The contribution of the vagus nerve in interleukin-1β-induced fever is dependent on dose

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Hansen, Michael K., Kevin A. O’Connor, Lisa E. Goehler, Linda R. Watkins, and Steven F. Maier. The contribution of the vagus nerve in interleukin-1β-induced fever is dependent on dose. Am J Physiol Regulatory Integrative Comp Physiol 280: R929–R934, 2001.—It has been suggested that proinflammatory cytokines communicate to the brain via a neural pathway involving activation of vagal afferents by interleukin-1β (IL-1β), in addition to blood-borne routes. In support, subdiaphragmatic vagotomy blocks IL-1β-induced, brain-mediated responses such as fever. However, vagotomy has also been reported to be ineffective. Neural signaling would be expected to be especially important at low doses of cytokine, when local actions could occur, but only very small quantities of cytokine would become systemic. Here, we examined core body temperature after intraperitoneal injections of three doses of recombinant human IL-1β (rh-IL-1β). Subdiaphragmatic vagotomy completely blocked the fever produced by 0.1 μg/kg, only partially blocked the fever produced by 0.5 μg/kg, and had no effect at all on the fever that followed 1.0 μg/kg rh-IL-1β. Blood levels of rh-IL-1β did not become greater than normal basal levels of endogenous rat IL-1β until the 0.5-μg/kg dose nor was IL-1β induced in the pituitary until this dose. These results suggest that low doses of intraperitoneal IL-1β induce fever via a vagal route and that dose may account for some of the discrepancies in the literature.

Proinflammatory cytokines [interleukin (IL)-1α and -β, tumor necrosis factor-α, and IL-6] are released by peripheral immune cells in response to pathogenic challenge (20). These cytokines play a local role at the site of infection in mediating immune defense, but they also signal the central nervous system, thereby initiating the brain-mediated components of host defense such as fever. Thus the peripheral administration of cytokines such as IL-1β leads to fever and other brain-mediated, host-defense responses (21) as well as a distinctive pattern of neural activation (4, 10) and neurochemical changes (7). Analogously, the peripheral blockade of receptors for IL-1 blocks or reduces the neural activation and host-defensive responses (6) that follow challenge with immune-activating agents such as lipopolysaccharide (LPS; a constituent of the cell walls of gram-negative bacteria). In addition, this immune-to-brain signaling induces behavioral changes characteristic of depressed mood (38) as well as other changes not typically associated with host defense (23).

Although it is clear that cytokines such as IL-1β signal the brain, the pathway(s) by which this communication is accomplished remains a matter of controversy. Because there are receptors for IL-1β and other cytokines in the brain (1), it is natural to suggest that blood-borne cytokines enter the brain and bind to their receptors, thereby initiating the neural cascade. However, cytokines are large peptides and are unlikely to cross the blood-brain barrier in significant quantities. This has led to suggestions that blood-borne cytokines are carried into the brain by specific active transport mechanisms (2), initiate signaling at regions of the brain where the blood-brain barrier is weak or absent (3), and bind to receptors on the inside of the cerebral vasculature, thereby leading to the release of other second-order messengers (e.g., prostaglandins) on the “brain side” of the vasculature (9).

Alternatively, it has recently been suggested that cytokines also communicate to the brain via a neural route. It has been argued that cytokines such as IL-1β bind to receptors located on afferent vagal fibers or structures closely associated with afferent vagal fibers (14), thereby activating afferent vagal fibers (12) that terminate in the nucleus of the solitary tract (NTS) and initiate the neural cascade. The most compelling evidence for vagal signaling comes from studies in which IL-1β has been injected intraperitoneally in animals in which the vagus has been severed at the subdiaphragmatic level. The frequent result has been that subdiaphragmatic vagotomy blocks both the changes in the brain (e.g., norepinephrine release) and the host-defense responses (e.g., fever) that would normally follow the IL-1β administration (see Ref. 22 for a review).

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However, the vagotomy experiments have been controversial, and a number of failures to find any effects of vagotomy on neurally mediated responses to IL-1β has been reported (see below). Although there is a large number of factors that might influence the outcome of abdominal deafferentation studies, the assumption that there exist both blood-borne and neural pathways of immune-to-brain communication suggests that cytokine dose is likely to be a critical factor. This is because only very small amounts of cytokine might be expected to become systemic after the intraperitoneal injection of small doses, thereby yielding a balance that relies on vagal communication. In contrast, larger quantities may become systemic after larger intraperitoneal doses, thereby shifting the balance to blood-borne signaling. Dose may even play the same role after intravenous injection, with most of the cytokine being retained in the liver after small intravenous doses (26), with the hepatic branch of the vagus then taking on a primary signaling role (33, 37). It is thus of interest that vagotomy has been reported to block the sleep-promoting effects of intraperitoneal recombinant human IL-1β (rh-IL-1β) only at a very low 0.1-μg/kg dose (16). However, blood levels of rh-IL-1β were not measured, and so it is difficult to interpret the effects of dose in this study. Fever has been the most often measured outcome of peripheral IL-1β, although IL-1β dose has not been manipulated in a vagotomy/fever study, and dose has varied over a wide range in reported studies. The present studies examined the effects of subdiaphragmatic vagotomy on the fever produced by different doses of intraperitoneal rh-IL-1β as well as the entry of rh-IL-1β into blood and the induction of pituitary IL-1β at these doses.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats (250 g at purchase; Harlan Sprague Dawley, Indianapolis, IN) were used in all studies. All animals (n = 76) were individually housed in plastic cages at 25 ± 1°C with a 12:12-h light-dark cycle (lights on at 0800), and standard rat chow and water were freely available. Care and use of the animals were in accordance with protocols approved by the University of Colorado Institutional Animal Care and Use Committee.

Subdiaphragmatically vagotomized (Vag) and sham-operated (Sham) rats were prepared under halothane anesthesia as previously described in detail (37). In addition, precalibrated radio transmitters (MiniMitter, Sun River, OR) for measuring core body temperature (CBT) were implanted in the peritoneal cavity at the time of surgery. During the immediate postaural period (~2 days), Sham and Vag rats were maintained on highly palatable food and received acetaminophen (0.5 mg/ml) in their drinking water. Verification of vagotomy was performed using food-intake analysis and stomach weight measurements as previously described (15).

Experimental testing occurred ~4 wk after surgery. At the time of experimental testing, all animals were gaining weight (Sham: 365 ± 5 g; Vag: 329 ± 8 g) and appeared healthy. Each rat (Sham: n = 24; Vag: n = 16) was injected with vehicle (sterile, pyrogen-free saline) on a control day, and CBT was measured for 6 h after the injection using standard telemetry techniques. On the next day, each rat was injected with one dose (0.1, 0.5, or 1 μg/kg) of rh-IL-1β (provided by the Biological Response Modifiers Program, National Cancer Institute). These doses were chosen on the basis of a pilot experiment designed to determine the minimum dose that would produce fever. A dose of 0.1 μg/kg produced fever, whereas a dose of 0.05 μg/kg did not. All injections were performed 2 h after light onset in an injection volume of 1 ml/kg. After the rh-IL-1β injections, CBT measurements were again taken for 6 h after which all rats were killed by decapitation. At the time of death (6 h after rh-IL-1β injection), pituitary samples were collected, snap-frozen in liquid nitrogen, and stored at −80°C until processed. In addition, pituitary samples were collected from a separate group of Sham and Vag rats (n = 8) that received saline injections on both the control and test day.

The pituitary samples were processed for endogenous rat IL-1β measurements as previously described (27). Briefly, the pituitary tissue was sonicated in a sonication buffer, centrifuged (10,000 rpm, 10 min, 4°C), and supernatants were collected and stored at −20°C until assayed. Bradford protein assays were performed to determine total protein concentrations. Pituitary IL-1β protein levels were measured using a commercially available ELISA kit (R & D Systems, Minneapolis, MN) as previously described (18). Cross-reactivity with rh-IL-1β is 1.6%.

In a second experiment, rats (n = 28) received either vehicle (pyrogen-free saline), 0.05, 0.1, or 0.5 μg/kg rh-IL-1β. Rats were killed 15 and 30 min later by decapitation, blood was collected in sterile tubes, and serum was obtained by centrifugation (3,000 rpm, 20 min, 4°C) and stored at −20°C until assayed. Serum rh-IL-1β protein levels were measured using a commercially available ELISA kit for human IL-1β (R & D Systems). There is no significant cross-reactivity with rat IL-1β.

The effects of vagotomy and rh-IL-1β on CBT were analyzed by repeated-measures ANOVA. The effects of rh-IL-1β on blood levels of human IL-1β and pituitary rat IL-1β were evaluated with a two-way ANOVA. Post hoc analysis was done, when appropriate, using the Student-Newman-Keuls multiple-comparison test. In all tests, an α-level of P < 0.05 was accepted as indication of statistical significance.

RESULTS

The rh-IL-1β produced a dose-dependent fever [F(2,21) = 14.33, P < 0.0001], with both the 0.5- and 1.0-μg/kg doses producing greater elevations in CBT than did the 0.1-μg/kg dose. The 0.5- and 1.0-μg/kg doses did not differ. Figure 1A presents the CBT after the control saline and the 0.1-μg/kg rh-IL-1β injections in Vag and Sham subjects. There was no difference in CBT between the Vag and Sham subjects on the saline day (P < 1.0), indicating that vagotomy did not alter basal CBT. The low dose of 0.1 μg/kg rh-IL-1β produced fever that was completely blocked by vagotomy. The 0.1-μg/kg rh-IL-1β injection, relative to saline, did not lead to increased CBT in the Vag subjects [F(1,7) = 3.70, P > 0.13], but it did produce fever in the Sham subjects [F(1,7) = 242.32, P < 0.0001]. Thus CBT after rh-IL-1β injection differed between Sham and Vag groups [F(1,11) = 6.58, P < 0.03]. Figure 1B presents the data for the low dose expressed as a difference between CBT on the saline and rh-IL-1β days for the Vag and Sham subjects. As is evident, the 0.1-μg/kg dose did not produce fever in the Vag subjects, but it did so in the Sham group [F(1,11) = 18.29, P < 0.002].
The pattern of data following 0.5 mg/kg rh-IL-1β was quite different (Fig. 2). Again, there were no differences between groups on the saline day ($F_{(1,0)}$). However, vagotomy produced only a marginal attenuation in the increase in CBT produced by rh-IL-1β. Sham subjects responded strongly to rh-IL-1β [$F_{(1,7)} = 84.18, P < 0.0001$], whereas the fever in Vag subjects was marginal [$F_{(1,4)} = 5.95, P < 0.08$]. The difference between Sham and Vag subjects after the rh-IL-1β injection was also marginal [$F_{(1,11)} = 3.65, P < 0.09$]. The data expressed as change from baseline (Fig. 2B) also indicate only a blunting of the increase in CBT produced by vagotomy [$F_{(1,11)} = 4.09, P < 0.07$].

The 1.0-µg/kg dose yielded a yet different pattern (Fig. 3). In this case, Vag animals displayed a slightly, but not significantly, lower CBT after saline injection than did Sham subjects [$F_{(1,12)} = 4.11, P < 0.07$]. For this dose, vagotomy had no effect at all on the fever after the rh-IL-1β injection. Both Sham [$F_{(1,7)} = 293.47, P < 0.0001$] and Vag [$F_{(1,5)} = 64.89, P < 0.0005$] groups responded to the rh-IL-1β, and the two groups showed an equal increase in CBT from baseline ($F < 1.0$).

Blood levels of rh-IL-1β 15 and 30 min after intraperitoneal injection of saline or rh-IL-1β are shown in Fig. 4. Measurable blood levels were present even after the 0.05-µg/kg dose that does not produce fever. A further small increase was evident after 0.1 µg/kg and a much larger increase after 0.5 µg/kg [$F_{(3,20)} = 74.21, P < 0.0001$].

The differences in blood levels after the 0.5-µg/kg dose compared with both the 0.05- and 1.0-µg/kg doses were significant, whereas the difference between the 0.05- and 1.0-µg/kg doses was not significant.

Pituitary IL-1β levels (Fig. 5) were increased after intraperitoneal injection of rh-IL-1β in a dose-dependent manner [$F_{(3,38)} = 22.92, P < 0.0001$]; there were large increases after 1.0 µg/kg, moderate increases after 0.5 µg/kg, and no increases at all after 0.1 µg/kg. Vagotomy neither altered pituitary IL-1β [$F_{(1,38)} < 1$] nor did it reduce the effect of rh-IL-1β injection ($F < 1.0$).

**DISCUSSION**

The present study demonstrates that whether or not subdiaphragmatic vagotomy will block the effects of intraperitoneal IL-1β on fever does indeed depend on the dose of IL-1β. Vagotomy completely blocked fever at a dose of 0.1 µg/kg, the lowest dose at which intraperitoneal IL-1β produced increased CBT in pilot work. Vagotomy had a partial effect at 0.5 µg/kg and no effect at all at 1.0 µg/kg. It is difficult to compare doses across studies because there are large differences in the biological activity of rh-IL-1β across different lots and

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**Fig. 1.** A: mean core body temperature for sham surgery (Sham) and vagotomized (Vag) subjects injected at time 0 with saline (Sal) or 0.1 µg/kg recombinant human interleukin-1β (rh-IL-1β). B: the difference between core body temperature after injection with rh-IL-1β and after Sal for Sham and Vag subjects.

**Fig. 2.** A: mean core body temperature for Sham and Vag subjects injected at time 0 with Sal or 0.5 µg/kg rh-IL-1β. B: the difference between core body temperature after injection with rh-IL-1β and after Sal for Sham and Vag subjects.
However, it can be noted that Hansen and Krueger (16) found vagotomy to almost completely block the somnogenic effects of 0.1 mg/kg, to only mitigate the effects of 0.5 mg/kg, and to have no effect after 2.5 mg/kg. It can also be noted that Porter et al. (29) and Schwartz et al. (31), who failed to detect an effect of vagotomy on intraperitoneal IL-1β-induced decreases in feeding, used a single dose of 2 mg/kg. Dose is also likely to be a critical issue in intraperitoneal LPS studies. Vagotomy has been reported to have no effect on intraperitoneal LPS-induced fever (5) and anorexia (29, 31), with the single dose used being 50 and 100 μg/kg, respectively. However, even 1.0 μg/kg ip LPS produces substantial fever (15). Indeed, the fever produced by 1.0 μg/kg ip LPS was as large as that produced by 50 μg/kg, although it was not as persistent. In keeping with the argument made here, vagotomy did not reduce the fever produced by 1.0 μg/kg ip LPS (15), and lower doses are currently being explored.

The argument being made is that at low intraperitoneal doses, very little IL-1β enters the circulation, and so the fever or other end point being measured is generated by abdominal vagal input to the brain. This input could arise either from direct IL-1β action in the abdomen (serosal or draining lymphatic actions) or by circulating IL-1β acting on vagal afferents in the liver or other organs. Under these conditions, subdiaphragmatic vagotomy would naturally have a major impact. At higher doses, significant quantities of IL-1β become systemic, and the end point is then produced by a combination of vagal and other signaling that result from blood-borne cytokines (e.g., entry at circumventricular organs). Here, vagotomy should have less and less impact the higher the dose. The blood levels of rh-IL-1β measured after 0.05, 0.1, and 0.5 μg/kg rh-IL-1β administration are consistent with this argument. The 0.05-μg/kg injection led to very little rh-IL-1β in blood; only 14 pg/ml were present at 15 min. To place this amount in context, normal blood levels of endogenous IL-1β in untreated rats are in the 25- to 50-pg/ml range (18). Thus, neither the blood level of rh-IL-1β nor the degree of vagal activation produced by 0.05 μg/kg is sufficient to produce fever. Although blood levels of rh-IL-1β were slightly higher (38 pg/ml at 15 min) after the 0.1-μg/kg dose, the difference was not statistically significant. Moreover, these levels were still quite small relative to normal basal levels of endogenous rat IL-1β, and so blood-borne rh-IL-1β was unlikely to be the source of the fever after this dose. Rather, the fever was likely generated by abdominal vagal activation, and thus subdiaphragmatic vagotomy completely blocked the fever. The 0.5-μg/kg dose led to a much larger quantity of circulating rh-IL-1β (164 pg/ml), and so vagotomy naturally had less impact. Although not measured, 1.0 μg/kg would have doubt-
lessly been followed by very high blood levels of rh-IL-1β, and so abdominal vagal deafferentation had no effect at all.

Pituitary IL-1β was measured because induction of IL-1β in the pituitary by intraperitoneal rh-IL-1β injection almost certainly is mediated via blood-borne transmission of the rh-IL-1β to the pituitary. Indeed, vagotomy had no effect on the induction of IL-1β in the pituitary at any dose. The important finding, however, was that the 0.1-μg/kg dose did not induce IL-1β in the pituitary. This supports the contention that the quantity of rh-IL-1β that enters the blood after this intraperitoneal dose is insufficient to produce signaling and that the fever that follows this dose is likely to be mediated independently of IL-1β in the blood. It might be argued that the present study examined only a 6-h time point and that pituitary IL-1β would have been elevated at some other time after the 0.1-μg/kg dose. This is, of course, possible.

This line of reasoning suggests that dose should be critical after intravenous administration as well. Small quantities of intravenously injected LPS, and perhaps rh-IL-1β as well, should be rapidly cleared from the circulation by the liver (26), and the liver is a particularly rich source of abdominal vagal afferents and local cytokine production and release from Kupffer cells. Thus intravenously injected substances would be able to generate a vagal signal to the brain at the liver (32). However, hepatic filtration would become saturated at higher doses, resulting in more sustained circulation of cytokines or LPS. Thus, as with intraperitoneally administered agents, subdiaphragmatic vagotomy should only be effective at low doses. Indeed, Romanovsky et al. (30) have reported that subdiaphragmatic vagotomy blocks the fever that follows 1.0 μg/kg iv LPS but not the fever that follows 10 to 1,000 μg/kg iv LPS. Indeed, experiments that have found vagotomy to have no effect after intravenous LPS (5, 35, 36) have employed doses ranging from 20 to 400 μg/kg, whereas a negative intravenous rh-IL-1β experiment (9) used a single dose of 1.87 μg/kg.

It should be noted that there is a large amount of additional evidence for vagal transmission of immune-to-brain signals. 1) IL-1-binding sites are located on structures associated with abdominal vagal terminals (14), and IL-1 receptor mRNA is present in the cell bodies of afferent vagal fibers (8). 2) Macrophages and other immune cells, some constitutively expressing IL-1β, are intermingled with and surround abdominal vagal fibers (13), thereby providing a mechanism whereby LPS or pathogenic agents can lead to local IL-1β production and release onto vagal terminals. 3) These lymphoid cells associated with the abdominal vagus rapidly increase IL-1 expression after intraperitoneal administration of immune-activating agents (13). 4) Intraperitoneally and intravenously administered IL-1β and LPS activate afferent vagal fibers as indicated by measurement of electrical activity (28) and c-fos expression in the cell bodies of afferent vagal fibers (11, 12). 5) There is a rapid increase in extracellular levels of glutamate in the NTS after intraperitoneal administration of LPS (25), and glutamate is known to be released by vagal terminals at their site of termination in the NTS (34). 6) Peripheral electrical stimulation of the vagus leads to neural alterations characteristic of peripheral immune activation by LPS and other agents (19).

The present data, along with those summarized above, indicate that the vagus nerve can carry the immune-to-brain signal that initiates neurally mediated host defense and that blood-borne extra vagal communication is not necessary. The critical importance of dose is consistent with the proposal that neural signaling routes are important early in an infection before significant blood levels of cytokines have developed or under conditions in which neither the infectious agent nor locally produced cytokines have become systemic (24). Later, when blood levels of cytokines or the infectious agent itself become systemic, blood-borne routes may come to play the dominant role. However, even here the vagus may play a role. Circulating cytokines will have access to vagal afferents in the liver and in regions such as the lungs that are not deafferented by subdiaphragmatic vagotomy. Whether vagal activation by blood-borne, rather than locally acting, cytokines is a factor in immune-to-brain communication remains to be determined.

Perspectives

The pathways used in cytokine-to-brain communication remain a topic of lively debate. Strong arguments have been made for both blood-borne and neural routes, but it seems clear that multiple-communication routes are used. This would seem entirely appropriate for a function so important for host defense during infection. The present results, along with those of Hansen and Kruger (16) and Romanovsky et al. (30), firmly suggest that whether vagotomy will block the effects of peripherally administered IL-1β or LPS depends on dose, and so subsequent studies of vagal deafferentation should be attentive to this issue. It is clear that it is possible to administer sufficiently large doses of IL-1β or LPS such that subdiaphragmatic vagotomy is no longer effective in reducing the impact of the injected substance. An understanding of the functional significance of this dose dependency will require studies similar to those conducted here, however, with the use of infectious agents rather than bolus injections of cytokines or LPS. We (15, 24) and Romanovsky et al. (30) have suggested that vagal afferents are likely to be an especially important pathway early in an infection and in response to small challenges in the physiological range, and the present data are fully supportive of this suggestion.

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