letters to the editor

The following is the abstract of the article discussed in the subsequent letter:

Scammell, Thomas E., John D. Griffin, Joel K. Elmquist, and Clifford B. Saper. Microinjection of a cyclooxygenase inhibitor into the anteroventral preoptic region attenuates LPS fever. *Am. J. Physiol.* 274 (*Regulatory Integrative Comp. Physiol.* 43): R933–R935, 1998.—Considerable evidence supports the role of prostaglandins in fever production, but the neuroanatomic sites of prostaglandin synthesis that produce fever remain unknown. With the use of a novel microinjection technique, we injected the cyclooxygenase inhibitor ketorolac into the preoptic area (POA) to determine which preoptic regions produce the prostaglandins required for fever. Initial experiments demonstrated that intravenous ketorolac blocked the fever normally produced by lipopolysaccharide (LPS) 5 µg/kg iv. Microinjection of ketorolac into the POA had no effect on body temperature, and injection of artificial cerebrospinal fluid into the POA did not alter LPS fever. Injection of ketorolac into the anteroventral POA markedly decreased the fever produced by LPS, compared with injections into more rostral, caudal, or dorsal locations. These observations indicate that prostaglandin synthesis in the anteroventral preoptic region is necessary for the production of fever.

Febrile nonresponsiveness of vagotomized animals: is it due to endotoxin translocation from the gut and tolerance?

To the Editor: In their recent article, Scammell and colleagues (5) discuss afferent signals that drive preoptic prostaglandin E_2 synthesis and fever after intravenous injection of lipopolysaccharide (LPS). The authors examine the concept of these signals being carried to the brain by the vagus nerve and take a closer look at two studies supporting this concept: a study by Sehic and Blatteis (6) showing a blockade of the biphasic febrile response to intravenous LPS in guinea pigs and our study (3) demonstrating ablation of the monophasic LPS fever in rats. Concurring with our repeated warnings (2–4, 7), the authors caution that alternative interpretations of these vagotomy experiments are conceivable. The possibility of multiple interpretations stems from two facts. First, truncal subdiaphragmatic vagotomy often results in severe complications such as gross emaciation, thermoeffector insufficiency (for review, see Ref. 3), and bacterial translocation from the gut (1), to name a few. Second, these side effects of vagotomy may by themselves decrease the febrile responsiveness. However, many of the side effects are preventable with special care and do not occur in fostered vagotomized animals, whereas the febrile nonresponsiveness does (3). Experiments in well-nourished rats rejected the explanation of the vagotomy-induced febrile nonresponsiveness as being due to either malnutrition (3) or thermoeffector incompetence (2). In their article, Scammell et al. (5)

propose a new interpretation of attenuated fevers in vagotomized animals. The authors suggest that slowed motility of the denervated gut promotes translocation of intestinal flora and their constituents into portal circulation, ultimately resulting in tolerance to LPS. However, the existing data allow for rejection of this intriguing hypothesis.

The first sign of tolerance to LPS is attenuation of the second phase of biphasic fever (whether LPS or interleukin-1 induced), without tangible changes in the first phase and without changes in the monophasic response to a low pyrogen dose (8). Yet well-nourished rats with subdiaphragmatic truncal vagotomy do not respond to a monophasic fever-inducing dose of intravenous LPS, but show no abnormality in the second febrile phase when injected with a wide range of higher doses (4). This is exactly the opposite of what is expected in LPS tolerance.

Our recent study with selective subdiaphragmatic vagotomies (7) provides an even stronger argument against the translocation hypothesis. Indeed, if vagotomy were to result in the proposed sequela (intestinal dysfunction→subsequent bacterial translocation from the gut→eventual tolerance to LPS), it would be denervation of the intestine (and/or perhaps stomach) that is responsible for these events. However, the rats with a denervated intestine (selective celiac vagotomy) exhibited no suppression of LPS fever and neither did the animals with selective gastric vagotomy. In contrast, the animals with selective hepatic vagotomy displayed drastically reduced fevers. This is despite the fact that the hepatic branch is a small, primarily afferent nerve, minimally contributing to efferent innervation of the gut. Moreover, selective transection of the hepatic branch does not lead to those devastating changes in gastrointestinal functions that are common in animals with gastric and, to a lesser extent, celiac vagotomies. Obviously, subdiaphragmatic vagotomy induces febrile nonresponsiveness via a mechanism localized in the liver and/or portal vein, but not in the gut. We conclude that the hypothesis by Scammell and colleagues (5) does not satisfactorily explain the published results of vagotomy experiments (3, 4, 6) and, therefore, does not dismiss the current concept of vagal afferents conveying peripheral pyrogenic signals to the brain.

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REPLY

To the Editor: In our recent article (9), we described the attenuation of lipopolysaccharide (LPS) fever by inhibition of prostaglandin synthesis in the preoptic area. We and others have proposed that LPS and circulating cytokines may activate perivascular and meningeal cells, which then release prostaglandin E_2 into the preoptic area to initiate fever. As a small point in our discussion, we suggested that febrile unresponsiveness to LPS after vagotomy could be due to the development of tolerance to LPS. Specifically, bacterial translocation can occur after gastric or truncal vagotomies (2) and an increased portal bacterial load could produce LPS tolerance. Romanovsky presents well-reasoned arguments against this idea and in support of the vagal hypothesis, but we feel this perspective cannot account for many observations.

Romanovsky argues that LPS tolerance results in attenuation of the second phase of fever but has little effect on the first phase. This phenomenon has been shown in several studies in which animals were treated with LPS for several days, but we are unaware of any in which a low dose of LPS or bacteria was administered over several weeks, modeling the tolerance that may occur with vagotomy. Direct evidence such as measurements of portal LPS, Kupffer cell reactivity, and cytokine concentrations and bioactivity after vagotomy may help clarify this important concern.

The strongest evidence in support of the vagal hypothesis is Simons and colleagues (10) recent study that demonstrated an attenuation of fever to a very low dose of intravenous LPS (1 µg/kg) after hepatic but not celiac or gastric vagotomy. The authors effectively demonstrate that the hepatic branch of the vagus nerve accounts for much of the effect of subdiaphragmatic vagotomy. Still, it is important to point out that after LPS these hepatic vagotomy rats had persistently higher rectal temperatures than the vehicle controls. Restraint stress can increase portal concentrations of LPS and interleukin-6 (11), and it is possible that subtle stress fevers could be masking small LPS fevers. More importantly, these researchers have shown that slightly higher doses of LPS (10 µg/kg iv) produce normal fevers in vagotomized rats (8), indicating the existence of other, nonvagal signaling pathways

Several lines of evidence suggest an important signaling role for barrier cells at the blood-brain interface. Low doses of systemic LPS or interleukin-1 induce production of interleukin-1 and cyclooxygenase-2 (COX-2) on central nervous system (CNS) endothelial cells, perivascular microglia, and meningeal macrophages, indicating that these cells can respond to blood-borne pyrogens (1, 3, 7). In addition, LPS-induced activation of autonomic regulatory neurons as indicated by expression of c-Fos and corticotropin-releasing factor (CRF) is not blocked by vagotomy (5).

As we, Romanovsky, and others have previously suggested (4, 6, 8), pyrogens may signal the brain through multiple routes, depending on the site and intensity of inflammation. It is possible that the hepatic branch of the vagus may be one of the most sensitive signaling pathways. However, LPS-induced COX-2 expression in CNS barrier cells and the persistence of c-Fos and CRF after vagotomy indicates that nonvagal, vascular pathways also play an essential role in the febrile response. Future experiments may help clarify the relative contributions and interactions of these vagal and vascular mechanisms.

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