TRPV4 links inflammatory signaling and neuroglial swelling

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A perennial challenge in neuroscience research has been to elucidate the role of astrocytes, the most numerous cell type in the CNS, at all levels of brain function from development and cognition to trauma and death of the organism.1 The many types of astrocyte tend to have in common the regulation of water/ion transport, metabolic homeostasis and inflammatory signaling. Prominent expression of volume-sensitive ion channels and aquaporins ensures that even small activity-induced changes in extracellular ion concentrations result in astroglial swelling, which in turn impacts on the concentration/diffusion of ions and neurotransmitters across the extracellular space. Because astrocyte swelling, exacerbated by neuronal overexcitation, ischemia and/or inflammation can drive excitotoxic death of neurons in diabetes, seizures, stroke/ischemia and neurodegenerative/retinal diseases, it represents a prevalent source of surgical concern.1 We recently identified the osmosensitive TRPV4 (transient receptor potential isoform 4) channel in retinal glia as a potential target for polyunsaturated fatty acids (PUFAs) commonly associated with brain swelling and inflammation, and elucidated its role in Ca2+ homeostasis, swelling and reactive gliosis.2

Both normal and pathological CNS activity generate free PUFAs, with the predominant elevation of arachidonic acid (AA), a cell diffusible, C20:4n6 long-chain fatty acid product of phospholipase A2 (PLA2). Normally a constituent of membrane phospholipids, AA is released following Ca2+-dependent activation of PLA2 and/or combined activation of phospholipase C and diacylglycerol lipase, reaching extracellular concentrations up to 0.5 mM.3 AA is typically produced and released by astroglia but can be taken up into neurons where it affects a variety of intrinsic and synaptic signaling mechanisms. AA regulates cellular signaling as a standalone 2nd messenger and/or by acting through its thromboxane, leukotriene, prostaglandin and/or epoxyeicosatrienoic acid (EET) metabolites.3,4 The AA pathway was suggested to promote inflammation by exacerbating glial swelling, neuronal damage and CNS edema,5,6 however, the relationship between PUFA signaling, swelling and Ca2+ homeostasis is not well understood.

We defined the dynamic link between astroglial swelling, Ca2+ homeostasis and biosynthesis of fatty acids by showing that the large-scale calcium entry into retinal Müller cells, mediated by the glial swelling sensor, TRPV4, requires concomitant production of EETs. Somewhat paradoxically, as reported previously for brain astrocytes,6 AA itself enhanced the Müller glial response to hypotonic swelling (HTS) which however swelling was suppressed by inhibition of PLA2. We then tested the hypothesis that glial volume is regulated by a downstream metabolite of AA. Inhibition of cytochrome P450 (CYP450) suppressed swelling, as did Ca2+ removal from extracellular saline and chelation of cytosolic Ca2+ levels whereas AA and the CYP450 product, 5,6-epoxyeicosatrienoic acid (5,6-EET), caused large and sustained [Ca2+]i elevations in Müller cells. We used genetic ablation and pharmacological antagonists to show that the swelling-, AA- and 5,6-EET-sensitive pathway in Müller cells requires TRPV4 channel activation.

Keywords: arachidonic acid, cytotoxic edema, epoxyeicosatrienoic acids, inflammation, neurodegeneration, phospholipase A2, TRPV4

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TRPV4 agonists (GSK1016790A) induced sustained Ca\(^{2+}\) elevations that far surpassed the effects of previously known effectors of Ca\(^{2+}\) signaling in Müller glia but also initiated propagation of transcellular Ca\(^{2+}\) waves in dissociated cells and retinal slices. Consistent with the canonical mechanism proposed by Bernd Nilius’ group, the effects of the agonist on cation currents and/or \([\text{Ca}^{2+}]_{i}\) were mimicked by AA and 5\(\text{O}-\text{EET}\), and antagonized by genetic elimination or pharmacological suppression of TRPV4. Showing that TRPV4 represent the main osmosensor in Müller cells, selective antagonists inhibited HTS-induced Ca\(^{2+}\) elevations. Interestingly, inhibition of TRPV4, PLA2 or CYP450 also suppressed hypotonic swelling, suggesting that TRPV4-mediated volume sensing might contribute to a positive feedback loop that exacerbates swelling. These observations led us to test the hypothesis that TRPV4 channels represent a missing link in the pathophysiological chain composed of glial swelling, AA release and reactive gliosis.\(^6,7\) In vivo injections of GSK1016790A induced massive upregulation of the MAP kinase cascade and the gliotic marker GFAP, suggesting that TRPV4 activation is sufficient to trigger the reactive state (see Fig. 1). Given that Trpv4-/- Müller glia also exhibited a moderate degree of GFAP immunoreactivity in the absence of experimental interventions, we hypothesize that steady-state TRPV4 activity is required for maintaining a healthy retinal response to light-dependent changes in osmolyte concentrations and/or body temperature.

Figure 1. Hypo-osmotic stress simultaneously activates TRPV4 channels in neurons and astroglia. In astrocytes, HTS-induced membrane stretch stimulates PLA2, which signals via CYP450 to open the channel through EETs. The mechanism of activation of the neuronal TRPV4 remains to be determined, however, concomitant swelling-induced increase in AA and Ca\(^{2+}\) overload may result in neuronal injury.

Another intriguing aspect of the study was that the concomitant TRPV4-mediated signals observed in retinal ganglion cells (RGCs)\(^2\), the sole visual information conduit to the midbrain, \([\text{Ca}^{2+}]_{\text{RGC}}\) elevations, induced by TRPV4 agonists or HTS, were sustained, had markedly slower time-to-peak, and insensitive to PLA2/CYP450 inhibition. It is worth noting that the confinement of retinal TRPV4 expression to RGCs and Müller prostaglandins and activation of Toll-like receptors.\(^1,2,8\) It is possible that TRPV4 channels, in conjunction with deranged arachidonic signaling, play important roles in the development of CNS edema associated with pathological swelling of astrocytes. Our results thus represent a first step towards assigning functional significance to differential osmoregulation in neurons and glia and defining the relationships between lipid messenger signaling, neuronal activity, vascular function and mechanotransduction.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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