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# Altered Expression of Small-Conductance $Ca^{2+}$-Activated $K^+$ (SK3) Channels Modulates Arterial Tone and Blood Pressure

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***Abstract*—**The endothelium is a critical regulator of vascular tone, and dysfunction of the endothelium contributes to numerous cardiovascular pathologies. Recent studies suggest that apamin-sensitive, small-conductance, $Ca^{2+}$-activated $K^+$ channels may play an important role in active endothelium-dependent vasodilations, and expression of these channels may be altered in disease states characterized by vascular dysfunction. Here, we used a transgenic mouse ($SK3^{T/T}$) in which SK3 expression levels can be manipulated with dietary doxycycline (DOX) to test the hypothesis that the level of expression of the SK subunit, SK3, in endothelial cells alters arterial function and blood pressure. SK3 protein was elevated in small mesenteric arteries from $SK3^{T/T}$ mice compared with wild-type mice and was greatly suppressed by dietary DOX. SK3 was detected in the endothelium and not in the smooth muscle by immunohistochemistry. In whole-cell patch-clamp experiments, SK currents in endothelial cells from $SK3^{T/T}$ mice were almost completely suppressed by dietary DOX. In intact arteries, SK3 channels contributed to sustained hyperpolarization of the endothelial membrane potential, which was communicated to the arterial smooth muscle. Pressure- and phenylephrine-induced constrictions of $SK3^{T/T}$ arteries were substantially enhanced by treatment with apamin, suppression of SK3 expression with DOX, or removal of the endothelium. In addition, suppression of SK3 expression caused a pronounced and reversible elevation of blood pressure. These results indicate that endothelial SK3 channels exert a profound, tonic, hyperpolarizing influence in resistance arteries and suggest that the level of SK3 channel expression in endothelial cells is a fundamental determinant of vascular tone and blood pressure. (***Circ Res*. 2003;93: 124-131.)**

**Key Words:** endothelium ■ potassium channels ■ vascular tone ■ blood pressure

Blood pressure and flow are regulated by the constriction and dilation of resistance arteries, generally with internal diameters $<300$ $\mu$m.[1] Physiological stimulation through elevations in intravascular pressure or increased sympathetic activity promotes smooth muscle depolarization, intracellular $Ca^{2+}$ influx, and vasoconstriction. The resulting increase in total peripheral resistance within the vasculature increases blood pressure.[2]

The endothelium exerts a dilating influence that opposes arterial constriction. Activation of $K^+$ channels is thought to contribute to this influence through increased release of relaxing factors such as NO and prostacyclin ($PGI_2$) and through smooth muscle hyperpolarization.[3–5] The small-conductance $Ca^{2+}$-activated $K^+$ (SK) channel has received considerable attention as a potential mediator of these responses. SK channels are opened by intracellular $Ca^{2+}$ via an association with calmodulin[6] and are believed to play a role in the modulation of tissue excitability.[7] Of the three characterized SK channel isoforms (SK1, SK2, and SK3),[8] mRNA for SK2 and SK3 has been identified in endothelial cells.[9] Apamin, a toxin blocker of SK channels, either alone or in combination with charybdotoxin, a blocker of intermediate-conductance (IK) and large-conductance (BK) $Ca^{2+}$-activated $K^+$ channels, has been reported to inhibit arterial relaxations to various endothelium-dependent vasodilators, such as acetylcholine, bradykinin, substance P, and ATP in numerous vascular beds.[10–13] Acute apamin-sensitive relaxations are most often attributed to hyperpolarization of arterial smooth muscle,[14,15] which may involve myoendothelial gap junctional communication,[16] or the action of an undefined factor referred to as endothelium-derived hyperpolarizing factor.[17–19] Particular interest in endothelial SK3 channels has been heightened by recent observations that the vascular dysfunctions resulting from balloon catheter injury[20] and cirrhosis[21] are associated with changes in endothelial SK3 expression. However, previous approaches have not allowed

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for specific isolation of these channels for experimental study. Consequently, the role of endothelial SK3 channels in the regulation of cardiovascular function is not known.

In the present study, we tested the hypothesis that SK3 channels influence tonic endothelium-dependent vasoregulation and that manipulation of SK3 channel gene expression alters vascular tone and blood pressure. We used a transgenic mouse ($SK3^{T/T}$) harboring genetically targeted alleles for the SK3 channel, in which SK3 gene expression can be experimentally controlled by dietary doxycycline (DOX).[22] In the absence of DOX, the SK3 gene is ≈3-fold overexpressed in this mouse compared with the wild-type mouse, as evidenced by expression patterns in brain and uterine tissues, with no change in the normal temporal and cell-type–specific profile of SK3 channel expression. Addition of dietary DOX greatly suppresses or abolishes SK3 gene expression. Through acute and specific control of a single gene product, the functional impact of SK3 channel expression could be specifically assessed. Moreover, reversible upregulation and downregulation of SK3 expression allowed us to assess the effects of a range of SK3 expression levels in vivo.

## Materials and Methods

Conditional SK3 expression ($SK3^{T/T}$) was achieved through homologous recombination by insertion of a gene switch targeting the SK3 gene, as previously described.[22] A regulatory cassette was inserted into the 5′ untranslated sequence of the gene, encoding the binary tetracycline-sensitive transactivator (tTA) protein. The native SK3 promotor drives expression of the functional gene via tTA such that promoter function and tissue distribution are conserved, and experimental modulation of SK3 expression is achieved via dietary exposure to the tetracycline derivative DOX for at least 5 days. Homozygous SK3-targeted mice ($SK3^{T/T}$) and wild-type mice used for the present study were of the same background strain (c57BL/6). Genotypes were confirmed by reverse transcription (RT)–polymerase chain reaction (PCR). Mice were euthanized via intraperitoneal injection of sodium pentobarbital and subsequent decapitation, in accordance with the University of Vermont Institutional Animal Care and Use Committee and the National Institutes of Health's *Guide on the Humane Treatment of Experimental Animals*. Some mice were given 0.5 mg/mL DOX and 2% sucrose in the drinking water for at least 6 days.

### Western Blotting and Quantitative PCR

Homogenized mesenteric arteries were subjected to SDS-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane. After exposure to rabbit anti-SK3 (primary) antibody (Alomone Laboratories) and to goat anti-rabbit (secondary) antibody, visualization was achieved by chemiluminescence. SK3 protein was quantified relative to α-GAPDH. For PCR, the mesenteric artery (≈75 mg) was pulverized under liquid nitrogen. Total RNA was extracted in 200 μL TriReagent and converted to single-stranded cDNA using murine Moloney leukemia virus reverse transcriptase. Real-time PCR, using SYBR Green, was performed on each sample in duplicate for β-actin (85 bp) and SK3 (75 bp), and a standard curve for each amplicon was prepared.

### Immunofluorescence

Segments of mesenteric artery were fixed with 4% formaldehyde for 15 minutes. Cryosections and whole-mount artery preparations were permeabilized with 0.2% Triton X, blocked with 1% BSA, and stained using rabbit anti-SK3 (primary) and Cy5 anti-rabbit (secondary) antibodies. Immunofluorescence images were obtained with a laser confocal Bio-Rad microscope (excitation 650 nm and emission 670 nm for Cy5). For whole-mount arteries, cell nuclei were identified with the cyanine nuclear dye YOYO-1 (excitation 490 nm and emission 510 nm).

### Patch-Clamp Electrophysiology

Aortas were removed and placed in ice-cold $Ca^{2+}$-free solution containing (mmol/L) KCl 6, NaCl 134, $MgCl_2$ 1, glucose 10, and HEPES 10 (pH 7.4). Vessels were cut into rings and digested for 50 minutes at 37°C in isolation solution containing (mmol/L) KCl 6, NaCl 60, sodium glutamate 85, $MgCl_2$ 2, $CaCl_2$ 1, glucose 10, and HEPES 10 (pH 7.3) as well as 4 mg/mL protease from *Bacillus polymyxa* and 1 mg/mL hyaluronidase. Elastase (0.1 mg/mL) was included for the final 10 minutes. Rings were washed and slit open, and single endothelial cells were obtained by gentle trituration. The whole-cell configuration of the patch-clamp technique was used to record membrane currents. Whole-cell currents were amplified by an Axopatch 200B, low-pass–filtered (1 kHz), digitized (20-kHz sample rate), and analyzed using a Digidata 1322A interface and Clampex & Clampfit software (Axon Instruments). The bathing solution contained (mmol/L) KCl 6, NaCl 134, $MgCl_2$ 1, $CaCl_2$ 2, glucose 10, and HEPES 10 (pH 7.4) along with charybdotoxin (100 nmol/L) to block IK channels. The pipette solution contained (mmol/L) KCl 134, $MgCl_2$ 5.53, $CaCl_2$ 0.207, HEPES 10, and HEDTA 5, adjusted to pH 7.2. Free [$Ca^{2+}$] in the pipette (intracellular) was 3 μmol/L, and [$Mg^{2+}$] was 1 mmol/L, as calculated using WinMAXC software.[23]

### Membrane Potential Measurements

For endothelial cell measurements, mesenteric arteries (200 to 300 μm) were cut longitudinally and pinned, luminal side up, to the bottom of a small chamber lined with Sylgard. The chamber was continuously superfused with warm (37°C), gassed (95% $O_2$/5% $CO_2$) physiological saline solution (PSS) containing (mmol/L) NaCl 119, KCl 4.7, $NaHCO_3$ 24, $KH_2PO_4$ 0.2, EDTA 1.1, $MgSO_4$ 1.2, $CaCl_2$ 1.6, and glucose 10.6, pH 7.4. Sharp glass electrodes (≈100-MΩ resistance) containing 1 mol/L KCl were advanced to the intimal surface using a micromanipulator, and measurements were made using an electrometer (World Precision Instruments). Data were recorded via computer using Axotape and Dataq software. For some experiments, electrode tips were loaded with the fluorescent dye propidium iodide (1% in 1 mol/L KCl), allowing for unambiguous identification of endothelial cells.[24] Multiple measurements (3 to 6) from each artery were averaged before and after apamin and were counted as a single n. For smooth muscle measurements, mesenteric arteries (100 to 200 μm) were cannulated, pressurized, and superfused with PSS. Membrane potentials were measured using electrodes containing 0.5 mol/L KCl.

### Diameter Measurements

Mesenteric arteries were dissected in cold PSS. Artery segments (100 to 200 μm) were cannulated on glass pipettes, pressurized (with no flow), and superfused with warmed, gassed PSS as described above. Arterial diameter was measured using a video dimension analyzer (Living Systems Instrumentation) and displayed and recorded by computer using Axotape software. Denudation of the endothelium was accomplished by placing an air bubble in the vessel lumen for 3 minutes and was confirmed by the lack of acetylcholine-mediated dilation. All concentration-effect curves to phenylephrine (PE) were performed at an intravascular pressure of 20 mm Hg.

### Blood Pressure Measurements

Pressures were measured using a noninvasive tail-cuff monitor (NIBP-2, Columbus Instruments). Measurements were performed in 8 $SK3^{T/T}$ mice (4 males and 4 females, aged ≥4 months) over a 5- to 7-day period. Pressures were measured again after exposure to DOX (>2 weeks) and again after removal of DOX (>2 weeks) from the drinking water. The average of 10 measurements was taken as a representative pressure for each animal per day. Similar measurements were made in 8 wild-type mice before and after DOX exposure.

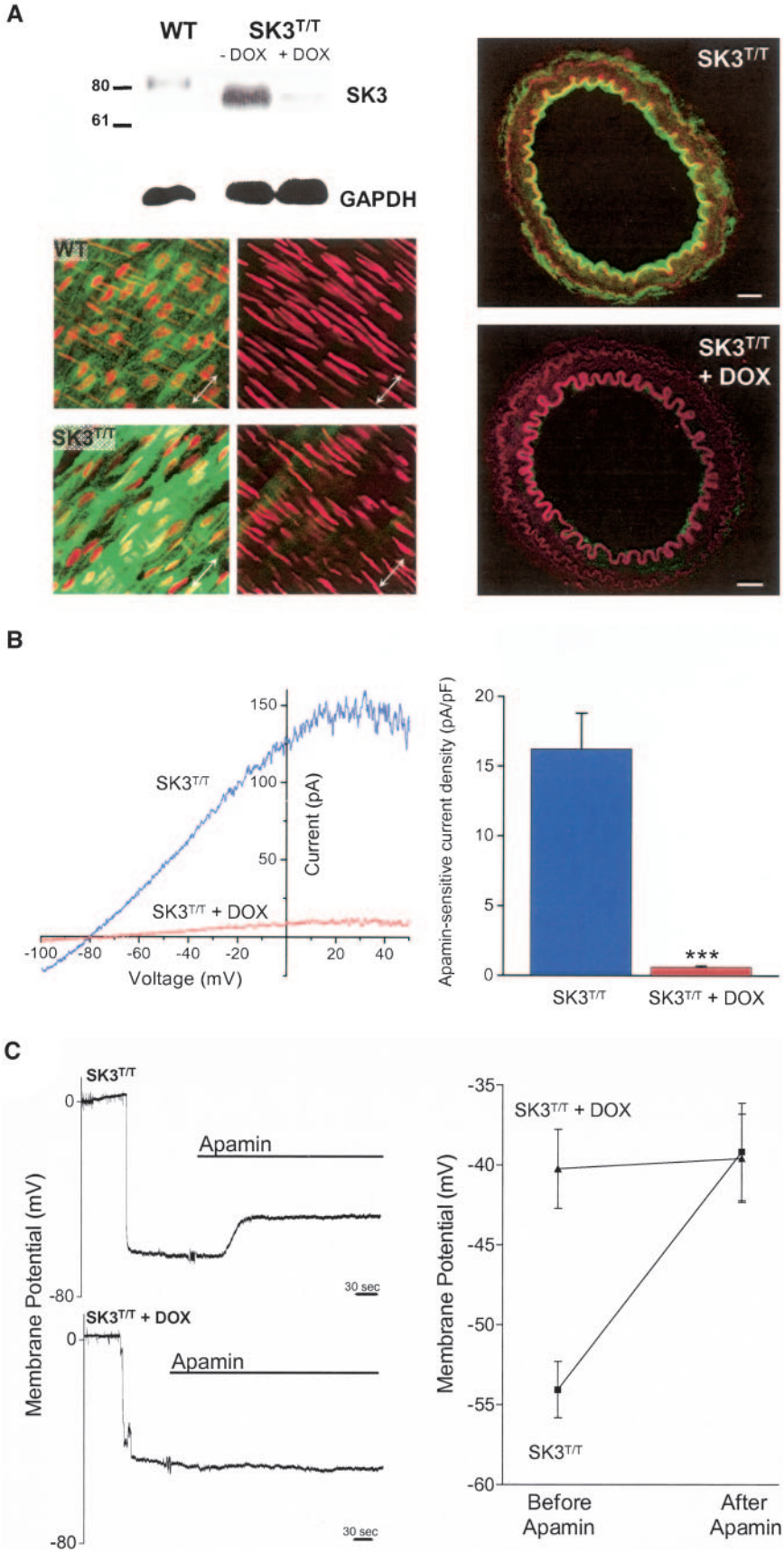


**Figure 1.** SK3 overexpression and suppression in endothelial cells. A, top left, Western blots from a wild-type (WT) control mouse, possessing only native alleles for the SK3 channel, an untreated $SK3^{T/T}$ mouse (−DOX), possessing two tTA-targeted alleles, and an $SK3^{T/T}$ mouse treated with 0.5 mg/mL DOX (+DOX) for 6 days. The apparent size difference between the SK3 proteins from $SK3^{T/T}$ and WT mice is due to a 40-amino acid deletion as previously described.[19] A, right, Immunofluorescence labeling of mesenteric artery cross sections from untreated ($SK3^{T/T}$) and DOX-treated ($SK3^{T/T}$+DOX) mice. SK3-labeled fluorescence is shown in green. Autofluorescence of the mesenteric artery wall (red) clearly defines the internal elastic lamina. Bar=20 $\mu$m. A, bottom left, En face confocal images of whole-mount mesenteric arteries from WT and $SK3^{T/T}$ mice. Endothelial (left) and smooth muscle (right) cell nuclei are shown in red. Substantial SK3-positive staining (green) was detected in endothelial cells, aligned with the axis of the vessel (arrows), but not in smooth muscle cells. B, Endothelial cell currents. B, left, Apamin-sensitive current-voltage relationships in endothelial cells from untreated (blue) and DOX-treated (red) $SK3^{T/T}$ mice. Currents were recorded in response to a 200-ms voltage ramp from −100 to 50 mV before and after exposure to apamin. Apamin-sensitive currents reversed close to the calculated electrochemical equilibrium potential for $K^+$, −83 mV (pipette $[Ca^{2+}]$=3 $\mu$mol/L). B, right, Mean apamin-sensitive current densities in endothelial cells ($V_m$=0 mV) from untreated (blue, n=7) and DOX-treated (red, n=7) $SK3^{T/T}$ mice. ***$P<0.001$. C, Endothelial cell membrane potentials. Continuous recordings (left) and summary data (right) show resting membrane potentials and effects of apamin on arteries from untreated and DOX-treated $SK3^{T/T}$ mice ($SK3^{T/T}$, n=6; $SK3^{T/T}$+DOX, n=5).

### Materials

SYBR Green, Cy5, and YOYO-1 were purchased from Molecular Probes. Hyaluronidase and elastase were obtained from Worthington, and anti-SK antibody was supplied by Alomone Laboratories. Unless otherwise noted, all other substances and reagents were purchased from Sigma.

### Data Analysis

Comparisons between groups were made with unpaired or paired *t* tests. Multiple-group data were subjected to 2-way ANOVA with repeated measures, and individual comparisons were made by Bonferroni post hoc test analysis where appropriate. Values of $P<0.05$ were considered statistically significant. Data are given as mean±SEM.

## Results

### Overexpression and Suppression of SK3 Channels in Resistance Arteries

SK3 protein levels were detected in small (≈200-μm) mesenteric arteries from wild-type mice and were elevated in arteries from $SK3^{T/T}$ mice (Figure 1A). Dietary DOX reduced SK3 protein to well below the levels found in wild-type mice. Quantitative RT-PCR showed that DOX exposure decreased SK3 mRNA levels by 97% in arteries from $SK3^{T/T}$ mice. In arteries from wild-type and $SK3^{T/T}$ mice, SK3 protein was largely localized to the endothelial cell layer that lines the arterial lumen, with little or no expression detected in the smooth muscle. SK3 immunofluorescence was essentially undetectable in DOX-treated mice. Thus, SK3 was highly expressed in the endothelium of mesenteric resistance arteries from $SK3^{T/T}$ mice and suppressed by dietary DOX.

### SK3 Channels Promote Tonic Hyperpolarization of the Endothelium

To determine whether SK3 protein levels in $SK3^{T/T}$ mice are correlated with the expression of functional channels in the vascular endothelium, we measured SK currents in freshly dissociated endothelial cells. SK currents, identified as apamin-sensitive (300-nM) $K^+$ currents, were ≈25-fold larger in endothelial cells from untreated $SK3^{T/T}$ mice compared with endothelial cells from DOX-treated mice (Figure 1B).

The measured currents suggested that SK3 channels should contribute substantially to the endothelial cell membrane potential. To test this hypothesis, we measured membrane potential in intact endothelium using microelectrodes. Blocking SK channels with apamin depolarized the intact endothelium by ≈15 mV in $SK3^{T/T}$ mesenteric arteries (−54.1±1.8 versus −39.2±3.0 mV, n=6), indicating that the SK conductance contributed substantially to the endothelial membrane potential (Figure 1C). Compared with $SK3^{T/T}$ mice, the membrane potential of endothelial cells from DOX-treated $SK3^{T/T}$ mice (n=5) was depolarized by ≈14 mV (−54.1±1.8 versus −40.2±2.5 mV, $P<0.001$). Apamin had no effect on membrane potential in these cells (−40.2±2.5 versus −39.6±2.8 mV), indicating that they were already considerably depolarized as a result of DOX-induced suppression of SK3 expression. These results indicate that SK3 channels promote tonic hyperpolarization of the endothelium depending on their level of expression.

### Tonic SK3-Mediated Endothelial Hyperpolarization Is Communicated to Arterial Smooth Muscle

We hypothesized that the SK3 channel effects on endothelial membrane potential may be translated to adjacent smooth muscle, either through direct communication via myoendothelial gap junctions or through the release of a diffusible factor. Apamin substantially depolarized the membrane potential of smooth muscle cells in intact $SK3^{T/T}$ arteries (Figure 2) by 11.9±1.2 mV at an intravascular pressure of 60 mm Hg. Removal of the endothelium led to similar smooth muscle depolarization (≈15 mV), which was not further affected by the addition of apamin, indicating that endothelial SK3 channels elicit tonic hyperpolarization of adjacent smooth muscle.

### Endothelial SK3 Channels Attenuate Arterial Tone

Tonic hyperpolarization of the smooth muscle via endothelial SK3 channels should decrease arterial tone by reducing $Ca^{2+}$ influx through voltage-dependent $Ca^{2+}$ channels in the smooth muscle cells.[25,26] To test the functional influence of endothelial SK3 expression in resistance arteries, we measured arterial tone at different levels of intravascular pressure. Pressure-induced increases in arterial tone ("myogenic tone") contribute substantially to the regulation of peripheral resistance and blood pressure.[27,28] Mesenteric arteries from $SK3^{T/T}$ mice exhibited little arterial tone (determined by the difference between active and passive diameters at a given pressure) to graded increases in intravascular pressure (Figure 3A), suggesting a tonic dilating influence of SK3 channels. Apamin caused a sustained constriction of $SK3^{T/T}$ arteries at low pressure and elevated arterial tone at all pressure levels tested. Suppression of SK3 expression with DOX

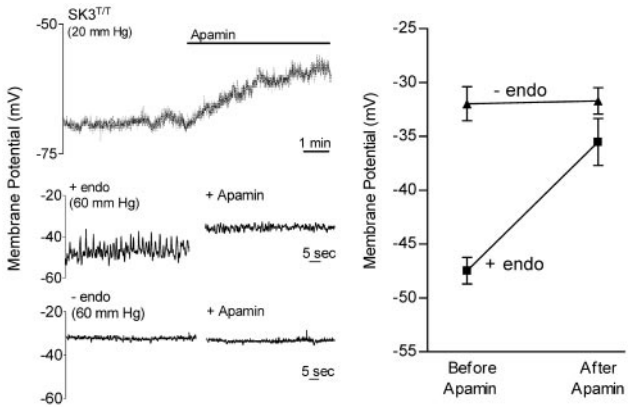


**Figure 2.** Influence of endothelial SK3 channels on vascular smooth muscle membrane potential. Continuous recording (top left) of smooth muscle membrane potential in a pressurized (20 mm Hg) $SK3^{T/T}$ artery, showing direct depolarization to apamin. Measurements were also made at 60 mm Hg (bottom left), a more physiological condition in which intravascular pressure normally elicits depolarization of smooth muscle and vasoconstriction. Apamin-induced constriction prevented continuous impalement at this pressure. Left, Individual recordings from intact ($SK3^{T/T}$) and endothelium-denuded ($SK3^{T/T}$ −endo) arteries before and after apamin. Right, Summary plot showing data recorded at 60 mm Hg in intact (+endo, n=6) and denuded (−endo, n=5) arteries.

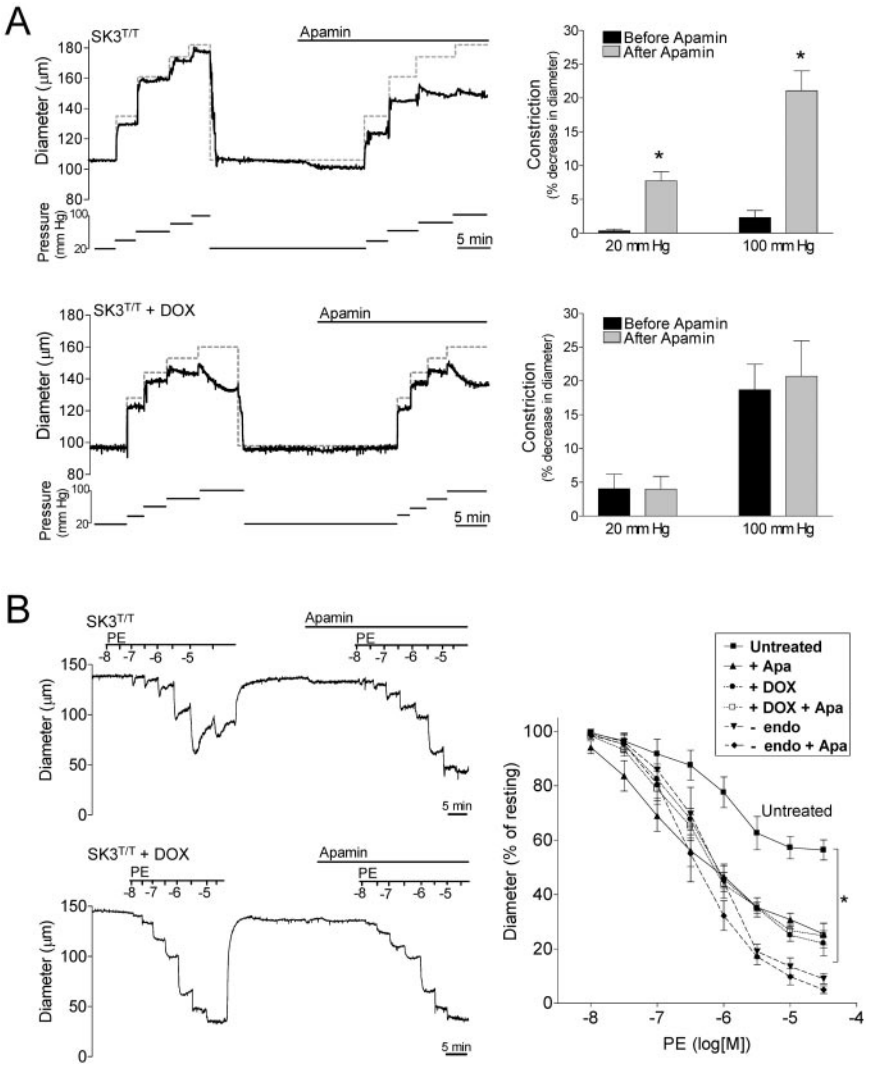


**Figure 3.** Influence of SK3 channels on vascular function. A, Pressure-induced (myogenic) constriction in mesenteric arteries from untreated $SK3^{T/T}$ mice (top) and $SK3^{T/T}$ mice receiving dietary DOX (bottom). Original traces (left) show diameter changes in response to increasing intravascular pressure in the absence or presence of the SK channel blocker apamin. Dotted lines show passive diameters (determined in $Ca^{2+}$-free PSS containing 2 mmol/L EGTA and 1 μmol/L nisoldipine) at each pressure. Pressure-induced constriction (right) was determined by the difference between active and passive diameters. *$P<0.05$ vs responses before apamin (n=6). B, PE-induced constrictions. On the left, original tracing shows mesenteric artery diameter changes in response to increasing concentrations of PE in the absence or presence of the SK channel blocker apamin. On the right, PE concentration-effect curves are shown under the following conditions: arteries from $SK3^{T/T}$ mice before and after apamin (untreated and +Apa, n=8), arteries from DOX-treated $SK3^{T/T}$ mice before and after apamin (+DOX and +DOX+Apa, n=8), and endothelium-denuded arteries from $SK3^{T/T}$ mice before and after apamin (−endo and −endo+Apa, n=7). *$P<0.05$ for maximal PE-induced constriction between untreated vs all other groups.

increased pressure-induced tone to a level similar to that observed in arteries exposed to apamin. Apamin had no effect on pressure-induced tone in the DOX-treated group. These results indicate that tonic SK3 channel activity attenuates myogenic tone.

Sympathetic nerve activity, through stimulation of α-adrenergic receptors on smooth muscle cells, is an important regulator of mesenteric artery function and vascular resistance. The α-adrenergic receptor agonist PE increased arterial tone of arteries from $SK3^{T/T}$ mice, and this PE-induced elevation of tone was markedly enhanced by apamin (Figure 3B), consistent with a decrease in the tonic dilating effect of endothelial SK3 channels. Suppression of SK3 expression by dietary DOX was equivalent to application of apamin, increasing the apparent sensitivity and maximal constriction in response to PE. Removal of the endothelium enhanced PE-induced tone, similar to the effect of blocking SK channels with apamin or suppressing SK3 expression with DOX. Apamin had no significant effect on PE-induced increases in arterial tone in the absence of endothelium, specifically implicating endothelial SK3 channels as the source of the dilating effect.

Together, our results indicate that SK3 channel activity increases the tonic vasodilating influence of the endothelium through hyperpolarization. This effect could involve enhanced release of the endothelial relaxing factors NO and $PGI_2$. However, blockade of NO and $PGI_2$ production with $N^{\omega}$-nitro-L-arginine (L-NNA, 200 μmol/L) and indomethacin (10 μmol/L), respectively, did not prevent the effects of apamin. Apamin constricted arteries from $SK3^{T/T}$ mice (8.5±2.2% constriction at 20 mm Hg, n=5) in the presence of L-NNA and indomethacin and increased PE-induced constrictions (from 24±6% to 64±2% at 1 μmol/L PE, $P<0.001$, n=5) to approximately the same extent as in the absence of L-NNA and indomethacin. Thus, the SK3-mediated dilating influence does not involve NO or $PGI_2$ release. Notably, in arteries from wild-type mice, apamin caused a significant, albeit less pronounced, enhancement of PE-induced constrictions (from 64±3% to 72±3% at 1 μmol/L PE, $P<0.05$, n=4) in the presence of L-NNA and indomethacin. These data suggest that SK3 channels contribute to the tonic NO- and $PGI_2$-independent dilating influence of the endothelium in wild-type arteries as well as $SK3^{T/T}$ arteries and that the magnitude of this dilating influence is related to the level of SK3 expression.

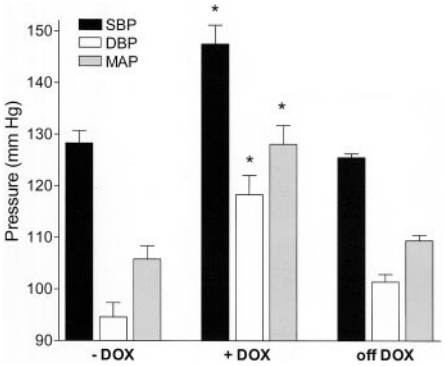


**Figure 4.** Effects of SK3 expression on blood pressure. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) in $SK3^{T/T}$ mice are shown before DOX exposure, 2 weeks after DOX exposure, and 2 weeks after removal of DOX (off DOX) from the diet. MAP was calculated using MAP=DBP+1/3(SBP−DBP). *$P<0.05$ vs pre-DOX values.

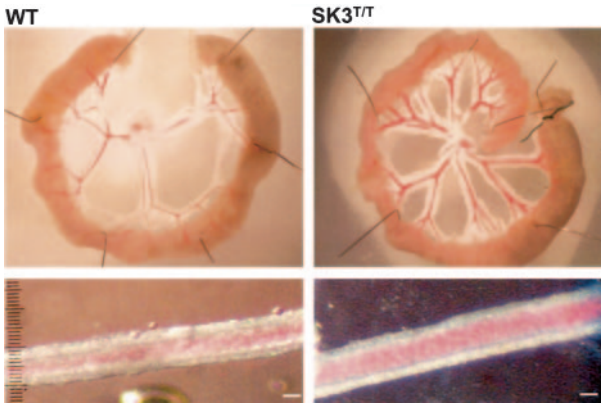


**Figure 5.** Influence of SK3 channel expression on vascular structure. Segments of small intestine and associated mesenteric vasculature from a WT mouse (left) and an $SK3^{T/T}$ mouse (right) are shown. Individual branches of mesenteric artery from anatomically identical sites are also shown (bar=50 $\mu$m). Animals were matched for sex and age (female, 20 weeks).

## Suppression of SK3 Expression Increases Blood Pressure

Because blocking SK3 channels or suppressing SK3 expression greatly increased the tone of resistance arteries in response to pressure and adrenergic receptor stimulation, we hypothesized that modulation of SK3 channel expression might influence blood pressure. Indeed, we found that suppression of SK3 expression with dietary DOX led to a marked increase in the systolic and diastolic pressures of $SK3^{T/T}$ mice,[29] which was reversed by removal of DOX (Figure 4). DOX had no effect on the blood pressures of wild-type mice. Thus, decreasing and increasing SK3 channel expression caused commensurate changes in blood pressure, consistent with changes in vascular tone and peripheral resistance.

## SK3 Channel Overexpression Increases Arterial Diameters

In addition to the direct functional effects of endothelial SK3 expression, we also observed SK3-dependent changes in vessel structure. Arteries from $SK3^{T/T}$ mice were dramatically different in appearance from their wild-type counterparts, exhibiting larger diameters and apparent differences in the degree of branching (Figure 5). The passive internal diameters of mesenteric arteries, for example, were ≈25% larger in $SK3^{T/T}$ mice than in wild-type mice (179±4 versus 142±13 $\mu$m, pressure 100 mm Hg, $P<0.05$, n=6) of the same age and sex. Coronary (septal) arteries, measured in situ from freshly excised hearts, were similarly larger in $SK3^{T/T}$ mice (internal diameters 101±4 versus 70±3 $\mu$m, $P<0.05$, n=5). Enlargement of other hollow organs from $SK3^{T/T}$ mice, including the

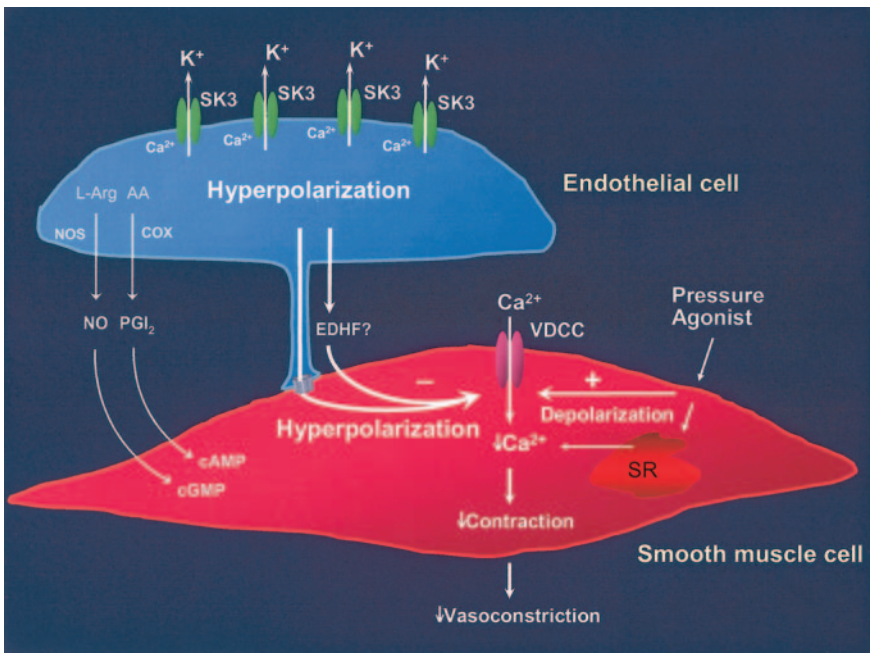


**Figure 6.** Proposed mechanisms by which SK3 channels in the endothelium regulate vascular tone. Increased expression of SK3 channels leads to hyperpolarization of the membrane potential of endothelial cells, which is likely communicated to smooth muscle cells through myoendothelial gap junctions or through the release of an unknown factor (endothelium-derived hyperpolarizing factor [EDHF]). Tonic hyperpolarization of the smooth muscle cell membrane potential decreases $Ca^{2+}$ entry through voltage-dependent $Ca^{2+}$ channels (VDCC), which leads to a decrease in smooth muscle intracellular $Ca^{2+}$ and thereby a decrease in the contractile response to physiological stimuli, such as pressure and sympathetic mediators. Other channels potentially contributing to vascular responses, including BK and IK channels, are not pictured. L-Arg indicates L-arginine; AA, arachidonic acid; NOS, NO synthase; SR, sarcoplasmic reticulum; and COX, cyclooxygenase.

urinary bladder,[30] has also been observed. Although the mechanism of such structural alterations is unknown, these observations suggest a role for SK3 channels in vascular development and/or remodeling and indicate an additional mechanism by which SK3 channels may contribute to cardiovascular function.

## Discussion

Through direct modulation of SK3 gene expression, we were able to focus exclusively on the SK3 channel and evaluate its impact in the vasculature. We found that the level of SK3 channel expression profoundly influences vascular function. Our evidence of SK3 channel expression in resistance artery endothelial cells is congruent with previous reports in large arteries.[9,20,21] In fact, the complete abolition of apamin sensitivity by DOX-induced gene suppression in our experiments suggests that only SK3 channels, and not other apamin-sensitive SK channel subtypes (SK1 and SK2), directly influence vascular tone. Moreover, our studies reveal an important pivotal role for SK3 channels in endothelium-dependent vascular regulation (Figure 6). In resistance arteries, tonic activity of endothelial SK3 channels induces a sustained hyperpolarization of the endothelial cell membrane potential, which is communicated to adjacent smooth muscle, likely through myoendothelial gap junctions[15] or ≥1 diffusible factor, as previously described.[31–33] Tonic smooth muscle hyperpolarization opposes the depolarizing and constricting effects of pressure and $\alpha$-adrenergic receptor stimulation, thereby modulating arterial tone and influencing blood pressure. Importantly, this tonic vasoregulation occurs in the absence of any exogenous endothelial stimulation, and its functional impact is inherently dependent on the degree of endothelial SK3 channel expression.

Overall, our findings suggest that the expression level of SK3 channels in the endothelium plays a fundamental role in determining arterial tone by setting arterial smooth muscle membrane potential. Modest but significant SK-dependent modulation of arterial tone was observed in wild-type arteries even in the absence of endothelial stimulation, and high or low SK3 expression in $SK3^{T/T}$ arteries exaggerated or abolished the tonic endothelial dilating influence, respectively. Thus, relative upregulation and downregulation of SK3 expression could provide a powerful and changeable means of modulating vasoconstriction under physiological conditions. This possibility is particularly exciting, considering that the tonic influence of the endothelium on vascular diameter is under the control of hormones such as estrogen,[34] and recent evidence indicates that SK3 gene expression is estrogen-regulated.[35,36] Indeed, estrogen deficiency not only reduces basal release of NO[37] but also attenuates acute endothelium-derived hyperpolarizations,[38] suggesting that regulation of endothelial SK3 expression may have an integral role in the cardiovascular effects of estrogen. Our findings also suggest that SK3 expression outside a normal physiological range may contribute to pathological states through extreme amplification or loss of the hyperpolarizing capacity of the endothelium. Indeed, attenuated vasoconstrictor responses in rats with cirrhosis are associated with elevated endothelial SK channel expression,[21] and blunted endothelium-dependent vasodilations of carotid arteries after balloon catheter injury appear to be linked to decreased SK3 and IK expression in regenerated endothelial cells.[20]

It should be noted that IK channels share many properties with SK channels,[39] and they have been implicated in acute endothelium-dependent dilations. Although the IK channel was not the focus of the present study, it may allow for additional fine-tuning of the tonic endothelial hyperpolarizing influence and warrants future study. Endothelium-dependent hyperpolarization has been implicated in flow-induced vasodilation in coronary arteries,[40] suggesting that endothelial channels such as SK3 and IK may play a particularly important vasoregulatory role in vivo. Under conditions of dynamic shear and blood flow, even moderate expression of SK3 and/or IK channels may have profound influences on vascular tone.

In the present study, we provide the first evidence that the expression level of an ion channel (SK3) in the vascular endothelium can profoundly influence cardiovascular function through a sustained hyperpolarization. It is likely that SK3 expression is tightly regulated by hormonal status and is altered with disease, having dramatic and dynamic effects on vascular function and structure. Consequently, altering SK3 expression within a normal physiological range may allow for flexible control of blood pressure and flow, whereas pathological upregulation or downregulation of SK3 expression may lead to severe hemodynamic dysregulation. Future studies will address the mechanisms and influence of dynamic SK3 channel regulation. Finally, these channels could be exploited clinically. Manipulation of endothelial SK3 channel expression may provide a novel therapeutic approach for the treatment of various vascular disorders, including hypertension.

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