Effect of Epithelial Sodium Channel Blockade on the Myogenic Response of Rat Juxtamedullary Afferent Arterioles

Zhengrong Guan, Jennifer S. Pollock, Anthony K. Cook, Janet L. Hobbs, Edward W. Inscho

Abstract—The mechanotransduction mechanism underlying the myogenic response is poorly understood, but evidence implicates participation of epithelial sodium channel (ENaC)-like proteins. Therefore, the role of ENaC on the afferent arteriolar myogenic response was investigated in vitro using the blood-perfused juxtamedullary nephron technique. Papillectomy was used to isolate myogenic influences by eliminating tubuloglomerular feedback signals. Autoregulatory responses were assessed by manipulating perfusion pressure in 30-mm Hg steps. Under control conditions, arteriolar diameter increased by 15% from 13.0 ± 1.3 to 14.7 ± 1.2 μm (P < 0.05) after reducing perfusion pressure from 100 to 70 mm Hg. Diameter decreased to 11.3 ± 1.1 and 10.6 ± 1.0 μm after increasing pressure to 130 and 160 mm Hg (88 ± 1 and 81 ± 2% of control diameter, P < 0.05), respectively. Pressure-mediated autoregulatory responses were significantly inhibited by superfusion of 10 μmol/L amiloride (102 ± 2, 97 ± 4, and 94 ± 3% of control diameter), or 10 μmol/L benzamil (106 ± 5, 100 ± 3, and 103 ± 3% of control diameter), and when perfusing with blood containing 5 μmol/L amiloride (106 ± 2, 97 ± 4, and 97 ± 4% of control diameter). Vasoconstrictor responses to 55 mmol/L KCl were preserved as diameters decreased by 67 ± 4, 55 ± 8, and 60 ± 4% in afferent arterioles superfused with amiloride or benzamil, and perfused with amiloride, respectively. These responses were similar to responses obtained from control afferent arterioles (64 ± 6%, P > 0.05). Immunofluorescence revealed expression of the α, β, and γ subunits of ENaC in freshly isolated preglomerular microvascular smooth muscle cells. These results demonstrate that selective ENaC inhibitors attenuate afferent arteriolar myogenic responses and suggest that ENaC may function as mechanosensitive ion channels initiating pressure-dependent myogenic responses in rat juxtamedullary afferent arterioles. (Hypertension. 2009;54:1062-1069.)

Key Words: myogenic response ■ epithelial sodium channel ■ amiloride ■ benzamil ■ juxtamedullary nephrons ■ autoregulatory response

Renal autoregulatory behavior maintains a relatively constant renal blood flow despite changes in renal arterial pressure, a vital renal function for preventing hypertension-induced renal injury. In kidneys, autoregulation is accomplished through the combined influences of the myogenic and tubuloglomerular feedback (TGF) mechanisms. The TGF response is a process by which the macula densa senses changes in distal tubule NaCl delivery and, in turn, modulates release of paracrine signals that alter afferent arteriolar tone. The myogenic response is an intrinsic property of preglomerular arterioles and afferent arterioles. Myogenic responses are characterized by vasoconstriction after an increase in transmural pressure or vasorelaxation after a decrease in transmural pressure. The myogenic response is inherent to vascular smooth muscle and independent of endothelium. Myogenic behavior is also observed in vascular elements of many other organs such as coronary, cerebral, and mesenteric arteries and cremaster arterioles and reflects an important mechanism for establishing ambient vascular tone and maintaining a relatively constant regional blood flow and capillary hydrostatic pressure. The cellular mechanisms by which an increase in arterial pressure triggers the myogenic response have been investigated intensively. It is well established that increasing local transmural pressure leads to membrane depolarization of vascular smooth muscle cells, activation of voltage-gated L-type calcium channels, and vasconstriction.

However, the mechanisms by which the mechanical stimuli lead to activation of myogenic signaling cascades remain poorly understood. Currently several hypotheses have been proposed for linking mechanical stimuli to the cellular events producing a myogenic response. These include involvement of stretch-activated cation channels, perturbation of the actin cytoskeleton, specialized membrane domains, or extracellular matrix proteins such as integrins. The role of stretch-activated cation channels in the myogenic response was indirectly demonstrated in isolated perfused hydronephrotic rat kidneys lacking TGF responses. In this model, pressure-mediated...
vasoconstriction of afferent arterioles was abolished during nonselective cation channel blockade by gadolinium and attenuated by reducing the extracellular sodium concentration. However, other studies showed that the myogenic response of mesenteric arteries, cerebral arteries, and afferent arterioles from hydronephrotic kidneys was unchanged by a low-sodium medium. Epithelial sodium channels (ENaC) consist of 3 subunits (α, β, and γ) and are heavily expressed in the apical membrane of epithelial cells in several organs, including the distal tubules in kidneys, where they facilitate Na\(^+\) reabsorption. Since the discovery that ENaC was a mechanosensor in Caenorhabditis elegans, similar mechanosensor roles have been postulated for ENaC in other species and cell types. Recent studies by Jernigan and Drummond suggest that ENaC may be part of a mechanosensitive ion channel complex involved in myogenic responses in isolated perfused rat cerebral and mouse intrarenal arteries. Single smooth muscle cells collected from these arteries were found to express \(\beta\) and \(\gamma\)-ENaC subunits. Furthermore, the selective ENaC blocker amiloride, or its more potent analogue, benzamil, attenuated or abolished myogenic vasoconstriction in isolated cerebral and intrarenal arteries.

Although all segments of the preglomerular vasculature contribute to myogenic control of whole kidney autoregulation, the afferent arteriole provides the principle resistance adjustment determining autoregulatory efficiency. A recent study using afferent arterioles from hydronephrotic rat kidneys showed that the myogenic response was unaltered by perfusion with amiloride or benzamil. To test the hypothesis that ENaC is involved in the myogenic response, we performed this study using the in vitro blood-perfused rat afferent arteriole. Glomeruli and microvessels were exposed by careful removal of connective tissue covering the inner cortical surface. After completion of the dissection, the chamber containing the prepared kidney was attached to the stage of a Nikon Eclipse E600FN microscope (Nikon) equipped with a Nikon water-immersion objective (40×). The perfusate was switched to the reconstituted blood from a sealed pressurized reservoir continuously gassed with 95% O\(_2\)/5% CO\(_2\). The surface of the kidney was super-fused with 37°C Tyrode buffer containing 1% BSA. The image of the perfused kidney was displayed on a video monitor via a high-resolution NC-70 Newvicon video camera (DAGE-MTI) and recorded on DVD for later analysis.

Perfusion pressure was monitored directly within the perfusion cannula via a polyethylene line connected to a pressure transducer (Model TNN005, Kent Scientific Corporation). Experiments were performed in vitro, using the blood-perfused juxtamedullary nephron technique, as described previously. For immunostaining of ENaC subunit protein expression, single segments of afferent arteriole were selected from the first 1/3 segment of afferent arteriole to the cortical surface. The ends of the large arteries were tied with 10-0 nylon suture to restore renal perfusion pressure. Glomeruli and microvessels were exposed by careful removal of connective tissue covering the inner cortical surface.

The ends of the large arteries were tied with 10-0 nylon suture to

**Materials and Methods**

**Animals**

Male Sprague-Dawley rats (total 86 rats, Charles River Breeding Laboratories, Raleigh, NC) were used in all experiments. The rats were fed a standard chow (Harlan Teklad) and had free access to water before all experiments. All procedures were approved by the Committee on Animal Use for Research and Education at the Medical College of Georgia.

**Kidney Preparation**

Experiments were performed in vitro, using the blood-perfused juxtamedullary nephron technique, as described previously. Briefly, 2 rats were anesthetized with an intraperitoneal injection (50 mg/kg) of pentobarbital sodium for each experiment. The right kidney from the kidney donor was cannulated via the superior mesenteric artery during continuous perfusion with Tyrode buffer containing 5.2% bovine serum albumin (BSA; Calbiochem) and L-amino acids. Blood was collected into a heparinized syringe (500 IU) via a carotid artery cannula and processed with blood collected from a blood donor rat. The kidney was harvested and sectioned along the longitudinal axis on the dorsal two-thirds of the kidney. The ends of the large arteries were tied with 10-0 nylon suture to

**Experimental Protocol**

Each protocol began with a 5-minute control period at a perfusion pressure of 100 mm Hg after an initial equilibration period (at least 10 minutes). After the control period, which established the baseline arteriolar diameter, a papillectomy was performed to eliminate TGF influences on autoregulatory responses. The arte-riole was refocused at the same site. After another 10-minute stabilization period, the superfusate was continued or switched to a similar solution containing either amiloride (5 or 10 µmol/L) or its analogue, benzamil (1 or 10 µmol/L), for a 30-minute incubation, and then pressure-mediated myogenic responses of afferent arterioles were assessed by lowering perfusion pressure from 100 mm Hg (control diameter) to 70 mm Hg, followed by stepwise increases in perfusion pressure from 70 to 160 mm Hg at 30-mm Hg intervals (5 minutes each). Perfusion pressure was returned to 100 mm Hg at the end of each protocol. At end of the experiment, some arterioles were exposed to a modified Tyrode buffer containing 55 mmol/L KCl (substituted for NaCl) and 1% BSA for 5 minutes to determine the effect of depolarization on afferent arteriolar diameter in the presence or absence of ENaC inhibitors.

Additionally, to determine the impact of luminal delivery of ENaC blockers on the myogenic response of juxtamedullary afferent arterioles, amiloride was added to the perfusate blood in 2 separate groups of rats. Similar to the above protocol, 10 minutes after papillectomy a fresh amiloride solution (100 or 500 µmol/L) was directly added into the perfusate blood to achieve an amiloride concentration of 1 or 5 µmol/L, respectively, while the kidney was superfused with 1% BSA solution. After 30 minutes incubation, pressure-mediated autoregulatory responses were assessed as described above.

**Immunofluorescence Staining in Single Preglomerular Microvascular Smooth Muscle Cells**

For immunostaining of ENaC subunit protein expression, single preglomerular microvascular smooth muscle cells were freshly isolated as described previously. Briefly, rats were anesthetized and the kidneys were perfused with 5.2% BSA followed by 0.5% Evan’s blue and removed in ice-cold physiological buffer solution (PBS). Preglomerular microvessels, consisting of interlobular arterioles and afferent arterioles, were carefully separated from the cortex under a stereomicroscope and transferred to an enzymatic digestion solution containing 0.05% dithiothreitol, 0.14% papain, and 0.4% BSA dissolved in PBS at 36.5°C. After a 20-minute incubation
period, the mixture was centrifuged (1000 rpm for 5 minutes) to collect the dispersed cells. The cells were fixed in 4% paraformaldehyde for 10 minutes and air-dried on positive charged glass slides at 37°C. The slides were rinsed in PBS and blocked in 5% normal goat serum for 1 hour. The cells were incubated overnight (4°C) with primary antibody for rat α, β, or γ-ENaC. To establish expression of all ENaC subunits in preglomerular microvascular smooth muscle cells and to control for variability in antibody specificity, antibodies for each subunit were obtained from 3 different sources including Chemicon (1:100; α-, β-, or γ-ENaC antibodies), Affinity BioReagents Inc, (1:100; β- and γ-ENaC antibodies), and a kind gift from Dr Heather Drummond (Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson; 1:4000; β and 1:1000; γ-ENaC antibodies).14 The cells were rinsed in PBS and incubated with secondary goat anti-rabbit IgG with an Alexa Fluor 594 tag (1:100, Invitrogen) for 1 hour and with the nuclear stain, DAPI (300 nmol/L, Invitrogen), for 5 minutes at room temperature. After a final rinse with PBS, samples were covered with Gel-Mount mounting medium and cover-slips. Negative controls were prepared by omitting the primary antibodies of each ENaC subunit with the rest of process identical. Images of immunostained cells were captured using an Olympus BX40F-3 fluorescence microscope equipped with an Olympus DP70 digital camera using DP Controller software (Olympus Optical Co Ltd).

Statistical Analysis
All values are expressed as a mean±SEM. Afferent arteriolar responses were normalized and analyzed as a percentage of the control diameter or as symmetrized percent changes.42 Because the trend of the 2 normalization techniques was not distinguishable, we only present the percentage of control diameter data in the Results section. Within-group analysis was conducted using one-way analysis of variance for repeated measures combined with the Newman-Keuls multiple range test. Differences between groups, within each series, were determined using a Student-Newman-Keuls multiple range test. Probability values <0.05 were considered to indicate significant differences.

Results
Effect of Papillectomy, Amiloride, or Benzamil on Baseline Diameter of Afferent Arterioles
In this study, arteriolar diameter measurements were made an average of 253±14 μm from the glomeruli (pooled data, n=47). Baseline afferent arteriolar diameters during the first 5 minutes (control period), 10 minutes after papillectomy, and after 30-minute incubation with ENaC inhibitors are provided in the Table. Baseline diameters of afferent arterioles during the control period were similar across all groups. Steady-state diameter of afferent arterioles remained relatively stable 10 minutes after papillectomy as shown in the Table. During the 30-minute incubation with ENaC inhibitors, the diameter also remained relatively stable except in the 10 μmol/L benzamil group, where diameter decreased significantly. To further analyze the effect of papillectomy on the responses of afferent arterioles, we pooled all data. Although the region of the afferent arterioles chosen for study was at a distance of 58% to 62% of arteriolar length away from the glomeruli (Table), there was still a transient vasodilation immediately after papillectomy. The arteriolar diameter increased 3.8±0.4% from an average of 13.7±0.02 to 14.2±0.05 μm in the first 5 minutes after papillectomy (n=42) but returned to the initial diameter (13.7±0.02 μm) after 10 minutes after papillectomy, suggesting that myogenic control may compensate for loss of TGF influences after papillectomy.

Inhibition of the Myogenic Response by Superfusion of Amiloride
The effect of superfusion with a potent ENaC inhibitor, amiloride, on pressure-mediated afferent arteriolar diameter responses is depicted in Figure 1. In control vessels, the afferent arteriole exhibited pressure-dependent vascular responses as perfusion pressure was varied between 70 and 160 mm Hg. Afferent arteriolar diameter averaged 13.0±1.3 μm at perfusion pressure of 100 mm Hg. Reducing perfusion pressure to 70 mm Hg increased arteriolar diameter to 14.7±1.2 μm (Figure 1A, P<0.05), which is 115±4% of the control diameter at 100 mm Hg (Figure 1B). The diameter decreased to 88±1 and 81±2% of the control diameter when the perfusion pressure was increased to 130 and 160 mm Hg, respectively (Figure 1B). The pressure-diameter relationship indicates an intact myogenic response. The profile of the myogenic response was unchanged by superfusion of 5 μmol/L amiloride (Figure 1). In contrast, kidneys superfused with 10 μmol/L amiloride exhibited an attenuated autoregulatory response. Afferent arteriolar diameter did not
change significantly when perfusion pressure was reduced to 70 mm Hg or increased up to 160 mm Hg (Figure 1). The relationship between pressure and diameter was relatively flat, indicating reduced myogenic activity.

Inhibition of the Myogenic Response by Superfusion of Benzamil

We also tested a more specific ENaC blocker, benzamil, on the pressure-mediated afferent arteriolar myogenic response. As shown in Figure 2, superfusion with a low concentration of benzamil (1 nM/L) did not alter afferent arteriolar responses to the changes in perfusion pressure. Afferent arteriolar diameter averaged 111 measurement, 96 ± 1, 86 ± 2 and 82 ± 2% of the control diameter at perfusion pressure of 70, 100, 130 and 160 mm Hg, respectively (Figure 2B). This pressure-diameter profile was similar to control kidneys. However, afferent arteriolar autoregulatory responses were significantly blunted by 10 nM/L benzamil (Figure 2). The relatively flat pressure-diameter relationship in the presence of benzamil is consistent with the impaired autoregulatory response observed in the presence of amiloride.

Inhibition of the Myogenic Response by Blood Perfusion With Amiloride

To determine whether intravascular delivery of ENaC inhibitors may inhibit the myogenic response, we added amiloride to the blood perfusing the vascular lumen rather than using adventitial exposure in the superfusate. As shown in Figure 3, perfusion with 1 nM/L amiloride had no detectable effect on pressure-mediated responses compared to control kidneys. However, 5 nM/L amiloride in the perfusate markedly attenuated the pressure-mediated afferent arteriolar vasoconstrictor response (Figure 3).

Intact Membrane Depolarization During ENaC Blockade

A central component of the myogenic response is activation of L-type calcium channels by depolarization. Consequently,
we verified the voltage-dependent vasoconstriction during ENaC inhibition. KCl (55 mmol/L) was applied to some kidneys at the end of experiments. As seen in Figure 4, although the myogenic response of afferent arterioles was inhibited by application of amiloride or benzamil, the vasoconstriction induced by exposure to KCl was intact during ENaC blockade. The reduction in arteriolar diameter in response to KCl was similar in each group in the absence and presence of ENaC inhibitors.

Expression of ENaC in Preglomerular Microvascular Smooth Muscle Cells

To assess ENaC expression, we isolated single smooth muscle cells from preglomerular microvessels. As shown in Figure 5A and 5B, all 3 subunits of ENaC (α, β, and γ) were detected in the smooth muscle cells freshly isolated from preglomerular microvessels. No staining was observed in negative control samples when the primary antibodies were omitted (Figure 5A and 5B). Expression of α, β, and γ-ENaC subunits were confirmed using specific antibodies from 3 different sources (the positive staining for β and γ-ENaC antibodies obtained from Affinity BioReagents Inc was not shown). Each cell was confirmed by the positive nuclear staining with DAPI (negative control DAPI staining not shown).

Discussion

This study provides novel insights into the involvement of ENaC on the mechanosensitive signal transduction pathway underlying the myogenic response in rat juxtamedullary afferent arterioles. Using the blood-perfused juxtamedullary nephron preparation combined with papillectomy, we observed that the pressure-mediated myogenic response of afferent arterioles was blunted by blockade of ENaC with amiloride, or its analogue benzamil, while vasoconstrictor responses to membrane depolarization with KCl remained intact. These observations suggest that inhibition of ENaC blunts the myogenic response of afferent arterioles at a site upstream of vascular smooth muscle membrane depolarization. Additionally, immunofluorescence studies provide direct evidence that all 3 ENaC subunits (α, β, and γ) are expressed in preglomerular microvascular smooth muscle cells. These studies support the hypothesis that ENaC may constitute part of a mechanosensitive ion channel complex triggering myogenic responses.

The kidney uses the myogenic and TGF mechanisms to accomplish whole kidney autoregulation of renal blood flow. Therefore, we performed a papillectomy to eliminate TGF influences and measurements were made near the midpoint of arteriolar length (at a distance of 58% to 64% away from the glomeruli) to isolate the myogenic responses from TGF influences. Under these conditions, pressure-mediated autoregulatory responses reflect myogenic control mechanisms. In the absence of ENaC inhibitors, increasing perfusion pressure caused appropriate decreases in afferent arteriolar diameter and decreasing perfusion pressure led to
predictable increases in afferent arteriolar diameter, indicating that pressure-mediated autoregulatory influences exist in this preparation after papillectomy. Amiloride (10 μmol/L) superfused on the inner cortical surface markedly attenuated pressure-mediated afferent arteriolar vasoconstriction. Benzamil is a benzyl-substituted amiloride analogue that is more potent than amiloride and that imposes a longer acting inhibition of ENaC than amiloride.22 Benzamil (10 μmol/L) also blunted pressure-mediated afferent arteriolar responses. The present findings suggest a role for ENaC in the myogenic signaling cascade that is consistent with the observations of others in different vascular beds.14,15,33,34

A central element of the myogenic signaling cascade is vascular smooth muscle membrane depolarization and activation of voltage-dependent Ca2+ channels.8 An early study in mouse neuroblastoma cells suggested that amiloride inhibited T-type Ca2+ channels dose-dependently with an IC50 of 30 μmol/L.43 Additionally, both amiloride (IC50 at 1700 μmol/L) and benzamil (IC50 at 56 μmol/L) inhibited L-type Ca2+ channels in GH3 anterior pituitary cells.44 Therefore, in the current study, KCl was applied to kidneys to verify that voltage-dependent contractile mechanisms were intact during ENaC inhibition. Data indicate that KCl-mediated vasoconstriction of afferent arterioles was unaffected by ENaC inhibition. This is strong evidence that the depolarizing function of afferent arteriolar smooth muscle is intact and that the inhibitory effect of amiloride or benzamil on afferent arteriolar myogenic responses acts upstream of membrane depolarization in the myogenic signaling cascade.

Early studies showed that amiloride and benzamil have a high affinity for ENaC with an IC50 in the submicromolar range.22,45 In addition to specific inhibition of ENaC, amiloride is also reported to inhibit other signaling mechanisms including the Na+/H+ exchanger, the Na+/K+/ATPase and Na+/Ca2+ exchange system.45–47 However, the nonspecific inhibitory effects of amiloride on these mechanisms require about 20-fold more amiloride than is required for ENaC blockade.58–51 For example, the IC50 of amiloride for inhibiting the Na+/Ca2+ exchanger is 1000 μmol/L whereas the IC50 of amiloride for inhibiting the Na+/H+ exchanger is 84 μmol/L.52 and is increased up to millimolar concentrations when Na+ concentrations reach 100 mmol/L.53 In addition, amiloride reportedly blocks transient receptor potential (TRP) channel currents.54–56 Several subfamilies of TRP are localized to vascular smooth muscle or endothelial cells, and they are implicated in vascular mechanotransduction in cerebral arteries and in modulation of afferent arteriolar reactivity.57–61 However, the concentration of amiloride required to block TRP channels is usually 10- to 100-fold greater (ranging from 100 to 1000 μmol/L) than the concentration applied in this study.34–36

Compared to isolated vessel studies,14,15 we found that we needed higher concentrations of amiloride and benzamil in the superfusion solution to block the afferent arteriolar myogenic response than were effective in isolated pressurized mouse intrarenal arteries. This discrepancy may be attributable to differences in participation of ENaC in myogenic responses in different segments of preglomerular vessels, or to species differences (rat versus mouse), or the route of drug delivery. If amiloride is applied only in the bath, a higher concentration is needed to block the myogenic response33,34 than when amiloride is applied on both sides of the vessel14,15 or to the adventitial side of the vessel.34 Further, Oyabe et al14 observed that amiloride acted slowly, and that the time required for the maximum inhibition of pressure-mediated cerebral arterial vasoconstriction was concentration-dependent. Low concentrations of amiloride needed longer incubation periods to inhibit myogenic responses in cerebral arteries. This could explain why lower concentrations of amiloride or benzamil had little effect on the myogenic response of afferent arterioles in the current study. Collectively, it is unlikely that the inhibition of pressure-mediated vasoconstriction of afferent arterioles by amiloride or benzamil reflects nonspecific effects.

Alternatively, it may reflect differences in the delivery systems used. In the current study, amiloride was given either luminally or to the adventitial side of the vessel. When amiloride was administered in the perfusate blood, 5 μmol/L amiloride was sufficient to diminish the myogenic response, similar to the inhibitory effect seen in mouse intrarenal arteries.15 This concentration is 50% lower than the concentration needed for adventitial treatment. Accordingly, amiloride is more effective when delivered via the vascular lumen. Possibly application of amiloride in the perfusate blood allows it to enter the capillary circulation and distribute more rapidly through the interstitial fluid space, thus enhancing vascular exposure and facilitating a lower concentration of amiloride to inhibit myogenic responses. Another possibility is that luminal amiloride inhibits endothelial ENaC and
enhances endothelial modulation of myogenic vasoconstrictor activities.\(^{62}\) It is important to note that myogenic responses are endothelium-independent, but vasoactive agents released by the endothelium can modulate vasoconstrictor responses.

In a recent study, the role of ENaC in the myogenic response of afferent arterioles was examined in hydrenephrotic rat kidneys.\(^{20}\) The myogenic response was unaltered by administering amiloride or benzamil or by reducing extracellular Na\(^+\) concentration from 140 to 100 mmol/L. Indeed, the myogenic response was enhanced by 1 \(\mu\)mol/L benzamil. Also, the vasorelaxation that occurred on restoring extracellular Na\(^+\) concentration to normal was attenuated by benzamil (10 \(\mu\)mol/L), suggesting that benzamil may partially inhibit Na\(^+\)/Ca\(^{2+}\) exchangers. In contrast, a more recent study showed that the myogenic response of rat cremaster first order arterioles was impaired by inhibitors of the Na\(^+\)/Ca\(^{2+}\) exchanger or by decreasing the extracellular Na\(^+\) concentration,\(^{9}\) although reducing extracellular Na\(^+\) concentration led to arteriolar constriction similar to that reported for arterioles from hydrenephrotic kidneys.\(^{20}\) The latter study suggests a contribution of the Na\(^+\)/Ca\(^{2+}\) exchanger to myogenic vasoconstriction in isolated rat cremaster arterioles.\(^{9}\)

Because ENaC are heavily expressed in distal tubular cells, one potential problem in detecting protein and mRNA expression of ENaC in the renal microvasculature is possible contamination of vascular tissue samples with distal tubular cells. However, the immunolabeling studies of ENaC using freshly isolated single smooth muscle cells from renal microvessels provides a reliable means to clearly localize ENaC expression specifically to the vascular smooth muscle cells. Clearly, both \(\beta\) and \(\gamma\)-ENaC are expressed in the smooth muscle cells collected from preglomerular microvasculature, whereas neither \(\beta\) nor \(\gamma\)-ENaC staining are present in the negative controls. This result is consistent with different sources of antibodies and also consistent with the findings of Jernigan and Drummond in mouse intrarenal arteries.\(^{15}\) However, we also detected expression of \(\alpha\)-ENaC in our cells. This differs from the observation of Jernigan and Drummond,\(^{15}\) where \(\alpha\)-ENaC expression was not detected in single cells isolated from mouse intrarenal arteries. The discrepancy may reflect differences in single cells from different preglomerular vascular segments or different species. Taken together, immunostaining of ENaC in preglomerular microvascular smooth muscle cells combined with different arteriolar sensitivity to ENaC blockers suggests that ENaC may act as mechanosensors in mediating myogenic autoregulatory responses in afferent arterioles.

In conclusion, this study presents immunofluorescent images establishing that preglomerular microvascular smooth muscle cells express ENaC subunits. Functional studies provide compelling evidence that pressure-mediated myogenic responses of afferent arterioles are attenuated by application of amiloride or benzamil, while KCl-induced vasoconstriction is preserved. These data suggest that ENaC blockers act upstream of membrane depolarization and support the hypothesis that ENaC may function as a mechanosensitive ion channel complex participating in myogenic responses of rat afferent arterioles.

Perspectives
The myogenic response is inherent in the renal, coronary, cerebral, and mesenteric vasculature. Impairment of myogenic control is a risk factor for renal injury, coronary artery disease, and stroke. A role for ENaC in the preglomerular myogenic response is a new concept. Afferent arterioles are the major vessels responsible for renal autoregulation. Therefore, it is important to understand the mechanisms of renal autoregulatory control. Better understanding of the mechanotransduction pathway underlying the myogenic response will provide a clearer understanding of vascular physiology and pathology and will aid in reducing end-organ damage during arterial pressure fluctuations.

Acknowledgments
We thank Dr Stephen W. Looney, Department of Biostatistics, Medical College of Georgia, for his advice on statistical analysis.

Sources of Funding
This study was supported by grants from the National Institutes of Health (DK 44628 and HL074167). Z.G. is the recipient of a postdoctoral fellowship from the Greater Southeast Affiliate of the American Heart Association.

Disclosures
None.

References


Effect of Epithelial Sodium Channel Blockade on the Myogenic Response of Rat Juxtamedullary Afferent Arterioles
Zhengrong Guan, Jennifer S. Pollock, Anthony K. Cook, Janet L. Hobbs and Edward W. Inscho

Hypertension. 2009;54:1062-1069; originally published online August 31, 2009;
doi: 10.1161/HYPERTENSIONAHA.109.137992
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/54/5/1062

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/