Published in final edited form as:

J Cardiovasc Pharmacol. 2012 January ; 59(1): 29–36. doi:10.1097/FJC.0b013e318233614c.

Requirement for functional BK channels in maintaining oscillation in venomotor tone revealed by species differences in expression of the $\beta 1$ accessory subunits

Hui Xu, MD, PhD, Sachin S Kandlikar, PhD, Erika B Westcott, PhD, Gregory D Fink, PhD, and James J Galligan, PhD

Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI, 48824

Abstract

We determined the possible role of large-conductance Ca^{2+} -activated K^+ (BK) channels in regulation of venous tone in small capacitance veins and blood pressure. In rat mesenteric venous smooth muscle cells (MV SMC), BK channel α - and $\beta 1$ -subunits were co-expressed, unitary BK currents were detected, and single channel currents were sensitive to voltage and $[\text{Ca}^{2+}]_i$. Rat MV SMCs displayed Ca^{2+} sparks and iberitoxin (IBTX)-sensitive spontaneous transient outward currents (STOCs). Under resting conditions *in vitro*, rat MV exhibited nifedipine-sensitive spontaneous oscillatory constrictions. Blockade of BK channels by paxilline and Ca^{2+} sparks by ryanodine constricted rat MV. Nifedipine caused venodilation and blocked paxilline-, KCl (20 mM) and BayK 8644-induced contraction. Acute inhibition of BK channels with IBTX *in vivo* increased blood pressure and reduced venous capacitance, measured as an increase in mean circulatory filling pressure in conscious rats. BK channel α -subunits and L-type Ca^{2+} channel $\alpha 1$ -C subunits are expressed in murine MV. However, these channels are not functional as murine MV lacked nifedipine-sensitive basal tone and rhythmic constrictions. Murine MV were also insensitive to paxilline, ryanodine, KCl and BayK8644, consistent with our previous studies showing that murine MV do not have BK $\beta 1$ -subunits. These data show that not only there are species-dependent properties in ion channel control of venomotor tone, but also that BK channels are required for rhythmic oscillations in venous tone.

Keywords

BK channels; venous smooth muscle cells; venomotor tone; L-type Ca^{2+} channels; rats vs mice

Introduction

The function of large conductance Ca^{2+} activated K^+ (BK) channels in arterial vascular smooth muscle cells (SMCs) is well established. Functional BK channels have also been detected in the large conduit portal vein from rat,¹ rabbit,² guinea pig,³ and BALB/c mice,⁴ and also rat inferior vena cava⁵ and horse penile veins.⁶ In these vessels, BK channels contribute to spontaneous phasic contractile activity and venous tone. BK channels also contribute to venous tone in human large conduit saphenous,^{7,8,9} and forearm veins^{10,11} by maintaining venous membrane potential and endothelial-NO-dependent dilation. Importantly, the channels may also regulate venous tone in capacitance rat small mesenteric veins (MV),^{12,13} which would contribute to blood pressure regulation.^{14,15}

Systemic capacitance veins contain ~70% of total blood volume, and over half of this blood is held in small splanchnic veins and venules.¹⁶ Therefore, systemic vascular capacitance depends largely on small veins in the splanchnic circulation.¹⁶ We and others have suggested that regulation of venous tone contributes to regulation of arterial pressure.^{14,17,18} Early studies suggested that impairment of BK channels *in vivo* not only increases MAP, but also decreases venous capacitance in pigs.¹⁹ Thus, it is important that further studies directly determine the contribution of venous BK channel function in regulating of venous tone in small capacitance veins.

Although there are some data indicating in the presence of functional BK channels in rat MV,^{12,13} there are no data directly demonstrating functional BK channels in capacitance venous SMC in rats. Thakali and co-workers²⁰ have shown that L-type Ca^{2+} channels in rat MV are “silenced” by intracellular Ca^{2+} . If so, it is unclear how BK channels could modulate venous function in the absence of functional L-type Ca^{2+} . Interestingly, we recently found that functional BK channels are almost absent in the majority murine (C57BL mice) venous system, BK $\beta 1$ -subunit expression was only detected in the large conductance portal and pulmonary veins. Previous data from our laboratory also demonstrate that, like BK channels, L-type Ca^{2+} channels also do not modulate murine venous tone.²¹ These data suggest that BK channel function is complex in the rodent venous system and requires further investigation to understand the role of these channels in the regulation of venomotor tone. These studies are especially important in term of clinical relevance because rodents are widely used in animal models for studies of cardiovascular diseases, particularly hypertension.

In the present studies, we used molecular, cellular and integrative approaches to demonstrate that BK channels are expressed and functional in rat MV. We also determined the functional interaction of BK/L-type Ca^{2+} channels in rat and murine MV. We compared the pharmacological and functional responses of rat and murine MV since murine MV from C57BL mice lack functional BK channels.²¹ The outcome from this study proves a better understanding of the contribution of BK channel function to venous tone.

Material and Methods

Animals

All of the studies were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23, revised 1996) and approved by the Michigan State University Institutional Animal Care and Use Committees. Male rats (≈ 350 g, Sprague Dawley) and C57BL/6 mice (≈ 25 g) were purchased from Charles River Laboratories, (Portage, MI). All animals were fed normal diet and were studied at 10–12 weeks of age

Analysis of BK channel expression in rat MV

Rats were euthanized with pentobarbital (80–100 mg/kg, ip). Rat MV were isolated as previously described.²² BK channel α - and $\beta 1$ -subunit expressions in rat MV SMCs were analyzed by immunocytochemistry. SMCs were incubated with rabbit anti-BKCa²⁺ $\beta 1$ (1:200; Alomone Labs) and mouse anti-BK channel α -subunits (anti-*slo 1*, 1:200, Antibodies Inc., Davis, CA) overnight at 4°C. SMCs were then incubated with sheep anti-mouse IgG conjugated to FITC (1:50) and goat anti-rabbit Ig conjugated to Cy3 (1:400) (Jackson ImmunoResearch) for 1 hour at room temperature. Images were obtained using a Nikon fluorescence microscope.

For Western blot analysis, we used a primary antibody targeted to BK channel α -subunits (anti-BKCa²⁺ 1.1, 1:500; Alomone Labs. Ltd., Jerusalem, Israel) and a secondary antibody

conjugated to horseradish peroxidase. The membranes were developed using an ECL kit (Amersham Pharmacia Biotech) and exposed to film (Hyperfilm-ECL, Amersham Pharmacia Biotech). SMC α -actin (1:5000; Oncogene) was used to normalize measurements of BK channel protein.

Expression of BK channel α -subunits and L-type Ca^{2+} $\alpha 1\text{-c}$ in murine MV segments were also analyzed by immunocytochemical staining (see supplemental materials).

Whole-cell and inside-out single channel recording of BK currents in rat MV myocytes

Patch-clamp recordings in freshly isolated rat MV myocytes were obtained as previously reported.²² The methods have been outlined in detail in Supplemental Materials.

Recording of Ca^{2+} sparks in rat MV (See Supplemental Materials)

Ca^{2+} sparks trigger STOCs (activation of BK channels) and cause membrane hyperpolarization and reduced vascular tone.²³ We determined if Ca^{2+} sparks are present in rat MV SMC by confocal microscopy (see Supplemental Materials).

Measurement of BK channel function and venous reactivity responses in isolated and pressurized MV

MV (outer diameter ~250 – 350 μm in rat, ~ 150 μm in mice without pressure) were mounted to a custom-made pressure myograph apparatus AND superfused with Krebs' solution equilibrated with compressed air (5% CO_2 -21% O_2 -74% N_2).²¹ Inner diameter (ID) was recorded using Diamtrak edge tracking software. Oxygenated Krebs' solution, maintained at 37°C, was circulated through the chamber. The lumen of the vessel was filled with Krebs' solution and maintained at pressures controlled by the height of the Krebs' solution reservoir. Pressure was increased from 0 to 5.5 cm H_2O (4 mmHg). Ca^{2+} - free Krebs' solution contained EGTA (2 mmol/L) without added CaCl_2 .

Measurements of Mean Circulatory Filling Pressure (MCFP)

Under isoflurane anesthesia (2–3%), rats were instrumented to allow repeated measures of MCFP in conscious undisturbed animals using methods described previously.^{14,17} Depth of anesthesia was assessed as stability of respiratory movement, pupil size and paw-pinch reflexes. Briefly, femoral arterial and venous catheters were implanted to allow measurement of arterial blood pressure (AP) and central venous pressure (CVP), respectively. A right atrial balloon catheter was advanced from the right jugular vein such that inflation of the balloon produced brief circulatory arrest to allow MCFP measurements. The ends of all of the catheters were tunneled subcutaneously and exited the rat between the scapulae into a stainless steel spring attached to the rat by a loosely fitted rubber jacket (Instech Solomon Inc, PA, USA). Antimicrobial prophylaxis and postoperative analgesia were achieved by administration of ticarcillin-clavulanate (200 mg/kg IV) and enrofloxacin (5 mg/kg IV) and buprenorphine (0.05 mg/kg SC), respectively. The rats were then loosely tethered in individual plastic cages to allow continuous access to all of the catheters without handling or disturbing the rat. Ticarcillin-clavulanate (200 mg/kg IV) and enrofloxacin (5 mg/kg IV) were administered daily for the duration of the experiment. Vascular catheters were flushed with heparinized saline (20 U/ml) each day, and the balloon catheter was briefly inflated daily to prevent adhesions to the atrial wall. Systolic, diastolic, and mean AP (MAP) and heart rate (HR) were recorded at the same time each morning for 20 minutes by connecting the exteriorized catheters to pressure transducers that were connected to digital pressure monitors (Digi-Med BPA-400, Micro-Med) linked to a computerized data acquisition system (DMSI-400, Micro-Med). The pressure transducers were calibrated daily against a column of water. One week after surgery, the right atrial

balloon catheter was inflated with 0.25 mL of saline for 5 seconds, resulting in a rapid fall in AP and a simultaneous rise in central venous pressure, both of which quickly reached a plateau. MCFP was computed from arterial plateau pressure (APP) and venous plateau pressure (VPP) using the following formula:

$$\text{MCFP} = \text{VPP} + (\text{APP} - \text{VPP}) / 60$$

1/60 represents the ratio of arterial to the venous compliance.^{14,17}

Statistical Analysis

Data are reported as means $\pm$ SE from *n* rats. Paired and unpaired t-tests were used to make single point comparisons. Current-voltage (*I-V*) relationships were determined by measuring steady-state current (i.e., at the end of the 200-ms test pulse) and compared between treatments. Comparisons of multiple points generated in *I-V* curves and concentration responses were accomplished using a two-way ANOVA with repeated measures followed by Student-Newman-Keuls test. Before and after IBTX treatment differences for MCFP and MAP were assessed by a 1-way ANOVA and posthoc multiple comparisons with Tukey's test. $P < 0.05$ was considered significant.

Results

Functional expression of BK channels in RMV

Fig. 1A shows representative immunofluorescence image demonstrating co-localization of BK channel α - and β 1-subunits in freshly isolated rat MV SMCs. Fig 1B shows a representative Western blot for protein expression of BK channel α -subunits in the tissues of rat mesenteric arteries (MA) and MV where expression of the BK channel α -subunit protein was similar in both blood vessels. Rat brain was used as a positive control.

Ca^{2+} sparks activate spontaneous transient outward currents in SMCs (STOCs) and STOCs, which provide a negative feedback mechanism that reduces contraction when Ca^{2+} enters SMCs following L-type Ca^{2+} channel activation. We observed STOCs in rat MV myocytes and the STOCs were blocked by the BK channel blocker, IBTX (0.1 μM) ($n=6$, Fig 1C), indicating that STOCs are due to activation of BK channels. In further studies of Fluo 4-loaded rat MV strips, Ca^{2+} sparks were detected and recorded from individual rat MV myocytes (Fig 1D). The presence of both sparks and STOCs in rat MV SMCs support data demonstrating the expression of BK channels and also suggest that, as in other types of VSMCs, rat MV STOCs may be triggered by the presence of Ca^{2+} sparks.

Characterization of BK currents in rat MV myocytes

K^{+} currents were measured in isolated rat MV myocytes using the whole cell patch clamp technique (Fig 2A). Whole cell *I-V* curves constructed by measuring peak I_o evoked by 200 ms duration step commands from an initial holding potential of -70 mV to $+80$ mV in 10 mV increments at 15 s intervals (Fig 2B). *I-V* curves were right-shifted after IBTX treatment (0.1 μM , 5 minutes, $n = 6$). The activation threshold for I_o was 0 mV (Fig. 2B). The amplitude of the current difference before and after treatment with IBTX was used to measure the BK currents. Peak I_o was reduced by IBTX from 2.5 ± 0.3 nA to 1.5 ± 0.1 nA ($P < 0.05$).

Unitary BK currents were recorded at patch potentials between -60 mV and $+100$ mV and at 0 and 1 $\mu\text{mol/L}$ $[\text{Ca}^{2+}]_i$ (Fig. 3A) using inside-out patches of rat MV SMCs. Unitary currents were absent in Ca^{2+} free medium (Fig. 3B). A linear fit of the current voltage

relationship revealed a unitary conductance of 220 ± 10 pS, (Fig. 3C ($[Ca^{2+}]_i = 1 \mu M$, $n=6$). These data indicate that BK channel activity in rat MV SMCs is $[Ca^{2+}]_i$ and voltage dependent.

BK channels regulate MV tone in rat, but not in mice

The next studies were designed to directly test BK channel function in isolated and pressurized small rat MV *in vitro*. Pressurized rat MV generated rhythmic, spontaneous contractions (Fig 4A). The paxilline ($0.5 \mu M$, BK channel blocker) and the ryanodine receptor antagonist, ryanodine ($10 \mu M$) caused constrictions in rat MV (Fig 4A, 4B, 4D, S2A), but not MMV (Fig 4C, 4D, S2B). The L-type Ca^{2+} channel blocker, nifedipine ($1 \mu M$) not only reversed the paxilline-induced contractions, but also caused venous dilation (Fig 4D, Fig S2A). NE ($1 \mu M$)-induced constrictions were similar in rat and murine MV (Fig 4D). Together, these data suggested that although BK channel α -subunits were expressed in murine MV (Fig. S1), these channels are non-functional.²¹

L-type Ca^{2+} channel-dependent venous tone and rhythmic contractions in rat MV

We next tested the role of Ca^{2+} channel function in both basal tone and rhythmic contractions in rat MV. Nifedipine ($1 \mu M$) not only blocked the rhythmic constrictions, but also caused a venous dilation in rat MV (Fig 5A, 5B). In murine MV, there were no rhythmic contractions and nifedipine did not cause murine MV dilation (Fig 5B, S2C). Ca^{2+} -free media also inhibited the rhythmic constrictions and caused venous dilation in rat MV, but not in murine MV (Fig 5D).

L-type Ca^{2+} channel function in rat, but not in murine MV

In the next studies, we determined the concentration-dependency of KCl-induced contractions in rat and murine MV with or without of nifedipine. KCl caused a concentration-dependent constriction in rat, but not in murine MV (Fig.5C, S3C). In rat MV, the maximal constriction occurred at 40 mM of KCl, and the concentration response curve was significantly right-shifted by nifedipine ($1 \mu M$), the EC 50 shifted from 24 ± 1 mM to 45 ± 2 mM (pD_2 1.4 ± 0.03 vs 1.7 ± 0.05 , $P < 0.05$). Nifedipine did not inhibit the contractions caused by 60 mM of KCl (Fig. 5C). L-Type Ca^{2+} channel agonist, BayK8644 ($0.1 \mu M$) constricted rat MV, and the constrictions were reversed by nifedipine ($1 \mu M$) (Fig 5D, S3A). BayK 8644 did not constrict murine MV (Fig 5D, S3B), although α_1 -C subunits were expressed in these blood vessels (Fig S1).

Venous BK channels contribute to regulation of venomotor tone and arterial blood pressure in rats

The next studies were designed to directly test BK channel function in regulating venomotor tone and blood pressure in rats. Venomotor tone was evaluated by measurements of MCFP in conscious rats. MAP and MCFP were measured before and after *i.v.* administration of IBTX ($0.3 \mu M/kg$ in 0.4 ml saline, over 1 min). The dose used was based on published protocols.^{19,24} MCFP was measured at 15 and 5 minutes before IBTX administration to establish control values, and then re-measured 5 and 15 minutes after IBTX administration. MAP was measured continuously. Fig. 6B shows that MCFP significantly increased from 7.2 ± 0.4 mmHg to 8.8 ± 0.6 mmHg 5 minutes after IBTX administration ($n=7$, $P < 0.05$). After 15 minutes, MCFP was not different from control values. MAP also increased after IBTX treatment from 112 ± 4 to 125 ± 2 mmHg ($P < 0.01$). MAP returned to baseline 5 minutes after IBTX administration (Fig. 6A). All animals recovered after 30 minutes. Heart rate did not change significantly after IBTX administration. Control animals were treated with saline (0.4 ml) only, and the MCFP and MAP were measured as described for IBTX

treatment. The same volume of saline injections did not change MCFP or MAP in control animals in our pilot studies.

Discussion

In the current studies, we show for the first time that SMCs from rat mesenteric *capacitance* veins express functional BK channels which regulate venous tone *in vitro* and *in vivo*. Similar BK channel regulation of venomotor tone does not occur in mice.

Previous work demonstrated that functional BK channels were detected in portal veins from rat,¹ rabbit,² guinea pig,³ and BALB/c mice.⁴ These studies are consistent with other studies showing that BK channels also modulate tone in human saphenous^{7,8,9} and forearm veins.^{10,11} Our data directly confirm expression of BK functional channels in rat MV SMCs as previous studies have suggested.^{12,13} In our immunocytochemistry and Western blot studies, both BK channel α - and β 1-subunits were expressed in rat MV myocytes. In our patch clamp studies, about 40% of the total outward current activated by *Em* depolarization was blocked by IBTX indicating that this fraction of outward current is carried by BK channels. We also found that rat MV BK channel activity is voltage and $[Ca^{2+}]_i$ -dependent; and the single channel conductance was about 220 pS similar to that found in arteries.²² BK channels mediate STOCs in rat MV myocytes and the STOCs are likely linked to Ca^{2+} sparks because the STOCs were blocked by ryanodine which blocks Ca^{2+} sparks in other vascular SMCs.²³ Our studies are the first to demonstrate Ca^{2+} sparks, STOCs and unitary BK channel activity in rat MV SMCs.

BK channels are activated by membrane depolarization and increases in free intracellular calcium (Ca^{2+} sparks).²³ In VSMCs, BK channels are composed of α -subunits that form the pore, and accessory β 1-subunits that modulate Ca^{2+} sensitivity of the α -subunits.²⁵ Blocking of both BK channels and Ca^{2+} sparks constricted pressurized rat MV indicating that BK channels modulate rat MV tone physiologically. These findings in rat MV differ from those in murine MV which do not have functional BK channels.²¹ Our current study showed that tone of murine MV *in vitro* is not altered by drug treatments which block BK channels or Ca^{2+} sparks, although BK channel α -subunit protein is expressed in murine MV. BK channel α -subunits can function without β 1-subunits, but generally, BK channel is harder to open because there is about a 30 – 40 mV positive shift in the voltage required to open BK channels in the absence of β 1-subunits in SMCs.²⁵ Murine MV SMCs do not express BK channel β 1-subunits.²¹ Our results indicate that the co-expression of BK channel α - and β 1-subunits is required for BK channel function in MV SMC.

BK channels regulate vascular tone through manipulation of voltage gated Ca^{2+} channel activity.^{23,25} VSMCs express two main types of voltage gated Ca^{2+} channels, L-type and T-type. Due to the unavailability of sufficiently selective blockers of T-type Ca^{2+} channels, we focused on function of L-type Ca^{2+} channels in MV. Rat MV generated rhythmic contractions in pressurized MV *in vitro*, which were blocked by nifedipine, indicating the expression of functional L-type Ca^{2+} . Nifedipine blocked rat MV constrictions caused by low concentration of KCl. Furthermore, the L-type Ca^{2+} channel activator, BayK 8644, constricted rat MV and this response was blocked by nifedipine. These data indicate that L-type Ca^{2+} channel can regulate resting and active tone of rat MV. This result is important because L-type Ca^{2+} channel function in veins is controversial. Several studies have reported that L-type Ca^{2+} channels function in the SMCs of conduit veins such as the saphenous and portal veins.^{26,27} Conduit veins also express functional BK channels and the two channels collaborate to produce venous rhythmic contractions of conduit veins.^{4,28} The role of BK channels in small venules (20–40 μ m) in the microcirculation is not as clear as some investigators report L-type Ca^{2+} channels are not functional in these small veins^{29,30}

while others show that L-type Ca^{2+} channels are important for venular tone.³¹ The possible absence of functional L-type Ca^{2+} channels in small venules is also supported by clinical data where L-type Ca^{2+} channels blockers cause pedal edema formation. This may be caused by an imbalance in arteriolar and venular dilation, which increases hydrostatic pressure in the capillary circulation and drives fluid shifts into the interstitial compartment.^{32,33} We found that the contractile properties of rat MV are similar to portal veins, as rat MV exhibited L-type Ca^{2+} channels-dependent basal tone and rhythmic contractions. Blocking of L-type Ca^{2+} channels completely inhibited KCl-induced rat MV constrictions at low KCl (20 mM) concentrations, and the concentration response curve was significantly right-shifted by nifedipine, indicating the presence of functional L-type Ca^{2+} channels in rat MV. Our results contrast to previous studies where it was shown that functional L-type Ca^{2+} channels are expressed in rat MV, but the channel activity is “silenced” by intracellular Ca^{2+} .²⁰ In this study it was reported that nifedipine did not block KCl (60 mM)-induced venoconstrictions which is confirmed by our data but we did find that constrictions caused by lower KCl (20 mM) were blocked by nifedipine, indicating the presence of functional L-type Ca^{2+} channels in rat MV. We also found that although murine MV express the $\alpha 1\text{-C}$ subunit of the L-type Ca^{2+} channels, these channels are not functional as KCl or BayK8644 did not constrict these veins.

Venous BK channels modulate venous tone and arterial blood pressure in rats *in vivo*: clinical significance?

The significance and the clinical relevance of arterial BK channel function have been well established, BK channel agonists have been developed as new and effective treatments for prevention and treatment of human diseases.^{34,35} Venomotor tone contributes to overall hemodynamic homeostasis in humans, and chronically increased venomotor tone exists in essential hypertensive patients.^{36,37} Venomotor tone is determined by myogenic factors, local and circulating hormones, and sympathetic nerve activity.^{37,38} In the present study, we show that BK channels also contribute to the regulation of venous tone in rats *in vivo*, since acute blockade with IBTX increased MCFP, a measure of venomotor tone.³⁴ The increased blood pressure caused by IBTX probably resulted from both arterial and venous constriction. Others have shown that blocking of BK channels *in vivo* increased total peripheral resistance by 30–60%, and caused 30% decrease in effective venous compliance (an index for venous tone) in pigs.¹⁹ The data indicated that activation of venous BK channel is an important mechanism maintaining venomotor tone *in vivo*. Limitations imposed by the technique used to measure MCFP make it difficult to match the time course of MCFP changes with MAP measurements. Peak MCFP changes may occur prior to the first measurement at 5 minutes post IBTX treatment. However, IBTX-induced increases in MCFP lasted longer than the elevation in MAP after IBTX administration. Taken together, the data indicated that BK channel function may play a prominent role in regulating venous tone *in vivo* in rats. In addition, the MCFP and MAP changes after IBTX administration *in vivo* may not be fully vasculature-dependent, since the BK channels in other system may also be inhibited by IBTX, for example, the BK channels in central nerve system which are also involved in regulation of blood pressure. Blocking BK channels *in vivo* can change hemodynamics by multiple mechanisms.

Both rats and mice are extensively used as animal models of cardiovascular diseases, but expression and function of venous BK channel differs in these animals. Our studies have indicated a lack of functional BK and voltage gated Ca^{2+} channels in the murine venous system especially capacitance veins.²¹ We have found that BK channel $\beta 1$ -subunit knock out mice are not hypertensive.²¹ The $\beta 1$ - subunit knock out would only alter arterial SMC function as veins in mice do not express functional BK channels. These data suggest that BK channel deficiency in arterial alone is not sufficient to cause hypertension and that

alterations in the function of capacitance veins may also be required to cause sustained elevations in blood pressure. The contribution of venous BK channel functions in regulation of blood pressure in human are still unclear, but other investigators have reported the presence of functional BK channels in human saphenous^{7,8,9} and forearm veins,^{10,11} and BK channel mediated venodilation is impaired in patients with chronic kidney failure.^{10,11} These studies, at least, indicated that venous BK channel dysfunction may involve in human diseases. However, there is still no evidence of functional BK channels in small capacitance mesenteric veins, although forearm veins could be distinguished as capacitance veins in humans.³⁹ Therefore, the future studies will require confirmation of the presence of functional BK channels in human capacitance veins as well as its role in the development of cardiovascular diseases.

Summary and Conclusion

These studies show that BK channel activity is important in the regulation of venomotor tone in rats but not mice. BK channels are expressed and produce IBTX-sensitive STOCs in isolated myocytes from these vessels, and our data also indicate that Ca^{2+} sparks in rat venous SMCs may be functionally coupled to the activity of BK channels in those cells. This work also demonstrates for the first time that BK channels regulate venous tone *in vivo*, as BK channel blockade with IBTX increases MCFP in chronically catheterized rats. These studies have also uncovered species-related differences in the role of BK channels: in contrast to the data from rats, murine MV do not have functional BK channels or L-type Ca^{2+} channels, despite the expression of the BK- α - and $\text{Ca} \alpha 1\text{-C}$ subunits, respectively.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by National Heart, Lung and Blood Institute grant numbers: P01 HL070687 and by Michigan State University Health and Biomedical Research Initiative Award for Dr. Hui Xu.

References

1. Cogolludo AL, Pérez-Vizcaíno F, López-López G, Ibarra M, Zaragoza-Arnáez F, Tamargo J. Propafenone modulates potassium channel activities of vascular smooth muscle from rat portal veins. *J Pharmacol Exp Ther*. 2001; 299:801–810. [PubMed: 11602697]
2. Morales E, Cole WC, Remillard CV, Leblanc N. Block of large conductance Ca^{2+} -activated K^{+} channels in rabbit vascular myocytes by internal Mg^{2+} and Na^{+} *J Physiol*. 1996; 495:701–716. [PubMed: 8887777]
3. Karle CA, Yao X, Kreye VA. A K^{+} single channel and whole-cell clamp study on the effects of levocromakalim in guinea pig portal vein cells. *Naunyn Schmiedeberg Arch Pharmacol*. 1998; 358:374–381. [PubMed: 9774226]
4. Saleh SN, Angermann JE, Sones WR, Leblanc N, Greenwood IA. Stimulation of Ca^{2+} -gated Cl^{-} currents by the calcium-dependent K^{+} channel modulators NS1619 [1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one] and isopimaric acid. *J Pharmacol Exp Ther*. 2007; 321:1075–1084. [PubMed: 17347326]
5. Raffetto JD, Ross RL, Khalil RA. Matrix metalloproteinase 2-induced venous dilation via hyperpolarization and activation of K^{+} channels: relevance to varicose vein formation. *J Vasc Surg*. 2007; 45:373–380. [PubMed: 17264019]
6. Martínez AC, Prieto D, Hernández M, Rivera L, Recio P, García-Sacristán A, Benedito S. Endothelial mechanisms underlying responses to acetylcholine in the horse deep dorsal penile vein. *Eur J Pharmacol*. 2005; 515:150–159. [PubMed: 15894308]

7. Milesi V, Aiello EA, Rebolledo A, Gomez Alvis A, Grassi de Gende AO. Role of a Ca^{2+} -activated K^{+} current in the maintenance of resting membrane potential of isolated, human, saphenous vein smooth muscle cells. *Pflugers Arch.* 1999; 437:455–461. [PubMed: 9914403]
8. Verónica M, Aiello EA, Rebolledo A, Alvis AG, Grassi de Gende AO. Role of a Ca^{2+} -activated K^{+} current in the maintenance of resting membrane potential of isolated, human, saphenous vein smooth muscle cells. *Pflugers Arch.* 1999; 437:455–461. [PubMed: 9914403]
9. Mauricio MD, Serna E, Cortina B, Novella S, Segarra G, Aldasoro M, Martínez-León JB, Vila JM. Role of Ca^{2+} -activated K^{+} channels on adrenergic responses of human saphenous vein. *Am J Hypertens.* 2007; 20:514–519. [PubMed: 17485013]
10. Martínez-León JB, Segarra G, Medina P, Vila JM, Lluch P, Peiró M, Otero E, Lluch S. Ca^{2+} -activated K^{+} channels mediate relaxation of forearm veins in chronic renal failure. *J Hypertens.* 2003; 21:1927–1934. [PubMed: 14508200]
11. Segarra G, Lluch P, Mauricio MD, Vila JM, Medina P, Martínez-León JB, Aldasoro M, Lluch S. Contractile hyporesponsiveness to norepinephrine of forearm veins in chronic renal failure. *Am J Hypertens.* 2006; 19:818–822. [PubMed: 16876681]
12. Nagakawa T, Yamazaki M, Hatakeyama N, Stekiel TA. The mechanisms of propofol-mediated hyperpolarization of in situ rat mesenteric vascular smooth muscle. *Anesth Analg.* 2003; 97:1639–1645. [PubMed: 14633534]
13. Stekiel TA, Contney SJ, Kokita N, Bosnjak ZJ, Kampine JP, Stekiel WJ. Mechanisms of isoflurane-mediated hyperpolarization of vascular smooth muscle in chronically hypertensive and normotensive conditions. *Anesthesiology.* 2001; 94:496–506. [PubMed: 11374612]
14. Fink GD, Johnson RJ, Galligan JJ. Mechanisms of increased venous smooth muscle tone in deoxycorticosterone acetate-salt hypertension. *Hypertension.* 2000; 35:464–469. [PubMed: 10642342]
15. Greenway CV, Lauth WW. Blood volume, the venous system, preload, and cardiac output. *Can J Physiol Pharmacol.* 1986; 64:383–387. [PubMed: 3730923]
16. Rothe CF. Mean circulatory filling pressure: its meaning and measurement. *J Appl Physiol.* 1993; 74:499–509. [PubMed: 8458763]
17. King AJ, Fink GD. Chronic low-dose angiotensin II infusion increases venomotor tone by neurogenic mechanisms. *Hypertension.* 2006; 48:927–933. [PubMed: 17000931]
18. Martin DS, Rodrigo MC, Appelt CW. Venous tone in the developmental stages of spontaneous hypertension. *Hypertension.* 1998; 31:139–144. [PubMed: 9449405]
19. Zanzinger J, Czachurski J, Seller H. Role of Calcium-dependent K^{+} channels in the regulation of arterial and venous tone by nitric oxide in pigs. *Pflugers Arch.* 1996; 432:671–677. [PubMed: 8764968]
20. Thakali KM, Kharade SV, Sonkusare SK, Rhee SW, Stimers JR, Rusch NJ. Intracellular Ca^{2+} silences L-type Ca^{2+} channels in mesenteric veins: mechanism of venous smooth muscle resistance to calcium channel blockers. *Circ Res.* 2010; 106:739–747. [PubMed: 20044515]
21. Xu H, Garver H, Galligan JJ, et al. Large-conductance Ca^{2+} -activated K^{+} channel $\beta 1$ -subunit knockout mice are not hypertensive. *Am J Physiol Heart Circ Physiol.* 2011; 300:H476–H485. [PubMed: 21131476]
22. Xu H, Jackson WF, Fink GD, Galligan JJ. Activation of potassium channels by tempol in arterial smooth muscle cells from normotensive and deoxycorticosterone acetate-salt hypertensive rats. *Hypertension.* 2006; 48:1080–1087. [PubMed: 17060504]
23. 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. [PubMed: 7570021]
24. Chen X, Patel K, Connors SG, Mendonca M, Welch WJ, Wilcox CS. Acute antihypertensive action of tempol in the spontaneously hypertensive rat. *Am J Physiol heart Circ Physiol.* 2007; 293:H3246–3253. [PubMed: 17933967]
25. Brenner R, Perez GJ, Bonev AD, Eckman DM, Kosek JC, Wiler SW, Patterson AJ, Nelson MT, Aldrich RW. Vasoregulation by the $\beta 1$ subunit of the calcium-activated potassium channel. *Nature.* 2000; 407:870–876. [PubMed: 11057658]

26. Magnon M, Gallix P, Caverio I. Intervessel (arteries and veins) and heart/vessel selectivities of therapeutically used calcium entry blockers: variable, vessel-dependent indexes. *J Pharmacol Exp Ther.* 1995; 275:1157–1166. [PubMed: 8531077]
27. Cox RH, Katzka D, Morad M. Characteristics of calcium currents in rabbit portal vein myocytes. *Am J Physiol.* 1992; 263:H453–463. [PubMed: 1380775]
28. Greenwood IA, Miller LJ, Ohya S, Horowitz B. The large conductance potassium channel beta-subunit can interact with and modulate the functional properties of a calcium-activated chloride channel, CLCA1. *J Biol Chem.* 2002; 277:22119–22122. [PubMed: 11994272]
29. Messing M, Van Essen H, Smith TL, Smits JF, Struyker-Boudier HA. Microvascular actions of calcium channel antagonists. *Eur J Pharmacol.* 1991; 198:189–195. [PubMed: 1650702]
30. Kushiro T, Watanabe N, Takahashi A, Koike M, Saito F, Otsuka Y, Kanmatsuse K. Different effects of L-type and L+N-type calcium channel blockers on hamster cheek pouch venules. *J Cardiovasc Pharmacol.* 2004; 44:672–675. [PubMed: 15550786]
31. Nakayama K, Horikawa N, Ogawa T, Kohno F, Ishii K, Kubo K, Imabeppu S. Arteriolar and venular vasodilating properties of benidipine hydrochloride, a 1,4-dihydropyridine Ca^{2+} antagonist with long-lasting action, assessed in rat mesenteric microcirculation. *J Cardiovasc Pharmacol.* 1999; 33:540–548. [PubMed: 10218723]
32. Messerli FH. Vasodilatory edema: a common side effect of antihypertensive therapy. *Curr Cardiol Rep.* 2002; 4:479–482. [PubMed: 12379167]
33. Weir MR, Rosenberger C, Fink JC. Pilot study to evaluate a water displacement technique to compare effects of diuretics and ACE inhibitors to alleviate lower extremity edema due to dihydropyridine calcium antagonists. *Am J Hypertens.* 2001; 14:963–968. [PubMed: 11587165]
34. Eichhorn B, Dobrev D. Vascular large conductance calcium-activated potassium channels: functional role and therapeutic potential. *Naunyn Schmiedeberg Arch Pharmacol.* 2007; 376:145–155. [PubMed: 17932654]
35. Félétou M. Calcium-activated potassium channels and endothelial dysfunction: therapeutic options? *Br J Pharmacol.* 2009; 156:545–562. [PubMed: 19187341]
36. Safar ME, London GM. Arterial and venous compliance in sustained essential hypertension. *Hypertension.* 1987; 10:133–139. [PubMed: 3301662]
37. Pang CCY. Autonomic control of the venous system in health and disease effects of drugs. *Pharmacology and Therapeutics.* 2001; 90:179–230. [PubMed: 11578657]
38. Monos E, Bérczi V, Nádasy G. Local control of veins: biomechanical, metabolic, and humoral aspects. *Physiol Rev.* 1995; 75:611–666. [PubMed: 7624396]
39. Haynes WG, Clarke JG, Cockcroft JR, Webb DJ. Pharmacology of endothelin-1 in vivo in humans. *J Cardiovasc Pharmacol.* 1991; 17 (Suppl 7):S284–286. [PubMed: 1725356]

Fig 1.

Functional expression of BK channels in rat MV. A, co-expression of BK channel α - and β 1-subunits in rat MV. Myocytes labeled with an anti-BK channel α -subunits (anti-*slo*) antibody (green) and anti-BKCa²⁺ β 1 (red). B, Western blot showing a band for the BK channel α -subunit (BKCa²⁺ 1.1) (100 kDa) in rat MA and rat MV. Positive control data obtained in protein extracts from rat brain tissue. C, Whole cell recordings of spontaneous transient outward currents (STOCs) in rat MV myocytes. Recording of whole cell currents held at -30 mV. IBTX blocked STOCs in MV myocytes. D, Spontaneous, transient and local increases in intracellular Ca²⁺ were detected indicating that Ca²⁺ sparks occurred in rat MV SMCs. * $P < 0.05$ vs. control.

Fig 2.

Whole cell BK channel current recordings from rat MV myocytes. *A*: original recording of I_o in a rat MV myocyte before and after treatment with IBTX (0.1 $\mu\text{mol/L}$) (holding potential = -70 mV). I_o was generated by depolarizing with a series of 200 ms step commands to +80 mV in 10 mV increments at 15 s intervals. IBTX reduced peak I_o by 40% of. *B*: Summary data of the effect of IBTX on the I_o - V curves. IBTX caused a rightward shift of the activation curve. Values are mean $\pm$ SE; $n=6$ cells. * $P < 0.05$ vs. control.

Fig 3.

Unitary BK channel current recordings using inside-out patches in rat MV myocytes. Unitary BK channel currents in normal $[Ca^{2+}]_i$ (1 $\mu\text{mol/L}$) (A) and Ca^{2+} free media (B) at patch potentials between -60 mV and $+100$ mV under symmetrical (140 mmol/L) K^+ conditions. Unitary currents were blocked in the absence of Ca^{2+} . C. Unitary BK channel currents in RMV myocytes at $+80$ mV, $[Ca^{2+}]_i = 1 \mu\text{mol/L}$. D. A linear fit of the current voltage relationship revealed a 227 ± 12 pS unitary conductance in rat MV myocytes ($[Ca^{2+}]_i = 1 \mu\text{mol/L}$, $n=6$ cells).

Fig 4.

Effects of paxilline and ryanodine on pressurized rat and murine MV *in vitro*. A. Recording of rat MVID changes after application of paxilline (Paxi). Rat MV generated rhythmic constrictions, and Paxilline constricts rat MV. B, recording of rat MVID response after application of ryanodine (Ryan). Ryanodine constricted rat MV too. C, recording of murine MVID response after application of paxilline. Murine MV did not respond to paxilline, but responded to norepinephrine (NE). D, Summarized data of the effect of NE, paxilline, ryanodine as well as paxilline plus either nifedipine (Nife) on responses of rat and murine MV. Values are means ± SE. * $P < 0.05$ vs. baseline levels.

Fig 5.

Role of Ca^{2+} channels in rat and murine MV. A, recording of rat MVID at resting situation and after application of nifedipine. Nifedipine caused venodilation and inhibited the rhythmic constrictions. B, Summarized data of the effect of nifedipine and extracellular Ca^{2+} free on resting rat and murine MV. C, Summarized data of the effect of nifedipine ($1\mu\text{M}$) on KCl-induced rat and murine MV contractions. KCl caused concentration-dependent constriction in rat, but not in murine MV. Nifedipine significantly right-shifted the concentration response curves of KCl in rat MV, but did not inhibit the maximal constriction. D, summarized data of effects of nifedipine on BayK 8644 (Bayk)-induced rat and murine MV constrictions. Murine MV did not respond to BayK 8644. * $P < 0.05$ vs. baseline levels.

Fig 6.

IBTX increases mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) in conscious rats. MAP and MCFP were measured before and after *i.v.* administration of IBTX (0.3 $\mu\text{M/kg}$ over 1 min). MCFP was tested at 15 and 5 minutes before IBTX administration to establish control values, and then re-measured 5 and 15 minutes after IBTX administration. Arterial blood pressure was measured continuously. Summarized data of the effects of IBTX on MAP (A) and MCFP (B). IBTX increases MCFP and MAP. MAP changes peaked at 2 minutes and recovered after 5 minutes after administration. MCFP was elevated at 5 minutes but returned to baseline by 15 minutes. Values are means $\pm$ SE; $n=7$. * $P < 0.05$ vs. baseline levels.