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Sensory Functions for Degenerin/Epithelial Sodium Channels (DEG/ENaC)

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ABSTRACT

All animals use a sophisticated array of receptor proteins to sense their external and internal environments. Major advances have been made in recent years in understanding the molecular and genetic bases for sensory transduction in diverse modalities, indicating that both metabotropic and ionotropic pathways are important in sensory functions. Here, I review the historical background and recent advances in understanding the roles of a relatively newly discovered family of receptors, the degenerin/epithelial sodium channels (DEG/ENaC). These animal-specific cation channels show a remarkable sequence and functional diversity in different species and seem to exert their functions in diverse

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sensory modalities. Functions for DEG/ENaC channels have been implicated in mechanosensation as well as chemosensory transduction pathways. In spite of overall sequence diversity, all family members share a unique protein topology that includes just two transmembrane domains and an unusually large and highly structured extracellular domain, that seem to be essential for both their mechanical and chemical sensory functions. This review will discuss many of the recent discoveries and controversies associated with sensory function of DEG/ENaC channels in both vertebrate and invertebrate model systems, covering the role of family members in taste, mechanosensation, and pain.

I. INTRODUCTION

The rapid advancements in molecular and genomic biology have resulted in a wealth of information about how genes and their protein products affect cellular and organismal functions, and how such functions evolved. These data also led to the realization that multiple independent protein families have often evolved to serve similar physiological functions. The complex relationship between protein structure and physiological functions highlights the importance of studying such relationships with integrative and comparative approaches.

One of the most diverse groups of proteins in terms of the relationship between protein structure and function are ion channels. These membrane-targeted proteins are found in all cell types, including prokaryotes, and are critical for maintaining the appropriate ionic gradients across all cellular barriers, including the plasma membrane and intracellular compartments (Ashcroft and ScienceDirect (Online service), 2000). This review focuses on a relatively newly discovered and enigmatic family of ion channels; degenerin/epithelial Na^+ channels (DEG/ENaC). DEG/ENaC proteins form nonvoltage gated, amiloride-sensitive cation channels (Bianchi and Driscoll, 2002; Garty and Palmer, 1997). DEG/ENaC channels comprise three to nine independent subunits, which can be either hetero- or homomultimers (Benson *et al.*, 2002; Canessa *et al.*, 1994b; Eskandari *et al.*, 1999; Jasti *et al.*, 2007; Kellenberger and Schild, 2002; Zha *et al.*, 2009b). In cases where members of the family have been characterized electrophysiologically, subunit composition was found to have a significant effect on the pharmacological and electrical properties of the channel, suggesting that subunit composition is a critical regulatory mechanism in these channels (Askwith *et al.*, 2004; Benson *et al.*, 2002; Chu *et al.*, 2004; Xie *et al.*, 2003; Zha *et al.*, 2009a; Zhang *et al.*, 2008).

Despite of the high diversity in the primary sequence of individual subunits, several structural constituents indicated that all members of the family have a similar protein topology (Bianchi, 2007; Bianchi and Driscoll, 2002; Corey and Garcia-Anoveros, 1996; Tavernarakis and Driscoll, 2000, 2001a).

The typical DEG/ENaC subunit has two transmembrane domains, two short intracellular domains and a large extracellular loop, which is a hallmark characteristic of the DEG/ENaC protein family topology (Fig. 1.1). The DEG/ENaC family seems to be animal specific and many different members have been identified in diverse species (Fig. 1.2).

In the few instances where the pharmacological, structural, and biophysical properties of specific DEG/ENaC subunits have been studied, the channels have been characterized as ligand-gated, voltage insensitive, depolarizing cation channels, which seem to be more selective for Na^+ over Ca^{2+} and K^+ (Garty and Palmer, 1997). The physical cloning of various DEG/ENaC subunits enabled the identification of selective agonists and antagonists for specific subunits. In addition, natural ligands and physical stimuli were found to activate or modulate channel functions. These include (1) peptides such as members of the invertebrate FMRFamide family (Askwith *et al.*, 2000; Green *et al.*, 1994; Lingueglia *et al.*, 1995; Xie *et al.*, 2003), mammalian FFamide and SFamide peptides (Deval *et al.*, 2003; Sherwood and Askwith, 2008, 2009), natural, and dynorphin-related opioid peptides (Sherwood and Askwith, 2009); (2) small increases in extracellular proton concentrations (Adams *et al.*, 1998b; Benson

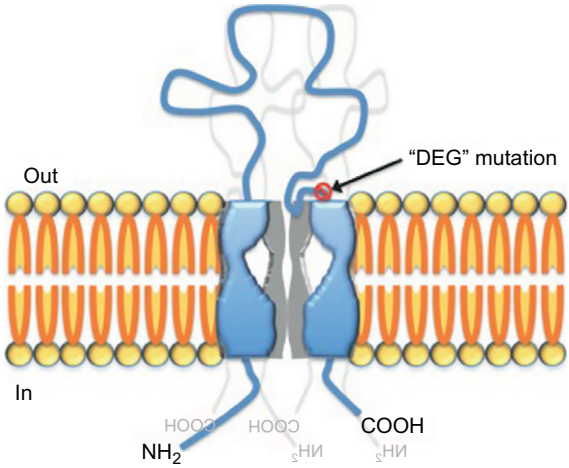

Figure 1.1. Topology of a typical DEG/ENaC channel. Each channel comprises three subunits (or multiples of three). Channels can be either homomeric or heteromeric protein complexes and are likely to include other accessory proteins. Each subunit comprises two transmembrane domains, two short intracellular domains (N terminus is typically longer than the C terminus), and an unusually large and highly structured extracellular domain. The "DEG mutation" represents an amino acid residue, which was shown to lock DEG/ENaC channels in a constitutively open state (Snyder *et al.*, 2000).

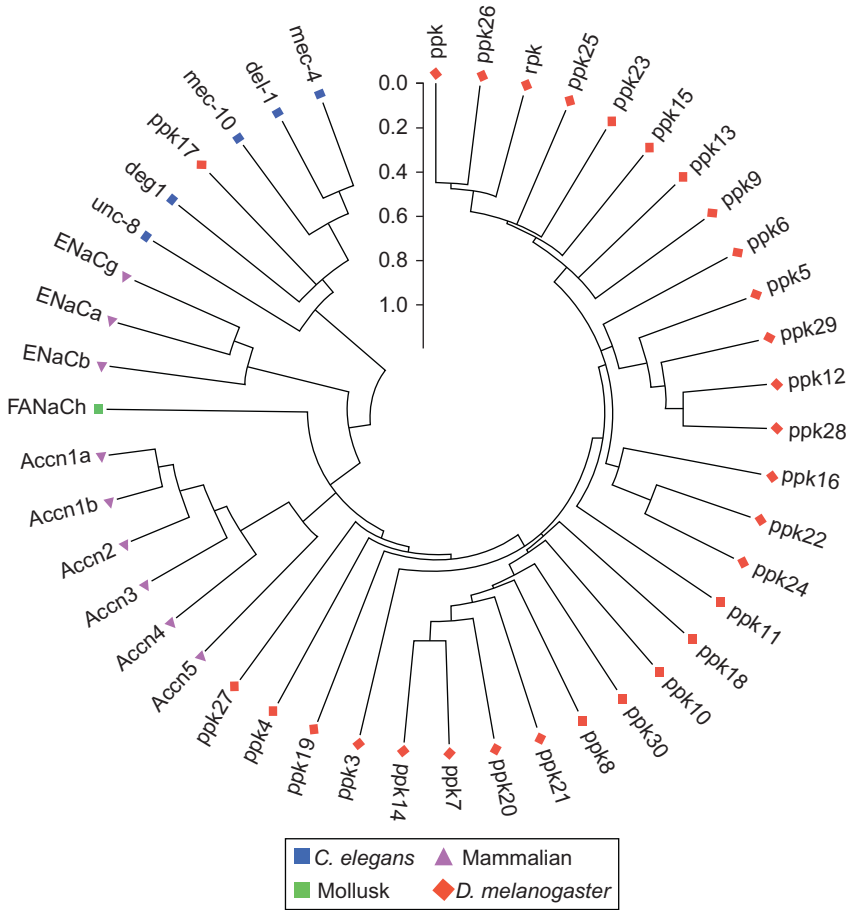

Figure 1.2. Molecular phylogenetic anaylsis of DEG/ENaC protein sequences. Evolutionary analyses were conducted in MEGA5 using default parameters (Tamura *et al.*, 2007). The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model (Jones *et al.*, 1992). The tree with the highest log likelihood (− 9302.3626) is shown. Initial tree(s) for the heuristic search were obtained automatically as follows. When the number of common sites is less than 100, or less than one-fourth of the total number of sites, the maximum parsimony method was used; otherwise BIONJ method with MCL distance matrix was used. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 45 amino acid sequences, which included representatives from *Drosophila* (*rpk*, and all *ppk* genes), *C. elegans* (*mec-4*, *del-1*, *mec-10*, *deg-1*, *unc-8*), mouse (Accn and ENaC genes), and the FMRFamide-gated channel from the pond snail (FaNaCh). All sequences were downloaded from the NCBI database, using the most updated reference sequence for each protein. All positions containing gaps and missing data were eliminated. There were a total of 86 positions in the final dataset.

et al., 2002; Price *et al.*, 2001; Waldmann *et al.*, 1997b; Xie *et al.*, 2003; Xiong *et al.*, 2004); (3) sulfhydryl compounds (Cho and Askwith, 2007); (4) small polyamines such as agmatine (Yu *et al.*, 2010); and (5) mechanical stimuli (Bazopoulou *et al.*, 2007; Lu *et al.*, 2009; O'Hagan *et al.*, 2005; Price *et al.*, 2001; Simon *et al.*, 2010; Tavernarakis and Driscoll, 2001a; Zhang *et al.*, 2004; Zhong *et al.*, 2010). Together, these data indicated that, like other ligand-gated ion channel families, DEG/ENaC channels have evolved to serve many different physiological functions, acting as ionotropic receptors to diverse extracellular stimuli.

Although amiloride-sensitive sodium currents from various epithelial tissues have been recorded since the early 1970s, the genes encoding for these channels, which were shown to be critical for regulating salt exchange in the kidney and blood pressure, were not identified until the early 1990s (Canessa *et al.*, 1993, 1994a; Lingueglia *et al.*, 1993a,b). The successful cloning of ENaC-coding genes was achieved by using expression cloning in *Xenopus* oocytes, which demonstrated that the mature ENaC comprises proteins from three highly related but independent genes. These genes were subsequently termed ENaCα, ENaCβ, and ENaCγ (Canessa *et al.*, 1994b). The existence of homologous channels in invertebrates was originally debated. Nevertheless, several studies suggested the existence of amiloride-sensitive sodium currents in the leech, *Xenopus*, and the pond snail, suggesting these channels were not mammalian specific (Green *et al.*, 1994; Weber *et al.*, 1992, 1993). Later, cloning of several DEG/ENaC-like proteins from the worm *Caenorhabditis elegans*, and the fruit fly, *Drosophila melanogaster* showed that the DEG/ENaC family is likely to be ubiquitously present in animal genomes (for a comprehensive review of the early studies, see Garty and Palmer, 1997).

Subsequently, several additional members of the DEG/ENaC superfamily have been cloned from mammalian models, including several acid-sensitive ion channels (ASIC/Accn) (Price *et al.*, 2000, 2001, 1996; Waldmann *et al.*, 1997b, 1996; Xie *et al.*, 2002). In contrast to ENaC-coding genes, which are transcriptionally enriched in epithelial tissues, members of the ASIC subfamily seem to be highly enriched in neuronal tissues, both centrally and peripherally (Lu *et al.*, 2009; Xie *et al.*, 2002). The completion of the sequencing of the human and other animal genomes revealed that mammals encode for eight to nine independent members of the DEG/ENaC protein superfamily. Surprisingly, the release of the completed genomes of the worm and the fruit fly revealed that the genomes of these invertebrates harbored a significantly larger number of independent DEG/ENaC-like genes (31 in the fruit fly and 30 in the worm), which also included several genes that can produce multiple variants due to alternative splicing and multiple transcriptional initiation sites (Bazopoulou *et al.*, 2007; Liu *et al.*, 2003a,b). Hence, DEG/ENaC genes represent one of the largest ion channel families in invertebrate genomes. The expansion of the DEG/ENaC

protein family in these animals suggests the hypothesis that DEG/ENaC ion channels have evolved to serve a much wider range of physiological functions in invertebrates relative to their roles in mammals. Alternatively, it may suggest that DEG/ENaC subunits in invertebrates are highly specialized; each subunit is performing a narrow slice of the physiological tasks performed by mammalian family members. Our group is focused on understanding the role of DEG/ENaC channels in invertebrate physiology, which we hope will help us to resolve these two alternative hypotheses.

Although members of the DEG/ENaC superfamily are easily recognized by their unique protein topology (Fig. 1.1), identifying the relationships between family members across distant species based on protein sequence alone is hampered by the poor overall sequence conservation of the extracellular loop domain. Hence, protein alignment analyses alone were not powerful enough to draw physiological homology conclusions (Fig. 1.2). Consequently, newly identified family members typically require physiological analyses *de novo*.

The best physiologically characterized members of the family are the three mammalian ENaC genes (Garty and Palmer, 1997; Horisberger and Chraibi, 2004). The mammalian ENaC channels are typically found at the apical membrane of epithelial cells where they play an essential role in regulating sodium gradients across epithelial barriers in a variety of tissues (Snyder, 2005; Snyder *et al.*, 1995; Voilley *et al.*, 1994). Mutations in ENaC subunits can lead to disorders such as Liddle's syndrome, which is a rare form of genetically inherited hypertension syndrome (Snyder *et al.*, 1995). ASIC represent the other major mammalian branch of the DEG/ENaC family (Waldmann *et al.*, 1999). These channels are enriched in peripheral and central neurons and are highly sensitive to changes in extracellular proton concentrations (Bassilana *et al.*, 1997; Waldmann *et al.*, 1997a; Wemmie *et al.*, 2002). ASIC channels seem to play a major role in several pH-dependent physiological processes in the brain that include seizure termination (Ziemann *et al.*, 2008), learning and memory (Askwith *et al.*, 2004; Wemmie *et al.*, 2002), and fear conditioning (Coryell *et al.*, 2009, 2007; Wemmie *et al.*, 2003; Ziemann *et al.*, 2009). Similar central neuronal roles have also been recently identified for DEG/ENaC channels in the worm model (Voglis and Tavernarakis, 2008). How these pH-sensitive channels affect neuronal functions is still a mystery. At least some of the functions might be mediated by direct, short-term effects on synaptic plasticity, possibly by sensing microchanges in pH that are associated with the low pH environment of the lumen of synaptic vesicles. In contrast to our understanding of the role of ASIC channels in the CNS, their roles in sensory functions are still controversial, which will be discussed in details below.

Despite the advances in understanding the role of DEG/ENaC signaling in the brain, its role in peripheral neuronal functions is still poorly understood. Nevertheless, recent work in invertebrate and mammalian models indicated that members of the DEG/EnaC superfamily are playing a major role in

chemosensation and mechanosensation, although the capacity in which they exert their sensory functions is still not well understood in most systems. This review focuses on the current state of research on the sensory roles of DEG/ENaC channels in diverse animal models.

The emerging interest in DEG/ENaC-dependent signaling has resulted in many studies of their functions in diverse species. As more and more individual subunits are being characterized, a complex picture is emerging in terms of the physiological roles of DEG/ENaC and their diverse gating mechanisms. Although the first DEG/ENaC channels cloned were characterized as a constitutively open sodium channels, later studies of the ENaC channel and the majority of other family members suggested that members of the family are likely acting as either classic ligand-gated ion channels (Horisberger and Chraibi, 2004) or mechanically gated channels (Bazopoulou *et al.*, 2007; Bianchi, 2007).

II. CHEMOSENSATION

A. Salt taste

Maintaining appropriate ionic homeostasis is critical for all organisms, especially in regard to sodium, which is kept in relatively high extracellular concentrations in most animal tissues. Animals actively regulate their sodium intake via food consumption (Geerling and Loewy, 2008). In agreement with the importance of sodium, studies in rodent models indicated that some taste cells are specialized for responding to NaCl while others are less specialized and can be activated by many different solutes, suggesting that they act as detectors of osmolarity rather than directly responding to specific ions (Frank *et al.*, 2008; Lundy and Contreras, 1999; Yoshida *et al.*, 2009). Interestingly, some of the NaCl responsive cells in the mammalian taste epithelium are also sensitive to amiloride, which essentially blocks their responsiveness to salt (Heck *et al.*, 1984). This amiloride sensitivity led to the hypothesis that ENaC channels might be involved in the salt taste signal transduction in mammals (Chandrashekar *et al.*, 2010; Yoshida *et al.*, 2009).

The first genetic evidence that DEG/ENaC signaling contributes to salt taste came from studies in the fruit fly (Liu *et al.*, 2003b). These studies used transgenic expression of RNAi or dominant-negative alleles targeting *ppk11* and *ppk19*, two independent DEG/ENaC encoding genes, which resulted in a reduced appetitive response to low concentrations of KCl and NaCl. The same manipulations had no effect on other taste modalities, suggesting these channels were not generic taste-related molecules.

Further support for salt sensing being genetically and cellularly independent of other taste modalities came from genetic studies in the mouse, which indicated that umami, sweet, and bitter sensing cells (type II taste cells) signal

through G-protein coupled receptors (GPCR) (Zhang *et al.*, 2003; Zhao *et al.*, 2003). In contrast, the same studies clearly indicated that GPCR-independent pathways are responsible for sensing sour and salt tastes (Zhang *et al.*, 2003).

Although the contribution of amiloride-sensitive sodium currents to salt taste is now generally accepted, other findings challenged these conclusions. These included studies showing that some NaCl responsive taste cells were not inhibited by amiloride in primary cultures. The receptor responsible for the amiloride-insensitive salt responses was recently identified as a variant of the TRP channel VR-1, a ligand-gated channel involved in the response to noxious heat and capsaicin (the "hot" compound in chili peppers; Lyall *et al.*, 2005). Other ligand-gated ion channels that have been recently implicated in amiloride-insensitive salt taste are P_2X2 and P_2X3 ATP receptors (Eddy *et al.*, 2009).

Despite the incomplete understanding of the role of ENaC signaling in salt taste, a recent study of a conditional ENaCα subunit knockout in the taste epithelia of mice resulted in animals that did not respond behaviorally or physiologically to a wide range of sodium concentrations, strongly supporting the primary role of ENaC signaling in mediating mammalian salt taste (Chandrashekar *et al.*, 2010).

In contrast to other taste modalities, the amiloride-sensitive taste cells, which are responsive to appetitive levels of NaCl, are likely represented by type I taste cells (Vandenbeuch *et al.*, 2008). These are surprising findings since these cells were previously assumed to act as nonexcitatory support taste cells that are not directly involved in taste transduction (Pumplin *et al.*, 1997). These findings raise an interesting problem in terms of how salt taste is coded by the nervous system if indeed Type I cells, which do not form synaptic connections (Finger *et al.*, 2000), are responsible for detecting salt taste via ENaC-dependent mechanisms (Vandenbeuch *et al.*, 2008).

B. Sour taste

The molecular identity of the mammalian sour receptor is still controversial (Dotson, 2010). Early studies in rodent models suggested that the sour-taste receptor acts as a sodium channel, which can be partially blocked by amiloride (Ugawa *et al.*, 1998). The subsequent cloning of the sensitive pH-gated members of the ASIC, which are members of the DEG/ENaC family, suggested that these channels might be the elusive sour-taste receptor (Shimada *et al.*, 2006; Ugawa, 2003; Ugawa *et al.*, 2003). Further analyses of the possible candidate ASIC channels involved in sour-taste transduction indicated that the sour-taste channel is possibly formed by heterodimerization of two alternatively spliced isoforms of the ASIC2 channel, ASIC2a and ASIC2b (Ugawa *et al.*, 2003). Neurophysiological characterizations of these channels in *Xenopus* oocytes showed that they had pH-dependent sodium currents that were very similar to the currents evoked

by low pH in taste buds in rats (Ugawa *et al.*, 2003). Further, immunohistochemical studies in rats showed that ASIC2 channels are enriched in a subpopulation of taste receptor cells that are responsive to acid stimuli (Lin *et al.*, 2004, 2002; Ugawa *et al.*, 1998). Surprisingly, studies of ASIC2 in the mouse model indicated that the channel was not expressed in taste receptor cells, and homozygous ASIC2 knockout mice showed normal appetitive and physiological response to acids (Richter *et al.*, 2004). These studies challenged the possible universal role of ASIC channels in sensing sour ligands.

Subsequent studies identified members of the polycystic kidney disease (PKD) genes as possible candidates for the "universal" mammalian sour-taste receptor (Chandrashekar *et al.*, 2009; Huang *et al.*, 2006; Ishii *et al.*, 2009; Ishimaru *et al.*, 2006; Kataoka *et al.*, 2008). Nevertheless, recent knockout models of PKD1L3, one of the two PKD genes implicated in sour taste, were shown to have normal sour-taste behaviors and physiology, challenging the role of PKD-like genes in sensing sour taste (Nelson *et al.*, 2010). These puzzling and conflicting data may suggest that multiple independent pathways, which are possibly different in different mammalian species, detect sour taste. Alternatively, it is possible that redundant, independent molecular mechanisms underlie sour taste. Further support for the redundancy model in humans comes from a recent study of sour ageusia (inability to detect low pH in ingested foods) in two individuals, which showed genetic mutations and reduced expression of both ASIC- and PKD-related proteins in sour-taste buds (Huque *et al.*, 2009).

C. Other

In contrast to the limited taste repertoire in vertebrates, insects seem to have evolved a gustatory system that responds to a wide spectrum of chemicals, which do not necessarily overlap with the five canonical taste modalities (sweet, bitter, umami, salt, and sour). One such striking example is the sensing of "water" taste in *Drosophila*. Two recent studies indicated that flies have a specific population of gustatory receptor neurons that directly respond to the taste of water. Further, these studies implicated DEG/ENaC signaling in water sensing by using either (1) a combination of pharmacology and reversed genetics approach (Chen *et al.*, 2010) or (2) a functional genomics approach to identify genes that are highly expressed in the proboscis of flies (Cameron *et al.*, 2010). Both studies identified *ppk28*, a member of the DEG/ENaC family in flies, as the molecular receptor for water. Specifically, these studies showed that *ppk28* is necessary for water detection, and that expression of *ppk28* in nonwater sensing gustatory receptor neurons was sufficient to confer water sensitivity, indicating that *ppk28* is likely the water receptor. The mechanism by which a DEG/ENaC channel like *ppk28* can detect water molecules is still unknown. As will be discussed below, the

possible role of DEG/ENaC channels in mechanosensory functions may suggest that *ppk28* senses water by detecting mechanical changes in membrane physical properties in response to changes in external osmolarity.

Insects also rely extensively on their chemosensory systems for detecting social signals underlying behaviors such as courtship, aggression, and aggregation. *Lounge lizard* (*llz/ppk25*), a member of the DEG/ENaC family in *Drosophila*, was shown to be expressed in chemosensory-related accessory cells that are specific to the male forelegs, and to contribute to male courtship behavior (Ben-Shahar *et al.*, 2010, 2007; Lin *et al.*, 2005). Although a direct role for *llz/ppk25* in sensing pheromones has not been demonstrated, data suggest that it might contribute to male courtship behavior (Lin *et al.*, 2005). Surprisingly, an independent study indicated that genetic ablation of *llz*-expressing cells had no effect on male courtship behaviors (Ben-Shahar *et al.*, 2010). Interestingly, *llz* represents a subfamily of several DEG/ENaC subunits in *Drosophila*, suggesting that several different subunits might be playing a role in social communication in insects. Consequently, more studies are required to establish *llz* and other similar DEG/ENaC subunits in signaling pathways underlying pheromonal sensing.

llz is expressed in nonneuronal sheath cells, suggesting that its putative effects on courtship, are not mediated by direct response to pheromones, or that the sheath, glia-related cells are also acting as nonneuronal sensory cells (Ben-Shahar *et al.*, 2010, 2007). Further support for this hypothesis came from a recent study in *C. elegans* in which *ACD-1*, a DEG/ENaC subunit, was shown to be expressed in chemosensory-related glia cells, and to contribute to acid avoidance behavior, as well as attraction to the amino acid lysine (Wang and Bianchi, 2009; Wang *et al.*, 2008). Together, these data indicate that the contribution of some DEG/ENaC subunits to chemosensation via *nonneuronal* sensory pathways might be more prevalent than previously thought.

III. MECHANOSENSATION

A. *C. elegans*

All organisms seem to have evolved on mechanisms to sense mechanical stimuli, and in most cases, physiological studies indicated that the mechanosensory complex acts as a cation channel (for a recent review, see Arnadottir and Chalfie, 2010). Yet, the molecular identities of the proteins responsible for sensing mechanical stimuli are still mostly unknown (Christensen and Corey, 2007; Corey, 2006). The difficulty in identifying the mechanosensory conducting channels is likely the result of functional redundancies in mechanosensory systems, which complicate genetic studies. In addition, the low number of conducting channels per each individual mechanosensitive cell has made biochemical

approaches for their isolation difficult. Nevertheless, in the past few years, researchers have identified at least some of the ion channels that underlie mechanosensory functions. The best examples to date are the classic screens for *mec* mutations in C. *elegans*, which have identified 15 genes that are defective in their response to gentle touch (Brown *et al.*, 2007; Chelur *et al.*, 2002; Cueva *et al.*, 2007; Goodman *et al.*, 2002; O'Hagan *et al.*, 2005). This discovery led to the identification of a sensory protein complex involved in the touch sensation of worms (O'Hagan *et al.*, 2005). Interestingly, two of the *mec* genes, *mec-4* and *mec-10*, were shown to be members of the DEG/ENaC family (Goodman and Schwarz, 2003), and mutations in the *mec-4*/*mec-10* complex led to a decrease in neuronal intracellular Ca^{2+} levels in response to gentle touch (Bianchi *et al.*, 2004; Brown *et al.*, 2007; Cueva *et al.*, 2007). Further characterization of the *mec-4* channel by using the *in vivo* whole-cell patch-clamp technique showed that the *mec-4* complex was responsible for conducting the mechanical stimulus in mechanosensory neurons, which mediate light touch (Brown *et al.*, 2007; Cueva *et al.*, 2007; Nelson *et al.*, 2010). To date, the *mec-4*/*mec-10* complex in C. *elegans* is one of the only *bone fide* examples of a eukaryotic, molecularly and genetically defined, mechanically activated ion channel. The only other well-established ionotropic mechanosensors are members of the TRPN subfamily of the transient receptor potential channels in flies and worms (Kang *et al.*, 2010; Lee *et al.*, 2010).

Identifying the DEG/ENaC mechanosensitive channel also led to the isolation of other conserved components of the mechanosensory transduction. These included intra- and extracellular components of the DEG/ENaC-dependent mechanosensitive protein complexes (for a comprehensive review, see Chalfie, 2009). For example, *mec-2*, a gene that encodes for a stomatin-like protein, was shown to be important for the function of the mechanosensory complex by modulating the *mec-4*/*mec-10* DEG/ENaC channel (Goodman *et al.*, 2002). This discovery led to studies showing that a stomatin-domain protein is also important for light mechanosensation in mammals (Fricke *et al.*, 2000; Huang *et al.*, 1995; Martinez-Salgado *et al.*, 2007; Price *et al.*, 2004). These comparative investigations indicated that at least some mechanosensory complexes are likely to be conserved across distant animal species, highlighting the value of studying these important questions in genetically tractable model organisms, using a comparative and integrative approaches.

How DEG/ENaC channels might exert their mechanosensory functions is still unknown. The current prevailing model for the mechanical activation of DEG/ENaC sensory complexes hypothesizes that the highly structured extracellular domain of some DEG/ENaC channels could interact with extracellular matrix proteins, while the short intracellular domains are likely to interact with constituents of the cytoskeleton. Upon deflection of the animal's outer surfaces, the pressure on the anchored extracellular domain results in a protein conformational changes that lead to the opening of the channel's pore (Fig. 1.3).

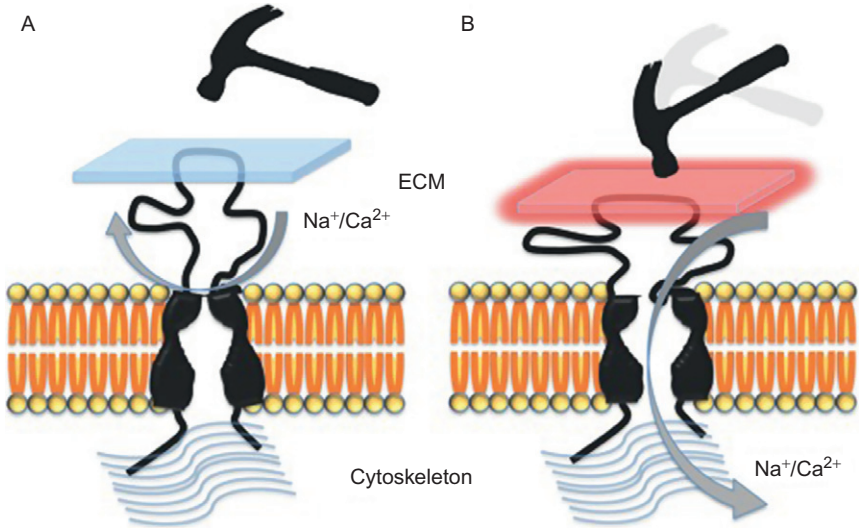

Figure 1.3. A model for the possible role of DEG/ENaC channels in the response to mechanical stimuli. (A) The large extracellular domain is attached to the extracellular matrix (ECM) either directly or possibly via other linker proteins. The short intracellular domains are attached directly or via other proteins to the cytoskeleton. (B) Upon mechanical pressure, the extracellular domain compresses, which results in opening of the pore, leading to influx of cations, which depolarizes the sensory cell. Currently, there are no conclusive data to support any of the models proposed for the mechanical gating of DEG/ENaC channels.

B. *Drosophila*

The identification of members of the DEG/ENaC family in mechanosensitivity screens in C. *elegans* led to several studies that attempted to identify mechanosensitive DEG/ENaC subunits in the nervous systems of other model organisms. Despite initial enthusiasm, whether DEG/ENaC channels are playing a mechanosensory role in other species is still debatable. However, recent work in *Drosophila* suggests that functional DEG/ENaC signaling is required for the function of mechanosensitive, nociceptive neurons in the larval stage; mutations in *ppk*, a DEG/ENaC subunit that is expressed in class IV mechanonociceptive multidendritic sensory neurons (Adams *et al.*, 1998a), were shown to affect locomotion (Ainsley *et al.*, 2003), and contribute to the sensation of harsh mechanical and thermal stimuli (Hwang *et al.*, 2007; Tracey *et al.*, 2003). Taking advantage of the power of *Drosophila* genetics and novel tools for *in vivo* activation of neurons, a subsequent study suggested that genetic disruptions of *ppk* were essential for the response to harsh mechanical stimuli (Zhong *et al.*, 2010).

Although these studies fell short of showing that *ppk* acts as a sensory channel, they did support an essential role for DEG/ENaC in the mechanosensory transduction pathway.

C. Mammals

One of the most central challenges still remaining in sensory biology is the identification of the elusive mammalian hair cell transduction channel essential for the conduction of auditory signals (Corey, 2006; Gillespie and Muller, 2009). Early observations suggested that the drug amiloride can block sodium currents in the chick hair cell, suggesting that the conducting channel might be an amiloride-sensitive ion channel (Jorgensen and Ohmori, 1988). The cloning of the amiloride-sensitive ENaC channels from hair cells and the emerging role of DEG/ENaC signaling in mechanosensation in worms further strengthened this hypothesis (Chalfie *et al.*, 1993). Despite the reports of the expression of various ENaC and ASIC channel subunits in mammalian hair cells, several lines of evidence emerged to suggest that DEG/ENaC channels are not likely to act as the hair cell transduction channel. First, the amiloride sensitivity of the hair cell channel is much higher than what was observed for DEG/ENaC channels in other epithelial tissues (Jorgensen and Ohmori, 1988). Second, the biophysical properties of DEG/ENaC channels are not consistent with the previously characterized biophysical parameters of the hair cell channel (Corey, 2006; Kellenberger and Schild, 2002). Third, knockouts of either the ENaCα (Rusch and Hummler, 1999) or ASIC2 channel in neonatal hair cells in mice did not result in any significant auditory or hair cell physiological impairments (Peng *et al.*, 2004; Roza *et al.*, 2004). To date, no direct evidence for the roles of ASIC1 or ASIC3 in hearing transduction have been reported. Nevertheless, it is now generally accepted that members of the DEG/ENaC channel family are not the mechanosensory channels responsible for mammalian hearing (Corey, 2006).

Although there is a lack of evidence to support a role for DEG/ENaC channels in the mammalian auditory signal transduction, it is possible that members of the family contribute to other types of mechanosensation. Genetic studies support the hypothesis that eukaryotic mechanosensation is mediated by ion channel receptors from multiple and independent protein families (Arnadottir and Chalfie, 2010).

Several ENaC and ASIC channels are expressed in subcutaneous mechanosensory structures, suggesting they might act as the mechanosensory transducer in the skin (Drummond *et al.*, 2000; Price *et al.*, 2000, 2001). Yet, genetic studies of these channels resulted in conflicting results. The first knockout (KO) model of ASIC2 (also called ACCN1 or BNaC1), a subunit that is enriched in the skin mechanosensitive neuronal fibers, indicated that the lack of DEG/ENaC signaling could lead to a mild reduction in the mechanosensitive

response of fast adapting mechanoreceptors in the skin (Price *et al.*, 2001). Yet, subsequent studies using independent ASIC2 and ASIC 3 KO models did not find any associations between ASIC functions and mechanosensitive currents in mammalian sensory neurons (Drew *et al.*, 2004; Roza *et al.*, 2004). Further, a transgenic model that expresses a dominant-negative form of ASIC3, which abolished most of the ASIC-like currents in DRG sensory neurons, also showed increased sensitivity to mechanical stimuli (Mogil *et al.*, 2005). Together, these findings suggest that ASIC-dependent signaling might play a role in the mammalian skin mechanosensation, possibly as an important modulator but not necessarily as the mechanotransducing channel itself.

Whether other mammalian DEG/ENaC subunits might act as subcutaneous mechanosensitive channels is still poorly understood. At least one report indicated that both β and γENaC subunits are expressed in medium and large number of DRG neurons that project to the mechanosensitive Merkel cells and Meissner-like corpuscles present in the rat footpad (Finger *et al.*, 2000). Surprisingly, the αENaC subunit was not present in these neurons, suggesting that a different DEG/ENaC subunit might be responsible for the sodium currents in these cells. In contrast to the human genome, which contains four independent genes that encode ENaC subunits, the rat genome has only three (Ji *et al.*, 2006; Le and Saier, 1996). These data suggest that in rodents the βENaC and γENaC subunits could form a mechanosensitive channel independent of the αENaC protein, possibly by heterodimerization with one of the ASIC subunits present in DRG neurons (Benson *et al.*, 2002; Xie *et al.*, 2002).

Mechanosensitive channels also play a role in regulating the vertebrate blood pressure, most likely by regulating the baroreceptive sensory response to change in aortic pressure (Chapleau *et al.*, 1995b; Cunningham *et al.*, 1995; Ma *et al.*, 2002). Arterial tension is sensed by specialized sensory neurons, which have their cell bodies in the nodus ganglion (Ma *et al.*, 2002; Snitsarev *et al.*, 2002). Previous reports indicated that rat nodus neurons are sensitive to mechanical stimuli in primary cultures as well as *in vivo* in response to glass probe stimuli or hypoosmotic buffers (Chapleau *et al.*, 1995a,b; Cunningham *et al.*, 1997; Kraske *et al.*, 1998; Snitsarev *et al.*, 2007; Sullivan *et al.*, 1997) and exhibit a mechanical stimulus-dependent increase in intracellular Ca^{2+} levels (Chapleau *et al.*, 1995b; Cunningham *et al.*, 1995). Further, these currents were blocked by gadolinium, a trivalent cation, which is thought to directly block mechanosensitive channels (Chapleau *et al.*, 1995a; Kraske *et al.*, 1998). Cell-attached patch-clamp studies indicated that the putative mechanosensitive channels in the rat nodus neurons act as nonselective, voltage-independent cation channels (Cunningham *et al.*, 1995). Although the molecular identity of the barosensitive channels in nodus neurons is still controversial, several studies suggest that DEG/ENaC signaling might contribute to their response to mechanical stimuli. First, RT-PCR studies indicated that all three ENaC subunits are expressed in nodus neurons

(Drummond *et al.*, 2000, 1998, 2001). Second, immunohistochemical localization of the γENaC subunit indicated that the channel is enriched at the sensory neurites of nodus neurons, which innervate the aortic arch (Drummond *et al.*, 2000). Further support for the possible role of ENaC signaling came from studies of the effects of benzamil, an amiloride analog, on the carotid reflex response, which showed dose-dependent inhibition of mechanosensitivity (Drummond *et al.*, 2001). Yet, genetic evidence that the ENaC channel is directly involved in regulating the arterial pressure reflex is still lacking.

Recently, the mammalian ASIC2 channel has emerged as a possible component of the baroreceptor complex and for the control of circulation pressure (Lu *et al.*, 2009). This study showed that all three ASIC subunits, including all their alternatively spliced isoforms, are expressed in nodus sensory neurons. Further, immunostaining of the nodus ganglion suggested that different neuronal populations expressed the ASIC2 subunit and either ASIC1 or ASIC3. These data supported the hypothesis that different sensory neuronal populations express receptors with different properties (Lu *et al.*, 2009). Studies of ASIC2 knockout mice showed significant baroreflex impairments by measuring the reflex response electrophysiologically. In contrast, ASIC2 transgenic mice showed hypersensitive arterial baroreception, further supporting the premise that ASIC2 signaling is a critical component of the baroresponse reflex in mammals (Lu *et al.*, 2009).

IV. PERIPHERAL PAIN

Although the general role of DEG/ENaC signaling in eukaryotic mechanosensation is still controversial, the data discussed above indicated that, at least in invertebrates, DEG/ENaC subunits are playing an important role in the function of mechanically activated sensory neurons, often in the context of mechanical and thermal nociceptive stimuli (Albeg *et al.*, 2010; Bounoutas and Chalfie, 2007; Chatzigeorgiou *et al.*, 2010; Chelur *et al.*, 2002; Goodman *et al.*, 2002; Roza *et al.*, 2004; Suzuki *et al.*, 2003; Tavernarakis and Driscoll, 2001a,b; Zhang *et al.*, 2004; Zhong *et al.*, 2010). Although a similar role for DEG/ENaC channels in mammalian mechanical and thermal nociceptive responses is still poorly understood (Askwith *et al.*, 2001; Drew *et al.*, 2004; Page *et al.*, 2004; Price *et al.*, 2000, 2001), roles for these channels in other forms of mammalian pain perception are starting to emerge. These studies have been discussed recently in comprehensive reviews and hence will be described here briefly (Deval *et al.*, 2010; Sluka *et al.*, 2009; Wemmie *et al.*, 2006).

The cloning of various ASIC subunits from mammalian genomes, and the discovery that some ASICs are highly sensitive proton receptors, led to studies that tested whether they might represent the nociceptive acid receptors that were

originally described physiologically in the early 1980s in free nerve endings of somatosensory neurons in rodents (Krishtal and Pidoplichko, 1981a,b). There are currently at least six known mammalian ASIC subunits, which are transcribed via alternative splicing from four independent genetic loci (ASIC1-4, sometimes referred to as ACCN1-4; Lingueglia, 2007). Not all subunits show acid activated currents as homomultimers in heterologous expression systems (Askwith *et al.*, 2004; Wemmie *et al.*, 2006), possibly indicating that some subunit combinations might either have a different gating mechanism, are missing other proteins for their functions *in vitro*, or that some *in vitro* expressed subunits do not actually assemble *in vivo*, and hence do not represent physiologically relevant channels. Expression studies of DRG nociceptive neurons in rodents indicated that all known ASIC subunits are expressed in these tissues, further supporting the hypothesis that at least some of these proteins are acting as the elusive peripheral pain-related acid receptors (Waldmann *et al.*, 1997b). Since the transient activation threshold of acid-evoked currents, and the channel's electrokinetics, are strongly affected by specific subunit compositions, the presence of all subunits in various DRG neurons supported a role for differential subunit expression as a mechanism for establishing diverse acid sensitivity threshold, as is expected from behavioral studies of pain response to acid stimuli *in vivo* (Askwith *et al.*, 2001; Benson *et al.*, 2002; Donier *et al.*, 2008; Xie *et al.*, 2003).

Among the various identified ASIC channels in DRG neurons, the ASIC3 homomeric channels show the highest pH sensitivities and hence were speculated to comprise the main acid sensing pain receptor in variety of different DRG and trigeminal sensory neurons (Deval *et al.*, 2008; Hattori *et al.*, 2009; Ikeuchi *et al.*, 2008, 2009; Walder *et al.*, 2010). Yet, ASIC3 knockout mice or transgenic animals that expressed an ASIC3 dominant-negative allele did not show a lower response to acidic pain relative to wild types (Mogil *et al.*, 2005; Price *et al.*, 2001). In fact, the ASIC3 knockout showed small but significant increase in responses to acid pain stimulus. Surprisingly, the data obtained in mouse models did not agree with pharmacological data that was obtained from human and rat investigations; several pharmacological studies suggested that general ASIC antagonists such as amiloride can block acid-induced pain in healthy human subjects (Ugawa *et al.*, 2002), and similarly, other ASIC antagonists such as A-317567 (Dube *et al.*, 2005) or the somewhat ASIC3 selective toxin APETx2 had significant effects on cutaneous pain in rats. These pharmacological findings were further validated with *in vivo* siRNA studies (Deval *et al.*, 2008). These conflicting data suggested that ASIC3 channels play a desensitizing role in pain sensory neurons in the mouse but might play a sensitizing role in human and rat neurons. These contradictory data from very close species such as the mouse and the rat suggest that ASIC3 is acting as the acid receptor in the periphery, but that the behavioral phenotypes observed in the mouse are due to its activity somewhere else in the pain circuit. Alternatively, it may suggest that

although ASIC3 is a highly sensitive acid receptor, it is not playing the role of a nociceptive receptor in cutaneous pain neurons, but rather that it is acting as a modulatory factor for other still unknown acid receptors. These alternative hypotheses could be sorted out by the development of conditional knockout models of ASIC3, which will target its deletion to specific populations of DRG neurons. Further, the recent developments in rat knockout technologies (Jacob *et al.*, 2010) will enable us to test the role of ASIC3 in rat pain with genetic rather than only pharmacological approaches. Regardless, these studies indicate the importance of studying DEG/ENaC signaling in multiple different species, since even under very similar physiological contexts, different species might utilize the same signaling pathways in contrasting manners.

V. CONCLUSIONS

Degenerin/epithelial sodium channels are emerging as important molecular players in animal sensory biology. Their possible role in mediating nociceptive behaviors in both invertebrates and vertebrates suggest that these channels evolved to serve such functions early in the metazoan radiation. One puzzling aspect of DEG/ENaC diversification is the large number of independent subunits present in invertebrate genomes relative to mammalian genomes. To my knowledge, no other ligand-gated ion channel families show such striking invertebrate–vertebrate dichotomy, suggesting these channels play special roles in invertebrate biology. As the significance of DEG/ENaC signaling in the mammalian nervous system becomes more apparent, the importance of developing novel models for studying DEG/ENaC signaling in genetically tractable models such as *Drosophila* and *C. elegans* should lead to the development of new understandings of how these channels exert their functions, what other proteins are playing a role in DEG/ENaC signaling, and how DEG/ENaC signaling affects neuronal physiology at both central and peripheral neurons, as well as in nonneuronal cell types.

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