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Transient Receptor Potential Vanilloid-4 Channels Are Involved in Diminished Myogenic Tone in Brain Parenchymal Arterioles in Response to Chronic Hypoperfusion in Mice

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Abstract

Aim: Adaptive responses of brain parenchymal arterioles (PAs), a target for cerebral small vessel disease, to chronic cerebral hypoperfusion is largely unknown. Previous evidence suggested that transient receptor potential vanilloid 4 channels may be involved in the regulation of cerebrovascular tone. Therefore, we investigated the role of TRPV4 in adaptations of PAs in a mouse model of chronic hypoperfusion.

Methods: TRPV4 knockout ($^{-/-}$) and wildtype (WT) mice were subjected to unilateral common carotid artery occlusion (UCCAo) for 28 days. Function and structure of PAs ipsilateral to UCCAo were studied isolated and pressurized in an arteriograph.

Results: Basal tone of PAs was similar between WT and TRPV4 $^{-/-}$ mice (22 ± 3 vs. $23\pm 5\%$). After UCCAo, active inner diameters of PAs from WT mice were larger than control (41 ± 2 vs. $26\pm 5\mu\text{m}$, $p<0.05$) that was due to decreased tone (8 ± 2 vs. $23\pm 5\%$, $p<0.05$), increased passive inner diameters (46 ± 3 vs. $34\pm 20\mu\text{m}$, $p<0.05$) and decreased wall-to-lumen ratio (0.104 ± 0.01 vs. 0.137 ± 0.01 , $p<0.05$). However, UCCAo did not affect vasodilation to a small- and intermediate-conductance calcium-activated potassium channel agonist NS309, the nitric oxide (NO) donor sodium nitroprusside, or constriction to a NO synthase inhibitor L-NNA. Wall thickness and distensibility in PAs from WT mice were unaffected. In TRPV4 $^{-/-}$ mice, UCCAo had no effect on active inner diameters or tone and only increased passive inner diameters (53 ± 2 vs. $43\pm 3\mu\text{m}$, $p<0.05$).

Conclusion: Adaptive response of PAs to chronic cerebral hypoperfusion includes myogenic tone reduction and outward remodeling. TRPV4 channels were involved in tone reduction but not outward remodeling in response to UCCAo.

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Keywords

carotid occlusion; cerebral circulation; hypoperfusion; myogenic tone; parenchymal arterioles; TRPV4

Introduction

Cerebral small vessel disease (CSVD), defined clinically as the presence of white matter lesions through magnetic resonance imaging, is a major cause of stroke and dementia.^{1–3} Despite affecting a substantial amount of people, the cause of CSVD is largely unknown because small vessels in the brain, including arterioles, capillaries, and venules are too small to image and study.⁴ Chronic hypoperfusion and the subsequent hypoxic injury are believed to be a major cause of CSVD, as evidenced by increased expression of hypoxia-inducing factor-1 in autopsy brains from patients with CSVD.⁵ Histological studies in autopsied brains showed that small perforating arteries and arterioles were partially occluded and had thicker walls with loss of smooth muscle cells and deposition of lipohyalinotic materials.^{6, 7} Based on these observations of the vessel wall at the end-stage of CSVD, it is predicted that increased blood-brain barrier permeability, loss of blood flow autoregulation, and/or partial lumen occlusion are major pathologies of CSVD.^{8, 9} However, early responses in cerebral small vessels to chronic hypoperfusion before wall damage and disease progression of CSVD are still largely unknown.

The hypoperfusion aspects of CSVD have been mimicked and studied in animal models. Brain parenchymal arterioles (PAs) were studied in the context of understanding the pathologies of CSVD because they are critical in providing adequate perfusion to the subcortical gray matter and white matter regions of the brain.^{10, 11} Recently we showed that brain parenchymal arterioles (PAs) had diminished myogenic tone after 4 weeks of unilateral common carotid occlusion (UCCAo) in normotensive rats.¹² Interestingly, decreased myogenic tone in PAs was not observed after UCCAo in stroke-prone spontaneously hypertensive rats (SHR-SP),¹² a widely used model of hypertensive CSVD.¹³ Because myogenic tone is the basal level of smooth muscle vasoconstriction in response to intraluminal pressure and is an important contributor to cerebrovascular resistance,^{14, 15} decreased myogenic tone in response to hypoperfusion may be an adaptive response to decrease vascular resistance and therefore increase blood flow to the brain parenchyma. The lack of this adaptive response of PAs in SHR-SP, on the other hand, may prolong chronic hypoperfusion that could lead to hypoxic injury in subcortical brain regions. It is therefore important to understand the mechanisms of this adaptive reduction of myogenic tone in PAs in response of chronic hypoperfusion because the lack of this adaptation may contribute to CSVD.

Transient receptor potential vanilloid 4 (TRPV4) channels are ion channels that may be involved in the regulation of myogenic tone in the cerebral circulation. TRPV4 channels are widely expressed at the endothelium and smooth muscle cells in blood vessels and in neurons, and non-neuronal cell types including astrocytes and microglia in the brain.¹⁶ TRPV4 channels are expressed at the endothelium of middle cerebral arteries and activation

of TRPV4 causes an increase in intracellular calcium.¹⁷ Increased intracellular calcium in vascular endothelial cells causes subsequent activation of small- and intermediate-conductance calcium-activated potassium channels (SK_{Ca}/IK_{Ca}), leading to endothelium-dependent hyperpolarization (EDH) and vasodilation.^{18–20} Our previous study showed that inhibition of SK_{Ca}/IK_{Ca} substantially increased myogenic tone of PAs, demonstrating SK_{Ca}/IK_{Ca} has important role in regulating myogenic tone of PAs.²¹ On the other hand, TRPV4 channels may be involved in the regulation of myogenic tone through their mechanosensitive properties when cellular shape changes occur due to mechanical force, such as shear stress, pressure, or cell swelling through osmolality changes.^{22–24} One previous study showed that lowering intraluminal pressure from 80 to below 50 mmHg caused reduction in myogenic tone in cremaster arterioles that was prevented by TRPV4 channel blocker RN1734.²² In addition, the same study showed that when lowering intraluminal pressure, increased intracellular calcium occurred and co-localized with endothelial TRPV4 channels.²² These results demonstrated that decreased pressure caused TRPV4-dependent reduction in myogenic tone in arterioles. However, whether reduced perfusion pressure caused by chronic hypoperfusion in the brain would activate TRPV4 channels and lead to reduced tone of PAs is not known.

In addition to pressure, a number of studies showed that TRPV4 channels are activated by changes in shear stress.^{25, 26} In a rat model of chronic cerebral hypoperfusion, enhanced shear stress caused TRPV4-dependent outward remodeling, also known as arteriogenesis, in cerebral collateral arteries.²⁶ Under hypoperfusion however, it is still possible that TRPV4 is activated by reduced shear stress because of the mechanosensitive properties of TRPV4 channels. Therefore, we further hypothesized that chronic hypoperfusion causes outward remodeling of PAs that involves TRPV4 channel activation. This hypothesis was based on the idea that outward remodeling is an adaptive response to hypoperfusion to increase dilator capacity of PAs, leading to increased blood flow. Therefore, in the current study, we investigated the role of TRPV4 channels in adapting to chronic hypoperfusion. We used TRPV4 knockout compared to wildtype (WT) C57BL6 mice subjected to UCCAO for 28 days and measured changes in vasoreactivity and structure in PAs.

Results

Effect of chronic hypoperfusion on active inner diameters and myogenic tone of PAs

Our previous study in normotensive rats showed that UCCAO for 4 weeks significantly increased active (in the presence of calcium) inner diameters and decreased tone of PAs.¹² Here, UCCAO significantly increased active inner diameters of PAs at all pressures (Figure 1a) and decreased tone in WT mice (Figure 1b). This confirmed chronic hypoperfusion caused similar changes in PAs in a mouse model of UCCAO as in rats.

Despite significant increase in inner diameters of PAs in WT mice, this was not observed in TRPV4^{-/-} mice (Figure 1a). Similarly, myogenic tone of PAs at 40 mmHg was not affected by UCCAO in TRPV4^{-/-} mice ($22 \pm 3\%$ vs. $29 \pm 2\%$), suggesting that the reduction in myogenic tone in response to chronic hypoperfusion required active TRPV4 channels.

Effect of chronic hypoperfusion on reactivity to vasoactive agents in PAs

SK_{Ca}/IK_{Ca} channels are expressed in endothelial cells of PAs and shown to inhibit myogenic tone.^{19, 21} In the current study, we found that PAs from control mice of both strains dilated to NS309 (Figure 2), confirming the presence of functional SK_{Ca}/IK_{Ca} channels. UCCAO did not significantly affect reactivity of PAs to NS309 in WT or TRPV4^{-/-} mice (Figure 2).

Furthermore, we tested the constriction of PAs to nitric oxide synthase (NOS) inhibitor L-NNA to investigate the role of nitric oxide (NO) in the regulation of myogenic tone. We found that some and not all PAs from control WT mice (4 in 5) constricted to L-NNA, suggesting heterogeneity in the role of NO in tone regulations in these vessels (Figure 3a). The trend was similar in PAs from UCCAO WT mice (3 in 5). This heterogeneity was observed in all groups and explained the high variability of the results. In addition, two-way ANOVA revealed that strain or UCCAO did not alter constriction of PAs to L-NNA. We further investigated smooth muscle responsiveness to NO. UCCAO did not significantly affect reactivity of PAs to the NO donor SNP in WT and TRPV4^{-/-} mice, confirmed by similar EC50: WT-control: $0.766 \pm 0.158 \mu\text{M}$; WT-CH: $0.555 \pm 0.188 \mu\text{M}$; TRPV4^{-/-}-control: $0.782 \pm 0.131 \mu\text{M}$; TRPV4^{-/-}-CH: $0.756 \pm 0.084 \mu\text{M}$. These results thus suggested that smooth muscle responsiveness to NO was not affected by chronic hypoperfusion or TRPV4 deficiency (Figure 3b).

Effect of chronic hypoperfusion on structure of PAs

Compared to PAs from control WT mice, passive inner diameters of PAs from control TRPV4^{-/-} mice were larger (Figure 4a). UCCAO increased passive inner diameters of PAs at all pressures studied in WT mice, suggesting hypoperfusion induced outward remodeling. UCCAO also increased passive inner diameters of PAs in TRPV4^{-/-} mice similar to WT mice (Figure 4a), suggesting that hypoperfusion-mediated outward remodeling was not related to TRPV4 channels. Wall thickness was not different in PAs from all groups of mice (Figure 4b). At 5 mmHg intraluminal pressure, wall thickness of PAs was $5.1 \pm 0.3 \mu\text{m}$ for WT controls, $5.4 \pm 0.2 \mu\text{m}$ for WT-CH, $5.5 \pm 0.2 \mu\text{m}$ for TRPV4^{-/-} control, and $6.0 \pm 0.1 \mu\text{m}$ for TRPV4^{-/-}-CH. Therefore, UCCAO decreased wall-to-lumen ratio in PAs from WT mice (Figure 5a) largely due to an increase in structural diameters of PAs, further demonstrating outward remodeling. However, UCCAO did not change wall-to-lumen ratio in PAs from TRPV4^{-/-} mice (Figure 5a). This unchanged wall-to-lumen ratio in PAs may be due to slightly thicker wall, which normalized increased inner diameter seen after UCCAO in TRPV4^{-/-} mice.

Remodeling of blood vessels may include changes of components at the vascular wall, such as smooth muscle, elastin, and collagen that can impact the distensibility. Therefore, we determined if outward remodeling of PAs by chronic hypoperfusion had an effect on distensibility of the vessel wall. We found that distensibility of vessel wall was not different between groups (Figure 5).

Discussion

Chronic cerebral hypoperfusion has been implicated as a major cause of CSVD,⁵ however, vascular adaptations of PAs, a target of CSVD, to chronic hypoperfusion are largely unknown. In the present study, we used a mouse model of UCCAO to show that chronic cerebral hypoperfusion caused decreased myogenic tone and structural outward remodeling in PAs. Importantly, decreased tone, but not outward remodeling, was prevented in PAs from TRPV4^{-/-} mice, suggesting activation of TRPV4 channels were involved in adaptive reduction of myogenic tone of PAs in response to chronic hypoperfusion. The underlying mechanism of this adaptive response of PAs to chronic hypoperfusion is important to understand because without extensive collateral perfusion in the brain parenchyma,¹¹ this adaptive response of PAs may be an effective way to reduce local vascular resistance and potentially restore blood flow to sub-cortical and white matter brain regions. On the contrary, the lack of adaptive vasodilation, as seen in TRPV4^{-/-} mice and previously in SHR-SP,¹² may lead to prolonged hypoperfusion and possibly ischemia/hypoxia to the white matter. In previous studies, downregulation of TRPV4 channel expression and impairment of TRPV4-dependent vasodilation were found in chronic hypertension.^{27, 28} Therefore, as loss of local autoregulation of cerebral blood flow is believed to be contributing to white matter lesions in CSVD,⁸ the lack of TRPV4-dependent reduction in tone in PAs to hypoperfusion may be one contributing factor to hypertensive CSVD.

TRPV4-mediated vasodilation involves an increase in endothelial cell intracellular calcium, leading to activation of SK_{Ca} and/or IK_{Ca} channels.^{18, 22, 27, 29} A previous study demonstrated that low pressure-induced reduction of myogenic tone was due to increased IK_{Ca}-dependent calcium events in endothelial cells that co-localized with TRPV4 channels.²² Based on these results, we speculate that endothelial IK_{Ca}-dependent potassium efflux and the subsequent hyperpolarization may possibly be an underlying mechanism for hypoperfusion-induced, TRPV4-mediated reduction of myogenic tone of PAs. An alternative pathway is that increased intracellular calcium in vascular endothelial cells activates NOS and subsequent vasodilation through NO-related mechanism.³⁰ This idea is based on a previous study showing that L-NAME, an NOS inhibitor, blocked TRPV4-mediated vasodilation in mesenteric arteries.³¹ However, this mechanism is unlikely because chronic hypoperfusion did not affect vasoconstriction of PAs to NOS inhibitor L-NNA, suggesting NO production was not increased after UCCAO.

Outward remodeling of PAs in response to hypoperfusion may be beneficial because enlarged structural lumen diameters increase vasodilator capacity and could allow PAs to dilate further to increase flow. In the present study, because wall thickness was not significantly affected by hypoperfusion and therefore increased wall-to-lumen ratio in PAs was mainly due to larger lumen diameters. Therefore, chronic hypoperfusion caused outward eutrophic remodeling of PAs, similar to our previous study in rats.^{12, 32} Increased shear stress has been shown to activate TRPV4 and causes outward remodeling in primary cerebral collateral arteries (at the circle of Willis).²⁶ Cerebral collateral arteries redirect cerebral blood flow during carotid occlusion and therefore experience increased flow and shear stress. In contrast, PAs are not collaterals and therefore UCCAO likely caused hypoperfusion and reduced shear stress in these vessels. Our data showing that although

wall-to-lumen ratio was normalized in PAs from hypoperfused TRPV4^{-/-} mice, TRPV4 was unlikely involved in outward remodeling in response to chronic hypoperfusion and reduced shear stress because increased lumen diameters were not normalized.

The majority of studies showed that TRPV4 channels are expressed in the endothelium of resistance vessels, including coronary, mesenteric, and middle cerebral arteries, and cremaster arterioles.^{17, 18, 22, 29, 33} However, TRPV4 channels may be expressed in other cell types in or closely associated with PAs. A previous study showed that TRPV4 was expressed in PAs, but did not specify cell type.³⁴ However, another study showed that TRPV4 was not expressed in PAs, but expressed in astrocytic end-feet associated with PAs.³⁵ TRPV4 may also be expressed in smooth muscle of PAs.³⁶ The results of these studies suggested that the location where the influence of TRPV4 channels on tone of PAs in response to chronic hypoperfusion remains unclear and possibly involves multiple cell types.

Although we showed reduced myogenic tone in PAs after 4 weeks of UCCAO, a previous study by Matin et al. found no change in myogenic tone of PAs in a different animal model of 8 weeks of bilateral carotid artery stenosis (BCAS).³⁴ One major difference of the two studies may be hemodynamic changes. It is possible that the engagement of cerebral collateral arteries in UCCAO to redirect flow to the ipsilateral side was absent in BCAS when blood flow through both carotid arteries was reduced. Therefore, chronic hypoperfusion in the Matin et al. study was more sustained, and with twice the duration (8 versus 4 weeks), one would expect different impacts on PAs. Matin et al. also showed that reactivity of PAs to a TRPV4 channel agonist GSK 1016790A after BCAS was decreased, which indicated lower expression or activity of TRPV4 and may explain the lack of TRPV4-dependent reduced myogenic tone of PAs in their study.³⁴

In conclusion, vascular adaptation of PAs to chronic cerebral hypoperfusion includes decreased myogenic tone and structural outward remodeling. TRPV4 channels appear to be involved in decreased tone, but not outward remodeling of PAs in response to chronic hypoperfusion. Further research is needed to understand the mechanisms by which chronic hypoperfusion causes outward remodeling of PAs, such as hypoxia inducible factor-1 α or vascular endothelial growth factor.^{37, 38} Understanding the mechanisms by which PAs adapt to chronic cerebral hypoperfusion may help our understanding of CSVD.

Materials and Methods

Animals

Male and female TRPV4 knockout (TRPV4^{-/-}) mice (20–30 g), originally developed by GlaxoSmithKline Pharmaceuticals (King of Prussia, PA, USA),³⁹ and genetically-matched WT control C57BL6 mice (Jackson Lab., Bar Harbor, ME, USA) were randomly divided into control or UCCAO groups (n=6–7/group). TRPV4^{-/-} mice were housed and bred in the Animal Care Facility at the University of Vermont, an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility. Genotyping of transgenic mice was performed by PCR from tail-clipping at weaning. All animals were maintained on a 12-hour light/dark cycle and allowed food and water ad libitum. All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Vermont

and complied with the National Institutes of Health guidelines for care and use of laboratory animals.

Animal model of chronic cerebral hypoperfusion

Chronic cerebral hypoperfusion was surgically induced by UCCAO for 28 days. This model has been previously shown to decrease cerebral perfusion by 40 % initially and remain below 25–30 % of baseline perfusion after 28 days in C57BL6 mice.⁴⁰ Animals were anesthetized by isoflurane (1.5–2 % in oxygen and air). A midline incision was made to expose the right common carotid artery, followed by ligation with 6–0 sterile silk suture proximal to the bifurcation of the internal and external carotid arteries. Animals received post-surgical observation and analgesic (buprenorphine, 0.05 mg/kg, s.c.) at 0, 6, 12 hours. The survival rate of these animals was 100 % after 28 days. Control animals were without any surgical procedure and no ligation of the carotid artery.

Preparation of isolated PAs and experimental protocol

Animals were decapitated under deep isoflurane anesthesia (4 % in oxygen) 28 days after UCCAO. The brain was removed and placed in cold, oxygenated artificial cerebrospinal fluid (aCSF). PAs branching between the M1 and M2 region of the MCA from the side of the ligation were carefully dissected and then mounted onto glass cannulas in an arteriograph chamber (Living Systems Instrumentation, St. Albans, VT, USA), as described previously.¹² The arteriograph chamber contained aCSF maintained at ~37 °C and bubbled with 10 % O₂, 5 % CO₂, and balanced N₂ to maintain pH at ~7.4. PAs were equilibrated at 20 mmHg for 1 hour to allow development of myogenic tone, after which intravascular pressure was increased stepwise to 100 mmHg to measure myogenic reactivity. Lumen diameters of PAs in each pressure was measured and recorded as active inner diameters. Intravascular pressure was then returned to 40 mmHg for the remainder of the experiment. Starting from the following experimental procedures, thromboxane A2 mimetic U46619 (10⁻⁷ M) was used to pre-constrict all PAs because some of these vessels did not develop sufficient spontaneous tone. In the presence of U46619, reactivity to a SK_{Ca}/IK_{Ca} agonist NS309 (10⁻⁸–10⁻⁵ M) was assessed. The bath was then washed with aCSF for 5 min to remove all reagents. NG-nitro-L-Arginine (L-NNA, 10⁻⁴ M), a nitric oxide synthase (NOS) inhibitor, was added to the bath, followed by cumulative doses of NO donor sodium nitroprusside (SNP, 10⁻⁸–10⁻⁵ M). At the end of the experiment, the bath was washed with zero calcium aCSF and structural measurements, including passive inner diameters and wall thickness, were obtained in fully relaxed arterioles at pressures from 200–5 mmHg.

Drugs and Solutions

Isolated vessel experiments were performed using aCSF (mM): NaCl 122.0, NaHCO₃ 26.0, KCl 3.0, NaH₂PO₄ 1.25, MgCl₂ 1.0, CaCl₂ 2.0, and glucose 4.0. Buffer solutions were made each week and stored without glucose at 4 °C. Glucose was added prior to each experiment. Zero calcium aCSF was made without the addition of CaCl₂. U46619, NS309, L-NNA, and SNP were purchased from Sigma (St. Louis, MO, USA). Stock solutions were made weekly and stored at 4 °C for L-NNA and SNP. Aliquots of U46619 and NS309 were made and kept frozen at –20 °C until use.

Data Calculations and Statistical Analysis

All results are presented as mean $\pm$ SEM. Tone was calculated at each pressure as a percent decrease in diameters from the fully relaxed diameters in calcium-free aCSF by the equation: $[1 - (\phi_{\text{tone}}/\phi_{\text{passive}})] \times 100\%$; where ϕ_{tone} is the inner diameters of the vessel with tone and ϕ_{passive} is the inner diameters of fully relaxed vessel. Percent constriction to L-NNA was calculated as a percent change in diameter from baseline by the equation: $[1 - (\phi_{\text{drug}}/\phi_{\text{baseline}})] \times 100\%$; where ϕ_{drug} is the diameter of vessel after drug exposure, and ϕ_{baseline} is the diameter before drug exposure. Percent reactivity to NS309 and SNP was calculated from the equation: $[(\phi_{\text{dose}} - \phi_{\text{baseline}})/(\phi_{\text{passive}} - \phi_{\text{baseline}})] \times 100\%$; where ϕ_{dose} is the diameter at a specific concentration of drug. Wall-to-lumen ration was calculated from the equation: wall thickness/inner diameters. Statistical power calculation was performed based on results from a similar previous study, with levels of $\alpha=0.05$ and $1-\beta=0.8$.¹² The calculation showed that the power of the study was 0.942 and 0.846 when the number of samples was 7 and 5, respectively. Differences between groups were determined by two-way analysis of variance with post-hoc Tukey's test using GraphPad Prism 7.0 (La Jolla, CA, USA). "Strain" and "Intervention" were set as Factors in statistical analysis. Differences were considered significant when $p < 0.05$.

Excluded Data

All data of structural measurements of PAs were included (n=6 in TRPV4^{-/-}-CH, and n=7 in other groups). However, data related to reactivity of PAs to pressure and pharmacological agents were removed in two WT-control experiments due to technical difficulties. Moreover, the same data from one hypoperfused WT and one hypoperfused TRPV4^{-/-} mice were removed because PAs in these experiments were not constricted to U46619, leading to no data on reactivity to NS309, L-NNA, and SNP.

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Figure 1.

Effect of chronic hypoperfusion (CH) on active inner diameters and myogenic tone of PAs. Graphs showing active inner diameters of PAs in response to stepwise increase in pressure (a) and percent tone of PAs at 40 mmHg (b) in WT and TRPV4^{-/-} mice. Results are mean $\pm$ SEM. *p<0.05 versus WT control mice; #p<0.05 versus WT-CH mice.

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Figure 2. Effect of chronic hypoperfusion (CH) on reactivity of PAs to NS309. Graphs showing reactivity of PAs to NS309 in WT and TRPV4^{-/-} mice. Results are mean $\pm$ SEM.

Figure 3.

Effect of chronic hypoperfusion (CH) on reactivity of PAs to NO. Graphs showing constriction of PAs to L-NNA in (a) and SNP (b) in WT and TRPV4^{-/-} mice. Results are mean $\pm$ SEM.

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Figure 4.

Effect of chronic hypoperfusion (CH) on structure of PAs. Graphs showing passive inner diameters (a) and wall thickness (b) of PAs from WT and TRPV4^{-/-} mice. Results are mean $\pm$ SEM. *p<0.05 versus corresponding controls.

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Figure 5.

Effect of chronic hypoperfusion on structural characteristics of PAs. Graphs showing wall-to-lumen ratio (a) and distensibility (b) of PAs in WT and TRPV4^{-/-} mice. Results are mean $\pm$ SEM. * $p < 0.05$ comparing WT-CH to WT-Control.

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