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FAT NERVES KEEP PAIN AT BAY

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Summary. Inflammatory pain is a debilitating condition and a severe health burden; physiologically it is a complex phenomenon with multiple contributing mechanisms. A new study published in EMBO Journal has discovered one such mechanism. This multidisciplinary investigation demonstrates that tissue inflammation results in local depletion of cholesterol in nociceptive nerves, causing a loss of lipid raft localization of a sodium channel $Na_v1.9$ and, ultimately, resulting in potentiation of its activity. The discovered effect contributes to the inflammatory overexcitability of peripheral nociceptive nerve terminals resulting in inflammatory hyperalgesia. Impressively, authors were able to offset this inflammatory pain mechanism by topical application of cholesterol-containing gels, opening a novel avenue for therapeutic intervention.

Cholesterol is generally perceived as an 'evil' molecule since high blood cholesterol levels are linked to severe cardiovascular disorders such as atherosclerosis, heart attack and stroke (Wald & Law, 1995). Yet, cholesterol is an essential component of an animal cell comprising ~30% or more of a mammalian cell plasma membrane; the human body produces about 1g of cholesterol daily (Sardesai, 2011). Physiological importance of cholesterol as an essential structural element of the plasma membrane has become evident with development of the 'lipid raft hypothesis' (Simons & Ikonen, 1997), which proposes that the lipid bilayer of the plasma membrane is not just a passive solvent, but a complex milieu laterally segregated by specific interactions between cholesterol, sphingolipids and integral membrane proteins. These molecules form stable and discrete assemblies ('rafts') within the membrane, often hosting specialized structures such as ion channels or G protein signaling complexes (Lingwood & Simons, 2010).

A study from Muriel Amsalem and colleagues found an unanticipated role for cholesterol in controlling sensitivity of peripheral somatosensory fibers specifically responsible for inflammatory pain (Amsalem, 2018). They found that tissue inflammation reduces cholesterol content in dorsal root ganglion (DRG) neurons, causing loss of lipid raft localization and subsequent potentiation of sensory-neuron-specific voltage-gated $Na_v1.9$ channels - important determinants of excitability in a subset of pain-sensing (nociceptive) nerves (Dib-Hajj et al, 2015). As a result, the affected neurons displayed enhanced excitability and firing rates, effects that contributed to inflammatory hyperalgesia observed in animal models of inflammatory pain. Importantly, the authors also demonstrated strong analgesic efficacy of transcutaneous cholesterol delivery in vivo; indeed cholesterol containing skin formulations prevented inflammation-mediated cholesterol loss and alleviated hyperalgesia in animal models of acute and chronic inflammatory pain.

The authors began their study by demonstrating that intraplantar injection of inflammation-induced agent λ-carrageenan or a cocktail of inflammatory mediators (histamine, bradykinin, ATP, prostaglandin E2 and norepinephrine) reduced cholesterol levels in skin biopsies by 15-20%; the same cocktail also reduced total cholesterol in DRG cultures. This reduction coincided with the development of mechanical and thermal hypersensitivity in injected paw in vivo, along with decreased action potential firing thresholds and increased firing frequencies in cultured DRG neurons. Even though the reduction in cholesterol levels was fairly modest, application of 'soluble' cholesterol (cholesterol in complex with methyl-betacyclodextrin, MβCD-chol) prevented the excitatory effect of inflammatory mediators seen accompanying cholesterol depletion. Another observation reinforcing the link between tissue inflammation, nerve cholesterol level and inflammatory pain was that cholesterol depletion with MβCD or cholesterol oxidase also produced excitatory effects when applied to cultured DRG neurons.

Likewise, hind paw injections of MβCD produced hyperalgesia; both in vitro and in vivo effects were prevented by MβCD-chol.

Interestingly, pro-algesic effects of cholesterol extraction (induced by hind paw MβCD injection) were significantly less pronounced (although still significant) in $Na_v1.9$ knock-out mice. In contrast, $Na_v1.8$ knock-out mice showed unaltered MβCD-induced hyperalgesia. These data pointed to the hypothesis that the excitatory effect of cholesterol depletion is partially mediated by $Na_v1.9$. In support of this idea, $Na_v1.9$-like currents recorded from cultured DRG neurons were strongly potentiated by the inflammatory mediators and by cholesterol extraction. These findings are in good agreement with previous literature on potentiation of TTX-resistant Na^+ channels by inflammatory mediators (Maingret et al, 2008; Ostman et al, 2008; Rush & Waxman, 2004; Vanoye et al, 2013). $Na_v1.9$ potentiation by GTPγS, a proxy for G-protein signaling, was also prevented by MβCD-chol (as was the potentiation induced by cholesterol extraction). The authors identified several putative cholesterol-binding motifs in transmembrane regions of $Na_v1.9$ protein and showed that three of these (tested as free peptides) can bind cholesterol in vitro. However this was not followed through so it is still unclear if a full-length, folded $Na_v1.9$ can directly bind cholesterol and if this binding is affected by tissue inflammation.

Following on from the putative role of cholesterol as a $Na_v1.9$ modulator, the authors discovered that $Na_v1.9$ localised to the cholesterol-rich 'raft' membrane fractions in whole DRG extracts, whereas inflammatory mediators induced translocation of the channels into non-raft fractions. Distribution of other raft marker proteins (caveolin-1 and flotilin) was unaffected by inflammatory mediators which indicated no obvious destruction of rafts themselves. This result was somewhat unexpected since MβCD treatment, even at lower concentrations as compared to those used by Amsalem and colleagues, does destroy lipid rafts in DRG neurons (Jin et al, 2013). Thus, the exact relationship between cholesterol levels, raft integrity and $Na_v1.9$ localization and activity has yet to be established.

How would inflammatory mediators deplete cholesterol levels in nociceptive nerve terminals though? The authors have tested (to some degree) a hypothesis that inflammation may result in local oxidative stress followed by the release of reactive oxygen species (ROS) and cholesterol oxidation (Gamper & Ooi, 2015). Accordingly, inflammatory mediators induced measurable ROS generation in cultured DRG neurons while antioxidant N-acetyl-cysteine (NAC) abolished $Na_v1.9$ potentiation in vitro and reduced mechanical hypersensitivity induced by plantar injection of inflammatory mediators in vivo. Thus, the ROS hypothesis seems plausible, even though at present there is no clarity which receptors (out of those activated by the cocktail) mediate ROS production in DRG and via which signaling mechanism(s).

What makes this study really fascinating is the fact that the authors were able to capitalize on their observations that MβCD-chol can reverse the majority of excitatory and pro-algesic effects of inflammatory mediators. They developed a formulation of cholesterol-containing transdermal gels and showed that topical application of such gels has significant analgesic activity against carrageenan-induced hyperalgesia and also reduced mechanical allodynia in a model of rheumatoid arthritis. While these experiments do not offer much further insight into mechanisms behind the proalgesic effects of cholesterol depletion, they do offer an exciting therapeutic opportunity for treatment of pains with inflammatory origin (e.g. arthritis pain, some back pain etc.) using topical cholesterol-containing formulations.

It is worth pointing out that fairly high concentrations of MβCD (40 mM) were used to deplete cholesterol in this study; it is likely that such treatment produced a greater degree of cholesterol

depletion as compared to that induced by inflammatory mediators or carrageenan. Furthermore, inflammatory mediators produce multiple other effects, via their respective receptors, in addition to mild reduction in cholesterol levels. Finally, $Na_v1.9$ KO only partially reducing MβCD-induced pain. Thus, while there is a convincing case that cholesterol depletion and subsequent potentiation of $Na_v1.9$ does contribute to inflammatory pain, multiple other mechanisms for inflammation-induced peripheral nerve overexcitability must exist (as acknowledged by the authors and explicitly summarised in their schematic). Nevertheless, the new role of cholesterol in modulation of pain transmission is a fascinating discovery which opens new avenues for pain relief.

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