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Circulation 2005;112;60-68; originally published online May 2, 2005;
DOI: 10.1161/01.CIR.0000156448.74296.FE
Circulation is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 0009-7322. Online ISSN: 1524-4539

Elevated Blood Pressure Linked to Primary Hyperaldosteronism and Impaired Vasodilation in BK Channel–Deficient Mice

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Background—Abnormally elevated blood pressure is the most prevalent risk factor for cardiovascular disease. The large-conductance, voltage- and Ca^{2+}-dependent K^+ (BK) channel has been proposed as an important effector in the control of vascular tone by linking membrane depolarization and local increases in cytosolic Ca^{2+} to hyperpolarizing K^+ outward currents. However, the BK channel may also affect blood pressure by regulating salt and fluid homeostasis, particularly by adjusting the renin-angiotensin-aldosterone system.

Methods and Results—Here we report that deletion of the pore-forming BK channel α subunit leads to a significant blood pressure elevation resulting from hyperaldosteronism accompanied by decreased serum K^+ levels as well as increased vascular tone in small arteries. In smooth muscle from small arteries, deletion of the BK channel leads to a depolarized membrane potential, a complete lack of membrane hyperpolarizing spontaneous K^+ outward currents, and an attenuated cGMP vasorelaxation associated with a reduced suppression of Ca^{2+} transients by cGMP. The high level of BK channel expression observed in wild-type adrenal glomerulosa cells, together with unaltered serum renin activities and corticotropin levels in mutant mice, suggests that the hyperaldosteronism results from abnormal adrenal cortical function in $BK^{-/-}$ mice.

Conclusions—These results identify previously unknown roles of BK channels in blood pressure regulation and raise the possibility that BK channel dysfunction may underlie specific forms of hyperaldosteronism. **(*Circulation*. 2005;112:60-68.)**

Key Words: blood pressure ■ ion channels ■ vasoconstriction ■ vasodilation ■ hyperaldosteronism

Increased arterial tone is a hallmark of elevated blood pressure. During hypertension, pressure-induced depolarization of vascular smooth muscle cells contributes to increased vascular tone by increasing Ca^{2+} influx through voltage-dependent Ca^{2+} channels.[1,2] In vitro evidence suggests that the unique large-conductance, voltage- and Ca^{2+}-activated K^+ (BK) channel limits Ca^{2+} entry and thereby arterial contraction by repolarizing smooth muscle cells and closing voltage-dependent Ca^{2+} channels previously opened by pressure or vasoconstrictors.[3] BK channels also mediate steady hyperpolarization and vasorelaxation as a result of transient outward currents carried by BK channels spontaneously activated by local release of Ca^{2+} from intracellular stores via ryanodine receptor channels.[4] The vascular BK channel consists of 4 α subunits that form the ion-conducting pore and 4 auxiliary β1 subunits. The β1 subunits, which are restricted to smooth muscle, maintain the normal high voltage and Ca^{2+} sensitivity of the pore-forming α subunit.[5] The role

Received August 4, 2004; revision received October 12, 2004; accepted October 25, 2004.

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The online-only Data Supplement can be found with this article at http://circ.ahajournals.org/cgi/content/full/01.CIR.0000156448.74296.FE/DC1.

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Circulation **is available at http://www.circulationaha.org** **DOI: 10.1161/01.CIR.0000156448.74296.FE**

of the BK channel auxiliary β_1 subunit in blood pressure regulation has been tested previously by deleting its gene in mice. The resulting loss of this subunit impaired the coupling between Ca^{2+} release and the activation of hyperpolarizing BK currents, leading to systemic hypertension.[6,7] Recent studies raise the possibility that changes in β1 subunit expression contribute to the development of hypertension in rat[8] and that gain of function mutation in the same subunit decreases the prevalence of diastolic hypertension in humans.[9] However, even in the absence of functional β1 subunits, the α subunit can still form functional channels, which might be activated at physiological potentials if their voltage and Ca^{2+} sensitivity are increased by other factors such as endothelial factors[10,11] and/or phosphorylation.[12,13] Thus, functional BK channels may be operative in blood vessels even when the β1 subunit is lacking. In addition, BK channels in tissues other than vasculature, such as the adrenal gland,[14] may also influence blood pressure regulation. Therefore, we used mice lacking the BK channel α subunit ($BK^{-/-}$[15] to evaluate the global impact of BK channels on blood pressure regulation.

Methods

Details are given in the online-only Data Supplement.

Mice

$BK^{-/-}$ mice were generated as described.[15] Wild type (WT) and $BK^{-/-}$ mice with the hybrid SV129/C57BL6 background (always F2 generation) were used. Either litter- or age-matched animals were randomly assigned to the experimental procedures undertaken in accordance with the German legislation on protection of animals.

Immunohistochemistry of Adrenal Gland

For immunofluorescence, on-slide 5-μm cryostat slices from nonfixed WT and $BK^{-/-}$ adrenal glands were incubated with anti-$BK\alpha_{(674–1115)}$. BK expression was analyzed with a confocal laser-scanning microscope (Biorad MRC1000 attached to Nikon Diaphot 300 and equipped with a krypton-argon laser). For peroxidase/DAB detection, 10-μm cryosections of WT and $BK^{-/-}$ adrenal glands were perfused with 4% paraformaldehyde and incubated with anti-$BK\alpha_{(674–1115)}$. Data analysis was performed with a Zeiss Axioplan 2 microscope equipped with a Zeiss Axio Cam digital camera.

Determination of Renin Activity, Corticotropin, and Cortisterone Levels From Serum

Plasma renin concentration was measured as the generation of angiotensin I (ng/mL per hour) when plasma samples were incubated with excess renin substrate (plasma with no intrinsic renin activity from a binephrectomized rat). Angiotensin I was measured by radioimmunoassay as previously described.[16] For the determination of corticotropin in plasma, we used a 2-site solid-phase immunoradiometric assay (IRMA) kit (Euria-acth IRMA kit) from Euro-Diagnostica AB. This assay measures intact corticotropin (1–39); in the assay used, the limit of detection was 5 pg/mL and the intra-assay variation <5%. Serum corticosterone was analyzed with an in-house specific radioimmunoassay, as described previously, and modified for microtiter plate scintillation proximity assay.[17]

Serum Electrolytes and Serum Aldosterone Levels

Serum was separated from nonheparinized blood collected by heart puncture from WT and $BK^{-/-}$ mice euthanized by inhalation of carbon dioxide. The serum concentrations of Na^+ and K^+ were measured by flame photometry. Serum concentrations of aldosterone were measured by radioimmunoassay.

Electrophysiology of Tibial Artery and Aortic Smooth Muscle Cells

For cell isolation, tibial artery, a fourth-order branch of the aorta, or aorta was incubated in Ca^{2+}-free physiological saline solution (PSS) containing papain at 37°C for 30 minutes. Then the solution was exchanged for PSS containing Ca^{2+}, collagenase type H, and hyaluronidase, and digestion was continued for another 10 minutes at 37°C. For measuring of outward membrane currents (whole-cell mode), the free Ca^{2+} concentration in the pipette solution was 300 nmol/L. The holding potential was −50 mV (arterial cells) and −20 mV (aortic cells), and test pulses of 300-ms duration were applied every 5 seconds. For measuring membrane potentials (whole-cell perforated patch), the pipette solution contained nystatin. For recording of macroscopic Ca^{2+} channel currents, cells were voltage-clamped at a holding potential of −60 mV, and the potential was stepped, for 300 ms every 5 seconds, in 10-mV increments up to 50 mV. The inward current was measured as peak inward current with reference to zero current.

Luminal Diameter Analysis of Small Arteries by Videomicroscopy

Tibial small arteries were equilibrated in buffer, and an intravascular pressure of 80 mm Hg was applied under nonflow conditions. The chamber was continuously perfused at a rate of 2 mL/min with buffer at 37.0±0.5°C. The small artery was allowed to equilibrate under videomicroscopic recording until a stable myogenic tone spontaneously developed after 15 to 20 minutes. At the end of each experiment, Ca^{2+}-free buffer was applied to determine maximal vessel diameters. No significant difference in maximal vessel diameter was detected between the 2 genotypes (WT, 69±3 μm; $BK^{-/-}$, 63±2 μm). All compounds were administered to the adventitial side of the pressurized small arteries. To exclude prostaglandin effects, the buffer contained 1 μmol/L diclofenac.

$[Ca^{2+}]_i$ Measurements in Aortic Smooth Muscle Cells

Single aortic smooth muscle cells (prepared as above) were loaded with 2.5 μmol/L fura 2-AM. For $[Ca^{2+}]_i$ measurement, cells were transferred to a glass coverslip coated with 0.01% poly-L-lysine solution and continuously superfused with PSS at 36°C at a flow rate of 2 to 4 mL/min. $[Ca^{2+}]_i$ measurements were performed with the use of the dual-wavelength microfluorescence technique. Two $[Ca^{2+}]_i$ transients were elicited consecutively with a 15-minute interval in between to allow refilling of intracellular Ca^{2+} stores.

Spontaneous Transient Outward Current and Ca^{2+} Spark Analysis

Cerebral arteries were placed in Ca^{2+}-free Hanks' solution supplemented with papain for 15 minutes at 36°C. The segments were then placed in Hanks' solution containing collagenase type F and H (ratio 30% and 70%, respectively) and 0.1 mmol/L $CaCl_2$ for 6 minutes at 36°C. After several washes in Ca^{2+}-free Hanks' solution, single cells were isolated. Spontaneous transient outward currents (STOCs) were measured in the perforated patch mode. Holding potential was set at −60 mV. The pipette solution contained 250 μg/mL amphotericin. To measure Ca^{2+} sparks, cells were incubated with fluo 3-AM for 30 minutes at room temperature in Ca^{2+}-free Hanks' solution. Ca^{2+} sparks were measured as local fractional fluorescence increases (F/F_0) by confocal fluorescence microscopy at room temperature. The baseline fluorescence (F_0) was determined by averaging line-scan images in the absence of Ca^{2+} sparks.

Long-Term Radiotelemetric Blood Pressure Analysis

Mean arterial blood pressure (MAP), heart rate (HR), and physical activity were analyzed in conscious male WT and $BK^{-/-}$ mice (n=7 for each genotype). Mice (aged 3 to 4 months), either litter- or age-matched, did not significantly differ in body weight (26.2±1.3 and 23.5±0.7 g, respectively). Mice were anesthetized with isoflurane. A ventral midline incision was performed before careful isolation of the left common carotid artery. For ligation

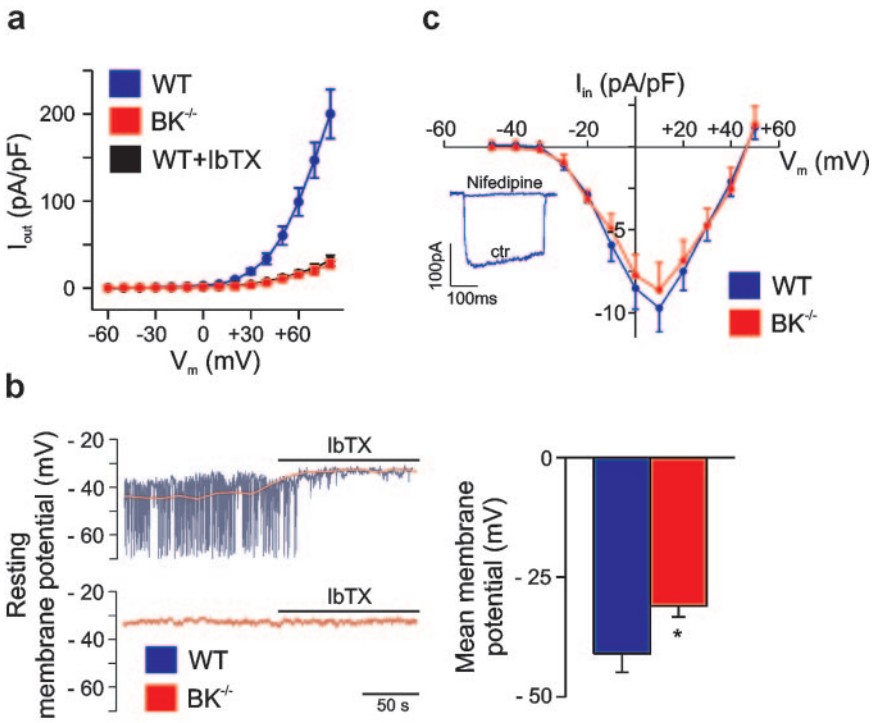

Figure 1. Resting membrane potential and current density of L-type Ca^{2+} current in tibial artery smooth muscle cells. a, Current-voltage relationship of K^+ outward currents in cells from 3 WT (blue circles) and 3 $BK^{-/-}$ (red squares) tibial arteries. Whole-cell currents were measured at 300 nmol/L $[Ca^{2+}]_i$ from a holding potential of −50mV without (blue circles, red squares) and with 300 nmol/L iberiotoxin (respective data points are covered by the red squares); n=11 (WT) and n=15 ($BK^{-/-}$) cells. b, Membrane potential recordings from WT and $BK^{-/-}$ tibial artery cells with and without iberiotoxin and mean±SEM membrane potential statistics (13 and 18 cells from WT and $BK^{-/-}$ mice, respectively); *$P<0.05$. c, Amplitudes of voltage-gated Ca^{2+} channel currents in WT and $BK^{-/-}$ cells do not differ. Currents were measured in the whole-cell configuration, and barium was used as charge carrier. Inward currents were evoked by step depolarizations (300-ms duration) of increasing amplitude from a holding potential of −60 mV up to +50 mV in 10-mV increments. Current-voltage relationships of peak inward currents are shown (n=11 from 3 WT and n=7 from 3 $BK^{-/-}$ mice). Mean±SEM current densities are plotted against the respective test potential. Inset, Averaged current tracings before (ctr) and after superfusion of a WT cell with 1 μmol/L nifedipine. Inward currents were activated by step depolarizations from −60 to +10 mV and maintained for 300 ms.

and retraction, 2 silk ligatures were passed under the vessel, one ≈0.8 cm caudal to the bifurcation of the interior and exterior carotid artery and another ≈0.5 cm rostral from the caudal ligature. A tiny incision in the carotid artery was made for insertion of the catheter. The inserted catheter tip was advanced to the thoracic aorta and fixed with suture. A subcutaneous pocket was performed along the right flank for placing the transmitter body. After subcutaneous fixation of the transmitter, the incision was closed with 6-0 silk. MAP, HR, and physical activity were recorded at days 5 to 7 after surgery, when mice have regained normal locomotor activity. Radiotelemetric signals were sampled for 1 minute at 5-minute intervals.

Results

Increased Myogenic Tone and Attenuated cGMP-Dependent Vasorelaxation in $BK^{-/-}$ Small Arteries

In smooth muscle cells from the tibial artery of WT mice, depolarization elicited large, iberiotoxin-sensitive outward K^+ currents, but no such currents were detected in cells from $BK^{-/-}$ cells (Figure 1a). Furthermore, $BK^{-/-}$ cells did not exhibit spontaneous hyperpolarizations and showed a more depolarized mean membrane potential (−31.0±2.3 mV) than WT cells (−41.0±3.9 mV). Depolarization was also observed in WT cells when BK channels were specifically blocked by iberiotoxin (Figure 1b). The BK channel has recently been found to be physically and functionally associated with the L-type Ca^{2+} channel.[18] In the arterial cells, however, the expression of nifedipine-sensitive L-type Ca^{2+} channels was not changed, as revealed by almost identical current densities in $BK^{-/-}$ and WT arterial cells, thereby excluding a compensatory downregulation of this channel in $BK^{-/-}$ arteries (Figure 1c). It is conceivable that the sustained depolarization of $BK^{-/-}$ arterial cells compared with WT cells could produce a sustained rise in cytosolic Ca^{2+} because of a "window current" caused by incomplete inactivation of L-type Ca^{2+} channels.[19] Interestingly, the steady state calcium window in smooth muscle cells was maximal at −30 mV,[19] which corresponds closely to the membrane potential in $BK^{-/-}$ cells.

Thus, the more depolarized membrane potential in $BK^{-/-}$ cells may influence small-artery tone, which is obligatorily dependent on pressure-evoked depolarization triggering Ca^{2+} entry through voltage-gated Ca^{2+} channels.[20] In fact, at physiological relevant pressures (80 and 120 mm Hg), tibial arteries from $BK^{-/-}$ mice showed increased myogenic tone compared with arteries from WT mice (Figure 2a). However, the myogenic response, ie, the change in myogenic tone induced by pressure steps, was not significantly different in $BK^{-/-}$ versus WT tibial arteries (Figure 2b), suggesting that BK channels do not determine the myogenic response per se. Apparently, membrane depolarization and increases in global $[Ca^{2+}]_i$ plus local Ca^{2+} sparks in response to transmural pressure are not sufficient to evoke a BK channel–mediated negative feedback regulation of pressure-induced contraction. Rather, BK channels seem to mediate essentially steady dilatation over a large pressure range, inasmuch as their deletion produced an almost parallel upward shift of the pressure-tone relationship (Figure 2a).

It is well established that the myogenic tone can be decreased by activation of the NO/cGMP/protein kinase G

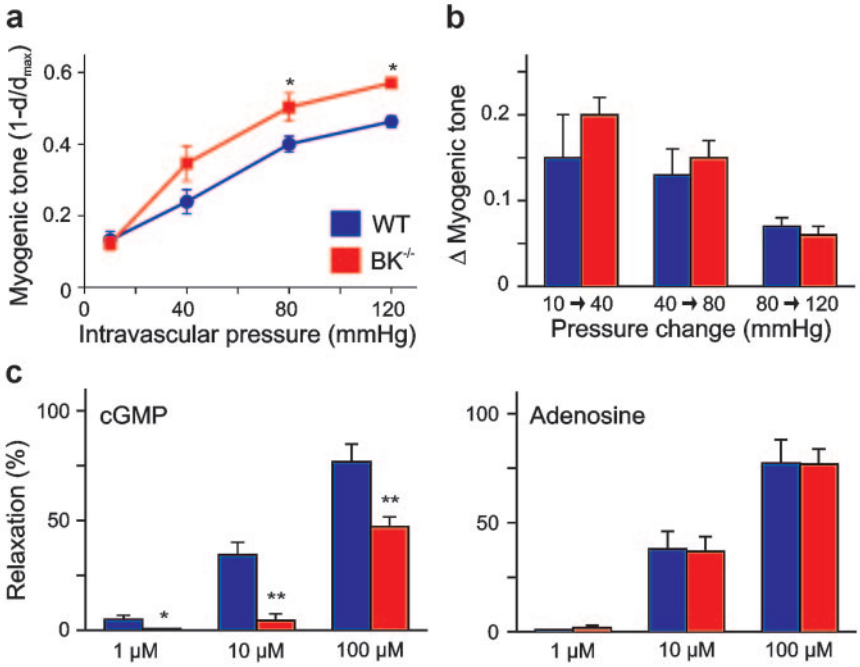

Figure 2. Increased myogenic tone and attenuated cGMP-dependent vasorelaxation in BK$^{-/-}$ small arteries. a, Effect of transmural pressure on myogenic tone in tibial artery. Myogenic tone was calculated as (1−d/d$_{max}$), where d is the vessel diameter at a certain pressure and d$_{max}$ the diameter in Ca^{2+}-free buffer at that pressure. b, Effect of pressure steps on myogenic tone change (Δmyogenic tone, ie, tone at end pressure minus tone at initial pressure of the step); n=4 WT and 4 BK$^{-/-}$ arteries. c, cGMP- and adenosine-induced relaxation of myogenic tone of tibial artery. Relaxation was calculated as drug-induced change of inner vessel diameter as a percentage of maximal diameter in Ca^{2+}-free buffer; n=6 arteries per genotype. All data are mean±SEM; *P<0.05; **P<0.01.

(PKG) and cAMP/protein kinase A (PKA) pathways.[21,22] Application of 8-pCPT-cGMP, a cell-permeable cGMP analogue that preferentially activates PKG, produced significantly less relaxation of BK$^{-/-}$ arteries than WT arteries (Figure 2c). In contrast, adenosine, which raises $[cAMP]_i$ via A_2 receptors,[23] was equally potent in WT and BK$^{-/-}$ arteries, suggesting that the cGMP/PKG, but not the cAMP/PKA, pathway requires BK channel activation to promote arterial relaxation (Figure 2c). The partial relaxation produced by 8-pCPT-cGMP in BK$^{-/-}$ arteries may involve other PKG substrates or cross-activation of PKA at high agonist concentrations.

Blood vessel relaxation by cGMP/PKG has been ascribed to suppression of Ca^{2+} transients.[24] To test this hypothesis, aortic smooth muscle cells from WT and BK$^{-/-}$ mice, basically exhibiting the same differences in membrane potential as cells from tibial artery (Data Supplement Figure), were stimulated with the α_1-adrenergic receptor agonist phenylephrine, and the resulting Ca^{2+} transients (due to Ca^{2+} release and influx via Ca^{2+} channels[25]) were measured in the absence of 8-Br-cGMP. The area under the curve (AUC) of the Ca^{2+} transients was almost identical in BK$^{-/-}$ (n=76) and WT cells (n=64) (2.1±0.1 and 2.2±0.2 arbitrary units, respectively). Additionally, the ratio between consecutively elicited Ca^{2+} transients (AUC 2/AUC 1) was not significantly different in BK$^{-/-}$ and WT cells (87±8% and 103±7%, respectively) (Figure 3). Preincubation with 8-Br-cGMP diminished the second transients in WT cells to 38±7% of control, whereas BK$^{-/-}$ cells were less affected (72±11%). The attenuated suppression in BK$^{-/-}$ cells suggests that BK channel activation via cGMP/PKG is important for relaxation, probably involving hyperpolarization-induced inhibition of voltage-gated Ca^{2+} channels. The small residual cGMP-induced suppression of Ca^{2+} transients in BK$^{-/-}$ smooth muscle cells may be caused by cGMP-mediated inhibition of Ca^{2+} release from IP_3-sensitive stores.[26]

The amount of Ca^{2+} in intracellular stores of aortic cells was apparently not changed by the absence of the BK channel. Caffeine at 10 mmol/L stimulated Ca^{2+} release from the sarcoplasmic reticulum to a similar extent in BK$^{-/-}$ (3.6±0.2 arbitrary units of AUC; n=108) and WT cells (3.4±0.2; n=92).

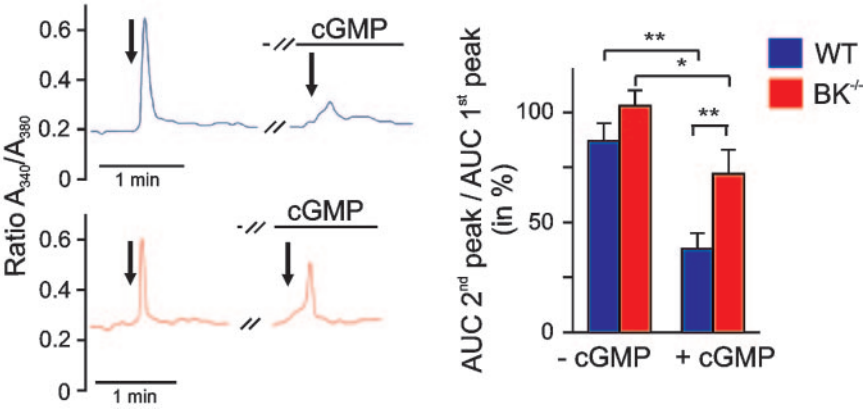

Figure 3. Reduced suppression of Ca^{2+} transients by cGMP in BK$^{-/-}$ aortic smooth muscle cells. Left, Consecutive $[Ca^{2+}]_i$ transients (first and second transients are shown), elicited by phenylephrine (10 μmol/L for 1 minute, arrows) at 15-minute intervals, in WT (blue) and BK$^{-/-}$ (red) aortic cells, after preincubation with or without 8-Br-cGMP (1 mmol/L, 5 minutes). Right, $[Ca^{2+}]_i$ transients were quantified as AUC (40 to 63 cells per genotype). All data are mean±SEM; *P<0.05; **P<0.01.

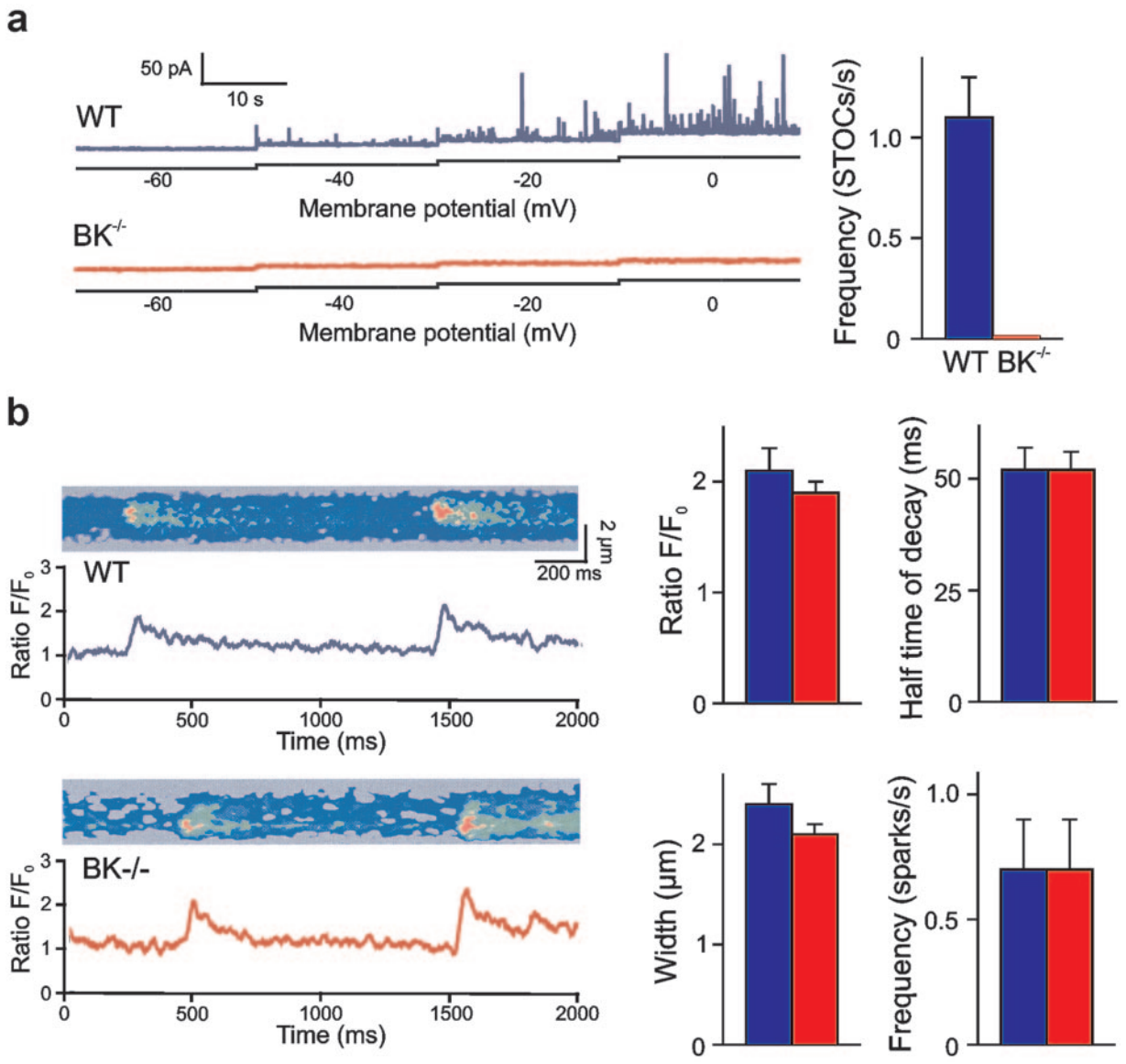

Figure 4. Lack of STOCs but presence of normal Ca^{2+} sparks in $BK^{-/-}$ cerebral artery smooth muscle cells. a, STOC activity in WT and $BK^{-/-}$ cerebral arterial cells recorded at increasing membrane potentials and STOC frequency at −20 mV from 5 to 7 WT and $BK^{-/-}$ cells. b, Confocal line scans of fluo 3–loaded WT and $BK^{-/-}$ cells and time course of corresponding Ca^{2+} sparks. Spark amplitudes were measured as local fractional fluorescence increases (F/F_0; F_0 is baseline). Spark duration was measured at half-maximal amplitude; n=5 to 12 cells per genotype. All data are mean±SEM; *$P<0.05$; **$P<0.01$.

Lack of STOCs in $BK^{-/-}$ Arterial Cells Did Not Affect Ca^{2+} Sparks

Transient activation of outward hyperpolarizing currents (STOCs), carried by BK channels, has been proposed as a mechanism for the paradoxical Ca^{2+} spark–mediated relaxation of small arteries.[27] Furthermore, STOCs have been proposed to provide a negative feedback mechanism to control Ca^{2+} spark dynamics through regulation of voltage-dependent calcium entry and resultant changes in sarcoplasmic reticulum Ca^{2+} loading and ryanodine receptor sensitization. We examined STOCs and Ca^{2+} spark activity in $BK^{-/-}$ cells. As expected, STOCs were completely absent in $BK^{-/-}$ cerebral arterial cells, even at depolarized membrane potentials that promote Ca^{2+} influx via voltage-gated Ca^{2+} channels (Figure 4a), implicating that STOCs are carried by BK channels. However, Ca^{2+} spark parameters were not affected in $BK^{-/-}$ cells, arguing against a feedback between STOCs and Ca^{2+} sparks via Ca^{2+} entry (Figure 4b). In summary, 2 mechanisms important for determining vascular tone were absent in $BK^{-/-}$ small arteries: (1) BK channel activity as a major effector of cGMP/PKG-mediated relaxation and (2) the steady hyperpolarizing effect in response to Ca^{2+} sparks. Hence, pathophysiological consequences for systemic blood pressure were expected in $BK^{-/-}$ mice.

Elevated Blood Pressure and Hyperaldosteronism in $BK^{-/-}$ Mice

MAP in the carotid artery, HR, and locomotor activity were measured by radiotelemetry in male mice. $BK^{-/-}$ mice were

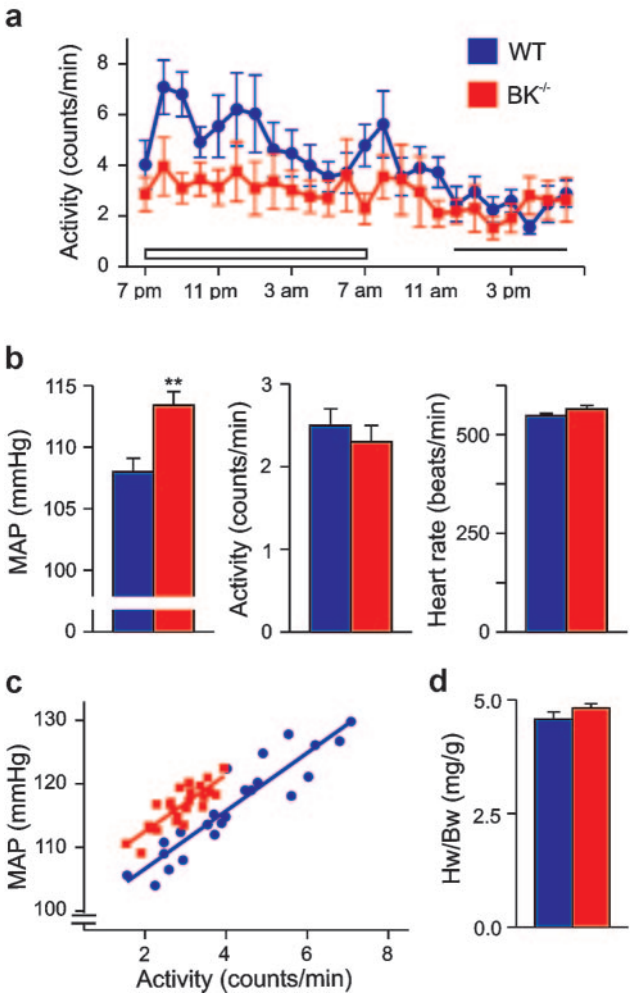

Figure 5. Elevated arterial blood pressure in $BK^{-/-}$ mice. a, Locomotor activity recorded by radiotelemetry for 24 hours at days 5, 6, and 7 after surgery. Bar indicates period of MAP and HR measurement shown below; open bar indicates dark phase. b, Mean values of MAP, HR, and activity from telemetric blood pressure analysis are calculated hour by hour between 12 AM and 6 PM, the period when WT and $BK^{-/-}$ mice exhibited comparable locomotor activity; n=7 per genotype. c, Correlations between locomotor activity and MAP. Linear regression lines were y=4.56x+97.5 (R^2=0.85) (WT) and y=4.44x+103.6 (R^2=0.69) ($BK^{-/-}$). d, Absence of cardiac hypertrophy in $BK^{-/-}$ mice. Statistics of heart weight (Hw)/body weight (Bw) (mg/g) from 10 to 11 mice per genotype are shown. All data are mean±SEM; *P<0.05; **P<0.01.

significantly less active during the dark phase as a result of motor impairment[15] (Figure 5a). Because physical activity affects MAP, we measured it during an interval (12 AM to 6 PM) when the 2 genotypes showed similar activity (Figure 5a, 5b). The $BK^{-/-}$ mice showed a significantly (5.4 mm Hg) higher MAP than WT mice (Figure 5b). Additionally, diastolic and systolic blood pressures were significantly elevated in the mutants, whereas HR did not differ. This excludes the possibility that higher sympathetic tone and HR caused MAP elevation. Analysis of the MAP of $BK^{-/-}$ and WT mice as a function of locomotor activity suggested that for any activity the MAP was shifted upward by 6.1 mm Hg in the mutants (Figure 5c). However, $BK^{-/-}$ mice did not show high-range locomotor activity because of their ataxia.[15] In contrast to $BK\beta_1$ knockouts,[6,7] the increase in blood pressure in $BK^{-/-}$ mice was not accompanied by significant cardiac hypertrophy in 4- to 6-month-old animals, as indicated by heart/body weight ratio (WT, 4.6±0.2 mg/g; $BK^{-/-}$, 4.8±0.1 mg/g) (Figure 5d). Nevertheless, the elevated MAP observed under resting conditions in $BK^{-/-}$ mice exhibiting HRs similar to those of WT mice supports the hypothesis that vascular BK channels may be important for the control of vascular tone and systemic blood pressure in vivo, mainly because of their effector role for both cGMP signaling and Ca^{2+} sparks.

However, blood pressure regulation also involves essential endocrine mechanisms such as the renin-angiotensin-aldosterone system, which might also be affected by BK channels.[28] Analysis of the serum electrolytes revealed a gender-independent decrease in the K^+ concentration ($[K^+]_{serum}$) in mutants compared with WT (male WT 8.3±0.3 mmol/L versus male $BK^{-/-}$ 6.5±0.8 mmol/L; female WT 9.2±0.2 mmol/L versus female $BK^{-/-}$ 7.5±0.4 mmol/L). In general, $[K^+]_{serum}$ values of WT mice were in agreement with previously published $[K^+]_{serum}$ values of mice with a genetic background similar to the $BK^{-/-}$ mice.[29] However, $[Na^+]_{serum}$ did not differ between the 2 genotypes (male WT 140±4 mmol/L versus male $BK^{-/-}$ 137±2 mmol/L; female WT 137±2 mmol/L versus female $BK^{-/-}$ 141±2 mmol/L) (Figure 6a). The lower $[K^+]_{serum}$ of $BK^{-/-}$ mice was associated with markedly increased serum aldosterone levels in both genders (male WT 185±24 pg/mL versus male $BK^{-/-}$ 573±108 pg/mL; female WT 236±33 pg/mL versus female $BK^{-/-}$ 597±132 pg/mL) (Figure 6b). Consistent with this phenotype, we found high levels of BK channel α subunit expression in the zona glomerulosa (Figure 6c). Here, these channels may control aldosterone production and/or release by influencing the membrane potential and hence the open probability of voltage-gated Ca^{2+} channels (L- and/or T-type) via the membrane potential.[30,31] In contrast to glomerulosa cells, cortisol-synthesizing cells from zona fasciculata were very weakly stained with the BK channel antibody (Figure 6c). In agreement herewith is the finding that corticosterone levels in the serum were not altered between WT and $BK^{-/-}$ mice (Figure 6d). A potential causative role of the renin/angiotensin system and/or the pituitary for the observed increase in aldosterone was also considered. The determination of serum corticotropin and serum renin activity, however, revealed no significant changes of these 2 parameters between both genotypes, suggesting that the hyperaldosteronism found in $BK^{-/-}$ mice was of a primary nature. Thus, elevation of blood pressure in $BK^{-/-}$ mice may be attributed to both vascular and hormonal dysfunctions.

Discussion

The deletion of the BK channel α subunit permitted the identification of physiological functions of this unique channel in regulating arterial blood pressure. $BK^{-/-}$ mice exhibit a moderate increase in blood pressure that was traced back to vascular and endocrine abnormalities. Our data indicate that the vascular abnormalities arise from lack of spontaneous outward currents that contribute to the mean resting membrane potentials in small and large vessels (Figure 1b; Data

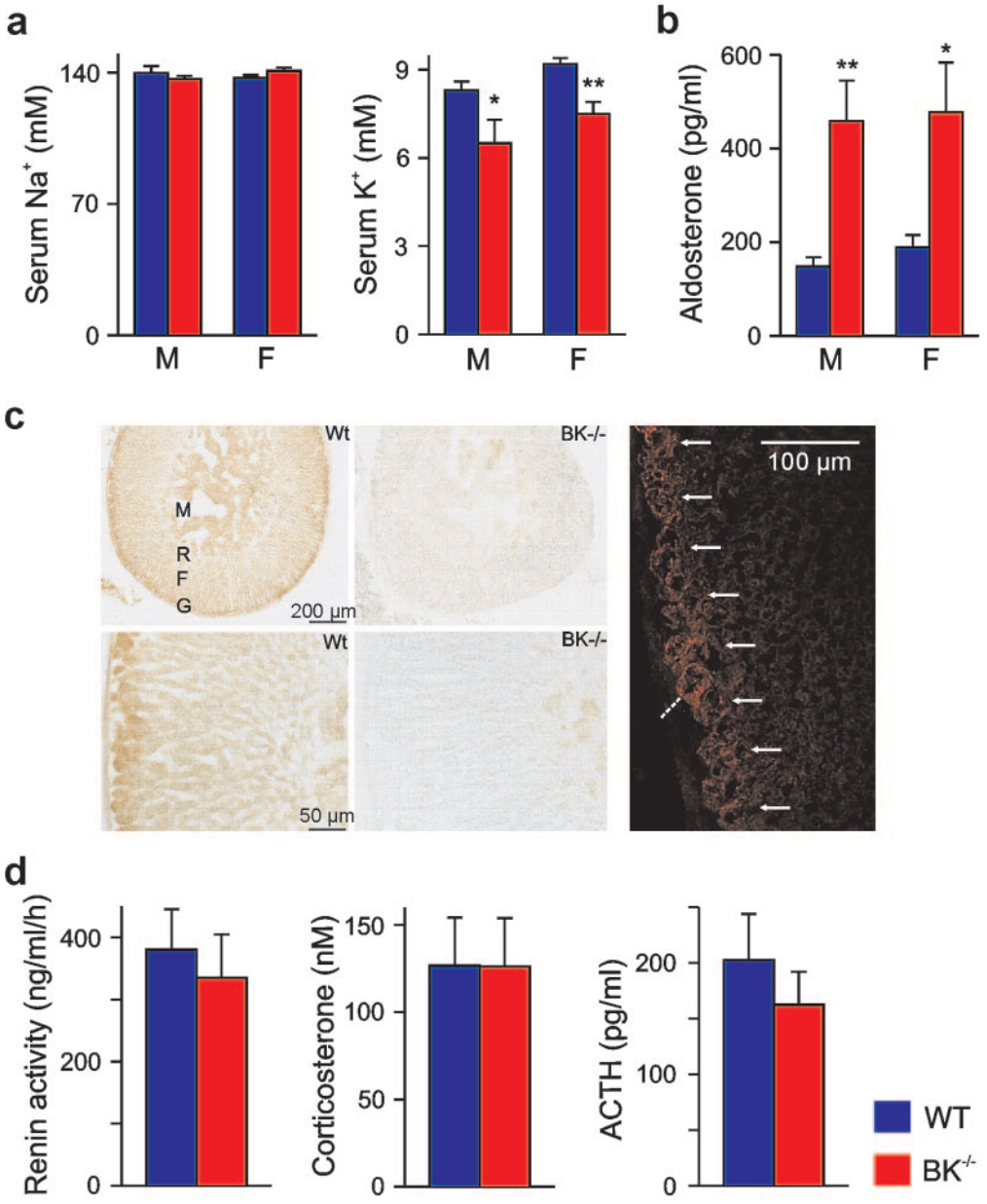

Figure 6. Primary hyperaldosteronism in $BK^{-/-}$ mice and BK channel expression in adrenal glomerulosa cells. a, Statistics of serum electrolyte concentrations from 6 to 9 male (M) and female (F) mice per genotype (WT, blue; $BK^{-/-}$, red). b, Statistical analysis of serum aldosterone from 6 to 9 mice per genotype and gender. c, Left, Immunohistochemical detection of BK channels in the adrenal gland by peroxidase/DAB staining. Prominent staining is found in the zona glomerulosa (G), very weak staining in the zona fasciculata (F) and reticularis (R), and intermediate staining in the adrenal medulla (M). Sections from $BK^{-/-}$ adrenal were not stained under identical conditions. Right, Confocal single optical section demonstrates specific BK immunofluorescence on the surface (dashed line) of zona glomerulosa cells (arrows) of adrenal cortex (bar=100 μm). $BK^{-/-}$ sections showed no staining (not shown). d, Statistical analysis of serum renin activity, corticosterone, and corticotropin (ACTH) levels from 8 to 16 mice per genotype. All data are mean±SEM; *$P<0.05$; **$P<0.01$.

Supplement Figure, panel b). Thus, the lack of BK channels may account for the observed membrane depolarization (Figure 1b), which will tend to increase myogenic tone, ie, vessel tone in response to intravascular pressure (Bayliss effect; Figure 2). The pathophysiology of an increased vascular tone in $BK^{-/-}$ mice apparently includes several mechanisms. First, we found that relaxation of myogenic tone by cGMP is impaired in $BK^{-/-}$ vessels. This finding indicates that the vascular BK channel is an important effector of the cGMP/cGMP kinase pathway. Previous studies, in which several mouse models with genetic ablations were used, have shown that this pathway contributes to basal blood pressure regulation.[24,32,33] By contrast, cAMP-mediated relaxation of small arteries involves effectors other than the BK channel, although cAMP kinase has also been shown to control BK channel activity in vascular smooth muscle cells.[34]

Second, BK channel deficiency abolished the transient outward K^+ currents that are induced by local Ca^{2+} release from internal stores or, alternatively, by Ca^{2+} influx through T-type Ca^{2+} channels, a mechanism that recently was pro-

posed to operate in coronary vessels.[35] Regardless of the Ca^{2+} source, the lack of transient K^+ currents should increase the open probability of voltage-gated Ca^{2+} channels, thus contributing to vasoconstriction of small arteries. In such arteries, myogenic tone in response to intravascular pressure was absent when L-type Ca^{2+} channels of the Cav1.2 type were inactivated specifically in smooth muscle.[36] This suggests that the increased myogenic tone in $BK^{-/-}$ arteries is mediated by enhanced Ca^{2+} influx through Cav1.2 channels, presumably because of the less negative membrane potential of arterial muscle cells and an attendant increase of the Cav1.2 "window current."[19] The subsequent increase of global $[Ca^{2+}]_i$, however, seems to be insufficient for stimulating the frequency or intensity of Ca^{2+} sparks evoked from intracellular stores (Figure 4b). Apart from these vascular dysfunctions, we found a marked hyperaldosteronism that probably contributes to the blood pressure elevation in $BK^{-/-}$ mice. Aldosterone, via the mineralocorticoid receptor, is the major regulator of ENaC expression and activity in the cortical collecting duct, thereby providing renal Na^+ reabsorption, which also necessitates increased water reabsorption to maintain Na^+ concentration at or near 140 mmol/L. Several rare forms of inherited hypertension are based on monogenetic defects associated with increased mineralocorticoid synthesis or dysfunctional aldosterone signaling (for a review, see Lifton et al[37]). We also found that BK channel protein is localized in the cortices of adrenal glands and is highest in zona glomerulosa cells therein. However, the physiological contribution of BK channels to aldosterone secretion from this cell layer is controversial.[28,38] Both T-type and L-type voltage-dependent Ca^{2+} channels are expressed in glomerulosa cells and have been implicated in steroidogenesis and aldosterone secretion.[30,31,39,40] Interestingly, both types have been described to be functionally associated with BK channels in other cell types.[18,35] Thus, loss of control of Ca^{2+} influx through Ca^{2+} channels in glomerulosa cells may be a plausible mechanism for the observed hyperaldosteronism in $BK^{-/-}$ mice. In support of this idea is the finding that neither the renin/angiotensin pathway nor pituitary hormones seem to evoke the hyperaldosteronism.

Considering that the $BK^{-/-}$ mice showed several synergistic mechanisms that all tend to increase blood pressure, it seems surprising that their blood pressure elevation was not more severe. The relative mildness of their blood pressure elevation was confirmed by the absence of significant cardiac hypertrophy in 4- to 6-month-old mutants (Figure 5d). We hypothesize that other peripheral or central regulators that are able to dampen blood pressure may be more active in $BK^{-/-}$ than in WT mice. This idea is supported by the finding that deletion of the less widespread regulatory BK channel $\beta 1$ subunit caused a more severe blood pressure elevation[6,7] (and consequently also significant cardiac hypertrophy in mutant mice[6]) than the deletion of the BKα subunit in the present study. The predominant expression of the BKβ_1 subunit in smooth muscle[5] suggests that $BK\beta_1^{-/-}$ mice rather than $BK\alpha^{-/-}$ mice represent the more selective "vascular" BK channel deletion, although lack of the BKβ_1 subunit leaves intact α subunits that can be still activated at physiological voltages by high local Ca^{2+}, cGMP/cGMP kinase, and additional pathways. Presumably, a selective deletion of only the vascular BKα subunit would produce an even more severe blood pressure elevation than $BK\beta_1^{-/-}$ deletion. However, in terms of revealing the basis of genetically anchored diseases and identifying susceptibility genes contributing to hypertension, the condition of a naturally occurring loss of function mutation of the BKα gene is best reflected by the general BK channel knockout as presented here.

In conclusion, the results of this study indicate that the systemic blood pressure phenotype of complete BK channel deletion is relatively mild—milder than expected—at least under resting conditions. We hypothesize that the renovascular role of the BK channel, which often acts as an "emergency brake",[41,42] may become more evident when studying microvasculature function and organ perfusions in $BK^{-/-}$ animals that are challenged with pathophysiological conditions such as ischemia.

Acknowledgments

We thank Dan-Yang Huang and Dr Volker Vallon for assisting us in flame photometry analysis, Isolde Breuning and Janina Smykowsky for excellent technical assistance, and Deutsche Forschungsgemeinschaft, Fonds zur Förderung der Wissenschaftlichen Forschung, The Wellcome Trust, Research Council of Norway, Thyssen-Stiftung and Schilling Foundation for financial support.

References

1. Harder DR, Smeda J, Lombard J. Enhanced myogenic depolarization in hypertensive cerebral arterial muscle. *Circ Res*. 1985;57:319–322.
2. Wellman GC, Cartin L, Eckman DM, Stevenson AS, Saundry CM, Lederer WJ, Nelson MT. Membrane depolarization, elevated Ca(2+) entry, and gene expression in cerebral arteries of hypertensive rats. *Am J Physiol*. 2001;281:H2559–H2567.
3. Brayden JE, Nelson MT. Regulation of arterial tone by activation of calcium-dependent potassium channels. *Science*. 1992;256:532–535.
4. Nelson MT, Cheng H, Rubart M, Santana LF, Bonev AD, Knot HJ, Lederer WJ. Relaxation of arterial smooth muscle by calcium sparks. *Science*. 1995;270:633–637.
5. Wallner M, Meera P, Ottolia M, Kaczorowski GJ, Latorre R, Garcia ML, Stefani E, Toro L. Characterization of and modulation by a beta-subunit of a human maxi KCa channel cloned from myometrium. *Receptors Channels*. 1995;3:185–199.
6. Brenner R, Perez GJ, Bonev AD, Eckman DM, Kosek JC, Wiler SW, Patterson AJ, Nelson MT, Aldrich RW. Vasoregulation by the beta1 subunit of the calcium-activated potassium channel. *Nature*. 2000;407:870–876.
7. Plüger S, Faulhaber J, Fürstenau M, Löhn M, Waldschütz R, Gollasch M, Haller H, Luft FC, Ehmke H, Pongs O. Mice with disrupted BK channel beta1 subunit gene feature abnormal Ca(2+) spark/STOC coupling and elevated blood pressure. *Circ Res*. 2000;87:E53–E60.
8. Amberg GC, Santana LF. Downregulation of the BK channel beta1 subunit in genetic hypertension. *Circ Res*. 2003;93:965–971.
9. Fernández-Fernández JM, Tomás M, Vazquez E, Orio P, Latorre R, Sentí M, Marrugat J, Valverde MA. Gain-of-function mutation in the KCNMB1 potassium channel subunit is associated with low prevalence of diastolic hypertension. *J Clin Invest*. 2004;113:1032–1039.
10. Fisslthaler B, Popp R, Kiss L, Potente M, Harder DR, Fleming I, Busse R. Cytochrome P450 2C is an EDHF synthase in coronary arteries. *Nature*. 1999;401:493–497.
11. Archer SL, Gragasin FS, Wu X, Wang S, McMurtry S, Kim DH, Platonov M, Koshal A, Hashimoto K, Campbell WB, Falck JR, Michelakis ED. Endothelium-derived hyperpolarizing factor in human internal mammary artery is 11,12-epoxyeicosatrienoic acid and causes relaxation by activating smooth muscle BK(Ca) channels. *Circulation*. 2003;107:769–776.
12. Han G, Kryman JP, McMillin PJ, White RE, Carrier GO. A novel transduction mechanism mediating dopamine-induced vascular relaxation: opening of BKCa channels by cyclic AMP-induced stimulation of the cyclic GMP-dependent protein kinase. *J Cardiovasc Pharmacol*. 1999;34:619–627.

13. Zhou XB, Arntz C, Kamm S, Motejlek K, Sausbier U, Wang GX, Ruth P, Korth M. A molecular switch for specific stimulation of the BKCa channel by cGMP and cAMP kinase. *J Biol Chem*. 2001;276: 43239–43245.
14. Payet MD, Bilodeau L, Drolet P, Ibarrondo J, Guillon G, Gallo-Payet N. Modulation of a Ca(2+)-activated K+ channel by angiotensin II in rat adrenal glomerulosa cells: involvement of a G protein. *Mol Endocrinol*. 1995;9:935–947.
15. Sausbier M, Hu H, Arntz C, Feil S, Kamm S, Adelsberger H, Sausbier U, Sailer CA, Feil R, Hofmann F, Korth M, Shipston MJ, Knaus HG, Wolfer DP, Pedroarena CM, Storm JF, Ruth P. Cerebellar ataxia and Purkinje cell dysfunction caused by Ca2+-activated K+ channel deficiency. *Proc Natl Acad Sci U S A*. 2004;101:9474–9478.
16. Millar JA, Leckie BJ, Morton JJ, Jordan J, Tree M. A microassay for active and total renin concentration in human plasma based on antibody trapping. *Clin Chim Acta*. 1980;101:5–15.
17. MacPhee IA, Antoni FA, Mason DW. Spontaneous recovery of rats from experimental allergic encephalomyelitis is dependent on regulation of the immune system by endogenous adrenal corticosteroids. *J Exp Med*. 1989; 169:431–445.
18. Liu G, Shi J, Yang L, Cao L, Park SM, Cui J, Marx SO. Assembly of a Ca(2+)-dependent BK channel signaling complex by binding to beta2 adrenergic receptor. *EMBO J*. 2004;23:2196–2205.
19. Fleischmann BK, Murray RK, Kotlikoff MI. Voltage window for sustained elevation of cytosolic calcium in smooth muscle cells. *Proc Natl Acad Sci U S A*. 1994;91:11914–11918.
20. Schubert R, Mulvany MJ. The myogenic response: established facts and attractive hypotheses. *Clin Sci (Lond)*. 1999;96:313–326.
21. Schubert R, Serebryakov VN, Mewes H, Hopp HH. Iloprost dilates rat small arteries: role of K(ATP)- and K(Ca)-channel activation by cAMP-dependent protein kinase *Am J Physiol*. 1997;272:H1147–H1156.
22. Sausbier M, Schubert R, Voigt V, Hirneiss C, Pfeifer A, Korth M, Kleppisch T, Ruth P, Hofmann F. Mechanisms of NO/cGMP-dependent vasorelaxation. *Circ Res*. 2000;87:825–830.
23. Kleppisch T, Nelson MT. Adenosine activates ATP-sensitive potassium channels in arterial myocytes via A2 receptors and cAMP-dependent protein kinase. *Proc Natl Acad Sci U S A*. 1995;92:12441–12445.
24. Pfeifer A, Klatt P, Massberg S, Ny L, Sausbier M, Hirneiss C, Wang GX, Korth M, Aszodi A, Andersson KE, Krombach F, Mayerhofer A, Ruth P, Fassler R, Hofmann F. Defective smooth muscle regulation in cGMP kinase I-deficient mice. *EMBO J*. 1998;17:3045–3051.
25. Furutani H, Zhang XF, Iwamuro Y, Lee K, Okamoto Y, Takikawa O, Fukao M, Masaki T, Miwa S. Ca2+ entry channels involved in contractions of rat aorta induced by endothelin-1, noradrenaline, and vasopressin. *J Cardiovasc Pharmacol*. 2002;40:265–276.
26. Schlossmann J, Ammendola A, Ashman K, Zong X, Huber A, Neubauer G, Wang GX, Allescher HD, Korth M, Wilm M, Hofmann F, Ruth P. Regulation of intracellular calcium by a signalling complex of IRAG, IP3 receptor and cGMP kinase Ibeta. *Nature*. 2000;404:197–201.
27. Jaggar JH, Porter VA, Lederer WJ, Nelson MT. Calcium sparks in smooth muscle. *Am J Physiol*. 2000;278:C235–C256.
28. Ganz MB, Nee JJ, Isales CM, Barrett PQ. Atrial natriuretic peptide enhances activity of potassium conductance in adrenal glomerulosa cells. *Am J Physiol*. 1994;266:C1357–C1365.
29. McDonald FJ, Yang B, Hrstka RF, Drummond HA, Tarr DE, McCray PB Jr, Stokes JB, Welsh MJ, Williamson RA. Disruption of the beta subunit of the epithelial Na+ channel in mice: hyperkalemia and neonatal death associated with a pseudohypoaldosteronism phenotype. *Proc Natl Acad Sci U S A*. 1999;96:1727–1731.
30. Hausdorff WP, Catt KJ. Activation of dihydropyridine-sensitive calcium channels and biphasic cytosolic calcium responses by angiotensin II in rat adrenal glomerulosa cells. *Endocrinology*. 1988;123:2818–2826.
31. Lotshaw DP. Role of membrane depolarization and T-type Ca2+ channels in angiotensin II and K+ stimulated aldosterone secretion. *Mol Cell Endocrinol*. 2001;175:157–171.
32. Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, Fishman MC. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. *Nature*. 1995;377:239–242.
33. Lopez MJ, Wong SK, Kishimoto I, Dubois S, Mach V, Friesen J, Garbers DL, Beuve A. Salt-resistant hypertension in mice lacking the guanylyl cyclase-A receptor for atrial natriuretic peptide. *Nature*. 1995;378:65–68.
34. Barman SA, Zhu S, White RE. Protein kinase C inhibits BKCa channel activity in pulmonary arterial smooth muscle. *Am J Physiol*. 2004;286: L149–L155.
35. Chen CC, Lamping KG, Nuno DW, Barresi R, Prouty SJ, Lavoie JL, Cribbs LL, England SK, Sigmund CD, Weiss RM, Williamson RA, Hill JA, Campbell KP. Abnormal coronary function in mice deficient in alpha1H T-type Ca2+ channels. *Science*. 2003;302:1416–1418.
36. Moosmang S, Schulla V, Welling A, Feil R, Feil S, Wegener JW, Hofmann F, Klugbauer N. Dominant role of smooth muscle L-type calcium channel Cav1.2 for blood pressure regulation. *EMBO J*. 2003; 22:6027–6034.
37. Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. *Cell*. 2001;104:545–556.
38. Lotshaw DP. Effects of K+ channel blockers on K+ channels, membrane potential, and aldosterone secretion in rat adrenal zona glomerulosa cells. *Endocrinology*. 1997;138:4167–4175.
39. Rossier MF, Python CP, Capponi AM, Schlegel W, Kwan CY, Vallotton MB. Blocking T-type calcium channels with tetrandrine inhibits steroidogenesis in bovine adrenal glomerulosa cells. *Endocrinology*. 1993;132: 1035–1043.
40. Spat A, Hunyady L. Control of aldosterone secretion: a model for convergence in cellular signaling pathways. *Physiol Rev*. 2004;84:489–539.
41. Gribkoff VK, Starrett JE, Dworetzky SI. Maxi-K potassium channels: form, function, and modulation of a class of endogenous regulators of intracellular calcium. *Neuroscientist*. 2001;7:166–177.
42. Hu H, Shao LR, Gu N, Chavoshy S, Tieb M, Behrens R, Laake P, Pongs O, Knaus HG, Ottersen OP, Storm JF. Presynaptic Ca2+-activated K+ channels in glutamatergic hippocampal terminals and their role in spike repolarization and regulation of transmitter release. *J Neurosci*. 2001;21: 9585–9597.

Supporting information: Figures

Supporting Fig. 1. Resting membrane potential in aortic smooth muscle cells. (**a**) Current-voltage relationship of K^+ outward currents in cells from 7 wt (circles) and 6 $BK^{-/-}$ (squares) mice. Whole-cell currents were measured at 300nM $[Ca^{2+}]_i$ from a holding potential of –20mV without and with 300 nM IbTx (respective data points are covered by the red squares). *Insets*: currents at +60 mV (step) from a holding potential of –20 mV of wt (top) and $BK^{-/-}$ (bottom) cells. The holding potential of –20 mV was used to inactivate K^+ outward currents other than BK. Similar results were obtained when using a holding potential of –50 mV, indicating that BK currents represent the major K^+ outward current in aortic cells. (**b**) Membrane potential recordings from wt and $BK^{-/-}$ aortic cells with and without IbTx, and mean membrane potential statistics (11 and 10 cells from wt and BK-/- mice, respectively)) ± SEM; **P*<0.05.

Supporting Information: In Detail Methods

Mice

BK channel-deficient mice ($BK^{-/-}$) were generated as described[15]. Wild type (wt) and $BK^{-/-}$ mice on the hybrid SV129/C57BL6 background (always F2 generation) were bred and maintained at the animal facility of the Pharmaceutical Institute, Department Pharmacology & Toxicology, University of Tübingen. Either litter- or age-matched animals were randomly assigned to the experimental procedures with respect to the German legislation on protection of animals.

Immunohistochemistry of adrenal gland

Immunhistochemistry was performed using on-slide 5 µm cryostat-slices from non-fixed wt and $BK^{-/-}$ adrenal glands. After preincubation with 10% normal donkey serum in buffer [1%

BSA, 0.5% Triton X-100, 0.05 M Tris-buffered saline (TBS)] and rinsing with TBS, the slices were incubated with anti-BK$\alpha_{(674-1115)}$ (1:1000 in buffer) and tagged with Alexa 555-conjugated donkey anti-rabbit IgG (1:1000 in buffer). BK channel immunofluorescence was analysed using a confocal-laser scanning microscope (Biorad MRC1000 attached to Nikon Diaphot 300 and equipped with a krypton-argon laser).

Immunohistochemical experiments applying the peroxidase/DAB detection were performed on 10 µm cryosections of wt and $BK^{-/-}$ adrenal glands perfused with 4% paraformaldehyde. Therefore tissue sections were permeabilized for 90 min with TBS (100 mM Tris, pH 7.2, 150 mM NaCl) containing 0.2% Triton X-100. After the endogenous peroxidase activity was blocked for 25 min with 0.9% H_2O_2 and 25% methanol in TBS the sections were incubated for 2h with 2% normal goat serum, 2% BSA, and 0.2% milk powder in TBS and 0.2% Triton X-100 (TBS-T). The anti-BK$\alpha_{(674-1115)}$ antibody was applied overnight (1:500 in TBS-T containing 1% BSA). Three rinses in TBS-T were performed before incubation with the secondary antibody (horseradish peroxidase-coupled goat anti-rabbit IgG, 1:400) for 2h. Finally the sections were washed with TBS and BK immunoreactivity was visualized using the common DAB method (Sternberg, 1979). Data analysis was performed using a Zeiss Axioplan 2 microscope equipped with a Zeiss Axio Cam digital camera.

Determination of renin activity, adrenocorticotropic hormone (ACTH, corticotrpin) and cortisterone levels from serum

Plasma renin concentration (PRC) was measured as the generation of angiotensin I (ng/ml/h) when plasma samples were incubated with excess renin substrate (plasma with no intrinsic renin activity from a binephrectomised rat). Angiotensin I was measured by radioimmunoassay as previously described[16]. For the determination of corticotropin (ACTH) in plasma we used a 2-site solid-phase immunoradiometric assay (IRMA)kit (Euria-acth IRMA kit)from Euro-diagnostica AB. Malmo, Sweden. This assay measures intact ACTH (1-

39) and in the assay used for this determination the limit of detection was 5pg/ml and the intra assay variation < 5%. Serum corticosterone was analysed using an in-house specific radioimmunoassay as described previously, and modified for microtitre plate scintillation proximity assay[17].

Serum electrolytes and serum aldosterone levels

Serum was separated from non-heparinized blood collected by heart puncture from wt and $BK^{-/-}$ mice sacrificed by inhalation of carbon dioxide. The serum concentrations of Na^+ and K^+ were measured by flame photometry (ELEX 6361, Eppendorf, Germany). Serum concentrations of aldosterone were measured by radioimmunoassay (Diagnostic Systems Laboratories, Sinsheim, Germany).

Electrophysiology of vascular smooth muscle cells

For cell isolation, tibial artery, a 4th order branch of the aorta, was carefully dissected and incubated in Ca^{2+}-free physiological saline solution (normal PSS; 130mM NaCl, 5.9mM KCl, 2.4mM $CaCl_2$, 1.2mM $MgCl_2$, 10mM HEPES, 11mM glucose [pH 7.4]) containing (in mg/ml) 0.7 papain, 1 (1,4)-dithio-D,L-threitol and 1 bovine serum albumin (BSA) at 37°C for 30 min. The buffer was then exchanged for PSS containing 0.05mM Ca^{2+}, 1mg/ml collagenase type H, 1mg/ml hyaluronidase, 1mg/ml BSA and digestion was continued for another 10 min at 37^{o}C, the remaining tissue was transferred to PSS and single cells were released by gentle trituration. The dispersed cells were kept at room temperature until electrophysiological measurements were performed. Membrane currents were measured in the whole-cell mode. For the recording of outward currents, the bath solution was PSS, the pipette solution contained (in mM) 136 KCl, 6 NaCl, 1.2 $MgCl_2$, 5 EGTA, 11 glucose, 3 dipotassium ATP and 10 HEPES (pH 7.4). The free Ca^{2+} concentration was adjusted to 300nM. The holding potential was -50mV and test pulses of 300 ms duration were applied every 5 s to potentials

ranging from -60 to +80mV. For recording of macroscopic Ca^{2+} channel currents, the bath solution contained (in mM) 130 tetraethylammonium chloride, 10 $BaCl_2$, 2 $MgCl_2$, 10 HEPES, 12 glucose and was buffered to pH 7.4 with tetraethylammonium hydroxide. The patch-pipette contained (in mM) 110 CsCl, 20 tetraethylammonium chloride, 2 $MgCl_2$, 10 ethylene glycol-bis(ß-aminoethyl ether)-N,N,N',N'-tetraacetic acid, 5 sodium ATP, 10 HEPES adjusted to pH 7.2 with CsOH. Cells were clamped every 5 s for 300 ms from a holding potential of –60 mV to +50 mV in 10 mV increments. The inward current was measured as peak inward current with reference to zero current. Membrane potentials were measured in the current-clamp mode by utilizing the whole-cell perforated-patch configuration of the patch-clamp technique. The bath solution was PSS, the pipette solution contained (in mM) 110 K aspartate, 30 KCl, 10 NaCl, 1 $MgCl_2$, 10 HEPES, and 0.05 EGTA (pH 7.2) and 250µg/ml nystatin. All signals were low-pass filtered at a cut-off frequency of 1 kHz and digitized at 5 kHz. Data acquisition and analysis was performed with an ISO-3 multitasking patch-clamp program (MFK, Niedernhausen, Germany).

Aorta. Isolation and electrophysiological characterization of aortic smooth muscle cells was performed in the way described above except that in the voltage-clamp experiments the holding potential was –20 mV and potentials ranged from –60 to +60 mV.

Luminal diameter analysis of small arteries by videomicroscopy

Tibial small arteries were equilibrated in buffer (in mM) 120 NaCl, 4.5 KCl, 1.2 NaH_2PO_4, 1 $MgSO_4$, 1.6 $CaCl_2$, 0.025 EDTA, 5.5 glucose, 26 NaHCO3 and 5 Na-HEPES (pH 7.4). After mounting the small artery a permanent transmural pressure of 80 mmHg was applied under non-flow conditions. The chamber was continuously perfused at a rate of 2 ml/min with buffer at 37.0±0.5 °C. The small artery was allowed to equilibrate until a stable myogenic tone spontaneously developed after 15-20 min. The myogenic tone decreased inner vessel diameter to 50 to 70% of the diameter obtained in Ca^{2+}-free buffer. At the end of each ex-

periment, Ca^{2+}-free buffer containing (in mM) 146 NaCl, 4.5 KCl, 1.2 NaH_2PO_4, 1 $MgSO_4$, 1 EGTA, 5.5 glucose, 5 Na-HEPES (pH 7.4) was applied to determine maximal vessel diameters. No significant differences in maximal vessel diameters were noticed between both genotypes (wt: 69±3 µm; BK-/-: 63±2 µm). All compounds were administered to the adventitial side of the pressurized small arteries. To exclude prostaglandin effects, buffer contains 1µM diclofenac.

$[Ca^{2+}]_i$ measurements in aortic smooth muscle cells

Single aortic smooth muscle cells were prepared as described above and kept on ice for 15 min to recover. Dispersed cells were loaded with 2.5µM Fura 2-AM for 60 min at room temperature. Excess buffer containing Fura 2-AM was removed by centrifugation at 1200 rpm for 3 min. Cells were washed with PSS, placed on ice for 10-15 min, slowly resuspended in 300µl PSS and kept on ice until use. For $[Ca^{2+}]_i$ measurement 10µl of the suspension were transferred on a glass coverslip coated with 0.01% Poly-L-Lysine solution and continuously superfused with PSS at 36°C and a flow rate of 2-4ml/min. $[Ca^{2+}]_i$ measurements were performed using the dual-wavelength microfluorescence technique. Two $[Ca^{2+}]_i$ transients were elicited consecutively with a 15 min interval in between to allow refilling of intracellular Ca^{2+} stores.

STOC and Ca^{2+} spark analysis in cerebral artery cells

Cerebral arteries were dissected, freed from connective tissue and thereafter placed in Ca^{2+}-free Hanks solution containing (in mM) 55 NaCl, 80 sodium glutamate, 5.6 KCl, 2 $MgCl_2$, 1 mg/ml BSA, 10 glucose and 10 HEPES (pH 7.4) supplemented with 0.5 mg/ml papain and 1 mg/ml dithiothreitol for 15 minutes at 36°C. The segments were then placed in Hanks solution containing 1 mg/ml collagenase type F and H (Sigma, ratio 30% and 70%, respectively) and 0.1mM $CaCl_2$ for 6 minutes at 36°C. After several washes in Ca^{2+}-free Hanks solution,

single cells were dispersed from artery segments by gentle trituration in Ca^{2+}-free solution and maintained in the same solution at 4°C until experiment starts. From these cells, STOCs were electrophysiologically measured in the perforated patch mode. Briefly, holding potential was set at -60mV while depolarizing test potentials were applied in 10mV steps from -60 to 0mV. The bath solution contained (in mM) 134 NaCl, 6 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES and 10 glucose (pH 7.4), whereas the pipette solution was composed of (in mM) 30 KCl, 110 K aspartate, 10 NaCl, 1 $MgCl_2$, 0.05 EGTA, 10 HEPES (pH 7.2), and 250µg/ml amphotericin.

To measure Ca^{2+} sparks, cerebral artery smooth muscle cells were seeded onto glass coverslips and incubated with the calcium fluorophor fluo-3/AM (10µM) and Pluronic acid (0.05%) for 30 minutes at room temperature in Ca^{2+}-free Hanks solution. Ca^{2+} sparks were measured as local fractional fluorescence increases (F/F_0) by confocal fluorescence microscopy at room temperature in a solution containing (in mM) 135 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 10 HEPES (pH 7.4). The baseline fluorescence (F_o) was determined by averaging line-scan images without occurring Ca^{2+} sparks. Ca^{2+} spark duration was determined as the time from peak to 50% of spark amplitude. All recordings were filtered and normalized.

Long-term telemetric blood pressure analysis

A radiotelemetric device (Data Sciences International, St. Paul, MN) was used for long-term analysis of mean arterial blood pressure (MAP), heart rate (HR) and physical activity in conscious male wt and $BK^{-/-}$ mice (n=7 for each genotype). Mice were three to four months old, either litter- or age-matched and did not significantly differ in body size and weight (26.2±1.3 g and 23.5±0.7 g, respectively). The mice were anaesthetized using a conventional isoflurane inhalation regime and placed on a heating pad. A ventral midline incision was performed prior to careful isolation of the left common carotid artery. For ligation and retraction, 2 silk ligatures were passed under the vessel, one about 0.8 cm caudal to the bifurcation of the inte-

rior and exterior carotid artery and another about 0.5 cm rostral from the caudal ligature. A tiny incision in the carotid artery was made for insertion of the catheter. The inserted catheter tip was advanced to the thoracic aorta and fixed with suture. A subcutaneous pocket was performed along the right flank for placing the transmitter body. After subcutaneous fixation of the transmitter the incision was closed using 6-0 silk. Inhalation anesthesia was stopped and mice were kept under infrared light for 2 hours. Their food and water intake, wound healing and morbidity was kept under surveillance. Five days after surgery, mice have regained normal locomotor activity. MAP, HR and physical activity were recorded at days 5-7 after surgery. Radiotelemetric data were continuously sampled for 1 minute at 5-min intervals and stored using the Dataquest ART data acquisition system (DSI).

Supporting Information
Fig. 1