Nociceptors for the 21st Century

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This review summarizes recent developments in the context of the neurochemical classification of nociceptors and explores the relationships between functionally and neurochemically defined subgroups. Although the complete picture is not yet available, several lines of intriguing evidence suggest that despite the complexity and diversity of nociceptor properties, a relatively "simple" neurochemical classification fits well with several recently identified molecular characteristics.

Advances in the cellular and molecular biology of nociceptors have profoundly revolutionized the field of pain research in the past decade, particularly with respect to the efforts to define biochemical markers and biophysical mechanisms of the "nociceptive phenotype(s)." At the start of the 1990s, the concept of two neurochemically different classes of small afferents became established, followed by recognition of the molecular diversity of neurotrophic factors and their receptors. Subgroups of sensory neurons were shown to depend on differential trophic support, and some trophic interactions were shown to be maintained in adulthood and to be involved in the modulation of nociceptor function. Workers in other fields established the molecular identity and properties of glutamate and neuropeptide receptors, many of which are involved in nociceptive signaling both in the spinal cord and periphery; the subtleties of their molecular organization are important for a better understanding of neurotransmission from nociceptors centrally, but are beyond the scope of this review.

By the middle of the decade an unexpected and bewildering array of ion channel subunits were identified, and nociceptors were shown to express several unique channels. Channels such as tetrodotoxin-resistant voltage-gated sodium channels and certain purinergic ligand-gated channels that are specifically expressed by nociceptors will surely provide unique opportunities for pharmacologic manipulation. Finally, the decade ended with the cloning of hypothetical transducers of noxious stimuli, including the vanilloid receptors and acid-sensing cation channels.

Neurochemical Classification of Nociceptor Subtypes

Based on the conduction velocity of their axons, nociceptors are commonly classified into Aδ- or C-nociceptor categories, with each group having subtypes that differ in their response characteristics. A dual classification also emerges when nociceptor cells are considered in terms of their neurochemical profiles. One major subpopulation of small dorsal root ganglion (DRG) neurons (presumed nociceptors) expresses neuropeptides such as calcitonin gene-related peptide (CGRP) or substance P (SP), whereas the other major group does not contain these neuropeptides.

Development of the concept of neurochemical subgroups of nociceptive afferents

Thomas Hokfelt's [1,2] group pioneered the characterization of neuropeptide expression in the DRG, with emphasis on neuropeptides such as CGRP, SP, and somatostatin. Their work demonstrated the existence of overlapping and nonoverlapping patterns of neuropeptide expression in small sensory neurons, presumably representing different classes of nociceptors. It is now well-known that neuropeptide expression in sensory neurons can be altered in situations of clinical relevance. After peripheral nerve injury, CGRP, SP, and somatostatin are down-regulated within a few days and a different set of neuropeptides is upregulated [3]. Neuropeptides expressed in intact DRGs were referred to as type 1 to underscore a distinction with those upregulated after injury (referred to as type 2). Generally, it is thought that type 1 neuropeptides have some role in nociceptive signaling whereas type 2 neuropeptides are presumed to be involved in regenerative processes; "intact" DRG neurons that express type 1 neuropeptides are often loosely referred to in the literature as "peptidergic" neurons. CGRP is the most "generalized" marker for this sensory neuron population because of its widespread expression in peptide-containing afferents [2].

The laboratory of Stephen Hunt intensively analyzed the nature of peptide-containing and other "fine" afferents. The peptide-containing group was identified by its expression of SP, which labels fewer "peptidergic" neurons than antibodies against CGRP. Importantly, it was found that the "nonpeptidergic" afferents could be labeled using an assay for a specific enzymatic activity, namely fluoride-resistant acid phosphatase (FRAP), which had been observed many years earlier in DRG neurons. In the spinal cord, SP-containing afferents were shown to project mainly to lamina I and the dorsal or outer half of lamina II (LIIo).

In contrast, FRAP-reactive afferents occupied the ventral half or inner lamina II (LIIi). Unfortunately, FRAP histochemistry is quite capricious, resulting in conflicting reports on the relative numbers, distribution, and central projections of these population of afferents. Despite these problems, the study of Hunt and Rossi [4] led to the proposal of parallel pathways established by peptide-containing and nonpeptide-containing nociceptors. However, this seminal idea remained in relative obscurity until recently.

Meanwhile, early studies by Dodd and Jessell [5] on the surface interactions that guide axonal elongation and targeting during development focused on the molecules responsible for specifying the spinal cord laminar projection patterns characteristic of different functional subgroups of sensory afferents, and by the mid 1980s a number of surface markers (mostly carbohydrate moieties of various glycolipids) were identified. Some, but not all, of these markers labeled the cell bodies and central terminals of small DRG neurons that contained neuropeptides.

Following this line of investigation, Alvarez *et al.* [6] found that one monoclonal antibody (LA4), directed against an α-galactose and α-fucose extended glycolipid, labeled a large subpopulation of small primary afferents that projects to LIIi. Quantitative analysis proved that the CGRP and LA4 positive populations represented roughly equivalent numbers of small afferents and together accounted for over 85% of all small sensory neurons. Moreover, the combined central projections of these populations occupied the full dorsoventral extension of lamina II, with slight overlap in mid-lamina II [6].

A number of lectins (oligosaccharide-binding plant proteins) also selectively label subpopulations of primary afferent neurons. One lectin, Griffonia (or Bandeiraea) simplicifolia isolectin B_4 (IB_4), labels a subpopulation of DRG neurons that largely overlap with the FRAP subpopulation, but little with peptide-containing primary afferents, and projects to LIIi [7]. The IB_4 lectin has a similar specificity as LA4 for galactose-extended oligosaccharides, but a side fucose is not necessary for its binding. Hence, the binding properties of IB_4 are more robust and perhaps also more generalized. Lectin-binding efficiency can be increased with divalent cations and this results in the labeling of neuropeptide-containing afferents as well [8]. This might explain why a larger overlap with CGRP and SP-containing DRG neurons is usually obtained with IB_4 lectin (around 20%) than with LA4 (usually 10% or less). However, the availability and ease of use of lectin labeling made this the marker of choice in most subsequent studies and we refer to this population as IB_4-binding for most of this review.

Early emphasis was (and still is in many medical textbooks) on the "peptidergic" sensory neuron as "the" nociceptor. However, large numbers of small DRG neurons do not express neuropeptides. It is also noteworthy that recent surveys of the peripheral innervation of cutaneous territories using very sensitive markers suggest that nonpeptidergic small afferent terminations outnumber those containing neuropeptides [9].

Taken together, these studies suggested that it would be appropriate to consider a relatively simple division of small "nociceptive" DRG neurons into two groups: one group that expresses a variety of neuropeptides and is usually labeled with CGRP-immunoreactivity and a second group that does not normally express neuropeptides but displays FRAP/LA4/IB_4 reactivities. The CGRP-containing DRG population is quite heterogeneous and includes neurons that give rise to unmyelinated and myelinated fibers, whereas the IB_4 population is rather homogeneous and is predominantly comprised of neurons with unmyelinated axons.

Caveats

There are, however, significant limitations to a simple duality of small-sized "nociceptive" afferents. For example, almost all visceral sensory neurons, which represent a sizable proportion of DRG neurons, contain CGRP and SP [10], and it is not clear that all of them subserve a typical "nociceptive" function. In addition, little is known about the extent to which the complement of "small" DRG cells includes cutaneous low threshold non-nociceptive afferents like thermoreceptors and C-mechanoreceptors. Moreover, some DRG neurons do not fit cleanly into the two neurochemical subgroups. One subpopulation of "peptidergic" afferents characterized by the expression of CGRP and somatostatin also exhibits immunoreactivity for α-galactose-specific monoclonal antibodies, strong IB_4 binding, and projects to the region of overlap between CGRP and FRAP/ IB_4/LA4 afferents in mid-lamina II [11]. Finally, some small DRG neurons do not contain neuropeptides or FRAP/IB_4/LA4 reactivity, but prominently express vanilloid or vanilloid-like receptors, suggesting a nociceptive function.

Trophic Support

A major breakthrough was the discovery of high-affinity nerve growth factor (NGF) binding or TrkA-immunoreactivity (TrkA is a high-affinity receptor for NGF) in adult DRG neurons that show "strong" expression of CGRP and/or SP [11–15] (interestingly, no TrkA expression is seen in CGRP/SP neurons that also coexpress somatostatin). Furthermore, very few IB_4 or LA4-binding afferents contained TrkA-immunoreactivity. NGF/TrkA is directly responsible for the normal development of neuropeptide expression, and for maintaining CGRP and SP expression in TrkA-expressing neurons in adult DRG [16], but exerts no trophic actions over the IB_4-binding afferents present in the adult. NGF is released in normal tissue by smooth muscle cells, basal keratynocytes, and fibroblasts among other cell types. During inflammation, NGF levels rise in the periphery [17], resulting in increased uptake and retrograde transport of NGF to the DRG where it can

upregulate CGRP and preprotachykinin gene expression (the precursor of SP and other related neuropeptides). The enhanced peripheral release of SP and CGRP, key molecules in the development of neurogenic inflammation, then promotes hyperalgesia. In addition to this slow "trophic" action, NGF released during inflammation also rapidly sensitizes nociceptors and causes hyperalgesia, either directly on TrkA-expressing nociceptor peripheral terminals or indirectly by inducing the release of other nociceptor-sensitizing agents by neighboring TrkA-expressing mast cells [18]. Hence, NGF exerts both "trophic" and "acute" influences on nociceptor activity after injury.

During development, however, NGF promotes the survival of both peptide-containing and IB_4-binding afferents [19]. In fact, all embryonic small afferents express TrkA, but IB_4-binding neurons down-regulate TrkA shortly after birth [20,21] and switch to glial cell line–derived neurotrophic factor (GDNF) dependence [22]. These cells then express members of the GDNF receptor complex, including the signal transducing domain, RET ("Rearranged in Transformation," a tyrosine kinase transmembrane receptor originally described as an oncogene), and one or more GDNF-family-receptor (GFR) ligand-binding domains [23•,24]. Little expression of any of these GDNF receptor components is detected in TrkA-positive DRG neurons. Similar to the role of NGF in maintaining CGRP/SP expression, GDNF is directly responsible for maintaining the IB_4-binding phenotype and somatostatin expression in adult DRGs. Thus, the two neurochemical populations of small afferents differ in their neurotrophin dependence in the adult but not during development.

Interestingly, in mice, elimination of the proapoptotic BCL-2 homologue BAX "rescues" small DRG neurons even in the absence of NGF or TrkA [25•]. However, in double null mutants (for BAX and NGF, or BAX and TrkA), although the cells survive, the lack of NGF/TrkA signaling prevents sensory axons from reaching peripheral targets. However, their central projections develop normally. Thus, during development, NGF promotes peripheral targeting and phenotype acquisition of both peptidergic and IB_4-reactive nociceptors. Interestingly, down-regulation of TrkA in IB_4-binding afferents occurs during a postnatal period (first 2 weeks in the rat) in which peripheral trophic factors fine tune the response characteristics of sensory afferents by directing the development of their peripheral transduction apparatus [26].

Response Properties

A fundamental question is whether the two major neurochemical subgroups of small afferents differ in their response characteristics to nociceptive stimuli. Functionally, nociceptors are identified as those sensory afferents that respond to potentially damaging stimuli (*ie*, they have high thresholds for mechanical or heat stimuli), or to the presence of tissue injury by responding to a variety of inflammatory mediators and chemicals. Nociceptors are divided into two groups according to conduction velocity (Aδ or C) and into further subgroups by virtue of their responses to different noxious stimuli, thresholds, sensitization, and adaptation, all of which can be further influenced by tissue location [27,28].

Most C-nociceptors are polymodal nociceptors (CPMs), meaning that they display relatively fast responses to noxious mechanical, thermal, and chemical stimuli. Because chemical mediators are frequently not tested, these units are also referred to as C mechano-heat nociceptors. The population of CPMs is not uniform—they display varying thresholds and responses to different modalities of noxious stimuli, and the structure of their receptor fields can also be different. In addition, a well-defined population of C-nociceptors that readily responds to noxious mechanical stimuli but is relatively unresponsive to noxious heat, has been frequently reported. In contrast, C-nociceptor units responsive to noxious heat but not to noxious mechanical forces are uncommon. Classifications according to chemical sensitivity are more complex because chemical actions are affected by interactions with the tissue environment surrounding the sensory terminals and can directly or indirectly alter the transduction mechanism. In addition, chemicals could affect ion channels related to membrane and firing properties independent of the transduction process. They can also induce the release of agents, like neuropeptides, from the nociceptors themselves. The reader is referred to recent reviews on this aspect of the nociceptor response [29,30].

In contrast to C-nociceptors, it is somewhat more straightforward to subdivide Aδ nociceptors into two categories that can be clearly differentiated according to their response to noxious heat. Type I Aδ nociceptors have relatively high thresholds to heat stimuli (> 53°C) but eventually sensitize to maintained noxious heat. Because they more readily respond to noxious mechanical stimuli they were originally named Aδ high threshold mechanoreceptors (AδHTMs). In contrast, type II units have lower thresholds (approximately 45°C) and more rapid responses to noxious heat, and are known as Aδ mechano-heat nociceptors (AδMHs). Finally, some C or Aδ nociceptors exhibit extraordinarily high thresholds to mechanical stimuli and are not responsive in uninjured tissue. However, they strongly sensitize during inflammation. These afferents have been referred to as "silent" or "sleeping" nociceptors and have been found and characterized in the skin of rodents, monkeys, and humans [31–33].

The correlation of this large diversity of nociceptor response characteristics with their neurochemical phenotype is a daunting enterprise. The direct approach, *ie*, to record the response properties from individual DRG neurons or afferent fibers in vivo and thereafter neurochemically characterize the recorded sensory afferent, is a simple concept but it is technically challenging. Consequently, sample size in these studies is usually low (with

one notable exception published by Lawson *et al.* [34]) and is also subject to well-known biases on sampling and classification due to search stimulus, fiber or cell body size, and/or recording stability. Nevertheless, studies in cat and monkey spinal cords have revealed the ultrastructure and immunoreactivity of the central terminations of a relatively large population of AδHTMs [35,36], one C-nociceptor that readily responded to noxious mechanical stimulation, and one C-fiber with no identifiable superficial receptive field using mechanical search stimuli [37]. Remarkably, all the central terminals from the C-nociceptor were filled with large dense-core vesicles that contained immunoreactivity for CGRP and SP, whereas none of the AδHTM's central terminals exhibited large dense-core vesicles or immunoreactivity for either of the two neuropeptides.

More recently, an extensive sample of sensory neurons were recorded from guinea pig DRG and combined with successful immunostaining against SP and CGRP [34,38]. The conclusions from this study also suggest that AδHTMs with superficial cutaneous receptive fields lack SP or CGRP-immunoreactivity. In contrast, all AδMH afferents from hairy skin contained neuropeptides, as did most Aδ nociceptors with "deep" cutaneous fields. Half of the CPMs with receptive fields in the surface of the skin contained SP and/or CGRP, whereas all CPMs with receptive tissues in deep skin displayed strong neuropeptide-immunoreactivity. It is important to note that whereas the SP-immunoreactive afferents were all nociceptors, a few of the CGRP-immunoreactive neurons displayed low threshold mechanoreceptor receptor properties, had receptive fields located in hair follicles, and conducted in the Aα/β range. This latter result confirmed an earlier more limited report that used a similar recording and labeling strategy in rat DRGs and found CGRP-immunoreactivity in 3 of 5 deep high threshold mechanoreceptors (HTMs), 1 of 1 skin HTMs, 1 of 12 hair follicle afferents, and none in other 11 skin low-threshold mechanoreceptors [39], again indicating that CGRP is expressed by a larger number of functionally distinct DRG neurons than SP, including some afferents that are not nociceptors.

Although very little attention has been devoted to the direct study of the nonpeptidergic population of afferents [39], some conclusions can be extrapolated from the previously mentioned studies. For example, AδHTMs and large numbers of C-nociceptors with superficial, probably epidermal, receptive fields are "nonpeptidergic" (however, some may not be IB_4-reactive either). This is in agreement with recent surveys of skin innervation that indicate a larger proportion of nonpeptide than peptide-containing fine afferents penetrate the epidermis [13]. Peptide-containing afferents are more common in the deeper dermis, particularly around blood vessels [13], whereas IB_4-binding afferents seem not to target vascular tissue extensively.

In a recent study of DRG neurons in culture, it was found that the amplitude of heat-evoked currents differed between IB_4-positive and IB_4-negative cells [40•]. In general, IB_4-positive neurons had smaller heat-evoked currents, supporting previous data in vivo indicating that some nociceptors with poor responses to acute noxious thermal stimuli belong to the nonpeptidergic population. Obviously, much more work and different approaches are needed to understand the response characteristics of different neurochemical types of primary afferents. The recent cloning of some of the molecular transducers that could mediate the response to noxious stimuli opened the possibility of directly localizing these receptor molecules to subpopulations of sensory afferents. Interestingly, the initial observations have uncovered even more complexity.

Transducers

Heat transduction, vanilloid receptors, and acid sensing

It has been known for some time that the responses to capsaicin (the active vanilloid compound in hot chili peppers) and noxious heat in primary afferents were correlated [41]. Moreover, injection of capsaicin to a patch of skin evokes thermal hyperalgesia [42]. The discovery of nonselective cation currents in DRG cells gated by capsaicin [43,44] and by noxious heat (threshold approximately 45°C) [41,45] suggested that a capsaicin receptor might be responsible for the transduction of noxious heat stimuli. This hypothesis was firmly established after the cloning of a vanilloid receptor (VR1) and the demonstration that VR1 confers capsaicin and noxious heat sensitivity to transfected cells by generating cation currents very similar to those found in DRG neurons [46, 47•].

Small DRG neurons giving rise to unmyelinated fibers express VR1, as do a few DRG neurons with myelinated fibers [47•,48]. VR1-expressing neurons comprise around 40% of the DRG population. Interestingly, 65% of CGRP-immunoreactive and 75% of IB_4-binding sensory neurons express VR1 [48], but detailed quantitative analysis of mRNA hybridization signals in situ suggest that IB_4-binding sensory neurons express VR1 at lower levels than peptide-expressing neurons [48]. This conclusion is in agreement with the observation that smaller heat-evoked currents are found in IB_4-binding sensory afferents, but it also points out that a sizable number of neuropeptide-containing afferents do not express VR1. In addition, VR1 is strongly expressed in a further small population of neurons (1% of the total DRG population), which does not express neuropeptides or IB_4-binding.

The vanilloid receptor (VR1) is one of several noxious heat transduction mechanisms found in DRG neurons, and it is known that only partial block of noxious heat-evoked responses is obtained in animals with VR1 gene deletions [49,50]. Other candidates include a mechanism mediated by a vanilloid receptor-like (VRL1) protein that shows a higher threshold for thermal stimuli (above 53°C) [51•], does not respond to capsaicin, and displays a different pharmacology than VR1.

Activation threshold differences between VR1 and VRL1 fit well with differences in the sensitivity to noxious

heat in cultured DRG neurons. Whereas smaller cells have thresholds of around 45°C (close to the thermal threshold of many C-polymodal nociceptors and type II AδHTMs) and are sensitive to capsaicin, larger cells have thresholds of around 51°C (close to the threshold for type I AδHTMs) and appear quite insensitive to capsaicin [52]. The pattern of expression of VRL1 suggests that its distribution is more restricted than that of VR1, comprising around 16.4% of DRG neurons, most of which are medium to large in size and express myelinated-fiber markers [51•]. Very few VRL1-expressing neurons exhibit IB_4-binding (1.7%) or SP-immunoreactivity (5%), and around a third of VRL1-positive neurons (36%) contained CGRP-immunoreactivity. Therefore, many VRL1-positive sensory afferents belong to neither the IB_4 or "peptidergic" populations, and may include type I AδHTMs.

Further transduction mechanisms are suggested by the description of a thermally-dependent internal release of Ca^{2+} that results in additional heat-evoked currents in DRG neurons [53]. Little is known about the expression patterns and significance of this intracellular transduction mechanism for heat stimuli.

Injured tissue undergoing inflammation is characterized by low pH, and protons (H+) have been shown to elicit both acute responses and sensitization of nociceptor terminals [54] and to generate cation currents in isolated DRG cells [55]. VR1 responds to solutions of low pH in the physiologic range of inflamed tissue [47•]. Low pH is also capable of lowering the threshold of VR1 for inducing heat-evoked currents, providing a mechanism for the known sensitizing action of low pH on both capsaicin and noxious heat responses. Hence, it has been proposed that VR1 might be a "polymodal" transducer because of its capacity to respond to various forms of injury via its acid sensitivity [47•]. In contrast to VR1, VRL1 exhibits no response to acid stimulation.

In addition, other families of cation channels gated by protons have been identified in DRG neurons. These channels are related to the degenerin/epithelial sodium channel family (DEG/ENaC) and include the acid sensing ionic channel (ASIC) isoforms α and β, dorsal root acid sensing ionic channel, and the modulatory subunit of the mammalian degenerin homologue, MDEG-2 [56]. ASICα is specifically expressed by small DRG neurons that express neuropeptides [57] or that are IB_4-negative [58]. ASICβ, an amino-terminal splice variant of ASIC, is located on a few small (unmyelinated) but many large (myelinated) primary afferents, none of which displayed IB_4-binding [58].

The exact role of these varieties of acid-sensing channels and mechanisms is at present unclear. Fast-inactivating proton-gated currents similar to those elicited by DEG/ENaC channels are found in 70% to 80% of DRG neurons. However, a proton-gated non-inactivating current with thresholds at pHs known to occur in inflamed tissue (usually above pH 6) is expressed by only 40% of DRG neurons and has been linked to nociception and capsaicin sensitivity [55] and is similar to VR1 [47•]. In contrast, DEG/ENaC currents are predominantly fast inactivating and open at lower pHs, but a role in nociception is nevertheless emphasized by the appearance of sustained currents in some DEG/ENaC channels after potentiation by the FF peptide, a neuropeptide released during inflammation [59]. Novel properties might arise when several DEG/ENaC channels are coexpressed. The distribution of these channels points to differences in the acid-sensing capabilities of peptide-containing nociceptive neurons that coexpress VR1 and members of the DEG/ENaC channel family and IB_4-binding neurons that express less VR1 and few DEG/ENaC channels.

Non-nociceptive functions for DEG/ENaC channels have also been proposed. The fact that ASICβ is predominantly expressed by large afferents, many of which are mechanoreceptors, and the molecular similarity to candidate degenerin-type mechanoreceptor transducers identified in *Caenorhabditis elegans* [60], prompted the suggestion that this channel could be involved in mechanosensitivity [58]. Interestingly, acid solutions are known to evoke mechanical hyperalgesia [54]. Further work should clarify whether or not this channel family is involved in mechanotransduction of noxious and/or innocuous stimuli.

Purinergic receptors and mechanotransduction

Mechanical injury releases ATP from damaged cells, and other sources of ATP in the periphery include sympathetic fibers and tumor cells, which contain very high concentrations of ATP [61]. ATP in the skin activates nociceptors and elicits pain [62]. Several purinergic receptor families are present in sensory neurons and can mediate the actions of released ATP. ATP can act through P2X ligand-gated cation channels or P2Y G-protein coupled receptors [63]. In addition, released ATP is quickly metabolized to adenosine that activates P1 adenosine receptors. DRG neurons express $P2X_1$ to $P2X_6$ receptor subtypes [64,65] in addition to G-protein coupled P2Y and P1 receptors. Most DRG neurons express one or other purinergic receptors, and consequently almost 90% of DRG neurons are responsive to ATP either by opening a cation current or increasing intracellular Ca^{2+}.

Several characteristics of the purinergic receptors point to potential roles in sensory transduction, and more specifically to mechanical sensitivity. For example, P2X receptor structure is also related to that of the degenerin family of putative transducers from *C. elegans* [60]. However, evidence of other roles in nociceptive neurotransmission at the level of the spinal cord have also been shown for P2X receptors [66,67].

One P2X channel, $P2X_3$, is almost exclusively expressed in the DRG [68,69]. The majority (> 94%) of DRG neurons expressing $P2X_3$ are IB_4-reactive and 20% of them express CGRP, including the majority of somatostatin-containing neurons. In contrast, very few $P2X_3$-expressing sensory neurons contained SP (3%) [70,71]. $P2X_3$ homomeric

cation channels differ somewhat from those expressed in small DRG neurons, but heteromerization with $P2X_2$ forms a channel that better mimics the characteristics of DRG channels [69]. $P2X_2$ and $P2X_3$ are highly co-localized in DRGs [72]. In addition, $P2X_2$ imparts strong pH-sensitivity (around pH 6) to heteromeric complexes with $P2X_3$ [73]. It is therefore plausible that $P2X_{2/3}$ heteromeric receptors are involved in the transduction of noxious stimuli and that this response is increased by low pH in the range typical of inflamed tissues.

Innocuous mechanoreception could involve different transducers, and stretch sensory neurons express ATP-mediated currents that differ from those produced by $P2X_3$ [74]. Furthermore, an mRNA isolated from DRG neurons that conferred mechanosensitivity when heterologously expressed in oocytes was found to encode $P2Y_1$ receptors and was specifically expressed by large DRG neurons [75]. Activation of $P2Y_1$ receptors after mechanical stimulation was due to the autocrine release of small amounts of ATP. The authors suggest the intriguing possibility that the high affinity of $P2Y_1$ for ATP is able to detect small concentrations of ATP released by low-threshold stimulation, whereas lower-affinity P2X receptors need higher amounts of ATP and therefore have higher thresholds (noxious) for mechanical stimulation.

Thus, several putative mechanotransducers have been suggested from the DEG/ENaC family and various classes of purinergic receptors. The early evidence points to a differential expression of different molecules by low-threshold mechanoreceptors ($P2Y_1$, ASICβ, P2X other than $P2X_3$), and peptide-containing (ASICα, few $P2X_3$) and nonpeptide containing nociceptors ($P2X_3$, little ASICα). Unfortunately, for most of these molecules there is yet no direct electrophysiologic data to prove a role in the mechanotransduction process. More recently, genetic approaches in *Drosophila* have identified the products of the gene *nompC* as the transducer of mechanical stimuli in the apical dendrites of neurons attached to mechanosensory bristles [76•]. Homology searches in *C. elegans* have identified similar gene-products and these are distinct from the degenerin family. This new family of mechanotransducers seems to be specifically located in ciliated mechanosensory neurons and are not present in nonciliated “touch” neurons. Mammalian homologues are unknown but their discovery is likely to be “around the corner.” *nompC* encodes a novel cation channel with several ankyrin repeats and similarity to the transient receptor potential family of channels. Intriguingly, both these characteristics are shared by the VR1 receptor [46].

Conclusions

The task of defining the "nociceptive phenotype" is incomplete, and the data so far gathered reveals diversity of physiologic, biochemical, and molecular features. Despite this diversity, there are remarkably consistent correlations between general functional properties, trophic interactions, transduction mechanisms, and the "peptide-containing" or "IB_4-reactive" neurochemical profiles of presumed nociceptive sensory neurons. It is expected that a better understanding of the molecules that mediate stimulus transduction will clarify the range of properties of different sensory afferents.

The function of this variety of nociceptors is also an important consideration and there is evidence for different targets both in the periphery and centrally. Obviously, a neuropeptide content endows this population with a broader range of possible actions both over spinal neurons and in the skin. In addition, there is evidence indicating a different complement of voltage-gated channels and these probably generate differences in the encoding properties of each population [40•]. Present evidence also suggests a larger homogeneity in phenotype in IB_4-binding sensory neurons than in neuropeptide-containing sensory neurons, which include several distinct subtypes. Finally, it is important to stress that some nociceptors might not easily fit the dichotomy of neuropeptide-expressing versus IB_4-reactive nociceptors, although these appear to be the two major neurochemical subgroups.

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Papers of particular interest, published recently,
have been highlighted as:

- • Of importance
- •• Of major importance

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