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Glutamate triggers intracellular Ca2+ oscillations and nitric oxide release by inducing NAADP- and InsP3-dependent Ca2+ release in mouse brain endothelial cells

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The neurotransmitter glutamate increases cerebral blood flow (CBF) by activating postsynaptic neurons and presynaptic glial cells within the neurovascular (NVU) unit. Glutamate does so by causing an increase in intracellular Ca2+ concentration ([Ca2+]i) in the target cells, which activates the Ca2+/Calmodulin-dependent NO synthase (NOS) to release NO. It is unclear whether brain endothelial cells also sense glutamate through an elevation in [Ca2+]i and NO production. The present study assessed whether and how glutamate drives Ca2+-dependent NO release in bEND5 cells, an established model of brain endothelial cells. We found that glutamate induced a dose dependent oscillatory increase in [Ca2+]i, which was maximally activated at 200 μM and inhibited by MCPG, a selective blocker of Group 1 metabotropic glutamate receptors. Glutamate-induced intracellular Ca2+ oscillations were triggered by rhythmic endogenous Ca2+ mobilization and maintained over time by extracellular Ca2+ entry. Pharmacological manipulation revealed that glutamate-induced endogenous Ca2+ release was mediated by inositol-1,4,5-trisphosphate-sensitive receptors and NAADP-gated two-pore channel 1 (TPC1). Constitutive SOCE mediated Ca2+ entry during ongoing Ca2+ oscillations. Finally, glutamate evoked a robust, although delayed increase in NO levels, which was blocked by pharmacologically inhibition of the accompanying intracellular Ca2+ wave. Of note, glutamate induced Ca2+-dependent NO release also in hCMEC/D3 cells, an established model of human brain microvascular endothelial cells. This investigation demonstrates for the first time that metabotropic glutamate-induced intracellular Ca2+ oscillations and NO release have the potential to impact on neurovascular coupling in the brain.

References

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Key words

Glutamate, endothelial cells, nitric oxide, neurovascular coupling, Ca^{2}+ oscillations.