Downregulation of the BK Channel β1 Subunit in Genetic Hypertension

Gregory C. Amberg, L. Fernando Santana

Abstract—The molecular mechanisms underlying increased arterial tone during hypertension are unclear. In vascular smooth muscle, localized Ca\(^{2+}\) release events through ryanodine-sensitive channels located in the sarcoplasmic reticulum (Ca\(^{2+}\) sparks) activate large-conductance, Ca\(^{2+}\)-sensitive K\(^{+}\) (BK) channels. Ca\(^{2+}\) sparks and BK channels provide a negative feedback mechanism that hyperpolarizes smooth muscle and thereby opposes vasoconstriction. In this study, we examined Ca\(^{2+}\) sparks and BK channel function in Wistar-Kyoto (WKY) rats with borderline hypertension and in spontaneously hypertensive rats (SHR), a widely used genetic model of severe hypertension. We found that the amplitude of spontaneous BK currents in WKY and SHR cells were smaller than in normotensive cells even though Ca\(^{2+}\) sparks were of similar magnitude. BK channels in WKY and SHR cells were less sensitive to physiological changes in intracellular Ca\(^{2+}\) than normotensive cells. Our data indicate that decreased expression of the BK channel β1 subunit underlies the lower Ca\(^{2+}\) sensitivity of BK channels in SHR and WKY myocytes. We conclude that the lower expression of the β1 subunit during genetic borderline and severe hypertension reduced BK channel activity by decreasing the sensitivity of these channels to physiological changes in Ca\(^{2+}\). These results support the view that changes in the molecular composition of BK channels may be a fundamental event contributing to the development of vascular dysfunction during hypertension. (Circ Res. 2003;93:965-971.)

Key Words: Ca\(^{2+}\) sparks ■ ryanodine receptors ■ sarcoplasmic reticulum ■ iberiotoxin

Hypertension, a major risk factor for cardiovascular disease, is the leading primary diagnosis in the United States.\(^1\) Increased arterial tone is a hallmark of hypertension. Evidence suggests that during hypertension, depolarization of vascular smooth muscle contributes to increased vascular tone by increasing Ca\(^{2+}\) influx through voltage-dependent Ca\(^{2+}\) channels.\(^2,3\) However, the molecular mechanisms underlying depolarization of smooth muscle during hypertension are poorly understood.

Large-conductance, Ca\(^{2+}\)-activated K\(^{+}\) (BK) channels are important regulators of vascular smooth muscle membrane potential.\(^4\) BK channels are activated by spontaneous, local Ca\(^{2+}\) release events (Ca\(^{2+}\) sparks) through ryanodine-sensitive channels in the sarcoplasmic reticulum.\(^5\) On activation by Ca\(^{2+}\) sparks, BK channels produce large currents that hyperpolarize and relax vascular smooth muscle, which causes vasodilation.\(^5,6\) Arterial smooth muscle BK channels are composed of pore-forming α and accessory β1 subunits.\(^7,8\) The β1 subunit, which is highly and uniquely expressed in smooth muscle,\(^9\) enhances the sensitivity of BK channels to activation by physiological changes in Ca\(^{2+}\).\(^10,11\) The functional significance of the β1 subunit has been demonstrated by the work of Brenner et al\(^9\) and Plüger et al.\(^12\) These authors found that genetically engineered mice lacking expression of the β1 subunit had vascular smooth muscle BK channels that were uncoupled from Ca\(^{2+}\) sparks. These mice also had increased vascular tone and were hypertensive.

Recently, we showed that a change in the molecular composition of BK channels contributes to vascular dysfunction in an angiotensin II infusion model of acquired, severe hypertension (systolic pressure >190 mm Hg).\(^13\) We found that angiotensin II reduced the expression of the BK channel β1 subunit in arterial smooth muscle. The resultant BK channels had reduced Ca\(^{2+}\) sensitivity, which significantly reduced their ability to respond to activating Ca\(^{2+}\) sparks. Because of this, these BK channels contributed little to vascular tone. An important issue raised by these observations is whether the uncoupling of BK channels from Ca\(^{2+}\) sparks is a general feature of hypertension and, if so, does it occur in mild hypertension.

In the present study, we examined the function of BK channels in a genetic model of hypertension (spontaneous hypertensive rats, SHR). An advantage of using the SHR is that hypertension develops in the absence of elevated angiotensin II.\(^14\) We found that BK channels from SHR were less sensitive to activation by Ca\(^{2+}\). Our data suggest that decreased β1 subunit expression resulted in BK channels with low Ca\(^{2+}\) sensitivity that were unable to respond normally to Ca\(^{2+}\) sparks. Surprisingly, we also found that the changes in BK channel molecular composition and function were present.
in borderline hypertensive animals. These observations indicate that the β1 subunit is necessary for physiological BK channel function in vascular smooth muscle and that reduction of β1 subunit function may be an integral component in the development of vascular dysfunction during hypertension.

Materials and Methods

Blood Pressure Measurements and Isolation of Arterial Myocytes

Blood pressures of conscious, age-matched adult male (12 to 16 weeks) Sprague-Dawley (SD), Wistar-Kyoto (WKY), and spontaneously hypertensive (SHR) rats were obtained by tail-cuff plethysmography as previously described.15 The animals were euthanized with a lethal dose of sodium pentobarbital (100 mg/kg; IP) as approved by the University of Washington Institutional Animal Care and Use Committee. Smooth muscle cells were prepared from basilar, posterior, and midcerebral arteries. Briefly, arteries were removed, cleaned of connective tissue, and placed in digestion buffer containing (in mmol/L) 130 NaCl, 1 KCl, 0.2 CaCl₂, 1 MgCl₂, 0.33 NaH₂PO₄, 3 pyruvate, 25 HEPES, and 22 glucose (adjusted to pH 7.4 with NaOH). We analyzed BK currents with an amplitude of three single BK channels was not observed at 80 mV.5,16,17 A similar criterion has been used previously.18,19 BK channel function and the severity of hypertension. The opening of three single BK channels per patch was estimated while patches were held at −40 mV in the presence of 10 μmol/L Ca²⁺, which maximizes the open probability (Pₒ) of these channels.16,19 All experiments were performed at room temperature (22 to 25°C).

[Ca²⁺] Imaging

Imaging of Ca²⁺ sparks was performed on cells loaded with the fluorescent Ca²⁺ indicator fluo-4-AM (5 μmol/L) using a BioRad Radiance 2100 confocal system coupled to a Nikon TE300 inverted microscope equipped with a Nikon 60× water immersion lens (numerical aperture=1.2). Ca²⁺ sparks were imaged using the line-scan mode of the confocal microscope. Images were analyzed using custom software written in IDL language (Research Systems Inc.). Ca²⁺ sparks were analyzed using a computer algorithm similar to the one described by Cheng et al.21

RNA Isolation and Real-Time RT-PCR

Total RNA was isolated from rat cerebral arteries using the TRIzol reagent (Invitrogen) as per the manufacturer’s protocol. Real-time RT-PCR was performed with the TaqMan 5′-nuclease assay on an ABI 7700 with the TaqMan One-Step PCR Mastermix (Applied Biosystems) using gene-specific primers during reverse transcription and PCR as previously reported.14 Primers and probes for real-time RT-PCR were as follows: α (GenBank accession No. NM 031828) sense NT 4100-4123, anti-sense NT 4157-4172, and probe NT 4134-4155; β1 (GenBank accession No. NM 019273) sense NT 683-708; anti-sense NT 740-762, and probe NT 710-732. A prede-veloped TaqMan assay reagent for 18S ribosomal RNA was obtained from Applied Biosystems. Samples were run in triplicate; RT(−) and nontemplate controls were included to detect nonspecific amplification.

Immunofluorescence Labeling

Immunofluorescent labeling of dispersed myocytes was performed as previously described22 using a BK channel β1 subunit-specific polyclonal antibody (Affinity Bioreagents, Inc). The secondary antibody was an Alexa Fluor 488-conjugated goat anti-mouse (highly cross-absorbed) from Molecular Probes. Immunofluorescent labeling of SD, WKY, and SHR myocytes was performed in parallel. Cells were imaged with our confocal system using identical laser power, pinhole aperture, and photomultiplier gain in all experiments. We performed three independent tests to verify the specificity of our labeling: Negative control experiments were performed where the primary (β1 subunit) antibody was substituted with either phosphate buffered saline or preabsorbed antibody (2 hours at room temperature with antigen supplied by Affinity Bioreagents, Inc). β1-Associated fluorescence was not detected for each condition (data not shown). Finally, β1-associated fluorescence was not detectable in dispersed cardiac myocytes (data not shown), as expected from previous observations showing a lack of BK channel expression in heart.23,24 β1-Associated immunofluorescence was quantified by measuring the intensity of pixels above a set threshold defined as the mean fluorescence intensity outside the cells (ie, background) plus four times its standard deviation.

Chemicals and Statistics

Unless stated otherwise, all chemicals were purchased from Sigma. Data are presented as mean±SEM. Statistical significance was evaluated by one-way analysis of variance with Tukey’s multiple comparison test; values of P<0.05 were considered significant.

Results

BK Currents, but not Ca²⁺ Sparks, Are Smaller in Borderline and Hypertensive Arterial Smooth Muscle

We assessed BK channel function in normotensive SD and SHR rats. The systolic blood pressure of SD and SHR rats used in this study was 117±3 and 195±5 mm Hg, respectively, as determined by tail-cuff plethysmography. We also examined a putative “borderline hypertensive” WKY rat substrain24 to determine if there is a relationship between BK channel function and the severity of hypertension. The systolic blood pressure of our WKY rats was 135±3 mm Hg.
These systolic pressures are consistent with other studies showing that the mean arterial pressure of WKY (≈110 mm Hg) is intermediate between that of SD (≈100 mm Hg) and SHR animals (≈135 mm Hg).24

We observed spontaneous BK current events in myocytes isolated from SD, WKY, and SHR arteries held at a membrane potential of −40 mV (Figure 1A). Although the frequency of these events was comparable at approximately 4 Hz (Figure 1B; *P < 0.05), BK current amplitudes were about 50% smaller in SHR than in SD myocytes (*P < 0.05, n = 700 events from 5 myocytes). Interestingly, the amplitude of BK currents was also approximately 50% smaller in WKY than in SD myocytes (P < 0.05; WKY and SHR BK current amplitudes were not different (*P < 0.05, n = 700 events from 5 myocytes). These data show that smooth muscle cells from mildly (WKY) as well as severely hypertensive (SHR) animals have smaller spontaneous BK currents than cells from normotensive (SD) animals.

Ca2+ sparks are the physiological activators of BK channels in vascular smooth muscle.5 Thus, it is possible that the smaller amplitude of BK currents in WKY and SHR myocytes result from smaller activating Ca2+ sparks. To test this hypothesis, we used confocal microscopy to detect Ca2+ sparks in SD, WKY, and SHR myocytes. Figure 1C shows surface plots of Ca2+ sparks from fluo-4–loaded SD, WKY, and SHR arterial myocytes. We found that Ca2+ sparks in SD (2.45 ± 0.05 F/F₀, n = 75), WKY (2.48 ± 0.06 F/F₀, n = 75), and SHR (2.58 ± 0.05 F/F₀, n = 75) cells were similar in amplitude (P > 0.05). In addition, Ca2+ spark half-time of decay was similar in SD, WKY, and SHR myocytes (56.7 ± 2.2, 53.4 ± 2.4, 54.7 ± 2.1 ms, respectively; P > 0.05). Consistent with our spontaneous BK current frequency data, there was also no difference in Ca2+ spark frequency (approximately 3 Hz) between SD, WKY, and SHR myocytes (Figure 1D; *P > 0.05, n = 5 myocytes each). It is important to note that the greater frequency of BK current events than Ca2+ sparks (4 versus 3 Hz) reported here likely reflects the occurrence of Ca2+ sparks outside of the imaged area. These data indicate that Ca2+ sparks are not altered during hypertension. Furthermore, these findings suggest that changes in Ca2+ spark activity cannot account for the smaller BK currents observed in WKY and SHR myocytes.

**β1 Subunit Function Is Lower in Borderline and Hypertensive Arterial Smooth Muscle**

If less Ca2+ spark activity does not account for the smaller BK currents observed in WKY and SHR myocytes, then differences in the functional properties of BK channels may underlie the difference in BK current amplitude in normotensive and hypertensive cells. To test this, we determined the apparent Ca2+ sensitivity of BK channels from SD, WKY, and SHR myocytes. We measured the open probability (Pₒ) of single BK channels in excised membrane patches at three different, physiologically relevant Ca2+ concentrations (0.1, 1, and 10 μmol/L). Figure 2A shows that at each Ca2+ concentration, BK channel Pₒ was less in WKY and SHR patches than in SD (P < 0.05; n = 8 patches); differences between WKY and SHR Pₒ values were not observed (P > 0.05). A reduction in the number of functional BK channels could also contribute to the smaller whole-cell BK currents in WKY and SHR myocytes. However, we found no difference in the number of BK channels per membrane patch in SD (3.8 ± 0.7 channels), WKY (3.7 ± 0.8 channels), and SHR (4.1 ± 1.2 channels) myocytes (P > 0.05, n = 15 patches).

**Figure 1.** Decreased BK current amplitudes in borderline and hypertensive arteries. A, Spontaneous whole-cell BK currents in SD, WKY, and SHR myocytes at a holding potential of −40 mV. Currents were obtained in the amphotericin-perforated patch configuration. B, Bar plots summarizing mean ± SEM BK current event amplitudes (left) and frequencies (right). C, Surface plots of averaged Ca2+ sparks from SD, WKY, and SHR arterial myocytes. D, Bar plots summarizing mean ± SEM Ca2+ spark amplitudes (left) and frequencies (right). *P < 0.05 vs SD.
In addition, the single-channel conductance of BK channels was not different between SD, WKY, and SHR cells (263±6, 253±8, 252±9 pS, respectively; P>0.05, n=6 patches). These data indicate that BK currents were smaller in WKY and SHR myocytes because of lower BK channel sensitivity to Ca$^{2+}$ and not because of a reduction in the number of BK channels or single-channel conductance.

The β1 subunit, which is expressed in arterial smooth muscle, increases the Ca$^{2+}$ sensitivity of BK channels. In addition to increasing Ca$^{2+}$ sensitivity, the β1 subunit also increases the open dwell time of BK channels and confers sensitivity to acute activation by tamoxifen. One possible mechanism underlying the lower Ca$^{2+}$ sensitivity of BK channels observed in WKY and SHR cells is lower β1 subunit function. Thus, if β1 subunit function is lower in WKY and SHR myocytes, then BK channels from these cells should have shorter open-time kinetics and be less-sensitive to activation by tamoxifen than BK channels from SD myocytes.

Figure 2B shows representative traces and corresponding open-time histograms from BK channels in excised patches from SD, WKY, and SHR myocytes. Histograms were fit with a single exponential function. BK channels from WKY [mean time constant (τ$_{open}$)=9.1±1.8 ms] and SHR (τ$_{open}$=8.9±2.1 ms) myocytes had shorter open times than BK channels from SD (τ$_{open}$=18.9±2.1 ms) myocytes, which is consistent with lower β1 subunit function in these cells (P<0.05, n=9 patches). Interestingly, WKY and SHR open times were similar (P>0.05).

Next, we examined the effects of tamoxifen (1 μmol/L; 100 nmol/L Ca$^{2+}$) on BK channels in SD, WKY, and SHR patches. Consistent with the observations by Dick et al, we found that application of tamoxifen (1 μmol/L) evoked nearly a 4-fold increase in the P$_{o}$ of BK channels in SD patches. Tamoxifen also caused a small decrease in the conductance of single BK channels. This property of tamoxifen resides in the BK channel α subunit and is a useful indicator of successful drug-channel interaction. In contrast to its effects on SD BK channel activity (see Figure 2C), tamoxifen (1 μmol/L) had no effect on the P$_{o}$ of WKY and SHR BK channels (P>0.05, n=6 patches). This is also consistent with less β1 function in WKY and SHR. Note that tamoxifen produced a decrease in the single-channel conductance of WKY and SHR BK channels, confirming the interaction of the drug with the channel. From these data (ie, lower Ca$^{2+}$ sensitivity, shorter open times, and insensitivity to activation by tamoxifen), we conclude that β1 function is less in WKY and SHR arterial myocytes relative to SD myocytes.

**Figure 2.** Properties of single BK channels indicate decreased β1 subunit function in borderline and hypertensive arteries. A, Ca$^{2+}$ sensitivity of BK channels in inside-out patches (HP=-40 mV) from SD, WKY, and SHR myocytes. Shown at left are representative BK channel records in the presence of 1 or 10 μmol/L Ca$^{2+}$. Bar plot to the right summarizes mean±SEM P$_{o}$ of BK channels at three Ca$^{2+}$ concentrations as indicated. B, Open-time analysis of BK channels in inside-out patches from SD, WKY, and SHR myocytes. Shown at left are representative single BK channel records at +40 mV in the presence of 1 μmol/L Ca$^{2+}$. Open-time histograms of these channels fit with a single-exponential function are shown in the center. Bar plot at right summarizes mean±SEM τ$_{open}$. C, Tamoxifen (Tam; 1 μmol/L) sensitivity of BK channels in inside-out patches (HP=-40 mV; 100 nmol/L free Ca$^{2+}$) from SD, WKY, and SHR myocytes. Shown at left are representative BK channel records before and after Tam. Bar plot at right summarizes the mean±SEM P$_{o}$ fold change in the P$_{o}$ of BK channels after the application of Tam. Dashed lines indicate open channels; ○ indicates open channels; and □, closed channels. *P<0.05 vs SD.

**Downregulation of β1 Subunits in Borderline and Hypertensive Arterial Smooth Muscle**

The next series of experiments examined the possibility that β1 expression was lower in WKY and SHR than in SD vascular smooth muscle. To test this hypothesis, we measured the amount of BK α and β1 transcripts in SD, WKY, and SHR arteries using real-time RT-PCR. We found no difference in the relative abundance of α transcripts in SD, WKY, and SHR arteries (Figure 3A; P>0.05, n=3 animals). These data are consistent with our observation of a similar number of BK channels in membrane patches from SD, WKY, and SHR myocytes. In sharp contrast (see Figure 3B), β1 transcripts were approximately 70% less abundant in WKY and SHR myocytes.
SHR arteries compared with SD (P<0.05, n=3 animals; β1 transcript was similar in WKY and SHR (P>0.05).

In light of these findings, we examined the expression of β1 subunit protein in SD, WKY, and SHR arteries. Different scenarios may explain the reduced β1 function observed in WKY and SHR myocytes relative to SD myocytes. For example, β1 protein expression may be reduced in WKY and SHR, as suggested by or real-time RT-PCR data, but in a nonuniform fashion. If the sites of reduced β1 expression were to occur near Ca2+ spark sites, this would effectively uncouple BK channels from Ca2+ sparks. Using the same logic, this result could be produced by differences in the spatial distribution of the β1 subunit (ie, away from Ca2+ spark sites) in the absence of a change in the level of β1 protein. Alternatively, β1 expression may be reduced equally throughout the plasma membrane in WKY and SHR cells. Although protein levels can be measured using Western blot analysis, this technique would not provide information regarding the spatial distribution of the β1 subunit. To bypass this limitation, we used immunofluorescence techniques and confocal microscopy to examine the expression and spatial distribution of the β1 subunit in SD, WKY, and SHR cells.

We dispersed and labeled SD, WKY, and SHR myocytes with a β1-specific antibody (see Materials and Methods). Figure 3C shows 3-dimensional plots of confocal images obtained from representative SD, WKY, and SHR myocytes. Note that β1-associated fluorescence in SD cells was intense and, as expected, limited primarily to the edges of the cell (ie, the plasma membrane). In SD arterial myocytes, the β1 subunit did not appear to be restricted to specific regions of the plasma membrane. Instead, this protein was broadly distributed along the surface membrane of vascular smooth muscle cells. We noticed, however, that SD cells did have regions of slightly elevated β1-associated fluorescence. The origin of these foci is unclear, but may reflect a nonuniform distribution of BK channels.28

In agreement with our real-time RT-PCR data, the intensity of β1-associated fluorescence was dramatically lower in WKY and SHR myocytes than in SD (Figure 3C). Note that β1-associated fluorescence is lower throughout the surface membrane of SHR and WKY cells relative to SD. The mean β1-associated fluorescence intensity was about 65% lower in SHR and WKY than in SD cells (P<0.05, n=50 cells each). These data suggest that the expression of β1 transcript and protein is lower in WKY and SHR than in SD smooth muscle. Furthermore, our data indicate that the lower levels of β1 protein in SHR and WKY than in SD smooth muscle is due to a general reduction in the expression of this protein across the surface membrane of these cells.

**Discussion**

This study demonstrates that BK channel function is reduced in arterial smooth muscle from a genetic model of severe (SHR) and borderline (WKY) hypertension. In comparison to normotensive (SD) smooth muscle cells, the amplitude of spontaneous BK currents in hypertensive cells was small despite a lack of difference in Ca2+ spark amplitude, frequency, or duration. Our data indicate that the Ca2+ sensitivity of BK channels in SHR and WKY is lower in SD cells. Lower expression of β1 in SHR and WKY relative to SD smooth muscle underlies the decreased Ca2+ sensitivity of BK channels in SHR and WKY cells. These data indicate that changes in the molecular composition of BK channels during genetic borderline and severe hypertension decrease this channel’s Ca2+ sensitivity, which decreases their coupling to Ca2+ sparks. Our results support the view that reduced β1 expression may be an important event in the development of vascular dysfunction during hypertension.

A series of recent studies provides insight into the functional consequences of reduced coupling between Ca2+ sparks and BK channels. Decreased BK channel activity depolarizes and constricts vascular smooth muscle.9,12,13 Ca2+ sparks are the physiological activators of BK channels.5 Accordingly, vasconstrictors acting through stimulation of protein kinase C decrease BK channel activity indirectly by inhibiting Ca2+ sparks.16 Conversely, increasing Ca2+ spark frequency or augmenting BK channel coupling to Ca2+ sparks causes vasodilation.17,29 Thus, decreased coupling of Ca2+ sparks to
BK channels would likely contribute to increased vascular tone and blood pressure in WKY and SHR arteries. An important finding in this study was that vascular smooth muscle from borderline hypertensive WKY rats (≈15 mm Hg increase in systolic pressure) had reduced β1 expression. Indeed, the reduction in β1 expression and BK channel Ca2+ sensitivity was similar in borderline hypertensive WKY myocytes as in myocytes from severely hypertensive SHR and angiotensin II–infused rats. It is intriguing to speculate that β1 downregulation and subsequent uncoupling of BK channels from Ca2+ sparks may be an early event in the development of hypertension.

Because BK channel dysfunction was similar in borderline and fully developed hypertension, an issue is raised about the relative importance BK channel–Ca2+ spark coupling in the determination of blood pressure. Three major lines of evidence indicate that BK channel coupling to Ca2+ sparks is important in controlling vascular tone and blood pressure. First, inhibition of Ca2+ sparks constricts cerebral arteries. Second, vasodilators enhance coupling, whereas vasoconstrictors decrease the activation of BK channels by Ca2+ sparks. Third, targeted deletion of the β1 subunit uncouples BK channels from Ca2+ sparks and is, by itself, sufficient to induce hypertension. It is therefore likely that the difference in blood pressures in WKY and SHR rats in the presence of similar BK channel dysfunction reflects differences in the progression of other hypertension-associated processes as well as differences in the success of compensatory mechanisms. The nature of these differences will be the focus of further investigation.

An important question raised by our prior study using angiotensin II–infused rats is whether β1 downregulation is caused by increased angiotensin II signaling and/or an increase in blood pressure per se. Interestingly, WKY and SHR are reported not to have increased plasma levels of angiotensin II. Taken together, these findings suggest that elevated levels of angiotensin II are not a prerequisite for downregulation of the β1 subunit. However, it is important to note that in humans, an angiotensin type-1 receptor polymorphism with enhanced responsiveness to angiotensin II has been associated with essential hypertension. Thus, renin-angiotensin activity may be increased during hypertension even in the absence of elevated angiotensin II plasma levels. At present, however, the signaling mechanisms that regulate expression of the β1 subunit in angiotensin II–infused and genetically hypertensive is unknown.

Although our data indicate that the molecular composition of BK channels is altered during genetic hypertension, the precise subunit stoichiometry of BK channels in hypertensive vascular smooth muscle cells is unclear. Comparison of the data shown in Figure 2A with that presented by Brenner et al. using β1-deficient mice, shows that the BK channels in the transgenic mouse have lower Ca2+ sensitivities than WKY and SHR BK channels. This suggests that, on average, not all BK channels in WKY and SHR cells are devoid of β1 subunits. Future experiments will examine the exact subunit stoichiometry of BK channels in normotensive and hypertensive vascular smooth muscle cells.

Coupling of BK channels to Ca2+ sparks has not been examined before in WKY and SHR. However, other investigators have examined the properties of single BK channels in aortic and cerebral arterial smooth muscles from these animals. In agreement with our findings, differences in the Ca2+ sensitivity of BK channels from WKY and SHR were not apparent. In light of our results, the similarity in Ca2+ sensitivity of BK channels in WKY and SHR cells suggests that β1 was reduced to a similar extent in both arterial smooth muscles. Inconsistent with our findings, these authors also report increased α subunit expression in SHR. The nature of these discrepancies is unclear. However, increased expression of the BK α subunit, in the absence of an increase in β1, would augment the disproportionately high ratio of BK α to β subunits during hypertension. Increased expression of the α subunit could therefore exacerbate BK channel uncoupling from Ca2+ sparks in hypertensive arteries. To conclude, we have demonstrated decreased expression of the BK channel β1 subunit and subsequent uncoupling of BK channels from Ca2+ sparks in the SHR model of genetic hypertension. Furthermore, we show that these changes are present in borderline hypertensive WKY rats. These results reiterate the importance of strong coupling between BK channels and Ca2+ sparks for physiological function and suggests that decreased expression of the β1 subunit is a common feature in hypertension.

Acknowledgments

This work was supported by grants NS34903 and HL07312. We would like to thank Dr Stephen M. Schwartz and Patti Polinsky for help with performing blood pressure measurements. We would also like to thank Rachel Hershman-Greven and Erin Sylvester for technical assistance and Scott Votaw for help with image analysis. We are also grateful to Dr Carmen A. Ufret for critically reading this manuscript.

References


Amberg and Santana  

**BK Channel Dysfunction During Hypertension**


Downregulation of the BK Channel β1 Subunit in Genetic Hypertension
Gregory C. Amberg and L. Fernando Santana

*Circ Res.* 2003;93:965-971; originally published online October 9, 2003;
doi: 10.1161/01.RES.0000100068.43006.36

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/93/10/965

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation Research* 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 *Circulation Research* is online at:
http://circres.ahajournals.org/subscriptions/