High Blood Pressure Upregulates Arterial L-Type Ca\textsuperscript{2+} Channels

Is Membrane Depolarization the Signal?

Aleksandra Pesic, Jane A. Madden, Miodrag Pesic, Nancy J. Rusch

Abstract—Long-lasting Ca\textsuperscript{2+} (Ca\textsubscript{L}) channels of the Ca\textsubscript{1.2} gene family contribute to the pathogenesis of abnormal arterial tone in hypertension. The physiological stimulus that enhances Ca\textsubscript{L} channel current in the vascular smooth muscle cells (VSMCs) remains unknown. The present study investigated if high blood pressure triggers an upregulation of vascular Ca\textsubscript{L} channel protein. Rat aortae were banded between the origins of the left renal (LR) and right renal (RR) arteries to selectively elevate blood pressure in the proximal RR arteries. After 2 days, the immunoreactivity on Western blots corresponding to the pore-forming \(\alpha_{1C}\) subunit of the Ca\textsubscript{L} channel was increased 3.25-fold in RR compared with LR arteries. This finding persisted at 28 days and was associated with abnormal Ca\textsuperscript{2+}-dependent tone and higher Ca\textsubscript{L} currents in the VSMCs exposed to high pressure. Based on microelectrode studies indicating that RR arteries were depolarized compared with LR arteries, further studies examined if membrane depolarization, an inherent response of VSMCs to high blood pressure, increased \(\alpha_{1C}\) expression. Isolated rat renal arteries were cultured for 2 days in low K\textsuperscript{+} (4 mmol/L) or depolarizing high K\textsuperscript{+} (30 mmol/L) media. Arteries preconditioned in high K\textsuperscript{+} showed a 5.47-fold increase in \(\alpha_{1C}\) expression, enhanced Ca\textsubscript{L} channel current, and elevated Ca\textsuperscript{2+}-dependent tone. These findings provide the first direct evidence that high blood pressure upregulates the Ca\textsubscript{L} channel \(\alpha_{1C}\) subunit in vivo and suggest that membrane depolarization is a potential signal involved in this interaction that may contribute to the development of abnormal vascular tone. (Circ Res. 2004;94:e97-e104.)

Key Words: calcium channels ■ \(\alpha_{1C}\) subunit ■ membrane potential ■ vascular smooth muscle ■ hypertension

Voltage-gated calcium influx through L-type Ca\textsuperscript{2+} (Ca\textsubscript{L}) channels of the Ca\textsubscript{1.2} gene family regulates the diameters of small arteries and arterioles, and maintains the myogenic tone of resistance vessels.\textsuperscript{1,2} At the protein level in arterial circulation have not been identified.\textsuperscript{5–9} This finding persisted at 28 days and was associated with abnormal Ca\textsuperscript{2+}-dependent tone and higher Ca\textsubscript{L} currents in the VSMCs exposed to high pressure. Based on microelectrode studies indicating that RR arteries were depolarized compared with LR arteries, further studies examined if membrane depolarization, an inherent response of VSMCs to high blood pressure, increased \(\alpha_{1C}\) expression. Isolated rat renal arteries were cultured for 2 days in low K\textsuperscript{+} (4 mmol/L) or depolarizing high K\textsuperscript{+} (30 mmol/L) media. Arteries preconditioned in high K\textsuperscript{+} showed a 5.47-fold increase in \(\alpha_{1C}\) expression, enhanced Ca\textsubscript{L} channel current, and elevated Ca\textsuperscript{2+}-dependent tone. These findings provide the first direct evidence that high blood pressure upregulates the Ca\textsubscript{L} channel \(\alpha_{1C}\) subunit in vivo and suggest that membrane depolarization is a potential signal involved in this interaction that may contribute to the development of abnormal vascular tone. (Circ Res. 2004;94:e97-e104.)

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The present study was designed to test the hypothesis that high blood pressure induces the expression of arterial Ca channel in vivo. To examine this issue, we banded the rat aorta between the origins of the right renal (RR) and left renal (LR) arteries to selectively expose the VSMCs of the proximal RR arteries to high blood pressure. As early as 2 days after banding, the RR arteries demonstrated an increased expression of the Ca channel α1C subunit compared with the LR arteries exposed to lower blood pressure. Furthermore, simply preconditioning isolated rat renal arteries in depolarizing media for 2 days increased α1C expression and the level of functional Ca channels. Our findings suggest a new paradigm for understanding the pathogenesis of hypertension, whereby the depolarizing influence of high blood pressure may trigger the upregulation of arterial Ca channels to further promote the development of abnormal vascular tone.

Materials and Methods

Animals
All procedures involving animals were approved by the institutional Animal Care and Use Committee. To perform interrenal aortic banding, Sprague-Dawley rats obtained from Harlan Laboratories (Madison, Wis) at 10 weeks of age were deeply anesthetized (1:10 vol:vol of ketajet-acepromazine, IM), a midline abdominal incision performed, and the aorta was visualized. A 22-gauge needle was placed adjacent to the abdominal aorta, and a strand of 3-0 silk suture was jointly tightened around the aorta and needle between the origin of the LR and RR arteries. The ligature was secured and the needle removed to permit the partial return of blood flow through the banded aorta. Sham-operated rats were exposed to the same surgery, but the aortic ligature was not tightened. After recovery, all rats were allowed free access to food and water. On the day of studies, rats were anesthetized again and the kidneys removed to obtain the renal arteries.

Vessel Culture
To determine the effect of membrane depolarization on vascular Ca channel expression, small renal arteries were microdissected from the renal parenchyma of normal Sprague-Dawley rats, and cultured for 2 days in control DMEM (4 mmol/L K+) or depolarizing DMEM containing 30 mmol/L K+. Both culture media contained a low concentration of 0.1% fetal bovine serum to discourage VSMC dedifferentiation.

Microelectrode Recordings
To determine if resting membrane potential (E<sub>m</sub>) differed between LR and RR arteries of aortic-banded rats, resting E<sub>m</sub> was recorded by glass microelectrodes in cannulated LR and RR arteries perfused at their respective in vivo blood pressure levels.

Analysis of Ca<sub>2+</sub> Channel Expression and Current
Membrane proteins were prepared from the intraparenchymal renal arteries of the left and right kidneys. The expression level of the pore-forming α1C subunit of the Ca channel was compared by Western blotting using a polyclonal antibody characterized in detail earlier. For patch-clamp studies, VSMCs from renal arcuate arteries were enzymatically isolated and studied within 6 hours. Whole-cell Ca<sup>2+</sup> channel current densities were assessed using standard pulse protocols and a patch-clamp station described previously. Experiments were performed at 25°C using 10 mmol/L barium chloride as the charge carrier to limit current rundown. The composition of the pipette solution was (in mmol/L) cesium glutamate 145, MgCl<sub>2</sub> 1, HEPES 10, EGTA 10, and Na<sub>2</sub>ATP 3 (pH 7.2). The bath solution contained (in mmol/L) BaCl<sub>2</sub> 10, TEA 135, MgCl<sub>2</sub> 1, HEPES 10, and glucose 10 (pH=7.4). In a subset of cells, 1 μmol/L nifedipine (Sigma) was used to verify the identity of Ca<sub>2+</sub> channel currents.

Vascular Reactivity Assays
To evaluate the contribution of Ca channels to vascular tone, concentration-dependent responses to the dihydropyridine agonist, Bay K8644 (Sigma), were measured in isolated renal arterial rings mounted for tension-recording. The level of nifedipine-sensitive basal tone mediated by the spontaneous opening of Ca channels was assessed in similar arterial rings.

Statistics
Unpaired t tests were used to evaluate significance of single point comparisons. Comparison of multiple points generated in patch-clamp and vessel reactivity studies were tested by two-way ANOVA with repeated measures, followed by a Duncan’s multiple range test. Significance was accepted at P<0.05. Sample sizes are indicated in the figure legends.

Results
Pressure Upregulates α<sub>1C</sub> in Arteries In Vivo
The aortae of rats were banded between the origins of the LR and RR arteries to selectively elevate blood pressure in the RR circulation (Figure 1A). Systolic pressures distal and proximal to the banded site were measured in anesthetized rats using femoral and carotid catheters at 2, 7, and 28 days after banding. In a total of 82 rats, systolic blood pressure below the banded site and perfusing the LR arteries remained low after banding, averaging 63±19 (n=11), 80±24 (n=15), and 86±19 mm Hg (n=56) at 2, 7, and 28 days, respectively. In contrast, systolic blood pressure proximal to the banded site that perfused the RR arteries in the same animals was elevated to 143±26, 157±15, and 195±30 mm Hg at 2, 7, and 28 days after banding, respectively (Figure 1B). Thus, the RR arteries of aortic-banded rats were exposed to similar genetic and humoral influences, but persistently higher levels of blood pressure than the LR arteries of the same animals.

Based on earlier studies demonstrating an increased availability of vascular Ca channels in the chronic stage of hypertension, Western blots were utilized to examine the effect of blood pressure elevation on the expression of Ca channel α1C subunits at 28 days after banding. These studies demonstrated a profound upregulation of α<sub>1C</sub> in the RR arteries exposed to high pressure, compared with the LR arteries distal to the banded site (Figure 1C). In contrast, the expression of an internal standard, smooth muscle α-actin, was similar between LR and RR arteries. Although arteries were generally pooled from several rats to provide higher protein yields, an enhanced expression of α<sub>1C</sub> in RR arteries was also detected in vascular proteins from single rats (Figure 1C, inset). In 6 Western blots using different protein batches, the immunoreactivity of the doublet bands at 200 and 240 kDa corresponding to the short and full-length forms of α<sub>1C</sub>, respectively, was increased 1.76-fold in RR compared with LR arteries. Although the expression of the α<sub>1C</sub> long form was consistently but mildly elevated by an average of 1.30-fold, expression of the α<sub>1C</sub> short form, which exists as a C-terminus truncation of the full-length protein, was increased by 2.52-fold in RR compared with LR arteries. Notably, the elevated levels of α<sub>1C</sub> in the RR arteries could not be attributed to a preexisting difference in protein expression between the LR
and RR circulations, or to components of the banding surgery unrelated to the induction of high blood pressure. Instead, five Western blots indicated that the natural expression level of \( \alpha_{1C} \) in RR arteries was slightly (16%) less than in LR arteries of sham animals (Figures 1D and 2). In sham-operated animals, a ligature was tied around the aorta but not tightened so that systolic blood pressure remained normal above (129±25 mm Hg) and below (124±22 mm Hg) the banded site at 28 days.

New studies were performed at 2 and 7 days after banding to determine the rapidity of the \( \alpha_{1C} \) response to high blood pressure. To ensure an adequate blood pressure stimulus, only renal arteries from animals that demonstrated a minimum pressure difference of 40 mm Hg across the banded site were used. In these animals, \( \alpha_{1C} \) expression was clearly increased in RR compared with LR arteries from 8 of 11 rats (73%) at 2 days after banding (Figure 1E). Similarly, the RR arteries from 11 of 15 rats (73%) showed an increased expression of \( \alpha_{1C} \) at 7 days (Figure 1F). In the RR arteries of the remaining animals (27%), no increase in \( \alpha_{1C} \) was detected at these earlier time limits (Figure 1G). Including all animals used for Western blot screening (n=59), the total immunoreactivity corresponding to the \( \alpha_{1C} \) protein was increased by 3.25-fold (day 2), 2.76-fold (day 7), and 1.76-fold (day 28) in RR arteries exposed to elevated blood pressure, inferring a rapid effect of high pressure on \( \text{Ca}^{2+} \) channel expression that peaked at 2 days but persisted at 28 days after banding (Figure 2).

**Figure 1.** A, Rat model of interrenal aortic banding. Blood pressure is selectively increased in the right renal circulation. B, Profile of systolic blood pressures in the left renal (LR) and right renal (RR) artery estimated by femoral and carotid catheters, respectively, at 2, 7, and 28 days after banding. \( \text{**Significant difference (} P<0.05 \text{)} \) between LR and RR values. C, Western blot comparing the 200- to 240-kDa doublet immunoreactive band associated with the \( \alpha_{1C} \) short and long forms between LR and RR arteries of 28-day banded rats. Expression of the \( \alpha \)-actin internal standard (45 kDa) was unchanged. Proteins were pooled from 4 animals. C, inset, Comparison of the \( \alpha_{1C} \) immunoreactive bands between LR and RR arteries of a single rat. D, Expression of \( \alpha_{1C} \) was not increased in RR compared with LR arteries of sham-operated rats at 28 days. Proteins were pooled from 3 rats. E and F, Comparison of \( \alpha_{1C} \) expression between LR and RR arteries of single rats at 2 days (E) and 7 days (F) after banding, respectively. G, At 2 and 7 days, \( \alpha_{1C} \) upregulation was not detected in a subset of rats.

**Pressure Increases Functional \( \text{Ca}^{2+} \) Channels**

Patch-clamp recordings were performed to determine if the appearance of \( \alpha_{1C} \) subunits in RR arteries was associated with more functional \( \text{Ca}^{2+} \) channels. In these studies, freshly isolated VSMCs from RR arteries showed higher levels of voltage-gated barium current (\( I_{\text{Ba}} \)) through \( \text{Ca}^{2+} \) channels than VSMCs of LR arteries (Figures 3A and 3B). In both preparations, \( I_{\text{Ba}} \) was sensitive to block by 1 \( \mu \)mol/L nifedipine. Cell capacitance was not significantly different between LR and RR VSMCs, averaging 21.9±0.5 (n=13) and 21.6±0.9 pF (n=8), respectively. Current-voltage (I-V) relationships indicated maximal \( I_{\text{Ba}} \) densities of 1.99±0.05 (n=13) and 4.82±0.26 pA/pF (n=8) in VSMCs from LR and RR arteries, respectively (Figure 3C). Thus, a 2.42-fold increase in functional \( \text{Ca}^{2+} \) channel current was detected in VSMCs exposed to high pressure in the RR circulation.
Tension-recording assays also detected more functional Ca\textsuperscript{2+} channels in RR arteries. In these studies, RR arteries developed more Ca\textsuperscript{2+}/pCa-dependent spontaneous tone than LR arteries, which was reversed by 1 \textmu mol/L nifedipine (Figure 4A). Furthermore, whereas LR arteries showed little responsiveness to increasing concentrations of the Ca\textsuperscript{2+} channel agonist Bay K 8644, RR arteries profoundly contracted, a response associated with an increased availability of Ca\textsuperscript{2+} channels (Figure 4B). Overall, RR arteries developed 2.8-fold more nifedipine-sensitive tone than LR arteries (Figure 4C; \( n = 6 \)). These data further demonstrate that the upregulation of \( \alpha_{ic} \) by high blood pressure in vivo increases the availability of functional Ca\textsuperscript{2+} channels in renal VSMCs.

Depolarization Upregulates \( \alpha_{ic} \) in Isolated Arteries

The literature provides few clues regarding possible signaling pathways that may link high blood pressure to Ca\textsuperscript{2+} channel expression. However, membrane depolarization has long been recognized as a functional alteration in VSMCs exposed to chronic hypertension.\textsuperscript{8,12} Thus, we considered the possibility that a reduced membrane potential (\( E_m \)) in VSMCs may represent an electrical signal that transduces the stimulus of high blood pressure into Ca\textsuperscript{2+} channel expression. First, we used microelectrode methods to confirm that the VSMCs of RR arteries exposed to high pressure were persistently depolarized, indicating that this electrical signal was available to modulate Ca\textsuperscript{2+} channel expression. Because the \( E_m \) levels of renal VSMCs cannot be recorded in vivo, LR and RR arteries were microdissected from 28-day banded rats (\( n = 10 \)), cannulated with glass pipettes, and perfused at average pressures of 73 ± 6 and 182 ± 7 mm Hg, respectively (Figure 5A). The perfusion pressure chosen for each artery reflected the blood pressure level measured below or above the banded site in that particular donor animal. Using this approach, microelectrode impalements revealed that VSMCs in the RR arteries were highly depolarized compared with LR arteries of the same animals (Figure 5B). The \( E_m \) values in RR and LR arteries from 10 rats averaged −39.5 ± 0.6 mV and −52.4 ± 0.5 mV, respectively (Figure 5C).

Subsequent studies examined if membrane depolarization, as an isolated stimulus, could reproduce the abnormal profile...
of elevated Ca₄ channel expression and function that was established by high blood pressure in RR arteries in vivo. Using a vessel culture approach, renal arteries were micro-dissected from normal rat kidneys, pooled, and cultured for 2 days in DMEM containing either low K⁺ (4 mmol/L) or high K⁺ (30 mmol/L) concentrations. Assuming a VSMC membrane selectively permeable to K⁺, the high K⁺ solution would be predicted to depolarize the renal VSMCs to −40 mV, although the relatively high Na⁺ permeability of VSMC membranes suggests that this value should be cautiously interpreted.²⁰ Five sets of arteries preconditioned in high K⁺ (Pre-K⁺) demonstrated a 5.47-fold increase in α₁C expression compared with control (Ctrl) arteries preconditioned in low K⁺ media for 2 days (Figure 6A). In contrast, α₁C was not upregulated in 2 sets of arteries preconditioned in DMEM containing 30 mmol/L sucrose (Figure 6B), indicating that the depolarizing rather than the osmotic challenge of high K⁺ triggered the upregulation of α₁C. Notably, Pre-K⁺ VSMCs showed increased levels of nifedipine-sensitive i₆Ba through Ca₄ channels compared with Ctrl VSMCs (Figure 6C, 6D). Average i₆-V relationships revealed peak i₆Ba densities of 2.66 ± 0.19 (n = 7) and 7.41 ± 0.40 pA/pF (n = 5) in VSMCs from Ctrl and Pre-K⁺ arteries, respectively, indicating a 2.78-fold increase in i₆Ba density (Figure 6E). In the same preparations, average cell capacitance values of 20.2 ± 1.0 and 24.5 ± 0.6 pF were not significantly different.

Interestingly, arteries preconditioned by depolarization for 2 days developed the contractile phenotype normally reserved for arteries exposed to high blood pressure in vivo.⁵,¹² Pre-K⁺ arteries demonstrated accentuated levels of nifedipine-
sensitive tone in tension-recording studies (Figure 7A), and contracted strongly to Bay K 8644 (Figure 7B), indicating a higher level of functional \(\alpha_{\text{IC}}\) channels. Overall, Pre-K\(^+\) arteries developed 3.23-fold more nifedipine-sensitive tone than Ctrl arteries (Figure 7C; \(n=6\)), and the maximal responses of Ctrl and Pre-K\(^+\) arteries to Bay K 8644 were 26±13% and 90±9% of the contraction elicited by 80 mmol/L KCl, respectively (Figure 7D; \(n=5\)).

**Discussion**

This study has two principal new findings. First, high blood pressure upregulates functional \(\alpha_{\text{IC}}\) channels in small rat renal arteries by promoting \(\alpha_{\text{IC}}\) subunit expression. Thus, we have identified a major physiological factor that influences the expression of vascular \(\alpha_{\text{IC}}\) channels in vivo. Second, the depolarization of VSMCs, a fundamental response of the vasculature to high blood pressure, is a powerful stimulus for \(\alpha_{\text{IC}}\) upregulation. We interpret this finding to suggest that \(\alpha_{\text{IC}}\) channel expression in VSMCs is coupled to the \(E_m\) level, and that pressure-induced depolarization of VSMCs is a possible mechanism for the upregulation of \(\alpha_{\text{IC}}\) subunits during hypertension.

**Pressure Dependence of \(\alpha_{\text{IC}}\) Channel Expression**

The present study directly demonstrates for the first time that high blood pressure dynamically increases the expression levels of the vascular \(\alpha_{\text{IC}}\) channel \(\alpha_{\text{IC}}\) subunit in vivo. The key observation that two different levels of \(\alpha_{\text{IC}}\) expression can be established in the left and right renal circulations of a single animal by exposing them to low and high perfusion pressures, respectively, strongly suggests that blood pressure is a powerful regulator of vascular \(\alpha_{\text{IC}}\) channel expression. Additionally, the VSMCs of RR arteries developed elevated \(\alpha_{\text{IC}}\) channel current and \(Ca^{2+}\)-dependent tone, implying the appearance of functional \(\alpha_{\text{IC}}\) channels. Interestingly, the \(\alpha_{\text{IC}}\) short form was particularly sensitive to pressure-induced upregulation at 28 days. This channel form results from a posttranslational proteolytic cleavage of the C-terminus and is missing critical residues required for \(\text{Ca}^{2+}\)-dependent phosphorylation of the \(\alpha_{\text{IC}}\) channel. Because the latter event mediates vascular relaxation, the induction of the \(\alpha_{\text{IC}}\) short form by high blood pressure may be relevant to the blunted cAMP-mediated vasodilator responses observed in hypertensive disease.

Notably, our finding of a causal, positive relationship between blood pressure and \(\alpha_{\text{IC}}\) channel expression may help to explain why arteries from diverse rat models of hypertension, which rely on different mechanisms to elevate blood pressure, share the common features of increased \(Ca_{\text{L}}\) current and \(Ca^{2+}\)-dependent tone. In these animals, high blood pressure may represent a common driving force for vascular \(\alpha_{\text{IC}}\) expression. In addition, although essential hypertension in humans evolves from diverse mechanisms, an elevated vascular resistance mediated by \(\alpha_{\text{IC}}\) channels is a hallmark finding, and isolated resistance arteries from these individuals show enhanced contractions mediated by \(\alpha_{\text{IC}}\) channels. Although rigorous evidence is lacking, these findings also are consistent with an elevated expression of vascular \(\alpha_{\text{IC}}\) channels.

**Depolarization as a Signal for \(\alpha_{\text{IC}}\) Channel Expression**

Membrane depolarization has long been recognized as a functional characteristic of VSMCs exposed to acute and chronic increases in blood pressure. For example, rat renal arteries depolarize by 15 mV during a pressure elevation from 60 to 140 mm Hg, a response that may be maintained during chronic hypertension. Indeed, microelectrode measurements in the present study provide new evidence that depolarization persists in renal VSMCs exposed to high blood pressure in vivo, and therefore, this electrical signal represents a possible event involved in \(\alpha_{\text{IC}}\) channel upregulation. To support this concept, we further demonstrated that simply preconditioning isolated renal arteries in high K\(^+\) media for 2 days triggered the appearance of \(\alpha_{\text{IC}}\) protein and functional \(\alpha_{\text{IC}}\) channels. Although a definitive link between blood pressure, VSMC depolarization, and the appearance of vascular \(\alpha_{\text{IC}}\) channels cannot be concluded from these data, the distinctive profile of \(\alpha_{\text{IC}}\) overexpression, increased \(\alpha_{\text{IC}}\) channel current, and abnormal \(\text{Ca}^{2+}\)-dependent tone induced by depolarization in isolated renal arteries was qualitatively indistinguishable from the profile of \(\text{Ca}^{2+}\) channel abnormalities induced by high blood pressure in the same arteries in vivo.

In this respect, several earlier reports indicate that abnormal patterns of electrical activity may regulate the expression of \(\alpha_{\text{IC}}\) channels in excitable cells from skeletal and cardiac tissues. For example, Pauwels et al originally demonstrated that exposing chick skeletal myotubes to high K\(^+\) triggered the appearance of dihydropyridine binding sites within 24 hours. More recently, atrial fibrillation in dogs and humans has been linked to a reduced \(\alpha_{\text{IC}}\) channel current in the affected myocytes, an abnormality that may contribute to impaired atrial contraction. However, in the latter condition, and also in other cardiac diseases including left ventricular hypertrophy and failure, the molecular basis of altered \(\alpha_{\text{IC}}\) channel availability is a matter of intense debate and only some investigators have detected changes in \(\alpha_{\text{IC}}\) protein corresponding to altered \(\alpha_{\text{IC}}\) channel availability. In contrast, the present study indicates that the expression level of the vascular \(\alpha_{\text{IC}}\) protein, a splice variant of the cardiac \(\alpha_{\text{IC}}\) channel, is highly responsive to the \(E_m\) level in isolated small arteries and also is dynamically regulated by blood pressure in vivo. These apparently diverse findings regarding the mechanisms of \(\alpha_{\text{IC}}\) regulation in vascular and cardiac cells may relate, in part, to the presence of different tissue-specific promoters in cardiac myocytes and VSMCs that drive \(\alpha_{\text{IC}}\) subunit expression.
that depolarization-induced increases in [Ca]i may influence CaL channel expression possibly by using a smooth muscle-specific α1c promoter. However, posttranslational events including changes in α1c trafficking and stability in the plasma membrane also may influence CaL channel availability, and at least two β subunits exist in VSMCs that may coassemble with α1c to form functional CaL channels.15,16,26 Regardless, the molecular composition of the CaL channel complex in resistance arteries from critical vascular beds has not been delineated, and the physiological factors that regulate α1c expression in the microvasculature remain primarily a mystery.

Study Limitations
Several limitations of the present investigation should be acknowledged. First, patch-clamp experiments were designed to evaluate the level of functional CaL channels in VSMCs, and detailed analyses of channel properties and kinetics were not included. These latter factors may have influenced the availability of CaL channel current in our experiments, although evidence for CaL channel defects in hypertension is lacking. Second, the experiments in the present study in which arteries were exposed to high K+ media, simply addressed the question of whether depolarization as an isolated signal could influence vascular α1c expression. These studies clearly could not recapitulate the complex spatiotemporal pattern of signaling events that high blood pressure initiates in small arteries, and other signaling components of the myogenic response should be viewed as potential modulators of CaL channel availability. Third, although our data indicated a positive relationship between blood pressure and α1c expression, the possibility exists that other physiological factors influenced α1c upregulation. In this regard, Gerzanich et al recently reported that angiotensin (Ang II) infusion in rats enhanced CaL channel current in cerebral VSMCs in the absence of α1c upregulation or blood pressure elevation, an event possibly related to mislocalization of endothelial nitric oxide synthase (eNOS). These findings clearly differ from those of the present study in aortic-banded rats, an animal model also characterized by elevated Ang II, in which LR arteries exposed to high Ang II levels but normal blood pressure showed no evidence of increased CaL channel function.

Pressure-Induced Upregulation of CaL Channels: A New Paradigm?
The fundamental reaction of small arteries to high blood pressure is the “myogenic response”, whereby the VSMCs strongly depolarize and constrict in an effort to normalize blood flow to distal tissues. In the renal circulation, this response will cause further rises in systemic blood pressure, but the enhanced Ca2+-dependent vascular tone is relied on at the local level to protect the kidneys from the damage that would occur if the high systemic pressure were transmitted unabated to the glomerular capillary beds. In fact, numerous studies have demonstrated that an increased myogenic tone mediated by CaL channels is central to the protection of the kidney from high systemic pressures, and that a loss of this protective vasoconstriction predisposes to progressive glomerular injury. Under these conditions, in which the integrity of the glomerular capillaries depends on a continued strong constriction of the renal circulation, the hypothesis that pressure-induced depolarization upregulates CaL channels to facilitate the voltage-dependent influx of activator Ca2+ for contraction is appealing from the physiological perspective.

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