Pflügers Archiv - European Journal of Physiology (2021) 473:133–134
https://doi.org/10.1007/s00424-020-02485-z

COMMENTARY

Reversible interruption of ER Ca^{2+} uptake inversely affects ACh-elicited exocytosis in mouse and bovine chromaffin cells

Arturo Hernández-Cruz[1]

Received: 14 October 2020 / Revised: 14 October 2020 / Accepted: 20 October 2020 / Published online: 27 October 2020

Adrenal medulla chromaffin cells (CCs) are invaluable cell models for neurosecretion studies [5]. Significant differences in Ca^{2+} dynamics and exocytosis exist in CCs from several mammalian species (bovine, rat, guinea pig, cat, human) [7]. Nonetheless, the interest in mouse CCs (MCCs) increased disproportionally after the advent of transgenic mouse models to explore, from the complexities of Ca^{2+} signaling and exocytosis to alterations of Ca^{2+} homeostasis in neurodegenerative diseases [5].

There is a consensus that bovine chromaffin cells (BCCs) express an efficient mechanism of $[Ca^{2+}]_i$ signal amplification by which the initial $[Ca^{2+}]_i$ elevation opens ryanodine receptors (RyRs) from the ER through which Ca^{2+} is released into the cytosol [2, 8]. An influential article [10] reported that MCCs lack RyR-mediated Ca^{2+}-induced Ca^{2+} release (CICR), but two recent studies in CCs from C57BL/6 mice [4, 11] conclusively support the presence of RyR and CICR. A differential role of mitochondria Ca^{2+} handling has also been reported: They sequester a more significant fraction of Ca^{2+} influx in MCCs than in BCCs [1].

Martínez-Ramírez et al., in this issue [9], compare the effects of acute, reversible SERCA pump inhibition by ciclopiazonic acid (CPA) on exocytotic responses and intracellular Ca^{2+} signals elicited by application to MCCs or BCCs of short ACh pulses at different intervals and extracellular $[Ca^{2+}]$. The results could not be more contrasting: *enhancement of exocytosis* during CPA treatment and *inhibition of exocytosis* upon CPA washout in MCCs; *inhibition of exocytosis* during CPA exposure, and *rebound exocytosis recovery* upon CPA washout in BCCs. These differences, found under identical recording conditions at 37 °C, appear genuine. Intriguingly, CPA only reduced ACh-induced Ca^{2+} signals slightly in both cell types. Why the reversible pharmacological inhibition of ER Ca^{2+} uptake affects so differently ACh-elicited CA exocytosis in MCCs and BCCs?

The authors suggest that the opposite effects of CPA on ACh-induced exocytosis in MCCs and BCCs result from different $[Ca^{2+}]_i$ handling by the ER and mitochondria, affecting vesicle traffic and refilling of the rapid release pool. During the SERCA's acute blockade, more Ca^{2+} is diverted to and taken up by the mitochondria through their Ca^{2+} uniporter. Conversely, upon restoration of ER Ca^{2+} uptake, mitochondria release Ca^{2+} through the Na^+/Ca^{2+} exchanger, which the ER eventually takes up. As mentioned above, more Ca^{2+} is sequestered by mitochondria in MCCs than in BCCs [1]. However, mitochondrial Ca^{2+} transport cannot explain interspecies differences because it is not affected by the CPA.

It has been demonstrated that the ER can either attenuate or potentiate depolarization-induced Ca^{2+} signals, depending on its Ca^{2+} content. When depleted, a fraction of Ca^{2+} entering the cell during stimulation is captured by the store, acting as a *Ca^{2+} sink*. Conversely, when the store is full, it acts as a *Ca^{2+} source* and releases Ca^{2+} by CICR, therefore amplifying Ca^{2+} entry [3, 6].

Thus, the different behavior could be explained by assuming that mouse and bovine CCs differ in the two opposing ER functions: CICR and Ca^{2+} uptake: MCCs display weak CICR and strong Ca sinking. The reverse is true for BCCs. Significantly, CPA inhibits both mechanisms. In MCCs, secretion is enhanced by CPA because Ca^{2+} sinking is reduced, and a larger fraction of the Ca^{2+} entering the cell becomes available to trigger exocytosis. Upon removal of CPA, Ca^{2+} sinking recuperates, and exocytosis diminishes back to control. In BCCs, by discontinuing Ca^{2+} uptake (and allowing ER Ca^{2+} leakage), Ca^{2+} content drops, and CICR is weakened, thus reducing exocytosis. After CPA wash out, CICR recovers

This article is a commentary to the original article https://doi.org/10.1007/s00424-020-02483-1

✉ Arturo Hernández-Cruz
ahernan@ifc.unam.mx

[1] Instituto de Fisiología Celular, Departamento de Neurociencia Cognitiva and Laboratorio Nacional de Canalopatías, Universidad Nacional Autónoma de México, CP 04510 México City CDMX, México

its strength, and exocytosis recuperates. See the summarizing scheme of Figure 9 in Martínez-Ramírez et al. [9]. The discordant Ca^{2+} signaling data is irrelevant in this context because Fura-2 reports "bulk $[Ca^{2+}]_i$" and the relevant Ca^{2+} signals for exocytosis occur locally, in the submembrane space.

One may wonder if SERCA inhibition would affect differently exocytosis elicited by other stimuli, namely high-K^+ depolarization, Ca^{2+} release by $InsP_3$-dependent mechanisms, or caffeine that directly opens RyRs. Also, given the crucial role of CICR in this context, it would be interesting to know if ryanodine, which blocks RyRs, affects exocytosis differently in MCC and BCC during SERCA inhibition.

The ER store's filling status depends on the cell's previous electrical activity, the expression level and activity of SERCA, and other Ca^{2+}-mobilizing proteins, the expression, and activity of RyRs, $InsP_3$ receptors etc. Variations of these parameters determine the cell's response under physiological and pathological scenarios. By clearly demonstrating differences between MCCs and BCCs in the same context, this study underscores the need to understand better the regulatory mechanisms that underlie these phenomena.

References

1. Alés E, Fuentealba J, García AG, López MG (2005) Depolarization evoques different patterns of calcium signals and exocytosis in bovine and mouse chromaffin cells: the role of mitochondria. Eur J Neurosci 21:142–150. https://doi.org/10.1111/j.1460-9568.2004.03861.x
2. Alonso MT, Barrero MJ, Michelena P, Carnicero E, Cuchillo I, García AG, García-Sancho J, Montero M, Alvarez J (1999) Ca^{2+}-induced Ca^{2+} release in chromaffin cells seen from inside the ER with targeted aequorin. J Cell Biol 144:241–254. https://doi.org/10.1083/jcb.144.2.241
3. Barry VA, Cheek TR (1994) A caffeine- and ryanodine-sensitive intracellular Ca^{2+} store can act as a Ca^{2+} source and a Ca^{2+} sink in PC12 cells. Biochem J 300(Pt 2):589–597. https://doi.org/10.1042/bj3000589
4. Calvo-Gallardo E, López-Gil Á, Méndez-López I, Martínez-Ramírez C, Padín JF, García AG (2016) Faster kinetics of quantal catecholamine release in mouse chromaffin cells stimulated with acetylcholine, compared with other secretagogues. J Neurochem 139:722–736. https://doi.org/10.1111/jnc.13849
5. Carbone E, Borges R, Eiden LE, García AG, Hernández-Cruz A (2019) Chromaffin cells of the adrenal medulla: physiology, pharmacology, and disease. Compr Physiol 9:1443–1502. https://doi.org/10.1002/cphy.c190003
6. Friel DD, Tsien RW (1992) A caffeine- and ryanodine-sensitive Ca^{2+} store in bullfrog sympathetic neurones modulates effects of Ca^{2+} entry on $[Ca^{2+}]$i. J Physiol 450:217–246. https://doi.org/10.1113/jphysiol.1992.sp019125
7. García AG, García-De-Diego AM, Gandia L, Borges R, García-Sancho J (2006) Calcium signaling and exocytosis in adrenal chromaffin cells. Physiol Rev 86:1093–1131. https://doi.org/10.1152/physrev.00039.2005
8. Lara B, Lopez MG, Villarroya M, Gandia L, Cleeman L, Morad M, Garcia AG (1997) A caffeine-sensitive Ca^{2+} store modulates K^+-evoked secretion in chromaffin cells. Am J Phys 272:C1211–C1221. https://doi.org/10.1152/ajpcell.1997.272.4.C1211
9. Martínez-Ramírez C, Gil-Gómez I, de Diego AMG, and García AG. (2020). Acute reversible SERCA blockade facilitates or blocks exocytosis, respectively in mouse or bovine chromaffin cells
10. Rigual R, Montero M, Rico AJ, Prieto-Lloret J, Alonso MT, Alvarez J (2002) Modulation of secretion by the endoplasmic reticulum in mouse chromaffin cells. Eur J Neurosci 16:1690–1696. https://doi.org/10.1046/j.1460-9568.11-2.02244.x
11. Wu P-C, Fann M-J, Kao L-S (2010) Characterization of Ca^{2+} signaling pathways in mouse adrenal medullary chromaffin cells. J Neurochem 112:1210–1222. https://doi.org/10.1111/j.1471-4159.2009.06533.x