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Toll-like receptors 2 and 4 modulate autonomic control of heart rate and energy metabolism

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ABSTRACT

Toll-like receptors (TLR) are innate immune receptors typically activated by microbial-associated molecular patterns (MAMPs) during infection or damage-associated molecular patterns (DAMPs) as a result of tissue injury. Recent findings suggest that TLR2 and TLR4 signaling play important roles in developmental and adult neuroplasticity, and in learning and memory. In addition, activation of TLR2 and TLR4 worsens ischemic injury to the heart and brain in animal models of myocardial infarction and stroke. TLR activation is also implicated in thermoregulation and fever in response to infection. However, it is not known whether TLRs participate in the regulation of the sympathetic and/or parasympathetic components of the autonomic nervous system (ANS). Here we provide evidence that TLR2 and TLR4 influence autonomic regulation of heart rate (HR) body temperature and energy metabolism in mice. We show that mice lacking TLR2 or TLR4 exhibit reduced basal HR, which results from an increase of parasympathetic tone. In addition, thermoregulatory responses to stress are altered in TLR2−/− and TLR4−/− mice, and brown fat-dependent thermoregulation is altered in TLR4−/− mice. Moreover, TLR2−/− and TLR4−/− mice consume less food and exhibit a greater mass compared to wild type mice. Collectively, our findings suggest important roles for TLR2 and TLR4 in the ANS regulation of cardiovascular function, thermoregulation, and energy metabolism.

1. Introduction

Toll-like receptors (TLR) comprise a family of 10–12 innate immune receptors in mammals. TLRs are typically activated during infection by microbial-associated molecular patterns (MAMPs; to be distinguished from the former term, *pathogen associated molecular patterns*, *PAMPs*, as it is acknowledged now that molecular patterns on non-pathogenic bacteria also activate TLRs) (Takeda and Akira, 2004). TLRs are also activated by damage-associated molecular patterns (DAMPs), a set of endogenous TLR ligands released in body tissues as a result of tissue injury (Yu et al., 2010). TLRs are expressed in a variety of immune-related cell types, as well as non-immune cells such as intestinal epithelial cells (Marques and Boneca, 2011), endothelial cells (Garrafa et al., 2011) as well as cells of the central nervous system (CNS) including microglia, astrocytes, oligodendrocytes and neurons (Bsibsi et al., 2002; Tang et al., 2008). TLRs and TLR-related signaling are increasingly implicated in developmental and adult neuroplasticity, including the regulation of neural progenitor cell proliferation (Lathia et al., 2008; Okun et al., 2010b; Rolls et al., 2007; Shechter et al., 2008), axonal guidance (Cameron et al., 2007; Ma et al., 2007, 2006), learning and memory (Okun et al., 2012, 2010a), and metabolism (Shechter et al., 2013).

Via counterbalancing actions of sympathetic and parasympathetic neurons, the autonomic nervous system (ANS) regulates body systems critical for many basic functions of the organism including heart rate (HR), blood pressure, thermoregulatory thermogenesis and energy metabolism (Cannon and Nedergaard, 2004) in both health and disease. In thermoregulatory thermogenesis, the parasympathetic innervations of brown adipose tissue

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(BAT) only appears in two minor BAT depots, but not in the major interscapular BAT depot. BAT non-shivering thermogenesis is mostly triggered by the release of norepinephrine from its sympathetic nerve terminals, stimulating β3-adrenoceptors (Bartness et al., 2010). Sympathetic control of brown adipose tissue is also essential for the cold acclimation-recruited norepinephrine-mediated thermogenesis (Cannon and Nedergaard, 2004). Activation of either TLR2 or TLR4 is implicated in thermoregulation and fever in response to infection (Romanovsky et al., 2006). Activation of members of the TLR family of receptors explains how secreted products of different microbes can evoke the same biological response, fever. Fever-inducing substances or pyrogens, such as Lipopolysaccharides (LPS) and cell wall components of Gram-negative bacteria such as *Escherichia coli*, are recognized by TLR4 (Poltorak et al., 1998). Similarly to TLR4 activation, Gram-positive bacterial MAMPs such as peptidoglycan, lipoteichoic acid, as well as products from mycobacteria, yeast or fungi that stimulate TLR2 (Akira, 2003) can evoke fever (Hubschle et al., 2006; Nakagawa et al., 2002). Although much less studied, stimulation of TLR3 using polyinosine–polycytidylic acid (poly (I:C)), a synthetic analogue of double-stranded RNA from viruses (Alexopoulou et al., 2001) is also capable of inducing fever (Nakagawa et al., 2002). Moreover, it is likely that most ligands that activate TLRs are pyrogens. However, it is not known whether this process is mediated by regulation of the sympathetic and/or parasympathetic components of the ANS.

Through its regulation via the parasympathetic/sympathetic controls, HR is an established indicator for numerous pathological conditions. Increasing autonomic parasympathetic control of HR is tightly linked to higher survival rates in patients with heart disease (Bigger et al., 1992). Utilizing rodents for the study of the different controls of HR is an important step toward understanding the contribution of genes affecting these traits in pathologies related to the ANS.

Heart failure occurs when there is an inability of the heart to pump enough blood to meet the requirements of the body's metabolizing tissues. Ischemic cardiomyopathy and hypertension-induced cardiac hypertrophy are the most common causes of heart failure (Brown et al., 2005; Gradman and Alfayoumi, 2006). Heart failure is associated with chronic inflammation (Anker and von Haehling, 2004) and up-regulation of TLRs in cardiac muscle (Birks et al., 2004; Frantz et al., 1999). TLR2 or TLR4 deficiency, however, prevents increase in myocyte and cardiac size following pressure overload (Favre et al., 2007; Ha et al., 2005). The acute activation of TLR signaling may be beneficial in the short term, but ongoing tissue damage which results in chronic activation of TLRs can lead to heart failure (Topkara et al., 2011). Both TLR antagonists and agonists have been shown to be protective in heart failure. Eritoran, a TLR4 inhibitor, has been shown to reduce cardiac hypertrophy in a mouse model of aortic constriction by inhibiting a TLR4-mediated inflammatory response (Ehrentraut et al., 2011a,b). Thus, patients with pressure overload induced heart failure may benefit from the inhibition of TLR4 signaling. However, the pharmacodynamics of eritoran requires administration by continuous infusion that would not be practical for the treatment of patients with chronic heart failure. Heart failure is associated with the activation of the sympathetic nervous system (Francis, 1989; Meredith et al., 1993) and this association contributes to increased mortality rates (Eichhorn and Bristow, 1996; Goldsmith, 1999). In addition, cardiovascular disease may lead to an inflammatory process within the brain and also play a key role in activation of the sympathetic nervous system (Zhang et al., 2010). Additional evidence link TLRs to both inflammatory processes and sympathetic control. In a mouse model of ischemia-induced heart failure, angiotensin II receptors in the brain stem contribute to the central sympathetic response through TLR4 and MyD88-mediated inflammatory response. In addition, intra-cerebroventricular infusion of the angiotensin receptor blocker Losartan reduces both inflammatory and sympathetic responses (Ogawa et al., 2011). Fluvastatin, an HMG-CoA reductase inhibitor, reduces the inflammatory response in patients with chronic heart failure with an inhibitory effect on monocyte TLR4-mediated immune response (Foldes et al., 2008; Navi et al., 2013).

While both the cardio-vascular and thermoregulatory response are attributes of the ANS, and TLR2 and TLR4 are both intimately linked to a myriad of heart and cardiovascular related pathologies as well as thermoregulatory alterations following inflammation, the mechanism that underlies these effects is not clear. We thus asked whether there is a link between TLR signaling and ANS regulation of HR, thermoregulation, and/or energy metabolism.

Here, we show that: (1) mice lacking TLR2 or TLR4 exhibit reduced HR; (2) thermoregulatory responses to stress are altered in TLR2−/− and TLR4−/− mice; (3) brown fat-dependent thermoregulation is altered in TLR4−/− mice; and (4) mice lacking either TLR2 or TLR4 consume less food and maintain a greater mass compared to WT mice. The above suggests that TLR2 and TLR4 influence energy intake and metabolism and help regulate key aspects of the ANS.

2. Materials and methods

2.1. Animals

Young adult male congenic TLR4−/− mice (B6.B10ScN-*Tlr4*$^{lps\text{-}del}$/JthJ) ($n = 20$), TLR2−/− mice (B6.129-*Tlr2*^{tm1Kir}/J) ($n = 20$) and genetically matched background WT mice (B6.B10) ($n = 20$) at identical ages were purchased from Jackson Laboratories (Bar Harbor, ME, USA). All experiments were completed using mice starting at 2 months old and lasted until mice were 5 months old. Animal care and experimental procedures followed NIH guidelines and were approved by the National Institute on Aging Animal Care and Use Committee. The different experimental paradigms conducted in this study are chronologically depicted in Scheme 1.

2.2. Drugs

The following drugs were used in this study: atropine methylnitrate, a blood–brain barrier-impenetrant competitive antagonist of muscarinic acetylcholine receptor types M1, M2, M3, M4 and M5 (MP Biomedicals; 2 mg/kg, (Griffioen et al., 2013)); the beta-1 adrenergic receptor antagonist atenolol a (MP Biomedicals; 2 mg/kg, (Griffioen et al., 2013)); the beta-3 adrenergic receptor agonist CL316243 (Tocris Bioscience; 1 mg/kg, (Fu et al., 2008)); and the beta-3 adrenergic receptor antagonist SR59230A hydrochloride (Tocris Bioscience; 0.5 mg/kg, (Bexis and Docherty, 2009)). All drugs were injected intraperitoneally, and the maximal injected volume of the drugs was 200 μl, diluted in phosphate buffered saline.

2.3. Telemetry

A telemetry system was used to continuously monitor physiological and behavioral parameters of mice in their home cages as described previously (Griffioen et al., 2011). Briefly, a transmitter, TA10ETA-F20 (Data Sciences International, St Paul, MN, USA), which monitors electrocardiogram (ECG), core body temperature, and general activity, was surgically implanted in each of the mice. Two biopotential leads were routed subcutaneously lateral to midline of the chest and secured to chest muscles with silk sutures (Ethicon). Telemetry data were continuously recorded in 2.5 min bins, every 10 min. A total of 30 mice (of the 60 mice used in this