

## Acetylcholine induces intracellular Ca<sup>2+</sup> oscillations and nitric oxide release in mouse brain endothelial cells

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Basal forebrain neurons increase cortical blood flow by releasing acetylcholine (ACh), which stimulates endothelial cells (ECs) to produce the vasodilating gasotransmitter, nitric oxide (NO). Surprisingly, the mechanism whereby ACh induces NO synthesis in brain microvascular ECs is unknown. An increase in intracellular Ca<sup>2+</sup> concentration recruits a multitude of endothelial Ca<sup>2+</sup> +-dependent pathways, such as Ca<sup>2+</sup> +/calmodulin endothelial NO synthase (eNOS). The present investigation sought to investigate the role of intracellular Ca<sup>2+</sup> + signaling in ACh-induced NO production in bEnd5 cells, an established model of mouse brain microvascular ECs, by conventional imaging of cells loaded with the Ca<sup>2+</sup> +-sensitive dye, Fura-2/AM, and the NO-sensitive fluorophore, DAF-DM diacetate. Overall, our data shed novel light on the molecular mechanisms whereby neuronally-released ACh controls neurovascular coupling in blood microvessels.

### Keywords

Mouse brain microvascular endothelial cells, bEND5 cells, acetylcholine, nitric oxide, Ca<sup>2+</sup> signaling, intracellular Ca<sup>2+</sup> oscillations, inositol-1,4,5-trisphosphate receptors, store-operated Ca<sup>2+</sup> entry, Orai2