Noxious mechanosensation – molecules and circuits

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Drugs that block mechanically-evoked pain would be useful for many common pain conditions, but appropriate drug development targets have yet to be defined. There is increasing evidence that both peripheral sensory neuron wiring patterns as well as the expression of transducing molecules are important for modality specific pain sensations. Progress in identifying the cell types, candidate transducing molecules and wiring patterns involved in mechanosensation has been dramatic over the past few years. Here we focus on potential mechano-transducing channels, and the relevant cell types and wiring patterns that provide clues for new analgesic drug development strategies.

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Introduction

Mechanically-evoked pain occurs in large numbers of people who often suffer ongoing poorly treated pain. The figures for osteoarthritis alone are staggering – most people over the age of 55 are inhibited in their movements to some extent by mechanically-evoked pain resulting from joint wear and tear [1]. The mechanisms of mechanotransduction and the cell types involved in mechanosensation are thus worthy of study and this has necessarily been carried out in animal models. A major problem in the field of mechanically-evoked pain is the precision of the terminology. Mechanical hyperalgesia – sensitisation to noxious stimuli – is distinct from allodynia where innocuous stimuli like the touch of a feather can become painful. The cells and mechanisms involved in these two events are probably different but there are difficulties in modelling these events in animals. There is agreement that squeezing the feet or tail of rodents with a Randall–Sellito apparatus provides a noxious stimulus. The withdrawal response from von Frey hairs is more problematic. Some groups define von Frey withdrawal thresholds of a few millinewtons as noxious, even though these stimuli are clearly not tissue damaging. As von Frey withdrawal thresholds are reduced in conditions of inflammatory pain, a situation of mechanical hypersensitivity, the ability to distinguish allodynia from mechanical hyperalgesia in animal behavioural models remains problematic (Figure 1).

Despite these difficulties, enormous progress has been made in understanding aspects of mechanosensation The specialised cell types found in the skin that are involved in sensing touch and vibration, as well as the evidence for subsets of sensory neurons that respond to different mechanical stimuli has been recently reviewed [2•].

First attempts to understand how sensory neurons were activated by mechanical stimuli came from Jon Levine's laboratory, where mechanical stimulation of cell somata was shown to result in inward currents [3••]. The great advantage of this system is that the cells can be voltage-clamped so that the characteristics of mechanically gated channels could be defined without recruiting voltage-gated channels as a consequence of depolarisation. In addition, the cells can be classified on the basis of their expression of various cellular markers associated with a particular function – for example expression of neuropeptides implicated in pain pathways.

In 2002 Drew *et al.* demonstrated that three different types of mechanically-gated channel could be identified in the cell bodies of sensory neurons *in vitro* [4]. All neurons associated with touch and proprioception express low threshold rapidly adapting mechanically-gated currents, whilst a mixed repertoire of high threshold intermediately adapting or slowly adapting currents and some rapidly adapting currents are associated with sensory neurons that express nociceptive markers. About a third of these neurons are mechanically insensitive using *in vitro* assays. These data were later confirmed [5] in a study of the terminals of sensory neurons in culture, where the channels normally reside *in vivo*. Whether the rapidly adapting currents in presumptive nociceptors are the same as those in low threshold mechanoreceptors associated with touch and proprioception is unknown.

Mechanotransducer candidates

Attempts to define the molecular nature of mechanosensitive channels have used two approaches. Firstly the behavioural and electrophysiological consequences of deleting candidate channels in mice have been investigated [6]. Secondly drug screening in an attempt to define selective blockers that may provide tools for purification

Figure 1
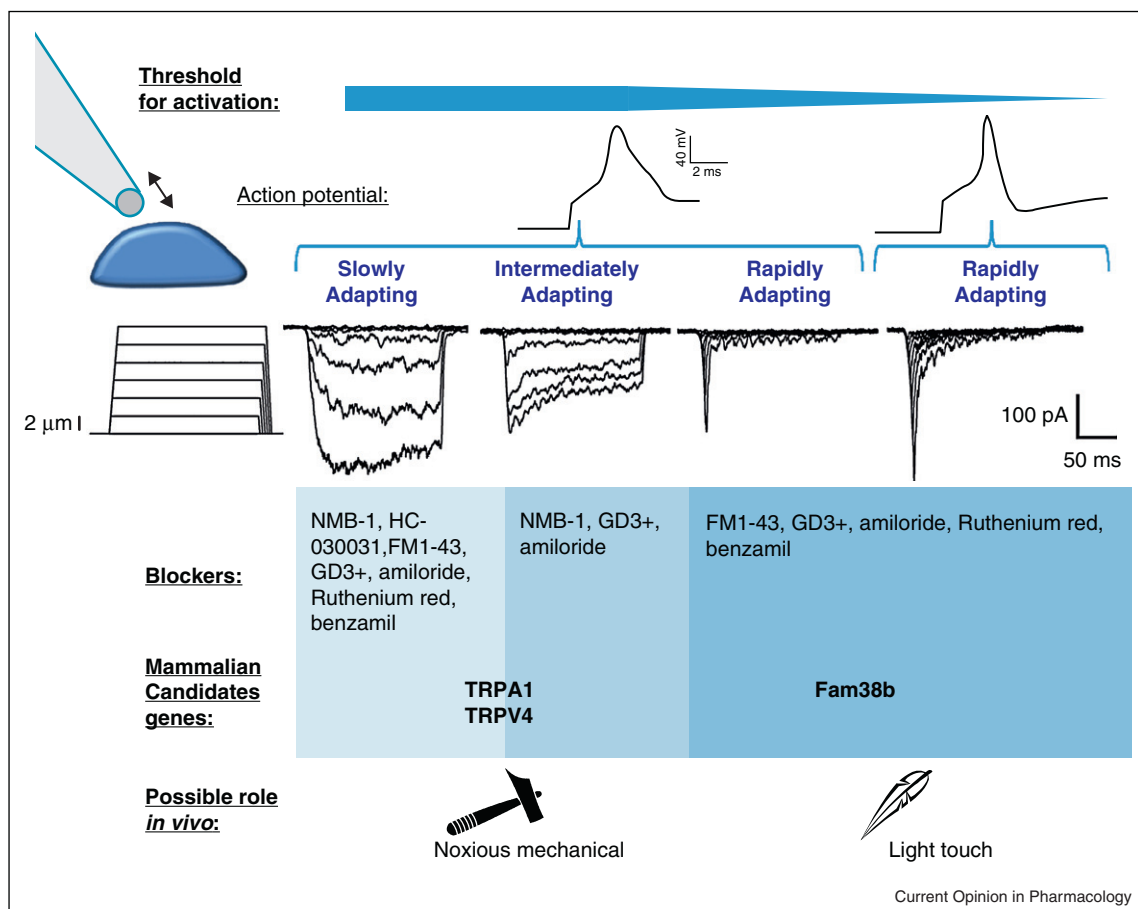
Mechanically activated currents in sensory neurons. Three different type of mechanically activated (MA) currents have been identified in cell bodies of sensory neurons [4]. The MA currents differ in the rate of adaptation and have distinct pharmacological profiles. The decay kinetics of rapidly adapting currents are best described by a bi-exponential fit, whilst intermediately adapting currents were best described by a mono-exponential fit. Activation of slowly adapting currents is slow in comparison to the other and is described by a mono-exponential fit. Small to medium cell diameter (<45 μm) neurons with broad action potentials that are associated with the detection of noxious stimuli display all three MA currents that have relatively high threshold for activation. Large diameter (>45 μm) neurons with narrow action potentials are associated with touch and proprioception as display predominantly rapidly adapting MA currents that have relatively low thresholds for activation. Displayed MA currents are representative for mouse sensory neurons at a holding potential of −70 mV.

or cloning has been carried out [7••]. Genetic studies of flies and worms have identified proteins that are bona fide mechanically gated ion channels. Transducing 'Mec' channels that are members of the epithelial sodium channel (ENaC) family have been described in C. elegans [8]. The mammalian homologues of these channels have been extensively investigated; none of the DRG ENac family members have provided a compelling case as mechanotransducers in sensory neurons [8,9]. Analysis of single or multiple ASIC knock-out mice shows that the mechanotransducing currents present on sensory neurons are not diminished [6].

A stronger case can be made for TRP channels as potential mammalian mechanosensors. The worm TRP family members OSM-9 and TRPA1 are involved in osmosensation and touch sensation and a role for TRP channels as direct mechanotransducers was demonstrated in mutagenesis studies of the TRP-4 channel, a member of the TRPN set that is not expressed in mammals [10••]. TRPV4 deletion in mice is associated with defective responses to noxious mechanical pressure and late-onset deafness [11]. TRPA1 is characterised by six transmembrane domains and has 14 N-terminal ankyrin repeats. TRPA1 is expressed in dorsal root ganglion, trigeminal ganglion neurons and hair cells. However hearing and touch are apparently normal in the null mutant [12]. Interestingly however, there is increasing evidence of a role for TRPA1 in noxious mechanosensation. The principal sensation associated with a gain of function TRPA1

mutation in man that leads to episodic pain syndrome (FEPS) is heavy unbearable pain conveying a feeling of pressure [13]. Deletion of TRPA1 in mice leads to the silencing of a set of small peptidergic sensory neurons that mainly express slowly adapting mechanosensitive currents on mechanical stimulation, and the same mice have behavioural deficits in response to the Randall–Sellito apparatus that provides noxious mechanical stimulation [14•,15]. A selective blocker of TRPA1 also inhibits mechanosensory currents in sensory neurons, and has useful analgesic effects in terms of inhibiting mechanical hypersensitivity in various models of pain associated with tissue damage [16]. TRPA1 is also required for normal mechano- and chemosensory function in specific subsets of vagal, splanchnic, and pelvic afferents [17]. All of these data are consistent with a role for TRPA1 as a mechanically-gated ion channel, but expression of such an activity has not yet been demonstrated using heterologous expression of TRPA1. This may reflect either loss of necessary accessory subunits to form the mechanotransducing complex, or the loss of a signalling molecule that is found in sensory neurons.

Other TRP channels have also been implicated in mechanosensation. TRPC1, C6 and TRPV4 have been linked with mechanical hyperalgesia associated with inflammation. Use of antisense oligonucleotides showed that TRPC1 and TRPV4 are required for mechanical hyperalgesia but not baseline mechanical thresholds, whilst TRPC6 plays a role in both mechanical and thermal hyperalgesia [18].

Recently two transmembrane proteins named FAM38a and b have been shown to confer mechanosensitivity when expressed in various cell lines [19••]. These proteins are additional candidates to mediate the stretch activated channel activity described in many cell types because Fam38a is blocked by GSMTX4, a tarantula toxin that is a stretch-activated channel blocker [20]. Fam38b re-named piezo-2 is also found in sensory neurons, where rapid inactivation kinetics make it a candidate for a mechanosensory role in non-nociceptive sensory neurons. In neonatal rat sensory neurons neither rapidly nor slowly adapting mechanosensitive currents are blocked by GSMTX4 [4], but there remains the possibility that a subset of rapidly adapting currents are blocked by the peptide in adult animals.

The behavioural consequence of deleting voltage-gated sodium channels has suggested that they may play selective role in noxious mechanosensation. The most obvious phenotype in mice where the voltage-gated sodium channels Nav1.7 or Nav1.8 have been deleted in sensory neurons is insensitivity to noxious mechanical pressure, whilst light touch is unaffected. Global deletion of Nav1.7 leads to a loss of all pain modalities and olfaction in both mice and men [21]. Does this suggest that these

sodium channels are involved in mechanotransduction? In fact, these observations provide an insight into the wiring patterns that are key to distinguishing different types of pain sensation, as the mechanosensitive currents present in sensory neurons are unaffected by sodium channel deletion. It seems that these particular sodium channel isoforms are specifically associated with action potential generation in neurons that respond to mechanical damage.

Similarities between hearing and mechanosensation
The mechanically gated channels present in sensory neurons and cochlear hair cells both depend upon the integrity of the actin cytoskeleton for function, suggesting that channel tethering to the cytoskeleton occurs in both systems [4]. Interestingly, FM1–43, a permeant inhibitor of all sensory neuron mechanosensory currents is also a selective blocker of cochlear hair cell mechanically-evoked currents, suggesting that some common elements are involved in hearing touch and pain transduction [22,23]. In behavioural studies this compound blocks both light touch and noxious mechanosensation, linking the channels described in sensory neurons in culture with mechanosensation. Fixable FM1–43 dye derivatives stain up mechanosensitive cells in both the DRG and cochlea, again highlighting potential similarities in mechanism in these 2 sets of mechanosensitive cells [24].

Mediators that change mechanical pain thresholds
Inflammatory mediators alter all pain thresholds including those for mechanical stimuli. Dissecting mechanisms of mechanical sensitisation thus requires an understanding of both general changes of excitability as well as specific effects on mechanotransduction. It appears that PKA mediated events act on neuronal excitability rather than primary mechanotransduction, whilst NGF and PKC mediated events can increase levels of expression of mechanosensitive channels. NGF has potent effects at the level of transcription on the expression of mechanosensitive ion channels in sensory neurons in culture [25]. G-protein mediated enhancement of mechanosensitivity by UTP and ATP that reduce thresholds for mechanically induced action potential firing has also been described [26]. The clinically relevant question in this area is how mechanical allodynia occurs, and how it can be blocked, and as with many other aspects of this area of study, the identification of the primary mechanotransduction mechanisms is essential for this question to be answered.

Towards mechanosensory wiring diagrams
A combination of neuronal silencing strategies, cell depletion and gene deletion studies have provided us with new insights into the particular cell types that discriminate between different mechanical stimuli. Deletion of vesicular glutamate receptors that are

required to pack synaptic vesicles with the neurotransmitter blocks glutamate release and silences glutamatergic neurons. There are 3 vesicular glutamate transporters that are expressed in neuronal subsets of dorsal root ganglion (DRG) neurons that transmit sensory information using the excitatory transmitter glutamate. Kullander deleted VGLUT2 with a Cre recombinase driven by the Nav1.8 promoter and found that noxious mechanical pain was specifically abolished whilst thermal pain was unaffected. Inflammatory mechanical hyperalgesia also depended upon this subset of sensory neurons [27]. Use of a tyrosine hydroxylase Cre that deletes the transporter in a different population of sensory neurons caused deficits in thermal pain, and lead to a dramatic increase in itching [28]. Seal *et al.* found that a subset of neurons in mouse DRG express the low-abundance vesicular glutamate transporter VGLUT3 and project to lamina I and lamina II in the spinal cord [29]. The deletion of the VGLUT3 gene also impaired mechanical but not thermal pain sensation and the mechanical hypersensitivity associated with tissue damage owing to a loss of signalling from unmyelinated, low-threshold mechanoreceptors.

Diphtheria toxin-A subunit blocks translation and kills neurons in which it is expressed and, when driven by a global promoter, can be unmasked by cell specific expression of a Cre recombinase. Killing all post mitotic sensory neurons that express the sodium channel Nav1.8 leads to a loss of responsiveness to noxious mechanical pressure and cold as well as the heightened sensitivity to thermal or mechanic stimuli associated with inflammatory pain [30]. Thus the neurons that respond to low threshold mechanical or thermal stimuli are Nav1.8 negative, whilst those that sensitise these responses during inflammatory pain are Nav1.8+. Similarly, Nav1.7 deletion in the Nav1.8 population abolishes inflammatory pain and noxious mechanosensation, whilst Nav1.7 expression in other Nav1.8-neuronal populations is required for thermal pain. Total Nav1.7 ablation causes anosmia and a pain free state without cell death, making this an interesting target for mechanical as well as other types of pain.

Further clues about the properties of neurons involved in noxious mechanosensation comes from the ablation of neurons that express the G protein-coupled receptor Mrgprd leading to reduced behavioural sensitivity to noxious mechanical stimuli, but not to heat or cold [31].

Other genetic approaches have exploited the expression of growth factor receptors, combined with mapping the terminals of sets of sensory neurons. Those cells that express the GDNF receptor c-ret early in development seem to be particularly interesting from the point of view of light touch and perhaps allodynia. These cells form neurons with rapidly adapting mechanically gated currents associated with Meissner corpuscles, and Pacinian corpuscles that terminate in layers III through V of the spinal cord and the medulla [32]

Drugs and mechanosensation

Neutralising anti-NGF monoclonal antibodies are potent analgesics for many types of mechanically-evoked pain. Both osteoarthritis and back pain are well treated by reducing levels of NGF, an inflammatory mediator as well as trophic factor involved in the development of the peripheral nervous system. However, these drugs are on hold because of potential serious side effect issues possibly caused by unwitting self harm to joints during analgesia, as a result of removing circulating NGF [1].

Small peptide blockers of mechanosensitive channels (GSMTX4 and NMB1) provide support for the view that different molecules are involved in stretch-activated and slowly adapting mechanosensitive currents respectively, but high affinity selective ligands useful for cloning or biochemical purification strategies have not been described. Interestingly GSMTX4 blocks mechanical hyperalgesia, whilst NMB1 is specific for noxious mechanical pain [4,17]. Given the fact that mechanical hyperalgesia can be blocked by the usual repertoire of anti-inflammatory drugs, the most fascinating topic remains the mechanisms involved in the establishment of allodynia, and how allodynia can be addressed pharmacologically. Studies of Fam38b knock-mice will be particularly interesting in this respect.

Acknowledgements

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