Journal Club

Editor's Note: With this issue, the *Journal* launches a new feature, Journal Club, that will appear on a regular basis. These short reviews of a recent paper in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to mimic the journal clubs that exist in your own departments or institutions. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

An Epac-Dependent Pain Pathway

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Review of Hucho et al. (http://www.jneurosci.org/cgi/content/full/25/26/6119)

High-threshold nociceptor sensory neurons in the peripheral somatosensory nervous system do not act as fixed detectors of external stimuli; instead, the sensitivity of their peripheral terminals can be dynamically modulated. Excessive stimulation, inflammation, and peripheral nerve injury can reduce the threshold and increase the responsiveness of nociceptors, the phenomenon of peripheral sensitization. Peripheral sensitization contributes to the clinical findings of hyperalgesia, where the response to noxious stimuli is enhanced, and allodynia, where innocuous stimuli become painful (Woolf and Salter, 2000). In their article in *The Journal of Neuroscience*, Hucho et al. (http://www.jneurosci.org/cgi/content/full/25/26/6119) dissect out a signal transduction cascade in nociceptor sensory neurons that contributes to epinephrine-induced mechanical hyperalgesia, an acute form of mechanical pain hypersensitivity brought on by activation of β-adrenergic receptors (β-ARs) that are present on primary sensory neurons. Activation of one particular protein kinase C (PKC) isomer, PKCϵ, is a key element of this hyperalgesia (Khasar et al., 1999). The downstream targets of this kinase in nociceptors remain under investigation but most likely include both voltage-gated sodium channels and the noxious heat transducing receptor TRPV1 (Woolf and Salter, 2000). Here, the Levine laboratory focuses on the signaling components that lie between the activation of the β-AR and the activation of PKCϵ. The authors examined the proportion of adult dorsal root ganglion (DRG) neurons in primary culture that responded to pharmacological perturbation with a translocation of PKCϵ to the plasma membrane [Hucho et al. (2005), their Fig. 1*A* (http://www.jneurosci.org/cgi/content/full/25/26/6119/FIG1)]. They then verified the effect of the same agents on hyperalgesia *in vivo*.

The authors begin with the extracellular signal, then work their way downstream in the signal transduction cascade. First, they determined that the β-AR agonist isoproterenol induced PKCϵ translocation [Hucho et al. (2005), their Fig. 1*B*]. The β_2-AR antagonist ICI 118,551 [(+)-1-[2,3-(dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol-hydrochloride] blocked the induction [Hucho et al. (2005), their Fig. 1*C*]. Next, because β_2-AR is a G-protein-coupled receptor, they tested cholera toxin, which activates the Gα_s subunit, and forskolin, which activates adenylate cyclase, showing that both induced PKCϵ translocation [Hucho et al. (2005), their Fig. 2*C* (http://www.jneurosci.org/cgi/content/full/25/26/6119/FIG2)]. Interestingly, forskolin acted with more rapid kinetics than cholera toxin to induce translocation, consistent with adenylate cyclase lying downstream of Gα_s activation. The canonical next step in this cascade would be the activation of PKA by the cAMP produced by adenylate cyclase. However, the PKA inhibitor CMIQ (4-cyano-3-methylisoquinoline) did not affect PKCϵ translocation [Hucho et al. (2005), their Fig. 2*A*]. The apparent lack of PKA involvement in the PKCϵ translocation led the authors to turn to Epac. Epac is a recently discovered protein that acts as a cAMP-responsive guanine nucleotide exchange factor, capable of activating the Ras family GTPases (Bos, 2003). Application of the Epac activator 8-(4-chlorophenylthio)-2′-*O*-methyl-cAMP (CPTOMe) to cultured DRG neurons induced PKCϵ translocation [Hucho et al. (2005), their Fig. 2*C*].

Because diacylglycerol activates members of the PKC family, the authors tested for the involvement of phospholipase D (PLD), phosphatidylinositol (PI)-specific phospholipase C (PLC), and phosphatidylcholine (PC)-specific phospholipase C, using appropriate inhibitors. Inhibition of PI-PLC or PLD reduced the isoproterenol-induced PKCϵ translocation, but inhibition of PC-PLC did not affect translocation. Thus PI-PLC and PLD act downstream of Epac. The PI-PLC inhibitor prevented PKCϵ translocation, whereas the PLD inhibitor reduced translocation to 30% of normal levels. Although these results may leave the intervening steps between Epac and the phospholipases somewhat up in the air, the authors have nevertheless established a framework for the signal transduction pathway.

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Next, they turned to ask two critical questions: (1) what cell types are responsible for Epac signaling and (2) do the cell-culture findings have *in vivo* relevance? To answer the first, they used double staining to demonstrate that PKCε translocation in response to isoproterenol occurs predominantly in DRG neurons that bind isolectin B4 (IB4), a marker for nonpeptidergic nociceptors. To answer the second, they used the same pharmacological approach to perturb epinephrine-induced hyperalgesia *in vivo*. First, they show that CPTOMe, the Epac activator, produced a degree of hyperalgesia similar to that seen for epinephrine and that this could be blocked with a PKCε inhibitor, εV1–2 [Hucho et al. (2005), their Fig. 5*A* (http://www.jneurosci.org/cgi/content/full/25/26/6119/FIG5)]. Second, the inhibitors of PI-PLC and PLD, but not inactive controls, completely blocked the epinephrine and CPTOMe-induced hyperalgesia. These findings are impressive, but it would be interesting to know whether these inhibitors affect other models of inflammatory hyperalgesia, such as those elicited by CFA or carageenan injection, or the formalin test. These models are less mechanistically specific than epinephrine injection, but they may represent more biologically plausible models for inflammatory hyperalgesia and would aid in assessing the generalizability of the findings.

The findings presented regarding the IB4(+) neuron specificity of this mechanism seem likely to be of particular importance. IB4 binding identifies the nonpeptidergic subset of unmyelinated small-diameter DRG neurons; IB4(+) DRG neurons also require glial-derived neurotrophic factor (GDNF) for trophic support and express the GDNF receptor c-RET. In contrast, the IB4(−) population of unmyelinated small DRG neurons express substance P and calcitonin gene-related peptide (CRGP), express trkA, and require NGF for trophic support. Surprisingly, little is known about the function of IB4(+) neurons, either in normal somatosensation or after inflammation. Hucho et al. (2005) point out that PKCε, Epac, and the β_2-AR are present in virtually all DRG neurons, ruling out this most simple explanation for the specificity they find. Interestingly, it was shown recently that the IB4(+) population has a cutaneous innervation pattern that is anatomically distinct from that of the CGRP(+) population: IB4(+) nerve endings terminate in the epidermal stratum granulosum, whereas CGRP(+) endings terminate in the stratum spinosum (Zylka et al., 2005). Together, these findings indicate that in addition to being distinct in terms of molecular markers, trophic requirements, and innervation of the skin and spinal cord, IB4(+) nociceptors and CGRP(+) nociceptors may differ in the signals that induce them to adopt a hypersensitized state.

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