Putting Out Fat's Fire with the Cholinergic Antiinflammatory Pathway

Michael M. Swarbrick

Diabetes and Obesity Program, Garvan Institute of Medical Research, Darlinghurst, Sydney, New South Wales 2010, Australia; and School of Medical Sciences, Wallace Wurth Building, University of New South Wales, Sydney, New South Wales 2052, Australia

A compelling body of research over the last two decades has established that chronic inflammation in adipose tissue makes a key contribution to insulin resistance in obesity (reviewed in Ref. 1). Adipocyte expansion in obesity is frequently accompanied by an induction of inflammatory mediators and cytokines, including (but not limited to) TNFα (2), IL-1β and IL-6, plasminogen activator inhibitor-1 (PAI-1), and monocyte chemoattractant protein-1 (MCP-1) (3). The majority of these inflammatory signals are produced by bone marrow-derived macrophages, which infiltrate adipose tissue as monocytes and become activated proinflammatory macrophages (4, 5). Macrophage-derived cytokines contribute to insulin resistance in adipose tissue by interfering with insulin signaling in neighboring adipocytes (6, 7). Accordingly, genetic ablation of inflammatory signaling in myeloid cells has been shown to protect mice from diet-induced glucose intolerance and insulin resistance (8), and antiinflammatory therapeutics have recently been found to improve glycemic control in patients with type 2 diabetes (9, 10).

However, it is not well studied in this context that the central nervous system, via an inflammatory reflex involving the vagus nerve (and its major neurotransmitter, acetylcholine), can exert a tonic inhibition on cytokine production in immune cells (reviewed in Ref. 11). The existence of this mechanism, termed the 'cholinergic antiinflammatory pathway,' was first suggested in studies of animal models of sepsis, where hyperthermia induced by ip administration of IL-1β could be blocked by subdiaphragmatic vagotomy (12). Moreover, direct electrical stimulation of the vagus nerve suppressed elevations of circulating TNFα in a rat model of endotoxic shock (13). This and other key observations at the time delineated a reflex arc originating in vagal afferent fibers, which gather sensory information pertaining to noxious stimuli (pressure, temperature, inflammation) from the viscera and relay this information to the nucleus tractus solitarius in the medulla oblongata, which coordinates autonomic functions. In concert with other nuclei, including the dorsal motor nucleus of the vagus, an appropriate response is initiated and transmitted along preganglionic vagal efferents (14). These vagal efferents inhibit innate immune responses via acetylcholine and the nicotinic acetylcholine receptor subunit α7 (α7nAChR), expressed by cytokine-producing immune cells (15). In this issue of *Endocrinology*, Hang Shi and colleagues (16) provide fascinating new evidence to demonstrate that chronic activation of the cholinergic antiinflammatory pathway by nicotine, a nAChR agonist, can ameliorate obesity-induced inflammation and insulin resistance in mice, and that these effects are dependent upon α7nAChRs.

Before this study cholinergic antiinflammatory signaling in adipose tissue had not been studied, and the issue of whether adipose tissue possesses significant parasympathetic innervation is itself controversial (17, 18). Regardless, the authors detected mRNA expression of both α7nAChR and the acetylcholine-degrading enzyme butyrylcholinesterase (BChE) in mouse white adipose tissue. Notably, α7nAChR expression was higher in adipose tissue-derived macrophages than in isolated adipocytes, and levels of BChE expression mRNA and protein in white adipose tissue showed dynamic changes in response to chronic nicotine treatment (up-regulated) and genetic or diet-induced obesity (down-regulated). It is quite likely

ISSN Print 0013-7227 ISSN Online 1945-7170
Printed in U.S.A.

doi: 10.1210/en.2011-0041 Received January 12, 2011. Accepted January 18, 2011.

For article see page 836

Abbreviations: BChE, Butyrylcholinesterase; MCP, monocyte chemoattractant protein; α7nAChR, nicotinic acetylcholine receptor subunit α7.

that these changes in BChE expression occurred in the nonadipocyte fraction of adipose tissue, as a recent study of human omental fat showed that BChE mRNA expression was significantly higher in the stromal-vascular fraction than in adipocytes (19).

Next, the authors administered nicotine chronically to *db/db* and diet-induced obese mice, at a dose that did not affect food intake or body weight. Nicotine reduced fed and fasting blood glucose concentrations in the *db/db* mice and ameliorated glucose intolerance and improved whole-body insulin action in the diet-induced obese mice. Nicotine treatment did not affect these variables in lean mice. Consistent with the above effects on glucose homeostasis, examination of the skeletal muscle, liver, and adipose tissue of *db/db* or diet-induced obese mice revealed that the insulin signaling defects in these tissues were nearly completely reversed by chronic nicotine treatment, to levels observed in wild-type chow-fed mice. The salutary effects of nicotine treatment extended to a suppression of serum TNFα concentrations and a reduced expression of inflammatory signals (including TNFα, IL-1β, IL-6, MCP-1) and the macrophage marker F4/80 in adipose tissue.

Wang *et al.* (16) then tested whether the beneficial effects of chronic nicotine treatment were dependent upon α7nAChR, the nicotinic acetylcholine receptor present upon macrophages, by studying α7nAChR-null mice. Mice lacking α7nAChR are viable and have no gross phenotypic abnormalities (20) and have been reported to have higher serum concentrations of TNFα, IL-1β, and IL-6 relative to wild-type mice after endotoxemic shock (15). As expected, isolated macrophages from α7nAChR-deficient mice showed greater stearic acid–induced induction of TNFα, IL-1β, and IL-6 mRNA expression relative to wild-type macrophages; and nicotine suppressed these effects in wild-type macrophages but not in those derived from α7nAChR-deficient mice.

The overall relevance of the cholinergic antiinflammatory pathway in insulin resistance was then investigated in whole-animal studies of glucose homeostasis, including hyperinsulinemic-euglycemic clamps. α7nAChR-deficient mice displayed mild hyperinsulinemia on a chow diet; while on a high-fat diet, glucose intolerance, insulin resistance, and insulin signaling in muscle were worsened by the lack of α7nAChRs. Examination of adipose tissue from α7nAChR-null mice revealed a dramatic increase in F4/80-positive, CD11c-positive (indicating classically activated) macrophage content; in mice on a high-fat diet, the effects of α7nAChR deficiency on activated macrophage content were even greater, and adipose tissue expression of TNFα and MCP-1 was doubled relative to wild-type mice. Lastly, insulin signaling in skeletal muscle and liver was significantly impaired in α7nAChR-knockout animals on a high-fat diet, relative to wild-type mice, and the expression of gluconeogenic enzymes in the liver of α7nAChR-null mice was increased. One caveat of the study by Wang *et al.* (16) is that definitive proof for the involvement of α7nAChR-mediated suppression of inflammation in insulin resistance will likely require either selective deletion of α7nAChR from macrophages or transfer of α7nAChR-deficient macrophages to wild-type mice.

By widening the scope of inflammatory conditions suppressed by the cholinergic antiinflammatory pathway to adipose tissue insulin resistance, these new findings may uncover new targets for the treatment and prevention of metabolic disease. Both cholinergic agonists and vagal nerve stimulation have been shown to ameliorate inflammation in animal models of sepsis, hemorrhagic shock, ischemia/reperfusion, pancreatitis, arthritis (21), and inflammatory bowel disease (22). Interestingly, human obesity is itself characterized by a reduced vagal tone, which increases with exercise and weight loss (23). There is some evidence to suggest that vagal function may predict inflammatory disease: a recent cross-sectional study of >600 healthy human adults obtained evidence for independent relationships between vagally-mediated heart rate variability and both white blood cell count and circulating concentrations of C-reactive protein (24).

Perhaps more importantly, this study also contributes to the emerging view that circuits originating in the central nervous system, including those involved in energy balance, not only influence glucose homeostasis directly but also indirectly by influencing macrophage infiltration, activation, and the production of proinflammatory signals in peripheral tissues. The nucleus tractus solitarius is one of the main areas receiving afferent vagal inputs in the cholinergic antiinflammatory pathway and is also a critical extra-hypothalamic site of leptin action for feeding behavior (25). Leptin itself has been recently shown to improve survival in a murine model of sepsis (26). It would be of considerable interest to examine the relationships between established central regulators of energy homeostasis and cholinergic antiinflammatory vagal outflow. In this regard, it is notable that intracerebroventricular and peripheral administration of a selective melanocortin-4 receptor agonist, ACTH-(1–24), increases neural efferent activity along the vagus nerve and blunts increases in both the hepatic expression and circulating concentrations of TNFα in a rat model of hemorrhagic shock (27, 28). Identification of central nervous system circuits linking excessive caloric intake with adipose tissue inflammation and insulin resistance might allow us to 'turn down the heat' in adipose tissue and alleviate the global conflagration of insulin resistance and type 2 diabetes (29).

Acknowledgments

Address all correspondence and requests for reprints to: Michael M. Swarbrick, Ph.D., Diabetes and Obesity Program, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, New South Wales 2010, Australia. E-mail: m.swarbrick@garvan.org.au.

Dr. Swarbrick is supported by a National Health and Medical Research Council (NHMRC) of Australia Program Grant "Pathways to Diabetes Prevention." The author wishes to thank Dr. Amanda Brandon and Dr. Tristan Iseli (Garvan Institute) for their constructive comments on the manuscript.

Disclosure Summary: The author has nothing to declare.

References

1. **Berg AH, Scherer PE** 2005 Adipose tissue, inflammation, and cardiovascular disease. Circ Res 96:939–949
2. **Hotamisligil GS, Shargill NS, Spiegelman BM** 1993 Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259:87–91
3. **Fain JN** 2006 Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. Vitam Horm 74:443–477
4. **Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H** 2003 Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 112:1821–1830
5. **Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante Jr AW** 2003 Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112:1796–1808
6. **Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM** 1996 IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science 271:665–668
7. **Rotter V, Nagaev I, Smith U** 2003 Interleukin-6 (IL-6) induces insulin resistance in 3T3–L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. J Biol Chem 278:45777–45784
8. **Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, Wynshaw-Boris A, Poli G, Olefsky J, Karin M** 2005 IKK-beta links inflammation to obesity-induced insulin resistance. Nat Med 11: 191–198
9. **Larsen CM, Faulenbach M, Vaag A, Volund A, Ehses JA, Seifert B, Mandrup-Poulsen T, Donath MY** 2007 Interleukin-1-receptor antagonist in type 2 diabetes mellitus. N Engl J Med 356:1517–1526
10. **Goldfine AB, Fonseca V, Jablonski KA, Pyle L, Staten MA, Shoelson SE** 2010 The effects of salsalate on glycemic control in patients with type 2 diabetes: a randomized trial. Ann Intern Med 152:346–357
11. **Tracey KJ** 2009 Reflex control of immunity. Nat Rev Immunol 9:418–428
12. **Watkins LR, Goehler LE, Relton JK, Tartaglia N, Silbert L, Martin D, Maier SF** 1995 Blockade of interleukin-1 induced hyperthermia by subdiaphragmatic vagotomy: evidence for vagal mediation of immune-brain communication. Neurosci Lett 183:27–31
13. **Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, Wang H, Abumrad N, Eaton JW, Tracey KJ** 2000 Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 405:458–462
14. **Goehler LE, Gaykema RP, Hansen MK, Anderson K, Maier SF, Watkins LR** 2000 Vagal immune-to-brain communication: a visceral chemosensory pathway. Auton Neurosci 85:49–59
15. **Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, Li JH, Yang H, Ulloa L, Al-Abed Y, Czura CJ, Tracey KJ** 2003 Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature 421:384–388
16. **Wang XF, Yang ZG, Xue B, Shi H** 2011 Activation of the cholinergic antiinflammatory pathway ameliorates obesity-induced inflammation and insulin resistance. Endocrinology 152:836–846
17. **Giordano A, Song CK, Bowers RR, Ehlen JC, Frontini A, Cinti S, Bartness TJ** 2006 White adipose tissue lacks significant vagal innervation and immunohistochemical evidence of parasympathetic innervation. Am J Physiol Regul Integr Comp Physiol 291:R1243–R1255
18. **Kreier F, Buijs RM** 2007 Evidence for parasympathetic innervation of white adipose tissue, clearing up some vagaries. Am J Physiol Regul Integr Comp Physiol 293:R548–R549; author reply R550–R552; discussion R553–R554
19. **Fain JN, Buehrer B, Bahouth SW, Tichansky DS, Madan AK** 2008 Comparison of messenger RNA distribution for 60 proteins in fat cells vs the nonfat cells of human omental adipose tissue. Metabolism 57:1005–1015
20. **Orr-Urtreger A, Göldner FM, Saeki M, Lorenzo I, Goldberg L, De Biasi M, Dani JA, Patrick JW, Beaudet AL** 1997 Mice deficient in the alpha7 neuronal nicotinic acetylcholine receptor lack alpha-bungarotoxin binding sites and hippocampal fast nicotinic currents. J Neurosci 17:9165–9171
21. **Tracey KJ** 2007 Physiology and immunology of the cholinergic antiinflammatory pathway. J Clin Invest 117:289–296
22. **Meregnani J, Clarencon D, Vivier M, Peinnequin A, Mouret C, Sinniger V, Picq C, Job A, Canini F, Jacquier-Sarlin M, Bonaz B** 11 November 2010 Anti-inflammatory effect of vagus nerve stimulation in a rat model of inflammatory bowel disease. Auton Neurosci 10.1016/j.autneu.2010.10.007
23. **Thayer JF, Lane RD** 2007 The role of vagal function in the risk for cardiovascular disease and mortality. Biol Psychol 74:224–242
24. **Thayer JF, Fischer JE** 2009 Heart rate variability, overnight urinary norepinephrine and C-reactive protein: evidence for the cholinergic anti-inflammatory pathway in healthy human adults. J Intern Med 265:439–447
25. **Hayes MR, Skibicka KP, Leichner TM, Guarnieri DJ, DiLeone RJ, Bence KK, Grill HJ** 2010 Endogenous leptin signaling in the caudal nucleus tractus solitarius and area postrema is required for energy balance regulation. Cell Metab 11:77–83
26. **Tschöp J, Nogueiras R, Haas-Lockie S, Kasten KR, Castaneda TR, Huber N, Guanciale K, Perez-Tilve D, Habegger K, Ottaway N, Woods SC, Oldfield B, Clarke I, Chua Jr S, Farooqi IS, O'Rahilly S, Caldwell CC, Tschöp MH** 2010 CNS leptin action modulates immune response and survival in sepsis. J Neurosci 30:6036–6047
27. **Guarini S, Bazzani C, Cainazzo MM, Mioni C, Ferrazza G, Vergoni AV, Schioth HB, Wikberg JE, Bertolini A** 1999 Evidence that melanocortin 4 receptor mediates hemorrhagic shock reversal caused by melanocortin peptides. J Pharmacol Exp Ther 291:1023–1027
28. **Guarini S, Cainazzo MM, Giuliani D, Mioni C, Altavilla D, Marini H, Bigiani A, Ghiaroni V, Passaniti M, Leone S, Bazzani C, Caputi AP, Squadrito F, Bertolini A** 2004 Adrenocorticotropin reverses hemorrhagic shock in anesthetized rats through the rapid activation of a vagal anti-inflammatory pathway. Cardiovasc Res 63:357–365
29. **Zimmet P, Alberti KG, Shaw J** 2001 Global and societal implications of the diabetes epidemic. Nature 414:782–787