Pericyte contractile responses to endothelin-1 and Aβ peptides: assessment by electrical impedance assay

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Introduction

- Cerebrovascular abnormalities, including reduced cerebral blood flow, are major contributors to cognitive decline and occur up to 10-20 years before the onset of symptoms of Alzheimer's disease (AD) (1, 2)
- Pericytes are vascular mural cells that regulate capillary calibre and thus blood flow
- Pericyte tone is regulated by chemical mediators such as endothelin-1 (EDN1), which signals by binding to EDN1 receptors, EDNRA and EDNRB (3)
- EDN1 is increased in AD and a recent mechanistic study showed that capillaries were constricted in Aβ-burdened regions in human brain slices via an EDN1-mediated mechanism (4)
- EDN1 signalling has been characterised in retinal capillary pericytes but its signalling in human brain pericytes is largely unexplored

Aims and hypothesis

Aims:

- To investigate whether EDN1 causes contraction of human brain vascular pericytes (HBVP)
- To determine the role of EDNRA and EDNRB in EDN1-mediated contraction in HBVP
- To determine the effects of Aβ peptides on EDN1-mediated contraction of HBVP

Hypothesis:

- We hypothesise that EDN1-mediated pericyte contraction is a major contributor to cerebral hypoperfusion in AD
- We hypothesise that EDN1-mediated pericyte contraction is dysregulated in the presence of Aβ

Methods

- Foetal HBVP (fHBVP; *ScienCell*) and adult HBVP (aHBVP; *Cell Systems*) were cultured and all experiments were performed at passage < 8
- An electrical impedance assay was used to detect pericyte morphological changes in real time (Figure 1)
- Change in calculated cell index was used to reflect rates of contraction

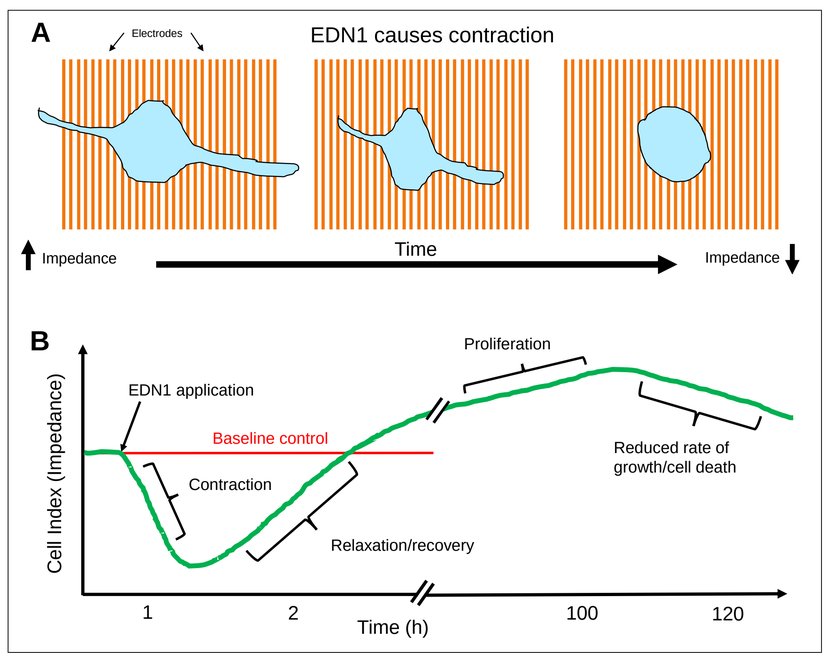

Figure 1. Graphical representation of cell contraction on impedance plates

(A) Graphical representation of cell contraction on impedance plates. Contraction results in less cell surface area in contact with culture dish electrodes and is read out as a reduction in cell index. (B) Example impedance trace readout from the xCELLigence software. Image B taken and adapted from ACEA Biosciences Inc.

Results

Human Brain Vascular Pericytes Express Pericyte Markers PDGFRβ, α-SMA and EDN1 receptors

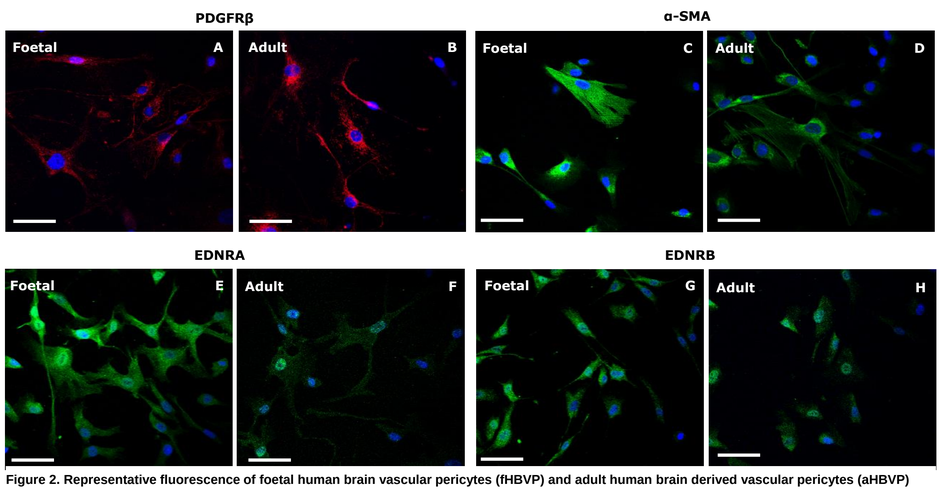

Figure 2. Representative fluorescence of foetal human brain vascular pericytes (fHBVP) and adult human brain derived vascular pericytes (aHBVP)

fHBVP and aHBVP pericytes express the canonical pericyte markers PDGFRβ (A-B), α-SMA (C-D) and both the endothelin-1 type A (EDNRA) (E-F) and type B (EDNRB) receptor (G-H). All counterstained with a nuclear marker (DAPI – blue). Magnification 40X. Scale bar represents 50 μM.

Results

Endothelin-1 mediates contraction of foetal human brain vascular pericytes through activation of the endothelin type A receptor

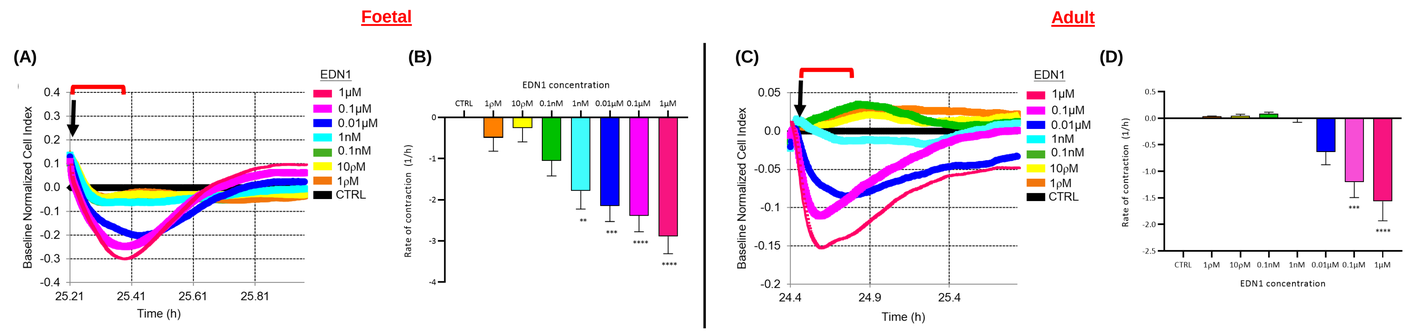

Figure 3. Rates of contraction of fHBVP and aHBVP in response to EDN1

(A) Representative electrical impedance measurements of the contractile response of fHBVP to EDN1. (B) One-way ANOVA and Dunnett's multiple comparison post-hoc test revealed the rate of contraction was significantly higher in wells where 1 nM (p = 0.0283), 0.01 μM (p = 0.0035), 0.1 μM (p = 0.0022) and 1μM (p = 0.0003) EDN1 were added compared to control wells. (C) Representative electrical impedance measurements of the contractile response of aHBVP to EDN1. (D) One-way ANOVA and Dunnett's multiple comparison post-hoc test revealed the rate of contraction was significantly higher in wells where 1 μM (p = 0.0008), 0.1 μM (p < 0.0001) EDN1 were added compared to control wells. The timeframe referred to in each graph is indicated by the red bracket. Arrow indicates time of EDN1 addition. Data show mean rate of contraction ±SEM.

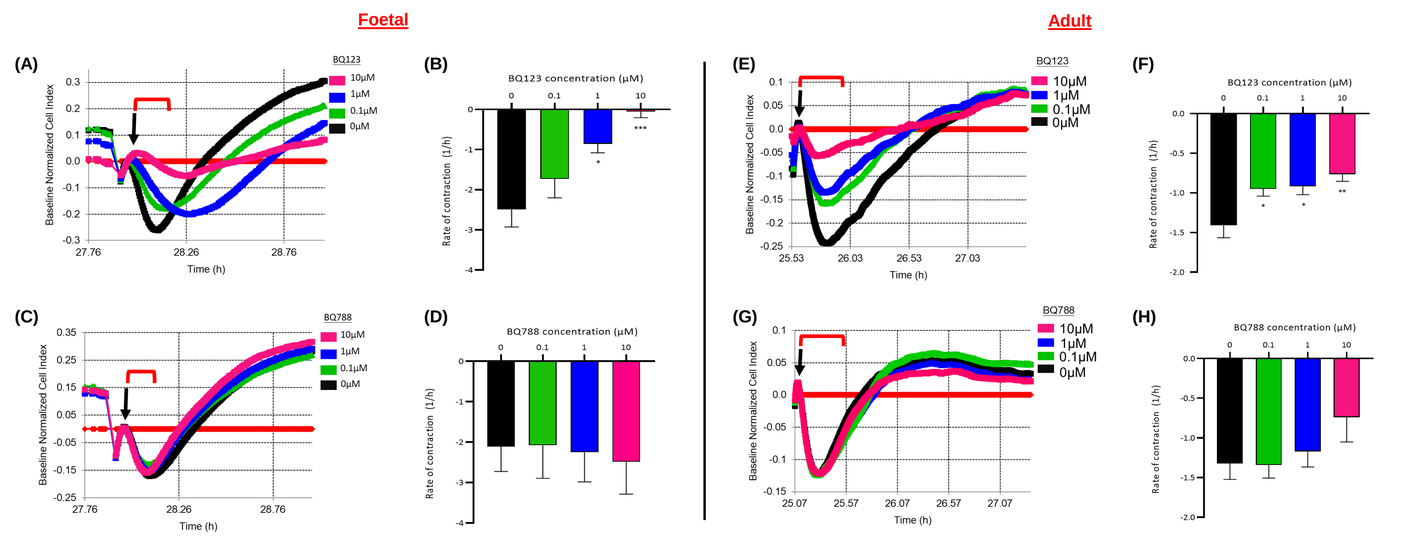

Figure 4. Rates of contraction of fHBVP and aHBVP in response to EDN1 in the presence of BQ123 and BQ788

(A) Representative electrical impedance measurements of the contractile response of fHBVP to EDN1 in the presence of BQ123, an EDNRA antagonist. (B) The rate of contraction was significantly reduced in cells to which 1 μM (p = 0.0158) or 10 μM (p = 0.0001) BQ123 had been added. (C) Representative electrical impedance measurements of the contractile response of fHBVP in the presence of BQ788, an EDNRB antagonist. (D) There was no significant difference in the rate of contraction between BQ788-treated and untreated cells. (E) Representative electrical impedance measurements of the contractile response of aHBVP to EDN1 in the presence of BQ123, an EDNRA antagonist. (F) The rate of contraction was significantly reduced in cells to which 0.1 μM (p = 0.0241), 1 μM (p = 0.0154) or 10 μM (p = 0.0016) BQ123 had been added. (G) Representative electrical impedance measurements of the contractile response of aHBVP in the presence of BQ788, an EDNRB antagonist. (H) There was no significant difference in the rate of contraction between BQ788-treated and untreated cells. The timeframe referred to in each graph is indicated by the red bracket. Arrows indicates the timepoint of EDN1 addition. The bars represent the mean values and SEM.

Endothelin-1-mediated contraction of foetal human brain vascular pericytes is influenced by 24-hour exposure to Aβ peptides

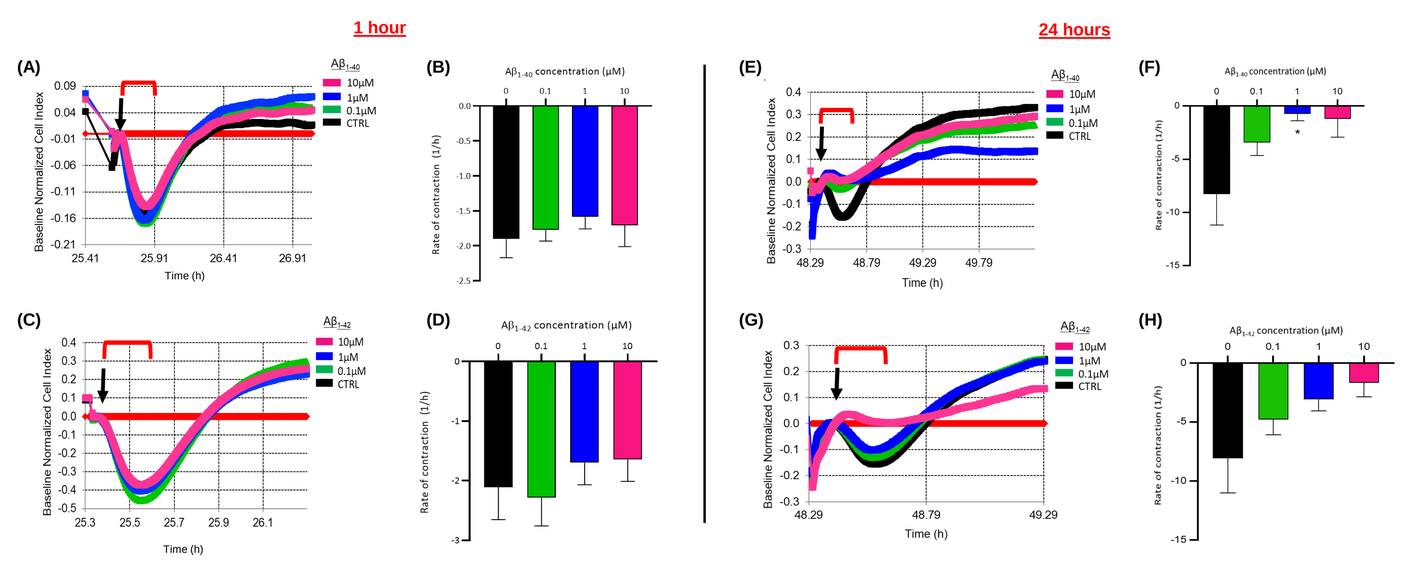

Figure 5. Rates of contraction of fHBVP in response to EDN1 after exposure to $Aβ_{1-40}$ and $Aβ_{1-42}$ 1 or 24 hours before

(A) Representative electrical impedance measurements of the contractile response of foetal human brain vascular pericytes (fHBVP) to EDN1 (100 nM) after 1 h incubation with $Aβ_{1-40}$ (n = 1). (B) There was no significant difference between the rate of contraction of treated and untreated cells. (C) Representative electrical impedance measurements of the contractile response of these fHBVP to EDN1 (100 nM) after 1 h treatment with $Aβ_{1-42}$. (D) The differences in the rate of contraction between treated and untreated cells were not statistically significant. (E) Representative electrical impedance measurements of the contractile response of fHBVP to EDN1 (100 nM) after 24 h incubation with $Aβ_{1-40}$. (F) There was a significant difference between the rate of contraction of untreated cells and those exposed to 1 μm (p = 0.0249) $Aβ_{1-40}$. (G) Representative electrical impedance measurements of the contractile response of these fHBVP to EDN1 (100 nM) after 24 h treatment with $Aβ_{1-42}$. (F) The differences in the rate of contraction between treated and untreated cells were not statistically significant. The timeframe referred to in each graph is indicated by the red bracket. Arrows indicates the timepoint of EDN1 addition. The bars represent the mean values and SEM.

Conclusions and Future Directions

Conclusions:

- EDN1 causes a dose-dependent contractile response in fHBVP and aHBVP although the response is less pronounced in aHBVP
- Contraction is primarily mediated through EDN1 binding to the EDNRA and not through EDNRB signalling
- In fHBVP, physiological concentrations of $Aβ_{1-40}$ dampens contraction

Future directions:

- To determine how Aβ peptides regulate EDN1-mediated pericyte contractility and explore differences between foetal and adult-derived cells

References

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4. Nortley R *et al.* 2019. Science. Vol 19;365(6450)