Electrical stimulation of afferent vagus nerve induces IL-1β expression in the brain and activates HPA axis

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Hosoi, Toru, Yasunobu Okuma, and Yasuyuki Nomura. Electrical stimulation of afferent vagus nerve induces IL-1β expression in the brain and activates HPA axis. Am J Physiol Regulatory Integrative Comp Physiol 279: R141–R147, 2000.—Possible roles of the afferent vagus nerve in regulation of interleukin (IL)-1β expression in the brain and hypothalamic-pituitary-adrenal (HPA) axis were examined in anesthetized rats. Levels of IL-1β mRNA and protein in the brain were measured by comparative RT-PCR and ELISA. Direct electrical stimulation of the central end of the vagus nerve was performed continuously for 2 h. The afferent stimulation of the vagus nerve induced increases in the expression of mRNA and protein levels of IL-1β in the hypothalamus and the hippocampus. Furthermore, expression of corticotropin-releasing factor mRNA was increased in the hypothalamus 2 h after vagal stimulation. Plasma levels of ACTH and corticosterone were also increased by this stimulation. The present results indicate that activation of the afferent vagus nerves itself can induce production of IL-1β in the brain and activate the HPA axis. Therefore, the afferent vagus nerve may play an important role in transmitting peripheral signals to the brain in the infection and inflammation.

Interleukin (IL)-1β is a proinflammatory cytokine produced not only in the immune system (e.g., lymphocytes and macrophage), but also in the brain (neuronal and glial cells). Peripheral or central application of IL-1β induces fever (22), inhibition of food intake (28) and gastric acid secretion (19, 43), and activation of the sympathetic (29) and hypothalamic-pituitary-adrenal (HPA) axis (4, 37). The effects induced by IL-1β mimic those produced by bacterial endotoxin lipopolysaccharide (LPS) (22). The expression of IL-1β in the brain is highly inducible by peripherally applied LPS (2, 25). IL-1β knockout mice exhibited an impaired acute-phase inflammatory response and were completely resistant to fever development and anorexia in response to inflammation induced by turpentine (44). These observations indicate that peripherally generated cytokines, such as IL-1β, mediate both the central and peripheral metabolic responses to endotoxin. However, the fact that IL-1β is a 17.5-kDa hydrophilic peptide that cannot cross the blood-brain barrier freely raises questions as to how these immune signals can act on the central nervous system (CNS). The precise mechanisms by which IL-1β signals the CNS are unknown, but possibilities include direct entry of IL-1β into the brain across the blood-brain barrier by a saturable transport mechanism (3), 2) interaction of IL-1β with circumventricular organs [organum vasculosum of the lamina terminalis (OVLT), area postrema, etc.,], which lack the blood-brain barrier (21), and 3) activation of afferent neurons of the vagus nerve (42).

Increasing evidence has suggested that the vagus nerve is an important neural pathway for communicating immune signals originating in the periphery to the brain. IL-1β immunoreactivity was expressed in dendritic cells and macrophages within connective tissues associated with the abdominal vagus after intraperitoneal injection of LPS (16), and systemic application of IL-1β increases hepatic (32) and gastric (23) branch of the vagus afferent nerve activity. Moreover, Ek et al. (11) demonstrated that circulating IL-1β stimulates vagal sensory activity via both prostaglandin-dependent and -independent mechanisms. Peripheral administration of IL-1β and LPS produce c-Fos activation in the nucleus of the solitary tract (NTS), which is the predominant termination site of afferent vagus nerves (7, 13). Subdiaphragmatic vagotomy has been shown to inhibit behavioral and neural effects of peripheral IL-1β or LPS including social exploration (5, 6), anorexia (8), fever response (17, 36, 40), stimulation of the HPA axis (15, 20), and IL-1β mRNA expression in the brain (18, 24). Furthermore, the selective transection of hepatic vagus nerve effectively inhibited a pyrogenic response induced by LPS (41). In contrast, there is inconsistent evidence showing that subdiaphragmatic vagotomy does not block c-Fos and corticotropin-releasing factor (CRF) expression in the brain (12) and suppression of food intake (34, 39) induced by the peripheral application of LPS or IL-1β.

In the present study, therefore, we examined possible roles of the afferent vagus nerve in regulation of IL-1β expression in the brain and HPA axis by means

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of direct electrical stimulation of central end of the vagus nerves from the cervical vagal trunk.

METHODS

Experimental animals. Adult male Sprague-Dawley rats weighing 350–400 g were maintained in a room at 22–24°C under a constant day-night rhythm and given food and water ad libitum. All animal experiments were carried out in accordance with National Institutes of Health Guide for Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee at Hokkaido University.

Animals were anesthetized with urethane (1.2 g/kg ip), and the femoral artery was cannulated for collecting blood samples and measuring systemic blood pressure and heart rate. The arterial catheter was connected to a pressure transducer (model AP-641G, Nihon Kohden, Japan), and arterial blood pressure and heart rate were recorded. For the electrical stimulation of the afferent vagus nerve, the carotid arteries were exposed by a midline incision in the neck and the left vagus nerve was freed from the artery. The left vagus nerve was cut, and the central end of the nerve was placed on a platinum ring electrode and stimulated continuously throughout the experiment. The stimulus parameter consisted of square-wave pulses of 0.5-ms duration, at 10 Hz, 1 mA, delivered by means of an electric stimulator (model SEN-3301, Nihon Kohden, Japan).

LPS from Escherichia coli endotoxin (055:B5, Sigma) was injected intraperitoneally at dose of 300 µg/kg dissolved in saline in an injection volume of 1 ml/kg. Animals were returned to their home cage and killed 2 h later.

RT-PCR. Total RNA was isolated using TRI-Reagent (Sigma). The quantity of the RNA obtained was checked by measuring optical density at 260 and 280 nm. cDNA was synthesized from 2 µg of total RNA by reverse transcription using 100 U of Superscript reverse transcriptase (GIBCO) and oligo(dT)_{12-18} primer in a 20-µl reaction containing 1× Superscript buffer (GIBCO), 1 mM dNTP mix, 10 mM DTT, and 40 U of RNase inhibitor. After incubation for 1 h at 42°C, the reaction was terminated by a denaturing enzyme for 15 min at 70°C. For PCR amplification, 1.2 µl of cDNA were added to 12 µl of a reaction mix containing 0.2 µM of each primer, 0.2 mM of dNTP mix, 0.6 U of Taq polymerase, and 1× reaction buffer. PCR was performed in a DNA Thermal Cycler (Perkin-Elmer 2400-R). The primers employed are as follows: IL-1β (upstream) 5’-CCT TCT TTT CCT TCA TCT TTG-3’; IL-1β (downstream) 5’-ACC GGT TTT CCA TCT TCT TCT-3’; CRF (upstream) 5’-GGG AAG GCA AAG AAA AGG A-3’; CRF (downstream) 5’-CCT GGA GTT GGG GGA CAG C-3’; GAPDH (upstream) 5’-AAA CCC ATC ACC ATC TTC CAG-3’; GAPDH (downstream) 5’-AGG GGC CAT CCA CAG TCT TCT-3’. The predicted PCR products of IL-1β, CRF, and GAPDH were 375, 391, and 361 bp, respectively. The PCR products (10 µl) were resolved by electrophoresis in an 8% polyacrylamide gel in 1× TBE (Tris-borate, EDTA) buffer. The gel was stained with ethidium bromide, and band densities were obtained by densitometric measurements using an FLA-2000 image analyzer (Fujifilm).

In the initial experiments, the optimal numbers of PCR cycles for IL-1β, CRF, and GAPDH were determined to be 36, 36, and 17, respectively. The amount of each amplified product was integrated and plotted graphically against the number of PCR cycles to determine whether the increase in intensity of the amplified product was linear to the number of PCR cycles.

To compare the expression of IL-1β and CRF mRNAs in the different experimental groups, the amount of IL-1β and CRF mRNA in each structure studied was estimated as the ratio (IL-1β or CRF/GAPDH).

ELISA for IL-1β. Each tissue was added to 0.9–1.0 ml of 20 mM Tris · HCl (pH 7.4) buffer containing (in mM) 0.5 phenylmethylsulfonyl fluoride, 0.5 benzamidine, 1.0 1,4-dithiothreitol, and 1.0 EDTA. Total protein was mechanically dissociated from tissue using an ultrasonic cell disrupter. Sonicated samples were centrifuged at 30,000 g for 30 min. Supernatants were removed and stored at −80°C until an ELISA was performed. Bradford protein assays were also performed to determine total protein concentrations in brain sonication samples. The ELISA for rat IL-1β was performed using a commercially available kit from Endogen (Woburn, MA). The detection limit was 16 pg/ml. This ELISA does not cross-react with rat IL-1α, rat tumor necrosis factor-α, mouse IL-1α, human IL-1α, human IL-1β, human pro-IL-1β (32 kDa), and human IL-1Ra.

Measurement of plasma level of ACTH and corticosterone. Arterial blood was collected in an EDTA tube via a cannula immediately after the end of the vagus nerve stimulation. Blood samples were centrifuged at 3,000 rpm at 4°C for 10 min, and aliquots were stored at −80°C until further use. Plasma ACTH was determined using an ACTH-RIA kit (Allegro, Nichols Institute Diagnostics, San Juan Capistrano, CA). The assay sensitivity was 1 pg/ml. This RIA kit does not cross-react with α-MSH, β-MSH, β-LPH, and β-endorphin. Plasma corticosterone was determined by RIA (30). The assay sensitivity was 0.2 ng/ml.

Statistics. Results are expressed as the means ± SE. Statistical analysis was performed with Student’s t-test.

RESULTS

Change in heart rate after electrical stimulation of the afferent vagus nerve. As shown in Fig. 1, continuous
electrical stimulation of the central side of the vagus nerve (10 Hz) markedly reduced heart rate. This reduction in heart rate lasted during the entire 2-h stimulation. This result clearly indicates a reduction in heart rate by vago-vagal reflex and that the afferent vagus nerves were effectively stimulated under the experimental conditions at 10 Hz, 0.5 ms, 1 mA.

Effects of the electrical stimulation of the afferent vagus nerve on IL-1β mRNA and protein levels. IL-1β mRNA does not abundantly exist in the brain, therefore, it may be difficult to detect without amplification by RT-PCR. In line with previous reports (18), we detected low basal levels of IL-1β mRNA expression in the hypothalamus and the hippocampus of sham-operated rats using comparative RT-PCR. In a preliminary experiment, electrical stimulation conditions of the vagus nerve were examined at several frequencies of 5, 10, and 20 Hz, with the most consistent increase in IL-1β mRNA in the hypothalamus observed at 10 Hz. Electrical stimulation of the afferent vagus nerve for 1 h (10 Hz, 0.5 ms, 1 mA) induced a slight increase in the expression of IL-1β mRNA in the hypothalamus (data not shown). Two hours after the stimulation, we observed significant increases in the expression of IL-1β mRNA in the hypothalamus and the hippocampus (Fig. 2). We also noted a slight increase of IL-1β mRNA in the cortex (data not shown). The expression of IL-1β mRNA in the hypothalamus after vagal stimulation was increased to 170% of that in the sham-operated group. This enhanced expression, however, was relatively less compared with that induced in the hypothalamus by LPS (300 μg/kg ip; Fig. 3). As measured by ELISA, IL-1β protein was significantly increased 2 h after vagal stimulation in the hippocampus, and a slight increase of this protein level was observed in the hypothalamus (Table 1). To determine whether or not the stimulation of the afferent vagus nerve increases peripheral IL-1β, we measured plasma levels of IL-1β protein. Plasma levels of IL-1β protein were not detected either in sham-operated or in the vagal-stimulated rats (Table 1), indicating that the increases in IL-1β protein in the brain originated from the brain, and not from periphery.

Effects of the electrical stimulation of the vagus nerve on the HPA axis. To elucidate the involvement of the vagus nerve in the HPA axis, we measured the expression of CRF mRNA in the hypothalamus and plasma levels of ACTH and corticosterone after vagal stimula-
tion. By using comparative RT-PCR, CRF mRNA in the hypothalamus was increased 2 h after vagal stimulation (Fig. 4). Furthermore, plasma levels of ACTH were markedly elevated 2 h after vagal stimulation (Fig. 5). We also observed increases in plasma levels of corticosterone 2 h after vagal stimulation (Fig. 5).

**DISCUSSION**

During peripheral inflammation, expression of IL-1β is induced in the brain (2, 25) and IL-1β induces symptoms of brain-mediated illness (22). It has been suggested that the afferent vagus nerve transmits peripheral inflammation signals to the brain. On the basis of subdiaphragmatic vagotomy experiments, however, inconsistent observations have been reported (12, 18, 24, 34, 36, 39). Recently, it has been reported that type 1 IL-1 receptor was detected in situ over neuronal cell bodies in the rat nodose ganglion (11). Such a result indicates that circulating IL-1 might activate vagal afferents, even at the level of nodose ganglion, and might explain the ineffectiveness of subdiaphragmatic vagotomy to block c-FOS and CRF expression in the brain (12) and suppression of food intake (34, 39) induced by peripheral administration of LPS or IL-1β. In the present study, we demonstrated that direct electrical stimulation of the afferent vagus nerve induced an increase in the expression of IL-1β mRNA in the hypothalamus and hippocampus. In addition, IL-1β protein was significantly increased in the hippocampus after the stimulation. On the other hand, plasma levels of IL-1β were not detected in sham-operated or vagal-stimulated rats. These results indicate that the origin of the IL-1β protein induced by the vagal stimulation may probably be the brain, but not peripheral tissue. These data demonstrate direct evidence that vagal afferent neurons play a critical role in transmitting peripheral signals to the CNS.

The vagal stimulation-induced increase in IL-1β mRNA in the brain was significant but rather less than that produced by intraperitoneal LPS. Adrenal glu-

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**Table 1. Effects of the stimulation of vagal afferent nerves on IL-1β protein levels of the hypothalamus, the hippocampus, and the plasma**

<table>
<thead>
<tr>
<th>IL-1β Protein (pg/100 μg total protein)</th>
<th>Sham</th>
<th>Vagal Stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamus</td>
<td>1.04 ± 0.07</td>
<td>1.18 ± 0.08</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.60 ± 0.04</td>
<td>0.73 ± 0.04*</td>
</tr>
<tr>
<td>Plasma</td>
<td>Not detectable</td>
<td>Not detectable</td>
</tr>
</tbody>
</table>

Values are means ± SE for n = 7 rats/group. Levels of interleukin (IL)-1β were measured at 2 h after vagal stimulation. *P < 0.05 (statistically significant difference from sham-operated rats).
cocorticoids are known to potently suppress IL-1\textsubscript{b} transcription and mRNA stability (26). We also observed that the stimulation of vagal afferent nerves induced increases in plasma levels of corticosterone. Thus it was possible that the production of IL-1\textsubscript{b} in the brain was suppressed by the increased level of corticosterone. Such an explanation is consistent with the observation that the stress-induced rise in corticosterone masks a robust and widespread increase in brain IL-1\textsubscript{b} (31). An alternative interpretation is postulated suggesting that to attain an efficacious expression of cytokine in the brain induced by peripheral immune signals, cooperation with the vagal afferent nerves and other pathways, such as direct interactions of cytokine with the brain, nonvagal afferents, and/or humoral pathways are needed.

It has been reported that peripheral application of LPS and IL-1\textsubscript{b} activates the HPA axis (4, 35, 37). Ericsson et al. (13) demonstrated that intravenous administration of IL-1\textsubscript{b} increases c-Fos and CRF mRNA in the paraventricular nucleus of hypothalamus through the activation of ascending catecholaminergic projections from the medulla oblongata. Furthermore, several independent lines of evidence have indicated that peripherally applied IL-1\textsubscript{b} activates afferent vagus nerves and neurons in the NTS (7, 27, 32).

Subdiaphragmatic vagotomy has been shown to suppress increases in ACTH secretion, but not corticosterone induced by intraperitoneally applied LPS or IL-1\textsubscript{b} (15, 20). Because plasma levels of corticosterone have been shown to be elevated by the direct action of IL-1\textsubscript{b} at the level of adrenal glands (1), the contribution of vagus nerve in LPS- or IL-1\textsubscript{b}-induced corticosterone response is difficult to estimate by means of vagotomy. Therefore, we examined the effect of direct stimulation of the afferent vagus nerves on HPA axis. In the present study, we demonstrated that vagal stimulation induced an increase in the expression of CRF mRNA in the hypothalamus and increases in plasma levels of both ACTH and corticosterone. These results indicate that the afferent vagus nerves may play a significant role in endotoxin- or cytokine-induced activation of the HPA axis.

In conclusion, we examined direct electrical stimulation of the afferent vagus nerves and demonstrated that activation of the vagal afferent neurons alone induced the production of IL-1\textsubscript{b} in the brain and activated the HPA axis. The vagus nerve may act as a pathophysiological component for a rapid-signaling pathway in the infection and inflammation.

**Perspectives**

It is interesting to note that the stimulation of afferent vagus nerves increased IL-1\textsubscript{b} transcript in the hippocampus. Recently, it was reported that IL-1\textsubscript{b} gene expression is substantially increased in the hippocampus during long-term potentiation (LTP), and blockage of the IL-1 receptor resulted in a reversible impairment of LTP maintenance (38). It has been suggested that the vagus nerve can modulate learning and memory. Subdiaphragmatic vagotomy attenuates memory retention produced by peripherally applied cholecystokinin octapeptide (14) or substance P (33). Furthermore, electrical stimulation of the vagus nerve enhances memory in rodents (9) and in human subjects (10). The present results permit us to speculate that the afferent vagus nerve may play a role in memory formation and/or maintenance through enhancement of IL-1\textsubscript{b} transcript in the hippocampus.

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