RESEARCH TRAINEE SHOWCASE

The University of Vermont
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Left to right: Osama Harraz, Ph.D. and Mark T. Nelson, Ph.D.

Department of Pharmacology

Trainee:
Osama Harraz, Ph.D., Postdoctoral Fellow

Mentor:
Mark T. Nelson, Ph.D., University Distinguished Professor and Chair of Pharmacology, UVM Larner College of Medicine

Research Area:
Vascular physiology and neuroscience

Poster:
"Piezo1 channels are mechanosensors in brain capillaries"

Dr. Harraz presented his poster at the July 2019 FASEB Smooth Muscle Summer Research Conference in West Palm Beach, Florida.

Piezo1 channels are mechanosensors in brain capillaries

Osama F. Harraz[1], Nicholas R. Klug[1], Amreen Mughal[1] and Mark T. Nelson[1,2]

[1] Department of Pharmacology, Larner College of Medicine, University of Vermont, Burlington, Vermont, USA; [2] Division of Cardiovascular Sciences, University of Manchester, Manchester, UK

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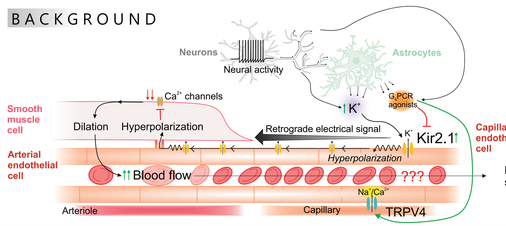
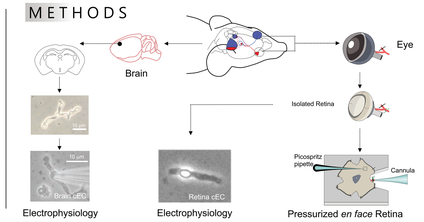
ABSTRACT

Cerebral blood flow (CBF) is exquisitely controlled to meet the ever-changing demands of active neurons in the brain. This activity-dependent blood delivery process is rapidly and precisely controlled through molecular mechanisms collectively termed 'neurovascular coupling' (NVC). Our recent work provides evidence that brain capillaries act as a neural activity-sensing network. In particular, brain capillary endothelial cells (cECs) are equipped with sensors of NVC agents released from neurons/astrocytes onto the abluminal side (outer wall) of a capillary. While capillaries can translate external signals into electrical and Ca^{2+} changes, control mechanisms from the lumen are less clear. The continuous flux of red blood cells and plasma through narrow-diameter capillaries imposes shear stress on the luminal (inner) capillary wall. Whether—and if so how—the ever-changing CBF could be mechanically sensed in capillaries is not known. Here, we propose that the mechanosensitive Piezo1 channels operate as mechanosensors in brain capillaries to ultimately regulate CBF. Patch clamp electrophysiology and immunohistochemical analyses confirmed the expression and function of Piezo1 channels in brain cECs. Mechanical or pharmacological activation of Piezo1 channels evoked currents that were sensitive to Piezo1 channel blockers. Using *Cdh5*-GCaMP8 mice, we observed that Piezo1 channel activation triggered Ca^{2+} signals in cECs in the brain and the retina. A novel ex vivo pressurized retina preparation was subsequently employed to explore the mechanosensitivity of capillary Piezo1-mediated Ca^{2+} signals. In conclusion, this study shows that Piezo1 channels act as mechanosensors in capillaries and that these channels initiate crucial Ca^{2+} signals in cECs. These observations will likely have profound significance for the control of blood flow in the CNS.

BACKGROUND

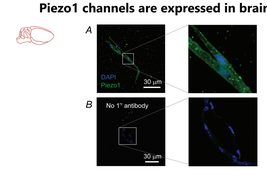


METHODS



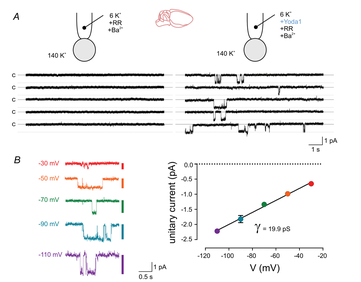
RESULTS

Piezo1 channels are expressed in brain cECs



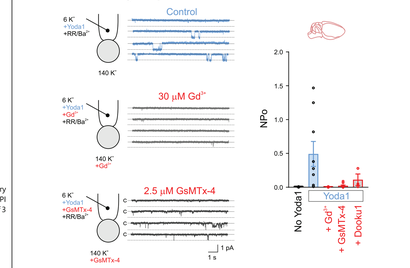
(A) Immunohistochemistry images demonstrate Piezo1 protein expression (green) in mouse brain capillary endothelial cells (cECs). The Piezo1 antibody NBP1-78446 (Novus) was used and nuclei were labeled with DAPI (blue). **(B)** Control staining where Piezo1 antibody was not added to fixed capillaries. Images are representative of 3 independent experiments performed on cECs from 3 different C57Bl/6 mice.

Yoda-1 evokes currents in brain cECs



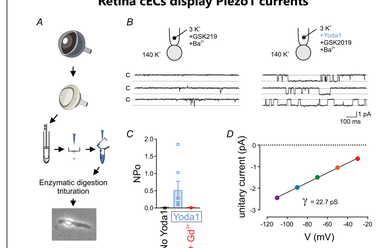
(A) Cell-attached recordings at V_p = −50 mV. Brain cECs were bathed in 140 mM K^+ solution and the $[K^+]$ in the pipette solution was kept at physiological levels (6 mM). Ruthenium Red (RR, 1 μM) and Ba^{2+} (100 μM) were added to pipette solution to block TRPV4 and Kir2.1 channels, respectively. The right traces were obtained from a cEC where Yoda1 (5 μM) was included in the pipette; frequent single channel events were detected. **(B)** In the cell-attached mode, different single unitary currents were recorded at different holding potential. Unitary current (i)-voltage relationship revealed a single channel conductance (γ) of ~20 pS.

Pharmacological profile of Yoda-1-induced currents



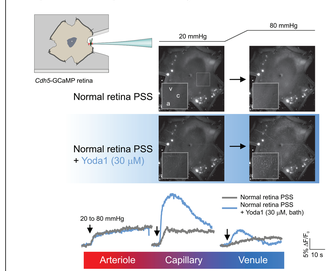
Traces and summary data of single-channel Yoda1-induced currents in brain cECs. Yoda1 (5 μM) was included in the pipette solution in the absence (control) or presence of Piezo1 inhibitors: Gd^{3+} (30 μM); GsMTx-4 (2.5 μM) or Dooku1 (10 μM). Scatter plot abd bar graph shows open probability (NPo) under each condition.

Retina cECs display Piezo1 currents



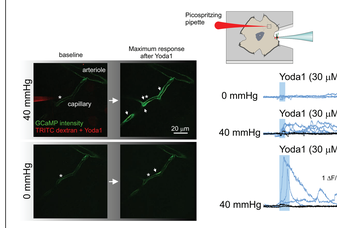
(A) Isolation procedure of retinal cECs. **(B)** Representative traces of single-channel openings in the absence or presence of Yoda1 (5 μM) in 3 mM K^+ pipette solution. GSK219 (1 μM, TRPV4 blocker) and Ba^{2+} (100 μM) were added to the pipette solution. **(C)** Open channel probability in the absence or presence of Yoda1 with or without Gd^{3+}. **(D)** Unitary current-voltage relationship shows a slope conductance of ~23 pS.

Capillaries are preferentially sensitiveto Piezo1 activation



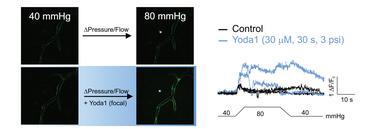
The pressurized *en face* retina preparation (diagram) obtained from *Cdh5*-GCaMP8 mouse. The pressure at the ophthalmic artery was elevated from 20 to 80 mmHg while the preparation was perfused with normal retina PSS (*upper*) or PSS supplemented with 30 μM Yoda1 (*lower*). Example endothelial Ca^{2+} responses to pressure elevation in different vascular segments (with or without Yoda1). Capillary endothelium was the most sensitive. Baseline Ca^{2+} displayed an increase in all vascular segments.

Piezo1 channel activation evokes a Ca^{2+} signal in capillaries



Focal application (picospritzing) of Yoda1 (30 μM) onto retinal capillaries in the *ex vivo* retina preparation. Yoda1 was applied for 3 or 7 s onto a capillary in a pressurized (40 mmHg) or an unpressurized (0 mmHg) *en face* retina. Vehicle was picospritzed as control.

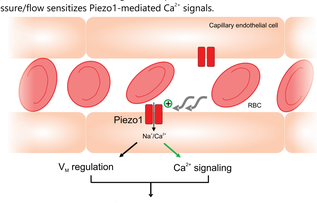
pharmacological Piezo1 activation enhances Ca^{2+} signals induced by Δ pressure/flow in retinal capillaries



Imaging field of a capillary in the pressurized retina preparation before and at peak Ca^{2+} response after elevating ophthalmic artery pressure (40 to 80 mmHg, *upper*) or the same pressure change along with focal application of Yoda1 (*lower*). Right: traces showing Ca^{2+} responses in the target capillary before and during the pressure change in the absence (control) or presence of Yoda1.

CONCLUSIONS

- Piezo1 channel is expressed and functional in CNS capillary endothelial cells.
- Piezo1 channel mediates Ca^{2+} signals in capillaries.
- Pressure/flow sensitizes Piezo1-mediated Ca^{2+} signals.



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