Calcium Channels Are Ganging Up in the Sarcolemma

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In this issue of Circulation Research, Navedo et al. provide provocative and exciting data, indicating that up to \(\sim 6\) L-type Ca\(^{2+}\) channels (LTCCs) (in this case Ca\(_{\text{L},1.2}\)) can physically interact with each other to gate in a coupled manner, producing large and long-lasting Ca\(^{2+}\) influx into smooth and cardiac muscle myocytes. This coupling appears to be relatively rare and transient, with a minority of overall LTCCs involved at any moment. However, coupling is favored by either replacement of calmodulin (CaM) from its binding location in the C-terminal LTCC domain, activation of protein kinase (PK)\(\gamma\), chronic hypertension (in vascular smooth muscle), and LTCC mutants that are associated with cardiac arrhythmias and hearing loss in humans (ie, Timothy syndrome, or long QT syndrome 8).

The fundamental observations here were made using total internal reflection fluorescence microscopy by measuring local submembrane Ca\(^{2+}\) events termed Ca\(^{2+}\) sparklets. These are attributable to Ca\(^{2+}\) entry via LTCC, unlike the sarcoplasmic reticulum (SR)/endoplasmic reticulum Ca\(^{2+}\) release events known as Ca\(^{2+}\) sparks or puffs (mediated by ryanodine or inositol 1,4,5 trisphosphate [InsP\(_3\)] receptor channels). Physiologically Ca\(^{2+}\) sparklets would be extremely small and brief events, because normal LTCC openings are \(< 1\) ms in duration, and current amplitude is only \(\sim 0.2\) pA (at physiological [Ca\(^{2+}\)]\(_o\), resulting in an integrated Ca\(^{2+}\) influx of only 0.1 femtocoulomb (or 300 Ca\(^{2+}\) ions). This would produce a very small hard to detect fluorescence signal. However, the Ca\(^{2+}\) sparklets here are much larger, which enhances detectability. They are larger because they are attributable to more rare persistent openings (like mode 2 openings favored by the LTCC agonist Bay K 8644),\(^6\) elevation of [Ca\(^{2+}\)]\(_i\), and measurements at negative voltages (where driving force is high). This group has previously shown using patch clamp that these Ca\(^{2+}\) sparklets are indeed caused by LTCC current (not SR Ca\(^{2+}\) release), can last hundreds of milliseconds, and are favored by PKC activation.\(^7\) Total internal reflection fluorescence imaging has the advantage (for these events) that one can sample the entire surface of the cell simultaneously, rather than just a small patch of membrane in cell-attached patch-clamp recording.

Here, the authors show that local Ca\(^{2+}\) sparklets and patch-clamp currents show quantal increments of 2 to 6 times the normal \(\Delta[\text{Ca}^{2+}]/\delta t\) and single Ca\(^{2+}\) current amplitude and that the apparent gating transitions of each channel occur essentially simultaneously (ie, coupled gating).\(^8\) This raises 3 obvious questions: (1) are these coupled gating events real; (2) why might they be of functional importance or relevance; and (3) how are they regulated?

**Coupled Gating of LTCC Seems Real**

I find the cumulative evidence of the authors compelling,\(^1\) especially because of the patch-clamp data, which has a similar phenotype as the Ca\(^{2+}\) sparklets and where channel opening and closing events are more discretely identifiable. It is also hard to imagine an artifact or technical limitation that would produce the striking coupled gating traces in Figure 2 of the article. The only thing missing is perhaps the absolute proof that these large currents (6 times the single LTCC current) are indeed attributable to LTCC and not to some other higher conductance myocyte channel that contaminates the record. However, because these events are rare, it may be harder to demonstrate clear nifedipine sensitivity (as they have done for normal Ca\(^{2+}\) channel openings). On this point (and in light of the other data), I am inclined to give them the benefit of the doubt. The conclusion that the large events are indeed coupled seems inescapable, because the relatively rare single channel events would virtually never superimpose, and as the authors point out, coupled events far outweigh that coincidental possibility. Moreover, Ca\(^{2+}\) entry through one LTCC would be expected to inhibit (rather than activate) a nearby LTCC (via Ca\(^{2+}\)-dependent inactivation),\(^7\) making this sort of chemical coupling very unlikely. Note that this is in striking contrast to coupled gating reported for SR Ca\(^{2+}\) release channels, ryanodine receptor (RyR),\(^8,9,9\) where controversy still remains, in part because it is known that Ca\(^{2+}\) release from one RyR will tend to activate its neighbors extremely rapidly, via Ca\(^{2+}\)-induced Ca\(^{2+}\) release.\(^10\) Note that LTCCs in smooth and cardiac muscle do not exhibit the paracrystalline array and neighbor abutment seen for RyR in striated muscle or Ca\(_{\text{L},1.1}\) channels in skeletal muscle.\(^11\) Finally, the fluorescence resonance energy transfer studies here suggest (but do not prove) direct physical interaction between LTCC monomers. They also led the authors to an intriguing initial working hypothesis that LTCCs interact via a C-tail-to-C-tail interaction. It will be interesting to see whether this model holds up on further study. The LTCC C terminal is a very busy regulatory zone, and this is an exciting new twist.

**Why Might Coupled LTCC Gating Be of Functional Importance or Relevance?**

The effect of coupled gating would be to increase the synchronous local Ca\(^{2+}\) influx. Although not addressed yet, this physical coupling could also increase the duration of opening, because one coupled channel may not as readily close if the others are still open. This might be an interesting angle for some follow-up studies. In any event, a larger local
Ca^{2+} influx might be advantageous in assuring local signaling fidelity. For example, where LTCC influx triggers SR Ca^{2+} release via RyR, a larger and longer Ca^{2+} influx would ensure SR Ca^{2+} release from each site. On the other hand, it has been argued that even a single LTCC opening, which would raise cleft [Ca^{2+}] to ~10 μmol/L (in tens of microseconds), may suffice to ensure the fidelity of E-C coupling in the heart, especially where multiple LTCC at the site each have a chance to independently activate the local RyR.5,12–14 Furthermore, with 20,000 SR junctions needing to fire at every heartbeat, the energetic cost of extruding extra Ca^{2+} that enters might favor keeping the local LTCC entry modestly above that required for high fidelity. My suspicion is that this coupled LTCC gating with locally synchronized Ca^{2+} entry may be most important for specific loci involved in the activation of local Ca^{2+} targets that require either very high or very sustained local [Ca^{2+}], to assure signaling fidelity. Identifying these specific Ca^{2+} targets and roles that require this sort of focal high [Ca^{2+}] signal would be a valuable extension of this work.

Moreover, if this apparently rare situation is to be physiologically important (as opposed to a biophysical curiosity), it must be influential to at least one Ca^{2+} signaling mechanism. This does not mean that a majority of the LTCCs of the cell would have to be recruitable to these clusters. Indeed, it may turn out that this clustering and coupling is driven by regulation discussed below to a locus where a small minority of LTCCs in the cell join for high fidelity discrete local Ca^{2+} signaling (independent of whole cell Ca^{2+} signals that activated muscle contraction).

How Is Coupled Gating of LTCC Regulated?

On this point, the authors already have several very intriguing leads, and they have tried to tie this in with the structure of the LTCC as well. CaM (which mediates Ca^{2+}-dependent inactivation) is thought to be normally bound to the LTCC,15 and that limits coupled gating.1 However, if the LTCC loses CaM, coupled gating is dramatically augmented. So, there may be some finite fraction of LTCC that lack CaM, and this may be regulated somehow, especially because there is intense competition among cellular sites for a limited pool of CaM (even in the unbound state).16 The authors of the present article also have data to suggest that the mechanism by which PKCα activates coupled gating is that it dislodges CaM from the LTCC (although that cannot be an obligate mechanism, because the CaM inhibitor W7 still enhances coupled gating when PKCα is knocked out).1 The anchoring protein AKAP150 also seems to enhance coupled gating, perhaps by bringing key signaling molecules to a subset of LTCCs at specific foci.

Finally, Navedo et al1 show that coupled gating seems to be more prominent in 2 pathophysiological models. Vascular smooth muscle cells from angiotensin II-induced hypertensive rats (which may tie into the PKCα effect above) show enhanced coupled gating, which could conceivably contribute to the clinical phenotype. The Timothy syndrome mutant LTCC also exhibited more coupled gating, weaker CaM association with the channel, and more C-tail-to-C-tail fluorescence resonance energy transfer than wild-type channels. It remains to be seen whether the coupled gating and homo-multimerization of LTCC α subunits really contributes to these clinical phenotypes, but the direction of the effects is right. These new results are provocative, and further studies will be required to further validate and understand the potential physiological and pathophysiological importance of this phenomenon. However, Navedo et al have gotten this idea off to an exciting start here.

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References

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