal administration of high doses, significant quantities of IL-1$\beta$ may enter the systemic circulation. When plasma levels of IL-1$\beta$ are relatively high, they may then be able to reach intact vagal afferents in other peripheral sites, such as in the lung. In support of this, mice inoculated with influenza virus exhibit symptoms of the acute phase response, yet there is no increase in the circulating levels of cytokines (9). Significant elevations of cytokine activity were, however, found in the lung lavage fluid; hence, it is likely, and even suggested, that cytokine signals may be conveyed to the brain by a neural pathway. In addition, it is possible that cytokines in the plasma may enter the brain in amounts sufficient to activate central somnogenic and pyrogenic structures; if plasma levels of cytokines were high, this mechanism could supplement vagal-mediated events. Cytokine entry into the brain may take place at sites where the blood-brain barrier is missing, e.g., the organum vasculosum laminae terminalis or area postrema, or via blood-to-brain transport mechanisms. Alternatively, it is possible that IL-1 or LPS stimulates fever were not blocked by vagotomy, thereby suggesting that in addition to the subdiaphragmatic vagus alternative pathways exist that contribute to the centrally controlled symptoms of the acute phase response.

Although these "other" vagal and nonvagal arguments remain speculative, recent data in the literature provide some support for these ideas. Thus vagotomy suppresses fever in guinea pigs in response to LPS or muramyl dipeptide when administered intraperitoneally but not when given by the intramuscular route (13). Furthermore, vagotomy attenuated the depression in social behavior in mice only when IL-1$\beta$ was given by the intraperitoneal route and not when administered intravenously or subcutaneously (5). In both studies, it was concluded that the vagus only conveys immune signals to the brain when injected into the abdominal cavity. However, in this study, when higher doses were injected into the abdominal cavity, sleep and fever were not blocked by vagotomy, thereby suggesting that in addition to the subdiaphragmatic vagus alternative pathways exist that contribute to the centrally controlled symptoms of the acute phase response.

EEG delta-wave amplitudes are thought to reflect the intensity of NREMS. For example, EEG SWA increases after sleep deprivation (29). In the present study, the highest dose of IL-1$\beta$ induced increases in EEG SWA during maximum sleep responses. This finding is consistent with studies showing that central or systemic injections of IL-1$\beta$ or microbial products, such as LPS, increase EEG SWA (19, 27). Similar increases in EEG SWA occur during the initial phase of the NREMS responses to infection (19). These changes in EEG SWA, like those occurring after sleep deprivation, are likely mediated in part by IL-1$\beta$ because inhibition of IL-1$\beta$ attenuates sleep deprivation-induced (35) and microbial product-induced (36) increases in EEG SWA. However, the highest dose of IL-1$\beta$ also caused a large initial suppression of EEG SWA and a long-lasting suppression in hours 7–23. Similar results were found in mice injected intraperitoneally with a high dose of IL-1$\beta$ (10) and suggest that the effects of IL-1$\beta$ on EEG SWA and duration of NREMS can be dissociated. Indeed, it is known that circadian factors modulate the effects of IL-1$\beta$ on EEG SWA and duration of NREMS and EEG SWA independently (27). Furthermore, there are many interactions between IL-1$\beta$ and classic neurotransmitters and humoral factors that could independently affect duration of NREMS
and EEG SWA. For example, IL-1β induces the release of growth hormone-releasing hormone, another well characterized sleep-promoting substance that also enhances EEG SWA (19). In contrast, intraperitoneal administration of IL-1β increases brain norepinephrine (NE) turnover rate; activation of brain NE systems is known to suppress EEG SWA (2). Therefore, it is likely that the enhancing and suppressing effects of IL-1β on EEG SWA are mediated by different molecular pathways and that these mechanisms are independent of those responsible for duration of NREMS.

It has been suggested that the lack of effects in Vx animals may be due to poor health (31). In the present study, it is very unlikely that the unresponsiveness of Vx rats to low and intermediate doses of IL-1β was due to malnutrition because the body weights of each group of rats were similar, and high doses of IL-1β were capable of inducing both sleep and fever in the same rats. Secondly, the fact that Vx rats had normal sleep patterns at the time of experimental testing indicates that the rats were not seriously distressed; it is well known that any distress seriously affects sleep. Finally, the finding that Vx rats respond to central injections of IL-1β (4) suggests that vagotomy itself does not impair the direct sensitivity of the brain to immune signals. The current data clearly demonstrate that the vagal nerve is an important element in the pathway by which peripheral cytokines signal the central nervous system to elicit various facets of the sickness syndrome.

Perspectives

Much evidence supports the hypothesis that brain IL-1 is critical for immune-cytokine interactions. The central administration of anti-IL-1 antibodies, the IL-1 receptor antagonist, or the IL-1 receptor fragment inhibits sleep (36) and behavioral responses (17), as well as fever (18), in response to peripherally injected immune stimuli. Furthermore, peripheral immune stimuli induce cytokine expression in the brain. Subdiaphragmatic vagotomy blocks the induction of IL-1β mRNA in the hippocampus and hypothalamus of LPS-treated mice (23). This evidence therefore suggests that the induction and subsequent release of IL-1β in the brain may be a final and critical step in the pathway by which vagally mediated immune signals result in centrally controlled symptoms of the acute phase response.

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