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Heather A. Drummond, Nikki L. Jernigan and Samira C. Grifoni

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# Sensing Tension

## Epithelial Sodium Channel/Acid-Sensing Ion Channel Proteins in Cardiovascular Homeostasis

Heather A. Drummond, Nikki L. Jernigan, Samira C. Grifoni

The epithelial sodium ( $\text{Na}^+$ ) channel (ENaC) plays a critical role in blood pressure regulation by controlling renal salt and water reabsorption. Channel overactivity can lead to severe hypertension and underactivity to salt wasting and hypotension.<sup>1</sup> In addition to their role in salt/water homeostasis, recent studies suggest that ENaC proteins, and their relatives, the acid-sensing ion channel (ASIC) proteins, may play more ubiquitous roles in cardiovascular regulation than considered previously. Recent evidence suggests that ENaC/ASIC proteins may act as mechanosensors and chemosensors in the cardiovascular system. ENaC/ASIC proteins are expressed in mechanosensing and chemosensing tissues, such as vascular smooth muscle cells (VSMCs), carotid body glomus cells, and sensory neurons innervating arterial baroreceptors, heart, and skeletal muscle. Disruption of the ENaC/ASIC channels alters myogenic constriction, arterial chemoreceptor and baroreceptor responses, and acid-induced responses in heart and skeletal muscle. This brief review summarizes the evidence supporting a role for ENaC and ASIC proteins in diverse systems of cardiovascular mechanosensing and chemosensing. Together, these studies suggest that ENaC/ASIC proteins contribute to cardiovascular homeostasis by mediating neural and local regulatory mechanisms.

### The Degenerin/ENaC/ASIC Family

ENaC and ASIC proteins are members of a protein family termed the degenerin (DEG)/ENaC/ASIC family. Members of this family are expressed in a wide range of species (nematode *Caenorhabditis elegans*, *Drosophila*, and mammals) and participate in diverse biological functions, including neurodegeneration, acid sensation, taste, learning and memory, proprioception,  $\text{Na}^+$ /water transport, and mechanosensation. All of the members of the DEG/ENaC/ASIC family share a highly conserved structure: intracellular N and C termini and 2 membrane-spanning domains separated by a large extracellular domain. Most DEG/ENaC/ASIC proteins form amiloride sensitive, nonvoltage, gated cation channels.<sup>1,2</sup>

### *C. elegans* DEGs

Members were first identified in the nematode, where a chemically induced mutation caused a subset of neurons to swell and lyse. This phenotype led to the first name of the family, Deg, short for degeneration. Subsequently, other *C. elegans* DEG genes expressed in neurons and muscle have been identified after genetic screens for proteins involved in touch responsiveness and proprioception, responses dependent on mechanotransduction.<sup>1,2</sup> These data provided the initial genetic link between the DEG/ENaC/ASIC channels and mechanotransduction.

### Mammalian ENaC and ASIC Proteins

In vertebrates, there are  $\geq 2$  subgroups of DEG/ENaC/ASIC proteins: ENaC and ASIC. Gain-of-function and loss-of-function mutations in ENaC channels are manifested in 2 diseases, Liddle's disease and pseudohypoaldosteronism type I, respectively. In Liddle's disease, certain mutations disrupt normal channel degradation, resulting in increased channel density, excessive salt/water retention, and severe hypertension. In pseudohypoaldosteronism type I, underactive channels produce salt wasting and hypotension. At least 5 different ENaC proteins have been identified in mammals ( $\alpha$ ENaC,  $\beta$ ENaC,  $\gamma$ ENaC,  $\delta$ ENaC, and  $\epsilon$ ENaC).  $\alpha$ ENaC,  $\beta$ ENaC, and  $\gamma$ ENaC proteins form a heteromultimeric channel critical in  $\text{Na}^+$  and water transport in the renal, colon, and lung epithelial.<sup>1,2</sup> The  $\delta$ ENaC and  $\epsilon$ ENaC subunits can substitute for  $\alpha$ ENaC or interact with  $\alpha\beta\gamma$ ENaC channels. Expression of the  $\delta$ ENaC subunit is limited to brain, pancreas, testes, ovary, and retinal cells.<sup>3-5</sup> Expression of the  $\epsilon$ ENaC subunit is limited to the brain, skeletal muscle, kidney, and urinary bladder in *Xenopus*.<sup>6</sup> ENaC channels are constitutively active, nonvoltage gated, and highly sensitive to amiloride ( $\alpha\beta\gamma$ ENaC  $\text{IC}_{50} < 100$  nmol/L;  $\alpha\delta\beta\gamma$ ENaC  $\text{IC}_{50} = 1$   $\mu$ mol/L;  $\delta\beta\gamma$ ENaC  $\text{IC}_{50} = 2.6$   $\mu$ mol/L) and its lipophylic analog benzamil.<sup>1</sup> Although  $\alpha$ ENaC protein is required to form the fully functional channel characteristic of the epithelial  $\text{Na}^+$  channel in renal epithelia,  $\beta$  and  $\gamma$ ENaC can form an  $\text{Na}^+$  conducting ion channel in the absence of  $\alpha$ ENaC.<sup>7</sup>

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ASIC and ENaC proteins are closely related. ASIC proteins (ASIC1, ASIC2, ASIC3, and ASIC4) can form homomultimeric and heteromultimeric channels that generally conduct Na<sup>+</sup>.<sup>1</sup> Although ASIC channels are also sensitive to amiloride, they tend to require higher doses than ENaC (10- to 100-fold). A specific inhibitor for ASIC1a is available (psalmotoxin); however, specific inhibitors for other ASIC channels are not available. A drop in extracellular pH gates most ASIC channels.<sup>1</sup> Until recently, ASICs were only identified in neuronal and neuroepithelial tissue, where they may contribute to acid taste, acid sensation, learning, and mechanosensation; however, recent evidence suggests that ASICs are also expressed in vascular smooth muscle.<sup>8</sup>

### Evidence of Mechanosensitivity and Chemosensitivity of ENaC/ASIC Channels in Isolated Systems and Epithelial Tissues

#### Mechanosensitivity

Early investigations into the mechanosensitivity of ENaC in heterologous and endogenous expression systems demonstrated that  $\alpha$ ENaC and  $\alpha\beta\gamma$ ENaC channels could be activated by the application of negative hydrostatic pressure.<sup>9-12</sup> Contrasting results were found in the *Xenopus* oocyte expression system in response to osmotic induced swelling and shrinking.<sup>13,14</sup> However, using the oocyte expression system, ENaC can be activated by a different mechanical stimulus, shear stress. In the cortical distal tubule, shear stress may be the appropriate stimulus to mechanically gate ENaC channels.<sup>15,16</sup> The reasons underlying the conflicting results in isolated expression systems are unknown; however, they may reflect several factors, including the following: (1) specificity of mechanical gating of  $\alpha\beta\gamma$ ENaC to the stimulus (ie, shear stress versus osmotic stretch); (2) presence of inhibitory substances such as ATP<sup>12</sup>; and (3) importance of the appropriate combination of intracellular and extracellular proteins necessary to gate the channel in response to stretch or strain. These findings suggest that, under certain conditions, mechanical forces can gate isolated ENaC channels. Currently, the mechanosensitivity of ASIC channels has not been addressed.

#### Chemosensitivity

Direct evidence of the chemosensitivity of homomeric and heteromeric ASIC channels is derived from studies in isolated expression systems, where extracellular acidosis (EC<sub>50</sub> pH range: 3.5 to 7.0) gates the channels, with the different channels having varying pH sensitivities. Although  $\alpha\beta\gamma$ ENaC channels are not gated by pH, the presence of the  $\delta$ ENaC subunit confers pH sensitivity with an EC<sub>50</sub> of pH 6.1 for  $\delta\beta\gamma$ ENaC and 6.5 for  $\delta\alpha\beta\gamma$ ENaC channels.<sup>1,17</sup> These findings suggest that ASIC channels, and ENaC channels containing  $\delta$ ENaC, can be gated by protons. Because protons are thought to signal chemoreflex responses initiated in carotid chemoreceptors and peripheral chemoreceptors, ENaC/ASIC proteins are considered candidates for these receptors.

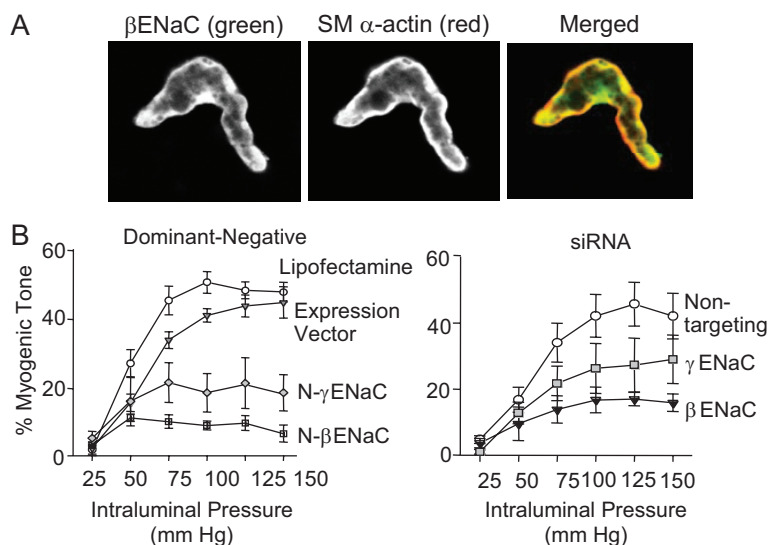
### Contribution of Neuron and Vascular Smooth Muscle ENaC/ASIC Proteins to Cardiovascular Homeostasis

In addition to their role in salt and water reabsorption, evidence suggests that ENaC/ASIC proteins contribute to cardiovascular homeostasis by functioning as mechanoreceptors in arterial baroreceptor neurons and VSMCs and as acid sensors in arterial chemosensors (carotid body glomus cells), myocardial tissue, and skeletal muscle. In this section, we discuss evidence implicating ENaC/ASIC proteins as mechanosensors and chemosensors in cardiovascular tissue.

If ENaC/ASIC proteins are to be considered as mechanosensors or chemosensors, then  $\geq 2$  criteria must be met. First, ENaC/ASIC proteins must be expressed at the site of mechanotransduction or chemoreception. Second, inhibition or disruption of ENaC/ASIC activity should inhibit the mechanosensitive or chemosensitive response. Because ENaC null mice are very ill or die shortly after birth, genetic evidence for ENaC involvement in mechanotransduction is lacking.<sup>18-20</sup> ASIC null mice thrive and have provided evidence for their involvement in mechanoreception and chemoreception. As an alternative to ENaC null mice, selective ENaC inhibitors, such as amiloride and benzamil, have been useful tools in determining ENaC involvement, because  $\alpha\beta\gamma$ ENaC can be blocked by as little as 100 nmol/L.<sup>1,21</sup> Thus, low doses of amiloride can discern the importance of ENaC channels from other transporters and ion channels.

#### Role in Neural Cardiovascular Mechanosensation

ENaC and ASIC molecules are expressed in specific sensory neuron populations in the dorsal root, trigeminal and nodose ganglia, and the afferent nerve terminals innervating certain somatic and visceral receptors.<sup>1,2,22-25</sup> One of these sites includes arterial baroreceptor nerve endings located in the aortic arch and carotid sinus. Arterial baroreceptors discharge in response to pressure-induced vessel wall stretch and play an important role in the beat-to-beat control of the cardiovascular system. Four lines of evidence suggest that ENaC/ASIC channels participate in arterial baroreceptor activation. First, baroreceptor neurons express at least  $\beta$ ENaC,  $\gamma$ ENaC, and ASIC2 molecules.<sup>22,26</sup> Second, ENaC inhibition prevents mechanically activated membrane depolarization and Ca<sup>2+</sup> transients in baroreceptor neurons.<sup>22,27</sup> Third, ENaC inhibition blocks pressure-induced changes in carotid baroreceptor activity and reflex-induced changes in systemic blood pressure. Fourth, ASIC2 null mice have reduced spontaneous baroreflex sensitivity.<sup>28</sup> Taken together these findings suggest that at least  $\beta$ ENaC,  $\gamma$ ENaC, and ASIC2 are expressed in baroreceptor neurons, where they may mediate mechanically initiated responses in vitro and in vivo. It is unknown whether  $\beta$ ENaC,  $\gamma$ ENaC, and ASIC2 associate to form a homogenous population of heteromeric channels (ie, all of the channels are composed of  $\beta$ ENaC,  $\gamma$ ENaC, and ASIC2) or if the subunits associate to form multiple heteromeric channels (ie, channels are composed of  $\beta$ ENaC/ $\gamma$ ENaC,  $\gamma$ ENaC/ASIC, or  $\beta$ ENaC/ASIC channels) or homomeric channels (ie,  $\beta$ ENaC,  $\gamma$ ENaC, or ASIC only). Although ENaC/ASIC proteins are expressed in nonbaroreceptor neurons in sensory ganglia, it is unknown whether ENaC/ASIC proteins act as mechanoreceptors in



**Figure 1.** VSMC ENaC. A, Localization of  $\beta$ ENaC (left) and smooth muscle  $\alpha$ -actin (middle) in a single VSMC dissociated from mouse renal arteries. Yellow coloring in the merged image (right) suggests colocalization of  $\beta$ ENaC and the cytoskeleton. B, Suppression of  $\beta$ ENaC/ $\gamma$ ENaC expression in isolated mouse renal interlobar artery segments inhibits vasoconstriction (percentage of myogenic tone) to increases in intraluminal pressure.

nonbaroreceptor cardiovascular mechanoreceptors, such as cardiopulmonary receptors.

### Local Control of Vascular Resistance: Pressure-Induced Vasoconstriction

In addition to neural mechanisms, ENaC/ASIC proteins may contribute to cardiovascular homeostasis by participating in local mechanisms regulating vascular resistance. Pressure-induced, or myogenic, constriction is an inherent response of certain vessels that allows resistance arteries to adjust tone in response to luminal pressure; vessels constrict to increases and dilate to decreases in pressure. The response is initiated by vessel wall stretch and is, thus, activated by a mechanical stimulus. The response may play a critical role in preventing the transmission of pressure waves to small, fragile microvasculature, particularly in the renal and cerebral circulations, and, thus, may protect against hypertension-induced injury.<sup>29,30</sup>

The importance of ENaC/ASIC proteins in pressure-induced vasoconstriction has been examined in cerebral and renal arteries. VSMCs express  $\beta$ ENaC,  $\gamma$ ENaC, and ASIC2.<sup>31–34</sup> In these VSMCs, ENaC/ASIC2 proteins are expressed at or near the cell surface, the predicted site of mechanotransduction of a VSMC stretch into a cellular signaling event (Figure 1A). Pharmacological inhibition of ENaC with amiloride or benzamil inhibits pressure-mediated constriction (at submicromolar and low micromolar concentrations) in the middle cerebral artery, renal interlobar, and renal afferent arterioles.<sup>31,32,35,36</sup> In a follow-up study, Jernigan and Drummond<sup>32</sup> used gene-specific silencing approaches, small-interfering RNA, and dominant negatives to determine the importance of  $\beta$ ENaC and  $\gamma$ ENaC in pressure-induced constriction (Figure 1B). Both approaches inhibited protein expression and pressure-induced vasoconstriction in isolated renal interlobar segments. Constriction in response to the  $\alpha$ -adrenergic receptor phenylephrine was not altered after ENaC inhibition or gene silencing, suggesting that vasoconstriction, per se, was not altered after ENaC inhibition.<sup>32,33</sup> Although these findings suggest that certain ENaC proteins may mediate pressure-induced constriction, the role of ASIC proteins has not been thoroughly examined. However, pre-

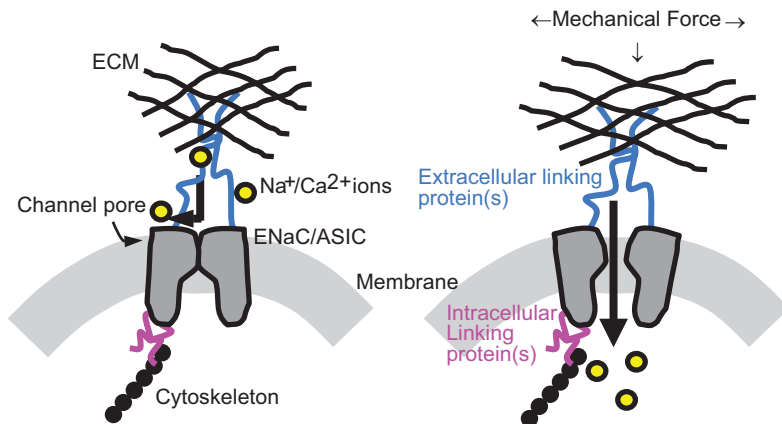
liminary studies suggest that pressure-induced constriction is absent in cerebral vessels of ASIC2 heterozygous null mice.<sup>34</sup> When we consider that ASIC2,  $\beta$ ENaC, and  $\gamma$ ENaC are expressed in similar VSMC populations; ASIC2 biochemically interacts with  $\gamma$ ENaC in other systems; and the loss of ASIC2,  $\beta$ ENaC, and  $\gamma$ ENaC produces the same phenotype (loss of pressure-induced constriction), the speculation that these proteins form a heteromultimeric channel seems reasonable.<sup>22,31–34,37,38</sup>

### Lack of Direct Electrophysiological Evidence of ENaC/ASIC Channels in VSMCs

Direct electrophysiological evidence of ENaC/ASIC channels in VSMCs is not available; however, one report of an epithelial-like  $\text{Na}^+$  current in VSMCs was found.<sup>39</sup> Similar to  $\alpha\beta\gamma$ ENaC, the channel reported in VSMCs is nonvoltage gated and has a 10 pS conductance and high  $\text{Na}^+:\text{K}^+$  selectivity. Unlike  $\alpha\beta\gamma$ ENaC, the channel is insensitive to amiloride (100  $\mu\text{mol/L}$ ). Although the amiloride characteristics of this channel are not consistent with the reported amiloride sensitivity of  $\alpha\beta\gamma$ ENaC and  $\beta\gamma$ ENaC channels in heterologous expression systems, this finding supports the potential presence of an ENaC-like  $\text{Na}^+$  channel in VSMCs. It is not clear why there is so little electrophysiological evidence of ENaC; however, one possibility is that investigators have not looked for them. Another possibility is the channels are electrically silent until gated by mechanical stimuli.<sup>40,41</sup>

### Can $\beta$ ENaC and $\gamma$ ENaC Form a Channel in the Absence of $\alpha$ ENaC?

In VSMCs and sensory neurons,  $\beta$ ENaC and  $\gamma$ ENaC appear to be the predominant ENaC proteins expressed, whereas  $\alpha$ ENaC is rare. Because  $\alpha$ ENaC is required to generate the fully functional, constitutively active ENaC channel found typically in epithelial tissue, are  $\beta$ ENaC and  $\gamma$ ENaC capable of forming a channel in the absence of  $\alpha$ ENaC? Evidence from Bonny et al<sup>7</sup> suggest that  $\alpha$ ENaC is not required for  $\beta$  and  $\gamma$ ENaC to form a channel. Bonny et al<sup>7</sup> demonstrated oocytes expressing  $\beta$ ENaC and  $\gamma$ ENaC generate amiloride



**Figure 2.** Proposed model of a mammalian mechanosensor. This model is based on the mechanotransducer model established in the nematode. The mechanosensing apparatus may be a large heteromultimeric complex. ENaC/ASIC proteins form the ion-transducing core of the mechanotransducer and are anchored to the extracellular matrix and cytoskeleton by associated linking proteins. The application of a mechanical stimulus, such as strain, allows an influx of Na<sup>+</sup>/Ca<sup>2+</sup>.

sensitive currents in the absence of  $\alpha$ ENaC, when provided a longer incubation period ( $\approx 6$  days). Channels formed by  $\beta$ ENaC and  $\gamma$ ENaC have a greater selectivity for Na<sup>+</sup> and conduct less current. In addition,  $\beta\gamma$ ENaC channels have a 10-fold higher inhibition constant for amiloride ( $\approx 2 \mu\text{mol/L}$  in  $\beta\gamma$ ENaC versus  $0.2 \mu\text{mol/L}$  in  $\alpha\beta\gamma$ ENaC). Thus, channels formed by  $\beta\gamma$ ENaC are not the same as channels formed by  $\alpha\beta\gamma$ ENaC. The finding by Jernigan and Drummond<sup>32</sup> that  $\approx 40\%$  of myogenic constrictor responses are blocked with  $1 \mu\text{mol/L}$  of amiloride is consistent with the amiloride inhibition constant for  $\beta\gamma$ ENaC channels.

Compared with  $\alpha\beta\gamma$ ENaC channels, trafficking of  $\beta\gamma$ ENaC channels to the surface membrane in *Xenopus* oocytes is delayed, which results in protein localization in the intracellular compartment. This may be the basis for the lack of current generated by  $\beta\gamma$ ENaC in heterologous expression systems.<sup>7</sup> In freshly dissociated VSMCs, trafficking of  $\beta$  and  $\gamma$ ENaC does not appear impaired because they are expressed at or near the cell surface (Figure 1).<sup>32,33</sup> The mechanism(s) mediating membrane localization of  $\beta$ ENaC and  $\gamma$ ENaC in the absence of  $\alpha$ ENaC is unknown; however, there are a few possible explanations. First, VSMCs may express another protein that associates with and stabilizes  $\beta$ ENaC and  $\gamma$ ENaC. Second, another pore-forming subunit may interact with  $\beta$ ENaC and  $\gamma$ ENaC, such as  $\delta$ ENaC or an ASIC protein. Third,  $\alpha$ ENaC 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  $\beta\gamma$ ENaC channels that reach the membrane. Regardless of the mechanism, in the absence of detectable levels of  $\alpha$ ENaC,  $\beta$ ENaC and  $\gamma$ ENaC appear to traffic to the cell surface of VSMCs in vivo and remain there after enzymatic dissociation.

### How Do ENaC/ASIC Proteins Transduce Mechanical Stimuli?

Although the studies presented in this review demonstrate that ENaC/ASIC proteins play a significant role in the mechanodependent responses, it is not entirely clear how ENaC/ASIC proteins transduce mechanical stimuli. A universal or "all-purpose" mechanotransducer model has been developed in the nematode for related DEG proteins.<sup>1</sup> In this model, the mechanosensor is a large heteromultimeric chan-

nel complex consisting of 5 basic components: (1) extracellular matrix proteins, (2) extracellular linking proteins, (3) pore-forming channels, (4) intracellular linking proteins, and (5) cytoskeleton proteins (Figure 2). Nematode members of the DEG/ENaC/ASIC family form the ion-conducting unit of the complex. The application of a mechanical force is transduced through the extracellular matrix to gate the channel. Thus, the interaction between the pore-forming proteins and the extracellular matrix is critical to channel gating. The cytoskeleton may also participate in transduction of the applied force, and, along with other extracellular proteins, may also stabilize the pore at the cell surface. We speculate that a similar model also applies to ENaC/ASIC proteins in mammalian mechanosensors. When the channel is gated open, Na<sup>+</sup> and possibly Ca<sup>2+</sup> entry leads to membrane depolarization and subsequent activation of downstream signaling events, leading to smooth muscle cell contraction or neuronal action potential generation.

### Role in Cardiovascular Chemosensation

In addition to their role in mechanosensing, ENaC/ASIC proteins may contribute to cardiovascular homeostasis via chemosensing mechanisms. A well-established characteristic of ASIC channels is their activation by drops in extracellular pH,<sup>1</sup> which has made them very attractive candidates for chemosensing processes in arterial chemoreceptors and muscle metaboreceptors in skeletal and cardiac tissue.

Several lines of evidence support a potential role for ASIC channels in arterial chemoreceptors. First, carotid body glomus cells express ASIC1 and ASIC3 and, to a lesser extent, ASIC2.<sup>42</sup> Second, carotid body glomus cells have pH-gated currents that resemble ASIC channels and are partially amiloride sensitive.<sup>42</sup> Third, cardiovascular responses to chemoreceptor stimulation with carotid artery occlusion are attenuated in ASIC3 and ASIC1/ASIC3 double-null mice.<sup>43</sup> Together these findings suggest that ASIC proteins may participate in pH sensing in arterial chemoreceptors.

Some investigators have suggested that ASIC proteins may be chemotransducers in muscle tissue that signal ischemic pain.<sup>25,44–47</sup> In cardiac tissue, ischemia-induced extracellular acidosis is part of the signaling event leading to the sensation of pain. Activation of cardiac sympathetic afferents leads to a sympathoinhibitory/vagal stimulatory effect to reduce cardiac work.<sup>48</sup> The first study to address the role of ASIC proteins in

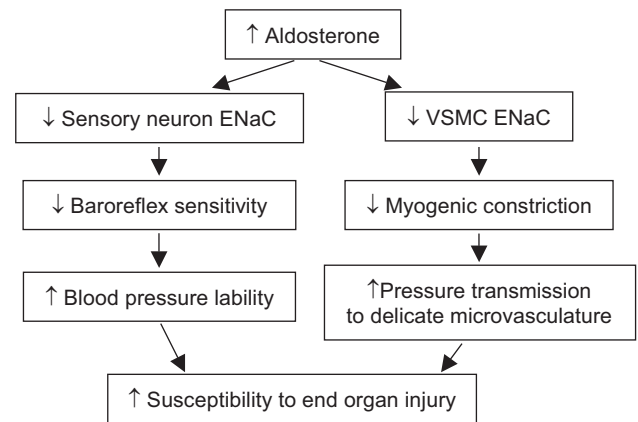
ischemic pain demonstrated that cardiac sensory neurons have substantial extracellular acid-evoked  $\text{Na}^+$  currents that resemble ASIC currents and are sensitive to amiloride.<sup>44</sup> Follow-up studies suggest an important role for ASIC3, because ASIC3 is highly sensitive to lactic acid, a mediator of ischemic pain, and acid-gated currents in cardiac sensory neurons are nearly identical to acid-gated currents of ASIC3 homomultimers.<sup>45,49</sup> Studies of cardiovascular responses to cardiac ischemia in genetically modified mice are needed to confirm the importance of ASIC proteins in sensing cardiac ischemia.

A similar role for ASIC3, as well as other ASIC proteins, in sensing changes in skeletal muscle pH has been proposed.<sup>25,46,47</sup> Similar to cardiac tissue, fine sensory afferents in skeletal muscle are activated with lactic acid, a byproduct of anaerobic muscle activity. Activation of these skeletal muscle afferents contributes to the cardiovascular and respiratory response to exercise. There are 3 lines of evidence supporting involvement of ASIC channels in this response. First ASIC proteins are localized in populations of small nociceptive neurons innervating skeletal muscle.<sup>25,50</sup> In particular, ASIC3 is expressed in fine nerve endings innervating skeletal muscle blood arterioles.<sup>25</sup> In addition, amiloride inhibits increases in blood pressure and heart rate to muscle contraction and intramuscular injection of lactic acid.<sup>46,47</sup> Although these findings support a role for ASIC proteins as pH sensors in muscle tissue and potential mediators of ischemic pain, involvement of ENaC channels cannot be ruled out, because  $\delta\text{ENaC}$  can confer pH sensitivity to ENaC channels, and ENaC proteins can interact with ASIC proteins to form channels. Future studies on ASIC/ENaC null mice are needed to elucidate the importance of specific ASIC (and possibly ENaC) proteins in muscle ischemic responses.

It is important to make a cautionary note: ENaC/ASIC channels are probably not the only cardiovascular mechanosensors and chemosensors. This protein family probably represents one of multiple signaling mechanisms for mechanosensing and pH sensing. Other ion channels are also involved, such as members of the 2-pore  $\text{K}^+$  channels and transient receptor potential channel families.<sup>51</sup>

### Aldosterone Regulation and Hypertension-Related Organ Injury

Results of recent clinical trials, Randomized Aldactone Evaluation Study and Eplerenone Heart Failure Efficacy and Survival Study, demonstrate the protective effect of aldosterone inhibition on cardiovascular function.<sup>52,53</sup> However, mechanisms of this protection are still unclear. One might consider, in the context of this review, a possible role for ENaC proteins. Aldosterone stimulates ENaC activity in epithelial tissue, yet its effect on vascular/neuronal ENaC expression is unknown.<sup>1</sup> Although sensory neurons and VSMCs might be expected to respond to aldosterone in a manner similar to epithelial tissue, this may not necessarily be true. Based on published studies and preliminary studies from our laboratory, we suspect that aldosterone may be a negative regulator of ENaC expression in sensory neurons and VSMCs.



**Figure 3.** Possible mechanism of aldosterone action on non-epithelial targets. Aldosterone inhibition of ENaC in neurons and VSMCs may suppress baroreflex sensitivity and myogenic constriction. These actions increase blood pressure lability and pressure transmission to delicate microvessels in the brain and kidney and, thus, increase susceptibility to end-organ injury. Protection against injury may contribute to the beneficial effect of aldosterone antagonism in clinical trials (Randomized Aldactone Evaluation Study and Eplerenone Heart Failure Efficacy and Survival Study).

Preliminary data from our laboratory suggest that aldosterone (1 nmol/L to 100  $\mu\text{mol/L}$ ) inhibits expression of  $\beta\text{ENaC}$  and  $\gamma\text{ENaC}$  in cultured sensory neurons. The effect is blocked by the aldosterone antagonists spironolactone and RU752. In addition, indirect evidence suggests that aldosterone inhibits ENaC in sensory neurons and VSMCs. Several investigations have linked elevated plasma aldosterone to reduced arterial baroreflex sensitivity and inhibition of myogenic tone in cerebral vessels. The studies discussed in this review suggest that baroreflex and myogenic control are mediated by ENaC.<sup>54–56</sup> Thus, aldosterone may suppress baroreflex and myogenic responses by inhibiting ENaC expression. These factors increase blood pressure lability and pressure transmission to the delicate microvasculature, which may result in susceptibility to pressure-related end-organ injury (Figure 3). We speculate that part of the cardioprotective effect of aldosterone antagonism in clinical trials may be because of its stimulatory effect on ENaC expression in sensory neurons and VSMCs, which may augment baroreflex sensitivity and prevent swings in systemic pressure and augment myogenic responsiveness and prevent the transmission of system pressure to the microvasculature.

How might aldosterone inhibit ENaC expression? Biochemical evidence suggests that aldosterone is capable of activating  $\geq 1$  negative regulatory pathway, the epidermal growth factor receptor-mitogen activated protein kinase pathway.<sup>57</sup> Recent studies suggest that aldosterone-mineralocorticoid binding leads to transactivation of the epidermal growth factor receptor, which acts as a “brake” that prevents overactivation of ENaC in epithelial tissue.<sup>58</sup> We speculate that this negative pathway is favored in VSMCs and sensory neurons, which results in the net inhibition of ENaC expression. Thus, aldosterone-mediated transactivation of the epidermal growth factor receptor is one mechanism that could mediate aldosterone inhibition of ENaCs in VSMCs and sensory neurons.

## Perspectives

A growing body of evidence suggests that ENaC/ASIC proteins play a more diverse role in cardiovascular homeostasis than recognized previously. Certain ENaC and ASIC proteins may also influence cardiovascular homeostasis by acting as mechanosensors that mediate arterial baroreflex responses and local control of vascular resistance. ASIC proteins may influence cardiovascular homeostasis by acting as chemosensors, detecting changes in arterial pH to mediate arterial chemoreflex and ischemic responses in cardiac and skeletal muscle and mechanosensors in baroreceptors. This is a very exciting time for this area of research; we are just beginning to understand the importance of ENaC/ASIC proteins as sensors in cardiovascular mechanoreception and chemoreception. How these channels interact with neighboring proteins to signal mechanical and chemical stimuli, contribute to the neural and local cardiovascular reflex responses, and contribute to the progression of cardiovascular disease, such as hypertension, remain to be determined.

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## Disclosures

None.

## References

- Kellenberger S, Schild L. Epithelial sodium channel/degenerin family of ion channels: a variety of functions for a shared structure. *Physiol Rev*. 2002;82:735–767.
- Syntichaki P, Tavernarakis N. Genetic models of mechanotransduction: the nematode *Caenorhabditis elegans*. *Physiol Rev*. 2004;84:1097–1153.
- Ji HL, Su XF, Kedar S, Li J, Barbry P, Smith PR, Matalon S, Benos DJ. Delta-subunit confers novel biophysical features to alpha beta gamma-human epithelial sodium channel (ENaC) via a physical interaction. *J Biol Chem*. 2006;281:8233–8241.
- Waldmann R, Champigny G, Bassilana F, Voilley N, Lazdunski M. Molecular cloning and functional expression of a novel amiloride-sensitive Na<sup>+</sup> channel. *J Biol Chem*. 1995;270:27411–27414.
- Brockway LM, Zhou ZH, Bubien JK, Jovov B, Benos DJ, Keyser KT. Rabbit retinal neurons and glia express a variety of ENaC/DEG subunits. *Am J Physiol Cell Physiol*. 2002;283:C126–C134.
- Babini E, Geisler HS, Siba M, Grunder S. A new subunit of the epithelial Na<sup>+</sup> channel identifies regions involved in Na<sup>+</sup> self-inhibition. *J Biol Chem*. 2003;278:28418–28426.
- Bonny O, Chraïbi A, Loffing J, Jaeger NF, Grunder S, Horisberger JD, Rossier BC. Functional expression of a pseudohypoaldosteronism type I mutated epithelial Na<sup>+</sup> channel lacking the pore-forming region of its alpha subunit. *J Clin Invest*. 1999;104:967–974.
- Grifoni SC, Jernigan NL, Hamilton G, Drummond HA. ASIC proteins regulate smooth muscle cell migration. *Microvasc Res*. 2008;75:202–210.
- Awayda MS, Ismailov II, Berdiev BK, Benos DJ. A cloned renal epithelial Na<sup>+</sup> channel protein displays stretch activation in planar lipid bilayers. *Am J Physiol*. 1995;268:C1450–C1459.
- Kizer N, Guo X-L, Hruska K. Reconstitution of stretch-activated cation channels by expression of the  $\alpha$ -subunit of the epithelial sodium channel cloned from osteoblasts. *Proc Natl Acad Sci U S A*. 1997;94:1013–1018.
- Palmer LG, Frindt G. Gating of Na channels in the rat cortical collecting tubule: effects of voltage and membrane stretch. *J Gen Physiol*. 1996;107:35–45.
- Ma HP, Li L, Zhou ZH, Eaton DC, Warnock DG. ATP masks stretch activation of epithelial sodium channels in A6 distal nephron cells. *Am J Physiol Renal Physiol*. 2002;282:F501–F505.
- Awayda MS, Subramanyam M. Regulation of the epithelial Na<sup>+</sup> channel by membrane tension. *J Gen Physiol*. 1998;112:97–111.
- Ji H-L, Fuller CM, Benos DJ. Osmotic pressure regulates  $\alpha\beta\gamma$ -rENaC expressed in *Xenopus* oocytes. *Am J Physiology*. 1998;275:C1182–C1190.
- Carattino MD, Sheng S, Kleyman TR. Epithelial Na<sup>+</sup> channels are activated by laminar shear stress. *J Biol Chem*. 2004;279:4120–4126.
- Satlin LM, Sheng S, Woda CB, Kleyman TR. Epithelial Na<sup>+</sup> channels are regulated by flow. *Am J Physiol Renal Physiol*. 2001;280:F1010–F1018.
- Waldmann R, Champigny G, Lingueglia E, De Weille JR, Heurteaux C, Lazdunski M. H<sup>+</sup>-gated cation channels. *Ann NY Acad Sci*. 1999;868:67–76.
- Barker PM, Nguyen MS, Gatzky JT, Grubb B, Norman H, Hummler E, Rossier B, Boucher RC, Koller B. Role of gammaENaC subunit in lung liquid clearance and electrolyte balance in newborn mice. Insights into perinatal adaptation and pseudohypoaldosteronism. *J Clin Invest*. 1998;102:1634–1640.
- Hummler E, Barker P, Gatzky J, Beermann F, Verdumo C, Schmidt A, Boucher R, Rossier BC. Early death due to defective neonatal lung liquid clearance in alpha-ENaC-deficient mice. *Nat Genet*. 1996;12:325–328.
- McDonald FJ, Yang B, Hrstka RF, Drummond HA, Tarr DE, McCray PB Jr, Stokes JB, Welsh MJ, Williamson RA. Disruption of the beta subunit of the epithelial Na<sup>+</sup> channel in mice: hyperkalemia and neonatal death associated with a pseudohypoaldosteronism phenotype. *Proc Natl Acad Sci U S A*. 1999;96:1727–1731.
- Kleyman TR, Cragoe EJ Jr. Amiloride and its analogs as tools in the study of ion transport. *J Membr Biol*. 1988;105:1–21.
- Drummond HA, Price MP, Welsh MJ, Abboud FM. A molecular component of the arterial baroreceptor mechanotransducer. *Neuron*. 1998;21:1435–1441.
- Drummond HA, Abboud FM, Welsh MJ. Localization of beta and gamma subunits of ENaC in sensory nerve endings in the rat foot pad. *Brain Res*. 2000;884:1–12.
- Fricke B, Lints R, Stewart G, Drummond H, Dodt G, Driscoll M, von Düring M. Epithelial Na<sup>+</sup> channels and stomatin are expressed in rat trigeminal mechanosensory neurons. *Cell Tissue Res*. 2000;299:327–334.
- Molliver DC, Immke DC, Fierro L, Pare M, Rice FL, McCleskey EW. ASIC3, an acid-sensing ion channel, is expressed in metaboreceptive sensory neurons. *Mol Pain*. 2005;1:35.
- Lu Y, Whiteis CA, Benson CJ, Chapleau MW, Abboud FM. Expression and localization of acid-sensing ion channels in mouse nodose ganglia. *FASEB J*. 2006;20:A775.
- Snitsarev V, Whiteis CA, Abboud FM, Chapleau MW. Mechanosensory transduction of vagal and baroreceptor afferents revealed by study of isolated nodose neurons in culture. *Auton Neurosci*. 2002;98:59–63.
- Sabharwal R, Stauss HM, Lazartigues E, Whiteis CA, Davisson RL, Price MP, Welsh MJ, Abboud FM, Chapleau MW. Abnormalities in baroreflex sensitivity and autonomic control in conscious ASIC2<sup>-/-</sup> mice. *FASEB J*. 2006;20:A1186.
- Bidani AK, Griffin KA. Pathophysiology of hypertensive renal damage: implications for therapy. *Hypertension*. 2004;44:595–601.
- Smeda JS, Payne GW. Alterations in autoregulatory and myogenic function in the cerebrovasculature of Dahl salt-sensitive rats. *Stroke*. 2003;34:1484–1490.
- Drummond HA, Gebremedhin D, Harder DR. Degenerin/epithelial Na<sup>+</sup> channel proteins: components of a vascular mechanosensor. *Hypertension*. 2004;44:643–648.
- Jernigan NL, Drummond HA. Vascular ENaC proteins are required for renal myogenic constriction. *Am J Physiol Renal Physiol*. 2005;289:F891–F901.
- Jernigan NL, Drummond HA. Myogenic vasoconstriction in mouse renal interlobar arteries: role of endogenous beta and gammaENaC. *Am J Physiol Renal Physiol*. 2006;291:F1184–F1191.
- Gannon KP, Galmiche L, Drummond HA. ASIC2 protein is required for pressure-induced constriction in mouse middle cerebral artery. *Am J Physiol Heart Circ Physiol*. 2008;Feb 22 [Epub ahead of print].
- Oyabe A, Masumoto N, Ueta K, Nakayama K. Amiloride-sensitive pressure-induced myogenic contraction in rat cerebral artery. *Fundam Clin Pharmacol*. 2000;14:369–377.
- Guan ZCA, Inscho E. Effect of ENaC blockade on the myogenic response of rat juxtamedullary afferent arterioles. *FASEB J*. 2007;21:595.15. Abstract.
- Meltzer RH, Kapoor N, Qadri YJ, Anderson SJ, Fuller CM, Benos DJ. Heteromeric assembly of acid-sensitive ion channel and epithelial sodium channel subunits. *J Biol Chem*. 2007;282:25548–25559.

38. Berdiev BK, Xia J, McLean LA, Markert JM, Gillespie GY, Mapstone TB, Naren AP, Jovov B, Bubien JK, Ji HL, Fuller CM, Kirk KL, Benos DJ. Acid-sensing ion channels in malignant gliomas. *J Biol Chem.* 2003; 278:15023–15034.
39. Van Renterghem C, Lazdunski M. A new non-voltage-dependent, epithelial-like Na<sup>+</sup> channel in vascular smooth muscle cells. *Pflugers Arch.* 1991;419:401–408.
40. Hughey RP, Bruns JB, Kinlough CL, Harkleroad KL, Tong Q, Carattino MD, Johnson JP, Stockand JD, Kleyman TR. Epithelial sodium channels are activated by furin-dependent proteolysis. *J Biol Chem.* 2004;279: 18111–18114.
41. Caldwell RA, Boucher RC, Stutts MJ. Serine protease activation of near-silent epithelial Na<sup>+</sup> channels. *Am J Physiol Cell Physiol.* 2004; 286:C190–C194.
42. Tan ZY, Lu Y, Whiteis CA, Benson CJ, Chapleau MW, Abboud FM. Acid-sensing ion channels contribute to transduction of extracellular acidosis in rat carotid body glomus cells. *Circ Res.* 2007;101:1009–1019.
43. Sabharwal R, Chapleau MW, Price MP, Welsh MJ, Abboud FM. Molecular mechanisms of baro- and chemoreceptor activation: evidence that ASIC1 and ASIC3 contribute to chemoreceptor activation. *Hypertension.* 2005;46:832. Abstract.
44. Benson CJ, Eckert SP, McCleskey EW. Acid-evoked currents in cardiac sensory neurons: a possible mediator of myocardial ischemic sensation. *Circ Res.* 1999;84:921–928.
45. Sutherland SP, Benson CJ, Adelman JP, McCleskey EW. Acid-sensing ion channel 3 matches the acid-gated current in cardiac ischemia-sensing neurons. *Proc Natl Acad Sci U S A.* 2001;98:711–716.
46. Li J, Maile MD, Sinoway AN, Sinoway LI. Muscle pressor reflex: potential role of vanilloid type 1 receptor and acid-sensing ion channel. *J Appl Physiol.* 2004;97:1709–1714.
47. Hayes SG, Kindig AE, Kaufman MP. Blockade of acid sensing ion channels attenuates the exercise pressor reflex in cats. *J Physiol.* 2007; 581:1271–1282.
48. Guyton AC, Hall JE. *Textbook of Medical Physiology.* 11th ed. Philadelphia, PA: Elsevier Saunders; 2006.
49. Immke DC, McCleskey EW. Lactate enhances the acid-sensing Na<sup>+</sup> channel on ischemia-sensing neurons. *Nat Neurosci.* 2001;4:869–870.
50. Sluka KA, Price MP, Breese NM, Stucky CL, Wemmie JA, Welsh MJ. Chronic hyperalgesia induced by repeated acid injections in muscle is abolished by the loss of ASIC3, but not ASIC1. *Pain.* 2003;106:229–239.
51. Clapham DE, Runnels LW, Strubing C. The TRP ion channel family. *Nat Rev Neurosci.* 2001;2:387–396.
52. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, Palensky J, Wittes J. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med.* 1999;341:709–717.
53. Pitt B, Remme W, Zannad F, Neaton J, Martinez F, Roniker B, Bittman R, Hurley S, Kleiman J, Gatlin M. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. *N Engl J Med.* 2003;348:1309–1321.
54. Wang W. Chronic administration of aldosterone depresses baroreceptor reflex function in the dog. *Hypertension.* 1994;24:571–575.
55. Yee KM, Struthers AD. Aldosterone blunts the baroreflex response in man. *Clin Sci (Lond).* 1998;95:687–692.
56. Rigsby CS, Pollock DM, Dorrance AM. Spironolactone improves structure and increases tone in the cerebral vasculature of male spontaneously hypertensive stroke-prone rats. *Microvasc Res.* 2007;73:198–205.
57. Gekle M, Freudinger R, Mildenerger S, Silbernagl S. Rapid actions of aldosterone on cells from renal epithelium: the possible role of EGF-receptor signaling. *Steroids.* 2002;67:499–504.
58. Grossmann C, Freudinger R, Mildenerger S, Krug AW, Gekle M. Evidence for epidermal growth factor receptor as negative-feedback control in aldosterone-induced Na<sup>+</sup> reabsorption. *Am J Physiol Renal Physiol.* 2004;286:F1226–F1231.