HHS Public Access

Author manuscript

Arterioscler Thromb Vasc Biol. Author manuscript; available in PMC 2016 November 21.

Published in final edited form as:

Arterioscler Thromb Vasc Biol. 2015 August ; 35(8): 1843–1851. doi:10.1161/ATVBAHA.115.305736.

Genetic Ablation of $Ca_V3.2$ Channels Enhances the Arterial Myogenic Response by Modulating the RyR-BK_{Ca} Axis

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Abstract

Objective—In resistance arteries, there is an emerging view that smooth muscle $Ca_V3.2$ channels restrain arterial constriction through a feedback response involving the large-conductance Ca^{2+}-activated K^+ channel (BK_{Ca}). Here, we used wild-type and $Ca_V3.2$ knockout ($Ca_V3.2^{-/-}$) mice to definitively test whether $Ca_V3.2$ moderates myogenic tone in mesenteric arteries via the $Ca_V3.2$-ryanodine receptor-BK_{Ca} axis and whether this regulatory mechanism influences blood pressure regulation.

Approach and Results—Using pressurized vessel myography, $Ca_V3.2^{-/-}$ mesenteric arteries displayed enhanced myogenic constriction to pressure but similar K^+-induced vasoconstriction compared with wild-type C57BL/6 arteries. Electrophysiological and myography experiments subsequently confirmed the inability of micromolar Ni^{2+}, a $Ca_V3.2$ blocker, to either constrict arteries or suppress T-type currents in $Ca_V3.2^{-/-}$ smooth muscle cells. The frequency of BK_{Ca}-induced spontaneous transient outward K^+ currents dropped in wild-type but not in knockout arterial smooth muscle cells upon the pharmacological suppression of $Ca_V3.2$ channel. Line scan analysis performed on en face arteries loaded with Fluo-4 revealed the presence of Ca^{2+} sparks in all arteries, with the subsequent application of Ni^{2+} only affecting wild-type arteries. Although $Ca_V3.2$ channel moderated myogenic constriction of resistance arteries, the blood pressure measurements of $Ca_V3.2^{-/-}$ and wild-type animals were similar.

Conclusions—Overall, our findings establish a negative feedback mechanism of the myogenic response in which $Ca_V3.2$ channel modulates downstream ryanodine receptor-BK_{Ca} to hyperpolarize and relax arteries.

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Disclosures

None.

The online-only Data Supplement is available with this article at http://atvb.ahajournals.org/lookup/suppl/doi:10.1161/ATVBAHA.115.305736/-/DC1.

Keywords

arteries; calcium-activated potassium channels; calcium channels; calcium signaling; ryanodine receptors; T-type calcium channels; vascular smooth muscle

The cardiovascular system comprises a muscular pump and a distribution network of arteries, veins, and capillaries. Within this integrated system, resistance arteries control the magnitude and distribution of tissue perfusion and respond to vasoactive stimuli, including mechanical forces, neurotransmitters, and metabolites.[1–5] Bayliss first described the inherent ability of resistance arteries to constrict to elevated pressure,[6] and studies have shown that the so-called myogenic response is intimately tied to depolarization and the activation of smooth muscle L-type Ca^{2+} channels. It is often presumed that $Ca_V1.2$ is the only Ca^{2+} channel of functional significance because dihydropyridines, L-type blockers, prominently attenuate myogenic tone.[3] This traditional perspective has begun to change with the identification of arterial T-type Ca^{2+} channels, including $Ca_V3.1$ and $Ca_V3.2$ subtypes.[7,8] Recent findings suggest that the former (ie, $Ca_V3.1$) modestly facilitates myogenic constriction at hyperpolarized voltages, whereas the latter (ie, $Ca_V3.2$) facilitates a negative feedback response restraining arterial constriction.[9,10]

Our recent observations have tied the paradoxical ability of rat cerebral arterial $Ca_V3.2$ channel to limit myogenic tone to the triggering of ryanodine receptors (RyR) on the sarcoplasmic reticulum. The RyR-mediated generation of Ca^{2+} sparks subsequently activates the large conductance Ca^{2+}-activated K^+ channels (BK_{Ca}), eliciting a hyperpolarization to counteract pressure-induced constriction.[10] Furthermore, the $Ca_V3.2$ conductance in the human cerebral circulation seems to mediate a similar physiological role.[10] Although the concept of a voltage-gated Ca^{2+} channel counterbalancing vasoconstriction is novel and intriguing, it is one delimited by 2 primary concerns. First, current work is heavily reliant on the presumed selectivity of Ni^{2+} to block $Ca_V3.2$ channels.[11] Second, there is a lack of corroborative observations, outside the cerebral circulation, in vascular beds known to acutely and sustainably regulate systemic blood pressure.

Here, we used wild-type and $Ca_V3.2$ knockout ($Ca_V3.2^{-/-}$) mice to definitively test whether $Ca_V3.2$ channel moderates myogenic tone in mesenteric arteries via the $Ca_V3.2$-RyR-BK_{Ca} axis and, more generally, whether this regulatory mechanism influences blood pressure regulation. Experiments ranged from cells to whole animals and encompassed the integrative use of myography, electrophysiology, Ca^{2+} imaging, and intravascular catheterization. Arteries displayed enhanced myogenic tone when $Ca_V3.2$ channels were genetically ablated or pharmacologically suppressed using Ni^{2+}. Subsequent analyses indicated that Ni^{2+} inhibited BK_{Ca} currents and Ca^{2+} sparks in wild-type but not $Ca_V3.2^{-/-}$ arteries. Although $Ca_V3.2$ channel moderated myogenic constriction, the blood pressure measurements of both animal types were similar. In conclusion, this study establishes a negative feedback response in which $Ca_V3.2$ channel modulates downstream activity of the RyR-BK_{Ca} complex to hyperpolarize and relax resistance arteries.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Genetic Ablation of $Ca_V3.2$ Enhances Arterial Myogenic Tone

Our earlier reports revealed the involvement of $Ca_V3.2$ channels in negative feedback control of rat cerebral arterial tone[10]; thus, channel deletion should enhance myogenic tone. Figure 1A and 1B demonstrates that C57BL/6 and $Ca_V3.2^{-/-}$ arteries were myogenically active and constricted as intravascular pressure increased from 20 to 100 mm Hg. Ca^{2+}-free saline (0 mmol/L Ca^{2+}+2 mmol/L EGTA) reversed myogenic constriction and evoked passive arterial dilation. Expressing data as a percentage myogenic tone (Figure 1C) revealed the predicted enhancement in $Ca_V3.2^{-/-}$ arteries (at 60 mm Hg: $Ca_V3.2^{-/-}$, 28%±2%; C57BL/6, 19% ±3%). Although myogenic tone was significantly different, 60 mmol/L K^+-induced vasoconstriction was similar in C57BL/6 (40%±2%) and $Ca_V3.2^{-/-}$ (43%±2%) pressurized arteries (Figure 1D). Note, the basal diameter of $Ca_V3.2^{-/-}$ arteries was smaller than that of C57BL/6, a finding consistent with variable myogenic tone. Interpretational cautious is, however, warranted because $Ca_V3.2^{-/-}$ mice display a lighter body mass than wild-type animals.[12] Quantitative PCR showed that mRNA expression of $Ca_V1.2$, $Ca_V3.1$, BK_{Ca}-α, or RyR2 was comparable in wild-type and $Ca_V3.2^{-/-}$ arteries (Figure 1E).

Micromolar Ni^{2+} Selectively Blocks $Ca_V3.2$ Currents

Mesenteric arterial smooth muscle cells from C57BL/6 mice express 3 subtypes of voltage-gated Ca^{2+} channels ($Ca_V1.2$, $Ca_V3.1$, and $Ca_V3.2$)[9], and thus, total inward current is representative of this ensemble of channels. To distinguish between the subcomponents, we used patch clamp electrophysiology in combination with defined pharmacology.[10] First, nifedipine (200 nmol/L) was applied to block L-type $Ca_V1.2$ channels and reveal a current predominated by T-type conductances (Figure 2A). Subsequent application of 50 μmol/L Ni^{2+}, which is presumed to be a selective $Ca_V3.2$ blocker,[10–14] reduced the nifedipine-insensitive T-type currents in C57BL/6 but not in $Ca_V3.2^{-/-}$ smooth muscle cells (Figure 2B and 2C). The absence of an effect of Ni^{2+} in $Ca_V3.2^{-/-}$ myocytes is consistent with this pharmacological selectivity. Note that the broad-spectrum T-type blocker (NNC 55–0396, 1 μmol/L) subsequently abolished the residual current[15] in both C57BL/6 and $Ca_V3.2^{-/-}$ myocytes because of the suppression of remaining $Ca_V3.1$ current (Figure 2C). Importantly, voltage dependence profiles demonstrated that the T-type current is available for activation at physiological membrane potentials (Figure I in the online-only Data Supplement). As noted previously, $Ca_V3.1$ mRNA levels were similar among the 2 groups of animals, whereas $Ca_V1.2$ was modestly but insignificantly lower in $Ca_V3.2^{-/-}$ arteries (Figure 1E).

$Ca_V3.2$ Activity Regulates BK_{Ca}-Mediated STOCs

Given the enhancement of myogenic constriction in $Ca_V3.2^{-/-}$ arteries, we next tested whether this T-type channel modifies myogenic reactivity through a negative feedback response that involves downstream BK_{Ca}.[10,12] We used perforated patch clamp electrophysiology to monitor BK_{Ca}-mediated spontaneous transient outward K^+ currents

(STOCs) in arterial smooth muscle cells from wild-type and knockout animals. In C57BL/6 cells held at the physiological voltage of −40 mV, Ni^{2+} significantly suppressed STOC frequency. In contrast, Ni^{2+} had no effect on STOCs when C57BL/6 cells were voltage-clamped at more depolarized potentials (−20 mV), a finding consistent with the voltage profile of $Ca_V3.2$ channel (Figure 3A). In $Ca_V3.2^{-/-}$ cells, Ni^{2+} had no effect at either −40 or −20 mV (Figure 3B). All STOCs were fully abolished by the application of the BK_{Ca} inhibitor paxilline (1 μmol/L;Figure II in the online-only Data Supplement). Message expression of the BK_{Ca} pore-forming subunit (BK_{Ca}-α) was similar in C57BL/6 and $Ca_V3.2^{-/-}$ arteries (Figure 1E). Further, Ni^{2+} had no effect on STOC amplitude under different experimental conditions (Figure 3C). Note, basal STOCs tended to fire at lower frequencies in $Ca_V3.2^{-/-}$ myocytes compared with C57BL/6 counterparts. Statistical analysis was not performed across groups as cells that did not fire sufficient STOCs were eliminated a priori from experimentation.

$Ca_V3.2$ Channel Controls Ca^{2+} Spark Generation

Given the ability of $Ca_V3.2$ channel to modulate BK_{Ca} current (Figure 3) and the reported correlation between RyR-mediated spark generation and BK_{Ca} activation,[10,16] we next explored the $Ca_V3.2$-Ca^{2+} spark relationship using Ca^{2+} imaging and line scan analysis of mouse mesenteric arteries (Figure 4). In C57BL/6 arteries loaded with Fluo-4, Ca^{2+} sparks were observed in 76% of the 454 line scans performed under control conditions. Depolarizing arteries (30 mmol/L K^+) increased spark activity (95%), whereas the subsequent addition of Ni^{2+} significantly reduced firing (52%; Figure 4A and 4B). In comparison, 30 mmol/L K^+ increased spark firing in $Ca_v3.2^{-/-}$ arteries from 58% to 94% (456 line scans) but Ni^{2+} failed to attenuate sparks (98%; Figure 4A and 4B). In depolarized wild-type C57BL/6 arteries, Ca^{2+} spark frequency was calculated to be 0.0347 and 0.0153 sparks/μm s in the absence and presence of Ni^{2+}, respectively; spark frequency in knockout tissues was distinctively insensitive to the application of Ni^{2+} (Figure 4C). The genetic absence of $Ca_V3.2$ channels was notably associated with lower percentage firing ($Ca_V3.2^{-/-}$, 58%; C57BL/6, 76%) and lower basal Ca^{2+} spark frequency when compared with wild-type arteries ($Ca_V3.2^{-/-}$, 0.0065±0.0014; C57BL/6, 0.0169±0.005 sparks/μm s). The amplitudes and spatiotemporal characteristics of Ca^{2+} sparks displayed no significant changes before and after the application of Ni^{2+} on C57BL/6 and $Ca_V3.2^{-/-}$ arteries (Figure 4D).

$Ca_V3.2$ Activity Restrains Myogenic Constriction by Altering BK_{Ca} Feedback

The application of Ni^{2+} (50 μmol/L) onto C57BL/6 arteries evoked vasoconstriction at intravascular pressure values between 20 and 60 mm Hg, and this vasomotor effect diminished at higher pressures; $Ca_V3.2^{-/-}$ arteries lacked a similar response (Figure 5A). The percentage of myogenic tone in wild-type C57BL/6 arteries increased after the application of Ni^{2+} (at 60 mm Hg: control 20%±4% versus Ni^{2+} 25%±1%), but was not altered in $Ca_V3.2^{-/-}$ arteries (control 30%±5% versus Ni^{2+} 28%±8%; Figure 5B). Coinciding with vasomotor data, membrane potential measurements showed that Ni^{2+} only depolarized C57BL/6 but not $Ca_V3.2^{-/-}$ pressurized arteries (60 mm Hg; Figure 5C). In C57BL/6 arteries, the BK_{Ca} blocker (paxilline, 1 μmol/L) evoked vasoconstriction and enhanced myogenic tone similar to that of Ni^{2+} (Figure 5D), an observation consistent with a common signaling axis between $Ca_V3.2$ and BK_{Ca} channels. When Ni^{2+} and paxilline

were sequentially added to the same wild-type artery, Ni^{2+} evoked vasoconstriction, whereas subsequent paxilline had no additional effect. Similar experiments using $Ca_V3.2^{-/-}$ arteries demonstrated a lack of vasomotor responses to Ni^{2+} but preserved responsiveness to paxilline (Figure 5E).

$Ca_V3.2^{-/-}$ Mice Display Normal Blood Pressure Responses

Pharmacological and genetic approaches suggested that $Ca_V3.2$ channel counterbalances myogenic constriction (Figure 1 and 5) and could as such influence blood pressure regulation. To explore this possibility, we catheterized carotid arteries of C57BL/6 and $Ca_V3.2^{-/-}$ mice to monitor blood pressure under resting conditions and in response to a vasopressor challenge. As depicted (Figure 6A), basal mean arterial pressure was similar in wild-type and knockout mice (C57BL/6, 100±2 mm Hg; $Ca_V3.2^{-/-}$, 103±5 mm Hg), a finding consistent with earlier reports in conscious animals.[17,18] Given that $Ca_V3.2$ channels seem to be involved in a feedback mechanism, we next assessed whether this conductance can alter mean arterial pressure responsiveness to a vasopressor challenge. The intravenous administration of phenylephrine (α_1-adrenoceptor agonist, 1–16 μg/kg body weight) evoked dose-dependent rises in mean arterial pressure, and these transient responses were similar among the 2 groups (Figure 6B). Analogous to in vivo experiments, phenylephrine (0.01–10 μmol/L)-induced vasoconstriction was similar in C57BL/6 and $Ca_V3.2^{-/-}$ mesenteric arteries (Figure 6C and 6D).

Discussion

This study used wild-type and $Ca_V3.2$ knockout mice to examine the purported contribution of $Ca_V3.2$ channels to a negative feedback response that counterbalances arterial tone development. Using mesenteric arteries, functional experiments illustrated that the genetic ablation or pharmacological suppression of $Ca_V3.2$ channel selectively enhanced myogenic constriction. Subsequent electrophysiological recordings revealed that $Ca_V3.2$ channel modulates downstream BK_{Ca}-mediated STOCs. Ca^{2+} imaging further demonstrated that Ca^{2+} spark generation is an intermediary step in the $Ca_V3.2$-BK_{Ca} functional axis. Finally, although $Ca_V3.2$ moderated myogenic tone, this regulatory mechanism did not influence resting blood pressure or vasopressor-induced responses. In summary, findings from this study establish a model by which $Ca_V3.2$ channel restrains myogenic constriction by driving a process where Ca^{2+} influx triggers Ca^{2+} sparks and downstream activation of BK_{Ca} currents (Figure III in the online-only Data Supplement).

Resistance arteries control tissue perfusion and respond to a range of vasoactive stimuli.[1–5] The integral ability of resistance arteries to respond to perturbations in arterial pressure, known as the myogenic response,[6] is intrinsic to vascular smooth muscle and plays an essential role in maintaining blood flow and capillary pressure to tissues, such as the brain and the heart.[19] This response is mechanistically linked to a rise in cytosolic $[Ca^{2+}]_i$ driven by a depolarization that activates L-type $Ca_V1.2$ channels. The traditional perspective that $Ca_V1.2$ is the only voltage-gated Ca^{2+} channel of functional significance[3] has shifted with the identification of 2 T-type Ca^{2+} channels,[7,8] the first being $Ca_V3.1$, which modestly facilitates myogenic tone at hyperpolarized voltages.[9] In contrast, $Ca_V3.2$ channels have

been reported to drive a paradoxical feedback response that limits arterial constriction.[10,12] The feedback response begins with Ca^{2+} influx through $Ca_V3.2$ channels triggering ryanodine receptors to initiate Ca^{2+} sparks. These discrete sarcoplasmic reticulum–driven events in turn activate BK_{Ca} channels to hyperpolarize and dilate resistance arteries.[10] Although an intriguing concept, data interpretation is singularly dependent on the presumed selectivity of Ni^{2+} as a pharmacological $Ca_V3.2$ blocker.[11] Current knowledge is also restricted to the cerebral circulation, and experiments have not extended to other vascular beds essential to blood pressure regulation.

One means to better probe the functional properties of arterial $Ca_V3.2$ channel is to use an animal model in which the channel has been genetically ablated.[12] In this regard, we performed pressure myography on arteries from $Ca_V3.2$ knockout ($Ca_V3.2^{-/-}$) mice to study the vascular phenotype. Consistent with the view that $Ca_V3.2$ channel mediates feedback vasodilation, we observed enhanced myogenic constriction in mesenteric arteries from $Ca_V3.2^{-/-}$ mice. This finding was somewhat akin to the functional observations in coronary arteries[12] and the enhancement of rat cerebral arterial myogenic tone noted in the presence of Ni^{2+}.[10] Our present findings overcome concerns raised by past studies that off-target effects of Ni^{2+} could theoretically have accounted for the vasomotor responses.[20–22] In particular, we were unable to elicit Ni^{2+}-sensitive current in $Ca_V3.2^{-/-}$ arterial myocytes, and Ni^{2+} also failed to constrict/depolarize arteries from knockout mice.

The unexpected ability of $Ca_V3.2$ channel to mediate a negative feedback response has been previously tied to downstream modulation of BK_{Ca} channels through intermediary activation of ryanodine receptors.[10] In arterial smooth muscle, BK_{Ca} is ubiquitously expressed and known to moderate membrane depolarization and arterial constriction.[23] To activate arterial BK_{Ca}, vasoactive stimuli must induce depolarization and elicit a discrete micromolar rise in $[Ca^{2+}]_i$ in the subsarcolemma taking the form of a Ca^{2+} spark.[24] Here, we present multiple lines of evidence implicating BK_{Ca} as the final downstream effector of $Ca_V3.2$ channel. First, electrophysiology revealed that BK_{Ca}-mediated STOCs were sensitive to Ni^{2+} in wild-type but not in $Ca_V3.2^{-/-}$ arterial myocytes. Second, pressure myography illustrated that adding Ni^{2+} or paxilline (BK_{Ca} inhibitor) to the superfusate comparably augmented myogenic tone. Intriguingly, applying paxilline to wild-type arteries pre-treated with Ni^{2+} had no additive effect, consistent with $Ca_V3.2$ and BK_{Ca} channels being linked through a common sequential pathway.

It has been long established that RyR activation is responsible for the initiation of BK_{Ca}-mediated STOCs in arterial smooth muscle,[16,24] and this recognized relationship led us to examine the nature of Ca^{2+} spark generation in mouse mesenteric arteries. Indeed, inhibitors of RyR (eg, ryanodine) has been shown to suppress the generation of STOCs in vascular smooth muscle irrespective of their origin.[24] Here and in consistency with $Ca_V3.2$ channel driving Ca^{2+} sparks and subsequently STOC generation, we used Ca^{2+} imaging and line scan analysis and observed that spark frequency decreased in wild-type arteries when $Ca_V3.2$ channels were inhibited with Ni^{2+}. We also found that firing of Ca^{2+} sparks was unaffected by this divalent cation in $Ca_V3.2^{-/-}$ arteries and that basal Ca^{2+} spark frequency was lower than those of wild-type arteries. These findings align well with recent work from the cerebral circulation where a variety of functional, structural, electro-physiological, and

computational observations draw a critical relationship between $Ca_V3.2$ and RyR and then BK_{Ca} (Figure III in the online-only Data Supplement).[10] Intriguingly, the preceding work is distinct from neuronal studies where T-type conductances have been suggested to directly activate Ca^{2+}-activated K^+ channels independent of RyR.[25–28]

With $Ca_V3.2$ channels playing an intimate role in limiting myogenic constriction, it is logical to argue that the loss of this conductance would alter peripheral resistance and systemic blood pressure regulation. We tested this supposition by catheterizing anesthetized mice and assessing blood pressure at rest and in response to a vasopressor challenge. Resting blood pressure was similar among wild-type and knockout mice, a finding consistent with earlier reports which used different monitoring approaches (eg, catheterization, tail cuff) in conscious animals over longer time spans (days to weeks).[17,18] Given the presumed role of $Ca_V3.2$ in feedback (rather than active vasodilation), we challenged blood pressure regulation using intravenous phenylephrine. This multidose challenge evoked comparable rises in blood pressure among the 2 animal groups; likewise, mesenteric arteries isolated from wild-type and $Ca_V3.2^{-/-}$ mice constricted similarly to this α_1-adrenergic agonist. These in vivo and in vitro results interestingly seem to rule out a direct modulatory role for vascular $Ca_V3.2$ channels in agonist-induced vasoconstriction.

It is not clear at present why blood pressure responses fail to change. Perhaps, the loss of smooth muscle feedback in $Ca_V3.2^{-/-}$ arteries is compensated by enhanced dilatory feedback from the endothelium. Other functional compensation may have occurred in this global knockout independent of vascular smooth muscle[29]; in this context, establishing a smooth muscle–specific $Ca_V3.2$ knockout model would be valuable. It should also be recognized that for the proposed $Ca_V3.2$/RyR/BK_{Ca} feedback mechanism to effectively drive a blood pressure response, it should be ideally present in a full range of vascular beds. Recent work documenting heterogeneity of BK_{Ca} activity,[30] because of differences in channel activity and Ca^{2+} spark generation, challenges this key assumption. Further investigation is warranted to address this intriguing anomaly.

In conclusion, this study showed that arterial $Ca_V3.2$ channels retain a unique ability to counterbalance myogenic constriction. This negative feedback response entails a modulatory paradigm in which Ca^{2+} flux through $Ca_V3.2$ channel triggers Ca^{2+} spark generation and then activates BK_{Ca} channels to hyperpolarize and relax arteries (Figure III in the online-only Data Supplement). This novel functional axis, recently described in the human cerebral circulation,[31] challenges the traditional view that voltage-gated Ca^{2+} channels solely facilitate arterial tone development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of Funding

This work was supported by an operating grant from the Canadian Institutes of Health Research (CIHR, MOP-69088 to D.G. Welsh). D.G. Welsh is the recipient of the Rorabeck Chair in Neuroscience and Vascular

Biology (University of Western Ontario). O.F. Harraz is a Vanier Scholar and was supported by salary studentships from Alberta Innovates Health Solutions (AIHS) and Achievers in Medical Sciences. A. Zechariah was supported by AIHS and Eyes High postdoctoral fellowships. Imaging was performed in the Loma Linda University School of Medicine Advanced Imaging and Microscopy Core that is supported by the National Science Foundation (MRI-DBI 0923559 to S.M. Wilson) and the Loma Linda University School of Medicine. The work was also supported in part by USPHS (United States Public Health Service) Grant (HD069746 to S.M. Wilson).

Nonstandard Abbreviations and Acronyms

BK_{Ca}	large-conductance Ca^{2+}-activated K^+ channel
$Ca_V3.2^{-/-}$	$Ca_V3.2$-deficient (knockout) mice
RyR	ryanodine receptor
STOC	spontaneous transient outward K^+ current

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Significance

Vascular smooth muscle cells express T-type Ca^{2+} channels along with L-type channels. Although the latter have been long implicated in arterial excitation–contraction coupling, studies have only recently begun to assess the role of T-type channels. Using an animal model in which $Ca_V3.2$, a T-type channel, was genetically deleted, we tested its role in arterial tone development. We demonstrate that resistance arteries from knockout animals paradoxically display enhanced responsiveness to arterial pressure. This enhancement was mechanistically attributed to the ability of $Ca_V3.2$ to modulate downstream K^+ channels, which hyperpolarize and relax arteries. This novel data challenge the traditional view that voltage-gated Ca^{2+} channels are singularly involved in the genesis of arterial constriction.

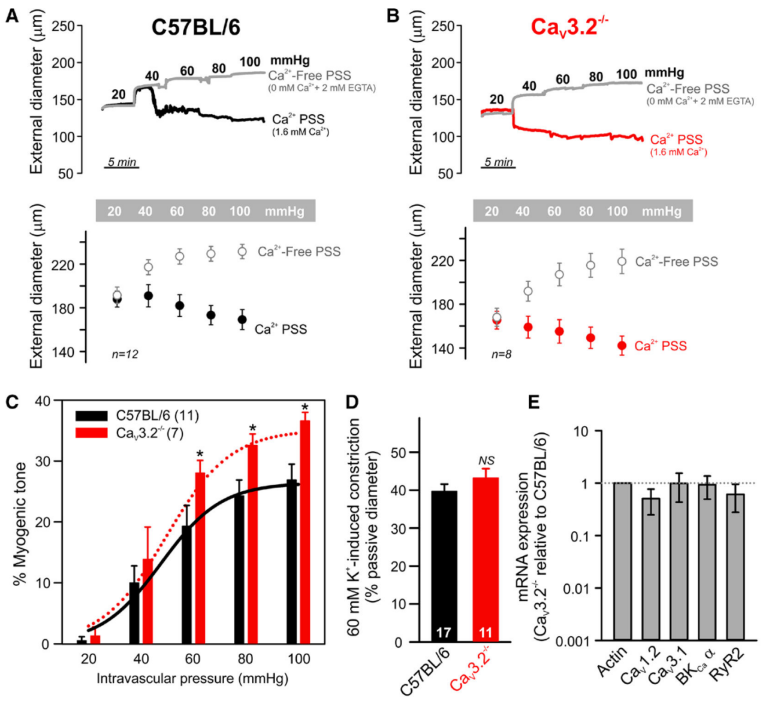

Figure 1.

$Ca_V3.2^{-/-}$ arteries display enhanced myogenic tone. **A**, Representative traces and summary data demonstrate that C57BL/6 mesenteric arteries constricted to elevations in intravascular pressure from 20 to 100 mm Hg in Ca^{2+} containing physiological saline solution (PSS). Maximum arterial diameters were obtained by replacing Ca^{2+} PSS with PSS with zero Ca^{2+}+2 mmol/L EGTA (n=12). **B**, Traces and averaged data of the myogenic response in $Ca_V3.2^{-/-}$ mesenteric arteries (n=8). **C**, Percentage myogenic tone was significantly higher in $Ca_V3.2^{-/-}$ (n=7) than in wild-type C57BL/6 (n=11) arteries (unpaired *t* test, **P*≤0.05). **D**, Vasoconstriction evoked by 60 mmol/L K^+ was similar in C57BL/6 and $Ca_V3.2^{-/-}$ pressurized (15 mm Hg) arteries (n=11–17, unpaired *t* test; NS denotes not significant). **E**, Messenger RNA of key genes ($Ca_V1.2$, $Ca_V3.1$, BK_{Ca}-α, and RyR2) were not different in C57BL/6 and $Ca_V3.2^{-/-}$ mesenteric arteries. Relative expression was calculated using 3 independent quantitative PCR reactions.

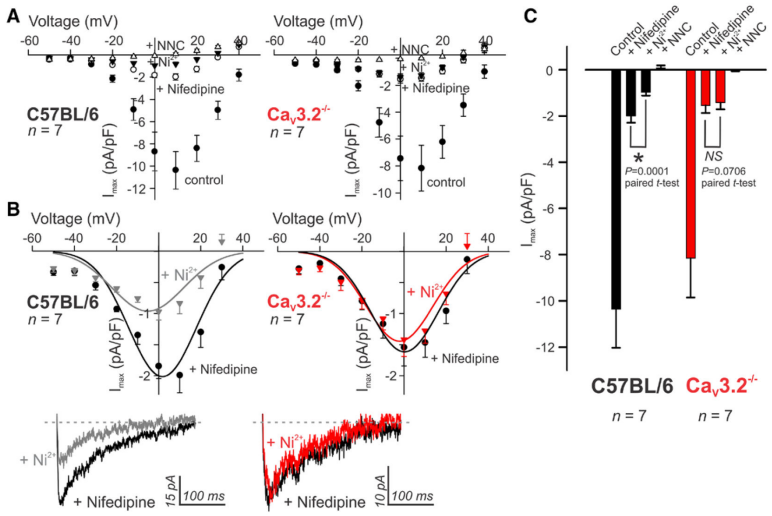

Figure 2.

Micromolar Ni^{2+} suppresses $Ca_V3.2$ current. **A**, Current–voltage (I-V) plots illustrate total inward currents in wild-type C57BL/6 and $Ca_V3.2^{-/-}$ smooth muscle cells and the effects of $Ca_V1.2$ and $Ca_V3.2$ blockade using nifedipine (200 nmol/L) and Ni^{2+} (50 μmol/L), respectively. All recordings used 10 mmol/L Ba^{2+} as the charge carrier. **B** and **C**, Representative traces and summary data illustrate the effect of Ni^{2+} on nifedipine insensitive T-type currents. Representative traces were evoked using a prepulse (−90 mV) followed by a test pulse (0 mV). Ni^{2+} significantly suppressed T-type currents in C57BL/6 but not $Ca_V3.2^{-/-}$ smooth muscle cells (n=7 each; $*P \leq 0.05$).

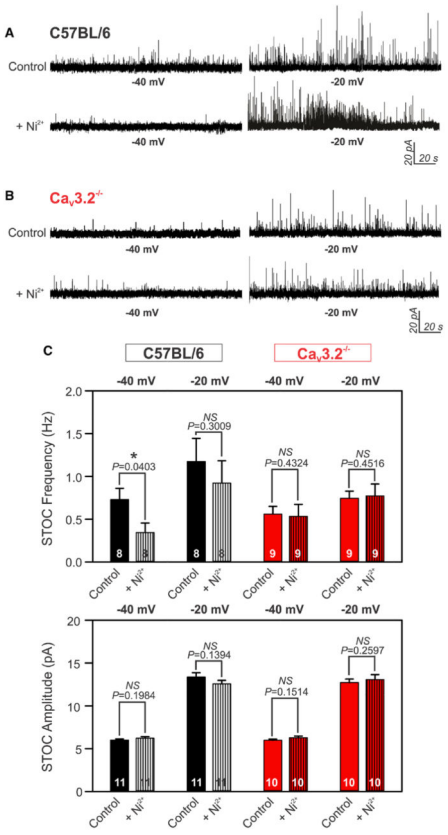

Figure 3.

$Ca_V3.2$ channel modulates BK_{Ca}-mediated spontaneous transient outward K^+ currents (STOCs). **A** and **B**, Representative traces of STOCs recorded at −40 and −20 mV in wild-type C57BL/6 (**A**) or $Ca_V3.2^{-/-}$ (**B**) arterial smooth muscle cells. Application of 50 μmol/L Ni^{2+} suppressed STOC frequency only at −40 mV in C57BL/6 cells with no noticeable effect at other conditions. **C**, Averaged bar graphs illustrate the effect of Ni^{2+} on STOC frequency (Hz) and amplitude (pA) at −40 or −20 mV in C57BL6 or $Ca_V3.2^{-/-}$ arterial myocytes (n=8–11, $*P \leq 0.05$, paired *t* test).

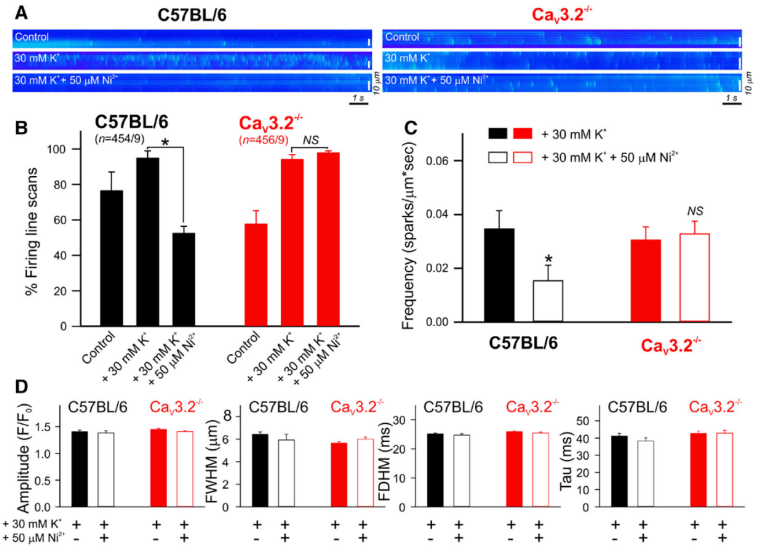

Figure 4.

$Ca_V3.2$ channel triggers Ca^{2+} spark generation. **A**, Representative line scan images (1.89 ms/line) of Fluo-4 loaded wild-type C57BL/6 or $Ca_V3.2^{-/-}$ mesenteric arteries showing Ca^{2+} sparks recorded before and after the sequential application of 30 mmol/L K^+ and 50 μmol/L Ni^{2+}. The latter suppressed Ca^{2+} spark generation in C57BL/6 but not $Ca_V3.2^{-/-}$ line scans. **B**, Percentage firing of Ca^{2+} sparks under control conditions and after the application of 30 mmol/L K^+ followed by Ni^{2+}. Line scans were recorded in C57BL/6 (n=454 line scans/9 animals) or $Ca_V3.2^{-/-}$ (red, n=456 line scans/9 animals) mesenteric arteries (*$P \leq 0.05$, paired *t* test). **C**, Bar graph of Ca^{2+} spark frequency (sparks/μm s) before and after the application of Ni^{2+}. $Ca_V3.2$ suppression significantly attenuated spark frequency in C57BL/6 (n=9, 454 line scans in total, *$P \leq 0.05$) but not $Ca_V3.2^{-/-}$ (n=9, 456 line scans in total) arteries. **D**, Bar graphs of averaged Ca^{2+} spark amplitude (F/F_0); full width at half maximum (FWHM, in μm); full duration at half maximum (FDHM, in ms); and time constants of decay (Tau, in ms) before and after Ni^{2+}.

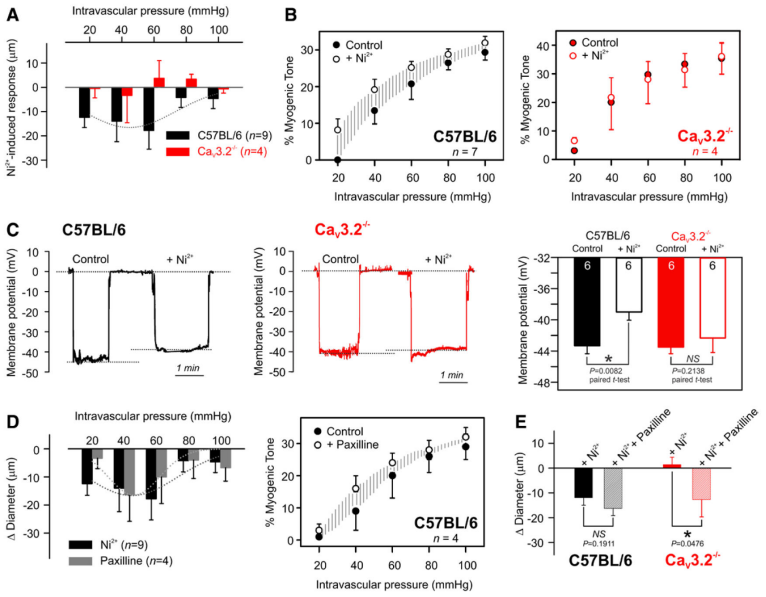

Figure 5.

$Ca_V3.2$ suppression constricts/depolarizes wild-type arteries via altering the vasomotor BK_{Ca} response. **A**, Averaged data demonstrate that the application of 50 μmol/L Ni^{2+} constricted pressurized C57BL/6 arteries (n=9) at pressure values 20 to 60 mm Hg with no significant effect on $Ca_V3.2^{-/-}$ arteries (n=4). **B**, $Ca_V3.2$ blockade enhanced percentage myogenic tone in wild-type C57BL/6 arteries, but displayed no detectable change in $Ca_V3.2^{-/-}$ arteries. **C**, Representative traces and summary data of membrane potential (mV) in C57BL/6 and $Ca_V3.2^{-/-}$ pressurized arteries (60 mm Hg) before and after the application of Ni^{2+} (n=6 each; paired *t* test; **P*≤0.05). **D**, Application of Ni^{2+} (50 μmol/L, n=9, as in **A**) or paxilline (1 μmol/L, n=4) on pressurized C57BL/6 arteries elicited similar constrictor responses. Percentage myogenic tone increased in response to BK_{Ca} blockade in C57BL/6 arteries (n=4). **E**, Changes in arterial diameter in response to Ni^{2+} followed by paxilline in pressurized C57BL/6 or $Ca_V3.2^{-/-}$ arteries (at 60 mm Hg, n=5–7, **P*≤0.05).

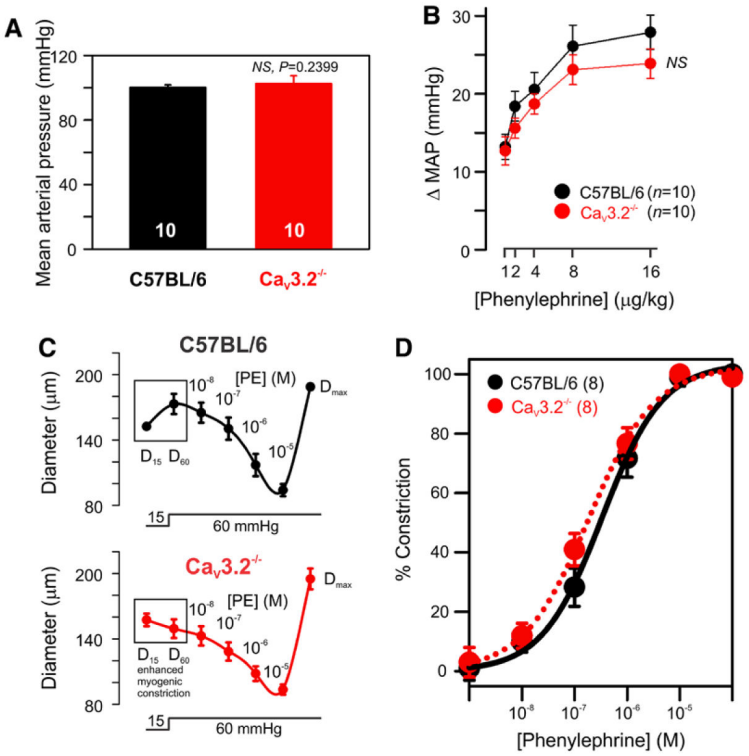

Figure 6.

$Ca_V3.2^{-/-}$ mice display normal resting blood pressure and responsiveness to phenylephrine. **A**, Resting mean arterial pressure of anesthetized C57BL/6 or $Ca_V3.2^{-/-}$ mice (n=10 each). **B**, Changes in mean arterial pressure (ΔMAP, mm Hg) in response to intravenous phenylephrine (1–16 μg/kg body weight) in C57BL/6 and $Ca_V3.2^{-/-}$ mice (n=10 each). **C**, Diameter responses of C57BL/6 and $Ca_V3.2^{-/-}$ mesenteric arteries to increased pressure from 15 to 60 mm Hg followed by cumulative application of phenylephrine (PE; 0.01–10 μmol/L). Maximum diameters (D_{max}) were finally achieved by perfusion of Ca^{2+} free PSS. **D**, Phenylephrine concentration response curve shows no significant differences between wild-type C57BL/6 and $Ca_V3.2^{-/-}$ arteries (n=8 each).