Focal cerebral ischemic stroke results in endothelial $\text{BK}_{\text{Ca}}$ expression and altered function, with no change in TRPV4, in middle cerebral artery

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Introduction

• Functional $\text{BK}_{\text{Ca}}$ are absent in healthy intact artery endothelial cells (ECs).
• Exception is gracilis artery of hypoxic rat (Hughes et al., 2010, AJPH, 299:H1349-50).
• EC isolation and culture from many (not all) arteries results in $\text{BK}_{\text{Ca}}$ expression / function.
• Above suggests a stress-induced phenotype (Sandow & Grayson, 2009, AJPH, 297:H11-7).
• $\text{K}_{\text{Ca}}$ and TRPV4 are critical for endothelial control of tone in many arteries.

Hypothesis

Stroke and hypoxia result in $\text{BK}_{\text{Ca}}$ and altered TRPV4 expression in intact middle cerebral artery (MCA) endothelium.

Aims

- Determine $\text{BK}_{\text{Ca}}$ and $\beta_1$ (pore-forming and regulatory subunits, respectively) and TRPV4 distribution / expression in intact MCA in a rat model of hypoxia and stroke.
- Determine $\text{BK}_{\text{Ca}}$ distribution / expression in human cerebral vessel control vs stroke.

Methods

MCA (ipsilateral) from 8-10 wk male SD rats was examined from the following models (n, # animals, parenthesis): 1. i. Control, untreated (4) and ii. saline treated (4); 2. Hypoxia (8%O2/1h/d, 5d; with pre-saline: 4); 3. Endothelin (120 pM bolus)-induced stroke (+normoxia; 4). 4. Endothelin (120 pM bolus)-induced stroke and hypoxia (8%O2/1h/d, 5d; 24h post-stroke; 4). Conventional confocal immunohistochemistry using characterized specific antibodies and image analysis (CellRIQ, Olympus) determined fluorescence intensity. The antibodies were; $\text{BK}_{\text{Ca}}$, Alomone APC-107, to mse aa 1184-1200; $\text{BK}_{\beta_1}$, Merek (Garcia, NJ), to bov aa 61-75 and 118-132; TRPV4 Abs (Antibody Verify). Ab controls used positive and negative expressing tissue, peptide block and Western blotting. Pressure myography and $\text{BK}_{\text{Ca}}$ and TRPV4 activators and blockers determined channel function. Human tissue was from the NSW Brain Bank. Protocols were approval by the UNSW ACEC/HREC.

Results

• Rat MCA $\text{BK}_{\text{Ca}}$/$\beta_1$: absent in control / even diffuse and punctate in stroke and hypoxia. • EC TRPV4 in all cohorts / even diffuse, intermittent punctate at membrane / increased in hypoxia and stroke and reduced in stroke + hypoxia (Fig 1).

Figure 1. Rat MCA $\text{BK}_{\text{Ca}}$/$\beta_1$, TRPV4 localization and expression in stroke and hypoxia.

• Rat MCA EC $\text{BK}_{\text{Ca}}$/$\beta_1$: absent in control / even diffuse and punctate in stroke and hypoxia. • EC TRPV4 in all cohorts / even diffuse, intermittent punctate at membrane / increased in hypoxia and stroke and reduced in stroke + hypoxia (Fig 1).

Figure 2. Human penetrating CA $\text{BK}_{\text{Ca}}$/$\beta_1$ localization in control and stroke.

• Human cerebral arteriole EC $\text{BK}_{\text{Ca}}$ absent in control, present in stroke; and in SM of control and stroke (Fig 2).
• Basal $\text{BK}_{\text{Ca}}$ controlling tone increased following stroke (Fig 3).
• TRPV4 contribution to tone same in control and stroke (Fig 3).
• No $\text{BK}_{\text{Ca}}$-TRPV4 link (IbTX had no effect on V4 agonist in control-stroke).

Figure 3. Functional properties of MCA of control and stroke rat.

Summary / Conclusion

• Stroke and hypoxia induce cerebral artery endothelium to express $\text{BK}_{\text{Ca}}$/$\beta_1$, altering tone.
• Stroke and hypoxia induce differential changes in endothelial and smooth muscle TRPV4 expression.
• Functional and human studies are ongoing: including which TRP in human stroke?
• $\text{BK}_{\text{Ca}}$ in isolated and culture ECs have limited relevance to intact vessel data.