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THE EXPRESSION OF TOLL-LIKE RECEPTOR 4, 7 AND CO-RECEPTORS IN NEUROCHEMICAL SUB-POPULATIONS OF RAT TRIGEMINAL GANGLION SENSORY NEURONS

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Abstract—The recent discovery that mammalian nociceptors express Toll-like receptors (TLRs) has raised the possibility that these cells directly detect and respond to pathogens with implications for either direct nociceptor activation or sensitization. A range of neuronal TLRs have been identified, however a detailed description regarding the distribution of expression of these receptors within sub-populations of sensory neurons is lacking. There is also some debate as to the composition of the TLR4 receptor complex on sensory neurons. Here we use a range of techniques to quantify the expression of TLR4, TLR7 and some associated molecules within neurochemically-identified sub-populations of trigeminal (TG) and dorsal root (DRG) ganglion sensory neurons. We also detail the pattern of expression and co-expression of two isoforms of lysophosphatidylcholine acyltransferase (LPCAT), a phospholipid remodeling enzyme previously shown to be involved in the lipopolysaccharide-dependent TLR4 response in monocytes, within sensory ganglia. Immunohistochemistry shows that both TLR4 and TLR7 preferentially co-localize with transient receptor potential vallinoid 1 (TRPV1) and purinergic receptor P2X ligand-gated ion channel 3 (P2X3), markers of nociceptor populations, within both TG and DRG. A gene expression profile shows that TG sensory neurons express a range of TLR-associated molecules. LPCAT1 is expressed by a proportion of both nociceptors and non-nociceptive neurons. LPCAT2 immunostaining is absent from neuronal profiles within both TG and DRG and is confined to non-neuronal cell types under naïve conditions. Together, our results show that nociceptors express the molecular machinery required to directly respond to pathogenic challenge independently from the innate immune system.

Key words: pain, cell signaling, sensory neuron, trigeminal ganglion, dorsal root ganglion, Toll-like receptor.

INTRODUCTION

Traditionally it is thought that pathogens interact with sensory neurons through indirect mechanisms involving the activation of an innate immune response and production of pro-inflammatory mediators (see Ren and Dubner, 2010 for review). These indirect mechanisms involve the release of inflammatory mediators and the peripheral sensitization of high-threshold sensory neurons, nociceptors, increasing excitability to threshold and sub-threshold stimuli and contributing toward the transition from acute to chronic pain states (for reviews see Marchand et al., 2005; Ren and Dubner, 2010). A complex web of interactions between neurons, non-neuronal cells and immune cells develops to maintain a state of pain hypersensitivity (Milligan and Watkins, 2009; Austin and Moalem-Taylor, 2010; Grace et al., 2011; Nicotra et al., 2012). Previous work on the impact of inflammatory mediators on nociceptor sensitization and pain generation has suggested that the degree of pain associated with infection is heavily influenced by the degree of immune activation (Marchand et al., 2005; Ren and Dubner, 2010). It is now understood that sensory neurons can directly detect and respond to pathogenic challenge independent of the innate immune system.

Toll-like receptors (TLRs) are a family of innate pattern recognition receptors that detect a wide range of exogenous pathogenic and endogenous damage-released ligands. Since the discovery by Wadachi and Hargreaves (2006) of TLR4 expression on trigeminal sensory neurons, a range of functional TLRs have been shown to be expressed by neurons in both the peripheral and central nervous system (Lafon et al., 2006; Mishra et al., 2006; Wadachi and Hargreaves, 2006; Cameron et al., 2007; Acosta and Davies, 2008; Barajon et al., 2009; Ochoa-Cortes et al., 2010; Diogenes et al., 2011; Ferraz et al., 2011; Qi et al., 2011; Due et al., 2012;

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Abbreviations: AF, alexa-fluor; DAMP, damage-associated molecular pattern; DAPI, 4′,6-diamidino-2-phenylindole; DRG, dorsal root ganglion; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IHC, immunohistochemistry; IL-1β, Interleukin-1β; LPS, lipopolysaccharide; LPCAT, lysophosphatidylcholine acyltransferase; MD, myeloid differentiation protein; mRNA, messenger ribonucleic acid; MyD88, myeloid differentiation primary response protein 88; NF200, neurofilament 200; P2X3, purinergic receptor P2X ligand-gated ion channel 3; PBS, phosphate-buffered saline; qPCR, real-time polymerase chain reaction; RP105, radioprotective 105; TG, trigeminal ganglion; TLR, Toll-like receptor; TRAM, TRIF-related adapter molecule; TRPV1, transient receptor potential vallinoid 1.

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Park et al., 2014; Tse et al., 2014a). The expression of TLRs in neurons, particularly primary sensory neurons, has uncovered a potential innate surveillance function with implications for both the acute nociceptive response and the maintenance of chronic pain states.

TLR4 and the co-receptor CD14 are expressed on rat and human transient receptor potential vanilloid 1 (TRPV1)-positive and TRPV1-negative nociceptors within the trigeminal ganglion (TG) (Wadachi and Hargreaves, 2006; Diogenes et al., 2011). Activation of TLR4, by *Porphyromonas gingivalis* lipopolysaccharide (LPS), on TG sensory neurons both sensitizes TRPV1 and potentiates capsaicin-induced calcitonin gene-related peptide (CGRP) release (Diogenes et al., 2011; Ferraz et al., 2011). A role for TLR4, expressed by TG neurons, in mediating pain induced by tissue damage has also been demonstrated (Ohara et al., 2013; Miller et al., 2014). The activation of TLR4, by *Escherichia coli* LPS, on murine dorsal root ganglion (DRG) neurons has been shown to increase neuronal excitability (Ochoa-Cortes et al., 2010; Due et al., 2012), nociceptin expression (Acosta and Davies, 2008) and the myeloid differentiation primary response protein 88 (MyD88)-dependent production of pro-inflammatory mediators (Ochoa-Cortes et al., 2010; Tse et al., 2014a). The ability of TLRs to influence the nociceptive response is not limited to TLR4. Activation of TLR3, 7 and 9 expressed by DRG neurons induces the production of pro-inflammatory mediators, up-regulates TRPV1 expression and sensitizes TRPV1 activation (Qi et al., 2011). Activation of TLR3 and TLR7 induces an itch response (pruritus) and directly activates DRG nociceptors (Liu et al., 2010, 2012). More recently the activation of TLR7, by endogenous microRNAs, has been shown to rapidly activate DRG neurons through mechanisms that involve TRPA1 (Park et al., 2014).

In the DRG, the reported percentage of total TLR4-positive neurons varies widely (28–60%; Acosta and Davies, 2008; Due et al., 2012; Tse et al., 2014a,b). While it has been shown that 19% of neurons in the maxillary region of the TG and 29% of neurons innervating the gingivomucosa express TLR4, mainly in small- to medium-sized neurons (Vindis et al., 2014), there are currently no quantitative data for TLR4 expression within the TG as a whole. Previous studies have suggested that the expression of TLR7 is limited to small/medium sized neurons that express TRPV1 and TRPA1 although no quantitative analysis has been performed (Liu et al., 2010; Qi et al., 2011; Park et al., 2014).

There is also some debate regarding the composition of the TLR4 receptor complex expressed by sensory neurons. In innate immune cells a functional TLR4-signaling complex consists of TLR4, CD14 and myeloid differentiation protein (MD)-2 (Akira and Takeda, 2004). DRG neurons reportedly express CD14 and MD-1 messenger ribonucleic acid (mRNA) and protein but little MD-2 and no radioprotective 105 (RP105) mRNA or protein (Acosta and Davies, 2008). Upon activation, neuronal TLR4 is reported to form an atypical co-receptor complex with CD-14 and MD-1 (Acosta and Davies, 2008). MD-1 classically interacts with the TLR4 homolog, RP105 to regulate TLR4 signaling (Ohto et al., 2011). More recent studies however have shown that DRG neurons do express MD-2 and RP105 mRNA and protein in addition to MD-1 and CD14 mRNA and protein (Ochoa-Cortes et al., 2010; Tse et al., 2014a). Additionally it was shown that the majority of TLR4-positive neurons co-localize with CD14 and MD-2, rather than MD-1 (Tse et al., 2014a).

Lysophosphatidylcholine acyltransferase 1 and 2 (LPCAT1 and LPCAT2) are two isoforms of a phospholipid-modifying enzyme that participate in membrane remodeling by mediating the acylation of lysophosphatidylcholine (see Shindou and Shimizu, 2009). The phospholipid/lysophospholipid composition of cellular membranes affects membrane function, including lipid raft functions (Stulnig et al., 2001), and therefore may impact upon multiple cellular signaling pathways including TLR4 signaling (see Triantafilou et al., 2011). In macrophages, LPCAT2 is activated by phosphorylation following TLR4-dependent LPS recognition (Morimoto et al., 2010) and LPCAT activity is essential for the translocation of TLR4 to lipid rafts and subsequent generation of a TLR4 signaling response (Jackson et al., 2008). LPCAT1 and LPCAT2 have been identified in a range of tissues although a high level of expression has been demonstrated in lung alveolar cells and immune cells for LPCAT1 and LPCAT2, respectively (Nakanishi et al., 2006; Shindou et al., 2007; Morimoto et al., 2010). Both LPCAT1 and LPCAT2 expression have been demonstrated in a sub-set of spinal neurons (Okubo et al., 2012). Whereas LPCAT1 expression is constitutive, LPCAT2 is an inducible form of the enzyme (Shindou et al., 2005). Indeed, LPCAT2 is up-regulated in microglia following nerve injury while LPCAT1 expression remains unchanged (Okubo et al., 2012). LPCAT2 expression has also been identified in peripheral sensory neurons as well as non-neuronal cells within the DRG following nerve injury (Hasegawa et al., 2010). While previous studies have identified the expression of LPCAT isoforms in injured DRG neurons, the expression of LPCATs naïve peripheral sensory neurons and the subsequent role they might play in the neuronal TLR response is unknown.

While it has been shown that nociceptors express a range of TLRs, a detailed analysis of TLR expression within multiple sensory neuron sub-populations has not been performed, particularly within the TG. A detailed analysis of TLR4 and TLR7 expression patterns within primary sensory neurons is a pre-requisite for further functional analysis of receptor activation. In the current study we explore the hypothesis that nociceptors possess the required molecular components to directly detect and respond to ligands of bacterial, viral and endogenous origin. Using well-defined neurochemical markers, we provide a semi-quantitative analysis of the expression of TLR4 and TLR7 within sensory neuron sub-populations. We also detail a gene expression profile of TLR signaling-associated components within the TG. Lastly, we describe the distribution of expression of two isoforms of a lysophosphatidylcholine acyltransferase (LPCAT) enzyme, LPCAT1 and LPCAT2 within sensory ganglia.