Perspectives on: Local calcium signaling

Natural inequalities: why some L-type Ca^{2+} channels work harder than others

Luis F. Santana and Manuel F. Navedo

Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195

The Journal of General Physiology

Introduction

Voltage-gated L-type Ca^{2+} channels are expressed in the surface membrane of neurons and muscle cells (Catterall, 2000). There, they regulate multiple processes including excitability, contraction, gene expression, and memory storage. L-type Ca^{2+} channels are heteromeric complexes comprising a pore-forming α1 subunit and accessory β, $\alpha_2\delta$, and γ subunits. The specific set of pore-forming and accessory subunits that form functional L-type Ca^{2+} channels varies between cell types. Of the four α1 subunits identified to date (Cav1.1–4), Cav1.2 is the principal pore-forming α1 subunit isoform expressed in neurons and atrial, ventricular, and smooth muscle. However, the specific set of accessory subunits ($\alpha_2\delta$, β, and γ) that associate with Cav1.2 varies in neurons and cardiac and smooth muscle.

The biophysical properties of L-type Cav1.2 channels have been extensively examined using electrophysiological approaches (for review see Catterall, 2000). These studies have revealed that Cav1.2 channels are activated by membrane depolarization to potentials >−45 mV, undergo voltage- and Ca^{2+}-dependent inactivation, and are sensitive to dihydropyridines. Despite these advances, examination of the spatial organization of functional L-type Ca^{2+} channels in neurons and muscle has been elusive. The reason for that is that conventional patch clamp techniques have limitations in providing information about the spatial organization of functional channels. For example, in the whole cell configuration, the activity of specific ionic channels is averaged over the entire surface membrane, providing limited information on possible regional variations in channel function. Recording single-channel activity solves the spatial-averaging issue, but only partially because the experimenter can sample only a small portion of the cell surface membrane in each trial. In a typical smooth muscle cell with a surface area of ≈1,000 μm^2, a 1–3-μm^2 cell-attached patch would record currents from only 0.1–0.3% of the entire sarcolemma per trial.

Recent advances in imaging technology have helped circumvent these limitations by allowing investigators to image relatively small Ca^{2+} signals resulting from Ca^{2+} influx via Ca^{2+}-permeable channels with high speed (>100 Hz) and spatial resolution over relatively large areas (>100 μm^2) of the cell membrane (Zou et al., 2002; Demuro and Parker, 2005; Navedo et al., 2005). Implementation of these "optical clamping" approaches has revealed an intriguing feature of voltage-gated Ca^{2+} channels: their activity is heterogeneous along the surface membrane of ventricular myocytes, smooth muscle as well as HELA and tsA-201 cells expressing Cav1.2 (Navedo et al., 2005, 2010a; Tour et al., 2007; McCarron et al., 2009). Here, we discuss these studies and their implications and propose a model for local control of Cav1.2 channel activity in neurons and cardiac and smooth muscle.

A model for subcellular variations in L-type Ca^{2+} channel activity

Three mechanistic models could explain subcellular variations in Cav1.2 channel function. In model 1, heterogeneous Ca^{2+} channel activity results from subcellular variations in the molecular composition of L-type Ca^{2+} channels within a cell. In model 2, the molecular composition of all L-type Ca^{2+} channels is identical, but their expression is restricted to a relatively small number of regions within the surface membrane. In model 3, L-type Ca^{2+} channels are broadly distributed throughout the cell. However, subcellular variations in Ca^{2+} sparklet activity results from regional variations in the activity of specific signaling events (e.g., relative activities of kinases and opposing phosphatases) that culminate in local variations in the open probability of Cav1.2 channels.

Let us evaluate model 1 first. Many studies using patch clamp electrophysiology suggest that the average amplitude, kinetics, voltage dependence, and pharmacological

Correspondence to Luis F. Santana: santana@uw.edu

Abbreviations used in this paper: AKAP, protein A kinase–anchoring protein; EC, excitation–contraction; ET, excitation–transcription.

The Rockefeller University Press $30.00
J. Gen. Physiol. Vol. 136 No. 2 143–147
www.jgp.org/cgi/doi/10.1085/jgp.200910391

properties of L-type Ca^{2+} channel currents are similar within a specific cell type (Catterall, 2000). Thus, subcellular variations in the molecular composition of L-type Ca^{2+} channels are unlikely culprits for heterogeneous Ca^{2+} influx activity within a cell. Model 1 should therefore be rejected.

To test the validity of model 2, we reviewed several studies using immunofluorescence approaches to determine the spatial distribution of L-type Ca^{2+} channels in neurons and cardiac and smooth muscle (Pratt et al., 2002; Moore et al., 2004; Navedo et al., 2005; Di Biase et al., 2008; Asghari et al., 2009). These studies suggested that Cav1.2 channels formed small clusters that were broadly distributed throughout the surface membrane of hippocampal neurons and cardiac and smooth muscle. This is consistent with model 3, but not with model 2 above. If there are L-type Ca^{2+} channels all over neurons and cardiac and smooth muscle cells and each channel within each cluster has a similar molecular composition, what causes some of them to open more frequently than others?

Recent work by our group provides insight into this conundrum. We found that PKA and PKCα activity contributed to heterogeneous L-type Ca^{2+} channel activity in cardiac and smooth muscle (Navedo et al., 2006, 2010a,b). The actions of these kinases on L-type Ca^{2+} channels are opposed by the Ca^{2+}-sensitive phosphatase calcineurin (also known as protein phosphatase 2B). However, what is the mechanism by which PKA, PKCα, and calcineurin produce regional variations in Ca^{2+} influx via L-type Ca^{2+} channels?

Work on protein A kinase–anchoring proteins (AKAPs) provides a potential answer to this question. One AKAP deserves particular attention: AKAP79 (the human orthologue of rodent AKAP150) because it binds to PKA, PKCα, calcineurin, and Cav1.2 channels and is expressed in neurons and muscle (Coghlan et al., 1995; Oliveria et al., 2007; Navedo et al., 2008). Five important observations suggest that local targeting of PKA and PKCα by AKAP79/150 to specific regions of the surface membrane allows these kinases and phosphates to regulate the phosphorylation state of nearby Cav1.2 channels, thereby modulating their open probability.

First, AKAP150 and PKCα colocalize to specific foci at or near the sarcolemma of arterial myocytes. Second, loss of AKAP150 prevents PKCα targeting to the sarcolemma of arterial myocytes. It also abolishes high local Cav1.2 channel activity (Navedo et al., 2008). Third, AKAP is required for PKA-dependent modulation of L-type Ca^{2+} channels in smooth muscle (Zhong et al., 1999). Fourth, calcineurin plays a critical role in the regulation of Cav1.2 channels by opposing the actions of PKCα and presumably PKA (Navedo et al., 2006). Fifth, only a subpopulation of L-type Ca^{2+} channels interacts with AKAP150 and the proteins associated with it in smooth muscle (Navedo et al., 2008). Collectively, these observations are consistent with model 3 above in which subcellular Cav1.2 channels are broadly distributed throughout the cell, but the activity of a subset of these channels is regulated by specific signaling events resulting in local variations in their activity.

Mechanisms for AKAP79/150-dependent regional variations in Cav1.2 channel activity

The mechanism by which AKAP79/150 induces high local Cav1.2 channel activity likely involves its conventional role in targeting PKA, PKCα, and calcineurin to specific regions of the surface membrane of neurons and myocytes. In response to an increase in cAMP, the activation of AKAP79/150-targeted PKA near a subset of L-type Ca^{2+} channels would increase channel activity and hence Ca^{2+} influx. Likewise, an increase in diacylglycerol or $[Ca^{2+}]_i$ could activate PKCα.

A recent study suggests a mechanism for local, Ca^{2+}-dependent activation of AKAP79/150-associated PKCα (Faux and Scott, 1997). In this mechanism, Ca^{2+} entering the cell binds to calmodulin. The resulting Ca^{2+}–calmodulin complex binds to AKAP79/150, releasing PKCα from the AKAP150 complex and allowing this kinase to phosphorylate nearby L-type Ca^{2+} channels. An interesting aspect of this model is that because PKCα is tethered to specific sites of the cell membrane via its interaction with AKAP79/150, a global increase in $[Ca^{2+}]_i$ is not required to induce the translocation of this kinase to the membrane and thus phosphorylation of Cav1.2 channels.

As noted above, high activity Ca^{2+} entry sites result from frequent openings of single L-type Ca^{2+} channels after PKA or PKCα activation. Many lines of evidence suggest that large amplitude, local $[Ca^{2+}]_i$ elevations could arise from random overlapping openings of adjacent L-type Ca^{2+} channels with high open probabilities. First, L-type Ca^{2+} channel function can be simulated using Markov models, which is consistent with the hypothesis that the gating of these channels is largely independent (Tanskanen et al., 2005). Second, the amplitude histogram of "Ca^{2+} sparklets"—a local $[Ca^{2+}]_i$ signal produced by Ca^{2+} influx via Cav1.2 channels (Wang et al., 2001)—has multiple peaks of decreasing amplitude. This suggests that "multiquantal Ca^{2+} elevations are likely produced by random overlapping openings of adjacent L-type Ca^{2+} channels with high open probabilities" (Navedo et al., 2005), as the probability of multiple independently gating channels opening simultaneously decreases when the number of channels involved increases.

However, it seems that not all Cav1.2 channels gate independently. A recent study suggested that small clusters of Cav1.2 channels could undergo coordinated openings and closings (i.e., "coupled gating") (Navedo et al., 2010a). Activation of PKCα increases the probability of coupled gating between Cav1.2 channels. AKAP150 is required for coupled gating between Cav1.2 channels; in its absence, Cav1.2 channel gating is exclusively stochastic even after the activation of PKCα.

In those regions of the cells where Cav1.2 channels and AKAP150 coexist, Cav1.2 channels could switch between independent and coupled gating modes. Furthermore, the number of channels opening and closing simultaneously varies with time and between sites within a cell (Navedo et al., 2010a). This coupling mechanism is fundamentally different from that of RYRs, in which tetramers of tightly coupled channels undergo stable openings under physiological conditions (Marx et al., 1998). Although the mechanisms underlying coupled gating between Cav1.2 are unclear, FRET analysis suggests the possibility that coupled gating between Cav1.2 channels may involve a rearrangement of calmodulin in the C terminal of these channels and transient interactions between a variable number of adjacent Cav1.2 channels via their C termini. Two recent reports suggesting dimerization of entire channels as well as a fragment of the C terminal of Cav1.2 channels in vitro give credence to this hypothesis (Wang et al., 2004; Fallon et al., 2009).

Coupled gating does not seem to be a unique feature of Cav1.2 channels. L-type Ca^{2+} channels (presumably Cav1.1) from skeletal muscle fibers have also been shown to undergo coupled gating in planar lipid bilayers (Hymel et al., 1988). Like Cav1.2 channels, coupled gating between skeletal muscle L-type Ca^{2+} channels was transient, involved a variable number of channels, and was promoted by activation of a protein kinase, PKA (Hymel et al., 1988).

AKAP79/150-associated L-type Ca^{2+} channels in excitation–contraction (EC) coupling and excitation–transcription (ET) coupling

In Fig. 1, we show a cartoon describing the potential role of AKAP150-associated L-type Ca^{2+} channels in EC and ET coupling. EC coupling is the process by which an action potential triggers an increase in $[Ca^{2+}]_i$ that activates contraction in muscle. During ET coupling, changes in the resting membrane potential or the frequency or waveform of the action potential in neurons and muscle are coupled to changes in gene expression.

First, we will focus on EC coupling in ventricular myocytes (Fig. 1). In these cells, sarcolemmal Cav1.2 channels and RYRs in nearby junctional sarcoplasmic reticulum form a functional unit called a "couplon" (Franzini-Armstrong et al., 1999). During an action potential, Ca^{2+} influx via Cav1.2 activates small clusters of RYRs via the mechanism of Ca^{2+}-induced Ca^{2+} release. The $[Ca^{2+}]_i$ signal resulting from the concerted opening of RYRs is called a "Ca^{2+} spark" (Cheng et al., 1993). Activation of multiple couplons during an action potential results in a cell-wide increase in $[Ca^{2+}]_i$ that activates contraction in ventricular myocytes. The probability of Ca^{2+}

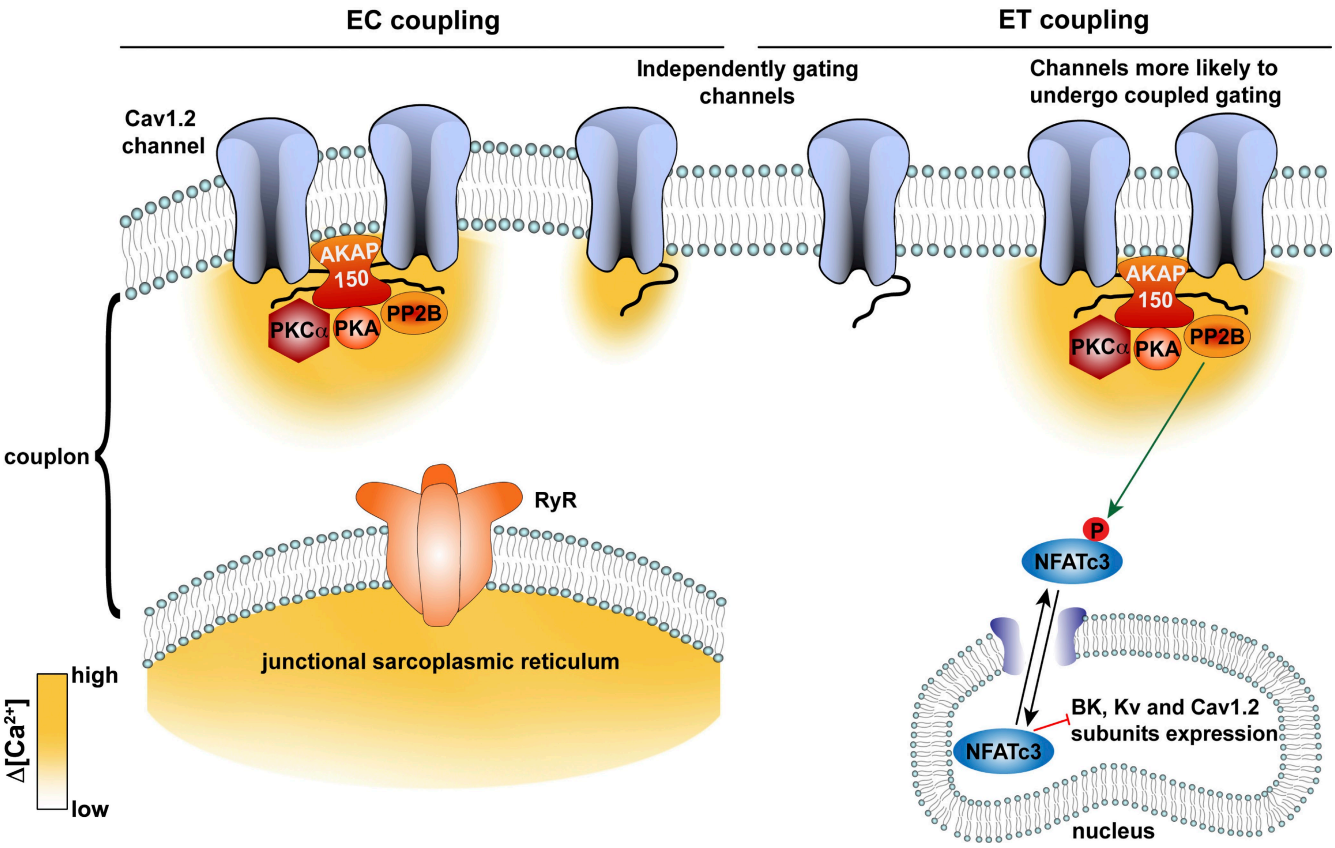

Figure 1. Cartoon of the proposed mechanisms by which AKAP150-associated Cav1.2 channels modulate EC coupling and ET coupling. See text for details.

spark occurrence (P_{Spark}) in a couplon is proportional to the open probability (P_o) of Cav1.2 channels and the square of the single Ca^{2+} channel current and square of the local $[Ca^{2+}]_i$ (Santana et al., 1996).

A recent study suggested that P_{Spark} approaches unity in specific sites within a cell at the relatively positive membrane potentials (≈+50 mV) attained during the plateau of the ventricular action potential (Inoue and Bridge, 2003). This is surprising because, at +50 mV, P_o is significantly <1 (≈0.7), and the current through a single L-type Ca^{2+} channel is very small (i.e., ≈0.2 pA with 110 mM Ba^{2+} as the charge carrier). Accordingly, Inoue and Bridge (2003) suggested that a cluster of at least two Cav1.2 channels must open simultaneously to be involved in the triggering of a couplon during the plateau of the action potential. Cav1.2 channels that are not associated with AKAP79/150 are expected to gate independently from each other at all times. Thus, we propose that in these couplons the high P_{Spark} is solely due to random overlapping openings of at least two contiguous Cav1.2 channels at positive membrane potentials. However, couplons with AKAP79/150-associated Cav1.2 channels could attain a P_{Spark} of ≈1 via two complementary mechanisms: independent as well as coupled openings of several Cav1.2 channels. Indeed, it is intriguing to speculate that P_{Spark} is higher in couplons with coupled Cav1.2 channels than in couplons with independently gating channels, as coordinated openings of several Cav1.2 channels are more likely to produce larger amplitude currents than a similar number of channels with equal P_o gating independently. Future studies should investigate these hypotheses.

In smooth muscle, Cav1.2 channels and RYRs are not functionally coupled (Essin et al., 2007). In these cells, activation of Cav1.2 channels during EC coupling results in an increase in $[Ca^{2+}]_i$ that directly activates contractile proteins. Thus, an increase in the activity of Cav1.2 channels due to an increase in coupled gating and/or independent openings would likely result in a further increase in Ca^{2+} influx and thus global $[Ca^{2+}]_i$ that activates contraction.

Two recent studies suggest that the signaling pentad composed of AKAP79/150, PKCα, PKA, calcineurin, and Cav1.2 channels is involved in ET coupling in neurons and muscle (Oliveria et al., 2007; Nieves-Cintrón et al., 2008). These studies suggest the following model for the local control of gene expression by the transcription factor NFAT. The opening of Cav1.2 channels associated with AKAP79/150 increases local $[Ca^{2+}]_i$, which activates nearby calcineurin. Upon activation, calcineurin dephosphorylates NFAT, allowing this transcription factor to translocate into the nucleus of neurons and myocytes where it modulates gene expression. In smooth muscle, NFAT modulates the expression of several genes, including Kv2.1 as well as the α and β1 subunit of large-conductance Ca^{2+}-activated K^+ channels (Amberg et al., 2004; Nieves-Cintrón et al., 2007; Layne et al., 2008). In cardiac myocytes, NFAT modulates the expression of Kv4.2, Kv4.3, and Cav1.2 channels (Gong et al., 2006; Qi et al., 2008; Xiao et al., 2008; Rossow et al., 2009) (Fig. 1). An interesting implication of this model is that AKAP79/150-associated Cav1.2 channels can be viewed as NFAT transcriptional control units. In this model, the level of NFAT activity is graded, at least in part, by the number of units activated.

Conclusions

To conclude, we propose a general model for inequalities in L-type Ca^{2+} channel activity within a cell. In this new model, the molecular composition of L-type Ca^{2+} channels is similar within each cell, endowing each channel a similar capacity for Ca^{2+} influx. In this context, regional variations in L-type Ca^{2+} channel activity are not the result of variations in their molecular composition or massive clustering. Instead, they are due to the association of a subpopulation of these channels with AKAP79/150 (or any other scaffolding protein) and associated proteins in specific regions along the surface membrane of neurons and cardiac and arterial myocytes. Together, L-type Ca^{2+} channels, calcineurin, PKA, PKCα, and AKAP150 form a signaling module that regulates local Ca^{2+} influx and NFAT-dependent gene expression in excitable cells.

This Perspectives series includes articles by Gordon, Parker and Smith, Xie et al., Prosser et al., and Hill-Eubanks et al.

We would like to thank Drs. Carmen Ufret-Vincenty and Bertil Hille for critically reading this manuscript.

This work was supported by National Institutes of Health grants HL085686, HL085870 (to L.F. Santana), and HL098200 (to M.F. Navedo), and American Heart Association (AHA) grant 0735251N (to M.F. Navedo). L.F. Santana is an Established Investigator of the AHA.

REFERENCES

Amberg, G.C., C.F. Rossow, M.F. Navedo, and L.F. Santana. 2004. NFATc3 regulates Kv2.1 expression in arterial smooth muscle. *J. Biol. Chem.* 279:47326–47334. doi:10.1074/jbc.M408789200

Asghari, P., M. Schulson, D.R. Scriven, G. Martens, and E.D. Moore. 2009. Axial tubules of rat ventricular myocytes form multiple junctions with the sarcoplasmic reticulum. *Biophys. J.* 96:4651–4660. doi:10.1016/j.bpj.2009.02.058

Catterall, W.A. 2000. Structure and regulation of voltage-gated Ca^{2+} channels. *Annu. Rev. Cell Dev. Biol.* 16:521–555. doi:10.1146/annurev.cellbio.16.1.521

Cheng, H., W.J. Lederer, and M.B. Cannell. 1993. Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. *Science.* 262:740–744. doi:10.1126/science.8235594

Coghlan, V.M., B.A. Perrino, M. Howard, L.K. Langeberg, J.B. Hicks, W.M. Gallatin, and J.D. Scott. 1995. Association of protein kinase A and protein phosphatase 2B with a common anchoring protein. *Science.* 267:108–111. doi:10.1126/science.7528941

Demuro, A., and I. Parker. 2005. "Optical patch-clamping": single-channel recording by imaging Ca^{2+} flux through individual muscle acetylcholine receptor channels. *J. Gen. Physiol.* 126:179–192. doi:10.1085/jgp.200509331

Di Biase, V., G.J. Obermair, Z. Szabo, C. Altier, J. Sanguesa, E. Bourinet, and B.E. Flucher. 2008. Stable membrane expression of postsynaptic CaV1.2 calcium channel clusters is independent of interactions with AKAP79/150 and PDZ proteins. *J. Neurosci.* 28:13845–13855. doi:10.1523/JNEUROSCI.3213-08.2008

Essin, K., A. Welling, F. Hofmann, F.C. Luft, M. Gollasch, and S. Moosmang. 2007. Indirect coupling between Cav1.2 channels and ryanodine receptors to generate Ca^{2+} sparks in murine arterial smooth muscle cells. *J. Physiol.* 584:205–219. doi:10.1113/jphysiol.2007.138982

Fallon, J.L., M.R. Baker, L. Xiong, R.E. Loy, G. Yang, R.T. Dirksen, S.L. Hamilton, and F.A. Quiocho. 2009. Crystal structure of dimeric cardiac L-type calcium channel regulatory domains bridged by Ca^{2+}* calmodulins. *Proc. Natl. Acad. Sci. USA.* 106:5135–5140. doi:10.1073/pnas.0807487106

Faux, M.C., and J.D. Scott. 1997. Regulation of the AKAP79-protein kinase C interaction by Ca^{2+}/calmodulin. *J. Biol. Chem.* 272:17038–17044. doi:10.1074/jbc.272.27.17038

Franzini-Armstrong, C., F. Protasi, and V. Ramesh. 1999. Shape, size, and distribution of $Ca^{(2+)}$ release units and couplons in skeletal and cardiac muscles. *Biophys. J.* 77:1528–1539. doi:10.1016/S0006-3495(99)77000-1

Gong, N., I. Bodi, C. Zobel, A. Schwartz, J.D. Molkentin, and P.H. Backx. 2006. Calcineurin increases cardiac transient outward K+ currents via transcriptional up-regulation of Kv4.2 channel subunits. *J. Biol. Chem.* 281:38498–38506. doi:10.1074/jbc.M607774200

Hymel, L., J. Striessnig, H. Glossmann, and H. Schindler. 1988. Purified skeletal muscle 1,4-dihydropyridine receptor forms phosphorylation-dependent oligomeric calcium channels in planar bilayers. *Proc. Natl. Acad. Sci. USA.* 85:4290–4294. doi:10.1073/pnas.85.12.4290

Inoue, M., and J.H. Bridge. 2003. Ca2+ sparks in rabbit ventricular myocytes evoked by action potentials: involvement of clusters of L-type Ca2+ channels. *Circ. Res.* 92:532–538. doi:10.1161/01.RES.0000064175.70693.EC

Layne, J., M.E. Werner, D. Hill-Eubanks, and M.T. Nelson. 2008. NFATc3 regulates BK channel function in murine urinary bladder smooth muscle. *Am. J. Physiol. Cell Physiol.* 295:C611–C623.

Marx, S.O., K. Ondrias, and A.R. Marks. 1998. Coupled gating between individual skeletal muscle Ca^{2+} release channels (ryanodine receptors). *Science.* 281:818–821. doi:10.1126/science.281.5378.818

McCarron, J.G., M.L. Olson, S. Currie, A.J. Wright, K.I. Anderson, and J.M. Girkin. 2009. Elevations of intracellular calcium reflect normal voltage-dependent behavior, and not constitutive activity, of voltage-dependent calcium channels in gastrointestinal and vascular smooth muscle. *J. Gen. Physiol.* 133:439–457. doi:10.1085/jgp.200810189

Moore, E.D., T. Voigt, Y.M. Kobayashi, G. Isenberg, F.S. Fay, M.F. Gallitelli, and C. Franzini-Armstrong. 2004. Organization of Ca^{2+} release units in excitable smooth muscle of the guinea-pig urinary bladder. *Biophys. J.* 87:1836–1847. doi:10.1529/biophysj.104.044123

Navedo, M.F., G.C. Amberg, V.S. Votaw, and L.F. Santana. 2005. Constitutively active L-type Ca^{2+} channels. *Proc. Natl. Acad. Sci. USA.* 102:11112–11117. doi:10.1073/pnas.0500360102

Navedo, M.F., G.C. Amberg, M. Nieves, J.D. Molkentin, and L.F. Santana. 2006. Mechanisms underlying heterogeneous Ca^{2+} sparklet activity in arterial smooth muscle. *J. Gen. Physiol.* 127:611–622. doi:10.1085/jgp.200609519

Navedo, M.F., M. Nieves-Cintrón, G.C. Amberg, C. Yuan, V.S. Votaw, W.J. Lederer, G.S. McKnight, and L.F. Santana. 2008. AKAP150 is required for stuttering persistent Ca^{2+} sparklets and angiotensin II-induced hypertension. *Circ. Res.* 102:e1–e11. doi:10.1161/CIRCRESAHA.107.167809

Navedo, M.F., E.P. Cheng, C. Yuan, S. Votaw, J.D. Molkentin, J.D. Scott, and L.F. Santana. 2010a. Increased coupled gating of L-type Ca^{2+} channels during hypertension and Timothy syndrome. *Circ. Res.* 106:748–756. doi:10.1161/CIRCRESAHA.109.213363

Navedo, M.F., Y. Takeda, M. Nieves-Cintrón, J.D. Molkentin, and L.F. Santana. 2010b. Elevated Ca^{2+} sparklet activity during acute hyperglycemia and diabetes in cerebral arterial smooth muscle cells. *Am. J. Physiol. Cell Physiol.* 298:C211–C220. doi:10.1152/ajpcell.00267.2009

Nieves-Cintrón, M., G.C. Amberg, C.B. Nichols, J.D. Molkentin, and L.F. Santana. 2007. Activation of NFATc3 down-regulates the β1 subunit of large conductance, calcium-activated K^+ channels in arterial smooth muscle and contributes to hypertension. *J. Biol. Chem.* 282:3231–3240. doi:10.1074/jbc.M608822200

Nieves-Cintrón, M., G.C. Amberg, M.F. Navedo, J.D. Molkentin, and L.F. Santana. 2008. The control of Ca^{2+} influx and NFATc3 signaling in arterial smooth muscle during hypertension. *Proc. Natl. Acad. Sci. USA.* 105:15623–15628. doi:10.1073/pnas.0808759105

Oliveria, S.F., M.L. Dell'Acqua, and W.A. Sather. 2007. AKAP79/150 anchoring of calcineurin controls neuronal L-type Ca^{2+} channel activity and nuclear signaling. *Neuron.* 55:261–275. doi:10.1016/j.neuron.2007.06.032

Pratt, P.F., S. Bonnet, L.M. Ludwig, P. Bonnet, and N.J. Rusch. 2002. Upregulation of L-type Ca^{2+} channels in mesenteric and skeletal arteries of SHR. *Hypertension.* 40:214–219. doi:10.1161/01.HYP.0000025877.23309.36

Qi, X.Y., Y.H. Yeh, L. Xiao, B. Burstein, A. Maguy, D. Chartier, L.R. Villeneuve, B.J.J.M. Brundel, D. Dobrev, and S. Nattel. 2008. Cellular signaling underlying atrial tachycardia remodeling of L-type calcium current. *Circ. Res.* 103:845–854. doi:10.1161/CIRCRESAHA.108.175463

Rossow, C.F., K.W. Dilly, C. Yuan, M. Nieves-Cintrón, J.L. Cabarrus, and L.F. Santana. 2009. NFATc3-dependent loss of $I_{(to)}$ gradient across the left ventricular wall during chronic beta adrenergic stimulation. *J. Mol. Cell. Cardiol.* 46:249–256. doi:10.1016/j.yjmcc.2008.10.016

Santana, L.F., H. Cheng, A.M. Gómez, M.B. Cannell, and W.J. Lederer. 1996. Relation between the sarcolemmal Ca^{2+} current and Ca^{2+} sparks and local control theories for cardiac excitation-contraction coupling. *Circ. Res.* 78:166–171.

Tanskanen, A.J., J.L. Greenstein, B. O'Rourke, and R.L. Winslow. 2005. The role of stochastic and modal gating of cardiac L-type Ca^{2+} channels on early after-depolarizations. *Biophys. J.* 88:85–95. doi:10.1529/biophysj.104.051508

Tour, O., S.R. Adams, R.A. Kerr, R.M. Meijer, T.J. Sejnowski, R.W. Tsien, and R.Y. Tsien. 2007. Calcium Green FlAsH as a genetically targeted small-molecule calcium indicator. *Nat. Chem. Biol.* 3:423–431. doi:10.1038/nchembio.2007.4

Wang, M.C., R.F. Collins, R.C. Ford, N.S. Berrow, A.C. Dolphin, and A. Kitmitto. 2004. The three-dimensional structure of the cardiac L-type voltage-gated calcium channel: comparison with the skeletal muscle form reveals a common architectural motif. *J. Biol. Chem.* 279:7159–7168. doi:10.1074/jbc.M308057200

Wang, S.Q., L.S. Song, E.G. Lakatta, and H. Cheng. 2001. Ca^{2+} signalling between single L-type Ca^{2+} channels and ryanodine receptors in heart cells. *Nature.* 410:592–596. doi:10.1038/35069083

Xiao, L., P. Coutu, L.R. Villeneuve, A. Tadevosyan, A. Maguy, S. Le Bouter, B.G. Allen, and S. Nattel. 2008. Mechanisms underlying rate-dependent remodeling of transient outward potassium current in canine ventricular myocytes. *Circ. Res.* 103:733–742. doi:10.1161/CIRCRESAHA.108.171157

Zhong, J., J.R. Hume, and K.D. Keef. 1999. Anchoring protein is required for cAMP-dependent stimulation of L-type $Ca^{(2+)}$ channels in rabbit portal vein. *Am. J. Physiol.* 277:C840–C844.

Zou, H., L.M. Lifshitz, R.A. Tuft, K.E. Fogarty, and J.J. Singer. 2002. Visualization of Ca2+ entry through single stretch-activated cation channels. *Proc. Natl. Acad. Sci. USA.* 99:6404–6409. doi:10.1073/pnas.092654999