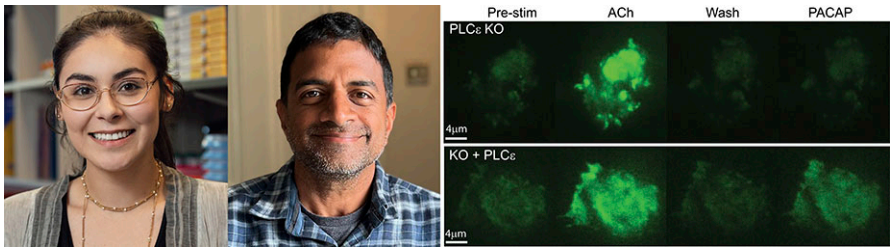
JGP Journal of General Physiology

RESEARCH NEWS

Two distinct pathways regulate chromaffin cell exocytosis

Ben Short

***JGP* study reveals how the neurotransmitter PACAP induces a secretory response in chromaffin cells that differs from the one induced by acetylcholine.**



Alina Morales (left), Arun Anantharam (center), and colleagues reveal that, acting through two distinct signaling pathways, the neurotransmitters ACh and PACAP elicit distinct Ca^{2+} signals and secretory responses in adrenal chromaffin cells. Imaging of cells expressing a fluorescent Ca^{2+} indicator (right) shows that the response to PACAP, but not ACh, is impaired in the absence of PLCε (top row).

In times of stress, chromaffin cells in the adrenal medulla regulate the function of peripheral organs by secreting epinephrine, norepinephrine, and a number of vasoactive peptides. The release of these hormones is controlled by sympathetic neurons that stimulate chromaffin cell exocytosis by releasing two different neurotransmitters, acetylcholine (ACh) and pituitary adenylate cyclase activating polypeptide (PACAP). In this issue of *JGP*, Morales et al. (1) reveal new details of the signaling pathway downstream of PACAP stimulation, and show that it results in a kinetically distinct secretory response.

In recent years, PACAP has emerged as an important regulator of chromaffin cell exocytosis that may be crucial for sustaining catecholamine release in periods of prolonged stress when the response to ACh becomes desensitized (2). In contrast to ACh-induced secretion, however, little is known about the pathway coupling PACAP to chromaffin cell exocytosis. "We wanted to figure out how PACAP works at the cellular level," explains Arun Anantharam from the University of Toledo.

Since exocytosis is triggered by increases in intracellular Ca^{2+} levels, Anantharam and colleagues, led by graduate student Alina Morales, began by comparing the Ca^{2+} signals evoked by ACh and PACAP. The researchers found that, whereas ACh elicits a large and rapid Ca^{2+} transient in chromaffin cells, PACAP induces smaller Ca^{2+} flickers that fluctuate with a variable time course and appear to depend on Ca^{2+} influx through L-type channels.

Morales et al. (1) then compared the secretory responses elicited by ACh and PACAP. The application of ACh to chromaffin cells evoked a burst of vesicle fusion events that declined in frequency over time. In contrast, PACAP induced a persistent, steady fusion of secretory vesicles. Moreover, the two neurotransmitters had different effects on the rate of release of the vesicles' contents. While catecholamines are released from fusing vesicles at the same, rapid rate in response to both ACh and PACAP, the subsequent release of peptide hormones such as NPY is slower in PACAP-stimulated cells.

PACAP has previously been suggested to promote chromaffin cell exocytosis via its receptor, PAC1R, which, through coupling to $G\alpha_s$, elevates cAMP levels and activates the GTPase exchange factor Epac (3). Confirming these findings, Morales et al. (1) found that treating chromaffin cells with an Epac-specific agonist induced Ca^{2+} fluctuations similar to those evoked by PACAP, whereas an Epac inhibitor blocked the response to PACAP.

Epac is known to activate Rap GTPases, which, in turn, can promote exocytosis by stimulating phospholipase C ε (PLCε). In collaboration with Alan Smrcka's group at the University of Michigan, Morales et al. analyzed chromaffin cells from PLCε-knockout mice and found that PACAP-induced Ca^{2+} fluctuations and exocytosis were inhibited in the absence of PLCε. In marked contrast, the response to ACh was completely unaltered in PLCε-deficient cells, highlighting the fact that ACh and PACAP signal via two different pathways to elicit kinetically distinct

bshort@rockefeller.edu.

patterns of secretion from chromaffin cells.

Exactly how the PACAP-induced activation of PLCε results in sustained vesicle release remains unclear. Anantharam notes that, unlike ACh, PACAP elicits only a slight depolarization of the chromaffin cell membrane, and the resulting Ca^{2+} fluctuations have a much smaller amplitude than the Ca^{2+} transients induced by ACh. "We now want to investigate how PACAP stimulation is coupled to changes in the excitability of the cell and subsequent vesicle release," Anantharam says.

References

1. Morales, A., et al. 2023. *J. Gen. Physiol.* 155. https://doi.org/10.1085/jgp.202213180
2. Eiden, L.E., et al. 2018. *Pflugers Arch.* 470:79–88. https://doi.org/10.1007/s00424-017-2062-3
3. Kuri, B.A., et al. 2009. *J. Neurochem.* 110: 1214–1225. https://doi.org/10.1111/j.1471-4159.2009.06206.x