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Differential effects of lipopolysaccharide on mouse sensory TRP channels

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ABSTRACT

Acute neurogenic inflammation and pain associated to bacterial infection have been traditionally ascribed to sensitization and activation of sensory nerve afferents secondary to immune cell stimulation. However, we recently showed that lipopolysaccharides (LPS) directly activate the Transient Receptor Potential channels TRPA1 in sensory neurons and TRPV4 in airway epithelial cells. Here we investigated whether LPS activates other sensory TRP channels expressed in sensory neurons. Using intracellular Ca^{2+} imaging and patch-clamp we determined the effects of LPS on recombinant TRPV1, TRPV2, TRPM3 and TRPM8, heterologously expressed in HEK293T cells. We found that LPS activates TRPV1, although with lower potency than for TRPA1. Activation of TRPV1 by LPS was not affected by mutations of residues required for activation by electrophilic agents or by diacylglycerol and capsaicin. On the other hand, LPS weakly activated TRPM3, activated TRPM8 at 25 °C, but not at 35 °C, and was ineffective on TRPV2. Experiments performed in mouse dorsal root ganglion (DRG) neurons revealed that genetic ablation of *Trpa1* did not abolish the responses to LPS, but remain detected in 30% of capsaicin-sensitive cells. The population of neurons responding to LPS was dramatically lower in double *Trpa1/Trpv1* KO neurons. Our results show that, in addition to TRPA1, other TRP channels in sensory neurons can be targets of LPS, suggesting that they may contribute to trigger and regulate innate defenses against gram-negative bacterial infections.

1. Introduction

The detection of pathogen-associated molecules relies on pattern recognition receptors (PRR) that identify specific molecular motifs, highly conserved across species [1]. Among these PRRs, Toll-like receptors (TLR) have been described as sentinels for distinct viral and bacterial components [2]. For instance, lipopolysaccharides (LPS), a major component of the wall of gram-negative bacteria, are recognized by TLR4 in antigen presenting cells and innate immune cells [3]. Upon LPS ligation, TLR4 activation triggers the production of pro-inflammatory cytokines and chemokines (TNFα, interleukin (IL)-6, IL-1) that induce immune-mediated inflammation through the recruitment of leukocytes [3,4].

Pain associated to infections was first described as sensitization and activation of nociceptors by inflammatory mediators found in the cytokine cocktail secreted by immune cells [5,6]. However, recent evidence indicates that bacterial components can directly sensitize and activate sensory afferent neurons. For instance, it has been proposed that bacterial-derived *N*-formyl peptides, pore-forming toxins and LPS produce pain by inducing depolarization and firing in nociceptive neurons [7–9]. Furthermore, we recently reported that the cation channel TRPA1 can be activated by LPS, leading to pain and neurogenic inflammation in mice [10] and to aversive responses in *Drosophila melanogaster* [11]. In the former study, we found that *E. coli* LPS induces a concentration dependent activation of mouse sensory neurons isolated from nodose and trigeminal ganglia. Up to the concentrations of 3 and 10 μg/ml the responses to LPS occurred exclusively in neurons responsive to the TRPA1 agonist cinnamaldehyde (CA). However, at higher concentrations (> 20 μg/ml), LPS induced responses in neurons that did not express TRPA1 (cinnamaldehyde-insensitive). Furthermore, the genetic ablation of *Trpa1* significantly reduced, but did not completely abolish, the responses to LPS. These data demonstrate that TRPA1 is not the only excitatory Ca^{2+} permeable channel mediating LPS effects.

Ligand promiscuity has risen as one of the key features of the TRP channel superfamily [12–16]. For instance, allyl isothiocyanate (AITC), initially described as a TRPA1 specific agonist, can also activate TRPV1 at higher concentrations, leading to aversive and pain responses and

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visceral irritation [17]. Furthermore, we have recently shown that epithelial TRPV4 is activated by LPS, inducing nitric oxide production and increased mucociliary beat frequency [18].

In this study, we tested the hypothesis that other sensory TRP channels may be involved in the TRPA1-independent effects of LPS. For this, we first characterized the effects of LPS on recombinant TRPV1, TRPV2, TRPM3 and TRPM8 channels heterologously expressed in HEK293T cells. We found that TRPV1 and TRPM3 are also sensitive to LPS, although at higher concentrations than for TRPA1. On the other hand, TRPM8 responded to LPS only at temperatures lower than physiological ones, and TRPV2 appeared to be LPS-insensitive. Using freshly isolated dorsal root ganglion neurons, we determined the relative roles of these channels *ex vivo*, by comparing the LPS-induced responses in sensory neurons isolated from wild type, *Trpa1* KO, *Trpv1* KO and double *Trpa1/Trpv1* KO mice. Together with our previous results [10], we conclude that TRPA1 and TRPV1 are the main contributors to the acute responses to LPS in sensory neurons at physiological temperatures.

2. Materials and methods

2.1. Reagents

Reagents were purchased from Sigma-Aldrich (Bornem, Belgium), unless stated otherwise.

2.2. Animals

Trpv1 knock-out (KO) mice were obtained from The Jackson Laboratory (http://jaxmice.jax.org/strain/003770.html) and *Trpa1* KO and double *Trpv1/Trpa1* KO mice have been described earlier [17,19]. All knockout strains were backcrossed at least nine times into the C57BL/6 J background, and C57BL/6 J mice were used as wild type (WT) controls. Mice of all genotypes were housed under identical conditions, with a maximum of four animals per cage on a 12-h light-dark cycle and with food and water *ad libitum*. Ten- to twelve-week-old male mice were used in all experiments. All animal experiments were carried out in accordance with the European Union Community Council guidelines and were approved by the local ethics committee (P021/2012).

2.3. Cell culture and transfection

Human embryonic kidney cells, HEK293T, were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal calf serum (FCS), 2 mM L-glutamine, 2 units/ml penicillin, and 2 mg/ml streptomycin at 37 °C in a humidity-controlled incubator with 10% CO_2. For intracellular Ca^{2+} imaging and whole-cell patch-clamp experiments, HEK293T cells were transiently transfected with mouse TRPM3α2, human TRPV2, TRPM8 or TRPV1 in the bicistronic pCAGGS/IRES-GFP vector, or with mouse TRPV1-S513Y or TRPV1-C157A in the bicistronic pIres2GFP vector, using Mirus TransIT-293 (Mirus Corporation; Madison, WI, USA). For whole-cell patch-clamp experiments with mouse TRPM3α2, a stably expressing HEK293T cell line was used.

2.4. Isolation and primary culture of dorsal root ganglion neurons

DRG neurons from adult (postnatal weeks 8–12) mice were cultured using a variant of a method previously described [20]. Briefly, DRGs were bilaterally excised under a dissection microscope, washed in 10% fetal calf serum Neurobasal A Medium (basal medium) and then incubated at 37 °C in a mix of collagenase of 1 mg/ml (Gibco, Gent, Belgium) and dispase of 2.5 mg/ml (Gibco) for 45 min. Digested ganglia were gently washed twice with basal medium and mechanically dissociated by mixing with syringes fitted with increasing needle gauges. Neurons were seeded on poly-l-ornithine/laminin-coated glass bottom chambers (Fluorodish WPI, Hertfordshire, UK) and cultured for 12–18 h at 37 °C in B27-supplemented Neurobasal A medium (Invitrogen, Gent, Belgium) containing GDNF of 2 ng/ml (Invitrogen) and NT4 of 10 ng/ml (Peprotech, London, UK).

2.5. Intracellular Ca^{2+} imaging experiments

Cells were incubated at 37 °C with 2 μM of Fura-2AM ester for 30 min before the recordings. Intracellular Ca^{2+} concentration ($[Ca^{2+}]$) was measured on an Olympus CellˆM system. Fluorescence was measured during excitation at 340 nm and 380 nm, and after correction for the individual background fluorescence signals, the ratio of the fluorescence at both excitation wavelengths (F_{340}/F_{380}) was monitored. Intracellular Ca^{2+} concentrations were calculated using the formula described below, as described by Grynkiewicz and colleagues [21]:

$$[Ca^{2+}] = K_{eff}\frac{R-R_{min}}{R_{max}-R}$$

where R denotes the ratio between the fluorescence intensities measured upon excitation at 340 nm (F_{340}) and 380 nm (F_{380}); R_{min} is the value of this ratio measured in Ca^{2+}-free medium containing 10 mM EGTA; R_{max} corresponds to the ratio measured using a high Ca^{2+} concentration (10 mM)-containing medium; and K_{eff} is the effective dissociation constant.

K_{eff} is determined by:

$$K_{eff} = K_D\frac{R_{max}+\alpha}{R_{min}+\alpha}$$

where K_D is the dissociation constant of Fura-2AM for Ca^{2+} and α is the 'isocoefficient'. The value of α is calculated from: $F_{isosb} = F_{340} + \alpha F_{380}$, and correspond to a value for which, the F_{isosb} becomes independent of intracellular Ca^{2+} [22].

Experiments were performed using the standard Krebs solution containing (in mM): 150 NaCl; 6 KCl; 10 HEPES; 1.5 $CaCl_2$; 1 $MgCl_2$; 10 glucose monohydrate; pH adjusted to 7.4 with NaOH. The data were classified semi-automatically using a function programmed in MATLAB (MathWorks, MA) and analyzed with Origin 7.0 (OriginLab Corporation, Northamptom, MA, USA).

2.6. Whole-cell patch-clamp experiments

Whole-cell membrane currents were measured with an EPC-10 patch-clamp amplifier and the Pulse (HEKA, Lambrecht/Pfalz, Germany) and Clampex (axon instruments, Sunnyvale CA, US) softwares. Currents were digitally filtered at 3 kHz, acquired 20 kHz and stored for off-line analysis on a personal computer. Cells were recorded in an extracellular solution containing (in mM): 156 NaCl; 10 HEPES; 1.5 $CaCl_2$; 1 $MgCl_2$; 10 glucose monohydrate; pH adjusted to 7.4 with NaOH. The pipette solution contained (in mM): 145 Cs-Aspartate; 10 EGTA; 10 HEPES; 1 $MgCl_2$; pH adjusted to 7.2 with CsOH. Non-transfected HEK293T cells were used as control. Whole-cell currents were elicited using 200 ms-long voltage ramps applied every 2 s from a holding potential of 0 mV from −110 mV to +110 mV. Time courses of current amplitudes were plotted as the current measured at −75 mV and +75 mV. Unless otherwise mentioned, all measurements were performed at 35 °C.

2.7. Data analysis and statistics

Electrophysiological data were analyzed using the WinASCD software (Guy Droogmans, KU Leuven) and Origin 9.0 (OriginLab). Origin 9.0 was also used for statistical analysis and data display. Pooled data are expressed as mean ± s.e.m.