Activation of Vagal Afferents after Intravenous Injection of Interleukin-1β: Role of Endogenous Prostaglandins

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Intravenous administration of interleukin-1 (IL-1) activates central autonomic neuronal circuits originating in the nucleus of the solitary tract (NTS). This mechanism(s) by which bloodborne IL-1 regulates brain functions, whether by operating across the blood–brain barrier and/or by activating peripheral sensory afferents, remains to be characterized. It has been proposed that vagal afferents originating in the periphery may monitor circulating IL-1 levels, because neurons within the NTS are primary recipients of sensory information from the vagus nerve and also exhibit exquisite sensitivity to blood-borne IL-1. In this study, we present evidence that viscerosensory afferents of the vagus nerve respond to intravenously administered IL-1β. Specific labeling for mRNAs encoding the type 1 IL-1 receptor and the EP3 subtype of the prostaglandin E2 receptor was detected in situ over neuronal cell bodies in the rat nodose ganglion. Moreover, intravenously applied IL-1 increased the number of sensory neurons in the nodose ganglion that express the cellular activation marker c-Fos, which was matched by an increase in discharge activity of vagal afferents arising from gastric compartments. This response to IL-1 administration was attenuated in animals pretreated with the cyclooxygenase inhibitor indomethacin, suggesting partial mediation by prostaglandins. In conclusion, these results demonstrate that somata and/or fibers of sensory neurons of the vagus nerve express receptors to IL-1 and prostaglandin E2 and that circulating IL-1 stimulates vagal sensory activity via both prostaglandin-dependent and -independent mechanisms.

Key words: nodose ganglion; viscerosensory; nucleus of the solitary tract; autonomic; cytokine; c-Fos; blood–brain barrier; neuroimmunomodulation; inflammation; acute-phase response
aferents in driving brain function after intravenous administration of inflammatory mediators have produced variable and inconclusive results (Katsura et al., 1988; Wan et al., 1994; Sehic and Blatteis, 1996; Ericsson et al., 1997; Romanovsky et al., 1997).

The present study was designed to examine whether vagal viscerosensory afferents have the endogenous capacity to monitor and respond to elevated plasma levels of IL-1 and/or prostaglandins. The responsiveness of viscerosensory vagal afferents to intravenously administered IL-1β, as well as the relative involvement of endogenous prostaglandins, were examined using electrophysiological techniques and immediate-early gene technology (Morgan and Curran, 1991) on sections through the nodose ganglion of indomethacin- or vehicle-pretreated rats. In situ hybridization histochemical labeling was used to detect mRNAs encoding either IL-1 or prostaglandin E2 (PGE2) receptors within the nodose ganglion. The results demonstrate that bloodborne IL-1β activates vagal sensory functions in a partially prostaglandin-dependent manner and that sensory neurons in the nodose ganglia express receptors for IL-1 and PGE2.

MATERIALS AND METHODS

Animals. Adult male Sprague Dawley rats (260–340 gm; B & K Universal, Sollentuna, Sweden) were used, and all were certified to be free of rodent pathogens. The rats were housed individually in our animal facility for a minimum of 9 d before the onset of experiments at constant room temperature and a 12 hr light/dark cycle (lights on at 6:00 AM). Food and water were provided ad libitum up until the day before the injection. To normalize interindividual variation in gastrointestinal status, the animals were deprived of food but allowed access to water ad libitum for 24 hr before the time of injection. Rats were accustomed to experimental conditions by daily handling for a minimum of 7 d before the onset of experiments. These studies were approved by the Animal Welfare Committee at the Karolinska Institute (Stockholm, Sweden).

Recording IL-1-induced changes in afferent activity of the gastric vagal nerve. Rats (n = 19) were initially catheterized via the trachea under general anesthesia using sodium pentobarbital (60–70 mg/kg, i.p.; Aptecksbolaget, Umeå, Sweden), and the respiration of the animals was artificially maintained with a respirator (model 683; Harvard Apparatus, Helliston, MA). The femoral vein was simultaneously cannulated to allow drug administration. The jugular vein was cannulated for constant infusion of pentobarbital and a muscle relaxant, gallamine triethiodide (Sigma, St. Louis, MO). Blood pressure was monitored continuously from the femoral artery and maintained above 90 mmHg (systolic) by administering 4% Ficoll 70 (Pharmacia, Uppsala, Sweden) as needed. Rectal temperature was maintained at 37.5 ± 0.1°C using a heating pad and an infrared lamp (ATB-1100; Nihon-Kohden, Tokyo, Japan). All surgical sites were cleansed with 70% ethanol and locally cooled with Luer-Lok 1 cm distal to the caudal end of the nodose ganglion. A 2% (w/v) dispersion of the fluorochrome true blue, a fluorescent marker for retrograde axonal transport (Sigma), was freshly prepared in pyrogen-free saline, and 0.05–0.1 ml was injected with a 100 μl Hamilton syringe directly into the vagal nerve trunk. Tracer transport continued for 7 d to backfill the sensory neurons. The rats were then subjected to the catheterization procedure described above.

Perfusion and tissue preparation. The time of perfusion was between 11:00 A.M. and 1:00 P.M. on the day of the HPA axis stress effects. After 2 d postsurgical recovery, the rats were preinjected intravenously with indomethacin (10 mg/kg) or with vehicle alone. One hour later, 2 μg/kg IL-1β or vehicle alone was injected in a total volume of 300 μl over 3 min. One hour after the last injection, rats were anesthetized and perfused transcardially with 36 m M Tris-HCl, and 36 m M sodium phosphate buffer, pH 7.4.

Intravenous administration of IL-1β to awake and freely moving rats. The procedures for administration of IL-1β to awake and freely moving rats has been described previously (Ericsson and Sawchenko, 1993). Briefly, indwelling catheters (PE-50; Becton Dickinson, Sparks, MD) containing sterile pyrogen-free heparin saline (500 U/ml) were surgically implanted in the right jugular vein of methoxyflurane-anesthetized rats. The catheter was positioned with the internal silastic tip in the atrium of the heart and then routed subcutaneously until its sealed end was exteriorized in the interscapular space. The present experiments were modified, however, in that the external part of the intrajugular catheter (50 cm in length) was protected from the animal by tunneling it through a flexible tubing and then anchoring it to a remote balancing device. This procedure completely avoided the need to approach the rat before and during the injections, reducing the potential risk of handling-related stress effects. After 2 d postsurgical recovery, the rats were preinjected intravenously with indomethacin (10 mg/kg) or with vehicle alone. One hour later, 2 μg/kg IL-1β or vehicle alone was injected in a total volume of 300 μl over 3 min. One hour after the last injection, rats were anesthetized and perfused transcardially with 36 m M Tris-HCl, and 36 m M sodium phosphate buffer, pH 7.4.

Tracer injections. Sensory neurons of the vagus nerve are located in the nodose and jugular ganglia. In the rat, these ganglia are fused to form a continuous continuous ganglionic mass in which vagal and gosstropillary sensory neurons are partly interspersed (Altschuler et al., 1989). Therefore, tract-tracing studies were performed to specifically label vagal sensory neurons in the nodose ganglion. Three rats were anesthetized with methoxyflurane, and the right vagus nerve was exposed via a ventral incision and dissected free of surrounding connective tissue ~1 cm distal to the caudal end of the nodose ganglion. A 2% (w/v) dispersion of the fluorochrome true blue, a fluorescent marker for retrograde axonal transport (Sigma), was freshly prepared in pyrogen-free saline, and 0.05–0.1 ml was injected with a 75N Hamilton syringe directly into the vagal nerve trunk. Tracer transport continued for 7 d to backfill the sensory neurons. The rats were then subjected to the catheterization procedure described above.

Preparation of radioactive labeled cRNA probes. The preparation of radioactively labeled cRNA probes encoding the type 1 IL-1 receptor (IL-1R1), the PGE2 receptor, and c-Fos was described as described previously (Altschuler et al., 1989). In brief, sense and antisense cRNA probes were transcribed in vitro with T3 or T7 RNA polymerase in the presence of [32P]-UTP (New England Nuclear, DuMedical, Sollentuna, Sweden). After unincorporated nucleotides were removed using Quick Spin columns (Boehringer Mannheim, Indianapolis, IN), the specific IL-1β protein (original specific biological activity in excess of 1 × 10^10 U/g protein; A375 assay) (Nakano et al., 1988) was generated from a recombinant human IL-1β cDNA fragment encoding the 152 residue mature form of IL-1β. After its receipt, the material was thawed on ice, diluted 1:1 in 200 m M Tris-HCl buffer, pH 7.4, containing 0.2% BSA and then aliquoted in 1.5 μg batches, and refrozen at −70°C. Before its injection, an IL-1β aliquot was thawed and diluted to the appropriate concentration, depending on the size of the rats to be injected, in 40 m M sodium phosphate buffer containing 0.01% ascorbic acid. Injections of vehicle alone were composed of 0.01% BSA, 0.01% ascorbic acid, 10 m M Tris-HCl, and 36 m M sodium phosphate buffer, pH 7.4.
activities of all the probes were 1–3×10<sup>6</sup> cpm/μg. IL-1Rt1 mRNA was transcribed from a 1.35 kb cDNA encoding part of the extracellular domain, as well as the entire transmembrane and cytoplasmic domains of the membrane-associated form of the IL-1Rt1 (Hart et al., 1993). In addition, an 885 bp cDNA fragment encoding part of the rat PGE2 receptor isoform, EP3<sub>a</sub>, was initially generated in our laboratory by reverse transcriptase-PCR using sequence-specific (Takeuchi et al., 1993) oligonucleotide primers. The EP3<sub>a</sub> cDNA clone encodes a large sequence (837 bp) common to all isoforms of the EP3 receptor and a short (46 bp) sequence unique to the EP3<sub>a</sub> isof orm (Takeuchi et al., 1993). Antisense probes transcribed from this sequence should hybridize to mRNAs encoding all EP3 isoforms. Radiolabeled antisense and sense c-Fos probes were generated from a cDNA clone (Curran et al., 1987) encoding the rat Fos protein. All restriction enzymes and RNA polymerases were obtained from Promega (Madison, WI).

In situ hybridization histochemistry. In situ hybridization histochemical analysis was used to detect cells expressing mRNAs encoding IL-1Rt1, the EP3 receptor, and c-Fos in the nodose ganglia from treated rats as described previously (Simmons et al., 1989). Briefly, slides with nodose ganglion sections were dried overnight in vacuo at room temperature. The sections were additionally post-fixed with 4% paraformaldehyde, pH 7.4, for 10 min, washed with PBS, and digested in preheated proteinase K solution (10 mg/ml proteinase K, pH 8.1). The slides were then rinsed in TE buffer (0.1 M Tris-HCl, pH 8.0, and 0.05 M EDTA, pH 8.0) for 2 min at 37°C. The sections were then rinsed in TE buffer (0.1 M Tris-HCl, pH 8.0, and 0.05 M EDTA, pH 8.0), dehydrated with ethanol, and dried in vacuo at room temperature overnight. Radioactively labeled cRNA probes (1–3×10<sup>6</sup> cpm/μg) were diluted in hybridization buffer [final concentrations of 41% (v/v) formamide, 247 mM NaCl, 8.2 mM Tris-HCl, pH 8.0, 0.82 mM EDTA, 0.82× Denhardt's solution (v/v) dextran sulfate, 111 mg/ml yeast RNA, and 0.2 mM DTT]. The final hybridization solution, 100–120 μl containing 10<sup>6</sup> cpm of either Fos, IL-1Rt1, or EP3R sense cRNA probe, was applied to each slide and covered with a coverslip. All probes were hybridized at 60°C for 16–18 hr. Slides were subsequently rinsed in 4× SSC (1× SSC: 150 mM NaCl and 15 mM sodium citrate, pH 7.0), and the tissue was incubated with 20 μg/ml RNAse A in 0.5× SSC at 37°C for 30 min. The slides were washed in decreasing concentrations of SSC, ending in a final stringency wash of 0.1× SSC for 30 min at 76–78°C. Sections were then dehydrated with ethanol, dried in vacuo, subsequently defatted in increasing concentrations of ethanol and xylenes, air-dried, and dipped in Kodak NTB-2 (Eastman Kodak, Rochester, NY) nuclear track emulsion. Slides were exposed for 14–30 d at 4°C and then developed with Kodak D-19 developer for 4 min at 14–15°C. Sections were counterstained with 0.1% cresyl violet, dehydrated with graded ethanol, and coverslipped. Tissue sections from rats that were preinjected with true blue into the vagal nerve trunk were subjected to the in situ hybridization histochemical procedure as described above, except that light exposure was minimized and sections were not counterstained. To protect the fluorescent dye from fading effects of xylenes and photochemicals, the sections hybridized at 60°C for 16–18 hr. Slides were subsequently rinsed in 4× SSC, 150 mM NaCl and 15 mM sodium citrate, pH 7.0, and the tissue was incubated with 20 μg/ml RNAse A in 0.5× SSC at 37°C for 30 min. The slides were washed in decreasing concentrations of SSC, ending in a final stringency wash of 0.1× SSC for 30 min at 76–78°C. Sections were then dehydrated with ethanol, dried in vacuo, subsequently defatted in increasing concentrations of ethanol and xylenes, air-dried, and dipped in Kodak NTB-2 (Eastman Kodak, Rochester, NY) nuclear track emulsion.

Quantitating IL-1-induced changes in c-Fos expression. Resting cells normally express c-Fos mRNA and protein only at nondetectable-to-low levels, whereas physiological activation rapidly elevates intracellular levels of the transcription factor (Morgan and Curran, 1991). In the present study, we tested whether sensory neurons within the nodose ganglion respond to intravenously injected IL-1β by measuring the cellular expression of c-Fos mRNA using in situ hybridization histochemistry. A minimum of 140 neurons were plotted from each nodose ganglion of vehicle/IL-1<sub>β</sub>, indomethacin/IL-1<sub>β</sub>, indomethacin/vehicle-, or vehicle/ vehicle-injected rats, using camera lucida. Only neurons with clearly visible nuclei were plotted. The area of each neuron was determined at high magnification under Nomarski-enhanced phase contrast optics. Plots and grain counts were done by an observer who was blind to the treatment conditions of each section. A neuron was considered to be specifically labeled for c-Fos mRNA if the silver grains overlaying it exceeded the mean background level ±2 SD (as measured over 20 similarly sized areas immediately surrounding the tissue section). This criterion was used by others who define specific labeling to be more than five times the background levels determined from tissue sections hybridized in parallel with a sense probe. Our procedure more accurately estimates background levels by eliminating variations between slides and probes, although there is still the risk of nonspecific hybridization of the probe to the tissue. Nevertheless, a pilot experiment revealed no significant differences between background levels determined by these two criteria. Specific labeling according to the conventional or our present technique was defined as more than 5 ± 0.6 or 5 ± 0.4 grains per neuron, respectively (20 circular areas with a diameter of 50 μm were analyzed per slide; n = 5).

Statistical analysis. Data are expressed as the mean ± SEM. Comparisons were initially made by ANOVA, followed by Scheff’s (c-Fos induction) or Dunnett’s multiple range (vagal discharge activity) post hoc tests.

RESULTS

Vagal sensory neurons express receptors for IL-1 and PGE2.

We measured the local expression of mRNAs encoding IL-1Rt1 and the EP3 subtype of the PGE2 receptor (EP3R) within the nodose ganglion by in situ hybridization histochemical analysis using 32P-labeled antisense cRNA probes. The IL-1Rt1-specific probe revealed labeling of weak-to-medium intensity exclusively over neuron-like cells in the rat nodose ganglion (Fig. 1A). These neurons were evenly distributed throughout the ganglion, and quantitative evaluation revealed that 26.5 ± 3.4% of the neurons in the nodose ganglion (n = 3) expressed mRNA for IL-1Rt1. Analysis for expression of the rat EP3R in nodose ganglion sections showed high levels of EP3R mRNA exclusively within neuron-like cells throughout the ganglionic mass (Fig. 1C,E). Ganglion sections, hybridized in parallel with 32P-labeled sense control probes for each of the receptors, did not show specific labeling above background levels (Fig. 1B,D). In addition, the expression of IL-1Rt1 and EP3R mRNAs in the nodose ganglia, both in terms of the number of receptor-expressing cells and in the relative intensity of labeling for each mRNA, did not visually differ between rats that had received injections of either recombinant IL-1β (2 μg/kg) or vehicle alone 60 min before perfusion fixation (data not shown).

In some rats (n = 3), the retrograde tracer true blue was injected into the vagus nerve to characterize the nodose ganglion neurons that eminate vagal sensory afferents. In situ hybridization analysis of ganglia from these rats showed specific labeling of EP3R mRNA exclusively localized (Fig. 1F) over neuronal cells in the nodose ganglion that were retrogradely filled with true blue (24.9 ± 5.2% of all the retrogradely labeled cells expressed EP3R mRNA; n = 3). These results demonstrate that EP3R-expressing cells in the nodose ganglia are vagal sensory neurons, which likely receive and relay information from the thoracic and/or abdominal compartments. It was difficult to obtain a reasonable signal using the IL-1Rt1 antisense probe in combination with true blue fluorescence because of a loss of sensitivity in the hybridization procedure, but the overall similarity in morphology and distribution between neurons expressing these two receptors strongly suggests that IL-1Rt1 mRNA is also expressed in vagal sensory neurons. Finally, attempts to analyze adjacent tissue sections that were independently labeled for IL-1Rt1 or EP3R mRNA did not reliably detect neuronal colocalization of these mRNA species.

Effects of blood-borne IL-1β on sensory neurons in the nodose ganglion.

Although in situ hybridization analysis showed only a few widely scattered neuron-like cells expressing c-Fos mRNA in the nodose ganglion of vehicle/vehicle-injected rats (11.10 ± 0.79%; n = 15) (Fig. 2), a substantial increase in their number was seen after IL-1β administration (2 μg/kg, i.v.; 28.40 ± 1.66%; n = 8; p < 0.0001) (Fig. 2). These IL-1-responsive neurons were generally
distributed throughout the nodose ganglionic mass but had a tendency to aggregate caudally (Fig. 2). Interestingly, IL-1 injections resulted in the widespread activation of a population of smaller cells with densely counterstained nuclei, presumably of glial or possibly vascular origin (Fig. 2). Rats pretreated with indomethacin (10 mg/kg, i.v.), a general inhibitor of prostaglandin synthesis, before the IL-1 challenge, revealed a significant reduction in the number of c-Fos-expressing neurons (21.70 ± 1.50%; $n = 9$; $p = 0.0093$, compared with vehicle/IL-1-injected rats) (Fig. 2). The indomethacin/IL-1-treated rats did, however, still show an elevated number of c-Fos-expressing neurons in the nodose ganglion compared with vehicle/vehicle-injected control rats ($p < 0.0001$) (Fig. 2). Rats injected with indomethacin/vehicle also exhibited a slight but nonsignificant increase in the number of c-Fos cells in the ganglion compared with those in the vehicle/vehicle control group (14.73 ± 1.04%; $n = 6$; $p = 0.28$).
Figure 2. Sensory neurons in the nodose ganglion are responsive to blood-borne IL-1. Photomicrographs in the top left panels show in situ hybridization analysis with a 35P-labeled cRNA antisense probe encoding the cellular activation marker c-Fos to 12 μm sections through the nodose ganglia of rats injected with 2 μg/kg human recombinant IL-1β (top photomicrograph) or vehicle alone (bottom photomicrograph) 60 min before perfusion fixation. Note the accumulation of black silver grains, corresponding to specific labeling for c-Fos mRNA, over neuron-like cells in IL-1- but not in vehicle-injected rats. Parallel sections hybridized with a c-Fos cRNA sense probe displayed background levels of silver grains throughout the ganglion (data not shown). Thick black or open arrows, respectively, indicate neurons that either do or do not express c-Fos mRNA. Thin black arrows indicate specific labeling for c-Fos mRNA over non-neuronal cells. Scale bar (in top photomicrograph), 25 μm. Top right panel shows results from the quantitative evaluation of neurons specifically expressing c-Fos mRNA in the nodose ganglia of vehicle/vehicle-, vehicle/IL-1-, indomethacin/IL-1-, or indomethacin/vehicle-injected rats. Silver grains were visually counted over neurons plotted with a camera lucida. The data are displayed in box plots in which the horizontal lines in each box correspond to the 25th percentile, the median, and the 75th percentile, and the range for each group is indicated by the extent of the vertical lines. *p = 0.0134; **p = 0.0093; ***p < 0.0001. The bottom panel is a schematic drawing showing the distribution of c-Fos-expressing cells within the nodose ganglion of IL-1- or vehicle-injected rats. Cells having a neuronal morphology and a clearly delineated nucleus were plotted throughout the ganglion using a camera lucida. Sensory neurons were defined as being specifically labeled or unlabeled with the c-Fos antisense mRNA probe and are represented by filled or open circles, respectively. The outline of each ganglion is shown with the caudal pole of the ganglion positioned to the right.
Intravenously administered IL-1β triggers increased discharge activity of gastric vagal afferents via a prostaglandin-dependent mechanism

Elevated cellular activity, as indicated by an increase in c-Fos expression, in vagal sensory neurons of IL-1-injected rats was matched by a sharp rise in discharge activity of vagal sensory afferent fibers. Electrophysiological analysis showed that the administration of 2 μg/kg human recombinant IL-1β caused a pronounced increase in the discharge activity of gastric vagal afferents (Fig. 3A,C). The response was immune within minutes after drug administration, manifested peak values (155 ± 9% of preinjection discharge levels) by 30 min (n = 5), and was still elevated (130 ± 12% of preinjection discharge levels) after 60 min. Vehicle-injected control rats (n = 6) did not display significant changes in afferent discharge activity compared with preinjection levels. Furthermore, administration of indomethacin before IL-1β injection completely blocked the IL-1-mediated rise in discharge activity (n = 5) (Fig. 3), indicating that these IL-1 effects on gastric afferents are dependent on endogenous prostaglandins. The initial injection of indomethacin alone did not alter the pattern of afferent discharge activity during the 60 min preceding the IL-1 injection (data not shown).

DISCUSSION

The activation of central autonomic neurocircuits during peripheral inflammatory processes raises fundamental questions about the mechanism(s) by which immune–neural communication occurs, whether via humoral effects across the blood–brain barrier or by activating peripheral sensory afferents. The present investigation explored the responsiveness of vagal sensory neurons in the nodose ganglion to intravenous IL-1β. Our data demonstrate that nodose ganglion neurons express IL-1Rt1 and EP3 receptors under basal conditions and that administration of IL-1β potently stimulates vagal sensory neurons in a partially prostaglandin-dependent manner, as measured by the expression of the cellular activation marker c-Fos and electrophysiological recordings.

Vagal sensory afferents are responsive to blood-borne IL-1

Central responses to immune challenges within the peritoneum depend on intact abdominal vagal afferents (Watkins et al., 1995b). Although this clearly suggests that vagal mechanisms mediate aspects of the systemic responses to immunological challenges within the abdominal cavity, the involvement of vagal viscerosensory afferents in mediating central responses to intravenous IL-1 or LPS remain controversial (Katsuura et al., 1988; Wan et al., 1994; Sehic and Blatteis, 1996; Ericsson et al., 1997; Romanovsky et al., 1997). Here, we demonstrate that intravenous infusion of IL-1β (2 μg/kg) causes nuclear induction of the transcription factor c-Fos within sensory neurons of the nodose ganglion. This marker of cellular activation has been used to identify activated central neural circuitries after an IL-1 challenge (Ericsson et al., 1994). The induction of c-Fos in nodose ganglion neurons in response to IL-1 strongly suggests that vagal sensory functions are triggered by this stimulus, which is further supported by our findings that gastric vagal afferents display rapid and lasting increases in discharge activity after an IL-1 challenge. This effect was also shown in animals whose gastric sympathetic nerves were crushed (data not shown), demonstrating that the IL-1-induced increase in gastric vagal afferent activity is not secondary to changes in the efferent activity of the vagus and/or sympathetic nerves. However, it remains to be analyzed whether the vagal responses to IL-1 were produced by recruitment of silent afferent fibers and/or by increases in spontaneous afferent firing rates.

These findings reveal that the activation of vagal viscerosensory afferents induced by IL-1β occurs not only when the cytokine is delivered to the abdominal cavity, but also when it is introduced into the general circulation. This is in agreement with findings that intravenous IL-1β stimulate hepatic afferents (Nijjima, 1996) and that selective hepatic vagotomy block fever responses to low levels of circulating LPS (Sehic and Blatteis, 1996; Simons et al., 1998). In contrast, abdominal vagotomies do not interfere with the activation of central neurocircuits, fever, or endocrine responses after intravenous administration of...
moderate-to-high doses of IL-1 or LPS (Katsuura et al., 1988; Wan et al., 1994; Ericsson et al., 1997; Romanovsky et al., 1997). Collectively, these findings suggest that abdominal vagal afferents mediate central responses to low levels of circulating IL-1 or LPS and that other mechanisms, including local signaling events across the blood–brain barrier and/or thoracic vagal and nonvagal afferents, are involved in mediating central responses to moderate-to-high plasma levels of IL-1 or LPS.

Neurons in the nodose ganglion receive viscerosensory information from pharynx, larynx, and the abdominal and thoracic compartments. Although these neurons do not display strict somatotopic organization within the ganglion, the sensory neurons receiving input from the abdominal organs are generally distributed in the caudal and middle portions of the ganglia (Sharkey et al., 1984; Gwyn et al., 1985; Green and Dockray, 1987), whereas those with afferent input from the larynx, esophagus, and the aortic depressor nerve tend to aggregate within the rostral pole of the ganglion (Altschuler et al., 1989; Hopkins and Armour, 1989; Uno et al., 1996). Our findings of c-Fos-labeled sensory neurons within the caudal, middle, and rostral divisions of the nodose ganglion are consistent with the simultaneous activation of gastric (present results; Kurosawa et al., 1997), hepatic (Niijima, 1996), and possibly thoracic vagal afferents in response to circulating IL-1. Extended studies are required to determine to what extent each vagal branch is involved in responding to an intravenous IL-1 challenge.

Prostaglandins mediate aspects of the vagal responses to peripheral inflammatory stimuli

Prostaglandins mediate central responses after peripheral cytokine administration. Pharmacological blockade of endogenous cyclooxygenase activity inhibits hyperthermia (Morimoto et al., 1989) and ACTH responses to intravenously administered IL-1β (Watanabe et al., 1990). Indomethacin pretreatment also reduces or prevents IL-1-mediated corticosterone secretion (Dunn and Chuluyan, 1992; Kandasamy et al., 1995), central c-Fos and hypothalamic CRF mRNA induction (Ericsson et al., 1997), behavioral responses (Crestani et al., 1991), and hypothalamic noradrenaline depletion (Dunn and Chuluyan, 1992). Moreover, infusion of IL-1 results in elevated plasma levels of PGE2 (Rondono et al., 1988; Watanobe et al., 1995), and intravenous administration of PGE2, like IL-1, induces ACTH secretion (Hollingsworth et al., 1995; Watanobe et al., 1995; Young et al., 1996). Although collectively these data indicate that prostaglandins participate in various aspects of the host systemic response to IL-1, limited evidence is available on how different prostaglandin subtypes influence central functions. Interestingly, several studies have demonstrated the contribution of prostaglandins in vagal afferent transmission. For example, PGE1, PGE2, and PGE2e exhibit multifaceted regulatory actions on vagal afferents transmitting cardiac, baroreceptor, and pulmonary sensory information (Kalix, 1979; Bergren et al., 1984; Panzenbeck et al., 1988; Taguchi et al., 1992; Lee and Morton, 1995). In addition, prostanoid attenuates baroreflex control of renal nerve activity via vagal afferents (Zucker et al., 1988), and tromboxane induces rapid, shallow breathing via vagal pulmonary receptors (Karla et al., 1992; Carrithers et al., 1994). Here, we show that prostaglandins are likely to contribute to vagal responses after administration of IL-1, because these responses were attenuated by indomethacin pretreatment. Similarly, increases in vagal hepatic afferent activity after intravenous administration of IL-1 are partly mediated via prostaglandins (Niijima, 1996). It remains to be determined whether prostaglandins exert their own independent actions on vagal sensory neurons or whether they augment the actions of IL-1. For instance, prostaglandins may influence the expression and/or function of IL-1 receptors on vagal afferents (see below), similar to the effects of PGE2 on sensitizing vagal responses to bradykinin (Staszewska-Barczak, 1983) and capsaicin (Lee and Morton, 1995). The effective blockade of IL-1-mediated increase in gastric vagal discharge activity in indomethacin-pretreated rats contrasts to the findings of a population of prostaglandin-independent IL-1-responsive neurons in the nodose ganglion. This suggests that distinct vagal viscerosensory afferents are differentially dependent on prostaglandins in their response to blood-borne IL-1. Collectively, these data show that prostaglandins regulate a variety of vagus-dependent processes and that they are likely to contribute in part to aspects of IL-1-mediated activation of vagal sensory functions.

Sensory neurons in the nodose ganglion express IL-1 and prostaglandin receptors

The partial involvement of prostaglandins in IL-1-induced vagal sensory activation suggests that cells in the nodose ganglion express both IL-1 and prostaglandin receptors. Evidence for this has been based on receptor-binding studies examining prostaclin binding on vagal afferents (Matsumura et al., 1995), as well as IL-1 receptor antagonist binding on glomus cells located within vagus nerve-associated paraganglia (Goehler et al., 1997). Paraganglia are distributed along the vagus nerve trunks in the abdomen and thorax, and they receive rich innervation from vagal afferents (Berthoud et al., 1995). It remains, however, to be ascertained whether these glomus cells actually express the transmembrane-signaling IL-1R1 and whether they interact with vagal sensory afferents. Here, we provide the first experimental data to show that vagal sensory neurons themselves synthesize mRNA encoding receptors for both IL-1 and PGE2. These findings demonstrate an alternative and more direct means by which IL-1 and PGE2 may regulate sensory functions of the vagus nerve.

Functional considerations

The hepatic vagal branch is implicated in driving centrally regulated systemic responses to peripheral immune stimuli, such as hyperalgesia (Watkins et al., 1994a) and hyperthermia (Simons et al., 1998). On the other hand, adrenocortical responses to intraperitoneal IL-1 are attenuated after subdiaphragmatic, but not hepatic, vagotomy (Fleshner et al., 1995), suggesting that gastrointestinal vagal afferents activate central neurocircuits involved in stress-related endocrine responses. In accordance, the kinetics of the electrophysiological responses in our study (i.e., discharge activity increases within 10 min and peaks 30 min after IL-1 administration) mimic the kinetics of cytokine-induced ACTH secretion (Rivier et al., 1989b) and hyperthermia (Simmons and Fewell, 1995). The IL-1-responsive viscerosensory neurons in the nodose ganglion identified in the present study are thus likely to participate in the central component of cytokine-responsive endocrine, nociceptive, and fever responses. Our current results, revealing that increased discharge activity of the gastric vagal branches to intravenous IL-1 is dependent on prostaglandins, extend our previous results, which showed that this vagal response is partly mediated via cholecystokinin (CCK) (Kurosawa et al., 1997). Interestingly, gastric vagal afferents are known to convey satiation signals to the CNS in a CCK-dependent manner (Smith et al., 1985). IL-1-induced anorexia is partly mediated via type A
CCK receptors in peripheral organs (Daun and McCarthy, 1993), and intravenous IL-1 sensitizes gastric afferents to the stimulatory actions of CCK on type A receptors (Bucinskaitė et al., 1997; Kurosawa et al., 1997). Moreover, IL-1-induced anorexia is blocked after systemically, but not centrally, administered cyclooxygenase inhibitors (Hellerstein et al., 1989; Uehara et al., 1989; Shimizu et al., 1991; McCarthy and Daun, 1992). These findings strongly suggest the involvement of gastric vagal afferents, CCK, and prostaglandins in mediating anorexie behaviors after IL-1 administration.

Conclusions

It is increasingly evident that the immune system has the capacity to affect central neural functions via several independent pathways. Our present findings reveal that there is a local production of IL-1 and PGE2 receptors in vagal sensory neurons and that blood-borne IL-1 stimulates vagal viscerosensory pathways in a partially prostaglandin-dependent manner. This reinforces the growing awareness that the vagus nerve functionally participates in relaying information of peripheral inflammatory insults to central autonomic regulatory centers.

REFERENCES


