Degenerin/ENaC channels

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26.1 INTRODUCTION
Degenerins (Deg) of Caenorhabditis elegans are the founding members of the Deg/ENaC ion channel family with the epithelial Na⁺ channel (ENaC) being the first mammalian homolog cloned (Bianchi and Driscoll, 2002; Cottrell, 1997; Garty and Palmer, 1997; Kellenberger and Schild, 2002; Lingueglia, 2007). As shown in the dendrogram in Figure 26.1, in mammals, this ion channel family includes acid-sensing ion channels (ASICs); the brain, liver, and intestine Na⁺ channel (BLINaC); and ENaC. The FMRFamide-gated Na⁺ channel (FaNaC) and pickpockets (Ppks) along with Deg channels are found in invertebrates. Homologs in lamprey (lASIC; Coric et al., 2005) and lungfish (nENaC; Uchiyama et al., 2012) represent important transitional precursors of mammalian ASIC and ENaC, respectively. The HyNaCs expressed in the primitive animal hydra are representative of ancestral Deg/ENaC channels that existed prior to the radiation of bilateria early in evolution (Durrnagel et al., 2010; Golubovic et al., 2007). As listed in Table 26.1, humans express nine genes that encode Deg/ENaC proteins: four encoding the α, β, γ, and δ NaC subunits, four encoding ASIC1–4 subunits, and one encoding the related BLINaC protein, which sometimes is referred to as ASIC5.

The goals here are to provide an informative discussion about the expression pattern, function, structure, and regulation of Deg/ENaC channels and the role these channels play in human physiology and pathology. In addition, the molecular evolution of the Deg/ENaC channel family is discussed briefly with emphasis on the initial appearances of the genes encoding ASIC and ENaC and how their expression preadapted the forebears of modern terrestrial vertebrates to conquer the land.

26.2 EXPRESSION AND FUNCTION OF Deg/ENaC CHANNELS
Deg/ENaC channels serve diverse functions in physiology. In some invertebrate animals as typified by the nematode C. elegans, some members of the Deg/ENaC channel family function as ionotropic molecular sensory transducers in peripheral sensory neurons involved in perception of the external environment.
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(Bianchi and Driscoll, 2002; Mano and Driscoll, 1999). In others, as typified by *Helix aspersa*, Deg/ENaC channels function as neuropeptide-gated channels in central neurons controlling excitation and synaptic transmission (Cottrell, 1997). In contrast, ENaC, which is expressed in epithelia of terrestrial vertebrates, functions during ion transport and consequently is involved in homeostatic control of fluid volumes and electrolyte content, making this channel a key effector in the regulation of blood pressure (Garty and Palmer, 1997; Kellenberger and Schild, 2002). Mammalian ASIC, which are expressed in both peripheral and central neurons, more closely parallel the function of invertebrate Deg/ENaC channels as sensory receptors and during neurotransmission in the postsynaptic cell (Bianchi and Driscoll, 2002; Sherwood et al., 2012). Seven Deg/ENaC homologs, the invertebrate FaNaC, HyNaC, Deg and Ppk, and the mammalian ENaC, ASIC, and BLINaC are discussed here as proteins representative of the family and these various functions.

### 26.2.1 Physiological Role of Deg/ENaC Proteins

Deg/ENaC proteins are ion channel subunits that contribute directly to a functional channel pore. Deg/ENaC channels preferentially conduct monovalent cations over divalent cations and anions with the majority of these channels being selective.

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**Figure 26.1** The Deg/ENaC ion channel family. In this dendrogram, lamprey, *N. forsteri*, mouse, and human are abbreviated as l, n, m, and h, respectively, and *H. aspersa*, *Lymnaea stagnalis*, and *Aplysia californica* are abbreviated as Ha, Ls, and Ac, respectively.

**Table 26.1** Deg/ENaC channels expressed in humans

<table>
<thead>
<tr>
<th>CHANNEL</th>
<th>ALTERNATIVE NAME</th>
<th>GENE</th>
<th>CHROMOSOME</th>
<th>LOCATION (Mb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASIC1</td>
<td>BNAC2</td>
<td>ACCN2</td>
<td>12</td>
<td>50.45–50.48</td>
</tr>
<tr>
<td>ASIC2</td>
<td>BNAC1, MDEG</td>
<td>ACCN1</td>
<td>17</td>
<td>31.34–32.5</td>
</tr>
<tr>
<td>ASIC3</td>
<td>DRASIC</td>
<td>ACCN3</td>
<td>7</td>
<td>150.75–150.75</td>
</tr>
<tr>
<td>ASIC4</td>
<td>BNAC4, SPASIC</td>
<td>ACCN4</td>
<td>2</td>
<td>220.38–220.4</td>
</tr>
<tr>
<td>BLINaC</td>
<td>hINaC, BASIC</td>
<td>ACCN5</td>
<td>4</td>
<td>156.75–156.79</td>
</tr>
<tr>
<td>αENaC</td>
<td>SCNEA</td>
<td>SCN1A</td>
<td>12</td>
<td>6.46–6.49</td>
</tr>
<tr>
<td>βENaC</td>
<td>SCNEB</td>
<td>SCN1B</td>
<td>16</td>
<td>23.29–23.39</td>
</tr>
<tr>
<td>γENaC</td>
<td>SCNEG</td>
<td>SCN1G</td>
<td>16</td>
<td>23.19–23.23</td>
</tr>
<tr>
<td>δENaC</td>
<td>SCNED, dNaCH</td>
<td>SCN1D</td>
<td>1</td>
<td>1.21–1.23</td>
</tr>
</tbody>
</table>
for Na⁺. The degree of selectivity varies among family members. For instance, ENaC has a 100-fold preference for Na⁺ over K⁺ (Kellenberger and Schild, 2002). ASIC and many invertebrate Deg/ENaC channels in comparison have less preference for Na⁺ (Cottrell, 1997; Kellenberger and Schild, 2002; Sherwood et al., 2012). Many of these less selective Deg/ENaC channels also conduct divalent cations. As a consequence, they contribute to the Ca²⁺ permeability of the plasma membrane. Due to their selectivity, Deg/ENaC channels conduct depolarizing inward currents under physiological conditions and ionic gradients. Figure 26.2a and b shows typical macroscopic inward Na⁺ currents conducted by ASIC1 activated in a rat CA1 hippocampal neuron and Ppk1 activated in a class IV multidendritic (md) peripheral sensory neuron from Drosophila melanogaster, both voltage clamped at typical resting membrane potentials (Boiko et al., 2013).

Deg/ENaC channels, like all channels, gate. While they share fundamental gating mechanisms as defined by structure, gating is differentially regulated among the distinct types of Deg/ENaC channels. For instance, ENaC is a noninactivating, constitutively active channel that gates independent of voltage or a ligand. In comparison, ASIC, BLINaC, FaNaC, and HyNaC are ligand-gated ion channels (Bianchi and Driscoll, 2002; Cottrell, 1997; Kellenberger and Schild, 2002; Sherwood et al., 2012). The prior is activated and subsequently desensitized by acid with protons serving as ligands at extracellular binding sites. Bile acids function as extracellular ligands for BLINaC, and small neuropeptides, as typified by RFamides, serve as ligands for FaNaC and HyNaC. Ppk and Deg channels also gate in response to stimuli although certain of these channels respond to mechanical rather than chemical cues. For instance, as shown in Figure 26.2c, force directly activates mechanoreceptor currents mediated by Deg-1 in ASH neurons (Geffeney et al., 2011). ASH neurons are ciliated polymodal sensory neurons in C. elegans involved in mechanosensation.

Because they respond to stimuli, ASIC, BLINaC, Ppk, Deg, FaNaC, and HyNaC channels function as ionotropic receptors capable of affecting the electrical properties and excitability of a cell in response to an extracellular signal. They also may influence cell signaling by affecting the cell entry of the second messenger Ca²⁺. In comparison, ENaC does not respond directly to a ligand. Thus, ENaC does not function as an ionotropic receptor but rather as a regulated gateway allowing Na⁺ to enter the cell in response to cell signaling. This enables ENaC to contribute to vectorial ion transport across epithelial barriers.

### 26.2.2 FaNaC: A PEPTIDE-GATED Na⁺ CHANNEL

FaNaCs are widely expressed in ganglia neurons of Mollusca, such as the giant pedal neurons of the mollusk (Cottrell, 1997; Davey et al., 2001; Kellenberger and Schild, 2002; Lingueglia et al., 1995, 2006; Perry et al., 2001). The function of ganglia neurons in the simple rope-ladder-like nervous system of the mollusk is akin to that of central motor neurons in the more sophisticated nervous systems of complex animals. In ganglia neurons, FaNaC functions as a neuropeptide-gated channel that conducts a depolarizing inward Na⁺ current upon activation. FaNaC activation is responsible for the fast excitatory action of neuropeptides in mollusks. As shown in Figure 26.3, FaNaC
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rapidly activates and then desensitizes to FMRFamide (Cottrell et al., 2001). This effect of FMRFamide on FaNaC brings the neuron to threshold, evoking a transient train of action potentials (Perry et al., 2001). Such observations are consistent with FaNaC functioning as an excitatory, ionotropic receptor in the postsynaptic cell enabling peptidergic neurotransmission.

FaNaC is the first channel recognized to be peptide gated (Cottrell, 1997; Lingueglia et al., 1995). The fast excitatory response to FMRFamide as mediated by FaNaC is distinct from slower responses in these same neurons. Like the ionotropic and metabotropic glutamatergic and purinergic receptors of mammals, this ionotropic RFamide receptor is the complement to G-protein-coupled metabotropic RFamide receptors in invertebrates. Interestingly, while mammals express metabotropic receptors for small neuropeptides to include RFamides, an ionotropic counterpart has not been identified to date in these animals. Emerging evidence suggests that ASIC, BLaNaC, or both perhaps serve such a function in mammals (Kellenberger and Schild, 2002; Lingueglia et al., 2006).

26.2.3 HyNaC: THE ANCESTRAL Deg/ENaC CHANNEL

HyNaC as expressed in Hydra magnipapillata is the closest to a primordial Deg/ENaC channel for which we currently have amino acid sequence and information about function (Durrnagel et al., 2010; Golubovic et al., 2007). Similar to FaNaC, HyNaC is directly gated by neuropeptides, in this case Hydra-RFamides I and II, and conducts a depolarizing inward Na⁺ current upon activation as shown in Figure 26.4. HyNaCs are expressed in cells localized to the base of tentacles. These cells are likely to be epitheliomuscular cells and are adjacent to neurons that produce Hydra-RFamides, consistent with these neuropeptides being the natural ligands for these channels (Durrnagel et al., 2010; Golubovic et al., 2007). This organization is consistent with HyNaC functioning as a postsynaptic ionotropic receptor involved in fast peptidergic neurotransmission, possibly directing tentacle contractions during feeding.

The appearance of genes encoding these early Deg/ENaC channels in primitive animals parallels the appearance of the first nervous systems evolving in cnidarians, including hydra. That these ancient nervous systems extensively used peptides as neurotransmitters fits with Deg/ENaC channels serving a key role in the origins of synaptic transmission. Moreover, that at least three distinct HyNaC genes and one pseudogene are present in an organism in which the nervous system first evolved suggests that the expression of Deg/ENaC channels contributed to preadaptations that enabled the emergence of these early nervous systems (Durrnagel et al., 2010). The presence of peptide-gated Deg/ENaC channels in the primitive nervous systems of Cnidaria and Mollusca, in addition, indicates that peptide control of gating is an ancient feature of this channel family that has been preserved during evolution, particularly in Protostomia. It currently is unclear whether this feature is also conserved in Deuterostomia or whether neuropeptides have been completely replaced by other ligands in more complex animals.

26.2.4 Deg: MECHANOSENSITIVE CHANNELS OF THE NEMATODE

C. elegans express at least six different Deg/ENaC proteins involved in mechanosensation and possibly up to 21 Deg/ENaC homologs all together (Bianchi and Driscoll, 2002; Kellenberger and Schild, 2002; Mano and Driscoll, 1999). Those involved in mechanosensation, to include Mec-4 and Mec-10, form ion channels that are directly gated by mechanical force (Arnadottir et al., 2011; Driscoll and Chalfie, 1991; Hong and Driscoll, 1994; Hong et al., 2000; O’Hagan et al., 2005). Degs originally were identified because mutations in these proteins, for instance, the mec-4(d) mutant as shown in Figure 26.5a, caused degeneration of specific mechanosensitive neurons (Blum et al., 2008; Hong and Driscoll, 1994; Hong et al., 2000). This class of mutation resulted from the gain of function where hyperactivation of Degs

Figure 26.4 Stimulation of HyNaC by RFamides. (a and b) Expression of HyNaC in cells at the base of tentacles in hydra. (c) Activation of macroscopic HyNaC currents by RFamide I and II. (Reprinted from Golubovic, A. et al., J. Biol. Chem., 282, 35098, 2007. With permission.)

Figure 26.5 Degs are involved in mechanosensation. (a) Picture of a C. elegans that expresses a Deg mutation that causes swelling of neurons (noted by white arrows). (Reprinted from Blum, E.S. et al., Cell Death Differ., 15, 1124, 2008. With permission.) (b) Crawling pattern of wild type and (c) C. elegans harboring the unc-8(f) loss of function mutation. (Reprinted from Tavernarakis, N. et al., Neuron, 18, 107, 1997. With permission.)
caused a constant influx of cations, leading to inappropriate membrane depolarization and ultimately necrotic cell death. In many respects, the cell death caused by hyperactivation of Degs mirrors the excitotoxic cell death that occurs in neurons of higher organisms in response to injury such as that in the brain during stroke (Bianchi and Driscoll, 2002; Sherwood et al., 2012).

Activation of Degs leads to an excitatory, depolarizing inward Na⁺ current (see Figure 26.2c; Geffeney et al., 2011). Consequently, the Degs expressed in touch receptors of C. elegans function as molecular mechanoelectrical transduction machines capable of transforming force into a bioelectrical signal. Similarly, Degs expressed in the motor neurons that control nematode locomotion, such as Unc-8, also respond to mechanical cues. In this instance, a channel formed of Unc-8 and Del-1, which is expressed in specialized synapse-free processes, is believed to be activated by stretch (Mano and Driscoll, 1999; Tavernarakis et al., 1997). This is thought to contribute to nematode proprioception by providing feedback on body posture to fine-tune motor neuron activity. Consistent with this, disruption of Unc-8, as shown in Figure 26.5b and c, disrupts the normal sinusoidal body wave of the moving worm (Tavernarakis et al., 1997).

Yet another Deg, Unc-105, possibly activated by mechanical cues, also is thought to contribute to proprioception by monitoring muscle stretch (Kellenberger and Schild, 2002). In contrast to Unc-8 and Del-1 expressed in neurons, Unc-105 is expressed in muscle. Gain-of-function mutations in Unc-105 cause muscle hypercontraction because muscle cells are inappropriately depolarized by a sustained cation influx conducted by the activated channel.

Degs, such as Deg-1, which are directly activated by a mechanical stimulus (see Figure 26.2c), also are critical to nociceptive responses in the nematode (Geffeney et al., 2011). In this role, these nociceptors function as molecular ionotropic mechanoelectrical transducers capable of transforming applied force into a bioelectrical signal that influences sensory neuron excitation to ultimately provoke appropriate behavioral responses.

Although there is abundant experimental evidence documenting the function of Degs in mechanosensory transduction in C. elegans, it is less clear if their mammalian orthologs respond to identical modalities. Because of the potential importance of this possibility to how mammals may perceive touch, sound, and pain, this area is the focus of many contemporary studies.

### 26.2.5 Ppk: Deg/ENaC CHANNELS OF INSECTS

Insects express the largest number of distinct Deg/ENaC genes. For instance, *D. melanogaster* expresses at least 16 and possibly up to 30 different Deg/ENaC genes referred to as Ppk (Adams et al., 1998; Bianchi and Driscoll, 2002; Darboux et al., 1998; Kellenberger and Schild, 2002; Liu et al., 2003a; Mano and Driscoll, 1999). Correspondingly, the products of these genes have diverse expression profiles, activating stimuli and physiological functions to include involvement in mechanosensory transduction and control of locomotion, liquid clearance from the trachea, detection of pheromones and courtship behavior, egg-laying, and salt taste (Boiko et al., 2012; Liu et al., 2003b; Rezaval et al., 2012; Thistle et al., 2012; Zhong et al., 2010). The Ppk channels involved in sensory transduction are expressed in peripheral sensory neurons. The function of Ppk channels expressed in central neurons is obscure. The Ppk channels involved in transport are expressed in epithelial cells.

Akin to the function of ENaC in mammalian airways, Ppk4 and Ppk11 are necessary for liquid clearance from the trachea of the fly (Liu et al., 2003a). The proper clearance of liquid in airways is as critical to air breathing in insects as it is to mammals. This function of Ppk4 and Ppk11 is consistent with these channels contributing to Na⁺ transport because such reabsorption provides the osmotic draw pulling water from the lumen of the trachea. As discussed in more detail later, involvement of Deg/ENaC channels in epithelial transport is retained in mammals, suggesting that this is a conserved function of the family.

The ability to detect salt is critical to the survival of terrestrial animals, including insects. In the fly, Ppk11 and Ppk19 are expressed in larval taste-sensing terminal organs and in adult taste bristles of the labelum, legs, and wing margins (Liu et al., 2003b). Disrupting these Ppk proteins decreases the ability of larvae to detect low concentrations of Na⁺ and attenuates the electrophysiological response to small amounts of salt. Moreover, disrupting Ppk11 and Ppk19 changes the behavior of both larvae and adults relative to salt. Such findings argue that these Ppk proteins function as critical gateways facilitating cell entry of Na⁺ into peripheral taste receptors. Activation of Ppk channels in taste receptors then is positioned to convey information about the presence of salt in the environment, ultimately driving appropriate behavioral responses.

Ppk channels, in addition, play a key role in reproduction in the fly. In females, Ppk channels are expressed in neurons that are part of a critical circuit that mediates postmating responses and egg-laying (Rezaval et al., 2012). In males, Ppk23 and Ppk29 are expressed in sensory neurons that influence through central circuits courtship behavior in response to sex-specific sensory cues (Thistle et al., 2012). Activation of these Ppk channels by pheromones in males is necessary and sufficient to promote courtship toward females. In this regard, Ppk23 and Ppk29 function as ionotropic sensory receptors responsive to chemical stimuli. It is reasonable to suggest that activation of these Ppk receptors excites sensory neurons to convey information about the presence of receptive females.

Ppk channels are also critical to normal fly locomotion and responses to noxious stimuli (Ainsley et al., 2003; Boiko et al., 2012; Zhong et al., 2010). Ppk1 is restrictively expressed in class IV md neurons of the fly. These are polymodal peripheral sensory neurons that form extensive dendritic networks that ramify beneath the epidermis and are required for normal proprioception and nociception. Class IV md neurons have much in common with polymodal neurons involved in sensing touch and noxious stimuli in the mammalian peripheral nervous system. As such, they have been used as a model to investigate mechanosensation. In class IV md neurons, Ppk1 conducts a transient depolarizing inward Na⁺ current. As shown in Figure 26.6, targeted stimulation of Ppk1 excites class IV md neurons bringing them to threshold, evoking a transient train of action potentials as defined by the gating pattern of the channel (Boiko et al., 2012). Thus, Ppk1 functions in class IV md neurons as an ionotropic molecular sensory transducer capable of sensing and transforming a stimulus into a change in neural activity. Details about the specific physiological stimuli
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that activate Ppk1 currently are obscure, but indirect evidence suggests that akin to certain Degs, Ppk1 may be activated directly by mechanical cues. In any case, neural output from class IV md neurons as generated by activation of Ppk1 ultimately influences locomotive behavior. Cellular ablation of class IV md neurons like disruption of Ppk1 expression and function abrogates normal wandering of larvae in the foraging stage. Appropriate larval wandering is critical to normal food-seeking behavior. It is interesting that loss of function of Ppk1 phenocopies gain of function at the level of behavior (Boiko et al., 2012). This parallels the effects of loss and gain of function of Deg channels in mechanosensitive neurons in *C. elegans* (Gonzales et al., 2009; Mano and Driscoll, 1999). Loss of Ppk1 function abolishes the ability to sense a stimulus and respond appropriately. Gain of Ppk1 function abolishes the ability to transform the sensing of a stimulus properly and thus also abrogates an appropriate cellular response. Such findings demonstrate that appropriately graded Ppk1 activation is critical to proper signaling from mechanosensitive neurons of the fly. Perhaps mammalian orthologs serve a similar function. This, though, remains controversial.

26.2.6 ASIC: ACID-SENSING Na⁺ CHANNELS EXPRESSED IN MAMMALIAN NEURONS

ASICs are also prominent members of the Deg/ENaC family. Although discussed here in brief, ASICs are also the focus of Chapter 25. Mammalian ASICs are widely expressed in neurons of both the central and peripheral nervous systems (Gonzales et al., 2009; Kellenberger and Schild, 2002; Lingueglia, 2007; Mano and Driscoll, 1999; Sherwood et al., 2012). Mammals express four ASIC genes with at least three of them, ASIC1–3, giving rise to several splice variants often referred to as a and b isoforms. ASIC1a, ASIC2a, and ASIC2b are richly expressed in the brain, chiefly in the hippocampus, cerebellum, cerebral cortex, striatum, and amygdala. They also are expressed in the spinal cord. The pituitary has strong ASIC4 expression. ASIC1a, ASIC2a, and ASIC3 are expressed in peripheral neurons with ASIC3 expressed primarily in small-diameter polymodal sensory neurons involved in sensation of pain and ASIC2a in medium- and large-diameter mechanosensory neurons, such as those in the dorsal root ganglia.

As their name implies, ASICs are activated by decreases in extracellular pH (see Figure 26.2a). Although it is clear that protons function as ligands that bind and activate ASIC, it is unclear if they are the only or even the primary physiological stimulus for every ASIC isoform. Active ASICs conduct excitatory, inward cation currents. Similar to FaNaC in postsynaptic cells and Ppk1 in sensory neurons, the magnitude of the depolarizing current conducted by ASIC is sufficient to evoke action potentials in mammalian neurons (Bianchi and Driscoll, 2002; Kellenberger and Schild, 2002; Lingueglia, 2007; Lingueglia et al., 2006; Sherwood et al., 2012).

![Figure 26.6](https://example.com/figure26.6)

*Figure 26.6* Ppk1 functions as an ionotropic molecular sensory transducer. (Top) Crawling pattern of three representative larvae, (middle) typical macroscopic Ppk1 currents in voltage-clamped md neurons, and (bottom) action potentials evoked by targeted activation of Ppk1 in current-clamped md neurons in (a) wild type, (b) Ppk1−/−, and (c) rescued flies and for (d) flies harboring the *Deg* gain-of-function mutation in Ppk1. (Reprinted from Boiko, N. et al., *J. Biol. Chem.*, 287, 39878, 2012. With permission.)
The possibility that ASICs are involved in mechanosensation in mammals has generated much controversy. Currently, such a role is supported primarily by indirect and subtle evidence (Bianchi and Driscoll, 2002; Borzan et al., 2010; Lingueglia, 2007; Lingueglia et al., 2006; Lu et al., 2009; Staniland and McMahon, 2009). This hypothesis, however, is attractive and persists because of parallels with the function of Deg and Ppk homologs in invertebrates. The most compelling support for such a conserved function in touch and mechanosensation comes from the analysis of skin mechanoreceptor responses in ASIC2 and ASIC3 knockout mice. Moreover, ASIC2a expression in mechanosensory neurons is localized to specialized cutaneous nerve termini involved in sensing mechanical cues. Deletion of ASIC2 disrupts responses in two types of low-threshold, light touch-sensitive mechanosensitive fibers (Bianchi and Driscoll, 2002; Price et al., 2000). In ASIC2 knockout mice, rapidly adapting (RA) and to a lesser extent slowly adapting (SA) fibers are defective in increasing firing frequency in response to stronger displacement force stimuli. The threshold sensitivity and response frequency of AM fiber mechanonociceptors, which respond to high-threshold stimuli, including pinching, are significantly reduced in ASIC2 knockout mice (Price et al., 2001).

Although exciting, these two knockout models thus far have provided only subtle support for a conserved role for mammalian Deg/ENaC channels in mechanosensation because deletion of ASIC protein failed to eliminate completely either responses to light or harsh touch or responses tonoxious mechanical stimuli. For instance, while defective, RA receptors in ASIC2 null mice maintain threshold sensitivity and other mechanoreceptors that express this channel appear normal (Bianchi and Driscoll, 2002; Price et al., 2000; Roza et al., 2004). Although such results could be explained by redundancy or overlapping function or that ASICs are modulatory but not necessary, additional research is needed before definitive conclusions can be made in this regard.

Expression of ASICs in neurons involved in sensing pain and activation by acidic pH is consistent with them playing a role in pain perception, particularly in response to tissue acidosis such as that arising from ischemia and inflammation (Bianchi and Driscoll, 2002; Chu and Xiong, 2013; Kellenberger and Schild, 2002; Leng and Xiong, 2012; Lingueglia, 2007; Sherwood et al., 2012). For instance, ASIC3 is expressed in sympathetic cardiac afferents, where compelling evidence supports their involvement in sensing nonadapting ischemic pain caused by acidosis (Chu and Xiong, 2013; Kellenberger and Schild, 2002; Leng and Xiong, 2012; Lingueglia, 2007; Sherwood et al., 2012). During cardiac ischemia, extracellular pH within affected areas decreases to values capable of activating ASIC3. It is reasonable to suggest that stimulation of ASIC3 excites these sensory neurons, evoking a train of action potentials that conveys the sensation of cardiac pain. Consistent with such a role, amiloride, the prototypical inhibitor of Deg/ENaC channels, has analgesic effects in a variety of animal pain models and inhibits activation of slowly conducting sensory fibers (C-type) by acid in the rat (Chu and Xiong, 2013; Kellenberger and Schild, 2002; Leng and Xiong, 2012; Staniland and McMahon, 2009; Xiong et al., 2008). Similarly, several different toxins that inhibit ASICs, including venom from the black mamba and psalmotoxin 1 from the tarantula, have strong analgesic action (Diochot et al., 2012; Leng and Xiong, 2012; Mazzuca et al., 2007; Sherwood et al., 2012). Psalmotoxin 1 in particular has very potent analgesic properties against mechanical, chemical, inflammatory, and neuropathic pain in rodents. It is believed that these actions are mediated at least in part by inhibition of ASIC1a in nociceptors. Other peptidergic toxins, such as APETx2 from the sea anemone, inhibit ASIC3 and mitigate pain (Kellenberger and Schild, 2002; Lingueglia, 2007). Conversely, the coral snake toxin, MitTx, activates ASICs that induce pain (Bohlen et al., 2011). Thus, ASICs expressed in sensory neurons are targets for peptide toxins and other agents that inhibit or provoke pain. This is consistent with ASICs functioning as ionotropic molecular sensory receptors capable of exciting peripheral neurons involved in pain sensation.

Several of the toxins that inhibit ASIC and work as potent analgesics when applied locally in the PNS, including psalmotoxin 1, also mitigate pain when applied to the central nervous system (CNS) (Bianchi and Driscoll, 2002; Kellenberger and Schild, 2002; Sherwood et al., 2012). This suggests that ASICs also have a role in synaptic transmission as mediated by central neurons. Perhaps, they act as excitatory ionotropic receptors in postsynaptic neurons because they conduct depolarizing currents upon activation. Particulars about the degree they do this and in which neurons are currently obscure. Moreover, the physiological stimulus that would activate ASICs in this regard is unclear. Perhaps, like FaNaC and HynNaC, they respond to small neuropeptides. This, however, is speculation at this time. Alternatively, synaptic vesicles are acidic, and a transient drop in extracellular pH is associated with synaptic transmission (Bianchi and Driscoll, 2002). Perhaps, release of a neurotransmitter acidifies the synaptic cleft enough to activate postsynaptic ASICs that contribute to or modulate transmission in some regard. Although speculative, this is in agreement with the postsynaptic localization of these channels. Moreover, in some but not all studies, ASIC1 knockout mice have aberrant long-term potentiation as hippocampal neurons in null mice are defective in their response to high-frequency stimulation. This defect correlates with specific learning deficits in mutant mice (Gloor et al., 1993; Wemmie et al., 2002; Wu et al., 2013). Together, the bulk of this evidence is consistent with ASIC1 functioning at synapses in the CNS.

26.2.7 ENaC: THE MAMMALIAN EPITHELIAL Na\(^+\) CHANNEL INVOLVED IN TRANSPORT

ENaC is expressed in the apical membrane of polarized epithelial cells involved in Na\(^+\) transport (Canessa et al., 1993, 1994; Lingueglia et al., 1993). This includes epithelial cells lining the gastrointestinal tract and renal tubule as well as the lungs and airways to name a few (Garty and Palmer, 1997; Kellenberger and Schild, 2002). Figure 26.7a shows the polarized expression pattern of ENaC in the apical membranes of principal cells of the distal renal nephron (Mironova et al., 2012). Sodium is maintained in disequilibrium across the plasma membranes of cells by the constant activity of the Na\(^+\)/K\(^+\)-ATPase pump combined with the barrier properties inherent to lipid bilayers. Such disequilibrium makes Na\(^+\) a functional osmolyte capable of influencing the movement of water via osmosis. Activation of ENaC decreases the resistance of the apical membrane of an epithelial cell to Na\(^+\), allowing this ion to...
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ENaC allows Na⁺ transport in epithelial cells. (a) Fluorescence micrograph of ENaC expression (red) in aquaporin 2 (AQP2, green) positive cells (principal cells) of the ASDN. Nuclei labeled blue. (Reprinted from Mironova, E. et al., Proc. Natl. Acad. Sci. USA, 109, 10095, 2012. With permission.) (b) A drawing of a principal cell in the ASDN of the renal tubule with ion channels and transporters labeled. (c) A representative current trace of ENaC in a cell-attached patch formed on the apical membrane of a principal cell in an isolated, split-open murine ASDN.

enter the cell down its electrochemical gradient. This results in depolarization and an increase in the intracellular concentration of Na⁺. In polarized epithelial cells, basolateral Na⁺/K⁺-ATPases then rapidly pump Na⁺ out of the cell, extruding this ion across the membrane opposite from where it entered. Consequently, as depicted in Figure 26.7b, ENaC is an integral component of the transcellular pathway enabling vectorial transport (absorption) of Na⁺ across polarized epithelial cells (Bonny and Hummler, 2000; Garty and Palmer, 1997; Hummler and Horisberger, 1999; Kellenberger and Schild, 2002; Rossier et al., 2002). Water follows via osmosis. Because ENaC is the major cell-entry pathway for Na⁺ across the apical membrane in epithelial cells that express this channel, its activity is limiting for Na⁺ and coupled water absorption across these cells.

As limiting for vectorial Na⁺ absorption, ENaC plays a key role in setting the volume and electrolyte content of fluid compartments. This allows ENaC to serve several important physiological functions. In airways, it influences mucus hydration and consequently viscosity (Mall et al., 2004; Rauh et al., 2013). Proper fluidity of airway mucus is critical to the removal of inhaled foreign substances via mucociliary clearance. Deeper in the lungs, activation of ENaC facilitates water reabsorption, dehydrating alveolar spaces to decrease compliance (Bonny and Hummler, 2000; Hummler and Horisberger, 1999; Rossier et al., 2002). This is critical to the mechanics of air breathing. Thus, ENaC provides critical service in the lungs that allows air breathing. This in part enables terrestrial vertebrates to live out of water and inhabit the land.

In the gut and kidneys, ENaC enables (re)absorption of Na⁺ and water into the body (Bonny and Hummler, 2000; Garty and Palmer, 1997; Kellenberger and Schild, 2002; Rossier et al., 2002). ENaC is also expressed in taste receptor cells of the tongue that ultimately influence NaCl-seeking behavior (Chandrashekar et al., 2010). Thus, in a broad sense, ENaC functions to bring in and retain sodium and water within an animal. This ENaC-dependent water conservation offers freedom from an immediate source of water and as such allows terrestrial life.

The Na⁺ reabsorbed through ENaC in the kidneys ultimately influences the distribution of water within body fluid compartments and provides a mechanism for controlling blood pressure in mammals. The circulatory systems of higher animals are closed, meaning that vascular volume influences pressure in these systems. Na⁺ and its conjugated bases, Cl⁻ and HCO₃⁻, are the primary extracellular osmolytes in animals. Thus, terrestrial vertebrates control extracellular fluid volume, including plasma, by controlling serum Na⁺. As is clear when considering the action of diuretics that suppress tubular Na⁺ reabsorption, including those like amiloride that target ENaC, serum Na⁺ and thus blood pressure are controlled in part by regulation of renal Na⁺ excretion. Renal sodium excretion is fine-tuned in the aldosterone-sensitive distal nephron (ASDN). ENaC is expressed in the apical plasma membrane of principal cells of the ASDN (Bonny and Hummler, 2000; Garty and Palmer, 1997; Hummler and Horisberger, 1999; Kellenberger and Schild, 2002; Lifton et al., 2001). Here, ENaC serves as the primary cell-entry pathway for Na⁺ reabsorption from the urine back into interstitial fluid and blood.

The activity of ENaC also influences the movement of Cl⁻ and K⁺ through their respective channels and transporters in epithelial cells. This is a secondary effect stemming from the depolarizing actions of ENaC. Activation of ENaC increases the electrochemical forces driving K⁺ efflux out of a cell. If an epithelial cell expresses active K⁺ channels in its apical membrane along with ENaC, then activation of the latter channel will promote K⁺ secretion from this cell. This explains in part why K⁺ secretion is tied to Na⁺ reabsorption in the distal nephron. Similarly, activation of ENaC decreases the electrochemical forces driving Cl⁻ from a cell. If an epithelial cell expresses a Cl⁻ channel along with ENaC in its apical membrane, then activation of ENaC suppresses secretion of Cl⁻, and, conversely, activation of the Cl⁻ channel suppresses Na⁺ absorption mediated by ENaC. This relation between Cl⁻ secretion and Na⁺ (re)absorption also ultimately determines whether a specific epithelial tissue secretes or (re)absorbs water.

26.2.8 BLINaC: THE MAMMALIAN Deg/ENaC CHANNEL EXPRESSED IN BOTH NEURONS AND EPITHELIAL CELLS

The expression of BLINaC is more restrictive than ASIC and ENaC. As shown in Figure 26.8, BLINaC is expressed primarily in epithelial cells of the bile duct and a subset of interneurons in the granular layer of lobules IX and X of the cerebellum.
The function of BLINaC in epithelia is unknown. However, inferences can be made considering its expression pattern and protein function. As a bile acid–gated Na+ channel expressed in the apical membrane of cholangiocytes, BLINaC likely functions in these cells in a manner similar to the function of ENaC in renal and pulmonary epithelial cells (Wiemuth et al., 2012, 2013). BLINaC likely is a regulated gateway controlling cell entry of Na+ from ductal fluid to influence the electrolyte content and volume of biliary secretions tuning these secretions to bile production.

The function of BLINaC in cerebellar interneurons also is unknown. As a ligand-gated depolarizing ion channel (Sakai et al., 1999; Schaefer et al., 2000; Wiemuth and Grunder, 2011; Wiemuth et al., 2012), BLINaC is positioned to influence the excitability of these neurons perhaps in response to neuropeptides. Although not definitive, that the open probability of BLINaC approaches zero in the absence of a ligand is most consistent with this channel serving some role in the development or modulation of the action potential in the postsynaptic neuron in response to a specific stimulus.

## 26.3 Evolution of Deg/ENaC Channels

Deg/ENaC channels represent an ancient ion channel family expressed by most if not all extant metazoan species (Golubovic et al., 2007). As shown in Figure 26.9, the genes encoding Deg/ENaC proteins are of primordial origin appearing first >600–700 million years ago, prior to the Radiata-Bilateria dichotomy. The rise of Deg/ENaC genes paralleled the emergences of neurons in the decentralized nerve net of primitive animals. The antiquity of this ion channel family is emphasized by the recent cloning of Deg/ENaC genes from the freshwater cnidaria *H. magnipapillata*, which has radial symmetry and a primitive nervous system (Durrnagel et al., 2010; Golubovic et al., 2007). Although the exact period and specific species in which Deg/ENaC channels first arose are obscure, the genes encoding these channels likely emerged subsequent to the appearance of multicellular animals because genes encoding Deg/ENaC proteins have not been identified to date in the genomes of bacteria, archaea, yeast, or any other unicellular eukaryotes.

Ancestral Deg/ENaC channels as represented by HyNaC served as rapid-response ionotropic receptors for neuropeptides. These receptors complemented the function of more common but slower responding metabotropic neuropeptide receptors in the nervous systems of primitive animals. By the time bilaterian animals emerged, Deg/ENaC channels had diversified. Deg/ENaC channels began appearing in epithelial cells as well as neurons with corresponding expansion of channel function. As represented by FaNaC of *H. aspersa*, some family members retained their ability to sense neuropeptides and function as ionotropic Rfamide receptors (Cottrell, 1997). Others, such as those appearing in ecdysozoans, evolved to respond to different stimuli enabling divergent cellular functions. For instance, certain Degs in *C. elegans* and Ppk channels in *D. melanogaster* specialized into ionotropic molecular sensory transducers in peripheral sensory neurons involved in perception of the external environment, while others specialized to function during epithelial transport and control of fluid volume and composition (Bianchi and Driscoll, 2002; Mano and Driscoll, 1999).

### 26.3.1 Emergence of ASIC

It is uncertain whether the complement of Deg/ENaC channels expressed in mammals retains the full scope of function and ligand sensitivity of their predecessors. For instance, none of the mammalian Deg/ENaC channels is as yet recognized to be sensitive to neuropeptides or definitively shown to be sensitive to...
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Mechanical stimuli. Nevertheless, by the emergence of Chordates, in particular vertebrate fish, including the lamprey, a specialized branch of Deg/ENaC channels sensitive to extracellular pH had appeared (Coric et al., 2005). Such ASICs are common to the central and peripheral nervous systems of all extant vertebrate animals and play key roles in neuron function to include synaptic transmission and sensory perception (Sherwood et al., 2012).

26.3.2 EMERGENCE OF ENaC

ENaC is restrictively expressed in epithelial cells of all air-breathing sarcopterygians and terrestrial vertebrates (Cottrell, 1997; Garty and Palmer, 1997; Uchiyama et al., 2012). This specialized Deg/ENaC channel plays a critical role in Na⁺ transport across epithelial barriers and thus influences hydration of mucus membranes and systemic electrolyte and water homeostasis. The genes encoding ENaC likely initially arose in the lobe-finned fish that served as the common ancestor of modern lungfish and terrestrial vertebrates. The sequences of the genes encoding ENaC proteins in the modern Dipnoi lungfish, Neoceratodus forsteri, and the Deg/ENaC proteins of living Coelacanth and Hyperoartia fish are the closest in existence to the ancestral ENaC progenitor (Uchiyama et al., 2012). Although the appearance of the genes encoding ENaC proteins is firmly established in the timeline of evolution, questions remain about whether the rise of these genes enabled animals to face better selection pressures arising from the need for water conservation or air breathing using lungs necessary for terrestrial living. Regardless of which selection pressure was initially preeminent, proper expression and function of ENaC allows modern mammals to live on land. This is highlighted by the facts that loss of ENaC function in the lungs causes wet lungs, respiratory distress, and consequent laborious breathing in neonatal animals, which ultimately leads to their death, and loss of ENaC function in the kidneys causes inappropriate renal sodium and water wasting and dehydration that also lead to neonatal death (Bonny and Hummler, 2000; Hummler and Horisberger, 1999; Rossier et al., 2002).

26.4 STRUCTURE OF Deg/ENaC CHANNELS

The structure of Deg/ENaC channels is discussed here in brief. This also is covered in Chapter 57. The seminal work in which the structure of chicken ASIC1 recently was resolved at the atomic level has greatly informed understanding of this family of proteins (Jasti et al., 2007). Deg/ENaC channels are obligatory trimers where each of the three component subunits contributes to a central pore (Jasti et al., 2007; Staruschenko et al., 2005). Certain family members form obligatory heterotrimers composed of three related but distinct subunits as typified by ENaC, which comprises one α, one β, and one γ subunit (Canessa et al., 1994; Staruschenko et al., 2005). In some tissues, the α subunit is
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26.4 Structure of Deg/ENaC channels

replaced by the δ subunit. A predicted structure for αβγ ENaC as based on the crystal structure of cASIC1 is shown in Figure 26.10 (Stockand et al., 2008). Other family members form obligatory homotrimers, and others form both homo- and heterotrimers. ASICs fall into this latter category.

Deg/ENaC homologs share approximately 25%–35% sequence identity and have a conserved subunit topology, which is shown in Figure 26.11. All Deg/ENaC subunits contain a large extracellular domain bound by two transmembrane (TM) domains, TM1 and TM2, with intracellular NH2- and COOH-termini (Gonzales et al., 2009; Jasti et al., 2007). The extracellular domain contains much secondary structure, with beta sheets and alpha helices from each of the three component subunits making extensive inter- and intrasubunit contacts. These secondary structures form five major extracellular domains in each subunit: the finger, knuckle, thumb, β-ball, and palm domains. Extracellular ligands bind in a pocket formed by the finger and thumb domains (Baconguis and Gouaux, 2012; Dawson et al., 2012). The pore of the channel is formed by the symmetry-related three helical TM2 domains, one from each subunit, as they run through the membrane in a linear manner.

The fully formed Deg/ENaC channel can be envisioned as a chalice where extracellular domains form a cup, TM domains a stem, and intracellular domains a base (Jasti et al., 2007). As shown in Figure 26.12, the pore is hourglass in shape with wide extracellular and intracellular vestibules and a narrowing in the middle. This pore is within the stem of the chalice. The extracellular mouth of the pore is joined to extracellular domains by short linker sequences referred to as the wrist.

26.4.1 PORE OF Deg/ENaC CHANNELS

The Deg/ENaC pore has threefold symmetry around its central axis perpendicular to the plane of the lipid bilayer. Ions enter and egress out of the extracellular and intracellular mouths of the pore proper through large vestibules that lay partially within the plane of the membrane. As shown in Figure 26.12, these vestibules have profound negative electrostatic potentials enabling them to act as cation reservoirs. This concentrating of cations around the mouths of the pore increases channel conductance (Gonzales et al., 2009).

The pore contains three Na+ binding sites that are occupied during permeation. Permeant ions move through the pore in a single-file manner where adjacent sites are not occupied at the same time due to charge repulsion (Gonzales et al., 2009). The main-chain carbonyl oxygen atoms from the symmetry-related G432, G436, G439, and G443 residues in cASIC1 coordinate Na+ at these binding sites. Ions in the pore are coordinated with trigonal antiprism geometry by three ligands on the upper triangular plane being staggered in comparison to those in the lower triangular plane. This arrangement provides the appropriate number of partial negative charges for coordination while perfectly accommodating the underlying threefold molecular
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Degenerin/ENaC channels, employs identical modes and means of permeation but shares no primary sequence with these selective, and has 2°, 3°, and 4° structure resembling that of Deg/ENaC channels. Interestingly, ENaC is about 10-fold more selective for Na⁺ than Gly. Perhaps, this introduces a steric constraint that better reduces both the Na⁺ conductance and Na⁺ to K⁺ selectivity. Several of the Gly residues involved in coordinating Na⁺ within the pore of cASIC1 are replaced by Ser in ENaC. As such, trigonal antiprism coordination is believed to represent the archetype molecular basis of permeation through cation-selective ion channels containing three component subunits (Gonzales et al., 2009). Indeed, the P2X4 channel, which is trimeric, cation selective, and has 2°, 3°, and 4° structure resembling that of Deg/ENaC channels but shares no primary sequence with these channels, employs identical modes and means of permeation and coordination of permeant ions within the pore (Gonzales et al., 2009).

Several of the Gly residues involved in coordinating Na⁺ within the pore of cASIC1 are replaced by Ser in ENaC. Interestingly, ENaC is about 10-fold more selective for Na⁺ than is ASIC. Although structurally similar, Ser, which contains an extra carbon and hydroxyl moiety, occupies a larger volume than Gly. Perhaps, this introduces a steric constraint that better accommodates Na⁺ compared to other cations in the permeation pathway of the more selective ENaC. Consistent with such a premise, mutations in and near the selectivity filter in ENaC reduce both the Na⁺ conductance and Na⁺ to K⁺ selectivity (Kellenberger et al., 1999a,b; Snyder et al., 1999).

26.4.2 GATE OF Deg/ENaC CHANNELS

ASIC and most if not all ligand-gated Deg/ENaC channels assume three principal conformations: closed, open, and desensitized. ENaC, which gates independent of a ligand, does not inactivate or desensitize, having only closed and open conformations. It is assumed that all Deg/ENaC channels employ a common gating mechanism to move from the closed to open state with ligand-gated channels having an additional change in conformation leading to the desensitized state. In contrast to its inhibition of rat ASIC1, psalmotoxin 1 activates chicken ASIC1 (Gruner and Augustinowski, 2012). Understanding gleaned from the crystallization of psalmotoxin 1 with active cASIC1 has allowed rationalization of how Deg/ENaC channels may gate (Baconguis and Gouaux, 2012). The channel is thought to be in the open state following the untwisting of TM domains around the central axis of the pore perpendicular to the plane of the lipid bilayer. Specifically, the upper palm and knuckle domains of the three component subunits provide a fixed structural scaffold on which the lower palm domains shift to induce radial and rotational movements of the TM domains via the wrist linker (Baconguis and Gouaux, 2012; Gruner and Augustinowski, 2012). The finger and thumb domains, which make major contributions to the ligand-binding domain of Deg/ENaC channels, modulate movement of the lower palm, allowing the binding of a ligand to influence gating through an allosteric mechanism (Dawson et al., 2012; Gruner and Augustinowski, 2012).

Crystallization of psalmotoxin 1 with a variant of cASIC1 that can only assume the desensitized conformation revealed that three toxin molecules bind the trimeric channel making contact with thumb domains and extending deeply into ligand-binding pockets to contact palm domains (Dawson et al., 2012). This bimodal binding locks the relative arrangement between thumb and palm in the desensitized state for mammalian ASIC1 and in the open state for functional cASIC1 (Dawson et al., 2012; Gruner and Augustinowski, 2012).

Desensitization is thought to be the manifestation of a constriction formed by the crossing of the three TM2 domains at D433 in cASIC1 occluding the pore (Gonzales et al., 2009; Jasti et al., 2007). This residue sits just below the large vestibule leading into the extracellular mouth of the pore. The Deg mutation that constitutively activates Deg/ENaC channels by locking them in long-lived open states is at 432 in cASIC1, one position upstream of D433 (Goodman et al., 2002; Waldmann et al., 1996). Placement of an amino acid larger than Gly at the Deg site would cause a steric clashing between the symmetry-related TM2 domains, providing a mechanism whereby mutations at this site perturb gating to lock the channel out of the desensitized conformation. It is interesting that Asn residues occupy the positions in ENaC subunits homologous to D433 in cASIC1. This is a signature feature of ENaC. Perhaps, this contributes to ENaC gating constitutively and being unable to enter a desensitized-like conformation.

26.5 Deg/ENaC CHANNELS IN HUMAN DISEASES

A pathological role for ASIC and ENaC in several different human diseases has been described. Not unexpectedly, these diseases contain a neuronal component for ASIC and an epithelial component for ENaC.

26.5.1 ROLE OF ASIC IN PATHOLOGY

Because of their sensitivity to pH, ASICs contribute to pathology resulting from ischemia, inflammation, and trauma that cause tissue acidosis (Bianchi and Driscoll, 2002; Leng and Xiong, 2012; Linguaglia, 2007; Staniland and McMahon, 2009). For instance, activation of ASIC1 by the metabolic acidosis induced...
by brain ischemia contributes to neuronal death associated with stroke. Abundant evidence now exists that Na\(^+\) and Ca\(^{2+}\) influx through ASICs activated by ischemic conditions contributes to anoxic depolarization, the rapid and pathological loss of membrane potential, which ultimately leads to cell death. Accordingly, inhibition of ASIC1 is an emerging therapy for stroke intervention and treatment of other neurological diseases associated with focal drops in pH and neuronal death (Chu and Xiong, 2013; Leng and Xiong, 2012).

Inappropriate activation of ASIC, moreover, contributes to the progression of multiple sclerosis (MS) (Friese et al., 2007; Vergo et al., 2011; Xiong et al., 2008). MS is an autoimmune neuroinflammatory disease of the CNS (Judge and Bever, 2006; Korenke et al., 2008; Solari et al., 2002). Hallmarks of this disease include CNS lesions marked by demyelination and axonal degeneration (Kornek et al., 2000; Lovas et al., 2000). Axonal degeneration in MS is caused by improper influx of Na\(^+\) and Ca\(^{2+}\) into neurons (Dutta and Trapp, 2007; Dutta et al., 2006; Lassmann, 2007; Nikolaeva et al., 2005; Petrescu et al., 2007; Stys, 2005; Waxman, 2006; Xiong et al., 2004). ASIC1 whose expression is increased in MS lesions by proinflammatory mediators serves as the gateway for this pathological influx of cations into diseased neurons (Vergo et al., 2011). Accordingly, inhibition of ASIC1 with amiloride and disruption of the ASIC1 gene provide a neuroprotective effect improving clinical symptoms by protecting both myelin and neurons from damage in an animal model of MS (Friese et al., 2007; Vergo et al., 2011).

### 26.5.2 Regulation of ENaC by Intracellular Signaling

Mammalian Deg/ENaC channels are targets for diverse types of extracellular ligands, systemic hormones, and intracellular signaling cascades. Complete coverage of this subject is beyond the scope of this chapter. Because disruption of normal regulation of ENaC is causative for certain inheritable diseases in humans, key signaling pathways and domains within the channel involved in critical posttranslational regulation are discussed here in brief.

Corticosteroid hormones, including the mineralocorticoid aldosterone, are important positive regulators of ENaC. Upon binding its cognate receptor, aldosterone stimulates ENaC in renal epithelial cells by transactivating the gene-encoding serum and glucocorticoid-inducible kinase (Sgk1) (Pearce, 2003; Snyder, 2005; Staub et al., 2000). The activity of ENaC is controlled in part by regulation of its expression in the apical membrane. The ubiquitin ligase, Nedd4–2, associates with ENaC leading to ubiquitination of the channel. This tagging of the channel targets it for internalization. Sgk1 phosphorylates Nedd4–2 at a 14–3–3 binding site. Phosphorylation of this binding site allows 14–3–3 to sequester Nedd4–2 away from ENaC. Consequently, untagged ENaC is left in the apical membrane where it is active. Thus, aldosterone increases the activity of ENaC in part via a disinhibition mechanism lessening channel retrieval.

ENaC subunits contain a PY motif of the consensus PPPxY in their cytosolic COOH-terminals (Kellenberger and Schild, 2002; Pearce, 2003; Snyder, 2005; Staub et al., 2000). This PY motif functions as a binding site for proteins containing the WW motif. For ENaC expressed in renal epithelia, Nedd4–2 is the primary binding partner to this site.

### 26.5.3 Pathology Arising from ENaC Dysfunction

Because it functions as a critical end effector of the renin–angiotensin–aldosterone system during feedback control of blood pressure, any mutation in ENaC or the upstream regulatory pathways governing channel activity that lead to hyperactivation in the ASDN elevates blood pressure. This elevation in blood pressure results from the disruption of normal renal sodium excretion. Inheritable diseases that fall into this category include Liddle’s syndrome, apparent mineralocorticoid excess, glucocorticoid-remediable aldosteronism, and Geller’s syndrome to name a few (Bonny and Hummler, 2000; Geller et al., 1998, 2000; Hummler and Horisberger, 1999; Lifton et al., 2001; Rossier et al., 2002). Certain dietary factors, such as glycyrrhetinic acid, also can lead to inappropriate activation of ENaC mimicking these inheritable hypertensive diseases. Because mutation of ENaC represents an end-organ defect where the channel is removed from normal feedback regulation, such channelopathy has hallmark elevated ENaC activity and concomitant elevations in blood pressure in the presence of decreased plasma renin activity, aldosterone levels, and serum potassium. Disease resulting from such gain of function of ENaC can be countered with amiloride and other drugs that inhibit the channel.

Most Liddle’s mutations in ENaC that cause elevations in blood pressure result from frame shifts, early truncations that disrupt the cytosolic COOH terminus of one of its component subunits, or both. Specifically, it is the disruption of the Nedd4–2 binding PY motif that causes disease by impairing normal retrieval of the channel from the apical membrane leading to gain of channel function.

A disease-causing point mutation in ENaC outside of the PY motif that activates the channel and elevates blood pressure also has been described. The Liddle’s mutation N530S in γ-ENaC increases channel activity but not membrane expression to cause disease (Hiltunen et al., 2002). N530, which corresponds to D433 in cASIC1, is one position downstream of the γ44–2 binding motif that causes disease by impairing normal retrieval of the channel from the apical membrane leading to gain of channel function.

Loss of function mutations in ENaC and its upstream regulatory pathways also result in inheritable forms of tubulopathy (Bonny and Hummler, 2000; Hummler and Horisberger, 1999; Lifton et al., 2001; Rossier et al., 2002). The majority of mutations resulting in loss of ENaC function completely disrupt normal expression of this channel in the ASDN where its absence compromises renal sodium reabsorption. Diseases caused by such mutations are termed pseudohypoaldosteronism (PHA) type I. Together, PHA type I represents a group of rare genetic diseases presenting with hallmark hyperkalemia and renal Na\(^+\) wasting in the presence of high aldosterone (Chang et al., 1996; Riepe, 2000).

The missense mutation, G37S, in human βNaC also causes PHA type I (Chang et al., 1996; Grunder et al., 1997).
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This Gly residue is in an HG motif conserved in the intracellular NH$_2$-terminal portions of all Deg/ENaC subunits. This motif is required for normal gating (Chang et al., 1996; Grunder et al., 1997, 1999). Substituting either the H or G residue in this motif decreases ENaC activity to where the channel is not active under physiological conditions (Kucher et al., 2011). A distinct missense mutation, S562P, in αNaC also causes familial PHA type I (Ripe et al., 2009). S562 occupies the third position in the selectivity-filter sequence, GSS, identifying the disruption of permeation as the most likely mechanism underlying loss of function (Kellenberger et al., 1999a,b; Sheng et al., 2001).

Because of its role in transport, ENaC also is involved in diseases of the lungs and airways. Loss of ENaC function in the lungs leads to fluid accumulation in alveolar spaces due to disruption of sodium reabsorption (Bonacci and Hummler, 2000; Hummler and Horisberger, 1999; Kellenberger and Schild, 2002; Rossier et al., 2002). This increases the compliance of the lungs, which increases the energy cost of breathing, leading ultimately to exhaustion and death. Bacterial toxins and inflammatory mediators released in response to bacterial invasion of the lungs also decrease the activity of ENaC, causing a similar wet-lung phenotype. Through a related mechanism, abnormal ENaC activity is thought to explain certain instances of fluid accumulation in the lungs at high altitudes. Moreover, glucocorticoids modulate the expression of ENaC in the lungs. The surge of glucocorticoids and the forces and processes accompanying birththing activate ENaC to dehydrate the lungs to facilitate air breath upon parturition. Premature infants often are provided exogenous glucocorticoids in part to speed this process.

In airways, Na$^+$ reabsorbed via ENaC dehydrates mucus, countering the effects of Cl$^-$ and coupled-water secretion as mediated by the cystic fibrosis transmembrane conductance regulator (CFTR). As such, gain of ENaC function in the lungs causes a cystic fibrosis–like phenotype with overly dry and sticky mucus similar to that resulting from loss of function of CFTR (Mall et al., 2004; Rauh et al., 2013). The reverse of this also happens in the gut. Pathological hyperactivation of CFTR by bacterial toxins de polarizes the apical membrane to diminish the electrochemical forces favoring cell entry of Na$^+$ via ENaC, impeding the absorption of water. This underpins the conversion of intestinal epithelial cells from absorptive to secretory during secretory diarrhea such as that seen in cholera (Bonacci and Hummler, 2000; Hummler and Horisberger, 1999; Kellenberger and Schild, 2002; Rossier et al., 2002).

REFERENCES


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