

MEHDI H. SHISHEHBOR, DO*

Department of Cardiovascular Medicine
Cleveland Clinic
Cleveland, OH

CARLOS ALVES, MD*

Department of Cardiovascular Medicine
Cleveland Clinic
Cleveland, OH

VIVEK RAJAGOPAL, MD*

Department of Cardiovascular Medicine
Cleveland Clinic
Cleveland, OH

Inflammation: Implications for understanding the heart-brain connection

Autonomic dysfunction is a strong correlate of morbidity and mortality in cardiovascular disease. While increased sympathetic stimulation drives many adverse events in coronary artery disease and heart failure,¹ beta-adrenergic blockade is associated with improved outcomes.^{2,3} Similarly, diminished parasympathetic tone is also associated with adverse outcomes in cardiovascular disease, suggesting a key role for this limb of the autonomic system in maintenance of cardiac homeostasis.⁴

The mechanism of parasympathetic protection, however, is not clearly understood. Although an anti-arrhythmic mechanism appears intuitive, such a mechanism has not been corroborated by animal studies.⁴ Recently, Tracey and colleagues provided new insight by demonstrating that parasympathetic stimulation in mice and in human macrophages results in a decreased release of mediators of systemic inflammation.^{5,6} Given the importance of inflammation in atherosclerosis and adverse remodeling in congestive heart failure, it is possible that parasympathetic tone assuages atherogenesis and deleterious cardiac remodeling by directly inhibiting inflammation.⁷⁻⁹

■ PARASYMPATHETIC NERVOUS SYSTEM AND CARDIOVASCULAR MORTALITY

Several studies have shown an inverse relationship between parasympathetic tone and cardiovascular mortality.

In the Autonomic Tone and Reflexes After Myocardial Infarction (ATRAMI) study,¹⁰ La Rovere and colleagues studied 1,284 patients with a recent (< 28 days) myocardial infarction by measuring heart rate variability and baroreflex sensitivity, both of which are markers of cardiac vagal tone.¹ Multivariate analysis showed that low heart rate variability and low

baroreflex sensitivity (both markers of decreased parasympathetic input) were independently associated with cardiac mortality.¹⁰

Similarly, Nolan and colleagues measured heart rate variability in 433 patients with congestive heart failure (New York Heart Association classes I to III).¹¹ Multivariate analysis showed that poor heart rate variability was an independent predictor of all-cause mortality and was the most powerful predictor of death secondary to progressive heart failure.

In exercise testing, correlates of parasympathetic tone, such as heart rate recovery and ventricular ectopy in recovery, are also independent predictors of mortality.^{12,13} Moreover, heart rate recovery has been associated with the metabolic syndrome, psychosocial stress, educational level, smoking, and obesity.¹⁴⁻¹⁷ In a recent study by Jouven et al, heart-rate profile during exercise was a strong predictor of sudden death among 5,713 asymptomatic working men.¹⁸

Taken together, the above studies indicate that the autonomic nervous system, particularly the parasympathetic nervous system, is integral to cardiovascular health, morbidity, and mortality.

■ PARASYMPATHETIC NERVOUS SYSTEM AND INFLAMMATION

The vagus nerve innervates the cardiovascular system in addition to other visceral organs such as the liver, spleen, and gut. Tracey and colleagues demonstrated that injection of lipopolysaccharides in animals that underwent vagus nerve stimulation resulted in reduced macrophage release of inflammatory cytokines (tumor necrosis factor [TNF]- α , interleukin [IL]-1 β , IL-18, and IL-6) and death without affecting release of IL-10, an anti-inflammatory cytokine.⁵ However, vagal nerve transection removed this protection. Furthermore, in human macrophage cultures, acetylcholine inhibited TNF- α release when the cultures were exposed to lipopolysaccharide. These results indicated that the vagus nerve plays a role in the anti-inflammatory response.⁵

* All authors reported that they have no financial relationships that pose a potential conflict of interest with this article. Dr. Shishehbor is supported in part by the National Institutes of Health, National Institute of Child Health and Human Development, Multidisciplinary Clinical Research Career Development Programs Grant K12 HD049091, and the NIH Loan Repayment Program.

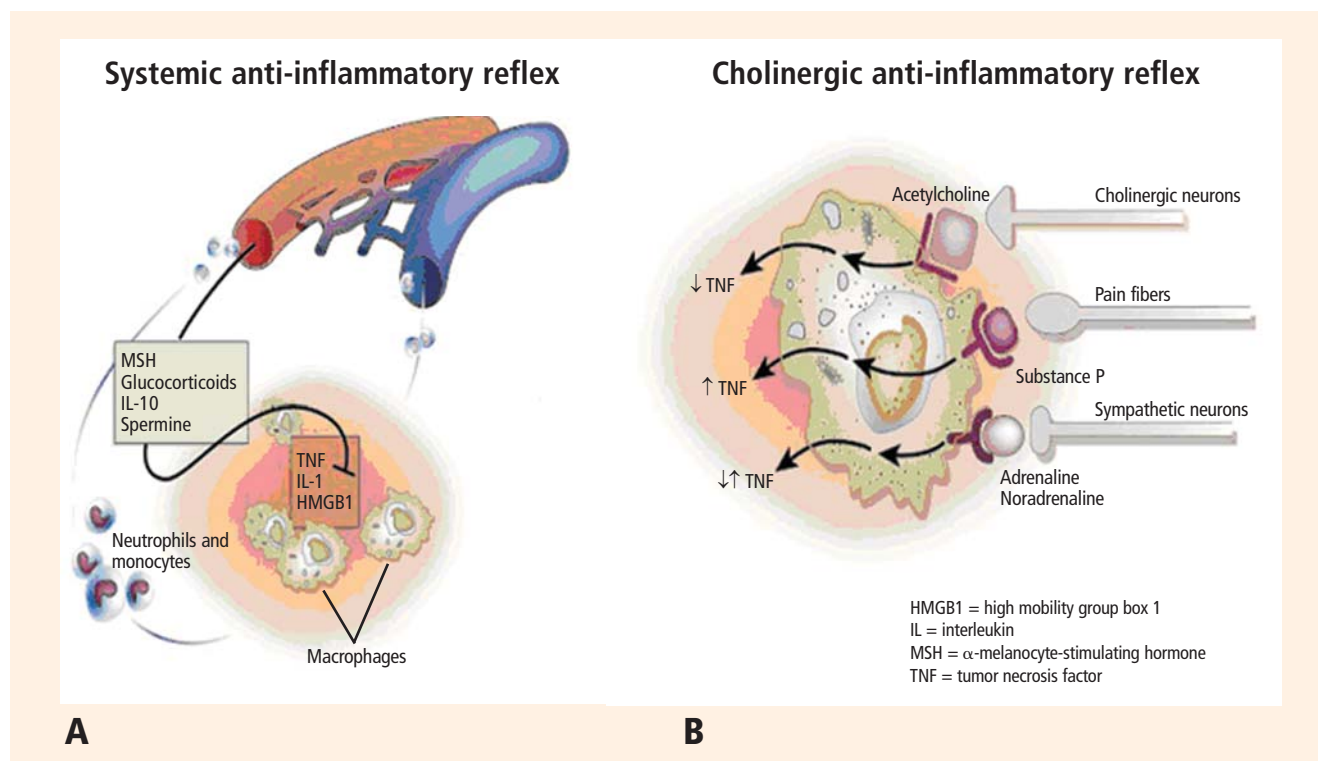


FIGURE 1. (A) The systemic anti-inflammatory reflex, which involves anti-inflammatory cytokines, glucocorticoids, and other humoral mediators, is slow and diffuse. (B) In contrast, the cholinergic anti-inflammatory reflex is quick and integrated. Reprinted by permission from Macmillan Publishers Ltd: Nature 2002; 420:853–859, copyright 2002 (www.nature.com).

In humans, abnormal heart rate variability, a measure of parasympathetic function, is significantly associated with elevated levels of inflammatory cytokines such IL-6 and C-reactive protein (CRP).¹⁹ Lanza et al showed that serum levels of CRP were significantly associated with abnormal heart rate variability in patients with unstable angina.²⁰ This association has also been observed in healthy subjects, patients with stable coronary artery disease, and patients with heart failure. Overall, in all these studies a significant inverse correlation has been observed between CRP levels and measurements of heart rate variability.

More recently, the mechanisms by which vagus nerve stimulation leads to an anti-inflammatory response have been described by Tracey and colleagues.^{6,21,22} Macrophages express nicotinic (cholinergic) receptors, composed of five $\alpha 7$ subunits, which are thought to be involved in the cholinergic anti-inflammatory reflex.⁶ In $\alpha 7$ subunit knockout mice, electrical vagal stimulation no longer prevented release of inflammatory cytokines, indicating that this receptor plays an important function in the vagally mediated anti-inflammatory response.²² In fact, $\alpha 7$ subunit knockout mice demonstrate a greater release of inflam-

matory cytokines in response to lipopolysaccharides than do wild-type mice.⁶ Furthermore, inhibition of this receptor in primary human macrophages stimulated with endotoxin (200 ng/mL) led to a significant reduction in high mobility group box 1 (HMGB1) inflammatory cytokines in a dose-dependent manner.⁶ Interestingly, atropine, a muscarinic antagonist, was not able to inhibit this reaction, but α -conotoxin, a nicotinic antagonist, inhibited the action of acetylcholine on this receptor,⁶ indicating that acetylcholine inhibits HMGB1 release through the nicotinic cholinergic pathway mediated by the $\alpha 7$ nicotinic acetylcholine receptor. Nicotine also reduces systemic levels of HMGB1 after induction of lethal endotoxemia in animals, and this inhibition led to a significant decrease in mortality in these animals in a dose-dependent fashion.⁶

■ CHOLINERGIC ANTI-INFLAMMATORY REFLEX

As shown in **Figure 1**, the systemic anti-inflammatory reflex (which involves anti-inflammatory cytokines, glucocorticoids, and other humoral mediators) is slow and diffuse, whereas the cholinergic anti-inflammatory reflex is quick, precise, and integrated, typically releas-

ing inflammatory cytokines at the site of inflammation.²¹ As illustrated in **Figure 2**, cytokines activate the afferent vagus fibers that travel to the nucleus tractus solitarius.²¹ Subsequently, efferent vagal nerve fibers activate the $\alpha 7$ nicotinic acetylcholine receptor on peripheral macrophages, leading to decreases in systemic inflammatory cytokines such as IL-6, TNF, and HMGB1.²¹ In addition, fibers communicating between the brainstem and hypothalamus stimulate release of adrenocorticotrophic hormone, leading to increased glucocorticoid secretion, which further suppresses inflammation.

■ JAK-STAT PATHWAY AND CHOLINERGIC ANTI-INFLAMMATORY RESPONSE

The Janus kinases (JAKs) and STAT (signal transducers and activators of transcription) class of transcription factors are the signaling pathway for a wide variety of extracellular signals, including many cytokines, lymphokines, and growth factors. These signal through a related superfamily of cell surface receptor tyrosine kinases that are associated with and activate the JAKs.²³ The JAK-STAT signaling pathway has been previously described as being involved in the signaling mechanisms of growth hormone, prolactin, epoetin alfa, thrombopoietin, granulocyte macrophage colony-stimulating factor, leptin, and various cytokines.

Mechanistically, once the ligand binds to the associated receptor, it induces the dimerization of the receptor and causes the reciprocal phosphorylation of tyrosine residues on the associated JAKs; this, in turn, phosphorylates tyrosine residues on the cytoplasmic tail of the receptor.^{23,24} These phosphorylated tyrosines serve as docking sites for the Src homology 2 (SH-2) domain of the various STAT proteins, and JAK then catalyzes the tyrosine phosphorylation of the receptor-bound STATs. Phosphorylation of the STATs at a conserved tyrosine residue induces SH-2-mediated homodimerization or heterodimerization, followed by translocation of the STAT dimer to the nucleus.^{23,24} It is there that the STAT dimers bind to specific DNA response elements in the promoter region of target genes to activate gene expression.

The vagus nerve inhibits peripheral inflammation centrally through muscarinic receptors and, more importantly, peripherally through cholinergic receptors, and acetylcholine released by efferent vagus nerve fibers inhibits peripheral macrophage activation. The molecular mechanism ultimately involves prevention of nuclear factor (NF)- κ B p65 nuclear translocation and activation, but the upstream media-

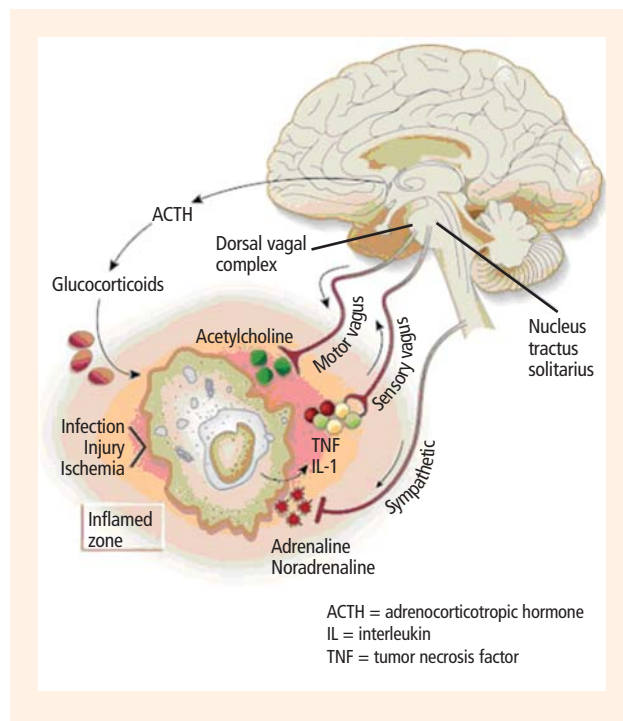


FIGURE 2. The cholinergic anti-inflammatory pathway. Inflammatory cytokines are released at the site of inflammation, activating afferent signals that are relayed to the nucleus tractus solitarius. Subsequently, efferent vagus nerve activates the $\alpha 7$ nicotinic acetylcholine receptor on peripheral macrophages, reducing systemic inflammatory cytokines. Additionally, fibers communicating between the brainstem and hypothalamus stimulate release of adrenocorticotrophic hormone, which increases glucocorticoid secretion, further suppressing inflammation. Reprinted by permission from Macmillan Publishers Ltd: Nature 2002; 420:853–859, copyright 2002 (www.nature.com).

tors linking the downstream effector vagal stimuli to macrophage deactivation have only recently been uncovered, by Tracey et al. They showed that the anti-inflammatory action of the nicotinic receptor's activation in peritoneal macrophages was dependent on activation of JAK2 by the $\alpha 7$ acetylcholine receptor subunit and on subsequent transactivation of the transcription factor STAT3.^{23,24} The anti-inflammatory effect of nicotine required the ability of phosphorylated STAT3 to bind and transactivate its DNA response elements.

Furthermore, an in vivo mouse model showed that stimulation of the vagus nerve ameliorated surgery-induced inflammation and postoperative ileus by activating STAT3 in intestinal macrophages.^{6,23} Another study described the effects of inhibiting STAT1 and STAT3 in peritoneal macrophages harvested from rats after being incubated and exposed to lipopolysaccharide.²⁵ It showed a powerful association between

lipopolysaccharide stimulation and HMGB1 mRNA synthesis, which decreased significantly ($P < .01$) after specific inhibition of STAT1 and STAT3 with fludarabine and rapamycin, respectively.²⁵

Studies have also implicated JAK2, STAT3, and STAT 4 in an experimental model of autoimmune arthritis and multiple sclerosis, in that a powerful inhibitor of IL-12 activation of JAK2, STAT3, STAT4, and tyrosine kinase 2 ameliorates the clinical condition of experimental rats by blocking T-cell proliferation and T helper 1 cell differentiation.²⁶

The data suggest that the molecular mechanism of the cholinergic anti-inflammatory pathway seems to involve the concurrent activation of JAK2 and STAT3 within macrophages, in a manner similar to, but independent of, activation of the anti-inflammatory cytokine IL-10, and which ultimately decreases the activation of a central proinflammatory transcription factor NF- κ B (p65).

THERAPEUTIC OPTIONS

The above-mentioned mechanisms bring into focus the importance of the anti-inflammatory properties of these pathways. Although nicotine's therapeutic use is limited by its short half-life in the body and its toxicity at low levels, other compounds are under development that have the same beneficial properties without the associated side effects. One of these, named CAP55, emerged as a leading cholinergic compound and has been shown to significantly inhibit TNF production by lipopolysaccharide-stimulated macrophages. In a recent report, CAP55 was found to inhibit both vascular cell adhesion molecule 1 and E-selectin expression by the endothelium and, hence, endothelial cell activation in vivo by 50%; it also reduced leukocyte activation and migration during acute inflammatory responses.²⁷ Further experiments delineating how this and other compounds affect clinical outcomes in animals—and eventually humans—are eagerly anticipated.

CONCLUSION

In the past few years, Tracey and colleagues have contributed significantly to our understanding of the cholinergic anti-inflammatory response, bolstering the belief that inflammation has important implications for the heart-brain connection. In vitro and animal studies have revealed a potential mechanistic relationship between the autonomic nervous system (especially the parasympathetic nervous system) and inflammation. The parasympathetic system, through the "cholinergic anti-inflammatory pathway," inactivates macrophages,

a critical inflammatory component of atherogenesis, vulnerable plaque, and injured myocardium. This inactivation leads to inhibition of inflammatory cytokines such as nitric oxide, reactive oxygen species, TNF, IL-1, and HMGB1. Many of these cytokines play a significant role in atherosclerosis formation and progression as well as plaque rupture.

Despite the preponderance of evidence linking inflammation to atherosclerosis and cardiovascular morbidity and mortality, few therapeutic options are available for the treatment of low-grade chronic systemic inflammation in coronary artery disease. Recently, novel medications such as CAP55 have shown some promise. As we maximize our therapeutic armamentarium against established risk factors such as hypertension and diabetes, new therapeutic options to treat novel risk factors such as inflammation are on the rise.

REFERENCES

1. Schwartz PJ, La Rovere MT, Vanoli E. Autonomic nervous system and sudden cardiac death. Experimental basis and clinical observations for post-myocardial infarction risk stratification. *Circulation* 1992; 85(Suppl 1):177-191.
2. Gheorghiade M, Goldstein S. Beta-blockers in the post-myocardial infarction patient. *Circulation* 2002; 106:394-398.
3. Jessup M, Brozena S. Heart failure. *N Engl J Med* 2003; 348:2007-2018.
4. Hull SS Jr, Vanoli E, Adamson PB, De Ferrari GM, Foreman RD, Schwartz PJ. Do increases in markers of vagal activity imply protection from sudden death? The case of scopolamine. *Circulation* 1995; 91:2516-2519.
5. Borovikova LV, Ivanova S, Zhang M, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 2000; 405:458-462.
6. Wang H, Yu M, Ochani M, et al. Nicotinic acetylcholine receptor $\alpha 7$ subunit is an essential regulator of inflammation. *Nature* 2003; 421:384-388.
7. Diwan A, Tran T, Misra A, Mann DL. Inflammatory mediators and the failing heart: a translational approach. *Curr Mol Med* 2003; 3:161-182.
8. Fan J, Watanabe T. Inflammatory reactions in the pathogenesis of atherosclerosis. *J Atheroscler Thromb* 2003; 10:63-71.
9. Shishehbor MH, Bhatt DL. Inflammation and atherosclerosis. *Curr Atheroscler Rep* 2004; 6:131-139.
10. La Rovere MT, Bigger JT Jr, Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. *Lancet* 1998; 351:478-484.
11. Nolan J, Batin PD, Andrews R, et al. Prospective study of heart rate variability and mortality in chronic heart failure: results of the United Kingdom Heart Failure Evaluation and Assessment of Risk Trial (UK-HEART). *Circulation* 1998; 98:1510-1516.
12. Cole CR, Blackstone EH, Pashkow FJ, Snader CE, Lauer MS. Heart-rate recovery immediately after exercise as a predictor of mortality. *N Engl J Med* 1999; 341:1351-1357.
13. Cole CR, Foody JM, Blackstone EH, Lauer MS. Heart rate recovery after submaximal exercise testing as a predictor of mortality in a cardiovascularly healthy cohort. *Ann Intern Med* 2000; 132:552-555.
14. Shishehbor MH, Baker DW, Blackstone EH, Lauer MS. Association of educational status with heart rate recovery: a popula-

- tion-based propensity analysis. *Am J Med* 2002; 113:643–649.
15. Seshadri N, Gildea TR, McCarthy K, Pothier C, Kavuru MS, Lauer MS. Association of an abnormal exercise heart rate recovery with pulmonary function abnormalities. *Chest* 2004; 125:1286–1291.
 16. Chen MS, Blackstone EH, Pothier CE, Lauer MS. Heart rate recovery and impact of myocardial revascularization on long-term mortality. *Circulation* 2004; 110:2851–2857.
 17. Cheng YJ, Lauer MS, Earnest CP, et al. Heart rate recovery following maximal exercise testing as a predictor of cardiovascular disease and all-cause mortality in men with diabetes. *Diabetes Care* 2003; 26:2052–2057.
 18. Jouven X, Empana JP, Schwartz PJ, Desnos M, Courbon D, Ducimetiere P. Heart-rate profile during exercise as a predictor of sudden death. *N Engl J Med* 2005; 352:1951–1958.
 19. Aronson D, Mittleman MA, Burger AJ. Interleukin-6 levels are inversely correlated with heart rate variability in patients with decompensated heart failure. *J Cardiovasc Electrophysiol* 2001; 12:294–300.
 20. Lanza GA, Sgueglia GA, Cianflone D, et al. Relation of heart rate variability to serum levels of C-reactive protein in patients with unstable angina pectoris. *Am J Cardiol* 2006; 97:1702–1706.
 21. Tracey KJ. The inflammatory reflex. *Nature* 2002; 420:853–859.
 22. Pavlov VA, Tracey KJ. The cholinergic anti-inflammatory pathway. *Brain Behav Immun* 2005; 19:493–499.
 23. Metz CN, Tracey KJ. It takes nerve to dampen inflammation. *Nat Immunol* 2005; 6:756–757.
 24. de Jonge WJ, van der Zanden EP, The FO, et al. Stimulation of the vagus nerve attenuates macrophage activation by activating the Jak2-STAT3 signaling pathway. *Nat Immunol* 2005; 6:844–851.
 25. Liu H, Yao YM, Dong YQ, Yu Y, Sheng ZY. The role of Janus kinase-signal transducer and transcription activator pathway in the regulation of synthesis and release of lipopolysaccharide-induced high mobility group box-1 protein. *Zhonghua Shao Shang Za Zhi* 2005; 21:414–417.
 26. Muthian G, Bright JJ. Quercetin, a flavonoid phytoestrogen, ameliorates experimental allergic encephalomyelitis by blocking IL-12 signaling through JAK-STAT pathway in T lymphocyte. *J Clin Immunol* 2004; 24:542–552.
 27. Saeed RW, Varma S, Peng-Nemeroff T, et al. Cholinergic stimulation blocks endothelial cell activation and leukocyte recruitment during inflammation. *J Exp Med* 2005; 201:1113–1123.

Address: Mehdi H. Shishehbor, DO, MPH, Cleveland Clinic, 9500 Euclid Avenue, JJ40, Cleveland, OH 44195; shishem@gmail.com.