AuPS/ASB Meeting - Adelaide 2010

Symposium: Calcium signalling

Monday 29th November 2010 - Hickinbotham Hall - 17:15

Chair: Grigori Rychkov & Greg Barritt

Calcium regulation of apoptosis in pancreatic acinar cells

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We have studied calcium regulation of induction of apoptosis in pancreas. In pancreatic acinar cells, the earliest events were found to be cytosolic calcium elevations due to release of calcium from intracellular stores. As a result of that calcium levels also increased in mitochondria aiding mitochondrial depolarization and mPTP. High mitochondrial calcium at the time of oxidant stress was found to be the crucial factor in the cell fate. When mitochondrial calcium was low, then apoptosis did not occur regardless of other stores' content. We also studied Bcl-2 family members, well known regulators of apoptosis involved in regulation of intracellular calcium homeostasis. Most interesting was a potential link between Bcl-2 family proteins and a passive calcium release from the intracellular stores. We found that BH3 mimetics induce calcium release from the ER that leads to the formation of calcium plateau. Inhibition of either IP3Rs or RyRs reduced but did not abolish BH3-elicited calcium release. Further, we have shown that loss of Bcl-2 protein decreases calcium release from the ER and increases cytosolic calcium clearance in pancreatic acinar cells.

Gerasimenko J, Ferdek P, Fischer L, Gukovskaya AS, Pandol SJ. (2010) *Pflügers Archiv European Journal of Physiology* **460(5)**: 891-900

Gerasimenko O, Gerasimenko J. (2010) *Methods in Molecular Biology* **591**: 201-10.

Baumgartner HK, Gerasimenko JV, Thorne C, Ferdek P, Pozzan T, Tepikin AV, Petersen OH, Sutton R, Watson AJ, Gerasimenko OV. (2009) *Journal of Biological Chemistry* **284(31)**: 20796-803.

The application of complementary luminescent and fluorescent imaging techniques to visualize nuclear and cytoplasmic Ca^{2+} signaling during *in vivo* differentiation of slow muscle cells in zebrafish embryos

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Intact zebrafish embryos were used as an *in vivo* animal model to investigate the role of Ca^{2+} signaling during the differentiation of slow muscle cells (SMCs) within forming skeletal muscle. Transgenic zebrafish were generated using an α-actin promoter that targeted apoaequorin expression specifically to muscle cells. Two distinct Ca^{2+} signaling periods (CSPs) were visualized in the developing SMCs: between ~17.5-19.5 hours post-fertilization (hpf) and after ~23 hpf, separated by a ~3.5 hour Ca^{2+} signaling quiet period. Further spatial characterization of these Ca^{2+} signals using confocal fluorescent microscopy and calcium green-1 dextran as a reporter, indicated that the earlier CSP displayed distinct nuclear and cytoplasmic components, whereas the later CSP was predominantly cytoplasmic. Both CSPs consisted of a series of oscillating Ca^{2+} waves generated at distinct frequencies, while the earlier CSP also displayed a slow rise then fall in the Ca^{2+} baseline-level. Imaging of cyclopamine- and forskolin-treated wild-type, or *smo*$^{-/-}$ mutant embryos, where SMCs do not form, confirmed the specific cell population generating the signals. Treating embryos with antagonists indicated that both IP_3Rs and RyRs are responsible for generating the temporal characteristics of the Ca^{2+} signaling signature, and that the latter plays a necessary role in SMC differentiation and subsequent myotome patterning (Cheung, *et al.*, 2010). Together, these data support and extend the proposition that specific spatiotemporal patterns of spontaneous Ca^{2+} signals might be used for different as well as combinatorial regulation of both nuclear and cytosolic signal transduction cascades, resulting in myofibrillogenesis in SMCs as well as myotome patterning (Webb & Miller, 2010).

Cheung, C.Y., Webb, S.E., Love, D.R., and Miller A.L. (2010) *International Journal of Developmental Biology, In Press.* DOI 10.1387/ijdb.103160cc

Webb, S.E., and Miller, A.L. (2010) *In: Calcium Signaling*, CHS Press (Eds. Berridge, M.J., Putney, J., Roderick, L., Bootman, M.D). *In Press.*

STIM:Orai stoichiometry and the trapping and activation of store-operated calcium channels

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The activation of store-operated Ca^{2+} entry (SOCE) by depletion of Ca^{2+} stores results from the redistribution of the ER Ca^{2+} sensor STIM1 and the CRAC channel protein Orai1 to ER-plasma membrane (PM) junctions where they form closely apposed clusters. Recent studies support a two-part diffusion-trap model for this process, in which the C-terminal polybasic domain of STIM1 binds to phosphoinositides in the junctional plasma membrane and the STIM1 CRAC activation domain (CAD) binds to Orai1, effectively trapping and activating mobile CRAC channels. Store depletion-induced oligomerization of STIM1 has emerged as the essential trigger for this sequence of events, as shown by the ability of artificial STIM1 crosslinking to elicit clustering at junctions and activate CRAC channels in the absence of store depletion. STIM1 traps and activates CRAC channels through the binding of the CRAC activation domain (CAD, aa 342-448) to the N- and C-termini of Orai1.

Crosslinking of individual CRAC channels by the isolated CAD protein fragment suggests that each channel probably contains four STIM binding sites. To determine the minimum number of binding events required to trap a CRAC channel at the ER-PM junction, we measured the junctional ratio of STIM1 to Orai1 as the expression level of Orai1 was increased relative to that of STIM1 in HEK 293 cells. At high Orai1 expression, the STIM:Orai ratio reached a minimum value of ~0.3, or 1.2 STIM/tetrameric CRAC channel, suggesting that a single STIM1 is capable of arresting a CRAC channel at the junction. To determine how CRAC channel activity varies with the number of binding sites occupied by STIM1, we measured I_{CRAC} density in HEK cells expressing a constant amount of STIM1 and increasing levels of Orai1. We found that CRAC channel activation is a highly nonlinear bell-shaped function of Orai1 expression, and that the minimum stoichiometry sufficient for trapping the channels at junctions fails to evoke significant activation. A simple cooperative gating model fitted to the data suggests that only CRAC channels with 4 sites occupied contribute significant current. This highly nonlinear activation of CRAC channels supports earlier conclusions based on current noise analysis (Prakriya & Lewis, 2006) that the slow development of whole-cell CRAC current after store depletion reflects the stepwise recruitment of individual channels from a silent to a high open-probability state as they enter ER-PM junctional sites.

Prakriya M, Lewis RS. (2006) *Journal of General Physiology* **128:** 373-86.