CHAPTER 6

ENaC Proteins in Vascular Smooth Muscle Mechanotransduction

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I. OVERVIEW

Mechanotransduction influences many aspects of biological function. In the cardiovascular system, mechanotransduction has a significant impact on vascular function. Multiple signal transduction pathways participate in the transmission of biomechanical forces into cellular signals, including integrins, adhesion molecules, cytoskeleton, and the activation of membrane-bound transporters and ion channels. In this chapter, the potential role of a novel family of ion channels with evolutionarily conserved involvement in mechanotransduction, the degenerin/epithelial Na^+ channel (DEG/ENaC) family, is

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discussed. Members of the family have been identified in a diverse range of species and tissue types. Most members of the DEG/ENaC family form cation selective ion channels, some of which are believed to form mechanically gated ion channels. While most research has focused on degenerins in sensory neuron mechanotransduction, emerging evidence suggests ENaC proteins may also participate in vascular smooth muscle mechanotransduction. This chapter addresses the potential role and physiological importance of ENaC proteins as mechanosensors in vascular smooth muscle.

II. INTRODUCTION

Mechanotransduction is the conversion of a mechanical force (pressure, strain, shear stress) into a biological response (channel activation, gene expression, contraction, and so on). It is a fundamental biological process, occurring in numerous cell types (epithelial, neuronal, and muscle; Sachs, 1988; Morris, 1990; Lingueglia *et al*., 1994; Ingber, 1997; Tavernarakis and Driscoll, 1997; Hamill and Martinac, 2001; Syntichaki and Tavernarakis, 2004). While mechanosensation influences many biological functions, including, bone growth, neuritogenesis, touch sensation, and hearing, it has significant impact on the cardiovascular system. For example, hemodynamic forces sculpt the developing heart and blood vessels (Riha *et al*., 2005). Mechanoreceptors in the heart, aortic arch, and carotid sinuses instantaneously regulate arterial blood pressure (Paintal, 1973; Sheperd and Mancia, 1986). Mechanical forces contribute to the regulation of vascular tone, local blood flow, and vascular remodeling (Davis and Hill, 1999; Ingber, 2006). Given the vast influence of mechanical forces on biological functions, it is not surprising that animal cells have evolved a variety of different signaling mechanisms including the transduction of mechanical forces into changes in gene expression and channel activity via the integrins, adhesion molecules, and the cytoskeleton, as well as activation of membrane-associated ion channels. Members of the transient receptor potential (TRP) and DEG/ENaC ion channel families have received attention for their potential involvement in mechanotransductory responses. Excellent reviews on the (1) involvement of the integrins and the cytoskeleton in mechanotransduction, (2) functioning of TRP channels as sensors, and (3) role of ENaC and acid-sensitive ion channel (ASIC) proteins in baroreception can be found elsewhere (Davis *et al*., 2001; Alenghat and Ingber, 2002; Lin and Corey, 2005; Martinez-Lemus *et al*., 2005; see also Chapter 21). The current chapter will focus on the evidence supporting a role for ENaC proteins in vascular smooth muscle cell (VSMC) mechanotransduction.

III. DEG/ENaC/ASIC PROTEINS ARE MEMBERS OF A DIVERSE PROTEIN FAMILY INVOLVED IN MECHANOTRANSDUCTION

A. *ENaC Proteins*

ENaC proteins are members of a large protein family, termed DEG/ENaC. Members of this family have been identified in the nematode, *Caenorhabditis elegans*; the fly, *Drosophila melanogaster*; the snail, *Helix aspersa*; and mammals. Members share a common structure that consists of short intracellular N- and C-termini and two membrane-spanning domains separated by a large extracellular domain. Most members of the DEG/ENaC family form homo- and/or heteromultimeric cation channels that are selective for Na^+, but some also conduct Ca^{2+} and other cations (Garcia-Anoveros and Corey, 1997; Benos and Stanton, 1999; Mano and Driscoll, 1999; Alvarez de la Rosa *et al.*, 2000; Kellenberger and Schild, 2002; Syntichaki and Tavernarakis, 2004).

ENaC proteins are commonly found in epithelial cells of the kidney, lung, and colon, where they play a rate-limiting role in Na^+ and water transport (Benos and Stanton, 1999; Mano and Driscoll, 1999; Kellenberger and Schild, 2002). In epithelial tissue, α-, β-, and γ-subunits form a heteromultimeric channel inhibitable by the diuretic amiloride. The stoichiometry of the ENaC channel is presumed to be $\alpha_2\beta_1\gamma_1$; however, an alternate stoichiometry of $\alpha_3\beta_3\gamma_3$ has also been proposed (Cheng *et al.*, 1998; Firsov *et al.*, 1998; Kosari *et al.*, 1998; Snyder *et al.*, 1998; Dijkink *et al.*, 2002). The channel is Na^+ selective, nonvoltage-gated and constitutively active. ENaC channels usually have long open and closed time(s); however, populations of channels with short opening times (50 ms) and long closed states have also been reported (Duchatelle *et al.*, 1992; Palmer and Frindt, 1996; Caldwell *et al.*, 2004). Gain-of-function mutations are associated with severe hypertension due to excess salt and water retention (Liddle's syndrome), while loss-of-function mutations are associated with salt wasting and hypotension (pseudohypoaldosteronism type I, PHA; Lifton, 1995; Luft, 1998, 2001; Pradervand *et al.*, 1999; Oh and Warnock, 2000; Kellenberger and Schild, 2002; Hummler and Vallon, 2005).

αENaC, βENaC, and γENaC are presumed to be the main players, but at least two other novel ENaC subunits have been identified with species-specific expression. Delta (δ)ENaC is expressed in the pancreas, testes, ovaries, and brain in human tissues (Waldmann *et al.*, 1995) and can substitute for αENaC and associate with βENaC and γENaC to form a channel. Similarly, in *Xenopus*, epsilon (ε)ENaC can replace αENaC and form a channel with βENaC and γENaC (Babini *et al.*, 2003). It remains to be determined, if these

subunits are also expressed in rodents since the rat and mouse homologues of those ENaC subunits have not been fully cloned.

B. Genetic Link to Mechanotransduction

ENaC proteins are members of a larger family of proteins with a strong genetic link to mechanotransduction. The importance of DEG/ENaC proteins in mechanotransduction originated from work in the nematode. Specific mutations in the *C. elegans* degenerins, which are expressed in neurons, hypodermis, and muscle, produce animals with an abnormal response to light touch or uncoordinated locomotion (Chalfie and Wolinsky, 1990; Driscoll and Chalfie, 1991; Gu *et al.*, 1996; Tavernarakis *et al.*, 1997; Mano and Driscoll, 1999; Syntichaki and Tavernarakis, 2004). The similarity between the *C. elegans* proteins and mammalian family members lead investigators to hypothesize that mammalian DEG/ENaC proteins may also be involved in mechanotransduction. Subsequently, research has provided genetic evidence that another group of DEG/ENaC proteins found in neural tissue and sensory epithelia, termed the ASIC, are required for the normal mechanotransduction in specific populations of sensory neurons (Ugawa *et al.*, 1998; Mano and Driscoll, 1999; Price *et al.*, 2000; Price *et al.*, 2001; Waldmann, 2001). Studies in ASIC null mice suggest some of the ASIC proteins are required for normal mechanotransduction in arterial baroreceptor neurons, touch receptors, and visceral mechanoreceptor (Price *et al.*, 2000, 2001; see Chapter 21). Thus, genetic evidence demonstrates *C. elegans* degenerins and mammalian ASIC proteins are required for normal mechanosensory responses.

Expression of ENaC proteins was originally considered to be limited to epithelial tissue. However, numerous studies show that ENaC expression can also be found at several important mammalian sites of mechanotransduction. In neural tissue, ENaC transcripts and proteins are found in certain hypothalamic nuclei, nodose, dorsal root, and trigeminal sensory ganglia (Drummond *et al.*, 1998; Fricke *et al.*, 2000; Chapleau *et al.*, 2001; Amin *et al.*, 2005; Yamamoto and Taniguchi, 2006). Further, ENaC proteins are expressed at the site of mechanotransduction in a wide variety of peripheral mechanoreceptor nerve endings. ENaC expression has also been detected in cells that are typically exposed to mechanical forces such as the placental trophoblasts, uroepithelia, osteoblasts, keratinocytes, and VSMCs (Kizer *et al.*, 1997; Drummond *et al.*, 1998; Kopp *et al.*, 1998; McCarter *et al.*, 1999; Garcia-Anoveros *et al.*, 2001; Mauro *et al.*, 2002; Drummond *et al.*, 2004; Jernigan and Drummond, 2006a). Genetic evidence demonstrating a link between ENaC proteins and mechanotransduction is not available as ENaC null mice die shortly following birth (Hummler and Rossier, 1996; Koyama *et al.*, 1999; Bonny and Hummler, 2000; Hummler and Beermann, 2000; Snitsarev *et al.*, 2002).

In *Drosophila*, degenerins are expressed in diverse tissues. Pickpocket is expressed in multidendritic neurons, a subset of neurons similar to peripheral sensory neurons that play a role in touch sensation and proprioception (Adams *et al*., 1998). Disruption of this degenerin alters rhythmic locomotion in *Drosophila* larvae (Ainsley *et al*., 2003). Additional degenerin proteins contribute to salt taste and are expressed in trachea and contribute to salt and water transport, much like ENaC proteins in airway epithelia (Liu *et al*., 2003a,b). This suggests that *Drosophila* degenerin proteins may function as mechanosensors in neurons and Na^+ transporters in epithelia.

C. *Mechanotransduction in* C. elegans

C. elegans geneticists have identified degenerin protein expression in diverse range of cell types (sensory neurons, motorneurons, interneurons, hypodermis, muscle) and involvement in diverse functions including neurodegeneration, proprioception, and control of locomotion and touch sensation. The molecular basis of the latter response, touch sensation, has been extensively characterized in the nematode (Sulston *et al*., 1975; Chalfie and Sulston, 1981; Chalfie *et al*., 1986; Chalfie and Au, 1989; Huang and Chalfie, 1994; Gu *et al*., 1996; Du and Chalfie, 2001). Most of our understanding of how mammalian DEG/ENaC proteins may participate in mechanotransduction is based on touch sensation in the nematode. A model of the mechanotransducing complex responsible for touch responses in the nematode is discussed in the following section. For an in-depth review of degenerin channel structure, molecular attributes, and role in mechanotransduction, the reader is referred to a comprehensive review (Syntichaki and Tavernarakis, 2004).

1. Model of *C. elegans* Mechanotransducer

A tethered model of the mechanotransducer in *C. elegans* has been proposed (Driscoll and Tavernarakis, 1997; Tavernarakis and Driscoll, 1997, 2001; Gillespie and Walker, 2001; Ernstrom and Chalfie, 2002; Syntichaki and Tavernarakis, 2004). In this model, the channel is fixed in the membrane and tethered intracellularly to the cytoskeleton and extracellularly to the extracellular matrix. Mechanical force is transmitted to the channel through the extracellular matrix and cytoskeleton to gate the channel. On the basis of extensive studies, a model of a *C. elegans* mechanosensor was developed. A cartoon of this model is shown in Fig. 1. The mechanosensor is composed of core and accessory components.

a. The Mechanosensor Core. The channel pore and critical associated proteins form the core of the mechanosensor (Driscoll and Tavernarakis, 1997; Tavernarakis and Driscoll, 1997, 2001; Ernstrom and Chalfie, 2002; Syntichaki and Tavernarakis, 2004). The channel pore is formed by MEC-4

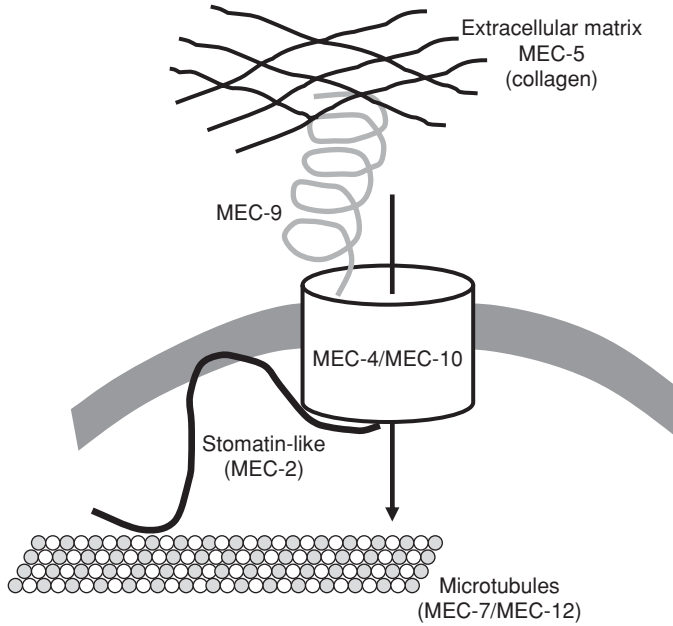

FIGURE 1 Model of the mechanotransduction complex in *C. elegans*. The proposed model of a mechanotransducer in *C. elegans* consists of a channel pore formed by degenerins MEC-4 and MEC-10. The stomatin-like protein MEC-2 links the channel pore is linked to the microtubules (MEC-7/MEC-12). The extracellular MEC-9 protein links the channel pore to collagen (MEC-5) in the extracellular matrix.

and MEC-10, members of the degenerin family and closely related to ENaC proteins (i.e., they have a similar structure of intracellular N- and C-termini, two membrane-spanning domains, and a large extracellular domain). Evidence suggests MEC-4 and MEC-10 can form a pore that conducts Na^+ (Goodman *et al.*, 2002). In addition to the pore-forming subunits, MEC-2 and MEC-6 are two intracellular proteins that contribute to the core of the mechanosensor. MEC-2 is a stomatin-like protein that is proposed to link the pore of the mechanotransducer to the membrane and cytoskeleton. Stomatin, also called Band 7, is a protein expressed in a diverse range of cell types in mammals. Along with other stomatin proteins, MEC-2 may stabilize the channel in the membrane. MEC-6 encodes a protein with similarity to paraoxonases and physically interacts with MEC-4 and MEC-10. MEC-6 is proposed to participate in channel assembly or stabilization. Although the precise role of MEC-6 is unknown, it is proposed to play a role in channel assembly and/or localization. While MEC-2 and MEC-6 do not form the pore

of the mechanosensor, they are critical to modulating gating properties (O'Hagan *et al.*, 2005).

b. Associated Proteins. Additional intracellular and extracellular proteins associate with the mechanosensor core to tether the channel with the cytoskeleton and extracellular matrix (Driscoll and Tavernarakis, 1997; Tavernarakis and Driscoll, 1997, 2001; Ernstrom and Chalfie, 2002; Syntichaki and Tavernarakis, 2004). Intracellular associated proteins include MEC-7 and MEC-12, which encode for β- and α-tubulin proteins, respectively. The tubulin proteins form microtubules and serve as an anchor/link for the mechanosensor channel to the cytoskeleton. MEC-2 may link the microtubules (MEC-7/MEC-12) to the pore (MEC-4/MEC-10). At least three different extracellular proteins (MEC-1, MEC-5, and MEC-9) are associated with the mechanotransducer. MEC-1 contains protein interaction domains (EGF-like domains and Kunitz repeats). A mammalian homologue of this protein has not been identified. MEC-5 is a collagen protein. A direct interaction between MEC-5 and degenerin proteins has not been proven; however, a genetic interaction has been shown. MEC-9 is a component of the extracellular matrix. It contains Kunitz type serine protease inhibitor domains. While the role of MEC-9 is undetermined, it is required for touch responses.

2. *C. elegans* UNC-105: A Muscle Mechanotransducer

While degenerin expression and function have been mostly studied in neuronal tissue, it is clear that degenerin expression is not limited to neuronal tissue. At least one degenerin protein is predominantly expressed in muscle tissue. UNC-105 is thought to be part of a mechanosensitive ion channel important in the control of locomotion (Liu *et al.*, 1996; Shreffler and Wolinsky, 1997; Garcia-Anoveros *et al.*, 1998). UNC-105 interacts with an extracellular collagen (LET-2), presumably to gate the channel (Liu *et al.*, 1996). UNC-105 is another example where a pore-forming degenerin (MEC-4/MEC-10 or UNC-105) can interact with an extracellular collagen (MEC-5 or LET-2) presumably to gate the mechanosensitive channel in response to mechanical stimuli. Although the role of degenerin proteins as mechanotransducers in muscle has received less attention, it is clear that degenerins are expressed in, and required for, stretch sensitivity in muscle tissue.

D. ENaC and Mechanotransduction

In addition to being related to proteins involved in mechanosensory responses, there is direct evidence that ENaC channels can be gated by mechanical factors such as pressure and shear stress. Further, a growing body of

functional evidence demonstrates ENaC proteins play an important role in mechanotransduction.

1. Mechanically Gated ENaC Activity

a. Stretch Activation of ENaC. Early investigations into the direct mechanosensitivity of ENaC in heterologous expression systems have yielded equivocal results. When expressed in a lipid bilayer, αENaC and $\alpha\beta\gamma$ENaC channels are activated by the application of negative hydrostatic pressure (Awayda *et al.*, 1995; Ismailov *et al.*, 1996a,b, 1997a,b). Similarly, expression of αENaC in a fibroblast cell line conferred the presence of stretch-activated cation channels (Kizer *et al.*, 1997) using an electrophysiological approach. However, contrasting results were found in the *Xenopus* oocyte expression system in response to osmotic-induced swelling and shrinking. Hypoosmotic-induced swelling either had no effect on or inhibited $\alpha\beta\gamma$ENaC current (Awayda and Subramanyam, 1998; Ji *et al.*, 1998) and hyperosmotic-induced shrinking inhibited $\alpha\beta\gamma$ENaC current. Thus, differences in mechanosensitivity of ENaC in heterologous systems may be due to the expression system itself (bilayer or fibroblast vs oocyte) or may reflect the ability of pressure- vs osmotic-induced stretch to generate membrane tension and activate ENaC channels.

Mechanical gating of ENaC channels has also been demonstrated in endogenously expressing tissue. Palmer and Frindt (1996) found that the application of negative pressure to isolated channels in cortical-collecting duct cells increased the open probability of native ENaC channels in 27% of patches. Subsequent work from Ma *et al.* (2002) suggests this low probability is likely due to inhibition by purinergic receptors. B lymphocytes also express a mechanically gated, amiloride-sensitive current (Achard *et al.*, 1996; Ma *et al.*, 2004). A study suggests B lymphocytes express αENaC and βENaC, but not γENaC; however, only αENaC appears to contribute to mechanically gated ENaC activity in these cells (Ma *et al.*, 2004).

b. Shear Stress Activation of ENaC. While studies evaluating activation of ENaC currents by pressure- vs hypoosmotic-induced stretch have provided equivocal results, experiments evaluating activation of ENaC to a different mechanical stimulus, shear stress, have provided more consistent findings (Satlin *et al.*, 2001; Carattino *et al.*, 2004, 2005; Morimoto *et al.*, 2006). In isolated rabbit cortical-collecting ducts, Na^+ reabsorption is dependent on tubular flow rate; increases in flow rate increase Na^+ reabsorption (Satlin *et al.*, 2001). In subsequent studies, Carattino *et al.* (2004) demonstrated shear stress activation of Na^+ current in oocytes expressing

$\alpha\beta\gamma$ENaC, a finding that provides direct evidence of the mechanosensitivity of ENaC. Further, residues within the pore participate in the transduction of shear stress (Carattino *et al.*, 2005). Shear stress likely activates $\alpha\beta\gamma$ENaC by increasing open probability because ENaC channels with a high intrinsic open probability were not activated by shear stress (Palmer and Frindt, 1996; Carattino *et al.*, 2004, 2005).

The contrasting effects of shear stress and osmotic-induced swelling on ENaC activation suggest mechanical gating of $\alpha\beta\gamma$ENaC may be stimulus specific (i.e., shear stress vs osmotic stretch). The reasons underlying modality-specific activation of $\alpha\beta\gamma$ENaC in oocytes are unknown, but could reflect the importance of the appropriate complement of intracellular and extracellular proteins necessary to gate the channel in response to stretch or strain. Alternatively, modality-specific activation of ENaC channels may be conferred by the specific ENaC subunits forming the channel. It is unknown if shear stress sensitivity is due to the presence of αENaC. Whether a channel formed by $\beta\gamma$ENaC can be activated by shear stress, or another mechanical stimulus such as strain, has never been addressed.

Constitutive activity is a common feature reported for ENaC channels. However, electrically silent channels may also be expressed at the membrane (Caldwell *et al.*, 2004; Morimoto *et al.*, 2006). Despite the decreased constitutive activity of this pool of channels, it is likely that these channels can be gated mechanically. Emerging evidence suggests proteolytic cleavage of ENaC subunits contributes to baseline activity of ENaC channels (Lewis and Alles, 1986; Palmer and Frindt, 1986; Vallet *et al.*, 1997; Jovov *et al.*, 2001; Caldwell *et al.*, 2004; Hughey *et al.*, 2004a; Olivieri *et al.*, 2005; Carattino *et al.*, 2006). Satlin *et al.* (2001) have proposed that uncleaved channels represent a pool of ENaC channels that can be put into action (Hughey *et al.*, 2004b). Interestingly, even though uncleaved, or protease resistant, channels exhibit reduced activity under basal conditions, they can still be gated by mechanical stimulation with shear stress (Morimoto *et al.*, 2006). This is a significant finding because it suggests that an ENaC channel that has little or no constitutive activity can be mechanically gated.

2. ENaC Proteins Are Expressed in Mechanosensitive Tissue and Activity Is Required for Mechanosensory Responses

If ENaC proteins are to be considered as mechanosensors, then at least two criteria must be met. First, ENaC proteins must be expressed at the site of mechanotransduction. Second, inhibition or disruption of ENaC activity should inhibit the mechanosensitive response. Since ENaC null mice die shortly after birth (Hummler and Rossier, 1996; McDonald *et al.*, 1999; Bonny and Hummler, 2000; Hummler and Beermann, 2000; Snitsarev *et al.*, 2002),

genetic evidence for ENaC involvement in mechanotransduction is lacking. As an alternative, selective ENaC inhibitors, such as amiloride and benzamil, are useful tools to determine ENaC involvement. Although most degenerin channels are sensitive to amiloride, $\alpha\beta\gamma$ENaC is the most sensitive, blocked by as little as 100 nM (Kellenberger and Schild, 2002). Other mammalian degenerins such as the ASIC channels require up to 100 μM (Kellenberger and Schild, 2002). Amiloride has been used as a probe for mechanosensitive channel in *Xenopus* oocytes and hair cells (Hamill *et al*., 1992; Rusch *et al*., 1994). However, the doses used to inhibit these channels were significantly greater than the K_i for $\alpha\beta\gamma$ENaC channels. The use of pharmacological inhibitors is limited as they cannot discern among the importance of the individual ENaC subunits in a given mechanosensory response. Thus, the development of animal models with tissue selective knockdown of ENaC proteins will be necessary to determine the importance of specific ENaC subunits in mechanotransduction.

ENaC proteins are expressed in populations of somatic and visceral sensory neurons in the dorsal root, trigeminal, and nodose ganglia. In mammals, ENaC proteins have been identified in the afferent nerve terminals innervating subsets of mechanoreceptors such as arterial baroreceptors, whiskers, larynx, tooth pulp, and touch receptors of hairless skin (Pacinian corpuscles, Merkel cells, Meissner corpuscles; Drummond *et al*., 2000; Fricke *et al*., 2000; Ichikawa *et al*., 2005; Yamamoto and Taniguchi, 2006). The specific ENaC proteins that are expressed in sensory neurons and their nerve endings vary, but include $\alpha\beta\gamma$ENaC and $\beta\gamma$ENaC. While a few studies have demonstrated that activation of mechanically gated currents or ion transients in sensory neurons are inhibited by amiloride, or its analogue benzamil, genetic evidence for a role of any ENaC protein in peripheral sensory neuron mechanotransduction is lacking (McCarter *et al*., 1999; Carr *et al*., 2001; Drummond *et al*., 2001; Snitsarev *et al*., 2002).

To date, expression of ENaC proteins has been reported in a variety of cells other than epithelial or neuronal and includes placental trophoblasts, chondrocytes, osteoblasts, endothelial cells, epidermal cells, and VSMCs (Kizer *et al*., 1997; Brouard *et al*., 1999; Trujillo *et al*., 1999; Golestaneh *et al*., 2001; Mauro *et al*., 2002; Driver *et al*., 2003; Page *et al*., 2003; Shakibaei and Mobasheri, 2003; Drummond *et al*., 2004; Jernigan and Drummond, 2006a). Mechanical factors influence responses in many of these cell types, in particular cardiovascular cells. Endothelial cells and VSMCs are continually exposed to mechanical forces such as shear stress, pressure, and strain and, as will be discussed in the following sections, recent investigations show ENaC proteins are expressed in VSMCs and are required for responses dependent on mechanical signaling.

IV. INVOLVEMENT OF ENaC PROTEINS IN VASCULAR SMOOTH MUSCLE MECHANOTRANSDUCTION

The VSMC is an excellent model to study ENaC proteins as mechanosensors for three reasons. First, VSMCs are known to express mechanosensitive ion channels of a largely unknown molecular identity. Second, quantitative assays for VSMC responses that are dependent on mechanical signaling are available. Pressure-mediated vasoconstriction (also referred to as myogenic constriction) is one widely used assay. Third, the mechano-dependent response, myogenic constriction, has physiological and pathophysiological significance. The evidence supporting a role for ENaC proteins in VSMCs and the physiological significance are discussed below.

A. ENaC Proteins in Pressure-Mediated Myogenic Constriction

1. What is Myogenic Constriction?

Myogenic constriction is an inherent characteristic of resistance vessels that is characterized by a decrease in luminal diameter in response to an increase in transmural pressure. The response is important in establishing basal vascular tone and autoregulation of blood flow. While myogenic constriction occurs in many vascular beds, it is an important regulatory mechanism for blood flow autoregulation in cerebral, mesenteric, and renal beds (Davis and Hill, 1999). Our understanding of the response is mechanical stimulation, produced by pressure-induced vessel wall strain, initiates a signaling pathway that leads to membrane depolarization and subsequent calcium influx via voltage-gated calcium channels, which in turns causes VSMC contraction (Harder, 1984; Meininger and Davis, 1992; Knot and Nelson, 1995; Davis and Hill, 1999; Hill *et al.*, 2006). The mechanism(s) transmitting vascular smooth muscle stretch into a cellular response is unknown, but several possible mechanisms have been postulated including extracellular matrix–integrin interactions, membrane-bound enzyme and second messenger systems, ion transporters and exchangers, or direct activation of mechano-sensitive ion channels on the vascular smooth muscle plasmalemmal membrane (Davis and Hill, 1999). Of these potential mediators, mechanosensitive ion channels have received the most attention. Recordings from dissociated VSM cells suggest mechanosensitive ion channels tend to be cation selective, with Ca^{2+} and Na^{+} as the principal conductors (Kirber *et al.*, 1988; Davis *et al.*, 1992; Wellner and Isenberg, 1993; Ohya *et al.*, 1998; Ernstrom and Chalfie, 2002). Stretch-mediated Na^{+} and/or Ca^{2+} entry depolarizes the membrane and leads to activation of voltage-gated cation channels. Activation of voltage-gated cation

channels produces a larger Ca^{2+} influx, which, in turn, stimulates the release of Ca^{2+} from intracellular stores. This large increase in cytosolic Ca^{2+} drives VSM cell contraction (Brayden and Nelson, 1992; Nelson *et al.*, 1997; Wu and Davis, 2001). TRP and ENaC channels are candidates for these channels.

2. Quantifying Myogenic Constriction

The myogenic response can be quantified using in isolated arteries (Fig. 2). In this approach, artery segments are dissected from surrounding tissue and mounted on glass pipettes (Fig. 2A). Vessels are filled and bathed with a Ca^{2+} containing physiologic salt solution. To determine pressure-induced constrictor responses, pressure in the vessel is raised in 25 mmHg increments and equilibrated for 5 min (Fig. 2B). At the end of each pressure step equilibration, an image of the vessel is collected for determination of internal diameter under "active" conditions. As shown in Fig. 2C, despite increasing pressure, arteries maintain or decrease diameter. This sequence is repeated in the absence of Ca^{2+} in the bathing solution. In the absence of external Ca^{2+}, arteries are unable to constrict and passively dilate (Fig. 2C). Myogenic tone at each pressure step is calculated as the myogenic tone (%) = (passive diameter−active diameter/passive diameter) × 100 and plotted, and a pressure–diameter curve constructed (Fig. 2D).

3. Importance of ENaC Proteins in Pressure-Mediated Vasoconstriction

a. ENaC Inhibition Abolishes Pressure-Mediated Vasoconstriction. The importance of ENaC proteins in pressure-induced vasoconstriction has been examined in two different preparations, rat middle cerebral and mouse renal interlobar arteries. In both arteries, pharmacological inhibition of ENaC with amiloride or benzamil inhibits pressure-mediated constriction (Oyabe *et al.*, 2000; Drummond *et al.*, 2004; Jernigan and Drummond, 2006a). In rat middle cerebral and mouse renal interlobar arteries, myogenic constriction is inhibited with submicromolar and low micromolar doses of benzamil (30 nM to 1 μM) and amiloride (1–5 μM; Drummond *et al.*, 2004; Jernigan and Drummond, 2006a). Approximately 40% of myogenic tone is blocked with 1-μM amiloride (Fig. 2C and D, representative of the response).

At submicromolar and low micromolar doses, amiloride and benzamil are selective ENaC inhibitors. Although higher doses of amiloride and benzamil can inhibit other ion transporters and channels such as the Na^+/Ca^{2+} exchanger, Na^+/H^+ exchanger, Na^+ and Ca^{2+} channels, and other degenerin channels, the doses used to evaluate the role of ENaC proteins in pressure-mediated constriction are selective for ENaC (100 nM to 5 μM; Kleyman and Cragoe, 1988; Kellenberger and Schild, 2002). To address the concern that ENaC inhibition

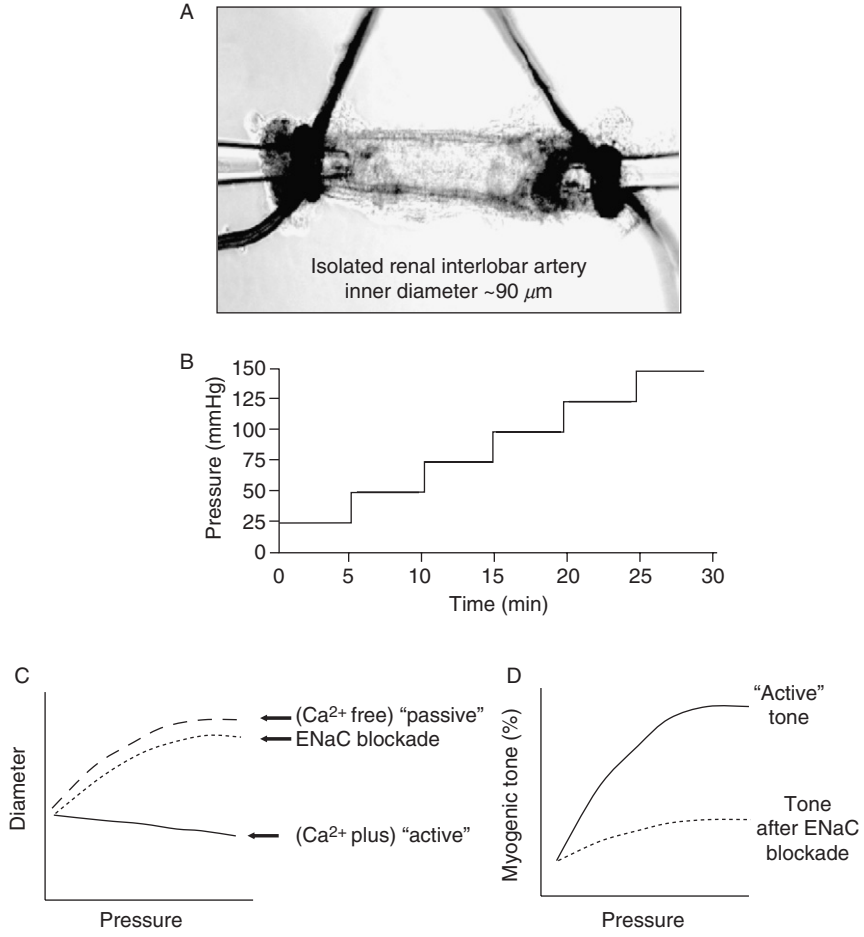

FIGURE 2 Assessment of myogenic constriction. (A) Artery segments, ~90–100 μm in diameter, dissected from surrounding tissue are mounted on two glass pipettes. (B) Arteries are exposed to stepwise (25 mmHg, 5 min) increase in luminal pressure. Artery diameter is determined at the end of each 5 min equilibration. (C) Cartoon of the myogenic response. The myogenic response is represented as the change in vessel diameter in response to changes in pressure. An artery with an active myogenic response maintains or decreases diameter when pressure increases (solid line). The passive response of an artery is determined by repeating the pressure steps using a Ca^{2+}-free bathing solution (dashed line). Following ENaC blockade with amiloride (5 μM) or benzamil (1 μM) in a Ca^{2+}-containing solution, the myogenic response is abolished. (D) Myogenic tone (passive diameter–active diameter/passive diameter) develops in an untreated artery. Following ENaC blockade, artery segments develop very little myogenic tone.

could be blocking the ability of the artery to constrict, vasoconstrictor responses to the α agonist phenylephrine were examined and found to be unchanged (Jernigan and Drummond, 2006a). These findings suggest ENaC inhibition (1–5 μM amiloride; 100 nM, 1 μM benzamil) specifically blocks pressure-mediated, but not agonist-induced vasoconstriction.

Similar to epithelial cells and heterologous expression systems, ENaC channels formed in VSMCs likely conduct Na^+ ions. In mouse renal interlobar arteries, step increases in pressure activate Na^+ transients that are abolished following ENaC inhibition with amiloride or benzamil (Jernigan and Drummond, 2006a). Although pressure also activates Ca^{2+} transients, the importance of Ca^{2+} moving through the pore vs release of intracellular Ca^{2+} was not determined.

b. ENaC Subunit Expression in VSMCs. In VSMCs freshly dissociated from mouse kidney and rat brain arteries, βENaC and γENaC, but not αENaC, expression is detected by RT-PCR and immunolabeling (Drummond *et al.*, 2004). Immunolocalization studies reveal βENaC and γENaC are found together and are concentrated at or near the membrane and frequently found localized in puncta. Since a similar punctate-staining pattern of MEC-4 along touch neuron processes has been suggested to reflect the presence of mechanotransducing ion channel complexes near the membrane, by analogy, localization of βENaC and γENaC in punctae along sarcolemma may reflect the distribution of mechanotransducing ion channel complexes along the smooth muscle membrane (Syntichaki and Tavernarakis, 2004).

c. Electrophysiological Evidence. Direct evidence of the ENaC channel in VSMCs is not available. However, Van Renterghem and Lazdunski (1991) reported an epithelial-like Na^+ current in VSMCs. Similar to αβγENaC, the channel reported in VSMCs is nonvoltage-gated and has a 10-pS conductance and high Na^+:K^+ selectivity. Unlike αβγENaC, the channel is insensitive to amiloride (100 μM). While the amiloride characteristics of this channel are not consistent with the reported amiloride sensitivity of αβγENaC and βγENaC channels in heterologous expression systems, this finding supports the potential presence of an ENaC-like Na^+ channel in VSMCs.

d. Can βENaC and γENaC Form a Channel in the Absence of αENaC? Evidence from Rossier's laboratory suggests αENaC is not required for βENaC and γENaC to form a channel; however, αENaC is required to form a *fully functional channel* (Bonny *et al.*, 1999). Bonny *et al.* (1999) demonstrated that oocytes expressing βENaC and γENaC generate amiloride sensitive currents in the absence of αENaC, when provided a longer incubation period

(~6 days). Channels formed by βENaC and γENaC have a greater selectivity for Na^+ and significantly less current. Additionally, βγENaC channels have a tenfold higher K_i for amiloride (~2 μM in βγENaC vs 0.2μM in αβγENaC). Thus, channels formed by βγENaC are not the same as channels formed by αβγENaC. The finding by Jernigan and Drummond that ~40% of myogenic constrictor responses are blocked with 1-μM amiloride is consistent with the amiloride K_i for βγENaC channels.

Compared to αβγENaC channels, trafficking of βγENaC channels to the surface membrane in *Xenopus* oocytes is delayed and results in protein localization in the intracellular compartment (Bonny *et al.*, 1999). The delayed trafficking of βγENaC channels may be the basis for lack of current generated by βγENaC in heterologous expression systems. In freshly dissociated VSMCs, trafficking of βENaC and γENaC does not appear to be impaired as they are expressed at or near the cell surface (Fig. 3; Drummond *et al.*, 2004; Jernigan and Drummond, 2006a). These findings suggest, βENaC and γENaC are trafficked to or near the surface in the absence of αENaC. The mechanism(s) mediating membrane localization of βENaC and γENaC in the absence of αENaC is unknown; however, there are a few possible explanations. First, VSMCs may express another protein that associates with and stabilizes βENaC and γENaC, perhaps a protein similar to the *C. elegans* degenerin, MEC-6 or MEC-2. Second, another pore-forming subunit may interact with βENaC and γENaC, such as δENaC, or an ASIC protein. Third, αENaC may be expressed in VSMCs, but we are unable to detect it and the small amount expressed is sufficient to stabilize the channel. Lastly, the presence of proteins within the dense extracellular matrix of blood vessels may help stabilize βγENaC channels that reach the membrane. Regardless of the mechanism, in the absence of detectable levels of αENaC, βENaC and γENaC appear to traffic to the cell surface of VSMC *in vivo* and shortly following enzymatic dissociation.

It is likely that ENaC proteins expressed in VSMCs from renal and cerebral circulations are not proteolytically cleaved. On the basis of Hughey *et al.* (2004b), demonstrating proteolytic cleavage of one subunit requires coexpression of all three subunits (αENaC, βENaC, and γENaC), it is likely that the absence αENaC in VSMCs prevents proteolytic cleavage of βENaC and γENaC. If βγENaC channels were not cleaved, they would be expected to be electrically silent (Hughey *et al.*, 2004b). Since electrically silent αβγENaC channels can be mechanically stimulated, it is probable to speculate that electrically silent cell surface βγENaC channels may also be activated by mechanical stimulation. However, this has not been directly evaluated.

On the basis of these findings, we speculate that βENaC and γENaC subunits are the predominant subunits forming ENaC channels in VSMC. Although the channels to traffic to the cell surface, they are probably electrically silent,

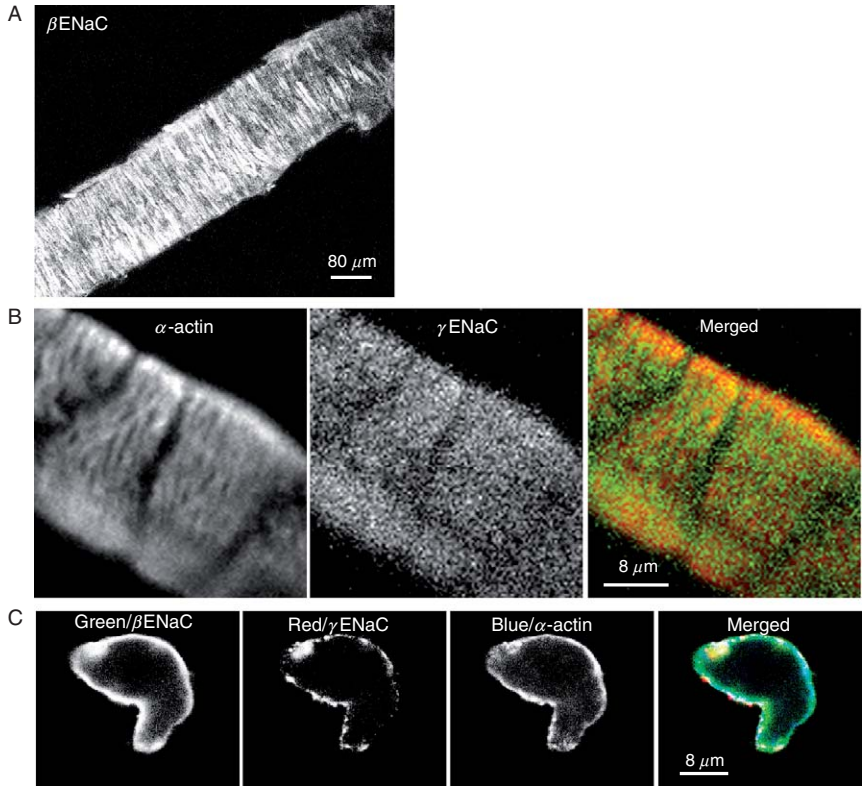

FIGURE 3 ENaC localization in vascular smooth muscle. (A) βENaC immunolocalization in an isolated rat middle cerebral artery segment. The horizontal banding pattern is characteristic of vascular smooth muscle. (B) High magnification image of γENaC localization with α-actin in another rat cerebral artery segment. At least two individual cells can be identified in the image. Note the highly punctate γENaC staining, suggestive of localization of mechanosensory complexes in the VSMCs. (C) ENaC localization in an individual VSMC enzymatically dissociated from a rat middle cerebral artery. Image is a single optical section taken through the middle of an isolated VSMC. Note that βENaC and γENaC are colocalized near the membrane.

since the channels are not likely to be proteolytically processed, but likely to be activated by mechanical stimuli.

e. Gene Silencing of β*ENaC and* γ*ENaC Inhibits Pressure-Mediated Vasoconstriction in Mouse Renal Interlobar Arteries.* Although amiloride and benzamil are great tools to screen for DEG/ENaC channel involvement, determining which specific DEG/ENaC proteins (i.e., βENaC or γENaC) are

involved requires selective gene silencing. Using two independent approaches to silence expression of specific ENaC genes, expression of dominant-negative ENaC isoforms and small interfering RNA (siRNA), Jernigan and Drummond (2006b) demonstrate suppression of βENaC or γENaC inhibits myogenic constriction. These findings suggest that both βENaC and γENaC subunits are required for mechanotransduction.

f. How Might ENaCs Transduce Vessel Strain In Vivo? The answer to this question is not known; however, they probably fit a model similar to *C. elegans*, where βENaC and γENaC form the pore of the mechanotransducing complex. The channel pore is likely anchored to the cytoskeleton and extracellular matrix in a similar manner. A potential intracellular protein associated the mechanosensor is stomatin. Stomatin is related to MEC-2, an essential component of the *C. elegans* mechanosensor. Stomatin colocalizes with βENaC and γENaC in sensory neurons of the trigeminal ganglia. Additionally, Price *et al.* (2004) have shown that stomatin interacts with and helps gate another degenerin family member. However, it is unknown if stomatin, or a related stomatin protein, regulates activity of ENaC channels or is required for mechanotransduction in VSMCs. ENaC channels are known to interact with cytoskeletal proteins including spectrin, anykrin, and actin (Rotin *et al.*, 1994; Jovov *et al.*, 1999; Zuckerman *et al.*, 1999; Berdiev *et al.*, 2001; Copeland *et al.*, 2001; Mazzochi *et al.*, 2006). In isolated expression systems, cytoskeletal proteins can regulate gating properties of ENaC channels (Achard *et al.*, 1996; Jovov *et al.*, 1999). Other investigators have suggested mechanotransduction in VSMCs requires a link between extracellular matrix proteins, integrins, and ion channels (Hill *et al.*, 2006); however, the identities of extracellular proteins that bind to ENaC are unknown. We speculate activation of ENaC proteins, by pressure or strain, leads to an influx of Na^+ and perhaps Ca^{2+} (Fig. 4). The cation influx depolarizes the membrane and activates voltage-gated Ca^{2+} channels to initiate the Ca^{2+}-signaling cascade and lead to vasoconstriction.

In addition to degenerins, evolving evidence suggests members of the TRP channel family may also form mechanosensitive channels in VSMC. TRPC6 and TRPM4 have both been implicated as mechanosensors and mediators of myogenic constriction in VSMCs (Welsh *et al.*, 2002; Earley *et al.*, 2004). In independent investigations using similar preparations, inhibition of ENaC function or TRP channel function produces a near total loss of myogenic function. If ENaC and TRP channels function independently of each other, then why does suppression of one mechanosensitive channel class abolish myogenic control? One might expect suppressing one channel would allow the other to compensate, at least partially. This leaves an alternative explanation that the function of ENaC and TRP channels are somehow linked.

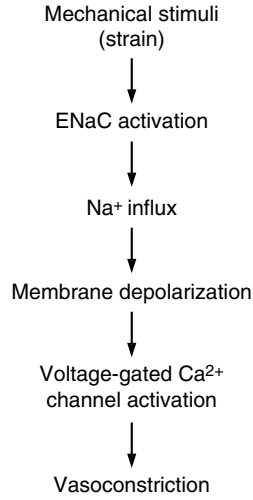

FIGURE 4 Proposed role of ENaC in myogenic constriction. Activation of ENaC channels by pressure-induced strain allows Na^+ influx and leads to membrane depolarization. Depolarization-induced activation of voltage-gated Ca^{2+} channels and Ca^{2+} influx stimulates release of intracellular Ca^{2+} stores, which leads to vasoconstriction.

Whether the channels are located in the same domain, linked directly, that is interacting via protein–protein interactions, or by a common signaling pathway, or some other way, have never been addressed.

g. What Is the Potential Physiological Importance of ENaC in Myogenic Constriction? Myogenic constriction contributes to the regulation of blood flow by allowing resistance arteries to adjust tone to luminal pressure; vessels constrict to increases and dilate to decreases in luminal pressure. In the kidney, myogenic constriction plays a critical role in protecting against hypertension-induced renal injury (Bidani *et al.*, 1987; Hayashi *et al.*, 1992; Van Dokkum *et al.*, 1999; Wang *et al.*, 2000; Loutzenhiser *et al.*, 2004) by preventing increases in blood pressure from being transmitted to the glomerulus, a primary determinant of renal injury (Bidani *et al.*, 1987; Griffin *et al.*, 2000; Bidani and Griffin, 2002; Griffin and Bidani, 2004). These findings suggest myogenic constriction may help protect the kidney from pressure-induced renal injury. It is important to note that the role of ENaC proteins in the myogenic constrictor was evaluated only in the larger middle cerebral and renal interlobar arteries. The role of ENaC proteins in myogenic constriction in the small resistance arterioles, the primary site of local blood flow regulation, was not determined. Thus, the role of ENaC proteins in

myogenic constriction in small resistance arterioles is unknown and remains an important area of future investigation. A better understanding of the physiological importance of ENaC proteins in vascular function will likely be accomplished through the evaluation of vascular function in tissue-specific knockout animal models as well as Liddle's and PHA type I patient populations.

V. SUMMARY AND FUTURE DIRECTIONS

In the kidney, the role of ENaC in the regulation of blood pressure through Na^+ and water homeostasis is well established. Numerous investigators suggested an additional role for ENaC proteins in mechanotransduction because they are members of a protein family required for mechanotransduction in *C. elegans*. Most investigations addressing the mechanosensitivity of ENaC demonstrate the ENaC channels can be gated by mechanical factors. In addition to their potential role in neural regulation of cardiovascular function, recent evidence also suggests ENaC may contribute to cardiovascular homeostasis by functioning as mechanosensors in VSMCs. However, whether ENaC proteins truly form mechanically gated channels in VSMC, how vascular ENaC channels contribute to the blood flow regulation *in vivo*, and if altered ENaC channel function contributes to cardiovascular disease remain important unanswered questions.

References

Achard, J. M., Bubien, J. K., Benos, D. J., and Warnock, D. G. (1996). Stretch modulates amiloride sensitivity and cation selectivity of sodium channels in human B lymphocytes. *Am. J. Physiol.* **270,** C224–C234.

Adams, C. M., Anderson, M. G., Motto, D. G., Price, M. P., Johnson, W. A., and Welsh, M. J. (1998). Ripped pocket and pickpocket, novel *Drosophila* DEG/ENaC subunits expressed in early development and in mechanosensory neurons. *J. Cell Biol.* **140,** 143–152.

Ainsley, J. A., Pettus, J. M., Bosenko, D., Gerstein, C. E., Zinkevich, N., Anderson, M. G., Adams, C. M., Welsh, M. J., and Johnson, W. A. (2003). Enhanced locomotion caused by loss of the *Drosophila* DEG/ENaC protein Pickpocket1. *Curr. Biol.* **13,** 1557–1563.

Alenghat, F. J., and Ingber, D. E. (2002). Mechanotransduction: All signals point to cytoskeleton, matrix, and integrins. *Sci. STKE* **119,** PE6.

Alvarez de la Rosa, D., Canessa, C. M., Fyfe, G. K., and Zhang, P. (2000). Structure and regulation of amiloride-sensitive sodium channels. *Annu. Rev. Physiol.* **62,** 573–594.

Amin, M. S., Wang, H. W., Reza, E., Whitman, S. C., Tuana, B. S., and Leenen, F. H. (2005). Distribution of epithelial sodium channels and mineralocorticoid receptors in cardiovascular regulatory centers in rat brain. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **289,** R1787–R1797.

Awayda, M. S., and Subramanyam, M. (1998). Regulation of the epithelial Na^+ channel by membrane tension. *J. Gen. Physiol.* **112,** 97–111.

Awayda, M. S., Ismailov, I. I., Berdiev, B. K., and Benos, D. J. (1995). A cloned renal epithelial Na^+ channel protein displays stretch activation in planar lipid bilayers. *Am. J. Physiol.* **268,** C1450–C1459.

Babini, E., Geisler, H. S., Siba, M., and Grunder, S. (2003). A new subunit of the epithelial Na^+ channel identifies regions involved in Na^+ self-inhibition. *J. Biol. Chem.* **278,** 28418–28426.

Benos, D. J., and Stanton, B. A. (1999). Functional domains within the degenerin/epithelial sodium channel (Deg/ENaC) superfamily of ion channels. *J. Physiol.* **520**(Pt. 3), 631–644.

Berdiev, B. K., Latorre, R., Benos, D. J., and Ismailov, I. I. (2001). Actin modifies Ca^{2+} block of epithelial Na^+ channels in planar lipid bilayers. *Biophys. J.* **80,** 2176–2186.

Bidani, A. K., and Griffin, K. A. (2002). Long-term renal consequences of hypertension for normal and diseased kidneys. *Curr. Opin. Nephrol. Hypertens.* **11,** 73–80.

Bidani, A. K., Schwartz, M. M., and Lewis, E. J. (1987). Renal autoregulation and vulnerability to hypertensive injury in remnant kidney. *Am. J. Physiol.* **252,** F1003–F1010.

Bonny, O., and Hummler, E. (2000). Dysfunction of epithelial sodium transport: From human to mouse. *Kidney Int.* **57,** 1313–1318.

Bonny, O., Chraibi, A., Loffing, J., Jaeger, N. F., Grunder, S., Horisberger, J. D., and Rossier, B. C. (1999). Functional expression of a pseudohypoaldosteronism type I mutated epithelial Na^+ channel lacking the pore-forming region of its alpha subunit. *J. Clin. Invest.* **104,** 967–974.

Brayden, J. E., and Nelson, M. T. (1992). Regulation of arterial tone by activation of calcium-dependent potassium channels. *Science* **256,** 532–535.

Brouard, M., Casado, M., Djelidi, S., Barrandon, Y., and Farman, N. (1999). Epithelial sodium channel in human epidermal keratinocytes: Expression of its subunits and relation to sodium transport and differentiation. *J. Cell Sci.* **112,** 3343–3352.

Caldwell, R. A., Boucher, R. C., and Stutts, M. J. (2004). Serine protease activation of near-silent epithelial Na^+ channels. *Am. J. Physiol. Cell Physiol.* **286,** C190–C194.

Carattino, M. D., Sheng, S., and Kleyman, T. R. (2004). Epithelial Na^+ channels are activated by laminar shear stress. *J. Biol. Chem.* **279,** 4120–4126.

Carattino, M. D., Sheng, S., and Kleyman, T. R. (2005). Mutations in the pore region modify epithelial sodium channel gating by shear stress. *J. Biol. Chem.* **280,** 4393–4401.

Carattino, M. D., Sheng, S., Bruns, J. B., Pilewski, J. M., Hughey, R. P., and Kleyman, T. R. (2006). The epithelial Na^+channel is inhibited by a peptide derived from proteolytic processing of its alpha subunit. *J. Biol. Chem.* **281**(27), 18901–18907.

Carr, M. J., Gover, T. D., Weinreich, D., and Undem, B. J. (2001). Inhibition of mechanical activation of guinea-pig airway afferent neurons by amiloride analogues. *Br. J. Pharmacol.* **133,** 1255–1262.

Chalfie, M., and Sulston, J. (1981). Developmental genetics of the mechanosensory neurons of *Caenorhabditis elegans*. *Dev. Biol.* **82,** 358–370.

Chalfie, M., and Au, M. (1989). Genetic control of differentiation of the *Caenorhabditis elegans* touch receptor neurons. *Science* **243,** 1027–1033.

Chalfie, M., and Wolinsky, E. (1990). The identification and suppression of inherited neurodegeneration in *Caenorhabditis elegans*. *Nature* **345,** 410–416.

Chalfie, M., Dean, E., Reilly, E., Buck, K., and Thomson, J. N. (1986). Mutations affecting microtubule structure in *Caenorhabditis elegans*. *J. Cell Sci. Suppl.* **5,** 257–271.

Chapleau, M. W., Li, Z., Meyrelles, S. S., Ma, X., and Abboud, F. M. (2001). Mechanisms determining sensitivity of baroreceptor afferents in health and disease. *Ann. NY Acad. Sci.* **940,** 1–19.

Cheng, C., Prince, L. S., Snyder, P. M., and Welsh, M. J. (1998). Assembly of the epithelial Na^+ channel evaluated using sucrose gradient sedimentation analysis. *J. Biol. Chem.* **273,** 22693–22700.

Copeland, S. J., Berdiev, B. K., Ji, H. L., Lockhart, J., Parker, S., Fuller, C. M., and Benos, D. J. (2001). Regions in the carboxy terminus of alpha-bENaC involved in gating and functional effects of actin. *Am. J. Physiol. Cell Physiol.* **281,** C231–C240.

Davis, M. J., and Hill, M. A. (1999). Signaling mechanisms underlying the vascular myogenic response. *Physiol. Rev.* **79,** 387–423.

Davis, M. J., Donovitz, J. A., and Hood, J. D. (1992). Stretch-activated single-channel and whole cell currents in vascular smooth muscle cells. *Am. J. Physiol.* **262,** C1083–C1088.

Davis, M. J., Wu, X., Nurkiewicz, T. R., Kawasaki, J., Davis, G. E., Hill, M. A., and Meininger, G. A. (2001). Integrins and mechanotransduction of the vascular myogenic response. *Am. J. Physiol. Heart Circ. Physiol.* **280,** H1427–H1433.

Dijkink, L., Hartog, A., van Os, C. H., and Bindels, R. J. (2002). The epithelial sodium channel (ENaC) is intracellularly located as a tetramer. *Pflugers Arch.* **444,** 549–555.

Driscoll, M., and Chalfie, M. (1991). The MEC-4 gene is a member of a family of *Caenorhabditis elegans* genes that can mutate to induce neuronal degeneration. *Nature* **349,** 588–593.

Driscoll, M., and Tavernarakis, N. (1997). Molecules that mediate touch transduction in the nematode Caenorhabditis elegans. *Gravit. Space Biol. Bull.* **10,** 33–42.

Driver, P. M., Rauz, S., Walker, E. A., Hewison, M., Kilby, M. D., and Stewart, P. M. (2003). Characterization of human trophoblast as a mineralocorticoid target tissue. *Mol. Hum. Reprod.* **9,** 793–798.

Drummond, H. A., Price, M. P., Welsh, M. J., and Abboud, F. M. (1998). A molecular component of the arterial baroreceptor mechanotransducer. *Neuron* **21,** 1435–1441.

Drummond, H. A., Abboud, F. M., and Welsh, M. J. (2000). Localization of beta and gamma subunits of ENaC in sensory nerve endings in the rat foot pad. *Brain Res.* **884,** 1–12.

Drummond, H. A., Welsh, M. J., and Abboud, F. M. (2001). ENaC subunits are molecular components of the arterial baroreceptor complex. *Ann. NY Acad. Sci.* **940,** 42–47.

Drummond, H. A., Gebremedhin, D., and Harder, D. R. (2004). Degenerin/epithelial Na^+ channel proteins. Components of a vascular mechanosensor. *Hypertension* **44,** 643.

Du, H., and Chalfie, M. (2001). Genes regulating touch cell development in *Caenorhabditis elegans*. *Genetics* **158,** 197–207.

Duchatelle, P., Ohara, A., Ling, B. N., Kemendy, A. E., Kokko, K. E., Matsumoto, P. S., and Eaton, D. C. (1992). Regulation of renal epithelial sodium channels. *Mol. Cell. Biochem.* **114,** 27–34.

Earley, S., Waldron, B. J., and Brayden, J. E. (2004). Critical role for transient receptor potential channel TRPM4 in myogenic constriction of cerebral arteries. *Circ. Res.* **95,** 922–929.

Ernstrom, G. G., and Chalfie, M. (2002). Genetics of sensory mechanotransduction. *Annu. Rev. Genet.* **36,** 411–453.

Firsov, D., Gautschi, I., Merillat, A. M., Rossier, B. C., and Schild, L. (1998). The heterotetrameric architecture of the epithelial sodium channel (ENaC). *EMBO J.* **17,** 344–352.

Fricke, B., Lints, R., Stewart, G., Drummond, H., Dodt, G., Driscoll, M., and von During, M. (2000). Epithelial Na^+ channels and stomatin are expressed in rat trigeminal mechanosensory neurons. *Cell Tissue Res.* **299,** 327–334.

Garcia-Anoveros, J., and Corey, D. P. (1997). The molecules of mechanosensation. *Annu. Rev. Neurosci.* **20,** 567–594.

Garcia-Anoveros, J., Garcia, J. A., Liu, J. D., and Corey, D. P. (1998). The nematode degenerin UNC-105 forms ion channels that are activated by degeneration- or hypercontraction-causing mutations. *Neuron* **20,** 1231–1241.

Garcia-Anoveros, J., Samad, T. A., Zuvela-Jelaska, L., Woolf, C. J., and Corey, D. P. (2001). Transport and localization of the DEG/ENaC ion channel BNaC1alpha to peripheral mechanosensory terminals of dorsal root ganglia neurons. *J. Neurosci.* **21,** 2678–2686.

Gillespie, P. G., and Walker, R. G. (2001). Molecular basis of mechanosensory transduction. *Nature* **413,** 194–202.

Golestaneh, N., Klein, C., Valamanesh, F., Suarez, G., Agarwal, M. K., and Mirshahi, M. (2001). Mineralocorticoid receptor-mediated signaling regulates the ion gated sodium channel in vascular endothelial cells and requires an intact cytoskeleton. *Biochem. Biophys. Res. Commun.* **280,** 1300–1306.

Goodman, M. B., Ernstrom, G. G., Chelur, D. S., O'Hagan, R., Yao, C. A., and Chalfie, M. (2002). MEC-2 regulates *C. elegans* DEG/ENaC channels needed for mechanosensation. *Nature* **415,** 1039–1042.

Griffin, K. A., and Bidani, A. K. (2004). Hypertensive renal damage: Insights from animal models and clinical relevance. *Curr. Hypertens. Rep.* **6,** 145–153.

Griffin, K. A., Picken, M. M., Churchill, M., Churchill, P., and Bidani, A. K. (2000). Functional and structural correlates of glomerulosclerosis after renal mass reduction in the rat. *J. Am. Soc. Nephrol.* **11,** 497–506.

Gu, G., Caldwell, G. A., and Chalfie, M. (1996). Genetic interactions affecting touch sensitivity in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **93,** 6577–6582.

Hamill, O. P., and Martinac, B. (2001). Molecular basis of mechanotransduction in living cells. *Physiol. Rev.* **81,** 685–740.

Hamill, O. P., Lane, J. W., and McBride, D. W., Jr. (1992). Amiloride: A molecular probe for mechanosensitive channels. *Trends Pharmacol. Sci.* **13,** 373–376.

Harder, D. R. (1984). Pressure-dependent membrane depolarization in cat middle cerebral artery. *Circ. Res.* **55,** 197–202.

Hayashi, K., Epstein, M., and Loutzenhiser, R. (1992). Enhanced myogenic responsiveness of renal interlobular arteries in spontaneously hypertensive rats. *Hypertension* **19,** 153–160.

Hill, M. A., Davis, M. J., Meininger, G. A., Potocnik, S. J., and Murphy, T. V. (2006). Arteriolar myogenic signalling mechanisms: Implications for local vascular function. *Clin. Hemorheol. Microcirc.* **34,** 67–79.

Huang, M., and Chalfie, M. (1994). Gene interactions affecting mechanosensory transduction in *Caenorhabditis elegans*. *Nature* **367,** 467–470.

Hughey, R. P., Bruns, J. B., Kinlough, C. L., Harkleroad, K. L., Tong, Q., Carattino, M. D., Johnson, J. P., Stockand, J. D., and Kleyman, T. R. (2004a). Epithelial sodium channels are activated by furin-dependent proteolysis. *J. Biol. Chem.* **279,** 18111–18114.

Hughey, R. P., Bruns, J. B., Kinlough, C. L., and Kleyman, T. R. (2004b). Distinct pools of epithelial sodium channels are expressed at the plasma membrane. *J. Biol. Chem.* **279,** 48491–48494.

Hummler, E., and Rossier, B. C. (1996). Physiological and pathophysiological role of the epithelial sodium channel in the control of blood pressure. *Kidney Blood Press. Res.* **19,** 160–165.

Hummler, E., and Beermann, F. (2000). Scnn1 sodium channel gene family in genetically engineered mice. *J. Am. Soc. Nephrol.* **11**(Suppl. 16), S129–S134.

Hummler, E., and Vallon, V. (2005). Lessons from mouse mutants of epithelial sodium channel and its regulatory proteins. *J. Am. Soc. Nephrol.* **16,** 3160–3166.

Ichikawa, H., Fukuda, T., Terayama, R., Yamaai, T., Kuboki, T., and Sugimoto, T. (2005). Immunohistochemical localization of gamma and beta subunits of epithelial Na^+ channel in the rat molar tooth pulp. *Brain Res.* **1065,** 138–141.

Ingber, D. E. (1997). Tensegrity: The architectural basis of cellular mechanotransduction. *Annu. Rev. Physiol.* **59,** 575–599.

Ingber, D. E. (2006). Cellular mechanotransduction: Putting all the pieces together again. *FASEB J.* **20,** 811–827.

Ismailov, I. I., Berdiev, B. K., Bradford, A. L., Awayda, M. S., Fuller, C. M., and Benos, D. J. (1996a). Associated proteins and renal epithelial Na^+ channel function. *J. Membr. Biol.* **149,** 123–132.

Ismailov, I. I., Awayda, M. S., Berdiev, B. K., Bubien, J. K., Lucas, J. E., Fuller, C. M., and Benos, D. J. (1996b). Triple-barrel organization of ENaC, a cloned epithelial Na^+ channel. *J. Biol. Chem.* **271,** 807–816.

Ismailov, I. I., Berdiev, B. K., Shlyonsky, V. G., and Benos, D. J. (1997a). Mechanosensitivity of an epithelial Na^+ channel in planar lipid bilayers: Release from Ca^{2+} block. *Biophys. J.* **72,** 1182–1192.

Ismailov, I. I., Shlyonsky, V. G., and Benos, D. J. (1997b). Streaming potential measurements in alphabetagamma-rat epithelial Na^+ channel in planar lipid bilayers. *Proc. Natl. Acad. Sci. USA* **94,** 7651–7654.

Jernigan, N. L., and Drummond, H. A. (2006a). Myogenic vasoconstriction in mouse renal interlobar arteries: Role of endogenous beta and gamma ENac. *Am. J. Physiol. Renal Physiol.* **296**(6), F1184–1191.

Jernigan, N. L., and Drummond, H. A. (2006b). Suppression of endogenous beta and gamma ENaC abolishes myogenic vasoconstriction in mouse interlobar arteries. *FASEB J.* 20, 2006 Experimental Biology meeting abstracts [on CD-ROM], Abstract #472.9.

Ji, H.-L., Fuller, C. M., and Benos, D. J. (1998). Osmotic pressure regulates $\alpha\beta\gamma$-rENaC expressed in *Xenopus* oocytes. *Am. J. Physiol.* **275,** C1182–C1190.

Jovov, B., Tousson, A., Ji, H. L., Keeton, D., Shlyonsky, V., Ripoll, P. J., Fuller, C. M., and Benos, D. J. (1999). Regulation of epithelial Na(+) channels by actin in planar lipid bilayers and in the *Xenopus* oocyte expression system. *J. Biol. Chem.* **274,** 37845–37854.

Jovov, B., Berdiev, B. K., Fuller, C. M., Ji, H. L., and Benos, D. J. (2001). The serine-protease trypsin cleaves C-termini of beta and gamma subunits of ENaC. *J. Biol. Chem.* **5,** 5.

Kellenberger, S., and Schild, L. (2002). Epithelial sodium channel/degenerin family of ion channels: A variety of functions for a shared structure. *Physiol. Rev.* **82,** 735–767.

Kirber, M. T., Walsh, J. V., Jr., and Singer, J. J. (1988). Stretch-activated ion channels in smooth muscle: A mechanism for the initiation of stretch-induced contraction. *Pflugers Arch.* **412,** 339–345.

Kizer, N., Guo, X.-L., and Hruska, K. (1997). Reconstitution of stretch-activated cation channels by expression of the α-subunit of the epithelial sodium channel cloned from osteoblasts. *Proc. Natl. Acad. Sci. USA* **94,** 1013–1018.

Kleyman, T. R., and Cragoe, E. J., Jr. (1988). Amiloride and its analogs as tools in the study of ion transport. *J. Membr. Biol.* **105,** 1–21.

Knot, H. J., and Nelson, M. T. (1995). Regulation of membrane potential and diameter by voltage-dependent K^+ channels in rabbit myogenic cerebral arteries. *Am. J. Physiol.* **269,** H348–H355.

Kopp, U. C., Matsushita, K., Sigmund, R. D., Smith, L. A., Watanabe, S., and Stokes, J. B. (1998). Amiloride-sensitive Na^+ channels in pelvic uroepithelium involved in renal sensory receptor activation. *Am. J. Physiol.* **275,** R1780–R1792.

Kosari, F., Sheng, S., Li, J., Mak, D. O., Foskett, J. K., and Kleyman, T. R. (1998). Subunit stoichiometry of the epithelial sodium channel. *J. Biol. Chem.* **273,** 13469–13474.

Koyama, K., Sasaki, I., Naito, H., Funayama, Y., Fukushima, K., Unno, M., Matsuno, S., Hayashi, H., and Suzuki, Y. (1999). Induction of epithelial Na^+ channel in rat ileum after proctocolectomy. *Am. J. Physiol.* **276,** G975–G984.

Lewis, S. A., and Alles, W. P. (1986). Urinary kallikrein: A physiological regulator of epithelial Na^+ absorption. *Proc. Natl. Acad. Sci. USA* **83,** 5345–5348.

Lifton, R. P. (1995). Genetic determinants of human hypertension. *Proc. Natl. Acad. Sci. USA* **92,** 8545–8551.

Lin, S. Y., and Corey, D. P. (2005). TRP channels in mechanosensation. *Curr. Opin. Neurobiol.* **15,** 350–357.

Lingueglia, E., Renard, S., Waldmann, R., Voilley, N., Champigny, G., Plass, H., Lazdunski, M., and Barbry, P. (1994). Different homologous subunits of the amiloride-sensitive Na^+ channel are differently regulated by aldosterone. *J. Biol. Chem.* **269,** 13736–13739.

Liu, J., Schrank, B., and Waterston, R. H. (1996). Interaction between a putative mechanosensory membrane channel and a collagen. *Science* **273,** 361–364.

Liu, L., Johnson, W. A., and Welsh, M. J. (2003a). *Drosophila* DEG/ENaC pickpocket genes are expressed in the tracheal system, where they may be involved in liquid clearance. *Proc. Natl. Acad. Sci. USA* **100,** 2128–2133.

Liu, L., Leonard, A. S., Motto, D. G., Feller, M. A., Price, M. P., Johnson, W. A., and Welsh, M. J. (2003b). Contribution of *Drosophila* DEG/ENaC genes to salt taste. *Neuron* **39,** 133–146.

Loutzenhiser, R., Bidani, A. K., and Wang, X. (2004). Systolic pressure and the myogenic response of the renal afferent arteriole. *Acta Physiol. Scand.* **181,** 407–413.

Luft, F. C. (1998). Molecular genetics of human hypertension. *J. Hypertens.* **16,** 1871–1878.

Luft, F. C. (2001). Molecular genetics of salt-sensitivity and hypertension. *Drug Metab. Dispos.* **29,** 500–504.

Ma, H. P., Al-Khalili, O., Ramosevac, S., Saxena, S., Liang, Y. Y., Warnock, D. G., and Eaton, D. C. (2004). Steroids and exogenous gamma-ENaC subunit modulate cation channels formed by alpha-ENaC in human B lymphocytes. *J. Biol. Chem.* **279,** 33206–33212.

Ma, H. P., Li, L., Zhou, Z. H., Eaton, D. C., and Warnock, D. G. (2002). ATP masks stretch activation of epithelial sodium channels in A6 distal nephron cells. *Am. J. Physiol. Renal Physiol.* **282,** F501–F505.

Mano, I., and Driscoll, M. (1999). DEG/ENaC channels: A touchy superfamily that watches its salt. *Bioessays* **21,** 568–578.

Martinez-Lemus, L. A., Sun, Z., Trache, A., Trzciakowski, J. P., and Meininger, G. A. (2005). Integrins and regulation of the microcirculation: From arterioles to molecular studies using atomic force microscopy. *Microcirculation* **12,** 99–112.

Mauro, T., Guitard, M., Behne, M., Oda, Y., Crumrine, D., Komuves, L., Rassner, U., Elias, P. M., and Hummler, E. (2002). The ENaC channel is required for normal epidermal differentiation. *J. Invest. Dermatol.* **118,** 589–594.

Mazzochi, C., Bubien, J. K., Smith, P. R., and Benos, D. J. (2006). The carboxyl terminus of the alpha-subunit of the amiloride-sensitive epithelial sodium channel binds to F-actin. *J. Biol. Chem.* **281,** 6528–6538.

McCarter, G. C., Reichling, D. B., and Levine, J. D. (1999). Mechanical transduction by rat dorsal root ganglion neurons *in vitro*. *Neurosci. Lett.* **273,** 179–182.

McDonald, F. J., Yang, B., Hrstka, R. F., Drummond, H. A., Tarr, D. E., McCray, P. B., Jr., Stokes, J. B., Welsh, M. J., and Williamson, R. A. (1999). Disruption of the beta subunit of the epithelial Na^+ channel in mice: Hyperkalemia and neonatal death associated with a pseudohypoaldosteronism phenotype. *Proc. Natl. Acad. Sci. USA* **96,** 1727–1731.

Meininger, G. A., and Davis, M. J. (1992). Cellular mechanisms involved in the vascular myogenic response. *Am. J. Physiol.* **263,** H647–H659.

Morimoto, T., Liu, W., Woda, C., Carattino, M., Wei, Y., Hughey, R., Apodaca, G., Satlin, L. M., and Kleyman, T. R. (2006). Mechanism underlying flow-stimulation of sodium absorption in the mammalian collecting duct. *Am. J. Physiol. Renal Physiol.* **291,** F663–F669.

Morris, C. E. (1990). Mechanosensitive ion channels. *J. Membr. Biol.* **113,** 93–107.

Nelson, M. T., Conway, M. A., Knot, H. J., and Brayden, J. E. (1997). Chloride channel blockers inhibit myogenic tone in rat cerebral arteries. *J. Physiol.* **502**(Pt. 2), 259–264.

O'Hagan, R., Chalfie, M., and Goodman, M. B. (2005). The MEC-4 DEG/ENaC channel of *Caenorhabditis elegans* touch receptor neurons transduces mechanical signals. *Nat. Neurosci.* **8,** 43–50.

Oh, Y. S., and Warnock, D. G. (2000). Disorders of the epithelial Na(+) channel in Liddle's syndrome and autosomal recessive pseudohypoaldosteronism type 1. *Exp. Nephrol.* **8,** 320–325.

Ohya, Y., Adachi, N., Nakamura, Y., Setoguchi, M., Abe, I., and Fujishima, M. (1998). Stretch-activated channels in arterial smooth muscle of genetic hypertensive rats. *Hypertension* **31,** 254–258.

Olivieri, O., Castagna, A., Guarini, P., Chiecchi, L., Sabaini, G., Pizzolo, F., Corrocher, R., and Righetti, P. G. (2005). Urinary prostasin: A candidate marker of epithelial sodium channel activation in humans. *Hypertension* **46,** 683–688.

Oyabe, A., Masumoto, N., Ueta, K., and Nakayama, K. (2000). Amiloride-sensitive pressure-induced myogenic contraction in rat cerebral artery. *Fundam. Clin. Pharmacol.* **14,** 369–377.

Page, K. R., Ashworth, C. J., McArdle, H. J., Finch, A. M., and Nwagwu, M. O. (2003). Sodium transport across the chorioallantoic membrane of porcine placenta involves the epithelial sodium channel (ENaC). *J. Physiol.* **547,** 849–857.

Paintal, A. S. (1973). Vagal sensory receptors and their reflex effects. *Physiol. Rev.* **53,** 159–227.

Palmer, L. G., and Frindt, G. (1986). Epithelial sodium channels: Characterization by using the patch-clamp technique. *Fed. Proc.* **45,** 2708–2712.

Palmer, L. G., and Frindt, G. (1996). Gating of Na channels in the rat cortical collecting tubule: Effects of voltage and membrane stretch. *J. Gen. Physiol.* **107,** 35–45.

Pradervand, S., Barker, P. M., Wang, Q., Ernst, S. A., Beermann, F., Grubb, B. R., Burnier, M., Schmidt, A., Bindels, R. J., Gatzy, J. T., Rossier, B. C., and Hummler, E. (1999). Salt restriction induces pseudohypoaldosteronism type 1 in mice expressing low levels of the beta-subunit of the amiloride-sensitive epithelial sodium channel. *Proc. Natl. Acad. Sci. USA* **96,** 1732–1737.

Price, M. P., Lewin, G. R., Mcllwrath, S. L., Cheng, C., Xie, J., Heppenstall, P. A., Stucky, C. L., Mannsfeldt, A. G., Brennan, T. J., Drummond, H. A., Qiao, J., Benson, C. J., *et al.* (2000). The mammalian sodium channel BNC1 is required for normal touch sensation. *Nature* **407,** 1007–1011.

Price, M. P., McIlwrath, S. L., Xie, J., Cheng, C., Qiao, J., Tarr, D. E., Sluka, K. A., Brennan, T. J., Lewin, G. R., and Welsh, M. J. (2001). The DRASIC cation channel contributes to the detection of cutaneous touch and acid stimuli in mice. *Neuron* **32,** 1071–1083.

Price, M. P., Thompson, R. J., Eshcol, J. O., Wemmie, J. A., and Benson, C. J. (2004). Stomatin modulates gating of acid-sensing ion channels. *J. Biol. Chem.* **279,** 53886–53891.

Riha, G. M., Lin, P. H., Lumsden, A. B., Yao, Q., and Chen, C. (2005). Roles of hemodynamic forces in vascular cell differentiation. *Ann. Biomed. Eng.* **33,** 772–779.

Rotin, D., Bar-Sagi, D., O'Brodovich, H., Merilainen, J., Lehto, V. P., Canessa, C. M., Rossier, B. C., and Downey, G. P. (1994). An SH3 binding region in the epithelial Na^+ channel (alpha rENaC) mediates its localization at the apical membrane. *EMBO J.* **13,** 4440–4450.

Rusch, A., Kros, C. J., and Richardson, G. P. (1994). Block by amiloride and its derivatives of mechano-electrical transduction in outer hair cells of mouse cochlear cultures. *J. Physiol.* **474,** 75–86.

Sachs, F. (1988). Mechanical transduction in biological systems. *Crit. Rev. Biomed. Eng.* **16,** 141–169.

Satlin, L. M., Sheng, S., Woda, C. B., and Kleyman, T. R. (2001). Epithelial Na(+) channels are regulated by flow. *Am. J. Physiol. Renal Physiol.* **280,** F1010–F1018.

Shakibaei, M., and Mobasheri, A. (2003). Beta1-integrins co-localize with Na, K-ATPase, epithelial sodium channels (ENaC) and voltage activated calcium channels (VACC) in mechanoreceptor complexes of mouse limb-bud chondrocytes. *Histol. Histopathol.* **18,** 343–351.

Sheperd, J.T, and Mancia, G. (1986). Reflex control of the human cardiovascular system. *Rev. Physiol. Biochem. Pharmacol.* **105,** 1–99.

Shreffler, W., and Wolinsky, E. (1997). Genes controlling ion permeability in both motorneurons and muscle. *Behav. Genet.* **27,** 211–221.

Snitsarev, V., Whiteis, C. A., Abboud, F. M., and Chapleau, M. W. (2002). Mechanosensory transduction of vagal and baroreceptor afferents revealed by study of isolated nodose neurons in culture. *Auton. Neurosci.* **98,** 59–63.

Snyder, P. M., Cheng, C., Prince, L. S., Rogers, J. C., and Welsh, M. J. (1998). Electrophysiological and biochemical evidence that DEG/ENaC cation channels are composed of nine subunits. *J. Biol. Chem.* **273,** 681–684.

Sulston, J., Dew, M., and Brenner, S. (1975). Dopaminergic neurons in the nematode *Caenorhabditis elegans*. *J. Comp. Neurol.* **163,** 215–226.

Syntichaki, P., and Tavernarakis, N. (2004). Genetic models of mechanotransduction: The nematode *Caenorhabditis elegans*. *Physiol. Rev.* **84,** 1097–1153.

Tavernarakis, N., and Driscoll, M. (1997). Molecular modeling of mechanotransduction in the nematode *Caenorhabditis elegans*. *Annu. Rev. Physiol.* **59,** 659–689.

Tavernarakis, N., and Driscoll, M. (2001). Degenerins. At the core of the metazoan mechano-transducer? *Ann. NY Acad. Sci.* **940,** 28–41.

Tavernarakis, N., Shreffler, W., Wang, S., and Driscoll, M. (1997). unc-8, a DEG/ENaC family member, encodes a subunit of a candidate mechanically gated channel that modulates *C. elegans* locomotion. *Neuron* **18,** 107–119.

Trujillo, E., Alvarez de la Rosa, D., Mobasheri, A., Gonzalez, T., Canessa, C. M., and Martin-Vasallo, P. (1999). Sodium transport systems in human chondrocytes. II. Expression of ENaC, Na+/K+/2Cl− cotransporter and Na+/H+ exchangers in healthy and arthritic chondrocytes. *Histol. Histopathol.* **14,** 1023–1031.

Ugawa, S., Minami, Y., Guo, W., Saishin, Y., Takatsuji, K., Yamamoto, T., Tohyama, M., and Shimada, S. (1998). Receptor that leaves a sour taste in the mouth. *Nature* **395,** 555–556.

Vallet, V., Chraibi, A., Gaeggeler, H. P., Horisberger, J. D., and Rossier, B. C. (1997). An epithelial serine protease activates the amiloride-sensitive sodium channel. *Nature* **389,** 607–610.

Van Dokkum, R. P., Alonso-Galicia, M., Provoost, A. P., Jacob, H. J., and Roman, R. J. (1999). Impaired autoregulation of renal blood flow in the fawn-hooded rat. *Am. J. Physiol.* **276,** R189–R196.

Van Renterghem, C., and Lazdunski, M. (1991). A new non-voltage-dependent, epithelial-like Na^+ channel in vascular smooth muscle cells. *Pflugers Arch.* **419,** 401–408.

Waldmann, R. (2001). Proton-gated cation channels—neuronal acid sensors in the central and peripheral nervous system. *Adv. Exp. Med. Biol.* **502,** 293–304.

Waldmann, R., Champigny, G., Bassilana, F., Voilley, N., and Lazdunski, M. (1995). Molecular cloning and functional expression of a novel amiloride-sensitive Na^+ channel. *J. Biol. Chem.* **270,** 27411–27414.

Wang, X., Ajikobi, D. O., Salevsky, F. C., and Cupples, W. A. (2000). Impaired myogenic autoregulation in kidneys of Brown Norway rats. *Am. J. Physiol. Renal Physiol.* **278,** F962–F969.

Wellner, M. C., and Isenberg, G. (1993). Stretch-activated nonselective cation channels in urinary bladder myocytes: Importance for pacemaker potentials and myogenic response. *EXS* **66,** 93–99.

Welsh, D. G., Morielli, A. D., Nelson, M. T., and Brayden, J. E. (2002). Transient receptor potential channels regulate myogenic tone of resistance arteries. *Circ. Res.* **90,** 248–250.

Wu, X., and Davis, M. J. (2001). Characterization of stretch-activated cation current in coronary smooth muscle cells. *Am. J. Physiol. Heart Circ. Physiol.* **280,** H1751–H1761.

Yamamoto, Y., and Taniguchi, K. (2006). Expression of ENaC subunits in sensory nerve endings in the rat larynx. *Neurosci. Lett.* **402,** 227–232.

Zuckerman, J. B., Chen, X., Jacobs, J. D., Hu, B., Kleyman, T. R., and Smith, P. R. (1999). Association of the epithelial sodium channel with Apx and alpha-spectrin in A6 renal epithelial cells. *J. Biol. Chem.* **274,** 23286–23295.