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

**Francis, Joseph, Shun-Guang Wei, Robert M. Weiss, and Robert B. Felder.** Neural regulation of the proinflammatory cytokine response to acute myocardial infarction. *Am J Physiol Heart Circ Physiol* 287: H791–H797, 2004; doi:10.1152/ajpheart.00099.2004.—Within minutes of acute myocardial infarction (MI), proinflammatory cytokines increase in the brain, heart, and plasma. We hypothesized that cardiac afferent nerves stimulated by myocardial injury signal the brain to increase central cytokines. Urethane-anesthetized male Sprague-Dawley rats underwent ligation of the left anterior descending coronary artery (LAD) or sham LAD ligation after bilateral cervical vagotomy, sham vagotomy, or application of a 10% phenol solution to the epicardial surface of the myocardium at risk. MI caused a significant increase in tumor necrosis factor (TNF)-α and interleukin (IL)-1β in the plasma and heart, which was blunted by vagotomy. MI also caused a significant increase in hypothalamic TNF-α and IL-1β, which was not affected by vagotomy. In contrast, epicardial phenol blocked MI-induced increases in hypothalamic TNF-α and IL-1β without affecting increases in the plasma and heart. These findings demonstrate that the appearance of proinflammatory cytokines in the brain after MI is independent of blood-borne cytokines and suggest that cardiac sympathetic afferent nerves activated by myocardial ischemia signal the brain to increase cytokine production. In addition, an intact vagus nerve is required for the full expression of proinflammatory cytokines in the injured myocardium and in the circulation. We conclude that the sympathetic and parasympathetic innervation of the heart both contribute to the acute proinflammatory response to MI.

**Acute Vagotomy Activates the Cholinergic Anti-Inflammatory Pathway**

To the Editor: With great interest, we have read the paper by Francis et al. In this study, the role of the sympathetic and parasympathetic nervous system in the proinflammatory cytokine response was studied in a rat model of myocardial infarction. The authors suggest that the release of proinflammatory cytokines in the brain after myocardial infarction is independent of blood-borne cytokines and that cardiac sympathetic afferent nerves activated by myocardial ischemia signal the brain to increase cytokine production. Second, the authors suggest that an intact vagus nerve is required for the full expression of proinflammatory cytokines in the injured heart as well as in the circulation, because vagotomy resulted in a marked decrease of systemic and cardiac cytokine release. In the discussion, the authors acknowledge that this is quite surprising, because previous reports have suggested that the vagus nerve exerts anti-inflammatory effects during inflammatory syndromes. We believe that this surprising finding can be explained by the design of the experiments and propose this explanation to the authors and suggest additional experiments to evaluate whether this is indeed the case.

Recently, it has been shown that the vagus nerve provides a “cholinergic anti-inflammatory pathway” during endotoxemia (1). In these experiments, vagus nerve stimulation (VNS) resulted in decreased cytokine release, whereas vagotomy resulted in an increase in cytokine release. The vagus nerve downregulates inflammation by decreasing the release of tumor necrosis factor (TNF)-α by macrophages. In studies in which vagus nerve activity was stimulated by intracerebroventricular injection of CNI-1493, systemic but also cardiac, TNF-α release was inhibited during subsequent endotoxemia (1). In line with this, as-yet-unpublished data from our group show that the same cardiac TNF-α release is also inhibited during electrical VNS in this model, suggesting that this pathway is present in the heart as well. These anti-inflammatory effects of VNS are mediated by an interaction of acetylcholine, the principle neurotransmitter of the vagus nerve, with macrophage nicotinic acetylcholine receptors expressing the α7-subunit (3). In the above-mentioned studies, vagotomy was performed several days before endotoxemia was induced; the reason for this is that when a vagotomy is performed and the vagus nerve is manipulated, the nerve dumps acetylcholine at distal nerve endings. In fact, experiments by our group show that when a vagotomy is performed 30 min before endotoxemia, this results in a decrease in subsequent TNF-α release (Fig. 1). In contrast, when a vagotomy is performed 3 days before endotoxemia, this results in an increase of TNF-α release as described above (Fig. 1).

Because the authors performed a vagotomy 1 h before the myocardial infarction, it is therefore conceivable that the effects observed by vagotomy might be overshadowed by the effects of the release of acetylcholine by the vagus nerve induced by manipulating and cutting the nerve. In our opinion, it cannot be ruled out that the release of acetylcholine and not the effects of the absence of vagus nerve activity during experimental myocardial infarction were studied. We would suggest repeating the vagotomy experiments and prolonging the time between vagotomy and experimental myocardial infarction.

Fig. 1. Endotoxemia was induced in male Lewis rats by intravenous injection of 10 mg/kg *Escherichia coli* 0111:B4 lipopolysaccharide (LPS); controls received saline. Either 30 min (A) or 3 days (B) earlier, a unilateral left cervical vagotomy (VGX) was performed. Rats were killed 2 h after LPS injection. Plasma tumor necrosis factor (TNF)-α levels are shown of control rats as well as endotoxic rats subjected to sham surgery (not involving manipulation of the vagus nerve, LPS) or vagotomy (VGX). T: time. n = 8 rats/group. *P < 0.05 vs. LPS.
Letters To The Editor

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REPLY

To the Editor: In our recent study (3) of the interactions between the autonomic nervous system and the proinflammatory cytokines in acute myocardial infarction, we were surprised to find that the rats that had undergone bilateral cervical vagotomy before infarction had lower levels of tumor necrosis factor (TNF-α) and interleukin (IL)-1β in cardiac tissue and plasma. This result was opposite to what might be expected based on previous studies demonstrating a nicotinic receptor-mediated “cholinergic antiinflammatory pathway” that modulates endotoxin-induced release of cytokines by macrophages, as outlined in the accompanying letter by Drs. van Westerloo and van der Poll. The vagotomy data also failed to confirm our working hypothesis that activation of cardiac vagal afferents by ischemic injury might signal the brain to increase cytokine synthesis.

Drs. van Westerloo and van der Poll pose a reasonable challenge to our assumption that vagotomy eliminated the influence of the vagus nerves. They contend that instead of eliminating the vagal influence, we actually stimulated it by manipulating and sectioning the vagus nerves, thereby inducing the release of acetylcholine at distal nerve endings—effectively activating the “cholinergic antiinflammatory pathway” and preempting the cytokine response to myocardial ischemia. In support of that argument, they present a figure showing that the plasma TNF-α response to lipopolysaccharide (LPS) is moderately reduced (but notably still present) 30 min after a unilateral vagotomy and that the LPS response is exaggerated when rats were tested 3 days after a unilateral vagotomy. Data at time points more appropriate to our experimental preparation, i.e., 1–3 h after vagotomy when the coronary artery was occluded, are not provided. Their interpretation of these data is speculative: they provide no direct evidence to support the conclusion that the smaller response at the earlier time point is due to acetylcholine release at the time of vagotomy. An alternative interpretation of their findings might be that the early result is the effect of vagotomy alone, whereas the later result reflects the combined effect of vagotomy and a compensatory overexpression of receptors or other mediators of the cellular response to LPS. Rat strain must also be considered. Our study was done in Sprague-Dawley rats, whereas their data were obtained in the Lewis rat, which is known to have an altered hypothalamic-pituitary-adrenal axis activity, a deficient glucocorticoid response, an increased susceptibility to inflammatory stress, and an altered cytokine profile (e.g., see Ref. 12). Overall, the lack of experimental detail precludes an adequate appraisal of these unpublished results.

Nevertheless, their hypothesis raises a number of questions that are difficult to address based on the extant literature. First, and perhaps most salient, is the fundamental question of whether acute myocardial ischemia and endotoxemia are equivalent stimuli. Endotoxin acts on macrophages and monocytes to trigger the synthesis and release of proinflammatory cytokines (9). Macrophages express the α7-subunit of the nicotinic receptor responsible for modulation by vagal release of acetylcholine (11). Vagal modulation of the endotoxin-induced cytokine response would therefore be anticipated. In contrast, myocardial infarction induces a more gradual but sustained synthesis of proinflammatory cytokines in myocytes of ischemic and nonischemic myocardium (5, 7) in addition to invoking an inflammatory response. To our knowledge, the α7-subunit of the nicotine receptor that appears to be critical to the acetylcholine modulation of macrophage cytokine release has not been demonstrated on myocytes, and so we have no reason to suspect that vagal release of acetylcholine would affect cytokine production by myocardium. Yet, in our study, vagotomy prevented the myocardial (and presumably the myocyte) cytokine response.

Macrophages and mast cells are present in the heart and most certainly play an important role in inflammatory response to myocardial ischemia (4, 10). However, several unproven assumptions are implicit in the hypothesis that a vagotomy-induced burst of acetylcholine might quell the inflammatory response to myocardial infarction at its point source in the heart. These assumptions include 1) macrophages recruited and activated by myocardial ischemia (not by endotoxin) are under vagal control; 2) cardiac macrophages are the primary source of the proinflammatory cytokines measured in heart tissue 2 h after the initiation of coronary occlusion and 3 h after vagotomy; and 3) sectioning of the preganglionic (cervical) vagal nerves evokes sufficient discharge of acetylcholine by the postganglionic vagus nerve terminals in the left ventricle to suppress subsequent (1.3 h later) macrophage cytokine release at the site of tissue injury.

Even if these assumptions were true, it would be reasonable to inquire further into the nature of acetylcholine regulation of cytokine release. Acetylcholine is a rapid signaling molecule, rapidly inactivated by acetylcholinesterase. Critical cardiovascular homeostatic processes depend on those brisk temporal characteristics. In the setting of ongoing vagal activity, the impact of a transient burst of acetylcholine is difficult to predict, but would be expected to be short lived. On the other hand, it is conceivable that a transient increase in the acetylcholine signal might induce a more prolonged change in molecular processes regulating macrophage cytokine synthesis or release. Could such an effect be sustained for the several hours of our protocol? While this scenario seems inconsistent with other vagally mediated cardiac effects, it deserves serious consideration. Unfortunately, studies (1, 8, 11) describing nicotinic receptor-mediated inhibition of macrophage cytokine release have not reported a time course for that effect.

Vagal afferent and efferent systems are strongly activated during acute myocardial infarction. Myocardial infarction provokes substantial and sustained increases in cardiac acetylcholine content (6) that are likely more important physiologically than any transient increases that might be induced by vagotomy. In the ischemic left ventricle, where the acetylcholine content is the highest during infarction, this response occurs...
whether the vagi are intact or sectioned; Kawada et al. (6) attributed this to chemical stimulation of postganglionic nerve terminals by products of ischemia. If the vagus nerves are intact, a reflex-mediated increase in acetylcholine also occurs in the noninfarcted ventricular myocardium (6). Under these conditions, the hypothesis that acetylcholine inhibits cytokine release in the injured heart would predict an absence of cytokine expression by macrophages in both the neurally intact and vagotomized animals. We observed a reduction in cardiac cytokine levels only in the vagotomized rats. Other pertinent observations from the study by Kawada et al. (6) are that J baseline values of acetylcholine in the left ventricle were similar before coronary occlusion, whether the preganglionic vagus nerves were intact or sectioned (although the time from vagotomy to coronary occlusion was not stated); and 2) acetylcholine levels in the left ventricle returned to baseline within 15 min after a 60-min occlusion was released.

Might vagotomy-induced release of acetylcholine into other (noncardiac) vagally innervated tissues account for the reduced plasma TNF-α and IL-1β response to myocardial ischemia? Our data do not address that question, nor do the preliminary results presented by Drs. van Westerloo and van der Poll. The plasma TNF-α data from the two studies early after vagotomy look similar, but the contributing mechanisms are likely quite different. Beyond the differences in rat strains, the nature of the inciting stimulus, and the cytokine-producing cell populations involved, there are differences in regional versus systemic patterns of response to these two different inflammatory stimuli. LPS evokes a response from widely distributed cells of the innate immune defense system that are armed and ready to respond in a programmed manner. In stark contrast, the response to myocardial ischemia emanates from a small circumscribed nidus of tissue injury that engages the inflammatory/immune response in a more gradual fashion and in the company of other excitatory neurohumoral systems (especially the renin-angiotensin-aldosterone system) that may influence its expression. The complexity of interacting systems that determine the circulating levels of TNF-α after myocardial infarction is illustrated, for example, by the ability of mineralocorticoid receptor blockade in the brain to normalize plasma TNF-α (2).

We were at least as surprised as our colleagues, Drs. van Westerloo and van der Poll, by the outcome of these experiments–by the effects of the sympathetic deafferentation as well as the vagal denervation. We appreciate their thoughts and this opportunity to expand our own thinking about possible mechanisms for the autonomic-immune interactions in myocardial ischemia and heart failure. In our opinion, however, the explanation for the effects of the vagotomy in our study remains uncertain. We are not convinced that vagal modulation of the LPS-induced cytokine response, a classic diffuse immune system stimulus, is a suitable model for direct comparison with vagal modulation of the cytokine responses to acute myocardial infarction. We suspect that the vagal contribution to injury and inflammation may differ depending on the inciting stimulus and coexisting factors. We anticipate that the role of the vagus nerve in regulating the inflammatory response to myocardial ischemia will be defined more clearly by future studies.

REFERENCES


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