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Impaired trafficking of β1 subunits inhibits BK channels in cerebral arteries of hypertensive rats

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Abstract

Hypertension is a risk factor for cerebrovascular diseases, including stroke and dementia. During hypertension, arteries become constricted and are less responsive to vasodilators, including nitric oxide (NO). The regulation of arterial contractility by smooth muscle cell (myocyte) large-conductance calcium (Ca^{2+})-activated potassium (BK) channels is altered during hypertension, although mechanisms involved are unclear. We tested the hypothesis that dysfunctional trafficking of pore-forming BK channel (BKα) and auxiliary β1 subunits contributes to changes in cerebral artery contractility of stroke-prone spontaneously hypertensive rats (SP-SHRs). Our data indicate that the amounts of total and surface BKα and β1 proteins is similar in unstimulated arteries of age-matched SP-SHRs and normotensive Wistar-Kyoto (WKY) rats. In contrast, stimulated surface-trafficking of β1 subunits by NO or membrane depolarization is inhibited in SP-SHR myocytes. Protein kinase C α (PKCα) and PKC βII total protein and activity were both higher in SP-SHR than WKY rat arteries. NO or depolarization robustly activated Rab11, a small trafficking GTPase, in WKY rat arteries, but weakly activated Rab11 in SP-SHRs. BIM, a PKC inhibitor, and overexpression of a PKC phosphorylation-deficient Rab11A mutant (Rab11A S177A) restored stimulated β1 subunit surface-trafficking in SP-SHR myocytes. BK channel activation by NO was inhibited in SP-SHR myocytes and restored by Rab11A S177A expression. Vasodilation to NO and lithocholate, a BKα/β1 channel activator, was inhibited in pressurized SP-SHR arteries and reestablished by BIM. In summary, data indicate that spontaneously active PKC inhibits Rab11A-mediated β1 subunit trafficking in arterial myocytes of SP-SHRs, leading to dysfunctional NO-induced BK channel activation and vasodilation.

Subject Codes

Cell Signaling/Signal Transduction; Ion Channels/Membrane Transport; Vascular

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Disclosures

None

Keywords

Hypertension; smooth muscle; cerebral arteries; large-conductance calcium-activated potassium channel; trafficking; Rab11; protein kinase C

Introduction

Hypertension is a risk factor for several cerebrovascular diseases, including stroke and dementia. During hypertension, cerebral arteries become constricted.[1–3] Cerebral arteries of hypertensive subjects are also less responsive to vasodilators, including nitric oxide (NO).[4] These pathological alterations can restrict regional brain blood flow, leading to insufficient perfusion to neurons and other brain cell types. Mechanisms that lead to these pathological changes in arterial contractility during hypertension are incompletely understood.

Membrane potential, is a principal regulator of arterial contractility.[5] Depolarization triggers the opening of voltage-dependent L-type Ca^{2+} ($Ca_V1.2$) channels in arterial smooth muscle cells, leading to Ca^{2+} influx and vasoconstriction.[6] In contrast, the opening of potassium channels, such as large conductance Ca^{2+}-activated potassium (BK) channels, leads to membrane hyperpolarization and vasodilation.[6, 7] Discrepancy exists in literature studying pathological changes in cerebral artery myocyte BK channels in hypertensive subjects. Studies have proposed that BK channels upregulate in arterial myocytes during hypertension, eliciting compensatory vasodilation.[1] In contrast, a reduction BK channel activity is described to contribute to vasoconstriction in hypertension.[8–10] This inconsistency in findings highlights a necessity for additional studies.

Arterial smooth muscle cell BK channels are composed of a pore-forming α subunit (BKα) and auxiliary β1 and γ subunits.[11–13] The regulated trafficking of BK channel subunits is a functional mechanism to control arterial contractility.[12, 14] In resting arterial myocytes, virtually all BKα protein is located in the plasma membrane, whereas a large proportion of β1 is intracellular and contained within Rab11A-postive recycling endosomes.[12] Nitric oxide (NO), a vasodilator, activates Rab11A, which stimulates rapid anterograde β1 subunit trafficking.[12] Membrane depolarization, also stimulates rapid β1 subunit surface trafficking through Ca^{2+}-dependent activation of Rho kinase (ROCK) 1 and 2.[15] These surface-trafficked β1 subunits then associate with plasma-membrane-localized BKα subunits, increasing their apparent Ca^{2+} sensitivity and activity.[12, 14] Endothelin-1, a vasoconstrictor, activated protein kinase C (PKC), which phosphorylated Rab11A at serine 177.[16] This mechanism reduced surface β1 trafficking, leading to BK channel inhibition in arterial myocytes and vasoconstriction.[16] In contrast to the regulated trafficking of β1 protein by Rab11A, BKα subunit anterograde trafficking is controlled by Rab4A-positive early endosomes in arterial myocytes.[17] Angiotensin II, a vasoconstrictor, stimulated PKC-dependent internalization and degradation of BKα subunits, which reduced transient BK currents and stimulated vasoconstriction.[17] These studies demonstrated that plasma membrane BK channel subunit composition is subject to modulation by physiological stimuli to control arterial contractility. Whether the trafficking of BK channel subunits is

altered during cardiovascular diseases, leading to changes in BK channel functionality is unclear.

Here, we examined BK channel subunit trafficking in cerebral artery myocytes of stroke-prone spontaneously hypertensive rats (SP-SHRs), using age-matched WKY rat arteries as controls. Our data indicate that spontaneously active PKC blocks the stimulated surface trafficking of β1 subunits. This inhibitory mechanism prevents BK channel activation by NO in arterial myocytes of SP-SHRs. A PKC inhibitor and overexpression of a mutant Rab11A that cannot be phosphorylated by PKC at serine 177 (Rab11A S177A) restored the stimulated surface trafficking of β1 subunits, BK channel activation and vasodilation mediated by NO in SP-SHR arteries. These data indicate that activated PKC blocks β1 subunit surface trafficking, which inhibits BK channels and prevents vasodilation in cerebral arteries of SP-SHR.

Methods

The techniques and data that support the results of this study are available from the corresponding author upon reasonable request.

Tissue preparation

Animal protocols were reviewed and approved by the Animal Care and Use Committee at the University of Tennessee Health Science Center. Male WKY rats and SP-SHRs (12 weeks) were euthanized with sodium pentobarbital (150mg/kg, i.p.). The brain was removed and placed in a HEPES-buffered physiological saline solution (PSS) containing (in mmol/L): 134 NaCl, 6 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 glucose (pH 7.4). Cerebral arteries were dissected from the brain. Smooth muscle cells were isolated using papain and collagenase, as previously described.[18]

Arterial surface biotinylation

Intact arteries were biotinylated as previously described[12, 19]. Briefly, cerebral arteries were placed in 1 mg/ml EZ-Link Sulfo-NHS-LC-LC-Biotin and 1 mg/ml EZ-Link Maleimide-PEG_2-Biotin (Pierce) in HEPES-buffered physiological saline solution for 1h. PBS with 100 mmol/L glycine was added to quench free biotin at the end of the incubation. Biotinylated arteries were homogenized in 1% Triton X-100 lysis buffer. The lysate was incubated with avidin beads (Thermo Fisher Scientific), then centrifuged at 13,000g. The beads were washed with PBS and protein samples were eluted with Laemmli buffer (Bio-Rad) and boiled. Protein samples were analyzed using Western blotting.

Western blotting

Protein samples were run on SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. Blots were blocked with 5% nonfat dry milk and then probed with either rabbit polyclonal anti-BKβ1 (1:500, Abcam), mouse monoclonal anti-BKα (1:500, Neuromab, UC Davis), mouse monoclonal anti-actin (1:10,000, Millipore), rabbit polyclonal anti-PKCα (1:500, Cell Signaling), rabbit polyclonal anti PKCβ (1:500, Santa Cruz), rabbit polyclonal anti-phospho PKCα/βII (Thr638/641, 1:500, Cell Signaling), rabbit monoclonal anti-rab11A

(1:500, Cell Signaling), mouse monoclonal anti-active Rab11 (1:500, NewEast Biosciences) or mouse monoclonal anti-cytochrome C (1:500, Santa Cruz) antibodies overnight at 4°C. The blots were washed before incubating with horseradish peroxidase-conjugated secondary antibodies. After incubating with a chemiluminescent detection kit, blots were imaged using a Bio-Rad ChemiDoc Touch imaging system. Band intensity was quantified using Quantity One software (Bio-Rad). Blots were often washed using stripping buffer (Thermo Scientific) and re-probed with other antibodies. Total proteins were calculated by summation of surface and intracellular proteins, as done previously.[15, 20]

Transfection of Intact Cerebral Arteries

A serine 177-to-alanine mutated Rab11A sequence (Rab11A S177A) was generated and sub-cloned into pcDNA3.1(+) (GenScript USA Inc., Piscataway, NJ). Cerebral arteries were transfected with empty pcDNA3.1 vectors, pcDNA3.1 encoding Rab11A, or pcDNA3.1 encoding Rab11A S177A using tandem-pulse electroporation (CUY21Vivo-SQ electroporator; Bex), as previously described.[21] Arteries were then cultured in serum-free Dulbecco's modified Eagle's media supplemented with 1% penicillin-streptomycin under standard culture conditions for 2 days.

Patch Clamp Electrophysiology

BK channels were recorded using the inside-out patch clamp configuration. The bath and pipette solution both contained (in mmol/L): 130 KCl, 10 HEPES, 5 EGTA, 1.6 HEDTA, 1 $MgCl_2$, and 10 μmol/L free Ca^{2+} (pH 7.2). Currents were recorded using an Axopatch 200B amplifier and Clampex 10.3 at a holding potential of −40mV or +80mV. To study the effects on NO-mediated trafficking on BK channel properties, myocytes were first exposed to SNP, after which inside-out patches were pulled. SNP was then washed out before recording BK channels. Lithocholate was added directly to inside-out patches. Currents were filtered at 1 kHz, digitized at 5 kHz and analyzed using Clampfit 10.3 (MDS Analytical Technologies).

Pressurized artery myography

Artery segments (1–2 mm length) were cannulated at each end in a perfusion chamber (Living Systems Instrumentation, St. Albans, VT). Experiments were performed using a physiological saline solution (PSS) which contained (in mmol/L): 112 NaCl, 4.8 KCl, 24 $NaHCO_3$, 1.8 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PQ_4 and 10 glucose that was gassed with 21% O_2, 5% CO_2 and 74% N_2. The endothelium was denuded by introducing air bubbles into the vessel lumen for ~1 minute followed by wash with PSS. The chamber was continuously perfused with PSS and maintained at 37 °C. Intravascular pressure was measured using a transducer. Arterial diameter was measured using a CCD camera attached to a Nikon TS100-F microscope and the edge-detection function of IonWizard (Ionoptix, Milton, MA). Myogenic tone (%) was calculated as: $100 \times (1 - D_{active}/D_{passive})$, where D_{active} is active arterial diameter and $D_{passive}$ is the diameter determined in Ca^{2+}-free PSS supplemented with 5 mmol/L EGTA.

Statistical analysis

Statistical analyses were performed using Origin v6.0 and GraphPad Prism v4.0. Values are indicated as mean ± SEM. Student's t-test was used to compare paired and unpaired data from two populations. ANOVA with Newman-Keuls post hoc test was used for multiple group comparisons. $P < 0.05$ was considered as significant. Power analysis was performed to verify that the sample size gave a value of >0.8 if P was >0.05.

Results

β1 subunit surface trafficking is impaired in cerebral arteries of SP-SHRs

MAPs were 123±3 and 190±4 mmHg in male WKY rats and SP-SHRs, respectively (n=6, $p<0.05$). Arterial biotinylation followed by Western blotting was performed to measure both the surface to intracellular distribution and total protein of BKα and β1 in cerebral arteries of WKYs and SP-SHRs. BKα and β1 subunit total (surface+intracellular) proteins were similar in cerebral arteries of WKYs and SP-SHRs (Fig. 1A, C). BKα and β1 proteins were also similarly distributed in cerebral arteries of WKYs and SP-SHR with >85 % of BKα present in the plasma membrane and >90 % of β1 located intracellularly (Fig. 1A, C). Cytochrome C, a mitochondrial protein, was detected only in the intracellular fraction of WKY and SP-SHR arteries, indicating that biotin did not label intracellular proteins, as shown previously (Figure 1D).[16] These data indicate that BKα and β1 subunit total protein and cellular distribution are similar in unstimulated cerebral arteries of WKYs and SP-SHR.

We tested the hypothesis that trafficking of BK channel subunits induced by vasoactive stimuli is altered in cerebral arteries of SP-SHR during hypertension. Responses were measured to SNP, a nitric oxide donor, or membrane depolarization, which was induced by increasing extracellular K^+ to either 30 or 60 mM (10 min each). SNP, 30 mM K^+ and 60 mM K^+ increased surface β1 protein ~2.2-, 2.3- and 2.5- fold, respectively, in WKY arteries (Fig. 1A, C). In contrast, SNP, 30 mM K^+ or 60 mM K^+ did not alter surface β1 protein levels in SP-SHR arteries (Fig. 1A, C). SNP, 30 mM K^+ or 60 mM K^+ (10 min) did not alter surface levels of BKα in both WKY and SP-SHR arteries (Fig. 1A, C). These data indicate that SNP- and depolarization-induced β1 subunit surface trafficking is functional in myocytes of WKY rat arteries, but is absent in myocytes of SP-SHR arteries.

BK channel activity is reduced due to impaired β1 subunit trafficking in myocytes of SP-SHRs

To investigate the functional consequences of inhibited β1 subunit trafficking, BK channel P_O was measured in inside-out patches pulled from cerebral artery myocytes. P_O was measured at −40mV, a physiological membrane potential, or at +80mV to stimulate maximal channel activity, using a free intracellular Ca^{2+} concentration of 10 μM. At −40 mV, the mean P_O of BK channels was similar in patches from control WKY and SP-SHR myocytes (Fig. 2A, B). Lithocholate, an activator of β1 subunit-containing BK channels, was applied directly to inside out-patches. Lithocholate increased BK channel P_O ~2.4-fold in patches pulled from WKY rat myocytes, but did not alter P_O in patches from SP-SHR myocytes (Fig. 2A, B). Myocytes were exposed to SNP (10 mins), after which patches were pulled and the P_O of BK channels measured in inside-out patches in the absence of SNP. Myocyte SNP-

treatment increased the P_o of BK channels ~2.5-fold in patches from WKYs, but did not alter the P_O in patches from SP-SHRs (Fig. 2A, B). Lithocholate increased BK channel P_O in patches from SNP-treated WKY rat myocytes such that it was ~4.1-fold higher than in the control condition (Fig. 2A, B). In contrast, lithocholate did not activate BK channels in patches from SNP-treated SP-SHR myocytes (Fig. 2A, B). These data suggest that in WKY rat myocytes, some β1 subunits are associated with surface BK channels and that NO stimulates an increase in the amount of surface β1 subunits associated with BKα, which increases P_O and activation by a β1 ligand. In contrast, in SP-SHR myocytes, surface β1 subunits do not appear to be associated with BKα and NO does not stimulate β1 subunit surface trafficking to activate BK channels.

Activated PKC inhibits β1 subunit surface trafficking in SP-SHR arteries

To evaluate the mechanism underlying inhibited β1 subunit trafficking, WKY and SP-SHR arteries were exposed to SNP in the presence or absence of BIM, a PKC inhibitor. SNP alone increased surface β1 protein in WKY arteries, but did not alter surface β1 in SP-SHR arteries (Fig 3A, B). BIM alone did not alter surface β1 protein in both WKY and SP-SHR arteries (Fig 3A, B). In WKY rat arteries, SNP similarly increased surface β1 when applied in the absence or presence of BIM (Fig 3A, B). BIM enabled SNP to increase surface β1 subunits ~2.7-fold in SP-SHR arteries (Fig 3A, B). Indeed, in the presence of BIM, the SNP-induced increase in surface β1 subunits was the same as in WKY arteries (Fig 3A, B). BKα distribution was not altered by SNP or BIM applied either alone or in combination in WKY or SP-SHR arteries (Fig 3A, B). These data indicate that a PKC inhibitor enables NO-induced β1 subunit surface trafficking in SP-SHR arteries.

Western blotting was performed to measure PKC protein and activity in WKY and SP-SHR arteries. PKC α and βII proteins were ~2.02- and 1.67-fold higher, respectively, in SP-SHR than WKY rat arteries (Fig 3C, D). An antibody that recognizes activated PKC α and βII indicated that the combined activity of these kinases was ~1.89-fold higher in SP-SHR than WKY rat arteries (Fig 3C, D). These data indicate that both the total amounts of PKC α and βII and their combined activity are higher in SP-SHR than WKY rat arteries.

Rab11A S177A restores β1 subunit surface trafficking in SP-SHR arteries

Rab11A-positive recycling endosomes traffic β1 subunits to the plasma membrane in arterial smooth muscle cells[12, 16]. To determine if attenuated β1 subunit trafficking is related to a reduced function of Rab11A, both total Rab11A and active Rab11 were measured in WKY rat and SP-SHR arteries. Western blotting demonstrated that total Rab11A in unstimulated SP-SHR arteries was ~48% of that in WKY rat arteries (Fig 4A, B). SNP and 30 mM K^+ increased active Rab11 ~1.96- and 2.14-fold, respectively, in WKY rat arteries (Fig 4A, B). In contrast, SNP and 30 mM K^+ increased active Rab11 only ~1.19- and 1.29-fold, respectively, in SP-SHR rat arteries (Fig 4A, B). These data indicate that Rab11A total protein is lower and Rab11 activation by SNP and membrane depolarization are both inhibited in SP-SHR arteries.

ET-1 activates PKC, which phosphorylates Rab11A at S177, leading to inhibition of β1 subunit surface trafficking in arterial myocytes.[16] Given that BIM restored β1 subunit

trafficking in SP-SHR arteries, we evaluated the function of Rab11A S177A. Arteries were transfected with an empty vector (mock), or vectors that express either Rab11A (Rab11A) or Rab11A S177A, a phosphorylation-deficient Rab11A mutant. Vectors encoding Rab11A or Rab11A S177A similarly increased Rab11A total protein to ~163% or 160% of the mock group (Fig. 4A, B). SNP similarly stimulated a ~3-fold increase in surface β1 subunits in WKY arteries overexpressing either Rab11A or Rab11A S177A (Fig 4C, D). SNP-induced surface trafficking of β1 subunits was impaired in SP-SHR cerebral arteries expressing Rab11A (Fig 4E, F). In contrast, Rab11A S177A expression enabled SNP-induced surface trafficking of β1 in SP-SHR arteries (Fig 4E, F). These data suggest that PKC-mediated phosphorylation of Rab11A S177 inhibits β1 surface trafficking in SP-SHR arteries.

Rab11A S177A restores BK channel activation in SP-SHR arterial myocytes

To examine the modulation of BK channel P_O by Rab11A S177, myocytes were isolated from WKY or SP-SHR arteries overexpressing Rab11A or Rab11A S177A. Myocytes were exposed to SNP (10 mins), after which inside-out patches were pulled and BK channels recorded at −40 or +80 mV in the absence of SNP. BK channel P_O was similar in WKY rat myocytes overexpressing either Rab11A or Rab11A S177A (Fig 5A, B). In patches from SP-SHR myocytes expressing Rab11A, mean BK channel P_O was ~60% of that in WKY myocytes expressing the same construct (Fig 5A, B). The expression of Rab11A S177A increased BK channel P_O in SP-SHR myocytes, such that it was the same as in WKY myocytes under the same condition (Fig 5A, B). The application of lithocholate to inside-out patches similarly increased BK channel P_O ~1.8-fold in WKY rat myocytes overexpressing either Rab11A or Rab11A S177A (Fig 5A, B). Lithocholate did not increase BK channel P_O in SP-SHR myocytes overexpressing Rab11A (Fig 5A, B). In contrast, Rab11A S177A expression enabled lithocholate-induced activation of BK channels in SNP-treated SP-SHR myocytes, such that P_O was the same as in WKY rat myocytes under the same condition (Fig 5A, B). These data indicate that Rab11A S177A expression restores SNP-induced BK channel activation in SP-SHR myocytes. These results support other evidence presented here that PKC-mediated phosphorylation of Rab11A S177 blocks BK channels in SP-SHR myocytes.

Impaired β1 subunit trafficking attenuates BK channel functionality in pressurized SP-SHR arteries

The impact of dysfunctional β1 subunit trafficking was investigated by measuring the contractility of pressurized cerebral arteries. At an intravascular pressure of 60 mmHg, endothelium-denuded SP-SHR arteries developed 36.1±1.8 % tone compared with 23.9 ±4.0 % tone in WKY arteries, or ~1.5-fold more (n=6, $P<0.05$). SNP and lithocholate increased the mean diameter of SP-SHR arteries by ~4 and 6 μm, respectively, responses that were ~26 and 48 % of those in WKY rat arteries (Fig. 6A, 6B). SNP (10 mins) increased lithocholate-induced dilation from ~6 to 20 μm in WKY rat arteries and from ~3 to 7 μm in SP-SHR arteries. Thus, lithocholate-induced vasodilation in the presence of SNP was ~2.8-fold more in WKY than SP-SHR arteries (Fig. 6B). BIM increased SNP-induced vasodilation in SP-SHR arteries to the same as in WKY rat arteries (Fig. 6B). BIM also increased lithocholate-induced vasodilation obtained in the presence of SNP in SP-SHR arteries to the same as that in WKY rat arteries (Fig. 6B). In contrast, BIM alone did not

alter WKY rat or SP-SHR artery diameter (Fig. 6B). BIM also did not alter vasodilation to SNP or lithocholate in WKY rat arteries (Fig. 6B). These data support our biochemical evidence that activated PKC inhibits mobilization of myocyte β1 subunits and greatly attenuates BK channel function in SP-SHR arteries.

Discussion

Here, we tested the hypothesis that hypertension is associated with altered trafficking of BK channel subunits in myocytes of cerebral arteries using SP-SHRs as a disease model and WKY rats as a control. Our data show that stimulated surface trafficking of β1 subunits by NO or membrane depolarization is inhibited in arterial myocytes of SP-SHRs. PKCα and βII total protein and activity were both higher in SP-SHR arteries. PKC inhibition or expression of Rab11A S177A, a PKC phosphorylation-deficient mutant, restored β1 subunit surface-trafficking in SP-SHR arteries. The impaired trafficking of β1 subunits prevents BK channel activation by NO in arterial myocytes of SP-SHRs. BK channel activation by NO is restored by Rab11A S177A in SP-SHR myocytes. PKC inhibition also enables NO-induced vasodilation by reestablishing the ability of NO to increase the abundance of β1 subunits associated with surface BKα. These data demonstrate that spontaneously active PKC blocks the ability of NO to stimulate β1 subunit surface trafficking in myocytes of SP-SHR, thereby preventing BK channel activation and vasodilation in cerebral arteries of SP-SHR.

Cerebral arteries from hypertensive subjects are depolarized and constricted.[2, 3] Endothelium-dependent vasodilation, including that produced by NO, is attenuated in vessels from multiple hypertensive animal models.[22–29] Given that BK channels regulate physiological arterial potential and contractility, we investigated whether pathological changes in BK channel trafficking alter arterial function during hypertension. We used SP-SHR, a rat genetic model of hypertension that is prone to stroke, to study the cellular distribution and regulation of BKα and β1 subunit total and surface abundance. Our data show that BKα and β1 total and surface proteins are similar in cerebral arteries of age-matched hypertensive SP-SHRs and normotensive WKY rat controls. Our data are in agreement with the findings of some, but not all, previous studies that measured either subunit message or total protein in cerebral arteries. Consistent with our results, Pabbidi reported that BKα and β1 total proteins were similar in cerebral arteries of a hypertensive model of Fawn Hooded rats, when compared with their normotensive genetic controls.[30] Other studies have reported that BKα mRNA is unaltered, but β1 mRNA was lower in cerebral arteries of angiotensin II-infused hypertensive Sprague-Dawley rats and mice and SHRs, when compared with their respective controls.[8, 9] Another study found that although BKα mRNA was similar, BKα protein was higher in cerebral arteries of SHR and WKYs.[31] Some of these different findings may relate to the approaches used to measure BK channel subunit expression or the animal models of hypertension that were examined. Regardless, our data show that the altered functionality of BK channels described here in SP-SHR cerebral arteries is not due to a change in the amount of BKα or β1 total proteins.

We previously described that in unstimulated arteries, low levels of β1 subunits are trafficked to the surface via a Rab11A-independent mechanism.[12] Our data here suggest that the surface-trafficking of these basal β1 subunits is not altered in SP-SHR. NO and

membrane depolarization stimulate Rab11A-dependent trafficking of β1 subunits to the plasma membrane in arterial myocytes, but through distinct signaling mechanisms: NO operates through PKG activation, whereas depolarization acts via the stimulation of ROCK.[12, 15] Regardless of the signaling mechanisms involved, surface-trafficked β1 proteins associate with plasma membrane-resident BKα, leading to channel activation.[12, 15] Here, NO and membrane depolarization failed to surface-traffic β1 subunits in cerebral arteries of SP-SHRs. Data show that although total Rab11A is lower in SP-SHR arteries, Rab11A overexpression did not reestablish β1 trafficking, consistent with the mechanism being inhibitory, rather than through the loss of Rab11A protein. The mechanism leading to a reduction in total Rab11A protein in SP-SHR arteries was not determined here, but would be relevant to determine in future studies. We recently described that endothelin-1 activates protein kinase C, which phosphorylates Rab11A at Serine 177.[16] This mechanism inhibits Rab11A, which blocks β1 subunit surface trafficking in arterial myocytes.[16] Given that NO stimulates Rab11A in normotensive rat arterial myocytes, it lead us to hypothesize that activated PKC may prevent β1 trafficking in arterial myocytes of SP-SHRs.[16] Here, active PKC α/βII and active Rab11 were measured using antibodies. Phosphorylation of PKC α and PKC βII on Thr638/641 increases catalytic activity[32, 33]. The phosphorylation state of PKCα/βII recognized by the antibody here is directly related to activity. Rab11 activity was measured using an antibody that recognizes the GTP-bound active form of Rab11, but does not recognize the GDP-bound inactive form. Data support our hypothesis proposed, including that PKCα and βII protein and activity are higher in SP-SHR than WKY arteries. SNP and membrane depolarization robustly activated Rab11 in arterial myocytes of WKY rats, but only slightly increased Rab11 activity in myocytes of SP-SHRs. BIM and Rab11A S177A, a Rab11A mutant that is unable to be phosphorylated by PKC at serine 177, enabled NO-induced trafficking of β1 subunits in SP-SHR arteries. Thus, spontaneously active PKC phosphorylates Rab11A at serine 177, which inhibits stimulated surface trafficking of β1 subunits by NO and membrane depolarization in SP-SHR myocytes. Rab11A S177 expression did not increase surface β1 subunits in unstimulated myocytes, suggesting that it is not PKC phosphorylation of Rab11A which maintains the basal intracellular localization of β1. Rather, our results suggest that in unstimulated myocytes, β1 subunits reside in recycling endosomes and an anterograde trafficking stimulus such as NO or membrane depolarization is necessary to mobilize these protein to the surface. Identifying the mechanism by which PKC is spontaneously activated in arteries of SP-SHRs was not a focus of our study, but several possibilities exist. An increase in the expression, translocation and activity of PKC has been implicated in the pathogenesis of hypertension and one or more of these signaling alterations may underlie PKC-mediated Rab11A inhibition in SP-SHR arterial myocyte.[34] Our data show that PKC is active in unstimulated arteries *in vitro*. The PKC-mediated inhibitory mechanism was also present in arteries that had been placed in serum-free media for 2 days. These data suggest that the mechanism of PKC activation occurs in the isolated arterial wall and that *in vivo* factors, such as circulating vasoconstrictors, are not required for this PKC activity and inhibition of β1 trafficking in arterial myocytes of SP-SHRs. PKC can be activated by many different intracellular signal elements, including diacylglycerol, Ca^{2+} and reactive oxygen species. Studies have shown that intracellular Ca^{2+} concentration and Ca^{2+} sparklet activity are increased in arterial myocytes during hypertension.[1, 10] Hypertension is also associated with an increase in the

bioavailability of vascular reactive oxygen species.[35] One or more of these multiple mechanisms may activate PKC, leading to the inhibition of β1 trafficking in arterial myocytes of SP-SHRs.

BK channels formed from four α subunits can contain between one and four β1 subunits.[36, 37] The α:β1-tetramer ratio shifts channel voltage- and Ca^{2+} dependence, with an increase in the number of β1 subunits elevating channel activity.[36] We applied lithocholate directly to inside-out patches to evaluate the presence of channels containing β1 subunits. Although surface β1 protein was similar in unstimulated WKY and SP-SHR myocytes, lithocholate activated BK channels only in patches from WKY rats and not in patches from SP-SHRs. Thus, plasma membrane β1 subunits do not activate BK channels in unstimulated SP-SHR arterial myocytes. Explanations for this finding include that a pathological signaling mechanism prevents low levels of β1 subunits from either activating or physically associating with BKα in in SP-SHR myocytes. SNP increased both BK channel P_O and lithocholate-induced channel activation in excised patches from WKY rat myocytes, indicating that NO increased the abundance of β1 subunits associated with BKα. In contrast, SNP did not activate BK channels or enable lithocholate-induced BK channel activation in SP-SHR myocytes, consistent with other data here that NO did not traffic β1 subunits to the surface in these cells. The overexpression of Rab11A did not enable NO- or lithocholate-activation of BK channels in SP-SHR arterial myocytes. In contrast, Rab11A S177A reestablished both NO- and lithocholate-activation of BK channels. These data suggest that PKC acting through Rab11A S177 inhibits β1 subunit surface-trafficking, thereby preventing NO from activating BK channels.

Arterial myocyte BK channels are activated by localized intracellular Ca^{2+} transients, termed Ca^{2+} sparks, that occur due to the release of sarcoplasmic reticulum Ca^{2+} through ryanodine receptors (RyRs).[6] A single Ca^{2+} spark activates multiple nearby BK channels, leading to a transient BK current.[6] Ca^{2+} spark frequency essentially determines transient BK current frequency, whereas the effective coupling of BK channels to Ca^{2+} sparks is the primary factor which establishes the amount of current produced by each BK transient.[7] We have previously shown that endothelin-1 activates protein kinase C, which inhibits β1 subunit surface trafficking, leading to BK channel inhibition and a decrease in transient BK currents in arterial myocytes.[16] Ca^{2+} spark frequency was similar, whereas the coupling between Ca^{2+} sparks and BK channels was attenuated in cerebral artery myocytes of hypertensive rats.[8] Here, activated protein kinase C blocks β1 subunit surface-trafficking, which would reduce the effective coupling of BK channels to Ca^{2+} sparks and reduce transient BK currents.

Functional data also support the signaling mechanism identified here using biochemistry and electrophysiology. In pressurized WKY rat arteries, SNP and lithocholate both stimulated dilation, SNP increased lithocholate-induced vasodilation and BIM did not alter these responses. In contrast, in SP-SHR arteries, SNP and lithocholate-induced dilations were small, SNP did not increase lithocholate-induced dilation, BIM increased SNP-induced vasodilation and enabled SNP to augment dilation to lithocholate. Importantly, BIM increased SNP-induced vasodilation such that it was the same as in WKY rat arteries. These data indicate that PKC-mediated inhibition of β1 subunit trafficking underlies dysfunctional

NO-induced vasodilation in SP-SHR cerebral arteries. As discussed above, studies have reported either no change or alterations in the abundance of BK channel subunits in hypertensive animal models. The upregulation of BK currents in arterial myocytes during SHRs is reported to elicit compensatory vasodilation.[31] In contrast, a reduction in β1 subunits has been described to decrease BK channel activity, producing vasoconstriction in angiotensin II-infused hypertensive Sprague-Dawley rats and mice and SHRs.[8–10] Our data indicate that BKα and β1 total protein is unaltered and myogenic tone is higher in pressurized arteries of SP-SHRs than WKY rats. It was not a focus of this study to investigate the underlying mechanisms for increased myogenic tone and whether BK channels contribute to this vasoconstriction or oppose it. BIM alone did not alter the diameter of either WKY rat or SP-SHR arteries, suggesting that the net effect of PKC activation does not contribute to the increase in myogenic tone. Other mechanisms, including those mediated through voltage-dependent K^+ (K_V) and $Ca_V1.2$ channels contribute to this pathological vasoconstriction.[1] Our data show that NO-induced vasodilation is attenuated due to PKC-mediated inhibition of β1 subunit trafficking in cerebral arteries of SP-SHRs.

Findings of our study may be relevant for human cardiovascular diseases. Single nucleotide polymorphisms (SNPs) in BKα and β1 genes contribute to human cardiovascular diseases, including hypertension.[38] More than 140 SNPs have been reported in or nearby the β1 subunit gene. An E65K polymorphism in β1 produces a "gain-of-function" mutant which increases BK channel Ca^{2+}-sensitivity and is associated with lower prevalence for diastolic hypertension.[39] BK channel apparent Ca^{2+}-sensitivity is lower in β1 subunit knockout mice, which leads to membrane depolarization, vasoconstriction and systemic hypertension.[11] These studies provide a link between altered β1 subunit function, vascular reactivity and blood pressure. Whether SNPs in β1 subunits interfere with protein surface trafficking is unclear, but would be relevant to investigate given findings reported here.

Perspectives

In summary, we show that that activated PKC blocks the surface trafficking of β1 subunits by Rab11A, which prevents BK channel activation in arterial myocytes of SP-SHRs. Blocking this PKC-mediated inhibitory mechanism restores the stimulated surface trafficking of β1 subunits, enabling BK channel activation and vasodilation. The identification of both this dysfunctional signaling pathway and a mechanism to block it may lead to the development of novel therapies that reduce vascular disease in hypertensive subjects.

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Abbreviations

BK	large-conductance calcium-activated potassium
cGMP	cGMP-dependent protein kinase
NO	nitric oxide
PKC	protein kinase C
SNP	sodium nitroprusside
BIM	bisindolylmaleimide
$\mathbf{P_O}$	open probability.

References

1. Joseph BK, Thakali KM, Moore CL, Rhee SW. Ion channel remodeling in vascular smooth muscle during hypertension: Implications for novel therapeutic approaches. Pharmacol Res. 2013; 70:126–138. [PubMed: 23376354]
2. Harder DR, Brann L, Halpern W. Altered membrane electrical properties of smooth muscle cells from small cerebral arteries of hypertensive rats. Blood Vessels. 1983; 20:154–160. [PubMed: 6831059]
3. Harder DR, Smeda J, Lombard J. Enhanced myogenic depolarization in hypertensive cerebral arterial muscle. Circ. Res. 1985; 57:319–322. [PubMed: 4017200]
4. Pires PW, Dams Ramos CM, Matin N, Dorrance AM. The effects of hypertension on the cerebral circulation. American journal of physiology. Heart and circulatory physiology. 2013; 304:H1598–1614. [PubMed: 23585139]
5. Nelson MT, Patlak JB, Worley JF, Standen NB. Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. Am. J. Physiol. 1990; 259:C3–18. [PubMed: 2164782]
6. Jaggar JH, Porter VA, Lederer WJ, Nelson MT. Calcium sparks in smooth muscle. Am. J. Physiol. 2000; 278:C235–C256.
7. Dopico AM, Bukiya AN, Jaggar JH. Calcium- and voltage-gated BK channels in vascular smooth muscle. Pflugers Arch. 2018; doi: 10.1007/s00424-018-2151-y
8. Amberg GC, Bonev AD, Rossow CF, Nelson MT, Santana LF. Modulation of the molecular composition of large conductance, Ca^{2+} activated K^+ channels in vascular smooth muscle during hypertension. J. Clin. Invest. 2003; 112:717–724. [PubMed: 12952920]
9. Amberg GC, Santana LF. Downregulation of the BK channel b1 subunit in genetic hypertension. Circ. Res. 2003; 93:965–971. [PubMed: 14551242]
10. Nieves-Cintron M, Amberg GC, Nichols CB, Molkentin JD, Santana LF. Activation of NFATc3 down-regulates the beta1 subunit of large conductance, calcium-activated K^+ channels in arterial smooth muscle and contributes to hypertension. The Journal of biological chemistry. 2007; 282:3231–3240. [PubMed: 17148444]
11. Brenner R, Perez GJ, Bonev AD, Eckman DM, Kosek JC, Wiler SW, Patterson AJ, Nelson MT, Aldrich RW. Vasoregulation by the b1 subunit of the calcium-activated potassium channel. Nature. 2000; 407:870–876. [PubMed: 11057658]
12. Leo MD, Bannister JP, Narayanan D, Nair A, Grubbs JE, Gabrick KS, Boop FA, Jaggar JH. Dynamic regulation of b1 subunit trafficking controls vascular contractility. Proc. Natl. Acad. Sci. U. S. A. 2014; 111:2361–2366. [PubMed: 24464482]
13. Evanson KW, Bannister JP, Leo MD, Jaggar JH. LRRC26 is a functional BK channel auxiliary gamma subunit in arterial smooth muscle cells. Circ. Res. 2014; 115:423–431. [PubMed: 24906643]

14. Leo MD, Jaggar JH. Trafficking of BK channel subunits controls arterial contractility. Oncotarget. 2017; 8:106149–106150. [PubMed: 29290926]
15. Leo MD, Zhai X, Muralidharan P, Kuruvilla KP, Bulley S, Boop FA, Jaggar JH. Membrane depolarization activates BK channels through ROCK-mediated β1 subunit surface trafficking to limit vasoconstriction. Sci Signal. 2017; 10
16. Zhai X, Leo MD, Jaggar JH. Endothelin-1 stimulates vasoconstriction through Rab11A Serine 177 phosphorylation. Circulation research. 2017; 121:650–661. [PubMed: 28696251]
17. Leo MD, Bulley S, Bannister JP, Kuruvilla KP, Narayanan D, Jaggar JH. Angiotensin II stimulates internalization and degradation of arterial myocyte plasma membrane BK channels to induce vasoconstriction. Am. J. Physiol Cell Physiol. 2015; 309:C392–402. [PubMed: 26179602]
18. Jaggar JH. Intravascular pressure regulates local and global Ca2+ signaling in cerebral artery smooth muscle cells. Am. J. Physiol. 2001; 281:C439–C448.
19. Bannister JP, Adebiyi A, Zhao G, Narayanan D, Thomas CM, Feng JY, Jaggar JH. Smooth muscle cell a2d-1 subunits are essential for vasoregulation by CaV1.2 channels. Circ. Res. 2009; 105:948–955. [PubMed: 19797702]
20. Bannister JP, Bulley S, Leo MD, Kidd MW, Jaggar JH. Rab25 influences functional CaV1.2 channel surface expression in arterial smooth muscle cells. Am J Physiol Cell Physiol. 2016; 310:C885–893. [PubMed: 27076616]
21. Narayanan D, Bulley S, Leo MD, Burris SK, Gabrick KS, Boop FA, Jaggar JH. Smooth muscle cell transient receptor potential polycystin-2 (TRPP2) channels contribute to the myogenic response in cerebral arteries. J. Physiol. 2013; 591:5031–5046. [PubMed: 23858011]
22. Yang D, Gluais P, Zhang JN, Vanhoutte PM, Feletou M. Endothelium-dependent contractions to acetylcholine, ATP and the calcium ionophore A 23187 in aortas from spontaneously hypertensive and normotensive rats. Fundam. Clin. Pharmacol. 2004; 18:321–326. [PubMed: 15147283]
23. Luscher TF, Aarhus LL, Vanhoutte PM. Indomethacin improves the impaired endothelium-dependent relaxations in small mesenteric arteries of the spontaneously hypertensive rat. Am. J. Hypertens. 1990; 3:55–58. [PubMed: 2302329]
24. Cordellini S. Endothelial dysfunction in DOCA-salt hypertension: possible involvement of prostaglandin endoperoxides. Gen. Pharmacol. 1999; 32:315–320. [PubMed: 10211585]
25. Jimenez R, Lopez-Sepulveda R, Kadmiri M, Romero M, Vera R, Sanchez M, Vargas F, O'Valle F, Zarzuelo A, Duenas M, Santos-Buelga C, Duarte J. Polyphenols restore endothelial function in DOCA-salt hypertension: role of endothelin-1 and NADPH oxidase. Free Radic. Biol. Med. 2007; 43:462–473. [PubMed: 17602962]
26. Zhou MS, Kosaka H, Tian RX, Abe Y, Chen QH, Yoneyama H, Yamamoto A, Zhang L. L-Arginine improves endothelial function in renal artery of hypertensive Dahl rats. J. Hypertens. 2001; 19:421–429. [PubMed: 11288812]
27. Heitzer T, Wenzel U, Hink U, Krollner D, Skatchkov M, Stahl RA, MacHarzina R, Brasen JH, Meinertz T, Munzel T. Increased NAD(P)H oxidase-mediated superoxide production in renovascular hypertension: evidence for an involvement of protein kinase C. Kidney Int. 1999; 55:252–260. [PubMed: 9893134]
28. Quaschning T, Hocher B, Ruhl S, Kraemer-Guth A, Tilgner J, Wanner C, Galle J. Vasopeptidase inhibition normalizes blood pressure and restores endothelial function in renovascular hypertension. Kidney Blood Press Res. 2006; 29:351–359. [PubMed: 17139187]
29. Choi H, Allahdadi KJ, Tostes RC, Webb RC. Augmented S-nitrosylation contributes to impaired relaxation in angiotensin II hypertensive mouse aorta: role of thioredoxin reductase. J. Hypertens. 2011; 29:2359–2368. [PubMed: 22025239]
30. Pabbidi MR, Mazur O, Fan F, Farley JM, Gebremedhin D, Harder DR, Roman RJ. Enhanced large conductance K+ channel activity contributes to the impaired myogenic response in the cerebral vasculature of Fawn Hooded Hypertensive rats. American journal of physiology. Heart and circulatory physiology. 2014; 306:H989–H1000. [PubMed: 24464756]
31. Liu Y, Hudetz AG, Knaus HG, Rusch NJ. Increased expression of Ca^{2+}-sensitive K^+ channels in the cerebral microcirculation of genetically hypertensive rats: evidence for their protection against cerebral vasospasm. Circ. Res. 1998; 82:729–737. [PubMed: 9546382]

32. Newton AC. Protein kinase C: structural and spatial regulation by phosphorylation, cofactors, and macromolecular interactions. Chem Rev. 2001; 101:2353–2364. [PubMed: 11749377]

33. Edwards AS, Faux MC, Scott JD, Newton AC. Carboxyl-terminal phosphorylation regulates the function and subcellular localization of protein kinase C betaII. The Journal of biological chemistry. 1999; 274:6461–6468. [PubMed: 10037738]

34. Salamanca DA, Khalil RA. Protein kinase C isoforms as specific targets for modulation of vascular smooth muscle function in hypertension. Biochem Pharmacol. 2005; 70:1537–1547. [PubMed: 16139252]

35. Montezano AC, Dulak-Lis M, Tsiropoulou S, Harvey A, Briones AM, Touyz RM. Oxidative stress and human hypertension: vascular mechanisms, biomarkers, and novel therapies. Can J Cardiol. 2015; 31:631–641. [PubMed: 25936489]

36. Wang YW, Ding JP, Xia XM, Lingle CJ. Consequences of the stoichiometry of Slo1 a and auxiliary b subunits on functional properties of large-conductance Ca^{2+}-activated K^{+} channels. J. Neurosci. 2002; 22:1550–1561. [PubMed: 11880485]

37. Knaus HG, Garcia-Calvo M, Kaczorowski GJ, Garcia ML. Subunit composition of the high conductance calcium-activated potassium channel from smooth muscle, a representative of the mSlo and slowpoke family of potassium channels. J. Biol. Chem. 1994; 269:3921–3924. [PubMed: 7508434]

38. Kohler R. Single-nucleotide polymorphisms in vascular Ca^{2+}-activated K^{+}-channel genes and cardiovascular disease. Pflugers Arch. 2010; 460:343–351. [PubMed: 20043229]

39. Fernandez-Fernandez JM, Tomas M, Vazquez E, Orio P, Latorre R, Senti 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. [PubMed: 15057310]

Novelty and Significance

What is New?

- The stimulated surface-trafficking of BK channel β1 subunits by either nitric oxide or membrane depolarization is inhibited in cerebral artery smooth muscle cells of stroke-prone spontaneously hypertensive rats (SP-SHR).
- Spontaneously active protein kinase C inhibits β1 subunit trafficking, which attenuates BK channel activation in arterial smooth muscle cells of SP-SHRs.
- PKC inhibition or overexpression of a phosphorylation-incapable Rab11A mutant (Rab11AS177A) restores stimulated β1 subunit trafficking and BK channel activation.
- PKC inhibition restores vasodilation to NO by enabling β1 subunit trafficking in cerebral arteries of SP-SHRs.

What is Relevant?

- Hypertension is associated with vasoconstriction, attenuated NO-mediated vasodilation, and alterations in BK channel functionality, although interplay between these pathological modifications and mechanisms involved are unclear.
- The regulated surface-trafficking of BK channel α and β1 subunits in smooth muscle cells is a mechanism recently described to control arterial contractility.
- Whether BK channel subunit trafficking is altered during cardiovascular diseases and leads to changes in functionality is unclear.

Summary

Our data indicate that spontaneously active PKC inhibits β1 subunit trafficking in smooth muscle cells and is responsible for dysfunctional NO-induced BK channel activation and vasodilation in cerebral arteries of SP-SHRs.

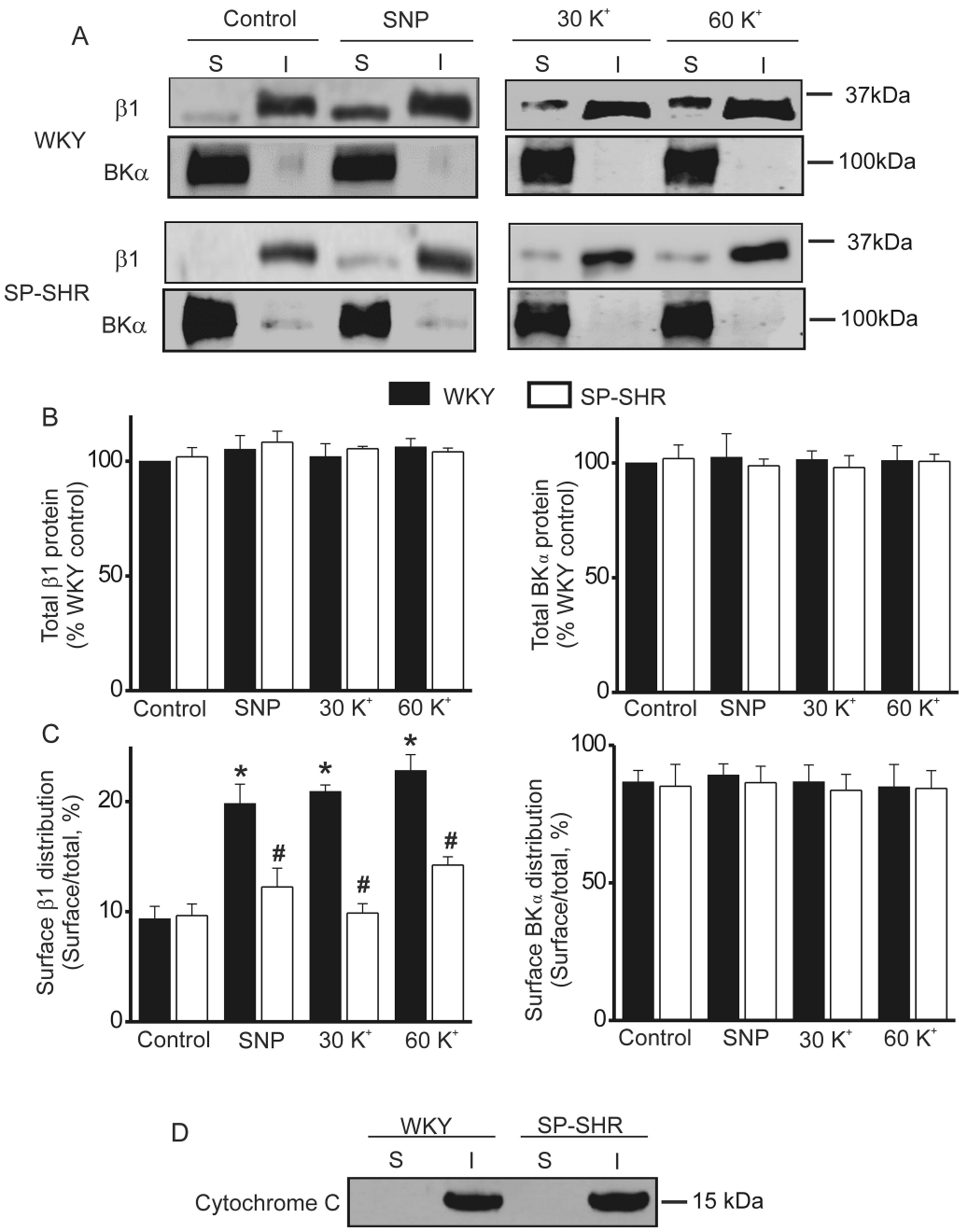

Figure 1.

NO-induced and depolarization-induced β1 subunit trafficking is impaired in cerebral arteries of SP-SHRs. (A) Representative Western blots showing surface (S) and intracellular (I) β1 subunits and BKα subunits. Sodium nitroprusside (SNP, 10 μmol/L). (B) Total BKα and β1 proteins are unaltered by SNP, 30 mmol/L K^+ and 60 mmol/L K^+ (n=5 for each). (C) The regulation of surface β1 and BKα subunits by SNP, 30 mmol/L K^+ and 60 mmol/L K^+ (n=5). * $p<0.05$ vs. control in WKY; # $p<0.05$ vs. the same condition in WKY. (D) Representative Western blot illustrating that cytochrome C is detected only in the non-

biotinylated (intracellular) fraction of WKY and SP-SHR arteries (representative of 4 experiments).

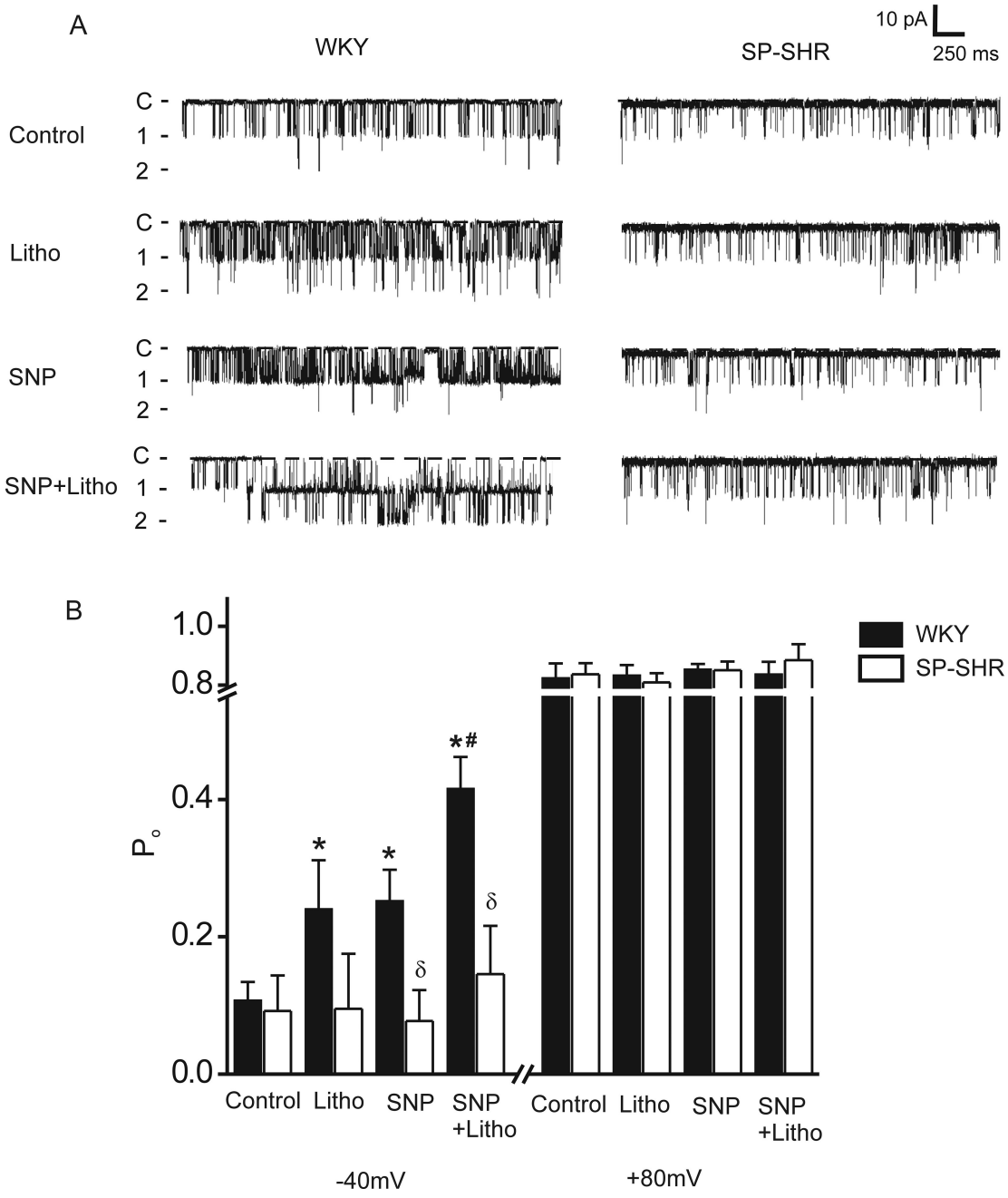

Figure 2.

BK channel activation is attenuated in cerebral artery myocytes of SP-SHRs. (A) Representative traces of BK channels recorded at −40 mV in inside-out patches from untreated myocytes and those exposed to SNP (10 μM, 10min) in the presence and absence of lithocholate (Litho, 45 μM) that was applied directly to the patch. (B) Mean Po of BK channels under indicated conditions at −40 and +80 mV (n=6 for each). * $p<0.05$ vs. control in WKY; # $p<0.05$ vs. SNP in WKY; δ $p<0.05$ vs. the same condition in WKY.

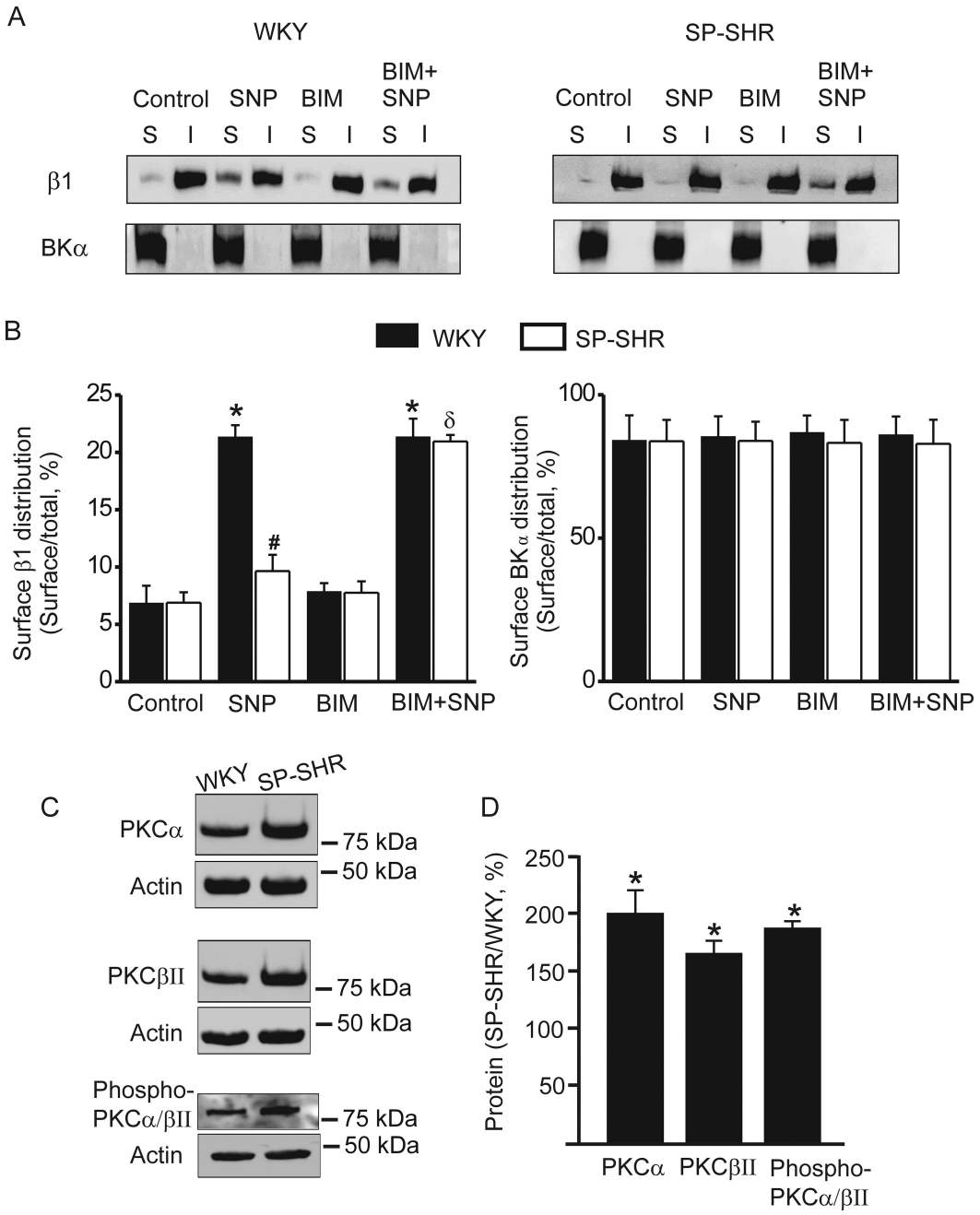

Figure 3.

BIM restores NO-induced β1 subunits trafficking in cerebral arteries of SP-SHR. (A) Representative Western blots illustrating surface (S) and intracellular (I) β1 and BKα subunits in WKY rat and SP-SHR arteries in control, SNP (10 umol/L, 10 min), BIM (10 umol/L, 1 h) and BIM+SNP. (B) Mean data of surface β1 and BKα proteins (n=5 for each). * $p<0.05$ vs. control in WKY; # $p<0.05$ vs. SNP in WKY; δ $p<0.05$ vs. control in SP-SHR. (C) Western blots illustrating total PKCα, total PKCβII and phosphoPKCα/βII in WKY rat and SP-SHR arteries. (D) Mean data (n=4 for each). * $p<0.05$.

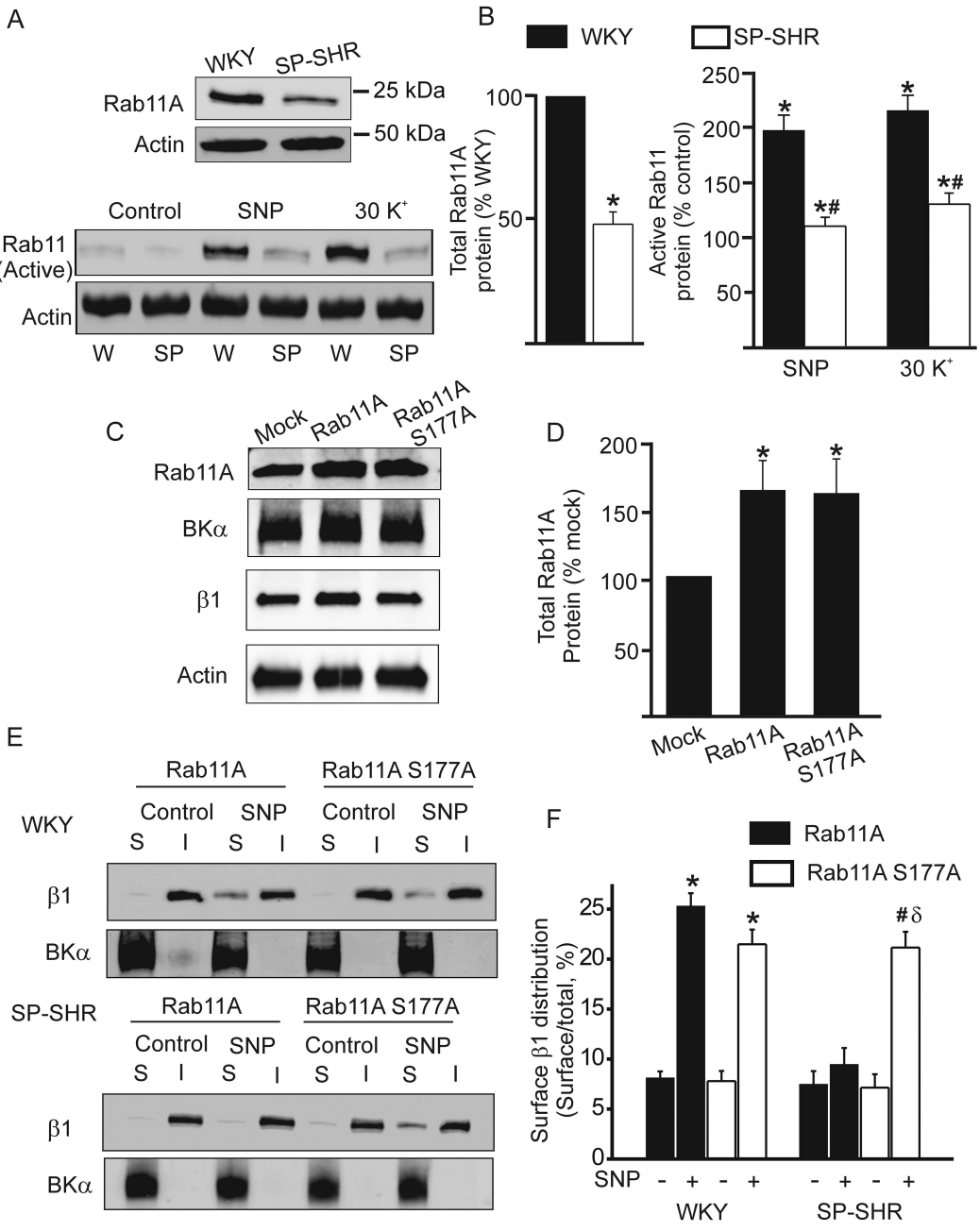

Figure 4.

Rab11A S177A restores SNP-induced surface-trafficking of β1 subunits in cerebral arteries of SP-SHRs. (A) Representative Western blots illustrating total Rab11A and active Rab11 and modulation by SNP (10 μmol/L, 10 min) and 30 mmol/L K^+ (10 min) in WKY and SP-SHR arteries. W=WKY, SP=SP-SHR (B) Mean data (n=4 for each). * $p<0.05$ versus control, # $p<0.05$ versus WKY. (C) Western blots of Rab11A, BKα, β1 and actin proteins in arteries transfected with empty vectors (mock) or plasmids that encode Rab11A or Rab11AS177A. (D) Mean data of total Rab11A protein compared with mock control in arteries overexpressing Rab11A or Rab11A S177 (n=5). * $p<0.05$ vs. mock (E) Representative

Western blots illustrating surface (S) and intracellular (I) β1 and BKα subunits in WKY rat and SP-SHR arteries in control or SNP (10 μmol/L, 10 min). (F) Mean data of surface protein of β1s and BKα subunits. (n=5 for each) * $p<0.05$ vs. control (no SNP) in WKY; # $p<0.05$ vs. control (no SNP) in SP-SHR; δ $p<0.05$ vs. SNP in SP-SHR Rab11A.

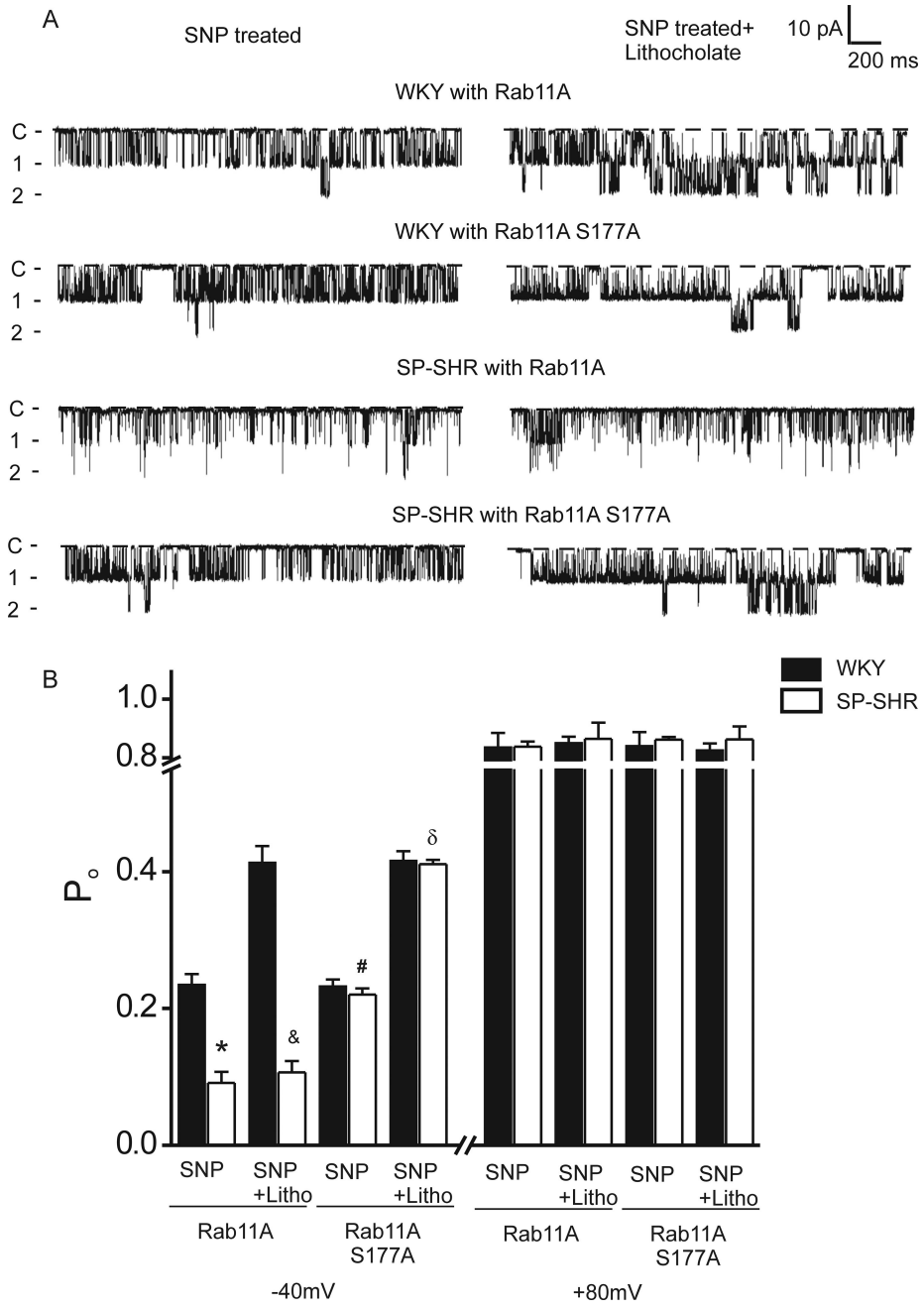

Figure 5.

Rab11A S177A increases BK channel Po by enabling β1 subunit-mediated activation in SP-SHR myocytes. (A) Representative recordings of BK channels in inside-out patches from myocytes overexpressing Rab11A or Rab11A S177A and modulation by lithocholate (Litho, 45 μM). (B) BK channel mean Po at −40 and +80 mV (n=6 for each). * $p<0.05$ vs. SNP in WKY Rab11A; & $p<0.05$ vs. SNP+litho in WKY Rab11A; # $p<0.05$ vs. SNP in SP-SHR Rab11A; δ $p<0.05$ vs. SNP+Litho in SP-SHR Rab11A.

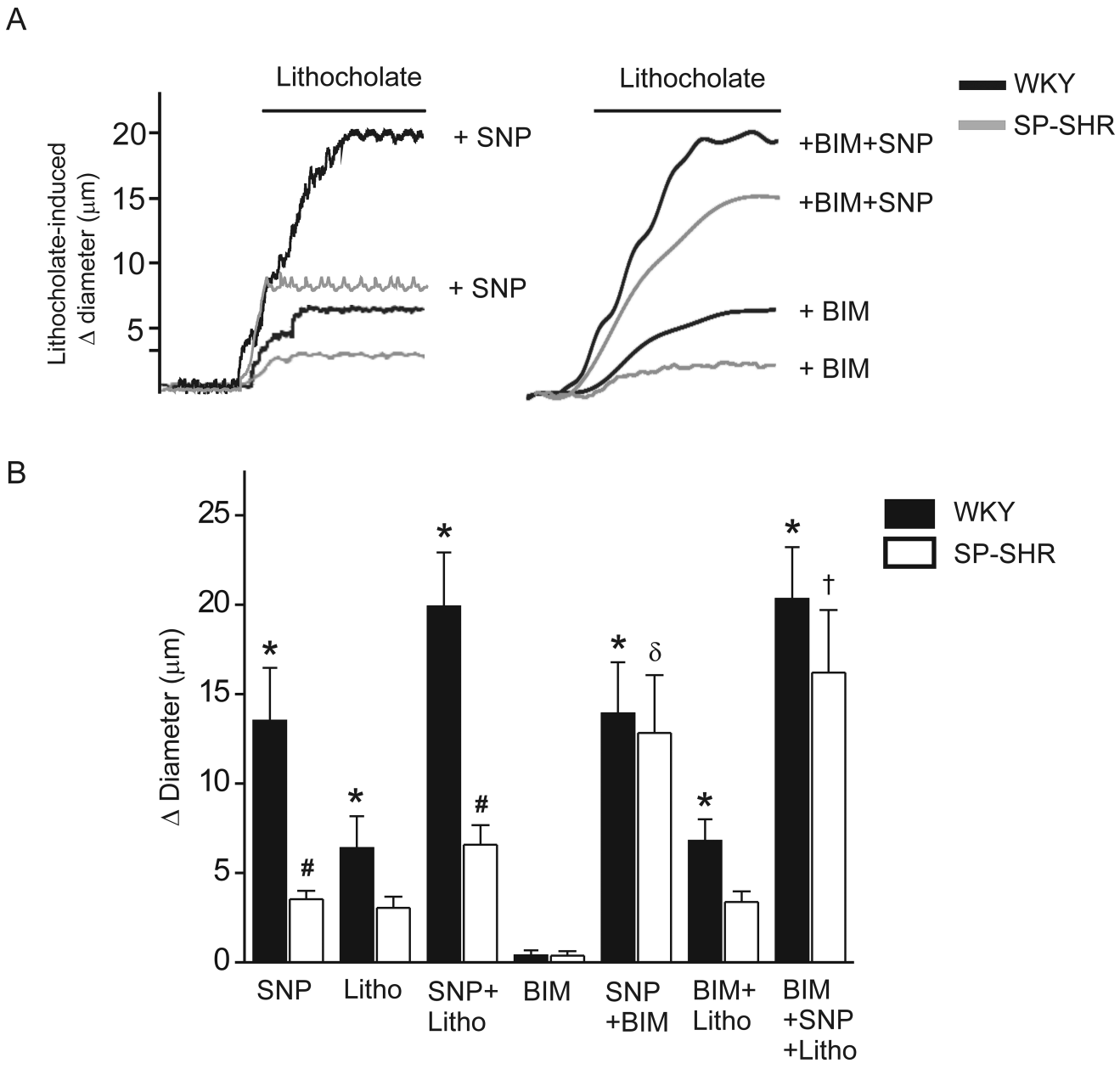

Figure 6.

BIM enables NO-mediated vasodilation through β1 subunits in pressurized cerebral arteries of SP-SHRs. (A) Representative traces illustrating lithocholate (45 μmol/L)-induced vasodilation in the presence and absence of SNP (10 μmol/L) and modulation by BIM (10 μmol/L) in pressurized (60 mmHg) cerebral arteries from WKY rats (black) and SP-SHRs (gray). (B) Mean data (n=6 for each). * $p<0.05$ vs. control in WKY; # $p<0.05$ vs. WKY in the same condition; δ $p<0.05$ vs. SNP in SP-SHR; † $p<0.05$ vs. SNP + Litho in SP-SHR.