

Growing Pains: The Cytoskeleton as a Critical Regulator of Pain Plasticity

Inflammatory mediators act on peripheral sensory neurons to produce pain and hypersensitivity after tissue injury. In this issue of *Neuron*, Dina et al. report that inflammatory mediators, such as epinephrine and prostaglandins, appear to couple to specific G protein-coupled receptor signaling pathways through plastic interactions with the cytoskeleton.

A fundamental issue of pain research revolves around mechanisms involved in modulating sensory transduction and information transfer to produce hypersensitivity or reduce nociceptive thresholds after tissue or nerve injury. Basic scientists and physicians need to understand plasticity not only in pain sensation responsible for acute pain, but also in the maintenance and refinement of the hypersensitive state in chronic pain. In this issue of *Neuron*, Dina and colleagues (2003) examine a novel role for the cytoskeleton in inflammatory mediator signaling as well as plastic switches in the cytoskeleton and downstream signaling cascades after tissue injury.

Tissue injury typically produces inflammation characterized by the pentad of rubor (redness), calor (heat), tumor (swelling), dolor (pain), and functio lasa (loss of function). Injury or infection often initiates inflammation, which involves a complex interaction of blood vessel endothelial cells, white blood cells, sympathetic efferents, and nociceptive afferents. In response to injury, all of these cellular players release “inflammatory mediators,” which increase blood flow and vascular permeability as well as activate or sensitize sensory neurons. Many mediators act indirectly to sensitize sensory neurons, but several studies have identified that epinephrine and prostaglandin E₂ (PGE₂) directly act on sensory neuron G protein-coupled receptors (GPCRs) to enhance sensory transduction and excitability leading to pain hypersensitivity (Julius and Basbaum, 2001).

Knowledge of GPCR and other signaling mechanisms in sensory neurons has grown significantly in the past few years; it is now clear that several kinase-mediated pathways modulate sensory neurons to produce hypersensitivity. Specifically, PGE₂ acts solely via protein kinase A (PKA), while epinephrine relies on PKA, protein kinase C ϵ (PKC ϵ), and the ERK1 and ERK2 mitogen-activated protein kinases (Aley et al., 2001; Khasar et al., 1999). Utilizing agents that disrupt actin microfilaments, neurofilaments, and microtubules, Dina and coworkers found that cytoskeletal disruption reduces hypersensitivity induced by intradermal epinephrine injection but has little effect on PGE₂-induced hypersensitivity. There are obvious concerns of potential widespread disturbances in neuronal structure and function when using these agents, so it is somewhat surprising that cytoskel-

etal disruption produced such specific effects on sensory plasticity. These data underscore a modern vision of the cytoskeleton not as a static scaffold designed simply to maintain cell structure, but rather as a dynamic, plastic system involved in compartmentalizing and modulating cell signaling. In sensory neurons, the cytoskeleton acts as a scaffold for epinephrine signaling involving β -adrenergic receptors and downstream coupling to PKA, PKC, and ERK1/2 and their effectors. Conversely, PGE₂ receptors (EP receptors) lie in a distinct signaling compartment, functioning independently of the cytoskeleton and relying solely on PKA activation.

With the work of Dina et al., the molecular mechanism of epinephrine-mediated hypersensitivity and its dependence on cytoskeletal integrity is now of paramount interest. Models will diverge on the basis of whether cytoskeletal integrity is required for activation of protein kinases or for modulation of downstream effectors, such as the TTX-resistant sodium channel (TTX_R). Since cytoskeletal disruption inhibits hypersensitivity produced by direct activation of either PKC ϵ or ERK1/2, the cytoskeleton must be involved in the coupling of these kinases to downstream effectors. These data do not exclude an additional role for the cytoskeleton in linking β -adrenergic receptors to PKC ϵ or ERK1/2. Conversely, since cytoskeletal disruption fails to reduce hypersensitivity mediated by direct activation of PKA, the cytoskeleton primarily links β -adrenergic receptors to PKA and does not participate in coupling PKA to its effectors. The divergence in susceptibility of epinephrine and PGE₂-mediated hypersensitivity to cytoskeletal disruption also provides indirect support of this model. If PKA coupling to downstream effectors were dependent on cytoskeletal integrity, then cytoskeletal disruption would inhibit PKA-mediated hypersensitivity regardless of the upstream GPCR. Therefore, the cytoskeleton selectively couples β -adrenergic receptors, but not prostaglandin EP receptors, to PKA in naive sensory neurons (Figure 1).

Several relevant interactions linking cytoskeletal elements, PKA, PKC ϵ , and ERK1/2 signaling molecules have been identified. PKC ϵ , but not other PKC isozymes, selectively binds to and may also be activated by filamentous actin (F-actin) (Prekeris et al., 1998). PKA often forms signaling scaffolds with other molecules via A-kinase anchoring proteins (AKAPs) (Colledge and Scott, 1999). AKAP79 is expressed in sensory neurons and can potentially support interactions between β -adrenergic receptors, PKA, PKC ϵ , and F-actin (Faux et al., 1999; Fraser et al., 2000; Gomez et al., 2002; Rathee et al., 2002). Finally, β -adrenergic receptor-mediated activation of ERK1/2 utilizes several signaling scaffolds set on the cytoskeleton. A previous study in sensory neurons found that epinephrine activation of ERK1/2 requires Gi and Ras activation, but not PKA (Aley et al., 2001). Thus, β -adrenergic receptors might switch from Gs to Gi coupling and then couple to Src and Ras via focal adhesion kinase (FAK) activation or growth factor receptor transactivation using G $\beta\gamma$ subunits. A role for G protein-coupled receptor kinase, β -arrestin, and clathrin-mediated internalization with

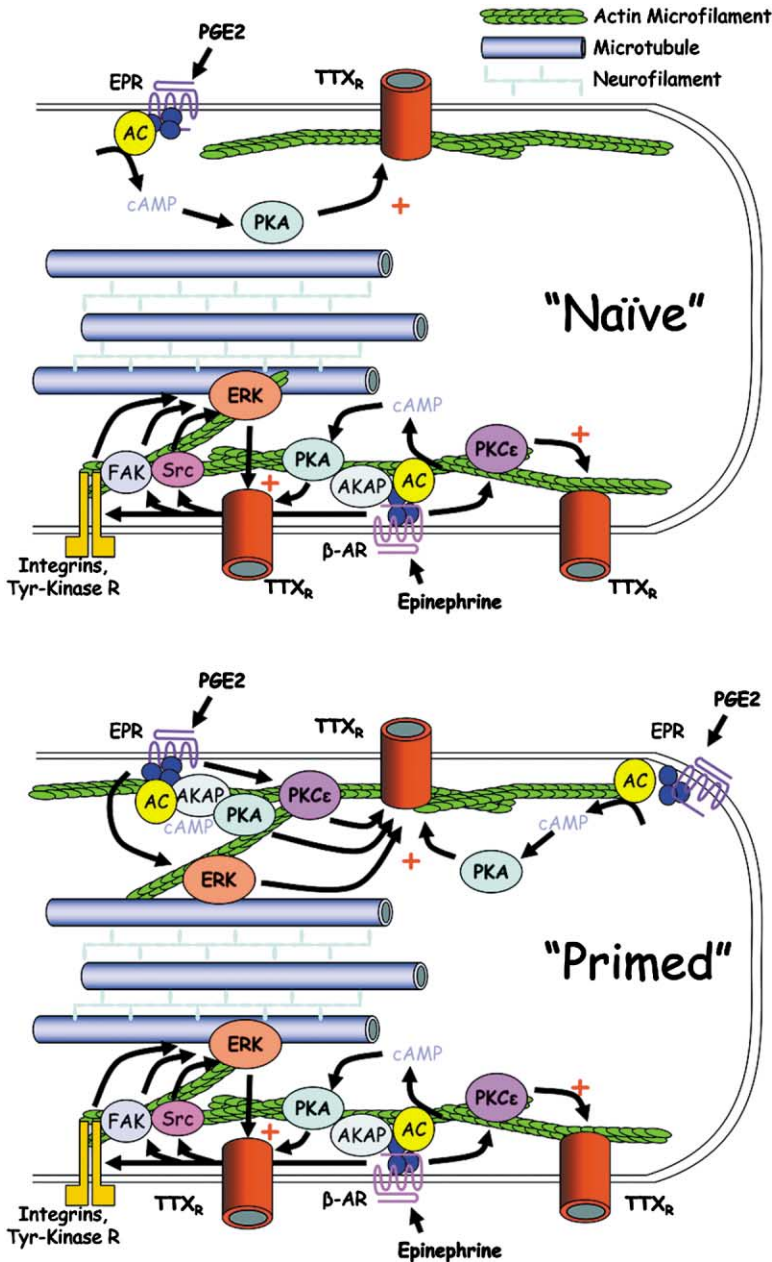


Figure 1. Plasticity in Cytoskeleton-Anchored Signal Transduction Elements within Sensory Neuron Terminals May Mediate Altered Pain Plasticity in Sensory Neurons "Primed" by Injury
An alternate model is proposed in the text.

subsequent coupling to Src and Ras is also possible (Luttrell et al., 1999). All of these mechanisms rely heavily on cytoskeletal integrity and plasticity, especially actin microfilaments (Della Rocca et al., 1999; Merrifield et al., 2002).

As described earlier, coupling of PKC ϵ and ERK1/2 to their effectors must also rely on a cytoskeletal scaffold. Future studies should springboard from the work of Dina and colleagues to examine potential interactions between PKC ϵ and ERK1/2 and their downstream effectors, such as TTX $_R$ (Khasar et al., 1999). For example, coimmunoprecipitation studies between TTX $_R$ subunits and PKA, PKC ϵ , and ERK1/2 in the absence or presence of cytoskeletal disrupting agents could potentially bolster the model depicted in Figure 1.

The most intriguing aspect of this story lies not in the

cytoskeleton-cell signaling interaction, but in its plasticity. Dina et al. use an interesting model of "hyperalgesic priming" to examine how cytoskeletal signal transduction may change after tissue injury. In this model, rat hindpaws are initially injured with intradermal carageenan. Animals recover over a 5-day period and then are reinjected with inflammatory mediators. After such priming, PGE $_2$ -mediated hypersensitivity becomes long lived and converts from solely activating PKA to resembling epinephrine-induced hypersensitivity depending upon PKA, PKC ϵ , and ERK1/2 activation. More importantly, cytoskeletal disruptors now abrogate PGE $_2$ -mediated hypersensitivity. While several studies have demonstrated that cytoskeletal plasticity modulates cellular or subcellular shape and function, these results are relatively novel in demonstrating specific switches in signal-

ing cascades and their cytoskeletal dependence. Such plasticity in cell signaling after tissue injury may represent a key, unique mechanism in converting acute to chronic pain. Activation of sensory neurons during acute bouts of pain following injury may slowly lead to transcriptional and posttranslational changes modifying the composition of the cytoskeleton and its signaling interactions, such as with prostaglandin receptors as shown in the “primed” state in Figure 1. An alternate model that should be considered includes the possibility that hyperalgesic priming induces a change in cytoskeleton-based active transport processes, and that this change leads to the increased transport of components of the signaling pathway involving PKA, PKC ϵ , and ERK1/2 signaling to the compartment where EP receptors mediate their effects. In this model, there would be a requirement for constant active transport of signaling molecules necessary for epinephrine-induced sensitization and for EP receptor-mediated sensitization in the primed state. Future studies will certainly endeavor to determine the basis for this dependence on cytoskeletal integrity. These modifications in cytoskeletal signaling may amplify nociceptive sensory neuron activity leading to chronic pain even in the absence of significant tissue injury or inflammation. Thus, a possible therapeutic modality for chronic pain may attempt to maintain cytoskeletal signaling in a “quiescent” rather than a “primed” state.

While basic science theory rarely finds its way to the bedside immediately, the results of Dina and colleagues may already elaborate on the pathophysiologic basis of a current medical therapy. Gout is a condition resulting from the deposition of urate crystals in joints, leading to a painful, inflammatory arthritis, and is the most common cause of inflammatory arthritis in men over the age of 40. Colchicine, a microtubule inhibitor, is the oldest treatment of acute gout. The traditional view is that colchicine inhibits microtubule-based inflammatory cell chemotaxis, phagocytosis, and generation of leukotrienes (Emmerson, 1996). However, the work of Dina et al. (2003) suggests that colchicine may also work directly at the level of sensory neurons, reducing their response to inflammatory mediators in joints. Interestingly, in a controlled study of colchicine in gout, pain scores fell before clinical scores related to joint inflammation after colchicine treatment (Ahern et al., 1987). Thus, colchicine may produce analgesic effects independent of inflammation. Recent success of colchicine in a clinical trial with osteoarthritis, a predominantly noninflammatory condition, also supports this notion (Das et al., 2002). The hypothesis that colchicine modulates sensory neuron function in arthritic conditions is exciting and should drive future research into the neuronal cytoskeleton as a possible therapeutic target for acute and chronic pain.

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A Form of Presynaptic Coincidence Detection

In this issue of *Neuron*, Sjöström et al. provide evidence for a novel presynaptic mechanism for coincidence detection in induction of timing-dependent LTD. In their scheme, simultaneous activation of presynaptic NMDA receptors and CB1 endocannabinoid receptors induces a long-lasting reduction in presynaptic transmitter release.

In spike timing-dependent synaptic plasticity (STDP), the direction and magnitude of synaptic modification depends critically on the relative timing of the pre- and postsynaptic spikes. This form of Hebbian plasticity was first demonstrated in vitro (Bi and Poo, 2001), and its functional consequences have been explored both theoretically (Abbott and Nelson, 2000) and experimentally (Allen et al., 2003; Fu et al., 2002). A standard description of STDP is an asymmetric window representing synaptic modification as a function of the pre-/postsynaptic interspike interval (Bi and Poo, 2001). While the basic asymmetry is preserved across a wide range of glutamatergic synapses, the width of the window varies considerably (e.g., compare Froemke and Dan, 2002, with Debanne et al., 1998), which may be important for neural computation (Abbott and Nelson, 2000). Thus, knowing what cellular processes determine the temporal window is of great interest not only from a mechanistic point of view but also at the functional level. To determine the