Instructions for Authors

1) Abstracts should contain the title; a list of author(s); the institution(s) where the investigation was performed and the text. **The Title must be in capital letters. For the Institute, please, specify the city only (no address), in italics, and the Country; use the asterisks in the case of multiple memberships.**

2) Underline the presenting author. **List of authors (initial of the first name followed by surname, uppercase only initials).**

3) **Text should be divided into 4 sections: objectives, materials and methods, results, conclusions.**

4) The recommended font is time new roman and font size 12. Kindly set the language to English.

5) Type the entire abstract single-spaced without margins at the top of sides. **Do not include bibliographic citations, tables or figures. Text can be maximum 2700 characters long, including spaces. Use only the acronyms and abbreviations common to the other state in full in the first quote**

7) Add the correct email address and mailing address, including phone, fax number, of the presenting author at the end of the abstract.

Example:

ACETYLCHOLINE INDUCES NITRIC OXIDE PRODUCTION BY INDUCING INTRACELLULAR Ca^{2+} OSCILLATIONS IN MOUSE BRAIN ENDOTHELIAL CELLS

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Objectives: 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 Ca^{2+} concentration ($[Ca^{2+}]_i$) recruits a multitude of endothelial Ca^{2+}-dependent pathways, such as Ca^{2+}/Calmodulin endothelial NO synthase (eNOS). The present investigation sought to investigate the role of intracellular Ca^{2+} signaling in Ach-induced NO production in bEnd5 cells, an established model of mouse brain microvascular ECs.

Materials and methods: Changes in $[Ca^{2+}]_i$ were monitored from bEnd5 cells loaded with the Ca^{2+}-sensitive dye, Fura-2/AM (2 μM, 30 min), by using a CCD camera. NO was measured by loading the cells with the NO-sensitive fluorochrome, DAF-FM diacetate (10 μM, 1 hour).

Results: Ach induced dose-dependent asynchronous Ca^{2+} oscillations in bEnd5 cells, 300 μM being the most effective dose to generate a prolonged (up to 1 hour) Ca^{2+} burst. Ach-evoked Ca^{2+} oscillations did not arise in the absence of external Ca^{2+} but rapidly resumed on Ca^{2+} restitution to the bath. However, nicotine, a selective agonist of the Ca^{2+}-permeable nicotinic receptors, did not cause any detectable increase in $[Ca^{2+}]_i$. Pharmacological manipulation indeed revealed that Ach-induced Ca^{2+} spikes in bEnd5 cells are triggered by the interaction between intracellular Ca^{2+} release from $InsP_3$ receptors ($InsP_3Rs$) SOCE. SOCE was then amplified by Ca^{2+} release through $InsP_3Rs$ and ryanodine receptors

(RyRs), thereby shaping the Ca^{2+} spikes. Consistently, the pharmacological depletion of the ER Ca^{2+} store with cyclopiazonic acid (CPA), a selective inhibitor of Sarco-Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA), revealed the expression of a BTP2- and La^{3+}-sensitive SOCE in bEnd5 cells. Next, we found that Ach-induced NO production was hindered by L-NAME, a selective NOS inhibitor, and BAPTA, a membrane permeable intracellular Ca^{2+} buffer. Moreover, Ach-elicited NO synthesis was blocked by the pharmacological abrogation of the accompanying Ca^{2+} spikes.

Conclusions: Ach stimulates bEnd5 cells by inducing a burst of intracellular Ca^{2+} spikes which is patterned by the interplay between ER-dependent Ca^{2+} mobilization and SOCE. Ach-elicited Ca^{2+} 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.

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