Why Pain Gets Worse: The Mechanism of Heat Hyperalgesia

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Pain differs from other senses in many ways; one of the most striking is that the intensity of pain increases with time in the presence of a painful stimulus, a process that is referred to as sensitization or hyperalgesia. With all the other senses, the perceived intensity decreases with continuous exposure to a constant stimulus. For instance, when you emerge from a cinema onto a sunny street you are at first dazzled, but soon adapt to the new and higher ambient light level. If vision behaved like pain the opposite would happen—the dazzling light of the street would become ever brighter. The reason for the difference is clear enough. Light adaptation allows us to operate over a wide range of ambient light intensities, a necessary property for any species that may at one moment be in bright sunlight and the next in a dark cave (possibly containing a bear). Pain, on the other hand, cannot be ignored as it often signals tissue damage, and it is essential for the integrity of the organism that a painful stimulus should become ever more pressing until the subject takes some action to remove its cause.

Thus hyperalgesia fulfills a valuable survival function, but it also has a major downside. When inflammation is chronic, for instance in an arthritic joint, hyperalgesia can be disabling because the only action the subject can take is to remain immobile, not a strategy with positive implications for survival in an animal, nor for emotional or economic well being in a human. Many of the early investigations in empirical pharmacognosy, otherwise known as folk medicine, were directed toward alleviating the pain resulting from inflammation. These investigations were conducted in a manner that we would now regard as highly unscientific, but they did score some notable successes. Amongst them were the discovery of two potent analgesics, in the shape of salicylic acid from willow bark, and opium from poppies. The descendants of both are, of course, still in widespread use today.

Modern science has taken a more rational strategy toward understanding hyperalgesia. We now recognize that it is not intrinsic to the pain-sensitive nerve terminals themselves, but instead depends on the release of extracellular proinflammatory mediators from stressed or damaged tissue. These mediators are many and diverse in origin, but amongst the best studied are bradykinin, ATP, prostaglandin E2, and (surprisingly) nerve growth factor (NGF). NGF is of course a potent growth factor, which promotes the targeting of outgrowing nerve fibers in the developing organism, but it also seems to play a role in the adult organism, as a proinflammatory mediator amongst its other roles (Lewin et al., 1993).

The paper by Stein et al. (2006), on p. 509 of this issue, reports a highly interesting advance in our understanding of the molecular basis of hyperalgesia. The paper focuses on the mechanism by which the heat-activated ion channel, transient receptor potential vanilloid 1 (TRPV1), is sensitized by NGF. TRPV1 is one mechanism (although not the only one) by which we detect mild levels of painful heat, and its activation at around 43°C is likely to be responsible for the transition in the English language between the word "warm," which has pleasant connotations and is used to describe heat below ~43°C, and "hot," which signifies an unpleasant heat sensation above this threshold temperature. TRPV1 is strongly activated by increases in temperature >43°C, a property that sets it apart from most other ion channels, in which channel opening is only weakly temperature sensitive. The activation of heat-sensitive ion channels in sensory neurons was first characterized in work by Cesare and McNaughton (1996), and the relevant channel (at first named VR1) was subsequently cloned by the group of David Julius (Caterina et al., 1997). An important property of TRPV1 is that the temperature threshold is lowered by proinflammatory mediators such as bradykinin (Cesare and McNaughton, 1996), in a process called heat hyperalgesia. This property is the basis of the heat hyperalgesia caused by inflammation, because this aspect of hyperalgesia is abolished in mice from which TRPV1 has been deleted (Caterina et al., 2000; Davis et al., 2000).

As with any highly evolved function, heat hyperalgesia is a complex process to which several different mechanisms can contribute. Which mechanism is recruited depends on the identity both of the inflammatory mediator and of the intracellular signaling pathways

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Abbreviations used in this paper: NGF, nerve growth factor; PIP_2, phosphatidyl inositol 4,5-bisphosphate; TRPV1, transient receptor potential vanilloid 1.

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activated downstream of its binding to a receptor in the neuronal membrane. In roughly historical order, the proposed mechanisms are as follows:

(1) Phosphorylation by PKCε. Bradykinin, ATP, and many other inflammatory mediators activate PKC, which in turn sensitizes the heat-activated current in neurons, which we now recognize as being carried by TRPV1 (Cesare and McNaughton, 1996). The main isoform involved is PKCε (Cesare et al., 1999), and serines 502 and 800 are the critical sites for the sensitizing effect (Numazaki et al., 2002; Bhave et al., 2003) (NB residue numbers are from the rat TRPV1 sequence).

(2) Removal of Inhibition by Phosphatidyl Inositol 4,5-Bisphosphate (PIP_2). An alternative pathway for the action of bradykinin, NGF, and other mediators that activate phospholipase C, leading to the metabolism of PIP_2, was proposed by Chuang et al. (2001). The idea here is that PIP_2 inhibits TRPV1 by binding directly to the ion channel, and that removal of PIP_2 by PLC would lead to sensitization. This idea was supported by experiments showing that application of either PLC or a PIP_2-sequestering antibody directly to TRPV1 expressed in membrane patches caused potentiation, and that deletion of a proposed PIP_2 binding domain in the C-terminal tail has a similar effect (Prescott and Julius, 2003).

(3) Phosphorylation by PKA. Prostaglandins and other inflammatory mediators activate PKA, which in turn phosphorylates TRPV1. The main effect seems to be via serine 116, and to involve a reduction in desensitization rather than a sensitization per se (Bhave et al., 2002).

(4) Phosphorylation by Src. Work in our group has recently identified a novel process by which NGF sensitizes TRPV1 by rapidly increasing the number of ion channels in the cell membrane (Zhang et al., 2005). The incorporation of new channels depends on activation of PI3 kinase, which in turn activates the nonreceptor tyrosine kinase Src. Phosphorylation by Src of a single critical residue in TRPV1, tyrosine 199, triggers the rapid movement of TRPV1 channels to the surface membrane.

(5) Upregulation of Channel Expression. NGF has the more long-term effect of upregulating de novo expression of TRPV1. The pathway involved is the Ras-MAPK pathway rather than the PI3K-Src pathway outlined above (Ji et al., 2002; Bron et al., 2003).

The role of mechanism 2, the relief of inhibition by PIP_2, is a particular focus of the paper by Stein et al. (2006). Doubt already had been cast on this model by work from Qin's group showing that stimulating the production of PIP_2 enhances rather than inhibits recovery of TRPV1 from desensitization (Liu et al., 2005), and work from our lab, which showed that sensitization of TRPV1 by NGF can be explained by phosphorylation of TRPV1 by the tyrosine kinase Src, a mechanism completely independent of PIP_2 (Zhang et al., 2005). However, one rather obvious experiment that does not appear to have been done before is to apply PIP_2 directly to the cytoplasmic face of an isolated membrane patch. Stein et al. have performed this experiment, and the result is striking. Instead of inhibiting TRPV1, as predicted by the PIP_2 inhibition model, PIP_2 dramatically potentiates the activation of TRPV1 by capsaicin. The effect of PIP_2 itself is not completely reversible, presumably because the highly hydrophobic PIP_2 molecule remains in the membrane patch, but the potentiation produced by a version of PIP_2 with shorter carbon tails is readily reversible. In a second experiment, endogenous PIP_2 was removed by the highly cationic molecule polylysine. Instead of the potentiation expected in the original PIP_2 model, the capsaicin-activated current was inhibited, and the inhibition could be reversed by subsequent addition of PIP_2. The basic tenet of the PIP_2 model therefore does not seem to hold up; PIP_2 does not inhibit TRPV1, but instead potently enhances its activation. A similar enhancement of a number of other members of the TRP family of ion channels by PIP_2 has been described (for review see Hardie, 2006). Thus, in the end, TRPV1 seems to be similar to many other TRP channels, in that its activation is potentiated by PIP_2, rather than being an "outlier" that is inhibited by PIP_2.

Stein et al. next investigated the cellular signaling pathways by which activated TrkA potentiates TRPV1. Several largely independent signaling pathways radiate from activated TrkA. Three well-characterized downstream pathways involve PLCγ, PI3 kinase, and Ras-MAP kinase (Kaplan and Miller, 2000). In agreement with earlier studies (Bonnington and McNaughton, 2003; Zhuang et al., 2004; Zhang et al., 2005), Stein et al. found that the effect of NGF was abolished by wortmannin, an inhibitor of PI3 kinase. They make one new observation, though, which was not present in earlier accounts; they show that the p85β subunit of PI3K interacts directly with TRPV1 via its SH2 domain. Yeast 2-hybrid experiments using the N terminus of TRPV1 as bait identify the p85β subunit of PI3K as a binding partner; coimmunoprecipitation experiments show that TRPV1 and p85β bind to one another; and experiments in which various domains of p85β were isolated and tested for their ability to bind to TRPV1 in coimmunoprecipitation experiments show that only the SH2 domain bound TRPV1 to any significant extent. However, two aspects of the binding are unexpected: first, the binding was not found to be promoted by NGF, as might have been expected if PI3K is targeted to TRPV1 after activation of TrkA; and second, Stein et al. were unable to find any evidence that TRPV1 was phosphorylated on tyrosine residues. This last result is surprising because

the function of SH2 domains is to bind to phosphotyrosine residues, and the fact that the SH2 domain of p85β binds to TRPV1 thus seems to imply that there must be some phosphotyrosine residues present in TRPV1. Zhang et al. (2005) have shown that tyrosines in TRPV1 are phosphorylated and that the phosphorylation is enhanced when TrkA is activated by NGF.

The final major area covered by Stein et al. relates to the mechanism by which NGF causes potentiation of TRPV1. Previous studies have identified a change in the activation properties of TRPV1 after phosphorylation by PKCε: the temperature threshold for activation is lowered, and the dose–response relation for capsaicin shifts to lower capsaicin concentrations (mechanism 1 above; see Vellani et al., 2001). A second effect has been reported more recently: the number of TRPV1 channels in the membrane was found to be enhanced, after exposure to NGF, by a rapid translocation of TRPV1 channels from a cytoplasmic pool (Zhang et al., 2005). Stein et al. confirmed that the maximum current activated by a saturating concentration of capsaicin is increased by NGF. In addition they demonstrated that TRPV1 is rapidly translocated to the membrane by an elegant experiment in which the density of GFP-tagged channels in the membrane was directly visualized using total internal reflection (TIRF) microscopy.

What happens downstream of PI3K is still an open question. Work in our group shows that Src is activated, binds to TRPV1, and phosphorylates residue Y199 (Y200 in the human sequence); this phosphorylation could be a signal for translocation of TRPV1 to the membrane. Stein et al. suggest that the binding of PI3K to TRPV1 itself mediates translocation of TRPV1 to the surface membrane. Clearly there is a need for further experiments.

A satisfying degree of agreement is now beginning to emerge on most aspects of potentiation of TRPV1. Of the mechanisms outlined above, numbers 1 and 3–5 are still looking strong. In the case of mechanism 2, however, the emerging consensus is that the PIP_2 mechanism is not important in heat hyperalgesia, in direct contradiction to previous studies (Chuang et al., 2001; Prescott and Julius, 2003). The Stein et al. article raises the tantalizing possibility that PIP_2 may yet play a role, but as a potentiator of TRPV1 rather than as an inhibitor. The physiological role of this mechanism remains to be elucidated.

REFERENCES

Bhave, G., W. Zhu, H. Wang, D.J. Brasier, G.S. Oxford, and R.W. Gereau. 2002. cAMP-dependent protein kinase regulates desensitization of the capsaicin receptor (VR1) by direct phosphorylation. *Neuron*. 35:721–731.

Bhave, G., H.J. Hu, K.S. Glauner, W. Zhu, H. Wang, D.J. Brasier, G. S. Oxford, and R.W. Gereau. 2003. Protein kinase C phosphorylation sensitizes but does not activate the capsaicin receptor transient receptor potential vanilloid 1 (TRPV1). *Proc. Natl. Acad. Sci. USA*. 100:12480–12485.

Bonnington, J.K., and P.A. McNaughton. 2003. Signalling pathways involved in the sensitisation of mouse nociceptive neurones by nerve growth factor. *J. Physiol.* 551:433–446.

Bron, R., L.J. Klesse, K. Shah, L.F. Parada, and J. Winter. 2003. Activation of Ras is necessary and sufficient for upregulation of vanilloid receptor type 1 in sensory neurons by neurotrophic factors. *Mol. Cell. Neurosci.* 22:118–132.

Caterina, M.J., M.A. Schumacher, M. Tominaga, T.A. Rosen, J.D. Levine, and D. Julius. 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*. 389:816–824.

Caterina, M.J., A. Leffler, A.B. Malmberg, W.J. Martin, J. Trafton, K.R. Petersen-Zeitz, M. Koltzenburg, A.I. Basbaum, and D. Julius. 2000. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science*. 288:306–313.

Cesare, P., and P.A. McNaughton. 1996. A novel heat-activated current in nociceptive neurons, and its sensitization by bradykinin. *Proc. Natl. Acad. Sci. USA*. 93:15435–15439.

Cesare, P., L.V. Dekker, A. Sardini, P.J. Parker, and P.A. McNaughton. 1999. Specific involvement of PKC-epsilon in sensitization of the neuronal response to painful heat. *Neuron*. 23:617–624.

Chuang, H.H., E.D. Prescott, H. Kong, S. Shields, S.E. Jordt, A.I. Basbaum, M.V. Chao, and D. Julius. 2001. Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. *Nature*. 411:957–962.

Davis, J.B., J. Gray, M.J. Gunthorpe, J.P. Hatcher, P.T. Davey, P. Overend, M.H. Harries, J. Latcham, C. Clapham, K. Atkinson, et al. 2000. Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. *Nature*. 405:183–187.

Hardie, R.C. 2006. TRP channels and lipids: from *Drosophila* to mammalian physiology. *J. Physiol.* DOI:10.1113/jphysiol.2006.118372.

Ji, R.R., T.A. Samad, S.X. Jin, R. Schmoll, and C.J. Woolf. 2002. p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. *Neuron*. 36:57–68.

Kaplan, D.R., and F.D. Miller. 2000. Neurotrophin signal transduction in the nervous system. *Curr. Opin. Neurobiol.* 10:381–391.

Lewin, G.R., A.M. Ritter, and L.M. Mendell. 1993. Nerve growth factor-induced hyperalgesia in the neonatal and adult rat. *J. Neurosci.* 13:2136–2148.

Liu, B., C. Zhang, and F. Qin. 2005. Functional recovery from desensitization of vanilloid receptor TRPV1 requires resynthesis of phosphatidylinositol 4,5-bisphosphate. *J. Neurosci.* 25:4835–4843.

Numazaki, M., T. Tominaga, H. Toyooka, and M. Tominaga. 2002. Direct phosphorylation of capsaicin receptor VR1 by protein kinase Cε and identification of two target serine residues. *J. Biol. Chem.* 277:13375–13378.

Prescott, E.D., and D. Julius. 2003. A modular PIP2 binding site as a determinant of capsaicin receptor sensitivity. *Science*. 300:1284–1288.

Stein, A.T., C.A. Ufret-Vincenty, L. Hua, L.F. Santana, and S.E. Gordon. 2006. Phosphoinositide 3-kinase binds to TRPV1 and mediates NGF-stimulated TRPV1 trafficking to the membrane. *J. Gen. Physiol.* 128:509–522.

Vellani, V., S. Mapplebeck, A. Moriondo, J.B. Davis, and P.A. McNaughton. 2001. Protein kinase C activation potentiates gating of the vanilloid receptor VR1 by capsaicin, protons, heat and anandamide. *J. Physiol.* 534:813–825.

Zhang, X., J. Huang, and P.A. McNaughton. 2005. NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. *EMBO J.* 24:4211–4223.

Zhuang, Z.Y., H. Xu, D.E. Clapham, and R.R. Ji. 2004. Phosphatidylinositol 3-kinase activates ERK in primary sensory neurons and mediates inflammatory heat hyperalgesia through TRPV1 sensitization. *J. Neurosci.* 24:8300–8309.