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Acetylcholine induces nitric oxide production by inducing intracellular Ca2+ oscillations in mouse brain endothelial cells

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Basal forebrain neurons control intracortical arterioles by releasing acetylcholine (Ach), which stimulates endothelial cells (ECs) to produce the vasodilating gasotransmitter, nitric oxide (NO). Surprisingly, the mechanism by which Ach induces NO synthesis in brain ECs is still unknown. An increase in intracellular Ca2+ concentration ([Ca2+]i) recruits a multitude of endothelial Ca2+-dependent pathways, such as Ca2+/Calmodulin endothelial NO synthase (eNOS). The present investigation sought to investigate the role of intracellular Ca2+ signaling in Ach-induced NO production in bEnd5 cells, an established model of mouse brain microvascular ECs. Ach induced dose-dependent asynchronous Ca2+ oscillations in bEnd5 cells. Ach-evoked Ca2+ oscillations did not arise in the absence of external Ca2+ but rapidly resumed on Ca2+ restitution to the bath. However, nicotine, a selective agonist of the Ca2+-permeable nicotinic receptors, did not cause any detectable increase in [Ca2+]i. Pharmacological manipulation indeed revealed that Ach-induced Ca2+ spikes in bEnd5 cells are triggered by the interaction between intracellular Ca2+ release from InsP3 receptors (InsP3Rs) SOCE. Next, we found that Ach-induced NO production was hindered by L-NAME, a selective NOS inhibitor, and BAPTA, a membrane permeable intracellular Ca2+ buffer. Moreover, Ach-elicited NO synthesis was blocked by the pharmacological abrogation of the accompanying Ca2+ spikes. Ach stimulates bEnd5 cells by inducing a burst of intracellular Ca2+ spikes which is patterned by the interplay between ER-dependent Ca2+ mobilization and SOCE. Ach-elicited Ca2+ spikes result in NO production and are, therefore, predicted to control local CBF in mouse brain. Future experiments will assess whether this signaling pathway is altered in neurodegenerative disorders, such as Alzheimer's Disease.

References

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Keywords

bEnd5 cells; [Ca2+]I; acetylcholine; SOCE; mouse brain.