Mechanisms of peripheral pain sensitization

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Tissue injury and inflammation lead to enhanced pain sensations (hyperalgesia) involving changes in nociceptive signaling events at both the peripheral and central nervous system levels. In the peripheral nervous system a variety of molecules released during injury act upon several ion channels and receptors to influence their response properties in maladaptive ways to promote chronic pain. Over the years one of the key targets that has emerged is the capsaicin receptor, TRPV1, a ligand gated non-selective cation channel activated by capsaicin, heat, and protons and centrally involved in the transduction of noxious stimuli by small diameter sensory afferents. TRPV1 responsiveness has been shown to be acutely enhanced by bradykinin, ATP, and neurotrophins such as nerve growth factor (NGF) and artemin (Art) as well as by a member of the TGFβ family, activin. Recent studies in a number of laboratories have focused on sensitization by NGF and the underlying signaling events upon activation of its primary receptor trkA, a receptor tyrosine kinase. We have recently demonstrated that adult, but not neonatal, sensory neurons exhibit this sensitization and that it reflects a developmental switch in signaling pathways rather than in expression of TRPV1 or trkA. The link between trkA activation and TRPV1 sensitization was initially proposed to involve phospholipase C (PLC) mediated reduction of PIP2 levels thus disinhibiting TRPV1. However this mechanism remains controversial and other signaling pathways are likely to be involved.

Utilizing adult rat and mouse DRG neurons as well as CHO cells coexpressing trkA and TRPV1, we have reinvestigated the signaling events underlying acute sensitization of TRPV1 by NGF using biochemical, electrophysiological, pharmacological, mutational, gene array and genetic knockout approaches. Selective pharmacological interference with p42/p44 mitogen activated protein kinase (MAPK) or phosphoinositide-3-kinase (PI3K), but not PLC abrogates sensitization of capsaicin responses in both systems. Co-expression of TRPV1 with either wild-type human trkA or the PLC signal deficient Y785F trkA mutant reconstitutes NGF sensitization. In contrast, TRPV1 coexpressed with either the MAPK signaling deficient Y490A or PI3K signaling deficient Y751F trkA mutants exhibits weaker sensitization by NGF. Western blot analysis of p42/p44 and Akt phosphorylation confirmed the biochemical specificity of the pharmacological agents and the trkA mutants. Gene array and real time PCR analysis of RNA from neonatal *vs* adult rat DRG neurons suggests an upregulation of both p42/p44 and PI3K in adults that parallels the development of NGF sensitivity. Moreover, NGF sensitization of capsaicin was greatly reduced in neurons isolated from mice in which the p85α regulatory subunit of PI3K had been deleted by homologous recombination. Inhibition of src kinase (c-src) expression or function also abrogates some of the NGF sensitization. Finally, inhibition p42/p44 phosphorylation does not prevent activin induced sensitization of TRPV1. These data suggest that PI3K, MAPK, and src pathways, but not the PLC pathway underlie the acute sensitization of TRPV1 by NGF. Furthermore, activin and NGF induce similar sensitization of TRPV1, but likely through distinct signaling pathways.