PERSPECTIVES

Calcium controls cardiac function – by all means!

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Sidney Ringer discovered in the 1880s that calcium is essential for contraction of the heart, and we have been using calcium channel blockers for treatment of patients for decades. Still, the many roles of calcium in the cell continue to astonish us. It seems like a paradox that the cell needs to keep a very low cytosolic calcium concentration to survive, but still uses calcium as one of the most ubiquitous intracellular signalling molecules. The ratio of the calcium concentration in the external cell environment to that in the cytosol is close to 20.000 (twenty thousand) which requires the close control of calcium access to the cell and an efficient means of pumping calcium out. If this control is lost, cells cannot survive and necrosis or apoptosis will ensue. In the heart, loss of control of calcium can occur during reperfusion after ischaemia and a dreaded complication is the so-called stone heart. Even so, the heart uses calcium in a positive feedback loop to trigger contraction. For each beat a small amount of calcium enters the cell through L-type calcium channels (LTCCs). This calcium binds to intracellular calcium release channels (ryanodine receptors, RyR2) that are located in the sarcoplasmic reticulum (SR). They will open and release the calcium that is contained in the SR, giving rise to the calcium transient that causes contraction when calcium is bound to troponin C. This is calcium-induced calcium release. Since the RyRs are calcium sensitive this is a self-amplifying process that only ceases because the calcium release ceases due to local depletion inside the SR. RyRs then close and the released calcium is pumped back into the SR by calcium pumps (SERCA). The small amount of calcium that entered through LTCCs is taken out of the cell primarily by the Na^+/Ca^{2+} exchanger (NCX). This neat orchestration of a potentially deadly agent is repeated for each beat and the cellular content of calcium is under tight control. However, calcium is also used for a plethora of other purposes, most of all signalling (Clapham, 2007). There are hundreds of proteins that bind calcium. One of the more important is calmodulin (CaM). When it binds calcium, the shape of the protein changes revealing hydrophobic residues that can bind to other proteins. Suffice here to mention two important targets for the Ca^{2+}–CaM complex: the calmodulin kinase (CaMK) family and the pore-forming α_{1C}-subunit of the LTCC ($Ca_v1.2$).

The predominant CaMK isoform in the heart is CaMKIIδ with splice variants δ_B and δ_C localized to the nucleus and the cytosol, respectively (Maier, 2009). CaMKIIδ can phosphorylate and alter properties of several proteins engaged in intracellular calcium cycling. The α_{1C}-subunit of the LTCC is phosphorylated at two sites, the RyR2 at several sites, but only Ser-2814 seems to be important for calcium release. Finally, SERCA activity is controlled by an inhibitory protein, phospholamban, which can also be phosphorylated by CaMKIIδ.

An essential point of control is the LTCC, which is regulated in several ways (Benitah *et al.* 2010). The channel is voltage gated, which means that it opens when the cell is depolarized during the action potential. Inactivation is both voltage and Ca^{2+} dependent. A Ca^{2+}–CaM complex forms locally when Ca^{2+} is released from the SR and speeds up inactivation by binding to the α_{1C}-subunit. Thus, when Ca^{2+} release from the SR is small, the channels stay open for a longer time, filling the SR with calcium again. The Ca^{2+}–CaM complex will also activate local CaMKIIδ that phosphorylates the channel causing the phenomenon of calcium-dependent facilitation. When heart rate is increased, facilitation will cause both a larger current amplitude and slower inactivation of the channel so that calcium transients gradually increase. By this mechanism facilitation is part of the positive force–frequency relationship seen in many species. The LTCC is also substrate for several other kinases (Benitah *et al.* 2010).

In a recent issue of *The Journal of Physiology*, Ronkainen *et al.* (2011) show that direct phosphorylation of the α_{1C}-subunit is not the only way in which CaMKIIδ controls the LTCC. Overexpression of either nuclear (δ_B) or cytosolic (δ_C) CaMKIIδ decreased the expression of the α_{1C}-subunit gene (*Cacna1c*) and the channel protein thereby opposing the facilitation effect of the kinase. In a series of elegant experiments, they show that the transcriptional repressor DREAM (binding to the downstream regulatory element, DRE) was translocated to the nucleus following calcium activation of CaMKIIδ and that elevated cytosolic calcium played an independent role since overexpression of DREAM alone was not sufficient. By a modelling approach they also show that the Ca^{2+}–CaMKIIδ–DREAM–α_{1C}-subunit cascade constitutes a negative feedback loop that prevents long-term calcium overload of the myocytes.

Also, we know that calcium and CaMKIIδ are involved in control of cardiac hypertrophy as seen in congestive heart failure. Both CaM and CaMKIIδ_B overexpression results in cardiac hypertrophy, probably through export from the nucleus of histone deacetylases thereby relieving gene repression (Maier, 2009). Furthermore, by its control of RyR2 activity, CaMKIIδ seems to play an important role in arrhythmogenesis, since increased calcium leak from the SR by RyR2 activation will promote spontaneous calcium waves.

Interestingly, other important calcium-handling proteins (NCX, RyR2, SERCA or other subunits of the LTCC) were not affected by DREAM activation (Ronkainen *et al.* 2011). Clearly, we need to know more about the control of expression of these proteins since they are also essential for the calcium homeostasis of the myocyte. When SR function is severely depressed in mice following conditional knock out of the SERCA2 gene, the sarcolemmal proteins NCX and LTCC are significantly upregulated and can sustain calcium transients that are of sufficient magnitude to maintain cardiac function for several weeks (Andersson *et al.* 2009). Hence, the many roles of calcium in the cardiac myocyte are still not clear and we need to know how they are orchestrated.

DOI: 10.1113/jphysiol.2011.210989

References

Andersson KB, Birkeland JA, Finsen AV, Louch WE, Sjaastad I, Wang Y, Chen J, Molkentin JD, Chien KR, Sejersted OM & Christensen G (2009). *J Mol Cell Cardiol* **47**, 180–187.

Benitah JP, Alvarez JL & Gómez AM (2010). *J Mol Cell Cardiol* **48**, 26–36.

Clapham DE (2007). *Cell* **131**, 1047–1058.

Maier LS (2009). *Front Biosci* **14**, 486–496.

Ronkainen JJ, Hänninen SL, Korhonen T, Koivumäki JT, Skoumal R, Rautio S, Ronkainen V-P & Tavi P (2011). *J Physiol* **589**, 2669–2986.