CHAPTER 15

Touch

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I. OVERVIEW

Light touch, a sense of muscle position, and the responses to tissue-damaging levels of pressure all involve mechanosensitive sensory neurons that originate in the dorsal root or trigeminal ganglia. A variety of mechanisms of mechanotransduction have been proposed. These range from direct activation of mechanically activated channels at the tips of sensory neurons to indirect effects of intracellular mediators, or chemical signals released from distended tissues, or specialized mechanosensory end organs. In this chapter, we describe the properties of mechanosensitive channels present in sensory neurons and the potential molecular candidates that may underlie this type of activity.

II. INTRODUCTION

In mammals, a number of different types of mechanoreceptors respond to distinct mechanical stimuli. There are four principle mechanosensory systems each with specialized receptor cells that have evolved to detect diverse forms of mechanical events. These are: (1) touch (detection of mechanical events impacting on the skin, including noxious mechanosensation), (2) kinesthesia, or the awareness of position, location, and orientation of the body and its parts (a branch of proprioception originating from receptors in the muscles, joints, and bones), (3) body motion and balance (a branch of proprioception originating in the inner ear), and (4) hearing (the detection of sound waves by hair cells of the inner ear). In this chapter, we will focus on touch, with some reference to kinesthesia. Excellent reviews on proprioception, balance, and hearing can be found in the literature (Day and Fitzpatrick, 2005; LeMasurier and Gillespie, 2005; Macefield, 2005; and Howard in Chapter 15).

Touch has been studied anatomically, electrophysiologically, pharmacologically, and most recently using brain imaging techniques (Hlushchuk and Hari, 2006). Remarkably, the primary transduction events that underlie this modality in mammals remain unknown despite extensive efforts to identify the channels and receptors that are likely to be involved. In this chapter, we focus on aspects of touch and noxious mechanosensation mediated by primary mammalian sensory neurons that innervate the skin. We review the specialized end organs present in the skin that have been implicated in mechanosensation, discuss the properties of channels present on sensory neurons that are mechanosensitive, and describe the candidate molecules that may underlie light touch and noxious mechanosensation.

III. STRUCTURE OF SKIN AND TOUCH RECEPTORS

Touch receptors are localized in the skin, the body's largest organ. Anatomically, the skin is the main part of the integumentary system that comprises the nails, hair, glands (sweat and sebaceous), and specialized nerve structures detecting tactile stimuli, temperature changes, and tissue damage. The skin accounts for 12–15% of the body weight, an average area of 1.8 m^2 in the adult human and a thickness of 2–3 mm on most of the body surface. The skin on the palms of the hands and the soles of the feet is particularly thick (owing to a high keratin content) and its surface is hairless and rich in *papillae*—ridges that are important for grip. The skin on the rest of the body is hairy, has fewer ridges, and is thinner and softer. Apart from being the site of tactile-, temperature-, and tissue-damage-evoked pain sensations, the skin also has a role as an anatomical barrier between external and internal environments preventing disease-causing microorganisms to enter the body, and plays a role in temperature regulation. The skin comprises two tissue layers, the epidermis (outer layer) and the dermis (inner layer). A third deeper layer, the hypodermis, connects the skin to the underlying bones and muscles.

A. Epidermis

The outermost layer of the skin is composed of several *strata* or stratified layers rimmed by an underlying basal membrane. From the outside to the inside, the *strata* making up the epidermis are the *corneum*, *lucidum*, *granulosum*, *spinosum*, and *basale* (Fig. 1A). The stem cells are produced in the innermost layers and differentiate while they move up to the distal layers. Apart from the *stratum basale*, the epidermis has no direct blood supply. Hence, the cells that migrate away from this layer are bound to die and when they eventually reach the *corneum* they are sloughed off, a process that takes 35–45 days and that is known as desquamation. In these different layers four types of cells are encountered: (1) Keratinocytes, whose role is to synthesize keratin, a protein that is the source of the skin's strength and flexibility and that waterproofs the skin surface. (2) Langerhans cells, derived from a macrophage-monocyte precursor in the bone marrow, constitute an epithelial component of the immune system and play a role in the recognition and processing of antigens in order to present them to either lymphocytes and/or macrophages. (3) Melanocytes produce melanin, the dark pigment that gives the skin its color and that acts as a sunscreen to protect the skin from ultraviolet light. (4) Merkel cells, involved with pressure sensation (see below).

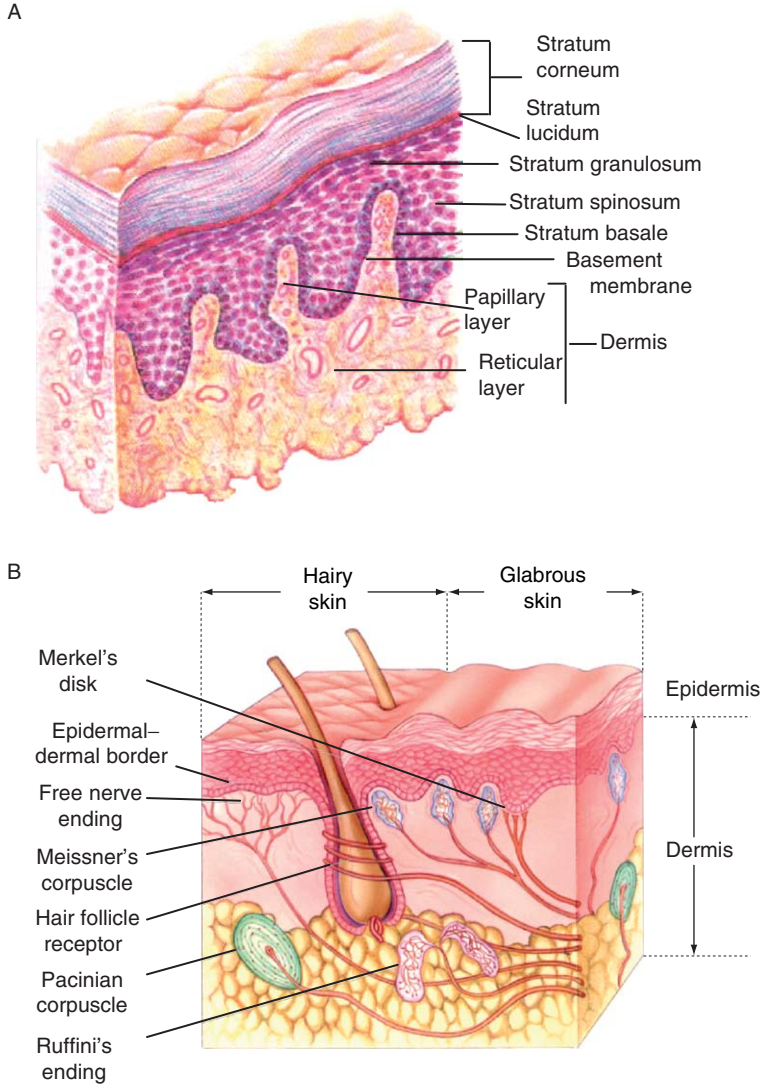

FIGURE 1 The skin and its receptors. (A) The layered structure of the skin. (B) The different types of skin receptors. Adapted from Bear, M. F., Connors, B. W., Paradiso, M. A. "Neuroscience: Exploring the Brain," 2nd ed. Lippincott Williams & Wilkins, 2nd Bk&Cdr ed. (March 15, 2002).

B. Dermis

The dermis which lies beneath the epidermis is the main part of the skin containing sensory nerve terminals, blood supply, smooth muscle, hair follicles, glands, and lymphatic tissue. The dermis is enriched in fibroblasts, adipocytes, and macrophages and is the site of production of excreted substances by glands. The dermis consists of two layers, one made of loose connective tissue (papillary layer) and the other made of dense connective tissue (reticular layer) (Fig. 1A). These two layers are very tightly connected and rich in collagen for strength, reticular fibers for support, and elastin fibers for flexibility. The papillary layer lies beneath the epidermis and contacts this layer through fingerlike projections called *papillae*. These *papillae* have different functions; some supply the epidermis with blood while others have a sensory function as they contain Meissner's corpuscles (see below). The skin on the palms of the hands and on the soles of the feet contain two rows of *papillae* resulting in the finger- and footprints which protect the skin from tearing and help gripping. The reticular layer is denser, contains less organized fibers and is rich in collagen, hence resisting stretch. Pacinian corpuscles, the sensory receptors for deep pressure (see below) are found in this layer. The dermis contains also sweat glands, hair follicles, lymph vessels, and smooth muscle.

C. Mechanosensory Receptors

Receptors in the skin can detect three different sorts of sensations: tactile sensations, temperature changes, and tissue damage resulting in pain. Tactile sensations can be divided into three modalities (touch, pressure, and vibration), temperature into two (hot and cold), whereas pain may be evoked by mechanical, thermal, or chemical stimuli. All these so-called "superficial" sensory modalities constitute, together with proprioceptive sensations (arising from muscles, joints, and ligaments) and visceral pain, the somatovisceral senses.

The terminals of the sensory nerves express peripheral receptors and they exist either in a free form or embedded in more complex and integrated anatomical structures. Free nerve endings generally express receptors for damage sensing and thermosensation, whereas the nerve terminals associated with specific end organs are specialized mechanoreceptors.

1. Specialized Mechanoreceptors

The skin can detect three different modalities of tactile sensations. Touch is detected by Meissner's corpuscles and hair follicle receptors. These two types of receptors respond to sudden light touch and therefore the critical

factor in activating them is the velocity rather than the intensity of the stimulus. Vibration is detected by nerve fibers terminating in Pacinian corpuscles. On the basis of their firing properties, these three classes of afferents are said to be low-threshold, rapidly adapting (or phasic) mechanoreceptors. Finally, pressure is sensed superficially by Merkel cell–neurite complexes and deeper in the skin by Ruffini's endings. The responses of sensory fibers connected to these structures are proportional to the pressure applied on the skin. Both Merkel's discs and Ruffini's endings are defined as low-threshold, slowly adapting mechanoreceptors, but they differ slightly in their mode of activation.

Meissner's corpuscles are low-threshold, rapidly adapting (or phasic) mechanoreceptors and are found in the dermal papillae of the glabrous skin, mainly hand palms and foot soles but also lips, tongue, face, nipples, and genitals (Fig. 1B). Anatomically, they consist of an encapsulated nerve ending, the capsule being made of flattened supportive cells arranged as horizontal lamellae embedded in connective tissue. There is one single nerve fiber per corpuscle. Any physical deformation of the corpuscle triggers a volley of action potentials that quickly ceases. When the stimulus is removed, the corpuscle regains its shape and while doing so produces another volley of action potentials. Due to their superficial location in the dermis, these corpuscles are particularly sensitive to touch and vibrations, being able to respond to low-frequency vibrations in the range of 20–40 Hz.

Hair follicle receptors (*G hairs*) are unmyelinated sensory nerve terminals which coil around the shaft of a hair within the external root sheath (Fig. 1B). They respond to hair motion and its direction by firing trains of action potentials at the onset and removal of the stimulus.

Merkel's discs consist of a Merkel cell in close apposition to an enlarged nerve terminal. A single sensory fiber can branch to contact up to 90 Merkel cells. Merkel's discs are found in the basal layer of the epidermis in fingers, lips, and genitals (Fig. 1B). Functionally, they are sensitive to very low-frequency vibrations (5–15 Hz) and respond to a low-intensity constant pressure by a nonadapting volley of action potentials (static response) for up to 30 min.

Ruffini's endings are thin cigar-shaped encapsulated sensory endings that detect pressure when the skin is stretched. They are broadly expressed in the dermis (Fig. 1B).

Pacinian corpuscles are low-threshold mechanosensors that display a very rapid adaptation in response to the indentation of the skin. They are also called acceleration detectors because they can detect changes in the strength of the stimulus and if, as happens in vibrations, the rate of change in the stimulus is altered, that is the acceleration of the skin movement changes, their response becomes proportional to this change. Pacinian corpuscles are

expressed in the deep dermis (Fig. 1B) as well as in vessel walls. Anatomically similar to Meissner's corpuscles, they are large ovoid corpuscles (1 mm in length) made of concentric lamellae of fibrous connective tissue and fibroblasts lined by flat modified Schwann cells. In the center of the corpuscle, in a fluid-filled cavity called inner bulb, terminates one single afferent unmyelinated nerve ending. Pacinian corpuscles sense gross pressure changes and most of all vibrations (in the range 150–300 Hz), which they can detect even centimeters away. They have a large receptive field on the skin's surface with a particularly sensitive center. Notably, these corpuscles function also as proprioceptive detectors and are therefore highly expressed in ligaments, muscles, and joint capsules.

2. Free Nerve Endings

In contrast to the majority of low-threshold mechanoreceptors (LTMs) that terminate in specialized end organs, the sensory terminals of nociceptive neurons (and a number of temperature receptors) exist as bare nerve endings in the skin. Temperature receptors react to local changes in skin temperature. Six ion channels expressed on sensory nerve terminals and belonging to the transient receptor potential (TRP) channel family have been ascribed a function in thermosensation (for a thorough review see Patapoutian *et al.*, 2003). The heat receptor function is supported by four channels from the vanilloid receptor subfamily: TRPV1, TRPV2, TRPV3, and TRPV4. TRPV3 and TRPV4 are activated by temperatures in the physiological range (above 33°C for TRPV3 and between 27 and 42°C for TRPV4) while TRPV1 and TRPV2, activated by temperatures above 42 and 52°C respectively, are the receptors for noxious heat. Two more TRP channels, the melastatin-related TRPM8 and the ankyrin repeat-rich TRPA1 are cold receptors. TRPM8 senses temperatures below 25°C whereas TRPA1 serves as a noxious cold receptor (activated by temperatures below 17°C). Between ~20 and 40°C, the temperature receptors adapt quite rapidly but more extreme temperatures are continuously sensed as hot and cold to protect the skin from damage.

Nociceptors are often polymodal, responding to chemical, mechanical, and temperature stimuli separately or together, when they are of a level of intensity that has the potential to cause tissue damage. Nociceptors are broadly of two classes, those that are peptidergic (i.e., they express the neuropeptides substance P and CGRP) and express TrkA and those that are nonpeptidergic, express c-Ret receptor complexes in adulthood and bind isolectinB4 (IB4). Both classes of nociceptors innervate the skin whereas nociceptive innervation of joints and the viscera is almost exclusively by peptidergic neurons (Keller and Marfurt, 1991; Ivanavicius *et al.*, 2004; Robinson *et al.*, 2004). Interestingly, in the skin, IB4-positive neurons appear to terminate more

superficially, in the stratum granulosum, than do peptidergic neurons that end in the stratum spinosum (Zylka *et al.*, 2005).

IV. PHYSIOLOGY OF MECHANORECEPTIVE NERVE FIBERS

Sensory nerves have their cell bodies in the dorsal root ganglia (DRG) or trigeminal ganglia and transmit sensory information into the dorsal horn of the spinal cord or the brain stem at the level of the pons, respectively. Sensory nerve fibers can be classified according to their conduction velocities (determined by their diameters and their degree of myelination) into three groups. The nociceptor and temperature-related fibers are mainly associated with two of these three classes of fibers. They are the C fibers (unmyelinated, diameter <1 μm, conduction velocity <1 m/s) and the Aδ fibers (thinly myelinated, $\sim$2.5 μm diameter, $<$15 m/s conduction velocity) which terminate in laminae I, II and I, II, V of the spinal dorsal horn, respectively. However, a review also highlights the existence of nociceptors functioning in the Aβ range (Djouhri and Lawson, 2004). The mechanosensation-associated fibers are predominantly Aβ fibers which are myelinated fibers with a large diameter ($\sim$10 μm) and a conduction velocity in man of up to 60 m/s. These fibers terminate in laminae III, IV, and V of the spinal cord.

A. Low-Threshold Mechanoreceptors

LTMs are generally associated with myelinated Aβ fibers, the largest and fastest type of sensory fibers. Nevertheless, two particular types of LTMs are also found on Aδ and C fibers (see later "D-hair" and "C-LT"). Microelectrode recordings from awake human subjects (microneurography) have greatly advanced our knowledge of the physiology of LTMs (Vallbo and Hagbarth, 1968). They have led to the identification of four distinct classes of Aβ-LTM afferents in the glabrous skin of the human hand (Macefield, 2005) that strictly correlate to the four types of mechanoreceptors encountered in this type of skin (Meissner's and Pacinian corpuscles, Ruffini's endings, and Merkel cells). Among the four types of LTM afferents, a further subclassification can be established according to their rate of adaptation. Two types of LTM fibers fire only at the onset of a sustained stimulus and are therefore called fast or rapid adapting (FAI or RA1 and FAII or RA11) while two other types fire throughout the stimulus and are referred to as slowly adapting (SAI and SAII; Fig. 2). FAI and FAII afferents are connected to Meissner's and Pacinian corpuscles, respectively, while SAI and SAII fibers innervate Merkel cells and Ruffini's endings, respectively. Type I afferents have small

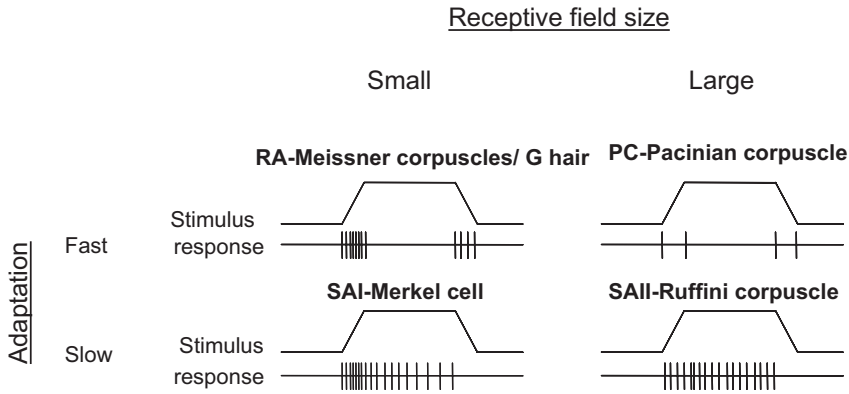

FIGURE 2 Response properties of low-threshold cutaneous mechanoreceptors. The stimulus trace represents a mechanical displacement. The response trace shows the pattern of action potentials (vertical lines) evoked by such a stimulus.

receptive fields that contain several "hot spots" of highest sensitivity corresponding to the individual Meissner's corpuscles and Merkel cells. In contrast, type II afferents display only one zone of maximal sensitivity in receptor fields which are large and unevenly shaped. Therefore, FAI afferents (Meissner) respond to light mechanical stimulation in a very delimited area whereas FAII (Pacinian) can sense brisk and transient stimuli applied remotely from the area of optimal sensitivity. The same applies for SA afferents as SAI fibers (Merkel) are sensitive to stimuli applied to a discrete zone of the skin while SAII (Ruffini) can be activated by lateral stretch of the skin. SAI fibers can be further differentiated from SAII afferents by their interspike intervals during their sustained firing not being constant, as opposed to the very regular pattern of interspike intervals observed in SAII receptors (Olausson *et al.*, 2000).

In human arm hairy skin, five types of large myelinated afferents are encountered. SAI and SAII afferents are the same as those in glabrous skin but three types of afferents make up the RA versant of the hairy skin (Macefield, 2005). These are the hair (also called G1 hairs) and field (or G2 hairs) units (Leem *et al.*, 1993; Lewin and Moshourab, 2004) and Pacinian corpuscles. Hair units (G1) respond to the movements of hairs and are linked to receptor fields that can be either well defined or irregular but that always contain several "hot spots" corresponding each to an individual hair. On the other hand, field units (G2) are sensitive only to actual skin contact and are connected to receptor fields similar to those of G1 receptors except that their "hot spots" are larger and less isolated.

It should be noted that the distribution of types of LTMs varies between different species and also between different parts of the body. Hence, it has been shown that the hairy skin of the human face was supplied by the same kind of SA and RA fibers as those found in the glabrous skin of the hand (Macefield, 2005).

The D-hair (Brown and Iggo, 1967), connected to large receptor fields, is the only known example of an LTM afferent belonging to the thinly myelinated class of Aδ fibers and most surprisingly constitutes the most sensitive form of LTMs, being able to respond to hair movements as small as 1 μm in the mouse (Lewin and Moshourab, 2004). It is considered a subclass of hair follicle receptor (Stucky *et al.*, 1998).

Finally, LTMs can also be found on unmyelinated C fiber afferents. The expression of these tactile C afferents or low-threshold C fibers (C-LT) is highly variable from one species to another but is high enough in humans to be considered as an important part of the touch receptor machinery responsive to caress-like and skin-to-skin contact between individuals that lead to pleasant sensations (Olausson *et al.*, 2002).

B. High-Threshold Mechanoreceptors

High-threshold mechanoreceptors (HTM) are the receptors for noxious mechanosensation. These receptors are predominantly found on two types of sensory nerve fibers that respond also to temperature variations, namely the thinly myelinated Aδ and unmyelinated C fibers (see above and Fig. 2).

Aδ fibers functioning as HTM encode the degree of skin indentation, firing throughout the duration of the stimulus, with little response to the moving part of the stimulus (Garell *et al.*, 1996) and are believed to be the receptors for fast mechanical pain. Structurally, they have free unmyelinated nerve terminals projecting to the deep epidermis where they associate with keratinocytes and Schwann cells and possess large receptor fields displaying "hot spots" of higher sensitivity (Lewin and Moshourab, 2004).

C fibers represent the predominant group of sensory nerve fibers innervating the skin. Most C fibers are polymodal receptors capable of responding to different stimuli, notably noxious mechanosensation and temperature change. Nevertheless, some of the C fibers sensitive to noxious mechanosensation are unresponsive to thermal stimuli. Hence, can we distinguish among C fiber afferents that respond both to mechanical and thermal stimuli—which are the C-mechanoheat (C-MH), the C-mechanocold (C-MC), and the C-mechanoheatcold (C-MHC) afferents—and afferents that respond

uniquely to painful mechanical stimulation, the C-mechanonociceptors (C-M) fibers (Lewin and Moshourab, 2004). It is not clear, however, whether there are any differences in mechanosensitivity between these different groups of C fibers. In addition, "silent" or "sleeping" nociceptors do not respond to a mechanical or thermal stimulation in physiological conditions but can acquire thermo- and/or mechanosensitivity following inflammation (Schmidt *et al*., 1995). They account for a significant part of C fibers and are also expressed in viscera and joints. These fibers are termed C-MiHi (Lewin and Moshourab, 2004).

V. QUANTITATING MECHANICAL RESPONSES IN ANIMAL MODELS

The two most commonly used behavioral tests of mechanosensation are the von Frey test (von Frey, 1894) and the Randall–Selitto assay (Randall and Selitto, 1957). The von Frey test involves applying a punctate stimulus to a given region of the rodent's body, usually the plantar surface of the hind paw, and recording the stimulus intensity that evokes a withdrawal reflex. Stimuli are typically applied using calibrated fibers with a specific bending force. The Randall-Selitto test uses a device that applies an ascending pressure ramp to either the animal's paw or tail and the point when a specific pain-related behavior is evoked (e.g., vocalization or writhing) is used as the pain threshold. Typically, thresholds in the Randall–Selitto test are around an order of magnitude greater than those observed in the von Frey test suggesting that different aspects of mechanosensory processing are being measured. This is further supported by observations of rodents that have had the majority of their nociceptors abolished by neonatal capsaicin treatment; these animals exhibit increased tolerance in the Randall–Selitto test but not in the von Frey test (Saumet and Duclaux, 1982; Nagy and van der Kooy, 1983; Shir and Seltzer, 1990). While experimentally induced changes in withdrawal thresholds in behavioral tests are consistent with affects on the transduction machinery of sensory neurons, care must be taken in extrapolating from such observations as changes in the electrical excitability of the neurons, the central synaptic properties of the neurons, or the general motivational/motor state of the animal may affect the outcome. It is reasonable to argue that the von Frey hair threshold is a measure of when the animal becomes aware of the presence of a mechanical stimulus that causes irritation, in contrast to the obvious pain evoked by a Randell–Sellito apparatus. However, there are no established tests for responses to light touch.

VI. ELECTROPHYSIOLOGICAL APPROACHES TO MECHANOSENSATION IN RODENTS

Mechanoreceptive neurons of the DRG synapse on to second order sensory neurons in the dorsal horn of the spinal cord. The activity of single spinal neurons (either superficial nociception-specific or deeper wide dynamic range neurons) can be recorded in the intact animal while stimuli are applied to the receptive field of that cell (Matthews *et al.*, 2006), thus giving a read out of the convergent input of multiple primary sensory neurons. For example, this approach has been used to show that administration of cannabinoid receptor agonists to the peripheral receptive field can inhibit responses to mechanical stimulation (Kelly *et al.*, 2003; Elmes *et al.*, 2004). In addition, it has been used to assess the thermal and mechanical sensitivity of null mutants with genes for sensory neuron ion channels ablated (Souslova *et al.*, 2000). Such studies have revealed a separation of the encoding of thermal and mechanical input by the somatosensory system (Matthews *et al.*, 2006).

A number of groups have developed techniques for recording from either DRG somata or nerve fibers in more or less intact preparations. The skin-nerve preparation (Reeh, 1986) is a technique for recording from teased single-nerve fibers of the saphenous nerve. The nerve is dissected out attached to an area of hind limb skin to which "natural" or electrical stimuli are applied. Using this technique, the firing properties of nerve fibers, classified by their conduction velocities and firing patterns to established subtypes of cutaneous afferents, can be recorded in response to calibrated mechanical stimuli. This approach has been used to assess the mechanosensitivity of nerve fibers in null mutant mice (Price *et al.*, 2000) and sensitization of nociceptive fibers to mechanical stimulation (Steen *et al.*, 1995).

Koerber and Woodbury (Woodbury *et al.*, 2001) developed a related approach in which the thoracic and/or sciatic nerves are dissected out with their spinal cord and peripheral cutaneous connections intact, and recordings are made from the DRG somata. In addition, Sally Lawson's group has extensively used *in situ* recordings from DRG neurons in anesthetized rodents and guinea pigs (Djouhri *et al.*, 1998). These approaches have the advantage that the recorded neuron can be phenotyped by immunocytochemical labeling and its projections examined.

Such approaches can be used to determine if genetic ablation of a candidate mechanotransducer effects mechanically evoked firing in cutaneous receptors. However, observed firing rates will be determined by the efficiency of transduction and the electrical properties of the nerve fiber, while it should be determined that the morphology of the peripheral terminals is unchanged. The electrical properties of the fibers can be tested directly (although

electrical stimulation of the skin may bypass the sensory terminal in exciting the nerve fiber) and terminal morphology can be observed with standard labeling procedures. Gary Lewin's group, using the skin-nerve preparation, has used a probe fixed to a linear stepping motor under computer control to apply defined mechanical stimuli to the receptive fields of neurons (Shin *et al*., 2003; Dubreuil *et al*., 2004), whereas other groups have used von Frey hairs to assess fibers mechanical thresholds (Albers *et al*., 2006) or broad classes of stimuli (e.g., brush, pinch, pinprick; Djouhri *et al*., 1998) to classify receptive properties.

VII. MECHANOSENSITIVE ION CHANNELS IN CULTURED SENSORY NEURONS

Ideally, recordings would be made of mechanically evoked receptor potentials from the nerve terminal. Loewenstein and coworkers in the 1960s used Pacinian corpuscles with the afferent nerve attached extracted from the cat mesentery to record such events (Loewenstein and Mendelson, 1965; Loewenstein and Skalak, 1966). Enzymatic removal of the corpuscle suggested that these structures act as a mechanical filter determining the dynamics of the mechanical stimulus that reach the mechanoreceptive nerve ending. Nonselective cation channels that conduct mainly sodium in physiological conditions mediate these receptor potentials. In addition, Katz (1950) and Ottoson and coworkers (Ottoson, 1964; Husmark and Ottoson, 1971), using extracellular electrodes, have characterized the receptor potentials of frog muscle spindles exposed to stretch. These responses are defined by an initial RA component followed by a static phase. Observations following the removal of sodium again suggested that this ion carries the majority of charge in normal Ringer's solution (Husmark and Ottoson, 1971). Receptor potentials were also decreased by removal of calcium and potassium (Ottoson, 1964; Husmark and Ottoson, 1971); however, these data are difficult to interpret due to the likely nonspecific affects on membrane potential that such manipulations would cause. Hunt and Ottoson (1975, 1976) also achieved similar results using muscle spindles from the cat tail, although the ionic basis of transduction was not investigated. No one has obtained similar results in rodents and certainly not from the bare nerve endings of nociceptors. One approach to this problem has been developed by Brock *et al.* (1998) who used a suction electrode attached to the guinea pig cornea to record activity in the terminals of nociceptors, they showed that sensory terminals could generate TTX-resistant action potentials in response to mechanical stimulation. Mechanical stimulation was achieved by "pushing the recording electrode gently against the corneal surface with a displacement

of the micromanipulator"; it remains to be seen if this stimulation technique can be used to quantify mechanosensitivity and applied to transgenic mice.

More recently, a number of groups have used cultured sensory neurons to study the responses of these cells to mechanical stimulation. These studies have used Ca^{2+} imaging or electrophysiology as the read out of the neuron's response. Such studies rely on the redistribution of ion channels expressed by neurons so that molecules normally localized at their peripheral terminals are found in the somatic cell membrane (Baccaglini and Hogan, 1983; Cesare and McNaughton, 1996; Reid *et al.*, 2002). In studies using ratiometric Ca^{2+} imaging neuronal stimulation using a rounded micropipette (Sharma *et al.*, 1995; Gotoh and Takahashi, 1999; Raybould *et al.*, 1999; Gschossmann *et al.*, 2000), fluid jet (Sullivan *et al.*, 1997), and hypoosmolarity-induced cell swelling (Viana *et al.*, 2001) has been shown to induce an increase in cytosolic Ca^{2+}. In each case, the rise in Ca^{2+} was dependent on extracellular Ca^{2+} suggesting that it was mediated via a calcium permeable membrane channel rather than release from intracellular stores. One problem with such studies has been the time course of the observed responses. In each case, the rise in Ca^{2+} levels has been slow (occurring over many seconds or even minutes) and the return to baseline has consistently occurred considerably after the end of the stimulus. How these slow responses relate to the rapid encoding of mechanical stimulation by nerve endings *in vivo* is unclear. Viana *et al.* (2001) showed that under voltage clamp, the $[Ca^{2+}]_i$ elevation produced by hypotonic stimulation was accompanied by the development of an inward current and a conductance increase. The time course and amplitude of the $[Ca^{2+}]_i$ response to hypoosmotic stimulation showed a close correlation with electrophysiological properties of trigeminal neurons. Fast $[Ca^{2+}]_i$ responses were associated with short duration action potentials characteristic of Aβ fibers. Subsequently, a role for GAP-43 in sensing osmolarity changes was demonstrated. By recruiting PKC to the cell membrane, GAP-43 can induce a rise in IP3 levels and intracellular calcium in response to hypoosmotic stimuli (Caprini *et al.*, 2003). However, GAP-43 is sparsely expressed in adult DRG neurons and this phenomenon is distinct from the influx of extracellular calcium in DRG cultures stimulated osmotically. Although some studies reported that calcium channel antagonists did not inhibit mechanically evoked responses (Sullivan *et al.*, 1997; Gotoh and Takahashi, 1999; Gschossmann *et al.*, 2000) as sensory neurons express a heterogeneous population of voltage-gated calcium channels (Yusaf *et al.*, 2001), it cannot be guaranteed that all of them will be blocked. Viana *et al.* (2001) showed that the calcium channel blocker Ni^{2+} does indeed inhibit swelling-evoked responses.

Calcium imaging studies have consistently reported that Gd^{3+}, a blocker of a range of mechanosensitive ion channels (Hamill and McBride, 1996), inhibited responses to mechanical stimulation. However, the effective blocking

concentration ranged from 5 μM (Gotoh and Takahashi, 1999) to 250 μM (Gschossmann *et al.*, 2000). Moreover, Gd^{3+} might also antagonize voltage-gated Ca^{2+} channels (Boland *et al.*, 1991). Gschossmann *et al.* (2000) reported that amiloride (100 μM) and κ-opioid agonists reduced mechanically evoked Ca^{2+} increases while not affecting increases induced by capsaicin. Drummond *et al.* (1998; using the stimulation method of Sullivan *et al.*, 1997) reported that increases in intracellular Ca^{2+} were antagonized by 100 nM amiloride.

Another variable that fluctuated widely between studies was the proportion of cells that responded, ranging from 25% (Raybould *et al.*, 1999) to 93% (Gotoh and Takahashi, 1999), although this may simply reflect differences in the degree of pressure applied using different stimulation protocols. The specificity of these mechanically evoked Ca^{2+} increases remains to be established through studies on populations of non-sensory neurons.

Electrophysiological studies of mechanotransduction by sensory neurons have used a number of stimulation protocols sometimes making comparison of the data difficult. Cho *et al.* (2002) investigated the expression of stretch-activated ion channels expressed by sensory neurons by applying negative and positive pressure to membrane patches of neonatal DRG neurons. This extensive study characterized two classes of MS ion channels; low-threshold (LT) channels (present in 26% patches) which had an activation threshold of around -10 to -20 mmHg and a $P_{1/2}$ of 60.6 mmHg and high-threshold (HT) channels (24% of patches) with a $P_{1/2}$ of 83.1 mmHg and a threshold of >60 mmHg. The activity of both channel types increased with ascending pressure and both nonselectively conducted cations with significant Ca^{2+} permeability. HT channels displayed a relatively linear current–voltage (I–V) relationship whereas LT channels were outwardly rectifying. Channel activity in both cases was inhibited by disruption of the cytoskeleton using either cytochalasin D (a disruptor of actin polymerization) or colchicine (a microtubule disruptor) and also by patch excision, which presumably disturbs the normal membrane–cytoskeleton interaction. Gd^{3+} inhibited HT and LT channels but neither were sensitive to amiloride nor arachidonic acid (AA). Finally, PGE2, acting via a PKA-dependent pathway, selectively potentiated the activity of HT ion channels, in part by decreasing their activation threshold.

At the whole-cell level, Cho *et al.* (2002) mechanically stimulated DRG neurons, in a method similar to that reported by Takahashi and Gotoh (2000), by applying positive pressure through the patch pipette. Both studies reported the activation of cationic currents in a subset of sensory neurons with diameters greater than 20 μm; Takahashi and Gotoh (2000), however, observed currents in a substantially higher proportion of cells ($\sim$75%) than did Cho *et al.* (2002; around 25%). A substantial lag was apparent between the application of the stimulus and the activation of the ensuing current

(ranging from 0.5 to 3 s). The reasons for this delay are unclear as the increase in pressure should rapidly result in membrane stretch and so the possibility of an intermediate biochemical gating mechanism cannot be excluded.

McCarter *et al.* (1999) and ourselves (Drew *et al.*, 2002, 2004; Di Castro *et al.*, 2006) have taken the approach of stimulating voltage-clamped neurons using a heat-polished glass probe. In their initial characterization, McCarter *et al.* (1999) reported that such a stimulus evoked nonselective cationic currents that were inhibited by Gd^{3+} and high concentrations of, the amiloride analogue, benzamil.

We used a piezoelectric crystal device to control a glass probe that is used to stimulate the center of the neuron's cell body (Fig. 3). The mechanically activated (MA) currents we observe using this approach have characteristics consistent with those reported by McCarter *et al.* (1999) reported. MA currents using this stimulation protocol are typically observed in >90% of neurons, consistent with the prevalence of mechanoreceptive neurons in the DRG. Currents activate at a specific membrane displacement and using a series of incremental ($\Delta = 2$ μm) mechanical steps showed that currents are proportional in amplitude to the stimulus size. We hypothesized that if this were an appropriate stimulation technique then different sensory neuron subpopulations would have differential responses to stimulation consistent with their presumptive *in vivo* functions. In two studies we found evidence to support this. First, we categorized neonatal rat neurons as presumptive nociceptors or LTMs based on their response to capsaicin. Nociceptors (i.e., capsaicin-sensitive neurons) displayed smaller MA currents that activated at higher thresholds than those seen in capsaicin-insensitive neurons (Drew *et al.*, 2002). The majority of currents were initially RA with a persistent late phase, although in a subset of capsaicin-insensitive neurons MA currents were observed that displayed SA kinetics. Among smaller neurons, it was observed that IB4-negative neurons generated MA currents but that IB4-positive neurons were largely refractory to mechanical stimulation. In a second study (Fig. 4), we classified adult mouse neurons according to size, action potential width, and capsaicin sensitivity (Drew *et al.*, 2004). Large neurons with narrow action potentials (i.e., LTM neurons) almost exclusively displayed rapidly MA currents that were larger than and kinetically distinct from MA currents in nociceptive neurons (wide action potentials, large or small cell body). Nociceptors (Fig. 5) displayed either SA responses or, what we termed, intermediately adapting currents that were clearly distinguishable from RA and SA currents. Action potential duration, a reliable indicator of a neuron's receptive properties *in vivo* (Djouhri *et al.*, 1998), was the strongest predictor of MA current properties; capsaicin sensitivity was associated only with wide action potential neurons but itself was not indicative of mechanosensitivity. Although a number of neurons did not respond within the stimulus range used, a greater proportion

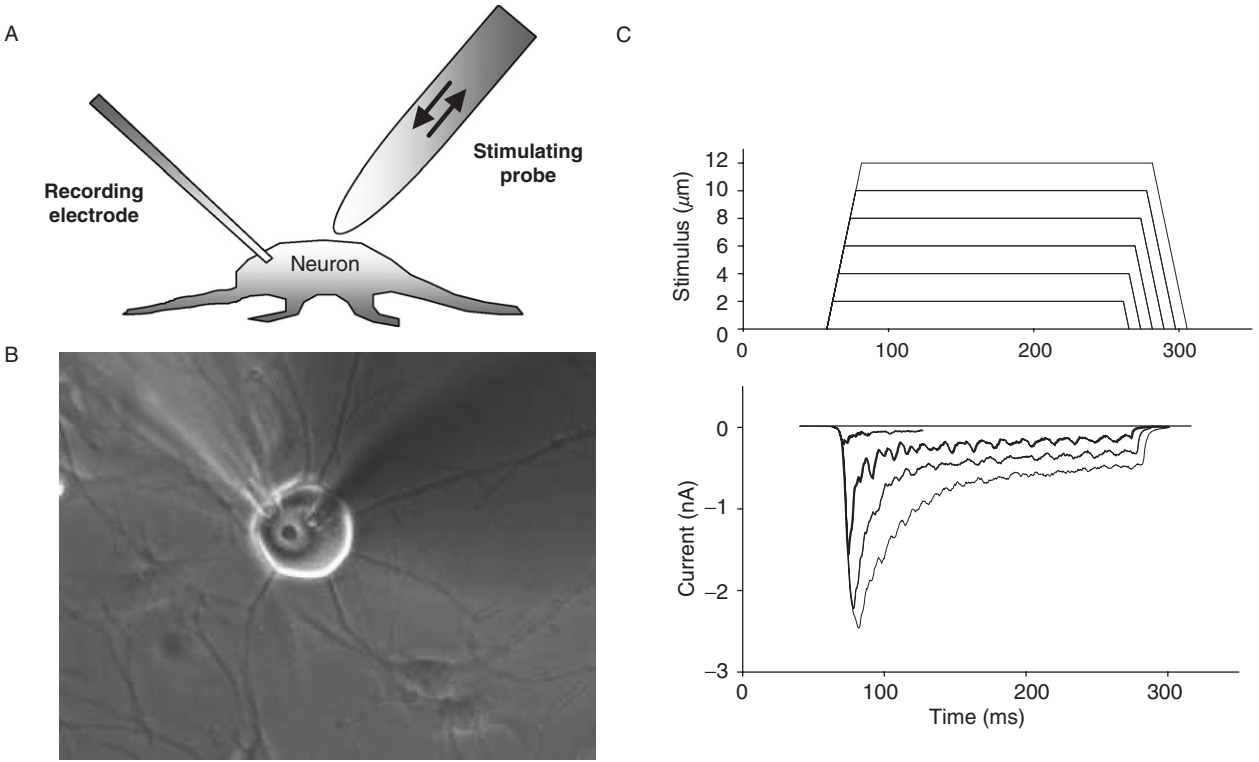

FIGURE 3 Technical setup and recordings. Mechanical stimulation of neuronal cell bodies was achieved using a heat-polished glass probe controlled by a piezocrystal drive. (A) Schematic diagram of the experimental setup and (B) Photo of a neuron during the application of a 6-μm stimulus. The recording electrode contacts the neuron on the top left and the stimulating probe comes from the top right of the picture to the center of the neuron. (C) Mechanical stimulation of cultured DRG neurons evokes graded cationic currents.

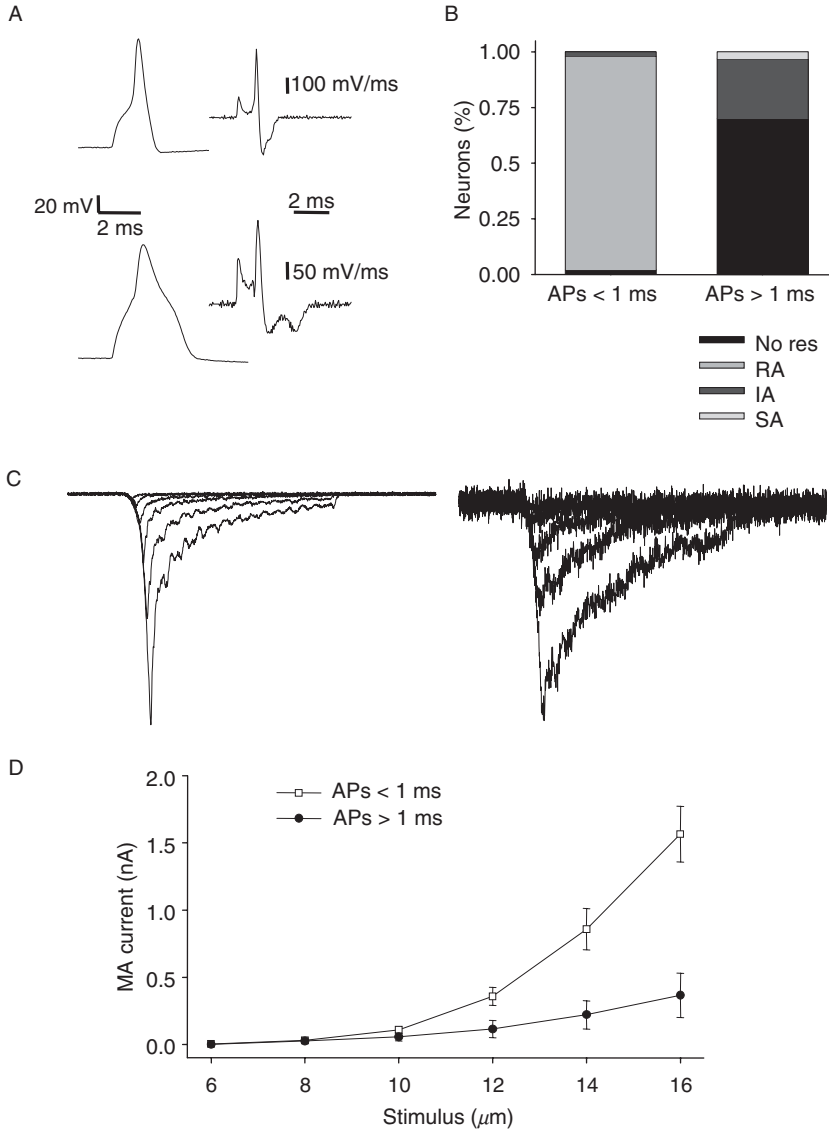

FIGURE 4 Action potentials and mechanically activated currents of large, wild-type mouse DRG neurons. (A) Examples of narrow (top) and wide (bottom) action potentials of large DRG neurons. Action potential traces are shown on the left and the differentials of these waveforms, which allow inflections to be more easily observed, are on the right. (B) Frequency histograms indicating the proportion of neurons with narrow and wide action potentials that respond to mechanical stimulation with rapidly adapting (RA), slowly adapting (SA), intermediately adapting (IA), or no (No res) currents. (C) Example traces of MA currents. Left: RA current

responded if the intensity was increased (unpublished data, LJD); no clear differences between IB4-positive and IB4-negative neurons were seen in adult mouse neurons. Interestingly, compression of the neurites of cultured sensory using a glass probe also evokes MA currents that are kinetically distinct and apparently associated with different cell types (Hu and Lewin, 2006). Overall, these data confirmed a separation of MA current characteristics with neuronal phenotype consistent with the *in vivo* function of these cells.

The MA currents we observe are mediated by nonselective cation channels permeant to calcium and magnesium that reverse at around 0 mV in quasi-physiological solutions and have a relatively linear *I–V* relationship. Replacement of external sodium with the impermeant organic monovalent cation *N*-methyl-D-glucamine reduced current amplitude to a significantly greater degree in nociceptors (by 80%) than in capsaicin insensitive neurons (by 68%). Currents in both cell types are inhibited by external Ca^{2+}, in a manner compatible with this cation acting as a permeant blocker, but to a markedly higher degree in non-nociceptors. This would suggest different channels are operational in the different cell types.

To test this further, we looked at current inhibition by low micromolar levels of Gd^{3+} and ruthenium red in different neuronal populations but the level of block by these compounds is indistinguishable between current types. We have found that FM1-43 (Gale *et al.*, 2001) acts as a permeant blocker of MS ion channels with greater potency at SA currents. These experiments therefore provide some evidence that molecularly distinct ion channels may transduce mechanical stimuli in different DRG neurons.

When we looked for signaling molecules that regulate MA currents, we found that nerve growth factor (NGF) and activation of PKC (but not PKA) acting through distinct mechanisms both increased mechanical responsiveness (Di Castro *et al.*, 2006). Working with neonatal and adolescent rat neurons, it was found that MA currents were potentiated by PKC activation selectively in IB4-negative nociceptors with a relatively rapid time course and in a tetanus toxin-sensitive manner, suggesting that PKC activation induced the insertion of extra MS channels into the cell membrane. NGF, again only acting in IB4-negative neurons consistent with an action on TrkA receptors, increased MA current amplitudes but with a slower time course. Inhibitors of mRNA transcription and protein translation blocked this action suggesting that NGF induces the synthesis of new MS ion channels. Again in this study IB4-positive neurons were essentially insensitive to mechanical stimulation and sensitivity was not increased by PKC activation or application of either NGF or GDNF.

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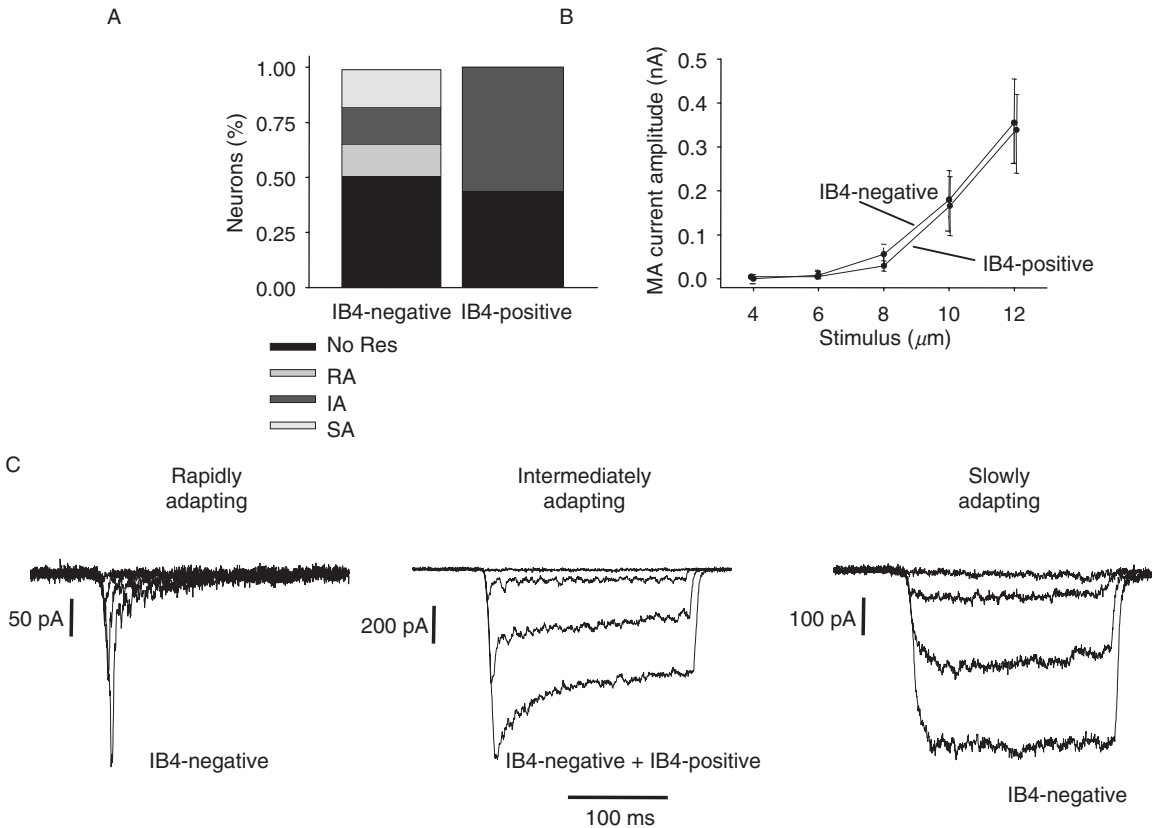
from a narrow action potential neuron. Right: intermediately adapting current from a neuron with a wide action potential. (D) Relationship between stimulus size and MA current amplitude in neurons with narrow and wide action potentials.


A
B
C
Neurons (%)
1.00
0.75
0.50
0.25
0.00
IB4-negative
IB4-positive
No Res
RA
IA
SA
MA current amplitude (nA)
0.5
0.4
0.3
0.2
0.1
0.0
IB4-negative
IB4-positive
4
6
8
10
12
Stimulus (μm)
Rapidly adapting
Intermediately adapting
Slowly adapting
50 pA
200 pA
100 pA
IB4-negative
IB4-negative + IB4-positive
IB4-negative
100 ms

Overall, these studies indicate that in cultured sensory neurons a number of different types of mechanical stimulation are capable of causing either an increase in intracellular Ca^{2+} or a cationic current, that is excitatory responses consistent with what occurs at the sensory terminal. An overview of these investigations, however, suggests that in many cases the transduction process activated by each stimulus type is distinct, and therefore it must be asked how many of these mechanisms are normally active in the sensory terminal in response to physiologically relevant mechanical events. It remains to be determined if the ion channels activated by cell swelling through positive pipette pressure correspond to those gated by compression of the cell by a glass probe. Both evoke a nonselective cationic current but the long activation latency associated with the former stimulus is in stark contrast to the submillisecond delay in gating by an external probe. Furthermore, in the Cho *et al.* (2002) study, this form of pipette pressure activated currents in only around 30% of neurons and neurons with diameters less than 30 μm, that is those most likely to be LTMs did not respond. Similarly, both classes of stretch-activated ion channels recorded at the single-channel level were absent from neurons over 25 μm in diameter arguing against a role in light touch sensation. In contrast, currents evoked by compression of the somatic cell membrane are largest and have the lowest threshold in large neurons, likely derived from LTMs. The initial rapid adaptation of these responses followed by a sustained component is similar to the kinetics of the receptor potential at cat muscle spindles (Hunt and Ottoson, 1975, 1976). RA currents (where MS ion channels close soon after opening) will encode both the magnitude and velocity of a mechanical stimulus; this latter aspect of a stimulus being important in both touch and in monitoring muscle position (Hunt and Ottoson, 1975, 1976). In addition, MA currents are mediated by nonselective cation channels (like all other mechanically evoked currents in DRG neurons) consistent with the limited data available on the ionic basis of transduction at the sensory terminal. Finally, MA currents evoked by membrane compression activate with very short latencies and also inactivate very rapidly when the stimulus is withdrawn; such rapidity is expected of a transduction mechanism that gives accurate information on mechanical stimuli and can encode high frequency vibrations. In contrast, LT ion channels (Cho *et al.*, 2002) remained active for a considerable time (>5 min) after the cessation of the stimulus.

FIGURE 5 MA currents exhibited by wild-type small to medium mouse DRG neurons. (A) Frequency histograms for responses of IB4-negative and IB4-positive neurons; responses were of four types-slowly (SA), rapidly (RA), or intermediately (IA) adapting currents, or no response (No res). (B) Stimulus-response relationships for pooled data from IA and SA currents (IB4-negative neurons) and IA currents (IB4-positive neurons). (C) Example traces of RA, IA, and SA currents.

On balance, the ion channels underlying MA currents evoked by external compression of the membrane represent the strongest candidates for being mechanotransducers in mammalian sensory neurons.

VIII. GATING MS ION CHANNELS IN DRG NEURONS

Estimating the force required to gate mechanosensitive ion channels is difficult especially for whole-cell stimuli. For example, increases in membrane tension in response to hypotonicity is distinct in different types of neurons depending on their membrane reserves and the degree of membrane insertion and retrieval during volume changes (Zhang and Bourque, 2003). Localized stimulation of neuronal somata with a glass probe will result in a focused area of membrane stretch surrounded by a concentric pressure gradient; as the stimulus size is increased, the area of neuronal membrane stretched above resting tension will expand and the membrane stretch at any point within that area will increase. Therefore, the population of MS ion channels in the membrane is exposed to a range of tensions and increasing the stimulus intensity will increase current amplitude due to activation of more channels (i.e., the area of suprathreshold membrane tension increases) and potentially by affecting the behavior (e.g., slowing the rate of closing/increasing the rate of reopening) of channels exposed to higher tensions (Drew *et al.*, 2004). [Cho *et al.* (2002) found that increasing membrane tension increased channel activity primarily by a reduction in the duration of long closings.] These concerns make kinetic analysis of channel opening and closing using such protocols limited; in addition, the relatively slow movement of the probe means that the area of sufficient stretch develops over several milliseconds. Finally, if sensory neurons differ in their amount of membrane reserve and degree of crenellation, then the degree of membrane stretch for a given displacement will vary.

The use of membrane patches allows more accurate prediction of the tension reaching the channel under observation (Cho *et al.*, 2002). Indeed, high-speed, pressure-clamp devices have been developed for the study of ion channels in membrane patches (Besch *et al.*, 2002), which allow for rapid actuation of membrane stretch that is important given the rapid activation and adaptation/inactivation of many MS ion channels.

Current models of the gating of mechanosensitive ion channels suggests that they are activated either by the direct sensing of membrane tension, as is the case for bacterial MS channels, or due to tethering to intracellular and/or extracellular structures that the channel moves relative to (Kung, 2005). Direct gating by membrane tension can be demonstrated when a known

protein is reconstituted in a lipid bilayer and gated by stretch. Unequivocal evidence of the second mechanism is more difficult because if gating is dependent on cytoskeletal elements it remains possible that these structures exert their effects indirectly by interactions with the cell membrane.

MA currents evoked by external membrane compression (Drew *et al.*, 2002) are inhibited by cytochalasin whereas stretch-activated ion channels (Cho *et al.*, 2002) in DRG neurons are reduced by both cytochalasin and colchicine. These data suggest that the activity of these channels may be reliant on cytoskeletal anchoring. However, actin disruption could exert its effects not by disturbing an interaction between the ion channel and an intracellular anchor but by reducing membrane tension in the vicinity of the channel by abolishing the normal interaction between the cortical cytoskeleton and the plasmalemma. The behavior of MS channels in their nonnative environment (e.g., in the cell soma or on the neurite) may be different from that at the sensory terminal if the resting membrane tension adjacent to the channel varies. It is not known if these specialized mechanosensory regions have local areas of prestressed membrane that could affect channel gating. If membrane tension is the key gating factor, then channel activation would be due to both the intrinsic sensitivity of the channel and the resting membrane tension determined by the cytoarchitecture of the terminal. These two factors will have coevolved for optimal activation of the ion channel in response to relevant stimuli and could mean that different membrane arrangements at subtypes of mechanoreceptor endings affect channel behavior. In addition to cytoskeleton–membrane interactions, the lipid composition of the membrane may also be important. For example, the cholesterol content of the membrane, particularly in microdomains, will affect local membrane stiffness (Lundbaek *et al.*, 2004). In addition, PIP2 in the cell membrane could be a key molecule in coupling MS channels to membrane stretch; Chemin *et al.* (2005) showed that high levels of PIP2 can gate the mechanosensitive ion channel TREK-1 and that this molecule appears to be necessary for mechanogating. Molecular cloning of the DRG mechanotransduction channel will facilitate the answering of these questions.

IX. CANDIDATE ION CHANNELS

Studies of invertebrate mechanosensation using screens of mechanosensitive mutants in *Caenorhabditis elegans* and *Drosophila melanogaster* (Ernstrom and Chalfie, 2002; Tracey *et al.*, 2003) have focused attention on two classes of ion channels as potential mechanotransducers in mammals: the DEG/ENaC (degenerin/epithelial sodium channels) and TRP superfamilies.

A. DEG/ENaC Ion Channels

1. DEG/ENaC Ion Channels in *C. Elegans* Mechanosensation

The current model of mechanotransduction in *C. elegans* is that an ion channel lies at the center of a multiprotein complex tethered to intracellular and extracellular structures. The ion channel is made up of two subunits, MEC-4 and MEC-10, of the DEG/ENaC family. These channels physically interact with MEC-2, a stomatin-like protein (Goodman *et al.*, 2002), and MEC-6, a single membrane pass protein, with a low homology to human paraoxonases (Chelur *et al.*, 2002). The functional ion channel is an amiloride-sensitive sodium channel. Evidence that this ion channel complex directly transduces mechanical stimuli came from Suzuki *et al.* (2003a) using a transgenic nematode line that expresses a cameleon Ca^{2+}-indicator protein. Low levels of mechanical stimulation evoked Ca^{2+} transients in wild-type touch receptor cells but not in those from mec-4, mec-6, and mec-2 loss-of-function mutants. Subsequently, O'Hagan *et al.* (2005) definitively made direct, *in situ* recordings of mechanically evoked receptor currents in patch-clamped touch receptor cells. These neurons generated rapidly activating and inactivating inward currents at the application and the withdrawal of a mechanical stimulus. Mechanically activated currents were absent in nematodes with loss-of-function mutations in mec-2, mec-4, and mec-6. Interestingly, mec-7 mutants displayed currents significantly smaller than those in wild-type animals, suggesting this microtubule is required for normal mechanosensitivity, but not for channel gating.

Mechanosensory functions have been proposed for two other nematode DEG channels, both of which are in the same subgroup as the MEC channels and have a conserved extracellular regulatory domain (Goodman and Schwarz, 2003). They are UNC-105 (Liu *et al.*, 1996), which is expressed in muscle cells, and UNC-8 (Tavernarakis *et al.*, 1997), which is expressed in sensory neurons, motor neurons, and interneurons. In *Drosophila*, Adams *et al.* (1998) identified PPK1, a DEG/ENaC homologue, expressed in the sensory dendrites of type II sensory receptors. When Ainsley *et al.* (2003) ablated the gene for PPK1, they found that mutants had normal larval touch sensitivity but showed locomotor abnormalities possibly due to a reduction in mechanosensory feedback during movement.

2. ASICs and Mammalian Mechanosensation

Following the extensive work defining the molecular components of the mechanotransduction complex in the touch receptors of *C. elegans*, the structurally related ENaCs and ASICs appeared to be plausible candidates for mammalian mechanotransducing channels. In particular, ASICs (acid sensing ion channels, so named as most members of this subfamily are activated by rapid drops in pH) were seen as strong candidate mechanosensors because

they are highly expressed in sensory neurons and two isoforms, ASIC3 and ASIC1b, are almost exclusively found in these cells. However, the evidence that these channels mediate mechanotransduction in mammalian sensory neuron is unconvincing.

The mechanically evoked firing patterns of cutaneous afferent fibers in ASIC1 (Page *et al*., 2004), ASIC2 (Price *et al*., 2000), and ASIC3 null mutants (Price *et al*., 2001) have all been characterized using the skin-nerve preparation. Overall, these analyses have shown very subtle differences between knockout animals and wild-type controls. The behavior of cutaneous mechanoreceptors in mice lacking the ASIC1 gene was entirely normal. In ASIC2 nulls, Aβ-fibers had reduced suprathreshold responses to mechanical stimuli; the decrease was small in SA LTMs, but in RA LTMs firing was reduced by around 50%. Conversely, in ASIC3 nulls, RA LTMs displayed increased rates of firing in response to mechanical stimulation whereas there was a reduction in the mechanosensitivity of Aδ-nociceptors.

While the phenotypes of ASIC2 and ASIC3 nulls could be consistent with a role for these channels in transduction, it is difficult to reconcile the broad expression of ASICs in the DRG with small changes in subpopulations of receptors. The voltage insensitivity of ASICs and the apparently normal membrane properties of ASIC2 and ASIC3 knockout (KO) neurons (Price *et al*., 2000; Drew *et al*., 2004) suggest such phenotypes are not due to decreased nerve excitability. However, the firing rates of fibers in response to electrical stimulation were not reported and ASIC3 mutants also showed reduced firing in response to heat stimuli. Additionally, a number of other papers have also cast doubt on the likelihood of ASICs transducing mechanical stimuli in sensory neurons. Roza *et al.* (2004), using a different strain of ASIC2 null mice to Price *et al.* (2000), failed to find a significant difference in mechanically evoked firing patterns in RA LTMs and this group also found indicators of auditory and intestinal mechanosensation to be unchanged in these animals. Additionally, transgenic mice expressing a dominant-negative form of ASIC3, which knocked down expression of any functional ASICs (as assessed by application of low-pH stimuli), had normal behavioral responses to mechanical stimuli (Mogil *et al*., 2005).

In a study of cultured DRG neurons from ASIC2 and ASIC3 KOs (Drew *et al*., 2004), MA currents evoked in neurons derived from LTMs (based on the generation of narrow action potentials) were found to be normal in both single KOs and cells lacking both ASIC2 and ASIC3. In addition, MA currents in nociceptive neurons were normal in the double KOs. The stretch-activated ion channels observed by Cho *et al.* (2002) in DRG neurons were insensitive to amiloride which blocks ASICs. These data together demonstrate that neither of these forms of mechanical stimulation activate ASICs in sensory neurons and no data has been published showing direct gating

of ASICs by pressure. While the caveat exists that ASICs would only be mechanosensitive when expressed in the correct environment at the nerve terminal, a number of evolutionary observations argue against them functioning in mechanosensation. First, the phylogenetic tree of structurally related ENaC/ASIC/DEG ion channels reveals a substantial diversification of these molecules in *C. elegans* and *D. melanogaster* that is not apparent in vertebrates and that those channels most strongly implicated in nematode mechanotransduction form a small subgroup that is distantly related to mammalian ASICs (Goodman and Schwarz, 2003). Second, ASIC homologues are present in zebrafish and are expressed across the nervous system but are absent from sensory neurons negating a role in sensory transduction (Paukert *et al.*, 2004). Furthermore, this group showed that proton sensitivity of ASICs arose recently in evolution (Coric *et al.*, 2005). This observation and uncertainty that pH falls substantially and rapidly enough to activate these channels in mammals means that the function of ASICs in both the peripheral and central nervous systems remains enigmatic. However, on balance, the available evidence suggests they are not mechanotransducing channels.

B. TRP Ion Channels

1. TRP Channels in Invertebrates

Genetic screens of invertebrates have identified a number of members of the TRP ion channel family as either mechanotransducers or essential for the function of mechanosensory cells (Lin and Corey, 2005). OSM-9 (Colbert *et al.*, 1997) and OCR-2 (Tobin *et al.*, 2002) appear to form a heteromeric ion channel expressed in the sensory processes of the ciliated ASH neurons in *C. elegans* that is required for the detection of nose touch by these cells. This channel appears to be polymodal in function, responding also to osmotic and chemical stimuli, and it remains to be determined if the channel is directly gated by each stimulus class or acts downstream of the true transducer. Also in the nematode, TRP-4 has been identified as the likely mechanosensory channel of a proprioceptive neuron, DVA (Li *et al.*, 2006). TRP-4 mutants exhibit abnormal movement and Ca^{2+} transients evoked by body bending in wild-type DVA neurons are absent in these animals.

TRP-4 is the *C. elegans* homologue of TRPN1, which was originally identified (as NompC) by Walker *et al.* (2000) as a candidate mechanotransduction channel in bristle receptors of *Drosophila*. Mutations causing premature stop codons in *nompC* led to a loss of all but a small nonadapting part of the mechanoreceptor potential recorded extracellularly from the bristle. Additionally, one mutant (cysteine to tyrosine at residue 1400, close

to the fourth predicted transmembrane domain) was isolated that increased significantly the rate of adaptation of mechanically evoked potentials, providing strong evidence that this channel is itself mechanically gated. There is no close mammalian homologue of TRPN1 but, surprisingly, Sidi *et al.* (2003) found that zebrafish selectively express an orthologue of TRPN1 in hair cells and that downregulation of its expression using morpholino-antisense oligonucleotides abolishes extracellularly recorded microphonic potentials. In addition, an orthologue of TRPN1 is found in *Xenopus*, where it is expressed in structures with mechanosensory functions, although its precise distribution was not consistent with it being the transduction channel itself (Shin *et al.*, 2005).

Drosophila nompC mutants have only moderate deficits in auditory responses (Eberl *et al.*, 2000). In *Drosophila*, responses to auditory stimuli are detected by structures analogous to bristle receptors in Johnston's organ, within the antennae of the fly. Transduction here is likely mediated by a heteromeric complex of two TRP channel subunits: Nanchung (NAN; Kim *et al.*, 2003) and Inactive (IAV; Gong *et al.*, 2004). NAN is related to OSM-9 whereas IAV is more closely related to OCR-4. In flies lacking functional genes for either channel, sound-evoked antennal afferent nerve activity is absent. These observations along with the distribution of these channels make them very strong candidates for being mechanogated transduction channels.

Finally, in *Drosophila*, the TRP ion channel, *painless*, has been implicated in noxious mechanosensation by Tracey *et al.* (2003). This group developed a genetic screen for studying nocifensive behavior in *Drosophila* larvae and showed that animals lacking functional *painless* expression showed defective behavioral responses to noxious temperatures and noxious pressure. Painless is expressed in a discrete punctate fashion on the dendrites of putative nociceptors, and the sensory deficits of mutants suggested that painless functions as a transducer of noxious stimuli in multiple modalities. However, channel activation by physical stimuli was not demonstrated and so it is possible that the protein acts up- or downstream of transduction.

2. TRP Candidates in Mammals

Several mammalian TRP channels have been described as being mechanically activated. Among them TRPV4, the closest mammalian homologue of OSM-9, seems to be a very attractive candidate as an HTM as it appears to be activated by both osmotic and mechanical stimuli (Liedtke and Kim, 2005) and is expressed in some DRG neurons from both LTM and HTM groups (Alessandri-Haber *et al.*, 2003; Suzuki *et al.*, 2003b). Studies have shown that

TRPV4 knockout animals display altered osmotic regulation and pressure sensation (Liedtke and Friedman, 2003; Mizuno *et al.*, 2003; Suzuki *et al.*, 2003c) while other works have described the involvement of TRPV4 in nociception in response to hyper- and hypoosmotic stimuli and in inflammation-induced mechanical hyperalgesia (Alessandri-Haber *et al.*, 2003, 2005, 2006). Nevertheless, it is important to keep in mind that if TRPV4 is effectively implicated in mechanosensation, its activation by mechanical and/or osmotic stimuli seems to be indirect and requires the synthesis of 5′,6′-epoxyeicosatrienoic acid [5′,6′-EET], a metabolite of AA (Vriens *et al.*, 2004). This suggests an activating mechanism for TRPV4 whereby a membrane mechanical sensor is coupled to the phospholipase A2 (PLA2) which in turn metabolizes membrane phospholipids to synthesize metabolites that in the end lead to the opening of TRPV4. Other members of the TRPV subfamily have been implicated in osmoregulation and mechanosensation processes. Birder *et al.* (2002) demonstrated that TRPV1 was necessary for normal bladder function in that it is essential to the purinergic release triggered by the mechanical distension of the bladder. An N-terminal splice variant of TRPV1 has been found to be expressed in the osmosensitive arginine-vasopressin-releasing neurons of the supraoptic nucleus, where it is proposed to be part of the central osmoreceptor (Sharif Naeini *et al.*, 2006). Finally, there is evidence that TRPV2 can be activated by an osmotic challenge in mouse aortic myocytes and can also be activated by membrane stretch when expressed in heterologous systems (Muraki *et al.*, 2003). Bearing in mind that TRPV1, TRPV2, and TRPV4 are all expressed in DRG neurons, all three channels may participate in mechanosensation.

Another candidate TRP channel is TRPA1, the mammalian homologue of the fly gene *painless* (Chapter 8, this volume). Corey *et al.* showed in 2004 that TRPA1 was expressed in the hair cells of the inner ear and that the mRNA for TRPA1 appeared at embryonic day E17, exactly matching the onset of mechanosensitivity in these cells. Furthermore, they also showed that the downregulation of the TRPA1 protein induced an alteration of the receptor cell function. This was seen as a major breakthrough as TRPA1 is known to be expressed in DRG neurons (although there is a debate over the proportion of neurons expressing it), where it is believed to act as a noxious cold sensor (Story *et al.*, 2003). But two recent studies using TRPA1 knockout mice oppose the assumption that TRPA1 might be the mechanosensor in both hair cells and DRG neurons. Bautista *et al.*, (2006) could not find any deficit in cold sensation and sound detection in these animals. On the other hand, Kwan *et al.* (2006) observed alterations in cold sensitivity but not in hearing and balance functions. Nevertheless, altered responses to punctate mechanical stimuli were reported, suggesting that TRPA1 might be part of a broader mechanosensitive complex in DRG neurons. Hence, the channel or

channel complex acting as the mechanotransducer of physiological and/or noxious tactile stimuli remains to be discovered.

Other mechanically activated mammalian TRP subunits might be involved but their expression in DRG neurons has yet to be determined. Among these channels are the polycystins TRPP2 and TRPC1. TRPP2 is a cation-permeable channel involved in the autosomal dominantly inherited polycystic kidney disease in which mutations in either the TRPP1 or TRPP2 gene cause the occurrence of cysts in the kidney and the liver (Delmas, 2005). TRPP2, in association with TRPP1 which is not a channel protein, form functional membrane channels tightly associated to actin filaments (Montalbetti *et al*., 2005) and located to the primary cilia of kidney epithelial cells where they function as calcium permeable channels opening in response to fluid flow (Nauli *et al*., 2003). TRP2 expression is widespread including high levels in the heart (Volk *et al*., 2003) as well as in the embryonic nodal cilia that determine the left–right body axis (McGrath *et al*., 2003). It is not known yet whether TRPP2 is directly activated by a mechanical stimulus but it has been already demonstrated that TRPP1 is able to activate TRPP2 when these two channels are associated (Delmas *et al*., 2004), suggesting that the mechanosensitivity of TRPP2 may depend on TRPP1. Nevertheless, the functionality of TRPP2 alone in responding to mechanical stimuli has also been shown in epithelial cells (Montalbetti *et al*., 2005). TRPC1 has also been shown to be mechanically activated (Maroto *et al*., 2005). In this study, the authors sought to describe the molecular identity of the mechanosensitive cation channel (MscCa) located in cytoskeleton-deficient membrane vesicles of the *Xenopus* oocytes. When the human TRPC1 was expressed in CHO-K1 cells, it also showed a mechanoresponsive behavior. These data demonstrated first that TRPC1 was mechanically activated but also that it did not need any connection to the cytoskeleton to be functional as opposed to TRPP2.

Two more mammalian TRP subunits may be considered as mechanosensor candidates. TRPML3 is expressed in hair cells and was shown to be the gene responsible for the semidominant mouse mutant varitint-waddler which displays early-onset hearing loss and vestibular defects (Di Palma *et al*., 2002). Finally, another kidney-located TRP channel, TRPM3, was shown to be activated by a decrease in osmolarity causing cell swelling (Grimm *et al*., 2003).

C. Mechanosensitive Potassium Channels

The two-pore potassium channels TASK and TREK-1 are known to be mechanically gated (Patel *et al*., 2001). TREK-1 is present in small diameter sensory neurons that are usually assumed to be nociceptors. TREK-1 is a

polymodal ion channel that is activated by lipids, membrane stretch, G-protein–subunits and heat. In addition, the volatile anesthetics nitrous oxide, xenon, and cyclopropane (that may act in part through inhibition of NMDA receptors) have been shown to be potent activators of TREK-1 at clinically relevant concentrations (Gruss *et al.*, 2004). Interestingly, these activators require the presence of a particular amino acid (Glu-306) that also has been implicated in channel activation by AA and membrane stretch.

A study of knockout mice showed a pronounced pain phenotype (Alloui *et al.*, 2006). Mice with a disrupted TREK-1 gene were more sensitive to painful heat and mechanical stimuli (albeit low-intensity stimuli in the von Frey test and not in the Randall–Selitto test) and showed enhanced hyperalgesia in conditions of inflammation, demonstrating a role for TREK-1 in inhibiting noxious input into the CNS. However, less obviously, osmotic stress in inflamed knockout animals resulted in a lowered pain phenotype. These observations suggest that TREK-1 may be physiologically activated by polymodal noxious stimuli and shape the form of the receptor potential. If this is the case then TREK-1 would act as an excitability brake, although it remains to be determined if general baseline neuronal excitability is decreased in these animals.

X. VOLTAGE-GATED CHANNELS AND MECHANOSENSATION

Measuring mechanically gated channels in sensory neurons in voltage clamp has been very informative, but in normal circumstances, sufficiently large mechanically evoked depolarizations will alter the activity of voltage-dependent channels possibly resulting in action potential generation. Interestingly, a number of voltage-gated channels (and some ligand-gated channels) are directly influenced by mechanical stimuli. NMDA receptors, for example, have been shown to be modulated by changes in osmolarity being potentiated in hypoosmotic conditions and inhibited by external hyperosmotic solutions (Paoletti and Ascher, 1994).

A. Sodium Channels

Of the nine voltage-gated sodium channel α-subunits (Nav1–9), three are strongly associated with expression in sensory neurons (Nav 1.7, Nav 1.8, and Nav 1.9), while most other subunits (apart from Nav1.4) are expressed to some extent in these cells. Interestingly, there is some evidence that mechanical stimuli can effect the peak current density of Nav1.5 in intestinal cells, and this effect depends on interactions of the C-terminus of the sodium

channel with the PDZ domains of syntrophin, an actin-binding protein (Ou *et al.*, 2003).

As voltage-gated sodium channels propagate action potentials, alterations in their threshold of activation have profound effects on peripheral pain thresholds, and may also be involved in the phenomenon of allodynia, where nonnoxious touch may be perceived as a painful stimulus (Wood *et al.*, 2004). Posttranslational modifications of sodium channels by inflammatory mediators involving phosphorylation have been shown to underlie some of these effects, for example in Nav1.8. This sodium channel is specifically associated with nociceptors, and its *I–V* relationship is shifted to more negative potentials and its peak current increased in response to PKA-mediated phosphorylation of serine residues in the second intracellular loop (Fitzgerald *et al.*, 1999). Of all sodium channels, Nav1.7 seems to play the most significant role in altering inflammatory pain thresholds and by analogy noxious mechanical thresholds, although the underlying mechanism is unknown (Nassar *et al.*, 2004). Nav1.7 is present at high densities at the terminals of nociceptive neurons (as well as sympathetic neurons) and its function has been addressed by the study of tissue-specific knockout mice. When Nav1.7 is deleted in nociceptors, thermal pain thresholds and non-noxious mechanosensation are apparently normal. Strikingly, noxious mechanosensation is almost completely abolished (Nassar *et al.*, 2004). In the Nav1.8 null mutant, a similar phenotype is apparent (Akopian *et al.*, 1999). Investigations of sensory processing in the spinal cord of Nav1.8 null mutants by Matthews *et al.* (2006) also revealed a selective deficit in mechanical over thermal input. This suggests that nerve fibers expressing Nav1.7 and Nav1.8 are necessary for noxious mechanosensation. In Nav1.8 null mutants, the mechanosensitivity of neuromas is also attenuated, consistent with this idea (Roza *et al.*, 2004). Capsaicin killing of TRPV1-positive neurons which include many nociceptors expressing Nav1.7 and Nav1.8 also results in a similar phenotype, without much effect on thermal pain thresholds (Hayes and Tyers, 1980). This suggests that there is either a quantitative difference in sensory coding for thermal and mechanical stimuli, where far fewer functional nociceptors are required to convey information about noxious heat, or suggests that a different set of fibers are involved in signaling the extent of noxious mechanical events from damaging levels of heat. An alternative explanation could be that the channel associated with noxious mechanosensation are expressed in a membrane domain at sensory nerve terminals close to Nav1.7 and Nav1.8. Uncoupling of the link between these sodium channels and mechanosensory channels would then lead to the specific loss of noxious mechanosensation. Identifying the proteins that interact with these sodium channels may thus provide us with clues to the identity of the noxious mechanotransducing channels.

B. Calcium Channels

An association between the expression of the voltage-gated calcium channel Cav3.2 in NT-4-dependent D-hair cells has been claimed recently, and pharmacological data accumulated to suggest this calcium channel may have a role to play in these highly mechanosensitive cells (Shin *et al.*, 2003). The authors suggest that this low-threshold calcium channel may be able to amplify small receptor potentials and account in part for the high sensitivity of D-hair receptors. A broader role for these channels in sensory neuron function is suggested by antisense studies, which demonstrated a pronociceptive role for these channels in hyperalgesia and allodynia (Bourinet *et al.*, 2005). Bouskila and Bostock (1998) showed direct effects of mechanical stimulation on calcium currents in sensory neurons. N-type calcium channel activity increased by over 70% when sensory neurons were subjected to a stream of buffer, while T-type currents show a small decrease.

XI. INDIRECT SIGNALING BETWEEN SENSORY NEURONS AND NONNEURONAL CELLS

Do the specialized end organs in which LTM afferent fibers terminate transduce mechanical stimuli and signal to sensory neurons (Ogawa, 1996)? While the function of Pacinian corpuscles as mechanical filters (Bell *et al.*, 1994) is well established, the possibility that Merkel cells transduce stimuli has been more contentious. Merkel cells have a number of attributes of a neurosecretory cell; they contain dense core vesicles close to the region apposing the nerve terminal (Haeberle *et al.*, 2004). This led to speculation that Merkel cells respond to mechanical stimulation and chemically communicate sensory information to the nerve terminal. Toxic ablation of these Merkel cells has given conflicting data (probably due to both incomplete eradication of Merkel cells and/or secondary damage to the sensory nerves); however, when Mills and Diamond (1995) ablated Merkel cells with near UV light and carefully mapped the affected touch domes, they showed that normally functioning SA1 fibers were present in the absence of Merkel cells. Moreover, Kinkelin *et al.* (1999) have shown that Merkel cells are almost entirely eliminated postnatally in p75 null mutant mice with no change in the physiology of SA mechanoreceptors.

A role for ATP in mechanosensation has been suggested by a number of studies that show that this mediator may be released from distorted tissue and act on the terminals of sensory neurons (for example, Cockayne *et al.*, 2000). ATP is released from *Xenopus* oocytes vesicular stores in response to mechanical stimulation in an exquisitely sensitive fashion (Maroto and

Hamill, 2001). Expression cloning in *Xenopus* oocytes lead to the identification of P2Y1 receptors as potential mechanosensors which were activated by low levels of ATP released from oocytes (Nakamura and Strittmatter, 1996). Interestingly, P2Y1 is expressed at high levels in large diameter sensory neurons. Cocultures of human keratinocytes with mouse DRG neurons have revealed that mechanical activation of the keratinocytes results in intracellular calcium waves that depend on the presence of extracellular ATP (Koizumi *et al*., 2004). A similar increase in calcium could be measured in the DRG neurons, and this depended on the presence of P2Y2 receptors, suggesting a role for keratinocyte-released ATP in signaling to sensory neurons. However, direct application of ATP to toad skin preparations support a modulatory rather than excitatory role for ATP. At high concentrations, ATP suppressed impulse discharge from SA mechanoreceptors without effect on RA mechanoreceptors (Fallon *et al*., 2002).

Finally, a role for the AA metabolite 5′,6′-epoxyeicosatrienoic acid (5′,6′-EET) in gating TRPV4 has been demonstrated (Vriens *et al*., 2004). As eicosanoids are membrane permeant, it is possible that external sources of this metabolite generated after the activation of PLA2 by tissue damage could activate TRPV4.

XII. CONCLUSIONS

Mechanically regulated electrical activity by touch and tissue damaging levels of pressure in sensory neurons seems to involve a variety of direct and indirect mechanisms and ion channels, and the involvement of specialized end organs in mechanotransduction complicates matters even more. Imaging studies are providing useful information about the events in the central nervous system associated with touch pain and allodynia (a pathological state where touch becomes painful; Naito and Ehrsson, 2006; Ruehle *et al*., 2006). In contrast, although a variety of TRP channels are potential candidate mechanosensors, and there is evidence of a role for TRPV4 in some aspects of mechanosensation, the channels underlying touch and noxious mechanosensation remain to be identified.

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