

## $\beta$ ENaC Acts as a Mechanosensor in Renal Vascular Smooth Muscle Cells That Contributes to Renal Myogenic Blood Flow Regulation, Protection From Renal Injury and Hypertension

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### ABSTRACT

Pressure-induced constriction (also known as the “myogenic response”) is an important mechanodependent response in small renal arteries and arterioles. The response is initiated by vascular smooth muscle cell (VSMC) stretch due to an increase in intraluminal pressure and leads to vasoconstriction. The myogenic response has two important roles as a mechanism of local blood flow autoregulation and protection against systemic blood pressure-induced microvascular damage. However, the molecular mechanisms underlying initiation of myogenic response are unresolved. Although several molecules have been considered initiators of the response, our laboratory has focused on the role of degenerin proteins because of their strong evolutionary link to mechanosensing in the nematode. Our laboratory has addressed the hypothesis that certain degenerin proteins act as mechanosensors in VSMCs. This article discusses the importance of a specific degenerin protein,  $\beta$  Epithelial Na<sup>+</sup> Channel ( $\beta$ ENaC), in pressure-induced vasoconstriction, renal blood flow and susceptibility to renal injury. We propose that loss of the renal myogenic constrictor response delays the correction of renal blood flow that occurs with fluctuations in systemic pressure, which

allows pressure swings to be transmitted to the microvasculature, thus increasing the susceptibility to renal injury and hypertension. The role of  $\beta$ ENaC in myogenic regulation is independent of tubular  $\beta$ ENaC and thus represents a non-tubular role for  $\beta$ ENaC in renal-cardiovascular homeostasis.

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**Key words:** Autoregulation; Epithelial sodium channel; Blood pressure; Mechanotransduction; Myogenic response

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### INTRODUCTION

Mechanotransduction in vascular tissues is a topic of physiologic and pathophysiologic importance. Chronic and transient mechanical forces contribute to development of atherosclerosis, angiogenesis, endothelial function, ischemia-reperfusion injury, myogenic constriction and hypertension. However, the molecular mechanisms underlying transduction of mechanical forces, particularly the transduction of transient mechanical forces into rapid changes in cellular function, remain unclear. Our laboratory has been investigating the molecular mechanism(s) underlying initiation of the myogenic response in renal vasculature.

### WHAT IS THE MYOGENIC RESPONSE AND WHY IS IT IMPORTANT?

The myogenic response. The myogenic response was initially described over 100 years ago<sup>[1]</sup>. The response is initiated by

intraluminal pressure-induced vessel wall stretch, which stretches VSMCs circumferentially arranged around the vessel<sup>[2,3]</sup>. In turn, VSMC stretch initiates a depolarization event, which is thought to activate secondary signaling pathways, which include but are not limited to, voltage gated  $\text{Ca}^{2+}$  channels.  $\text{Ca}^{2+}$  channel activation stimulates  $\text{Ca}^{2+}$  influx and triggers vasoconstriction (Figure 1A)<sup>[2,3]</sup>. The molecular mechanism underlying the initial depolarizing event remains unresolved.

Physiological significance of myogenic constriction: regulation of renal blood flow and protection from injury. The myogenic response is important because it participates in two processes. First, it is a mechanism of renal blood flow autoregulation, where blood flow is tightly controlled despite changes in systemic perfusion pressure between 50 and 150 mmHg<sup>[4,5]</sup>. Myogenic constriction is fast acting and adjusts vascular resistance to a change in perfusion pressure within 5-10 sec. The other mechanism of renal blood flow autoregulation, tubuloglomerular feedback (TGF), is slower and adjusts vascular resistance within 6-25 sec<sup>[6-9]</sup>. The fast nature of the myogenic response has led investigators to suggest another purpose of the myogenic response is the prevention of high systemic pressure transmission to the glomerular microvasculature, thus protecting microvasculature from pressure-related injury associated with hypertension, diabetes, and end stage renal disease<sup>[10,11]</sup>. While much is understood about signaling mechanisms underlying VSMC contraction, our understanding of the signaling mechanisms that transduce changes in intraluminal pressure into a cellular signaling event, i.e. the events that initiate myogenic constriction, is limited. We hypothesize that degenerin proteins may act as mechanosensors that transduce stretch into a cellular event.

## EVOLUTIONARY ROLE OF DEGENERIN PROTEINS AS MECHANOSENSORS

Degenerin proteins are a large family of proteins expressed in a diverse range of species, including the nematode, *Caenorhabditis elegans* (*C. elegans*), *Drosophila* and mammals. This family has strong evolutionary ties to mechanotransduction in neuronal and muscle tissues<sup>[12-21]</sup>. Members of this family share a common structure: intracellular N- and C- termini and a single large extracellular domain of approximately 400 residues separated by two membrane-spanning domains. Many of the degenerin proteins form homo- and heteromultimeric, non-voltage gated,  $\text{Na}^+$ /cation channels<sup>[14,15,19]</sup>.

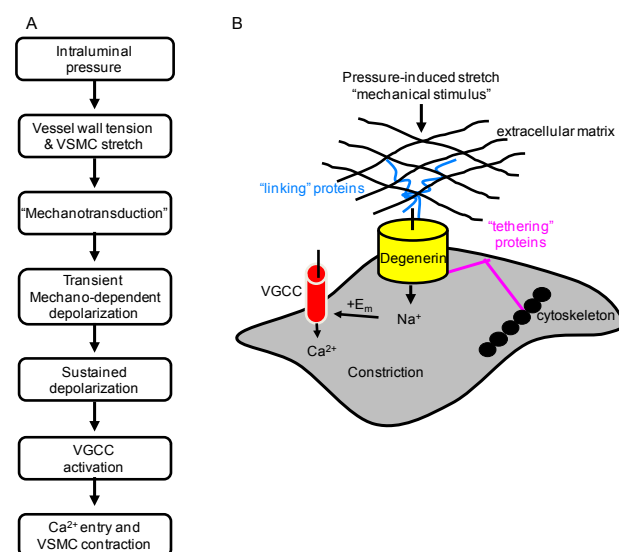
In mammals, two subfamilies of degenerin proteins have been identified: the Epithelial  $\text{Na}^+$  Channel (ENaC) and Acid Sensing Ion Channel (ASIC) proteins. ENaC proteins are known for their role in  $\text{Na}^+$  and water transport in the kidney, lung, and colon epithelia. In these tissues,  $\alpha$ ,  $\beta$  and  $\gamma$ ENaC proteins form a non-voltage gated,  $\text{Na}^+$  selective ion channel. The  $\alpha\beta\gamma$ ENaC channel is inhibited by submicromolar to low micromolar concentrations of the diuretic amiloride and its analog benzamil. ENaC proteins are also expressed at several important sites of mechanotransduction including somatic touch receptors in skin, osteoclasts, keratinocytes, arterial baroreceptor neurons, endothelial cells and VSMCs<sup>[16,22-26]</sup>. Because of their close evolutionary relationship to the *C. elegans* degenerins, expression in mechanosensitive tissues, and ability to form ion channels, ENaC proteins have been considered as likely components of mechanosensors in vertebrate tissue.

A model for a mammalian mechanosensor. Although a model of a mammalian mechanosensor has not been established, numerous genetic studies have led to the development of a mechanotransducer

model in *C. elegans* neuronal and muscle tissue<sup>[18]</sup>. The model consists of three essential components: (1) an ion-conducting pore; (2) extracellular matrix and proteins that may link the pore to the matrix; and (3) cytoskeleton and proteins that may tether the pore to the cytoskeleton. Degenerin proteins form the ion channel pore. The extracellular matrix participates in force transduction and helps stabilize the ion-conducting pore at the cell surface. The cytoskeleton may also participate in transduction of the applied force and stabilize the ion-conducting pore at the cell surface. Based on the concept of evolutionary conservation of function, we hypothesize the mammalian mechanosensor is similar to the nematode model (Figure 1B). Activation of the mechanosensor leads to influx of  $\text{Na}^+$  and/or  $\text{Ca}^{2+}$  through a degenerin ion channel, which leads to an initial membrane depolarization and subsequent activation of VGCC.

## DEGENERIN PROTEINS ARE EXPRESSED IN VSMCS AND MEDIATE RENAL MYOGENIC CONSTRICTION

To consider ENaC proteins as mechanosensors mediating pressure-induced constriction in blood vessels, ENaC proteins must be expressed in VSMCs and located at the site of mechanotransduction, near the cell surface and ENaC inhibition should abolish renal myogenic constriction. Therefore, initial studies focused on the expression/localization of ENaC proteins in VSMCs and the sensitivity of myogenic constriction to ENaC inhibition<sup>[23,27,28]</sup>. As shown in Figure 2A, VSMCs enzymatically dissociated from renal arterial segments express  $\beta$  and  $\gamma$ ENaC, but not  $\alpha$ , at or near the cell surface membrane<sup>[27]</sup>. The localization pattern is significant because a strain sensing mechanosensor might be predicted to be located near the cell surface. The lack of  $\alpha$ ENaC in VSMCs is also an important finding. It has been suggested that the lack of  $\alpha$ ENaC would render a  $\beta\gamma$ ENaC channel unable to conduct current in VSMCs, however, this is not entirely accurate as  $\beta$  and  $\gamma$ ENaC can form an amiloride-sensitive,  $\text{Na}^+$  conducting channel in the absence of  $\alpha$ ENaC<sup>[29]</sup>, albeit with reduced current. Additionally, the possibility of another subunit, such as an ASIC protein or unidentified ENaC, interacting with  $\beta$  and  $\gamma$ ENaC to form a channel has not been ruled out.



**Figure 1** Initiation of the myogenic response. A: The sequence of events leading myogenic constriction. B: Model role of degenerin proteins as a mechanotransducer in VSMCs.

Our laboratory has taken multiple approaches to determine the importance of ENaC proteins in renal myogenic constriction, which includes (1) pharmacological inhibition; (2) transient gene silencing and (3) genetically modified mice<sup>[27,30,31]</sup>. One of our methods for assessment of myogenic constriction is shown in Figure 2B-E. Renal interlobar artery segments are dissected from surrounding tissue and mounted on two pipettes (Figure 2A). Artery segments are exposed to a step-wise (25 mmHg, 5 min) increase in perfusion pressure (Figure 2C, with Ca<sup>2+</sup> containing and then Ca<sup>2+</sup> free extracellular solution). Under Ca<sup>2+</sup> containing conditions, artery segments constrict in response to the increase in pressure. However, under Ca<sup>2+</sup> free conditions, vessels will passively dilate in response to the increase in pressure (Figure 2D). Myogenic tone at each pressure step is calculated as the difference in diameter between Ca<sup>2+</sup> containing and Ca<sup>2+</sup> free conditions divided by Ca<sup>2+</sup> free diameter. A vessel segment with a myogenic response will exhibit an increase in tone with an increase in pressure. The pressure-myogenic tone relationship will be flat in a vessel with a weakened myogenic response (Figure 2E). If degenerin proteins are important in the transduction of myogenic constriction, then the relationship between pressure and myogenic tone should be altered following degenerin inhibition.

To determine if members of the degenerin protein family contribute to the transduction of the myogenic response, we initially used broad-spectrum degenerin inhibitors amiloride and its analog benzamil<sup>[27]</sup>. ENaC inhibition with benzamil abolishes myogenic constriction in renal interlobar arteries in a concentration dependent manner (Figure 2F). Amiloride, data not shown, elicited a similar inhibition of myogenic constriction. An important factor in the interpretation of these experiments is the selectivity of the ENaC inhibitors. At submicromolar and low micromolar doses, benzamil is a fairly selective inhibitor of ENaC. Furthermore, recent studies by Guan *et al.* and Nagasawa *et al.* suggest myogenic constriction in rat afferent arterioles is also sensitive to ENaC inhibition<sup>[32,33]</sup>. However, another study by Wang *et al* found benzamil did not inhibit myogenic constriction in renal arterioles<sup>[34]</sup>.

While pharmacological inhibition is a tool for screening for degenerin involvement, the contribution of specific subunits cannot be determined. Thus, to determine the importance of  $\beta$ ENaC and  $\gamma$ ENaC in renal myogenic constriction, we used a second approach, siRNA and dominant-negative constructs. siRNA and dominant-negative constructs specifically silence  $\beta$  or  $\gamma$ ENaC expression (~50-75%) in VSMCs in isolated mouse renal interlobar artery segments (Figure 2G and H)<sup>[31]</sup>. ENaC silencing inhibited pressure-induced constrictor responses by 40-80%, without altering the ability of the vessel to constrict to phenylephrine, suggesting the loss of vasoconstriction is specific to pressure rather than a generalized loss in the ability of the vessel to constrict.

In our third approach, we evaluated renal afferent arteriole myogenic constriction in a mouse model with reduced levels of  $\beta$ ENaC. The model, which is described in the following section, is characterized by a global reduction in  $\beta$ ENaC levels, including renal VSMCs. We examined myogenic constriction using the attached afferent arteriole-glomerulus preparation in which a single afferent arteriole attached to a glomerulus is dissected from the kidney and perfused at 60, then 120 mmHg<sup>[30]</sup>. The afferent arterioles from the  $\beta$ ENaC *+/+* mice develop significant myogenic tone in response to the step increase in pressure. However, myogenic tone is nearly abolished in renal afferent arterioles from  $\beta$ ENaC *m/m* mice, suggesting  $\beta$ ENaC is a critical to transduction of pressure-induced constriction (Figure 3A). Thus, three separate lines of evidence suggest certain ENaC proteins play a pivotal role in the myogenic response.

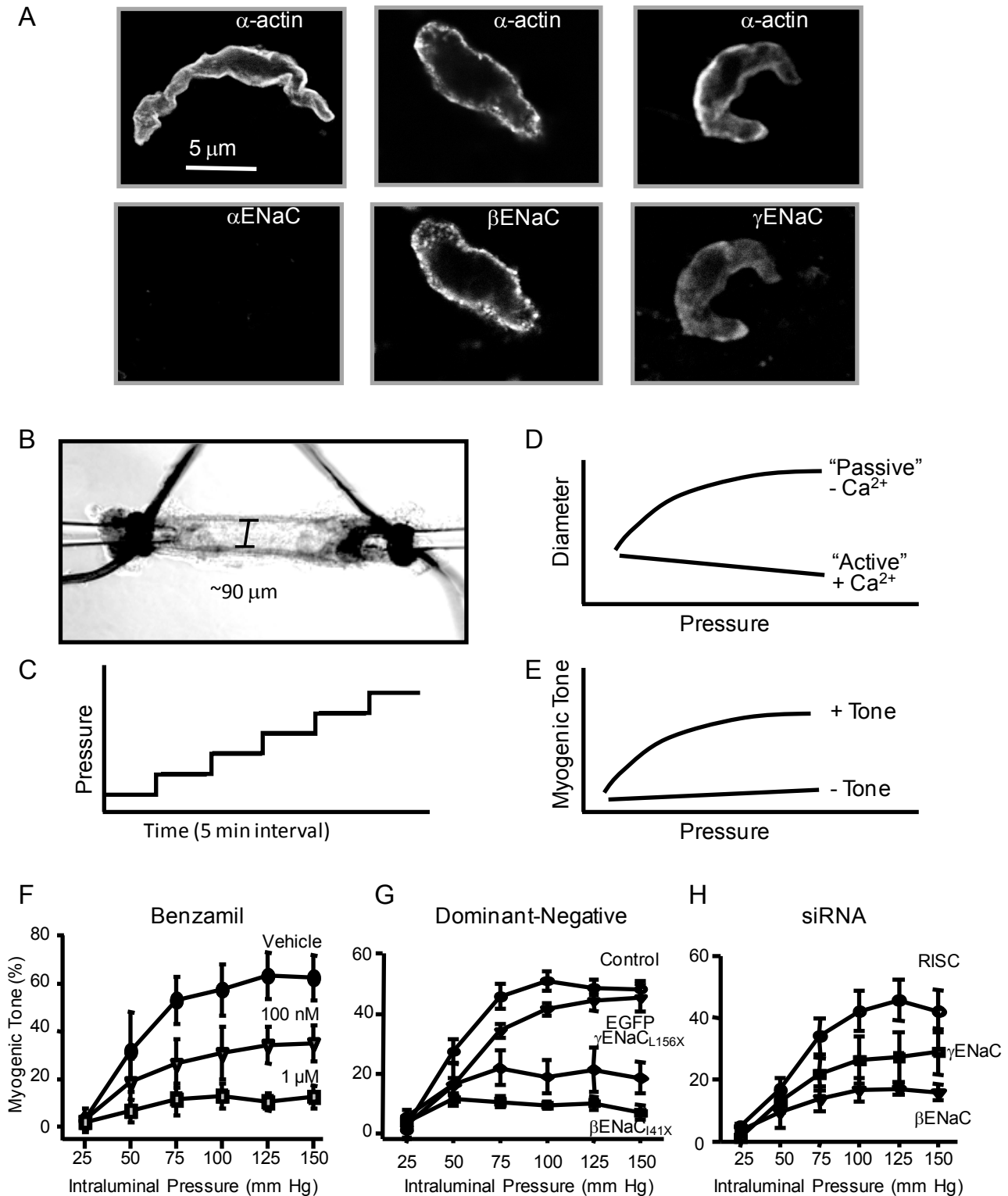
## DO DEGENERIN PROTEINS MEDIATE MECHANICALLY GATED CURRENTS?

While the studies addressed in the previous section demonstrate the importance of degenerin proteins to the myogenic response, they do not distinguish between a role as a mechanosensor that initiates the response or an amplifying mechanism. Early investigations into the mechanosensitivity of ENaC in heterologous systems were equivocal<sup>[16,35-38]</sup>. However, subsequent studies using endogenously expressing tissue are supportive of ENaC's mechanosensitivity. The application of negative pressure to isolated channels in cortical collecting duct cells can gate native ENaC channels, and shear stress can gate ENaC in both isolated rabbit cortical collecting duct and heterologously expressing oocytes suggest ENaC channels can be activated by mechanical forces<sup>[39-47]</sup>. However, not all mechanical forces are created equal and mechanosensors might be expected to exhibit modal specificity. For example, hypo-osmotic swelling and shear stress are mechanical forces, but they are not necessarily equivalent. Is it appropriate to expect osmotic swelling to activate a shear stress sensor? Based on our understanding of modal specificity in somatic touch receptors, probably not. Therefore, we should expect a mechanosensor that detects strain to be activated by stretch.

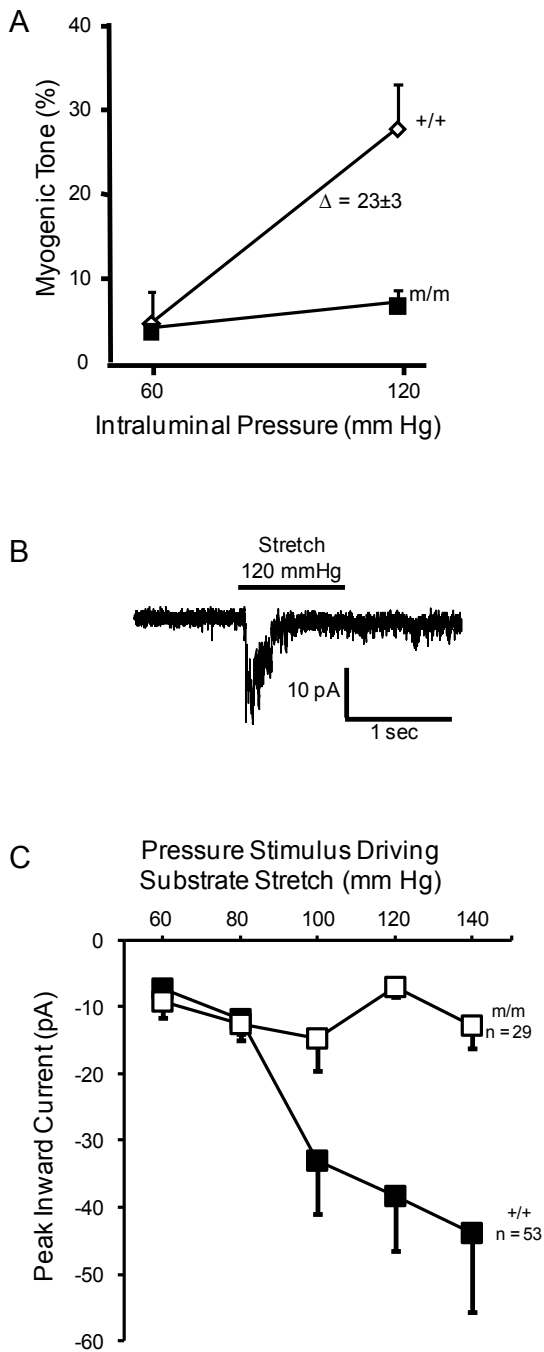
Since the importance of strain/stretch in activating a mammalian degenerin has not been addressed, our laboratory developed a novel in-vitro electrophysiologic assay to assess mechanically gated currents in isolated VSMCs using stretch<sup>[48]</sup>. Briefly, enzymatically dissociated renal VSMCs are plated on an elastomeric substrate coated with collagen. Cells are patched in a whole-cell configuration and the underlying membrane is stretched, which in turn, stretches the VSMCs along its long axis (~2  $\mu$ m). The stretch initiates a small but rapid, transient, Na<sup>+</sup> dependent, inward current (Figure 3B). As expected, the magnitude of the current is dependent on the magnitude of the stretch. The current is nearly abolished in renal VSMCs obtained from  $\beta$ ENaC *m/m* mice (Figure 3C). These later data are very important as they demonstrate that stretch gated currents in renal VSMCs are mediated by  $\beta$ ENaC.

## THE $\beta$ ENaC M/M MOUSE: A MODEL TO DETERMINE THE PHYSIOLOGICAL IMPORTANCE OF $\beta$ ENaC MEDIATED MYOGENIC CONSTRICTION

Our early studies using pharmacological and gene silencing approaches indicated an important role for  $\beta$ ENaC in myogenic constriction; however, understanding the consequences of long-term loss of myogenic constriction on cardiovascular health required a genetic model. For these studies, we used a model of reduced  $\beta$ ENaC ( $\beta$ ENaC *m/m*), rather than  $\beta$ ENaC knockout mice as they die shortly after birth. The  $\beta$ ENaC *m/m* model, developed by Bernard Rossier and Edith Hummler at the University of Lausanne, Switzerland<sup>[49,50]</sup>, was generated using standard gene targeting approaches in the course of generating a model of Liddle's syndrome (*increased*  $\beta$ ENaC) by the insertion a premature stop codon in the C-terminus coding region. However, the neomycin selection marker disrupts the  $\beta$ ENaC gene locus resulting in reduced  $\beta$ ENaC expression. Thus, a mouse model that under-, rather than over-expresses,  $\beta$ ENaC was generated. Mice homozygous for the mutation (*m/m*) express very low levels of  $\beta$ ENaC transcripts and/or protein in the lung, kidney and VSMCs, including renal afferent arterioles<sup>[30,38,50-52]</sup>. Importantly, myogenic



**Figure 2** Localization of  $\beta$ ENaC and  $\gamma$ ENaC in enzymatically dissociated renal VSMCs and in-vitro assessment of myogenic constriction. **A:** VSMCs were identified by labeling with  $\alpha$  smooth muscle actin (top row). We detected  $\beta$ ENaC and  $\gamma$ ENaC, but not  $\alpha$ ENaC, at or near the VSMC surface. The strongest labeling is consistently observed for  $\beta$ ENaC. Scale bar represents 5  $\mu$ m. **B:** Image of an isolated renal interlobar artery segment tied to inflow and outflow perfusion pipettes. **C:** To assess myogenic responsiveness vessels are exposed to a step-wise increase in perfusion pressure (25 mmHg step, 5 min each) from 25 to 150 mmHg under normal external  $Ca^{2+}$  and  $Ca^{2+}$  free solutions to determine active response and passive responses, respectively. **D:** The steady state diameter is plotted vs. pressure to obtain a pressure-diameter relationship under active vs. passive conditions. **E:** Myogenic tone is calculated as [(passive diameter - active diameter)/passive diameter] at each pressure step. A vessel with active myogenic tone will have an increase in tone with an increase in pressure. A vessel with reduced or absent myogenic tone will have a flattened relationship between pressure and tone. **F:** Effect of ENaC inhibition with benzamil on myogenic tone in renal interlobar arteries. **G:** Transient gene-silencing of  $\beta$ ENaC or  $\gamma$ ENaC using dominant-negative constructs of  $\beta$ ENaC or  $\gamma$ ENaC inhibits myogenic tone in renal interlobar arteries. **H:** Transient gene-silencing of  $\beta$ ENaC or  $\gamma$ ENaC using siRNA inhibits myogenic tone in renal interlobar arteries.



**Figure 3** Myogenic constriction in renal afferent arterioles and stretch gated currents in renal VSMCs are nearly abolished in mice with reduced levels of  $\beta$ ENaC ( $\beta$ ENaC m/m). A:  $\beta$ ENaC mediates myogenic constriction in renal afferent arteriole. Steady state vasoconstrictor responses to an increase in pressure in afferent arterioles in  $\beta$ ENaC +/+ and m/m animals ( $n=6$ , each group). Vasoconstrictor responses to adrenergic agonist norepinephrine (NE) were similar (not shown). B:  $\beta$ ENaC mediates stretch-gated whole cell currents in renal VSMCs. Stretch of the underlying elastomeric substrate elicits a transient inward current. C. The magnitude of the current response is dependent on the stimulus and the currents are nearly abolished in VSMCs from  $\beta$ ENaC m/m mice.

constriction in the renal afferent arteriole is abolished, as addressed in the previous section<sup>30</sup>. Since the afferent arteriole is the primary site of development of vascular resistance in the kidney,  $\beta$ ENaC mediated myogenic constriction likely contributes to control of whole kidney vascular resistance and blood flow regulation.

### Myogenic regulation of renal blood flow is attenuated in $\beta$ ENaC m/m mice

To address the physiologic and pathophysiologic importance of  $\beta$ ENaC mediated renal myogenic constriction in the regulation of whole kidney blood flow (RBF), we used the  $\beta$ ENaC m/m mouse model. We utilized the temporal separation between the onset of the myogenic mechanism (0-5 sec) and the TGF mechanism (6-25 sec) to determine the contribution of  $\beta$ ENaC to myogenic regulation of RBF<sup>30,52</sup>. In these studies, mice were instrumented with a carotid arterial catheter for blood pressure measurement and a renal flow probe for measurement of whole kidney blood flow (Figure 4A). A step increase in blood pressure was achieved with an occlusion of the lower abdominal aorta, just below the renal artery (Figure 4A, B). RBF increases and RVR decreases immediately following the step increase in pressure (Figure 4C and D). Within 5-10 sec, RBF begins to return to control levels in +/+ animals due to myogenically mediated increase in RVR. In contrast, RBF remains elevated and RVR reduced in the m/m mice, suggesting a loss of myogenic regulation.

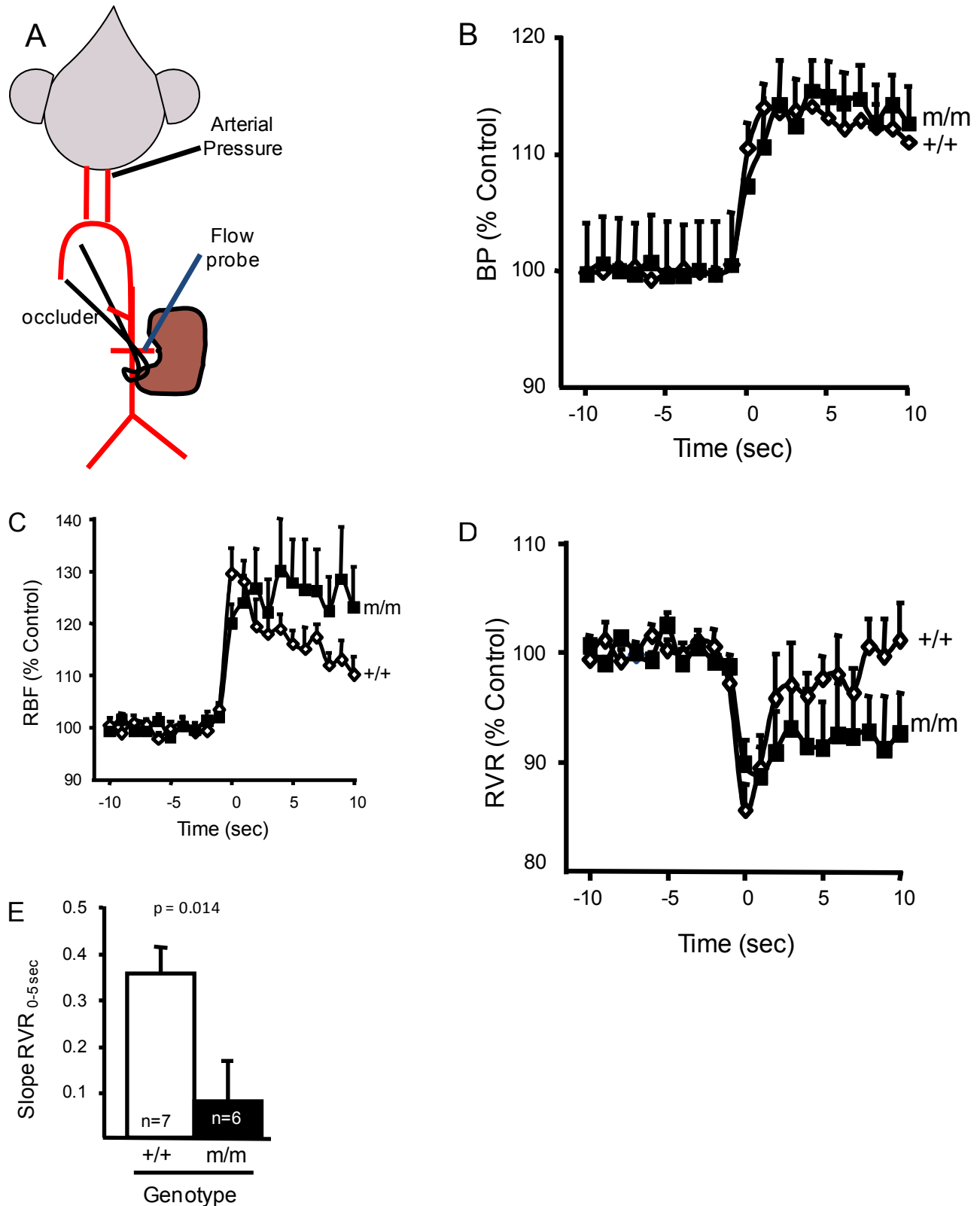
We quantified the speed of the myogenic mechanism by the determining the rate of change in whole kidney RVR during the first 5 seconds following a step increase in renal perfusion pressure (Slope  $RVR_{0-5sec}$ , Figure 4E). In both studies, we found a significant delay in the correction of RBF following a step increase in perfusion pressure in  $\beta$ ENaC m/m mice<sup>30,52</sup>. To minimize any confounding influence of TGF, we examined renal hemodynamic responses to a step increase in pressure following acute volume expansion to reduce the contribution of TGF to vascular resistance<sup>53-55</sup>. We found myogenic speed was suppressed nearly 80% (Figure 4E), findings that parallel our in-vitro findings (loss of myogenic constriction in afferent arterioles) in  $\beta$ ENaC m/m mice.

### Signs of renal inflammation and mild injury in the $\beta$ ENaC m/m mouse

Inappropriate regulation of RBF is linked to renal injury in hypertension and diabetes<sup>56,57</sup>. Normally, swings in systemic pressure are prevented from reaching delicate renal microvasculature because of autoregulatory mechanisms; when systemic pressure rises, autoregulatory mechanisms are activated leading to vasoconstriction, thus preventing transmission of higher systemic pressures to delicate microvessels<sup>56-59</sup>. Since the myogenic response responds rapidly to changes in perfusion pressure, it is considered an important mechanism to prevent transmission of pressure swings to the microvasculature. We considered the possibility that  $\beta$ ENaC m/m mice might have signs of renal injury and possibly, elevated blood pressure due to their reduced myogenic capacity. To address this possibility, we examined kidneys for indicators of renal injury and found signs of inflammation and mild renal injury characterized by increased levels of renal inflammatory cytokines ( $TNF\alpha$ ,  $IL1\beta$ ,  $IL6$ ), inflammatory cells (macrophages, lymphocytes), growth factors linked to pressure-dependent injury ( $TGF\beta$ ), and mild expansion of extracellular matrix<sup>51</sup>. We also found mean blood pressure is ~15 mm Hg higher than wildtype littermates (Figure 5)<sup>51</sup>. These findings demonstrate a link between altered  $\beta$ ENaC mediated myogenic function, renal injury and hypertension.

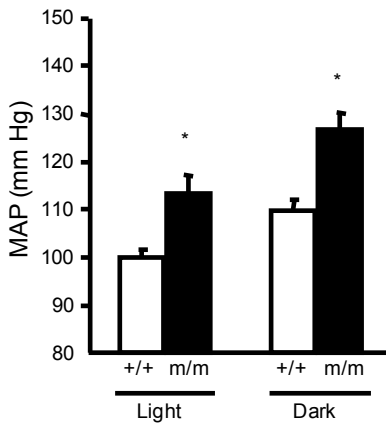
### Why isn't the $\beta$ ENaC m/m mouse hypotensive?

Because the  $\beta$ ENaC m/m mouse was generated using homologous recombination, reduced levels of  $\beta$ ENaC would be expected in all tissues, including renal tubular cells. Loss of tubular ENaC related salt and water transport would be expected to lead to reduced or normal blood pressure with compensatory up-regulation of sodium

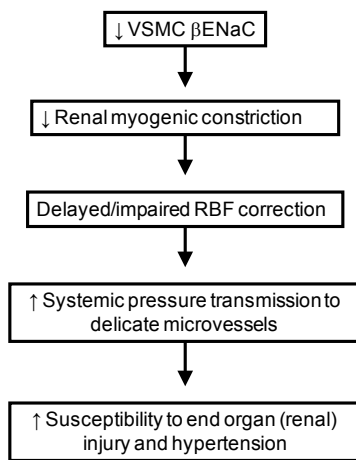


**Figure 4**  $\beta$ ENaC mediates myogenic regulation of whole kidney blood flow and renal vascular resistance. A: Schematic of animal preparation. Mice have an arterial carotid catheter for blood pressure measurement and a renal flow probe for whole kidney blood flow measurement. An occluder tie placed around the lower abdominal aorta is used to generate the step increase in pressure. B: Time course of the regulatory response of mean arterial pressure (MAP; B), renal blood flow (RBF; C), and renal vascular resistance (RVR; D), in wild type (+/+, filled symbols, n=6) and mutant (m/m, open symbols, n=7) 10 seconds before and 30 sec after the step increase in pressure. Data are presented as normalized changes in B-D to minimize variance. Following a similar increase in MAP, the transient increase in RBF and decrease in RVR are similar between +/+ and m/m mice. Immediately following the transient drop, RVR begins to increase in the +/+, but remains low in the m/m. E: The rate of increase in RVR during the first 5 sec following the drop in RVR (Slope RVR<sub>0-5sec</sub>) is significantly greater in +/+ vs. m/m mice (p=0.014). By 20-30 seconds following the step increase in MAP, RBF in the +/+ is corrected while RBF remains elevated in m/m animals. By 20-30 seconds following the step increase in MAP, the change in RVR from baseline is greater in the +/+ vs. m/m. Data are mean  $\pm$  SEM. \* Significantly different from  $\beta$ ENaC +/+ group at the p value indicated.

retaining hormones. Thus, at first consideration, our finding that blood pressure is elevated in the  $\beta$ ENaC m/m may seem counter-intuitive. However, when the elevated blood pressure data is taken in context with loss of myogenic autoregulation and presence of renal inflammation in the  $\beta$ ENaC m/m, renal injury dependent increase in blood pressure seems plausible (Figure 6).



**Figure 5** Blood pressure in normal salt (0.4% Na+) fed animals. Mean arterial pressure (MAP) for 12-hour light (L) -dark (D) cycles for over 5 days are shown in wildtype (+/+, n=8) and homozygous  $\beta$ ENaC mutant mice (m/m, n=7). Data are mean  $\pm$  SEM. \* Significantly different from wildtype (+/+) control animals,  $p < 0.05$ .



**Figure 6** Schematic of working hypothesis: the role of  $\beta$ ENaC as a mechanosensor and its role in cardiovascular pathophysiology. We hypothesize that  $\beta$ ENaC is a critical component of mechanosensors in renal VSMCs. Loss in  $\beta$ ENaC function in renal VSMCs leads to a loss in renal myogenic constriction and a delayed or impaired correction of renal blood flow following upward swings in blood pressure. Decreased myogenic capacity increases the opportunities and magnitude of systemic pressure transmission to the renal microvessels, which in turn, increases susceptibility to end-organ injury and hypertension.

**Future directions**

There is much that needs to be done to elucidate the role of degenerins in vascular function. Pressure-dependent renal injury is a leading cause of end-stage renal disease with substantial financial costs (Medicare Care costs exceeded \$26 billion in 2010)<sup>[60,61]</sup>. Understanding the degenerin mediated protection from injury will lead to development of approaches to prevent renal injury. Although the loss of renal pressure-dependent vascular function in  $\beta$ ENaC m/m mice does not lead to severe renal injury and hypertension, it is not clear if a “second hit”, such as high Na<sup>+</sup> diet, elevated angiotensin II, or added psychological stress would increase the severity of injury. Furthermore, information learned from the renal circulation may also apply to other myogenically active circulations, such as the cardiac and cerebral beds, which are often the targets of hypertension-induced injury.

Another important future direction is the determination of the identity of other proteins that form the heteromultimeric mechanosensor in VSMCs. Based on the *C. elegans* model, the mechanosensor is a large heteromeric complex in which the pore is tethered to the cytoskeleton and extracellular matrix. The identity of the other pore forming subunit(s) and the cytoskeleton and extracellular matrix proteins responsible for tethering the pore have not been identified in mammals. Understanding their identity and regulation by hormonal, inflammatory, and autocrine factors may provide additional insight into the prevention and treatment of renal injury. It is very likely that VSMC ENaC protein expression and/or function may be altered in hypertension because many of the “usual suspects” implicated in hypertension (i.e. endothelin, aldosterone, angiotensin II, inflammatory cytokines, reactive oxygen species, and nitric oxide, dietary salt) regulate epithelial ENaC expression<sup>[28,62-72]</sup>.

**Summary**

Our laboratory has considered degenerin proteins, specifically  $\beta$ ENaC, as a component of a VSMC mechanosensor because of the strong evolutionary link to mechanotransduction in *C. elegans*. This hypothesis is supported by multiple lines of in-vitro and in-vivo evidence including (1) appropriate protein localization; (2) disruption of myogenic constriction in isolated vessels using pharmacological, transient gene silencing and genetically modified animals; (3) disruption of stretch gated whole cells currents in renal VSMCs and (4) disruption of myogenically mediated whole organ blood flow *in vivo* (Table 1). Furthermore, the importance of degenerin mediated vascular function on cardiovascular health is becoming clear; loss of vascular degenerin function may inhibit the protective renal myogenic mechanism, thereby increasing susceptibility to pressure related renal injury and hypertension.

**ACKNOWLEDGEMENTS**

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Table 1 Publications demonstrating degenerin proteins, including $\beta$ ENaC, form mechanosensors that transduce renal myogenic regulation.	
Line of evidence	Citation
$\beta$ ENaC and $\gamma$ ENaC localized in rVSMCs at/near VSMC surface.	AJP, 289(4): F891-901, 2005; AJP, 291(6): F1184-91, 2006
$\beta$ ENaC localized in afferent arteriolar VSMCs	AJP, 302(11): F1486-93, 2012
Amiloride and benzamil inhibit myogenic constriction in renal interlobar arteries	AJP, 289(4): F891-901, 2005
Transient gene-silencing of $\beta$ ENaC or $\gamma$ ENaC blocks myogenic constriction in renal interlobar arteries	AJP, 291(6): F1184-91, 2006
Myogenic constriction in renal interlobar arteries and afferent arterioles is inhibited in mice with reduced levels of $\beta$ ENaC ( $\beta$ ENaC m/m).	AJP, 302(11): F1486-93, 2012
Myogenic regulation of whole kidney blood flow inhibited in $\beta$ ENaC m/m mice	AJP, 298(2): F285-92, 2010; AJP, 302(11): F1486-93, 2012
VSMC stretch gated currents abolished in $\beta$ ENaC m/m mice.	AJP, 304(12): F1428-37, 2011

## CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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