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Tackling Pain at the Source: New Ideas about Nociceptors

Minireview

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Coordinated and elaborate avoidance responses to potentially harmful stimuli are exhibited throughout the animal kingdom. Sherrington (1906) proposed that such "protective reflexes" are mediated by specialized detectors (now called nociceptors), which are "... excited by stimuli of such different modes as mechanical, thermal, conductive, thermal radiant, chemical and electrical... a group of excitants which has in relation to the organism one [common] feature... namely a noxious character." Sherrington recognized that tissue-damaging stimuli are also intimately associated with the sensation of pain, claiming that "[p]ain is the psychical adjunct of protective reflexes." The properties of this peripheral pain-signaling system are of clinical as well as academic interest because the treatment of pain remains such an unmet medical need.

Today, it is recognized that the large majority of small caliber cutaneous sensory fibers (that is, the thinly myelinated A and unmyelinated C fibers) in a variety of species including rat, monkey, and man are nociceptors. These species have small numbers of unmyelinated afferents (<10%) dedicated to signaling innocuous thermal information. Cutaneous nociceptors, as Sherrington posited, respond to mechanical, thermal, and chemical stimuli applied to their peripheral receptors. While essentially all combinations of sensitivity are found, polymodal nociceptors that respond to all of these stimulus types are numerically the most common. Thermal and mechanical responsiveness have been most studied. Skin temperatures in excess of 45°C elicit firing in many heat-sensitive nociceptors and are rated as painful in man. Typically, nociceptors respond to mechanical forces of only 10 mN or so, forces that, interestingly, are not generally rated as painful in humans. Our knowledge of chemical sensitivity is less complete, which is unfortunate given that many pain states in humans are probably causally associated with disturbances of the peripheral chemical milieu. A large number of endogenous molecules, such as bradykinin, serotonin, histamine, substance P, protons, and ATP, among others, that can excite nociceptors have been identified. However, the relative importance of these factors is still mostly unknown. Some chemical nociceptors are normally insensitive to mechanical or thermal stimuli. Such afferents—sometimes called silent afferents or sleeping nociceptors—are likely to be recruited only under pathophysiological conditions.

Thus, we have a fairly comprehensive description of nociceptor responsiveness, but in the broader context of neurobiology, our knowledge of nociceptors is quite limited. For instance, the factors that regulate the development, maintenance, and stability of these systems, as well as the molecules that mediate specific types of responses, are only now being explored. Perhaps unexpectedly, in view of their physiological properties, biochemical and anatomical differences that define two major classes of C fibers have recently been recognized (see Figure 1). The emerging functional properties of these two major classes may prove important in understanding peripheral pain mechanisms and are the focus of this minireview.

Criteria for Delineating Two Major Classes of Nociceptors

Development

Like all classes of peripheral neurons, sensory neurons require connections with their peripheral target tissues, a source of neurotrophins and other factors, for survival during development. Analysis of sensory neurons in gene-targeted mice has revealed a rather complete picture of neurotrophin requirements of sensory neurons. Importantly for nociception, 70%–80% of dorsal root ganglion (DRG) neurons express the nerve growth factor (NGF) receptor tyrosine kinase TrkA during development and require NGF for survival during embryonic life. This includes virtually all small diameter neurons with unmyelinated axons, most neurons expressing the nociceptive peptides calcitonin gene-related peptide (CGRP) and substance P, and most neurons projecting to laminae I and II of the superficial dorsal horn. Thus, during development, the survival of all small sensory neurons (known in the adult animal to be predominantly nociceptors) depends on NGF (Silos-Santiago et al., 1995, and references therein). The importance of this fact lies in the novel recent findings that this same molecule has been found to regulate important aspects of nociceptor function in maturity (discussed below).

As the animal matures, trophic factor sensitivity of sensory neurons changes. During the first 3 postnatal weeks in rat and mouse, about one-half of cells lose TrkA, so that by adulthood this receptor is only found in ~40%–45% of DRG neurons (see Molliver et al., 1997). These cells coexpress the neuropeptides CGRP and substance P. The cells that lose TrkA developmentally have a distinct histochemistry (they express the enzymes FRAP and TMP and bind the lectin IB4). These cells project centrally to the inner aspect of lamina II in the dorsal horn, unlike the TrkA neurons. During late embryonic life and over the first postnatal week, another receptor tyrosine kinase, Ret, is upregulated in the population of small cells that downregulates TrkA. These neurons then become sensitive to glial cell line–derived neurotrophic factor (GDNF) in the postnatal period (Molliver et al., 1997), a characteristic maintained in adulthood (Bennett et al., 1998b). An important question is whether these two groups of C fibers, with distinct trophic requirements and chemistries, have different functional roles. As yet, we have no definitive answer to this

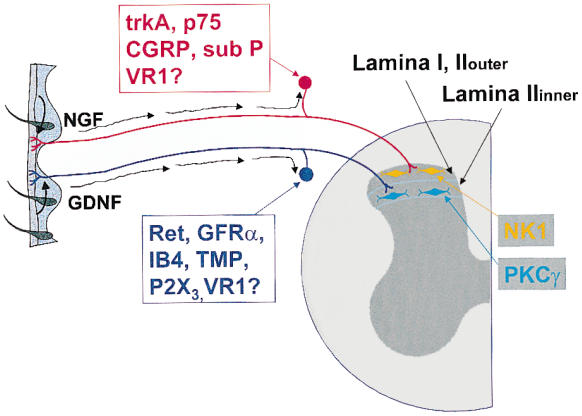

Figure 1. Two Major Nociceptor Classes

The diagram shows peripheral and central target fields, trophic factor dependence, and biochemical properties of the two major classes of cutaneous nociceptors. The CGRP/TrkA population represents roughly 40% of DRG neurons in rodents, whereas the IB4 population represents roughly 30%. Both populations of nociceptors are sensitive to capsaicin; these presumably express either VR1 or another member of the VR1 family. Which is the case cannot be taken as established until appropriate colocalization studies are performed.

question, but as we discuss below, there is at least suggestive new evidence that this may be the case.

Responsiveness

Our detailed physiological knowledge of nociceptors is now being supplemented by discovery of molecules conferring distinct response modalities.

THERMAL SENSITIVITY. The nociceptive stimulus capsaicin is the natural product of capsicum peppers and a prominent component of many hot and spicy foods. Capsaicin has a number of properties (ranging from its ability to destroy selectively virtually all unmyelinated DRG neurons in developing animals to its use as a non-damaging provoker of secondary hyperalgesia in man) that have made it an invaluable tool in the study of nociception. That distinct subsets of sensory neurons bear receptors for capsaicin has been suggested both by binding studies of radiolabeled analogs and because only small developing sensory neurons can be killed by exposure to this molecule.

Reasoning that the powerful pharmacological properties of capsaicin should reveal an important endogenous nociceptive pathway, Julius and colleagues set out to clone a capsaicin receptor (Caterina et al., 1997). By expression cloning, and using Ca^{2+} uptake as an assay, they identified a clone, VR1 (so named because of the presence of a vanilloid moiety), which showed homology to *Drosophila* TRP proteins, known to have a role in calcium homeostasis. Strikingly, cells transfected with VR1 exhibit a nonselective cation channel with high Ca^{2+} permeability that is activated by sudden increases in temperature. Prolonged exposure to capsaicin kills transfected HEK293 cells, analogous to the situation in vivo.

Thus, the capsaicin receptor is a channel that appears to confer responsiveness to heat as well as to chemical stimuli. Indeed, it is possible that the only endogenous "ligand" for this receptor is heat. As predicted, VR1 expression is confined to a subset of small sensory neurons in dorsal root and trigeminal ganglia. Surprisingly, no expression was seen in the nodose ganglion, which contains visceral capsaicin-sensitive neurons, raising the question of whether VR1 homologs exist. Indeed, expressed sequence tag (EST) data bases reveal a number of homologous human sequences.

The discovery of VR1 confirms an important element of the nociceptor concept in revealing selective expression of a key molecule related to signaling of specific types of sensory stimuli. Both IB4-binding and CGRP-expressing neurons respond to capsaicin in the adult. But since there may be receptors other than VR1 for capsaicin, it remains uncertain whether capsaicin receptors are differentially expressed by the NGF- and GDNF-responsive nociceptors.

CHEMICAL SENSITIVITY. The chemical sensitivity of nociceptors is a reflection of the expression of specific receptors, and advances here are occurring rapidly. Of particular interest is a recently cloned ATP receptor, P2X3, which is uniquely expressed in sensory neurons (Chen et al., 1995). Unlike other P2 receptors, P2X3 is highly expressed in the IB4-binding, GDNF-sensitive group of nociceptors but in only a small minority of the peptide-containing, NGF-sensitive neurons (Vulchanova et al., 1996, 1997, Soc. Neurosci., abstract). Hence, the IB4 group of nociceptors might exhibit a distinct responsiveness to ATP.

Nociceptors studied in vitro can be activated by ATP, and ATP applied to a blister base produces pain in man and nocifensive behavior in animals. Recently, Cook et al. (1997) showed that tooth-pulp afferents (believed to be almost exclusively nociceptive) respond to exogenous ATP with a pharmacology consistent with involvement of P2X3, unlike ATP-induced responses in large mechanosensitive trigeminal afferents. Another potential role of P2X3 is suggested by Gu and McDermott (1997), who examined ATP effects on P2X3 receptors expressed on the central rather than the peripheral terminals of afferent nociceptors. An electrophysiological analysis of a DRG–dorsal horn coculture system provided evidence that presynaptic P2X3 receptors might regulate glutamate release from nociceptors and thereby affect the central transmission of pain-related signals. Several potential sources of ATP, both peripheral and central, have been identified.

Although the evidence that ATP acts as an endogenous mediator of pain is still circumstantial, data on P2X3 that we do have further supports the idea of a dichotomy of nociceptor function.

MECHANICAL SENSITIVITY. Our understanding of the molecular mechanisms of mechanotransduction is still rudimentary. In *C. elegans*, a number of candidate genes have been identified. Some are members of the so-called mec family, which can show structural homology with sodium channels, putatively linking mechanical stimuli with electrical responses. However, very little is known of equivalent systems in mammals. Recently, it has been suggested that ATP receptors might function as important mechanotransducing receptors (Nakamura and Strittmatter, 1996), with perhaps different receptor types mediating responses in nociceptors (P2X3) and innocuous mechanoreceptors (P2Y1). The hypothesis envisages that mechanical stimuli release ATP, which

then acts on sensory neuron P2 receptors. While intriguing, current evidence is circumstantial.

Connectivity

A key distinguishing characteristic between the two major nociceptor classes is their central termination patterns. Thus, CGRP/TrkA–expressing DRG neurons project most heavily to lamina I and outer lamina II (IIo), whereas IB4-binding and Ret-expressing DRG neurons project most heavily to the inner aspect of lamina II (IIi). However, the functional significance, if any, of these different projection patterns is not clear. A major difficulty has been lack of information with regard to functions subserved by specific regions of the superficial dorsal horn.

Recently, two studies have either ablated specific classes of cells or eliminated molecules specific to different laminae, providing clues regarding the functional roles of these laminae. In one, a potent ribosomal toxin (saporin) coupled to substance P was infused into the spinal cord and specifically bound and destroyed cells expressing the substance P receptor (NK1) in lamina I (Mantyh et al., 1997). Importantly, such treatment had no effects on baseline behavioral responsiveness to nociceptive thermal and mechanical stimuli. However, hypersensitivity normally induced by capsaicin was almost completely abolished. These results support the emerging notion that substance P/TrkA–expressing sensory neurons projecting to lamina I are critical to hyperalgesic responses induced by inflammation.

The second addresses the potential importance of lamina IIi (Malmberg et al., 1997). Here, it was shown that the γ isoform of protein kinase C is specifically expressed in lamina IIi in the target field of Ret-expressing neurons but not in primary sensory neurons themselves. Elimination of PKC in these neurons by gene targeting had remarkable effects. Again, behavioral responses to acute thermo- and mechano-nociceptive stimuli were apparently normal. However, hyperalgesic responses induced by nerve injury were almost completely abolished.

These new findings suggest that both lamina I and its peptidergic nociceptive projection and lamina IIi and its nonpeptidergic projection may be critical to the establishment of chronic pain states. An intriguing possibility is that the former is particularly important in inflammatory conditions and the latter in neuropathic states.

Plasticity

Recently, it has emerged that nociceptors are also subject to functionally relevant regulation by neurotrophic factors. Following the important studies of Lewin and Mendell, a body of evidence has emerged showing that neurotrophic factors affect nociceptor function from peripheral to central terminals, and the evidence at hand suggests that the two major nociceptor classes may be regulated by different molecules.

Levels of NGF in the vicinity of sensory axon terminals are known to influence nociceptor function both acutely and chronically. Indeed the "gain" of the peripheral nociceptive system appears to be under tonic regulation by NGF via control of terminal arborizations and expression of receptor elements. Peripheral NGF deprivation is associated with depleted nociceptive terminals in the epidermis and reduced nociceptor sensitivity (Bennett et al., 1998a). Importantly, NGF levels are known to be increased in peripheral tissues in a variety of inflammatory conditions in both animals and humans. Experimentally increasing NGF levels results in a rapid thermal hyperalgesia. Some of this initial hyperalgesia arises because NGF releases algogens from other peripheral TrkA-expressing cells, in particular mast cells and sympathetic postganglionic neurons. That NGF upregulation contributes to inflammatory hyperalgesia has been determined independently by the laboratories of Woolf, Mendell, and McMahon, who have shown that in several animal models "antagonizing" NGF largely prevents the sensory abnormalities that normally develop (see references in Bennett et al., 1998a).

NGF exerts long-term effects on nociceptors via regulation of a variety of receptor proteins, including capsaicin, GABA, and bradykinin receptors, which of course influence nociceptor responsiveness. Furthermore, neurotransmitters/neuromodulators such as substance P and CGRP are also strongly regulated by NGF. More recently, it has become clear that NGF-sensitive nociceptors constituitively express low levels of another neurotrophin, brain-derived neurotrophic factor (BDNF), and that NGF can upregulate BDNF (see Michael et al., 1997). Many of these NGF-induced changes in primary sensory neurons can be expected to influence excitability in the dorsal horn. Indeed, NGF treatment increases activation-induced release of substance P and induces "central sensitization" that has been associated with persistent pain states (Malcangio et al., 1997).

Together, these findings demonstrate the importance of NGF in promoting a number of plastic changes believed important in the generation of chronic pain states. Given that almost one-half of the nociceptor population has receptor components for GDNF but not NGF, a natural question is whether GDNF and related factors exert as varied and important regulatory controls as NGF. For the most part, the experiments have not yet been undertaken, although evidence suggesting an important maintenance role for GDNF has recently been reported.

Sensory neurons show a wide range of changes in response to axonal injury. Provision of NGF can reverse axotomy-induced downregulation of the sensory neuropeptides substance P and CGRP. We now have direct evidence that GDNF can also reverse axotomy-induced changes in appropriate C fibers, such as decreased binding of the lectin IB4, decreased expression of the peptide somatostatin and the enzyme TMP, and decreased conduction velocity (Bennett et al., 1998b). An injury response that may be related to the emergence of neuropathic pain is anatomical rearrangement of the central terminals of large myelinated fibers so that they form synapses in a part of the dorsal horn that normally receives nociceptive input (Woolf et al., 1995). This change may allow inputs from innocuous mechanoreceptors to gain access to nociceptive spinal systems and may contribute to the phenomenon of touch-evoked pain that occurs in neuropathic states. There is reason to believe this anatomical remodeling is secondary to atrophic changes in central C fiber terminals (Woolf et al., 1995; Bennett et al., 1998b). Importantly, provision of trophic support to C fibers, with either NGF or GDNF, is sufficient to prevent dorsal horn remodeling after axonal injury (Bennett et al., 1998b).

Together, these observations show that NGF is a critical mediator of altered nociceptive properties in inflammatory conditions and that both NGF and GDNF are important in maintaining normal nociceptor properties. Given that nociceptive sensory neurons express receptors for a large number of neurotrophic molecules (see Molliver et al., 1997, and references therein), it can be expected that insights obtained to date represent only a preliminary glimpse at neurotrophic control of nociceptor function.

Conclusions and Therapeutic Implications

In this minireview, we have discussed new data highlighting the distinct properties of subsets of nociceptors that in turn raise questions regarding their functional roles. The concept of two major nociceptor classes—distinguished by their responses to chemical mediators and growth factors in the periphery, and by their central connectivity and pharmacology—may prove important for the understanding (and possibly the treatment) of chronic pain.

The most clear-cut implication of the distinction of nociceptor classes is in the setting of inflammation. Here, increases in NGF synthesis in the periphery, the associated upregulation of putative neuromodulators such as substance P and BDNF in TrkA-expressing nociceptors, and the consequent induction of central sensitization seem critical events underpinning abnormal pain sensitivity. The profound regulatory effects of NGF on nociceptor function and the powerful analgesic effects of anti-NGF on inflammatory hyperalgesia strongly suggest that interference with this ligand–receptor interaction provides a novel therapeutic opportunity. Given the current overwhelming clinical reliance on just two classes of analgesic drugs (nonsteroidal anti-inflammatory drugs [NSAIDS] and opiates), the distinct target offered by NGF represents a genuine conceptual advance.

The normal functions of the IB4-binding, GDNF-responsive class of nociceptors are, at the moment, mysterious. Some of their properties (lack of responsiveness to NGF in the periphery and less direct access to the NK1 pathway in the dorsal horn) suggest a very different role for these nociceptors. Other properties (potential responsiveness to ATP and the expression of the neuromodulator somatostatin in a subset of these cells) as yet do not imply clear functional roles. We are also still relatively ignorant of the conditions and factors regulating GDNF expression. However, a fascinating issue for the future relates to the importance of the IB4-binding neurons in chronic pain, perhaps particularly in neuropathic pain states. Clearly, GDNF or related molecules may also offer an important target in the development of novel analgesic therapies.

Selected Readings

Bennett, D.L.H., Koltzenburg, M., Priestley, J.V., Shelton, D.L., and McMahon, S.B. (1998a). Eur. J. Neurosci., in press.

Bennett, D.L.H., Michael, G.J., Ramachandran, N., Munson, J.B., Averill, S., Yan, Q., McMahon, S.B., and Priestley, J.V. (1998b). J. Neurosci., in press.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., and Julius, D. (1997). Nature *389*, 816–824.

Chen, C.-C., Akoplan, A.N., Sivllotti, L., Colquhoun, D., Burnstock, G., and Wood, J.N. (1995). Nature *377*, 428–431.

Cook, S.P., Vulchanova, L., Hargreaves, K.M., Elde, R., and McCleskey, W. (1997). Nature *387*, 505–508.

Gu, J.G., and MacDermott, A.B. (1997). Nature *389*, 749–753.

Malcangio, M., Garrett, N.E., Cruwys, S., and Tomlison, D.R. (1997). J. Neurosci. *17*, 8459–8467.

Malmberg, A.B., Chen, C., Susumu, T., and Basbaum, A.I. (1997). Science *278*, 279–283.

Mantyh, P.W., Rogers, S.D., Honore, P., Allen, B.J., Ghilardi, J.R., Li, J., Daughters, R.S., Lappi, D.A., Wiley, R.G., and Simone, D.A. (1997). Science *278*, 275–279.

Michael, G.J., Averill, S., Nitkuan, A., Rattray, M., Bennett, D.L.H., Yan, Q., and Priestley, J.V. (1997). J. Neurosci. *17*, 8476–8490.

Molliver, D.C., Wright, D.E., Leitner, M.L., Parsadanian, A. Sh., Doster, K., Wen, D., Yan, Q., and Snider, W.D. (1997). Neuron *19*, 849–861.

Nakamura, F., and Strittmatter, S.M. (1996). Proc. Natl. Acad. Sci. USA *93*, 10465–10470.

Sherrington, C.S. (1906). The Integrative Action of the Nervous System (New York: Scribner).

Silos-Santiago, I., Molliver, D.C., Ozaki, S., Smeyne, R.J., Fagan, A.M., Barbacid, M., and Snider, W.D. (1995). J. Neurosci. *15*, 5929–5942.

Woolf, C.J., Shortland, P., Reynolds, M., Ridings, J., Doubell, T., and Coggeshall, R.E. (1995). J. Comp. Neurol. *360*, 121–134.