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Short communication

Novel pathway for LPS-induced afferent vagus nerve activation: Possible role of nodose ganglion

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Abstract

The afferent vagus nerve has been suggested to be an important component for transmitting peripheral immune signals to the brain. However, there is inconsistent evidence showing that subdiaphragmatic vagotomy did not inhibit the brain mediated behavioral and neural effects induced by the peripheral application of lipopolysaccharide (LPS). LPS triggers innate immune cells through Toll-like receptor 4 (TLR4). In the present study, we found that TLR4 mRNA and protein was expressed in the rat nodose ganglion. Thus, it is suggested that LPS could activate afferent vagus nerve at the level of nodose ganglion, which exists centrally from the subdiaphragmatic level of vagus nerve. The results could provide evidence for the novel pathway of LPS-induced afferent vagus nerve activation.

Keywords: Afferent vagus nerve; Immune-to-brain communication; Toll-like receptor 4; Nodose ganglion

Infection and inflammation induce sickness responses such as anorexia, fever and depressed activity. It is becoming well accepted that the peripheral immune system can signal the brain during inflammation. However, the precise mechanisms by which the peripheral immune system can signal the brain has been the subject of much debate. The possibilities include: 1) the direct entry of cytokine into the brain across the blood–brain barrier by a saturable transport mechanism (Banks et al., 1991); 2) the interaction of cytokine with circumventricular organs which lack the blood–brain barrier (Blatteis et al., 1983; Katsuura et al., 1990); and 3) activation of afferent neurons of the vagus nerve (Watkins et al., 1995; Ek et al., 1998).

Recent evidence has suggested that afferent vagus nerve is an important component for transmitting peripheral immune signals to the brain. Subdiaphragmatic vagotomy has been shown to inhibit behavioral and neural effects of peripheral IL-1βor LPS, including social exploration (Bluthé et al., 1996, 1994), anorexia (Bret-Dibat et al., 1995), fever response (Hansen and Krueger, 1997; Romanovsky et al., 1997; Sehic and Blatteis, 1996), stimulation of the HPA axis (Gaykema et al., 1995; Kapcala et al., 1996), and IL-1β expression in the brain (Hansen et al., 2001; Layé et al., 1995). However, there is inconsistent evidence showing that subdiaphragmatic vagotomy does not block brain mediated behavioral and neural effects induced by the peripheral application of LPS (Porter et al., 1998; Schwartz et al., 1997). Furthermore, using subdiaphragmatic vagotomized rodents, several reports suggested that the role of afferent vagus nerve in transmitting peripheral inflammatory signals to the brain is dependent on experimental conditions such as dose (Hansen et al., 2001) or route (Goldbach et al., 1997) of the LPS injection. On the other hand, all of these

Abbreviations: IL-1β, Interleukin-1β; LPS, Lipopolysaccharide; HPA axis, Hypothalamic–pituitary–adrenal axis.

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experiments are done using vagotomized rodents at the subdiaphragmatic level. We thought that these inconsistencies might be in part because that LPS act on another area of afferent vagus nerves that exist centrally from the subdiaphragmatic level of vagus nerve. Therefore, in the present study, we hypothesized the possibility that LPS directly activate afferent vagus nerve from the nodose ganglion, which exist centrally from the subdiaphragmatic level of vagus.

Adult male Sprague Dawley rats weighing 400–500 g were maintained in a room at 22–24 °C with a constant day–night rhythm and given food and water ad libitum. All animal experiments were carried out in accordance with the NIH 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, i.p.) and the carotid arteries were exposed by a midline incision in the neck. After that, animals were sacrificed by bleeding from the carotid artery. Then, the nodose ganglion was quickly removed and dissected on an ice-cold plate. The samples were snap-frozen in liquid nitrogen and stored at − 80 °C. We pooled the sample (4–6 rats/sample) for RT-PCR and Western blotting analysis.

RT-PCR was performed as described previously (Hosoi et al., 2002). Total RNA was isolated using TRI REAGENT™ (SIGMA). cDNA was synthesized from total RNA by reverse transcription using 100 U of Superscript Reverse Transcriptase (GIBCO BRL) and Oligo $(dt)_{12-18}$ primer (GIBCO BRL) in a 20 μl reaction containing 1 × Superscript buffer (GIBCO BRL), 1 mM dNTP mix, 10 mM DTT, and 40 U of RNase inhibitor. Total RNA and Oligo $(dt)_{12-18}$ primer were incubated at 70 °C for 10 min prior to the reverse transcription. 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 was 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). Primers used were as follows: TLR4 upstream, 5′-cgc ttt cag ctt tgc ctt cat tac-3′ and TLR4 downstream, 5′-tgc tac ttc ctt gtg ccc tgt gag-3′; GAPDH upstream, 5′-aaa ccc atc acc atc ttc cag-3′ and GAPDH downstream, 5′-agg ggc cat cca cag tct tct-3′. The PCR products (10 μl) were resolved by electrophoresis in 8% polyacrylamide gel in 1 × TBE buffer. The gel was stained with ethidium bromide and were photographed under ultraviolet light.

For Western blotting, tissue samples were sonicated in a buffer containing 10 mM HEPES–NaOH (pH 7.5), 150 mM NaCl, 1 mM EGTA, 1 mM Na_3VO_4, 10 mM NaF, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM PMSF and 1% NP-40 for 30 s. The samples were centrifuged at 30,000 × g for 30 min at 4 °C, and the supernatants were collected. The samples were boiled with laemmli buffer for 3 min and fractionated by SDS-PAGE (60 μg/lane) and transferred at 4 °C to nitrocellulose membranes. The membranes were blocked and incubated with anti-TLR4 (Santa cruz: sc-10741) antibody at 4 °C overnight. The membranes were washed and then incubated with anti-horseradish peroxidase-linked antibody. Peroxidase was detected by chemiluminescence using an ECL system.

We investigated whether TLR4 is expressed in the rat nodose ganglion. As assessed by RT-PCR, we found that TLR4 mRNA was expressed in the nodose ganglion (Fig. 1A). In the absence of RT (reverse transcriptase) we did not detect the PCR product, indicating that there was no contamination by genomic DNA (Fig. 1A). Then, we next investigated whether TLR4 is expressed at the protein level. As a result, we found that TLR4 is expressed in the nodose ganglion at the protein level as assessed by Western blotting (Fig. 1B). Liver sample was done as positive control (Fig. 1B). These results indicate that TLR4 mRNA and protein were expressed in the nodose ganglion. Thus LPS is suggested to act at the level of nodose ganglion and we would like to purpose the model presented in the Fig. 2.

Increasing evidence has suggested that the vagus nerve is an important pathway for cytokine-to-brain communication. Subdiaphragmatic vagotomy has been shown to inhibit behavioral and neural effects of peripheral IL-1β or LPS (Bluthé et al., 1994, 1996; Bret-Dibat et al., 1995; Gaykema et al., 1995; Hansen and Krueger, 1997; Hansen et al., 1998; Kapcala et al., 1996; Layé et al., 1995; Romanovsky et al., 1997; Sehic and Blatteis, 1996). We previously reported that direct electrical stimulation of afferent vagus nerve induces IL-1β expression in the brain (Hosoi et al., 2000). On the other hand, an inconsistent observation has been reported regarding the contribution of afferent vagus nerve. It was

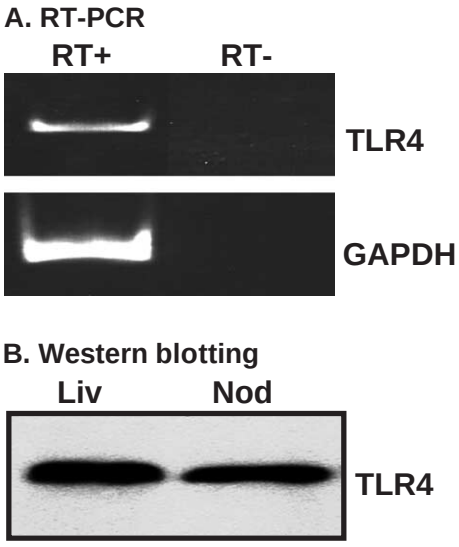

Fig. 1. Expression of TLR4 in the nodose ganglion. A) mRNA expression of TLR4 in the nodose ganglion as assessed by RT-PCR. Note the presence of a PCR product in the presence of RT (reverse transcriptase) but not in the absence of RT, indicating that there was no contamination by genomic DNA. The data are representative of 2 independent experiments. B) Protein expression of TLR4 in the nodose ganglion (Nod) as assessed by Western blotting. We also performed liver (Liv) sample as a positive control. The data are representative of 2 independent experiments.