

COMMENTARY

How Ca^{2+} influx is attenuated in the heart during a “fight or flight” response

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L-type Ca^{2+} channels are key actors in the various scenes that lead to cardiac contractility (Reuter, 1967; Beeler and Reuter, 1970; Benitah et al., 2010). Their activation during the cardiac action potential allows Ca^{2+} to enter myocytes (Beeler and Reuter, 1970; Ramos-Franco et al., 2016). This Ca^{2+} influx during systole results in an increase in myoplasmic Ca^{2+} concentration that leads to the activation of Ca^{2+} release channels known as ryanodine receptor 2 (RYR2) channels (Pessah et al., 1985; Imagawa et al., 1987). RYR2s are mainly located in the terminal cisternae of the SR (Seifert and Casida, 1986; Inui et al., 1987; Lai et al., 1988). An increase in the open probability (P_o) of RYR2 promotes Ca^{2+} release from the SR by a mechanism known as CICR (Ebashi and Endo, 1968; Fabiato and Fabiato, 1975; Fabiato, 1983). Ultimately, this large increase in myoplasmic Ca^{2+} concentration results in cellular contraction. In this issue of JGP, Morales et al. investigate the mechanisms involved in regulating Ca^{2+} influx during sympathetic stimulation and, in particular, the role of Ca^{2+} -dependent inactivation.

It has been known for more than 60 years that the autonomic nervous system modulates cardiac contractility (Lee and Shideman, 1959; Katz, 1967; Lindemann and Watanabe, 1985; Cohn, 1989; Henning, 1992). In fact, the sympathetic nervous system increases contractility by releasing the catecholamines epinephrine and norepinephrine, which induce a positive inotropic response (Lee and Shideman, 1959; Evans, 1986; Marks, 2013). When catecholamines bind to β -adrenergic receptors, they promote dissociation of a stimulatory G-protein α_s subunit and subsequent activation of adenylyl cyclase (Hildebrandt et al., 1983; Brum et al., 1984). This activation increases the intracellular concentration of cAMP, which promotes dissociation of the catalytic subunit of PKA (Krebs, 1972; Hayes and Mayer, 1981) and phosphorylation of multiple intracellular targets in the myocyte (Collins et al., 1981; Brum et al., 1984; Mundiña de Weilenmann et al., 1987; Suko et al., 1993; Valdivia et al., 1995; Fig. 1 C).

There are two critical proteins that increase cardiac contractility when phosphorylated. One is phospholamban; a protein that, under basal conditions, inhibits the SERCA2-mediated uptake of Ca^{2+} into the SR (Collins et al., 1981; Li et al., 1998; Valverde et al., 2006). Following adrenergic stimulation, phosphorylation of phospholamban at serine 16 by PKA (Chu et al., 2000) and at threonine 17 by CAMKII (Said et al., 2002) relieves its inhibitory effect on SERCA2. The relief of this inhibition increases the rate of Ca^{2+} transport from the cytosol to the SR, thus increasing the Ca^{2+} content of the SR.

A second protein that induces a positive inotropic effect when phosphorylated by PKA is the L-type Ca^{2+} channel ($\text{Ca}_v1.2$), which can be phosphorylated at two sites in the C terminus of the α_1 subunit. One site is serine 1928 (De Jongh et al., 1996; Mitterdorfer et al., 1996; Gao et al., 1997; Oliveria et al., 2007), located in the distal C terminus. The other site is serine 1700 (Harvey and Hell, 2013), located in the proximal C terminus (Fig. 1, A and C). However, the sites for PKA phosphorylation are still under discussion.

Interestingly, β subunits can be also phosphorylated. However, because $\alpha_1.2$ can interact with different β subunits, all of which are phosphorylated in different ways, it is unclear if PKA phosphorylation of β subunits has a major role in L-type Ca^{2+} channel function (Miriya et al., 2008; Yang et al., 2019).

In any event, PKA phosphorylation of L-type Ca^{2+} channels increases their P_o (Langer, 1983; Bean et al., 1984; Brum et al., 1984; Sperelakis, 1984). This increase in P_o results from a change in the modal gating of $\text{Ca}_v1.2$ (Yue et al., 1990; Delcour and Tsien, 1993; Shirokov et al., 1998). Under voltage-clamp conditions, the increase in P_o can be as large as three times (Yue et al., 1990). Therefore, the myocyte needs to have a mechanism that limits an excessive influx of Ca^{2+} upon phosphorylation.

There are two negative feedback mechanisms that can limit the positive inotropic actions of catecholamines. Specifically, $\text{Ca}_v1.2$ can reduce its own P_o by two different inactivation

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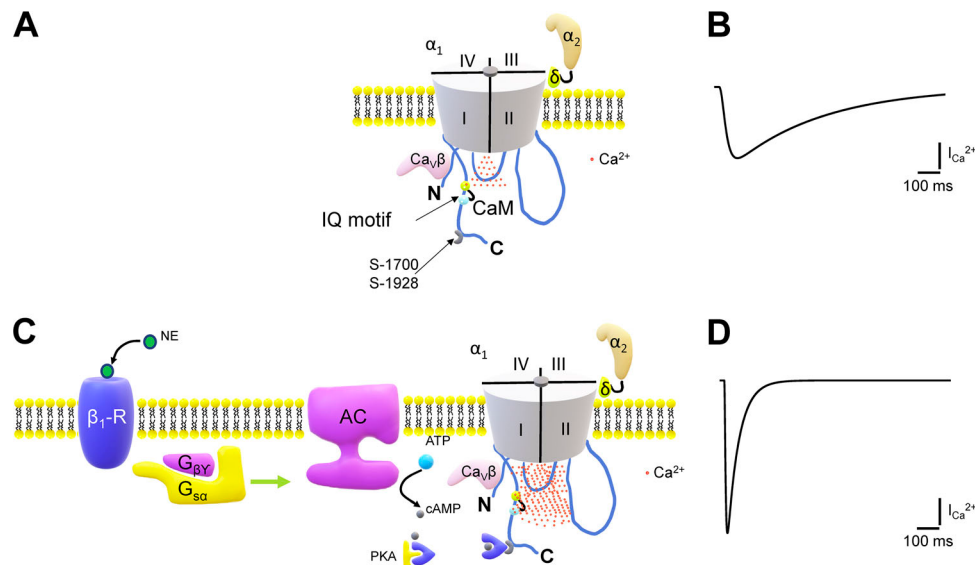


Figure 1. Overview of the molecular regulation of L-type Ca^{2+} currents. (A) The central molecular components of a $\text{Ca}_v1.2$ channel. The pore-forming subunit α_1 and the regulatory subunits α_2 , δ , and $\text{Ca}_v\beta$. The interaction of $\text{Ca}_v\beta$ and the N terminus of α_1 are essential in defining the VDI. On the other hand, CaM binding site at the IQ motif located at the C terminus is the protein locus involved in CDI. Interestingly, the PKA phosphorylation sites (S-1700 and S-1928) are also located at the C terminus. (B) The activation and inactivation of a numerically simulated L-type Ca^{2+} current. (C) The norepinephrine (NE) activation of the G-protein coupled receptor complex that finally leads to PKA phosphorylation of α_1 . The scheme illustrates that when α_1 is phosphorylated, there will be an increase in the Ca^{2+} current that will promote a local increase in the free Ca^{2+} concentration on the cytosolic face of the channel. This elevation in Ca^{2+} concentration will increase the probability of binding between Ca^{2+} and CaM, a critical event that promotes CDI. (D) Upon PKA phosphorylation of the α_1 subunit, there will be an increase both in the amplitude of the current and in the rate of CDI.

mechanisms; voltage-dependent inactivation (VDI; Cota et al., 1984; Kass and Sanguinetti, 1984; Lee et al., 1985; Zhang et al., 1994; Ferreira et al., 1997, 2003) and Ca^{2+} -dependent inactivation (CDI; Tillotson, 1979; Lipp et al., 1987; Lacampagne et al., 1996; Peterson et al., 2000). These inactivation mechanisms not only have physiological importance, but are also critical in preventing pathological events during catecholaminergic stimulation (Zhang et al., 2014). For example, in the absence of these mechanisms, excessive Ca^{2+} influx leads to SR Ca^{2+} overload in myocytes. This overload increases the probability of spontaneous SR Ca^{2+} release events during diastole. Thus, β -adrenergic stimulation can induce delayed diastolic depolarizations, which can trigger extrasystolic action potentials and eventually ventricular tachycardias and arrhythmias (Katra and Laurita, 2005; Curran et al., 2010; Ko et al., 2017).

VDI is mediated by the interaction between the pore-forming $\text{Ca}_v\alpha_1$ subunit and $\text{Ca}_v\beta$ subunits (Restituito et al., 2000; Wei et al., 2000; Kobrinsky et al., 2004; Jangsangthong et al., 2010; Fig. 1, A and C). On the other hand, CDI is primarily mediated by the Ca^{2+} sensor calmodulin (CaM; Zühlke et al., 1999; Peterson et al., 2000; Pitt et al., 2001). CaM has four helix-loop-helix domains (EF-hands) grouped within two lobes with low and high affinity for Ca^{2+} (Chin and Means, 2000). There is a Ca^{2+} -dependent CaM-binding sequence, the IQ motif, in the cytoplasmic C-terminal tail of the channel's α_1 subunit, which is critical for CDI (Peterson et al., 1999; Qin et al., 1999; Zühlke et al., 1999; Fig. 1, A and C). Although both inactivation mechanisms are physiologically relevant, there has been controversy about which of the two mechanisms have the larger impact on $\text{Ca}_v1.2$ inactivation during the cardiac action potential (Findlay,

2004; Grandi et al., 2010). Moreover, a phenomenon that is even less understood is what happens with the L-type channel inactivation during adrenergic stimulation (Morotti et al., 2012; Kumari et al., 2018).

In the current issue of JGP, Morales et al. (2019) use a novel conjunction of molecular biology, electrophysiological approaches, and mathematical modeling to investigate the mechanisms involved in controlling Ca^{2+} influx during catecholaminergic stimulation. Specifically, the authors test the hypothesis that CDI is the central mechanism limiting adrenergic stimulation of L-type Ca^{2+} current. This hypothesis, presented in Fig. 1, postulates that Ca^{2+} ions permeating through L-type Ca^{2+} channels will locally increase the cytosolic Ca^{2+} concentration and induce a certain degree of CDI in the absence of a sympathetic stimulus (Fig. 1, A and B). However, in the presence of a catecholaminergic stimulus, there will be an increase in current permeating through the Ca^{2+} channels due to an increase in $\text{Ca}_v1.2$ Po. This increase in Ca^{2+} current will not only augment Ca^{2+} influx into the myocyte, but also will increase the local cytosolic Ca^{2+} concentration. This local increase in Ca^{2+} will promote more CaM binding to Ca^{2+} , leading to an increase in CDI. Thus, catecholaminergic stimulation will lead to an increase the amplitude of the Ca^{2+} current and also accelerate the rate of inactivation of the channel (Fig. 1, B and D).

In their paper, Morales et al. (2019) explore the role of VDI by overexpressing the $\text{Ca}_v\beta_{2a}$ subunit, known to dramatically slow down VDI (Restituito et al., 2000; Wei et al., 2000). In a different set of experiments, the authors explore the relevance of CDI by inducing the expression of a mutated calmodulin (CaM_{34}), known to abolish CDI (Lee et al., 2003). The role of $\text{Ca}_v\beta_{2a}$

and/or the action of CaM34 are evaluated in experiments performed in neonatal cardiomyocytes. Specifically, myocytes were voltage clamped using the waveform of a self-action potential (sAP-Clamp; Banyasz et al., 2011, 2012), recorded from the same cell in control and isoproterenol-treated conditions.

In control cells, the authors show very nicely that application of 100 nM isoproterenol shortens the action potential by increasing the rate of inactivation of the L-type Ca^{2+} current recorded with sAP-Clamp (Figs. 1 and 2 in Morales et al. [2019]). This suggests that modifying the rate of inactivation of the L-type current has a critical effect on the duration of the action potential. Additionally, in experiments performed in the absence of isoproterenol, the authors demonstrate that molecular interventions that alter the rate of inactivation of L-type Ca^{2+} channels dramatically prolong the duration of the action potential and Ca^{2+} currents. The expression of CaM₃₄ increases action potential duration by more than five times (Fig. 5 in Morales et al. [2019]), and overexpression of $\text{Ca}_v \beta_{2a}$ increases action potential duration by more than three times (Fig. 6 in Morales et al. [2019]). These results confirm that the rate of inactivation of L-type Ca^{2+} currents defines the duration of the action potential in neonatal rat myocytes.

Figs. 8 and 9 in Morales et al. (2019) show the conclusive experiment designed to evaluate which of the L-type Ca^{2+} current inactivation mechanisms is dominant. The results presented in Fig. 8 illustrate that, in the absence of CDI induced by overexpression of CaM₃₄, isoproterenol has a significantly smaller effect than when VDI is impaired by the expression of $\text{Ca}_v \beta_{2a}$. This, along with isoproterenol's significantly larger effect in myocytes when CDI is not altered (Fig. 9), clearly indicates that CDI is the main mechanism for L-type Ca^{2+} channel inactivation during adrenergic stimulation.

As previously stated, these experiments were conducted in neonatal rat cardiomyocytes, a model that significantly differs from adult ventricular myocytes (Escobar et al., 2004; Pérez et al., 2005; Snopko et al., 2007). For example, neonatal myocytes have a reduced expression of $\text{K}_v 4.X$ channels (Kilborn and Fedida, 1990; Wang and Duff, 1997; Kobayashi et al., 2003) and the regulatory subunit KChIP (Kobayashi et al., 2003; Jia and Takimoto, 2006). These K^+ channels define a transient K^+ outward current (Ito; Guo et al., 1999; Teutsch et al., 2007; Rossow et al., 2009) that can reshape action potential repolarization. Thus, because of the presence of Ito, we can expect that both VDI and CDI will have a smaller effect on action potential duration in adult myocytes.

Previous studies have also shown that CICR is not critical for defining intracellular Ca^{2+} dynamics during excitation-contraction coupling in neonatal cardiac myocytes (Escobar et al., 2004), primarily because the tubular system is not fully developed (Di Maio et al., 2007). This reduced SR Ca^{2+} release in neonatal myocytes further supports the hypothesis presented by Morales et al. (2019): Ca^{2+} release from the SR would likely augment the effect of CDI (Lacampagne et al., 1996). Indeed, SR Ca^{2+} release is dramatically increased during adrenergic stimulation, not only due to a larger triggering signal, but also because the intra SR Ca^{2+} content is higher. The increase in luminal SR Ca^{2+} content is mediated by an increase in Ca^{2+} influx into the

myocyte during each action potential. In addition, SR Ca^{2+} content is further elevated due to phosphorylation of phospholamban, which in turn increases the SERCA2 transport rate. These findings suggest that the mechanism proposed by Morales et al. (2019) will be even more relevant in controlling Ca^{2+} influx during β -adrenergic stimulation in an adult heart.

In summary, Morales et al. (2019) use a novel and powerful approach to shed light upon a fundamental physiological and pathophysiological puzzle: how to prevent Ca^{2+} overload, and the pathological consequences of this overload, during a “fight or flight” response.

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References

- Banyasz, T., B. Horvath, Z. Jian, L.T. Izu, and Y. Chen-Izu. 2011. Sequential dissection of multiple ionic currents in single cardiac myocytes under action potential-clamp. *J. Mol. Cell. Cardiol.* 50:578–581. <https://doi.org/10.1016/j.jmcc.2010.12.020>
- Banyasz, T., B. Horvath, Z. Jian, L.T. Izu, and Y. Chen-Izu. 2012. Profile of L-type Ca^{2+} current and $\text{Na}^{+}/\text{Ca}^{2+}$ exchange current during cardiac action potential in ventricular myocytes. *Heart Rhythm.* 9:134–142. <https://doi.org/10.1016/j.hrthm.2011.08.029>
- Bean, B.P., M.C. Nowycky, and R.W. Tsien. 1984. Beta-adrenergic modulation of calcium channels in frog ventricular heart cells. *Nature.* 307:371–375. <https://doi.org/10.1038/307371a0>
- Beeler, G.W. Jr., and H. Reuter. 1970. Membrane calcium current in ventricular myocardial fibres. *J. Physiol.* 207:191–209. <https://doi.org/10.1113/jphysiol.1970.sp009056>
- Benitah, J.-P., J.L. Alvarez, and A.M. Gómez. 2010. L-type Ca^{2+} current in ventricular cardiomyocytes. *J. Mol. Cell. Cardiol.* 48:26–36. <https://doi.org/10.1016/j.jmcc.2009.07.026>
- Brum, G., W. Osterrieder, and W. Trautwein. 1984. Beta-adrenergic increase in the calcium conductance of cardiac myocytes studied with the patch clamp. *Pflügers Arch.* 401:111–118. <https://doi.org/10.1007/BF00583870>
- Chin, D., and A.R. Means. 2000. Calmodulin: a prototypical calcium sensor. *Trends Cell Biol.* 10:322–328. [https://doi.org/10.1016/S0962-8924\(00\)01800-6](https://doi.org/10.1016/S0962-8924(00)01800-6)
- Chu, G., J.W. Lester, K.B. Young, W. Luo, J. Zhai, and E.G. Kranias. 2000. A single site (Ser16) phosphorylation in phospholamban is sufficient in mediating its maximal cardiac responses to beta-agonists. *J. Biol. Chem.* 275:38938–38943. <https://doi.org/10.1074/jbc.M004079200>
- Cohn, J.N. 1989. Sympathetic nervous system activity and the heart. *Am. J. Hypertens.* 2:353S–356S. <https://doi.org/10.1093/ajh/2.12.353S>
- Collins, J.H., E.G. Kranias, A.S. Reeves, L.M. Bilezikjian, and A. Schwartz. 1981. Isolation of phospholamban and a second proteolipid component from canine cardiac sarcoplasmic reticulum. *Biochem. Biophys. Res. Commun.* 99:796–803. [https://doi.org/10.1016/0006-291X\(81\)91235-3](https://doi.org/10.1016/0006-291X(81)91235-3)

- Cota, G., L. Nicola Siri, and E. Stefani. 1984. Calcium channel inactivation in frog (*Rana pipiens* and *Rana moctezuma*) skeletal muscle fibres. *J. Physiol.* 354:99–108. <https://doi.org/10.1113/jphysiol.1984.sp015365>
- Curran, J., K.H. Brown, D.J. Santiago, S. Pogwizd, D.M. Bers, and T.R. Shannon. 2010. Spontaneous Ca waves in ventricular myocytes from failing hearts depend on Ca(2+)-calmodulin-dependent protein kinase II. *J. Mol. Cell. Cardiol.* 49:25–32. <https://doi.org/10.1016/j.yjmcc.2010.03.013>
- De Jongh, K.S., B.J. Murphy, A.A. Colvin, J.W. Hell, M. Takahashi, and W.A. Catterall. 1996. Specific phosphorylation of a site in the full-length form of the alpha 1 subunit of the cardiac L-type calcium channel by adenosine 3',5'-cyclic monophosphate-dependent protein kinase. *Biochemistry.* 35:10392–10402. <https://doi.org/10.1021/bi953023c>
- Delcour, A.H., and R.W. Tsien. 1993. Altered prevalence of gating modes in neurotransmitter inhibition of N-type calcium channels. *Science.* 259: 980–984. <https://doi.org/10.1126/science.8094902>
- Di Maio, A., K. Karko, R.M. Snopko, R. Mejía-Alvarez, and C. Franzini-Armstrong. 2007. T-tubule formation in cardiomyocytes: two possible mechanisms? *J. Muscle Res. Cell Motil.* 28:231–241. <https://doi.org/10.1007/s10974-007-9121-x>
- Ebashi, S., and M. Endo. 1968. Calcium ion and muscle contraction. *Prog. Biophys. Mol. Biol.* 18:123–183. [https://doi.org/10.1016/0079-6107\(68\)90023-0](https://doi.org/10.1016/0079-6107(68)90023-0)
- Escobar, A.L., R. Ribeiro-Costa, C. Villalba-Galea, M.E. Zoghbi, C.G. Pérez, and R. Mejía-Alvarez. 2004. Developmental changes of intracellular Ca2+ transients in beating rat hearts. *Am. J. Physiol. Heart Circ. Physiol.* 286: H971–H978. <https://doi.org/10.1152/ajpheart.00308.2003>
- Evans, D.B. 1986. Modulation of cAMP: mechanism for positive inotropic action. *J. Cardiovasc. Pharmacol.* 8(Suppl 9):S22–S29. <https://doi.org/10.1097/00005344-198611001-00003>
- Fabiato, A. 1983. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. *Am. J. Physiol.* 245:C1–C14. <https://doi.org/10.1152/ajpcell.1983.245.1.C1>
- Fabiato, A., and F. Fabiato. 1975. Contractions induced by a calcium-triggered release of calcium from the sarcoplasmic reticulum of single skinned cardiac cells. *J. Physiol.* 249:469–495. <https://doi.org/10.1113/jphysiol.1975.sp011026>
- Ferreira, G., J. Yi, E. Ríos, and R. Shirokov. 1997. Ion-dependent inactivation of barium current through L-type calcium channels. *J. Gen. Physiol.* 109: 449–461. <https://doi.org/10.1085/jgp.109.4.449>
- Ferreira, G., E. Ríos, and N. Reyes. 2003. Two components of voltage-dependent inactivation in Ca(v)1.2 channels revealed by its gating currents. *Biophys. J.* 84:3662–3678. [https://doi.org/10.1016/S0006-3495\(03\)75096-6](https://doi.org/10.1016/S0006-3495(03)75096-6)
- Findlay, I. 2004. Physiological modulation of inactivation in L-type Ca2+ channels: one switch. *J. Physiol.* 554:275–283. <https://doi.org/10.1113/jphysiol.2003.047902>
- Gao, T., A. Yatani, M.L. Dell'Acqua, H. Sako, S.A. Green, N. Dascal, J.D. Scott, and M.M. Hosey. 1997. cAMP-dependent regulation of cardiac L-type Ca2+ channels requires membrane targeting of PKA and phosphorylation of channel subunits. *Neuron.* 19:185–196. [https://doi.org/10.1016/S0896-6273\(00\)80358-X](https://doi.org/10.1016/S0896-6273(00)80358-X)
- Grandi, E., S. Morotti, K.S. Ginsburg, S. Severi, and D.M. Bers. 2010. Interplay of voltage and Ca-dependent inactivation of L-type Ca current. *Prog. Biophys. Mol. Biol.* 103:44–50. <https://doi.org/10.1016/j.pbiomolbio.2010.02.001>
- Guo, W., H. Xu, B. London, and J.M. Nerbonne. 1999. Molecular basis of transient outward K+ current diversity in mouse ventricular myocytes. *J. Physiol.* 521:587–599. <https://doi.org/10.1111/j.1469-7793.1999.00587.x>
- Harvey, R.D., and J.W. Hell. 2013. CaV1.2 signaling complexes in the heart. *J. Mol. Cell. Cardiol.* 58:143–152. <https://doi.org/10.1016/j.yjmcc.2012.12.006>
- Hayes, J.S., and S.E. Mayer. 1981. Regulation of guinea pig heart phosphorylase kinase by cAMP, protein kinase, and calcium. *Am. J. Physiol.* 240: E340–E349. <https://doi.org/10.1152/ajpendo.1981.240.3.E340>
- Henning, R.J. 1992. Vagal stimulation during muscarinic and beta-adrenergic blockade increases atrial contractility and heart rate. *J. Auton. Nerv. Syst.* 40:121–129. [https://doi.org/10.1016/0165-1838\(92\)90023-A](https://doi.org/10.1016/0165-1838(92)90023-A)
- Hildebrandt, J.D., R.D. Sekura, J. Codina, R. Iyengar, C.R. Manclark, and L. Birnbaumer. 1983. Stimulation and inhibition of adenylyl cyclases mediated by distinct regulatory proteins. *Nature.* 302:706–709. <https://doi.org/10.1038/302706a0>
- Imagawa, T., J.S. Smith, R. Coronado, and K.P. Campbell. 1987. Purified ryanodine receptor from skeletal muscle sarcoplasmic reticulum is the Ca2+-permeable pore of the calcium release channel. *J. Biol. Chem.* 262: 16636–16643.
- Inui, M., A. Saito, and S. Fleischer. 1987. Isolation of the ryanodine receptor from cardiac sarcoplasmic reticulum and identity with the feet structures. *J. Biol. Chem.* 262:15637–15642.
- Jangsangthong, W., E. Kuzmenkina, I.F.Y. Khan, J. Matthes, R. Hullin, and S. Herzog. 2010. Inactivation of L-type calcium channels is determined by the length of the N terminus of mutant beta(1) subunits. *Pflugers Arch.* 459:399–411. <https://doi.org/10.1007/s00424-009-0738-z>
- Jia, Y., and K. Takimoto. 2006. Mitogen-activated protein kinases control cardiac KChIP2 gene expression. *Circ. Res.* 98:386–393. <https://doi.org/10.1161/01.RES.0000201956.86258.e1>
- Kass, R.S., and M.C. Sanguinetti. 1984. Inactivation of calcium channel current in the calf cardiac Purkinje fiber. Evidence for voltage- and calcium-mediated mechanisms. *J. Gen. Physiol.* 84:705–726. <https://doi.org/10.1085/jgp.84.5.705>
- Katra, R.P., and K.R. Laurita. 2005. Cellular mechanism of calcium-mediated triggered activity in the heart. *Circ. Res.* 96:535–542. <https://doi.org/10.1161/01.RES.0000159387.00749.3c>
- Katz, A.M. 1967. Regulation of cardiac muscle contractility. *J. Gen. Physiol.* 50(6, Suppl):185–196. <https://doi.org/10.1085/jgp.50.6.185>
- Kilborn, M.J., and D. Fedida. 1990. A study of the developmental changes in outward currents of rat ventricular myocytes. *J. Physiol.* 430:37–60. <https://doi.org/10.1113/jphysiol.1990.sp018280>
- Ko, C.Y., M.B. Liu, Z. Song, Z. Qu, and J.N. Weiss. 2017. Multiscale Determinants of Delayed Afterdepolarization Amplitude in Cardiac Tissue. *Biophys. J.* 112:1949–1961. <https://doi.org/10.1016/j.bpj.2017.03.006>
- Kobayashi, T., Y. Yamada, M. Nagashima, S. Seki, M. Tsutsuura, Y. Ito, I. Sakuma, H. Hamada, T. Abe, and N. Tohse. 2003. Contribution of KChIP2 to the developmental increase in transient outward current of rat cardiomyocytes. *J. Mol. Cell. Cardiol.* 35:1073–1082. [https://doi.org/10.1016/S0022-2828\(03\)00199-8](https://doi.org/10.1016/S0022-2828(03)00199-8)
- Kobrinisky, E., K.J.F. Kepplinger, A. Yu, J.B. Harry, H. Kahr, C. Romanin, D.R. Abernethy, and N.M. Soldatov. 2004. Voltage-gated rearrangements associated with differential beta-subunit modulation of the L-type Ca(2+) channel inactivation. *Biophys. J.* 87:844–857. <https://doi.org/10.1529/biophysj.104.041152>
- Krebs, E.G. 1972. Protein kinases. *Curr. Top. Cell. Regul.* 5:99–133. <https://doi.org/10.1016/B978-0-12-152805-8.50010-1>
- Kumari, N., H. Gaur, and A. Bhargava. 2018. Cardiac voltage gated calcium channels and their regulation by β -adrenergic signaling. *Life Sci.* 194: 139–149. <https://doi.org/10.1016/j.lfs.2017.12.033>
- Lacampagne, A., C. Caputo, and J. Argibay. 1996. Effect of ryanodine on cardiac calcium current and calcium channel gating current. *Biophys. J.* 70:370–375. [https://doi.org/10.1016/S0006-3495\(96\)79580-2](https://doi.org/10.1016/S0006-3495(96)79580-2)
- Lai, F.A., K. Anderson, E. Rousseau, Q.Y. Liu, and G. Meissner. 1988. Evidence for a Ca2+ channel within the ryanodine receptor complex from cardiac sarcoplasmic reticulum. *Biochem. Biophys. Res. Commun.* 151:441–449. [https://doi.org/10.1016/0006-291X\(88\)90613-4](https://doi.org/10.1016/0006-291X(88)90613-4)
- Langer, G.A. 1983. Control of calcium movement in the myocardium. *Eur. Heart J.* 4 Suppl H:5–11.
- Lee, W.C., and F.E. Shideman. 1959. Role of myocardial catecholamines in cardiac contractility. *Science.* 129:967–968. <https://doi.org/10.1126/science.129.3354.967>
- Lee, A., H. Zhou, T. Scheuer, and W.A. Catterall. 2003. Molecular determinants of Ca(2+)/calmodulin-dependent regulation of Ca(v)2.1 channels. *Proc. Natl. Acad. Sci. USA.* 100:16059–16064. <https://doi.org/10.1073/pnas.2237000100>
- Lee, K.S., E. Marban, and R.W. Tsien. 1985. Inactivation of calcium channels in mammalian heart cells: joint dependence on membrane potential and intracellular calcium. *J. Physiol.* 364:395–411. <https://doi.org/10.1113/jphysiol.1985.sp015752>
- Li, L., G. Chu, E.G. Kranias, and D.M. Bers. 1998. Cardiac myocyte calcium transport in phospholamban knockout mouse: relaxation and endogenous CaMKII effects. *Am. J. Physiol.* 274:H1335–H1347.
- Lindemann, J.P., and A.M. Watanabe. 1985. Muscarinic cholinergic inhibition of beta-adrenergic stimulation of phospholamban phosphorylation and Ca2+ transport in guinea pig ventricles. *J. Biol. Chem.* 260:13122–13129.
- Lipp, P., S. Mechmann, and L. Pott. 1987. Effects of calcium release from sarcoplasmic reticulum on membrane currents in guinea pig atrial cardioballs. *Pflugers Arch.* 410:121–131. <https://doi.org/10.1007/BF00581904>
- Marks, A.R. 2013. Calcium cycling proteins and heart failure: mechanisms and therapeutics. *J. Clin. Invest.* 123:46–52. <https://doi.org/10.1172/JCI62834>
- Miriyala, J., T. Nguyen, D.T. Yue, and H.M. Colecraft. 2008. Role of CaVbeta subunits, and lack of functional reserve, in protein kinase A modulation

- of cardiac CaV1.2 channels. *Circ. Res.* 102:e54–e64. <https://doi.org/10.1161/CIRCRESAHA.108.171736>
- Mitterdorfer, J., M. Froschmayr, M. Grabner, F.F. Moebius, H. Glossmann, and J. Striessnig. 1996. Identification of PK-A phosphorylation sites in the carboxyl terminus of L-type calcium channel $\alpha 1$ subunits. *Biochemistry*. 35:9400–9406. <https://doi.org/10.1021/bi960683o>
- Morales, D., T. Hermosilla, and D. Varela. 2019. Calcium-dependent inactivation controls cardiac L-type Ca²⁺ currents under β -adrenergic stimulation. *J. Gen. Physiol.* jgp.201812236. <https://doi.org/10.1085/jgp.201812236>
- Morotti, S., E. Grandi, A. Summa, K.S. Ginsburg, and D.M. Bers. 2012. Theoretical study of L-type Ca(2+) current inactivation kinetics during action potential repolarization and early afterdepolarizations. *J. Physiol.* 590:4465–4481. <https://doi.org/10.1113/jphysiol.2012.231886>
- Mundiña de Weilenmann, C., L. Vittone, G. de Cingolani, and A. Mattiazzi. 1987. Dissociation between contraction and relaxation: the possible role of phospholamban phosphorylation. *Basic Res. Cardiol.* 82:507–516. <https://doi.org/10.1007/BF01907220>
- Oliveria, S.F., M.L. Dell'Acqua, and W.A. Sather. 2007. AKAP79/150 anchoring of calcineurin controls neuronal L-type Ca²⁺ channel activity and nuclear signaling. *Neuron*. 55:261–275. <https://doi.org/10.1016/j.neuron.2007.06.032>
- Pérez, C.G., J.A. Copello, Y. Li, K.L. Karko, L. Gómez, J. Ramos-Franco, M. Fill, A.L. Escobar, and R. Mejía-Alvarez. 2005. Ryanodine receptor function in newborn rat heart. *Am. J. Physiol. Heart Circ. Physiol.* 288: H2527–H2540. <https://doi.org/10.1152/ajpheart.00188.2004>
- Pessah, I.N., A.L. Waterhouse, and J.E. Casida. 1985. The calcium-ryanodine receptor complex of skeletal and cardiac muscle. *Biochem. Biophys. Res. Commun.* 128:449–456. [https://doi.org/10.1016/0006-291X\(85\)91699-7](https://doi.org/10.1016/0006-291X(85)91699-7)
- Peterson, B.Z., C.D. DeMaria, J.P. Adelman, and D.T. Yue. 1999. Calmodulin is the Ca²⁺ sensor for Ca²⁺-dependent inactivation of L-type calcium channels. *Neuron*. 22:549–558. [https://doi.org/10.1016/S0896-6273\(00\)80709-6](https://doi.org/10.1016/S0896-6273(00)80709-6)
- Peterson, B.Z., J.S. Lee, J.G. Mulle, Y. Wang, M. de Leon, and D.T. Yue. 2000. Critical determinants of Ca(2+)-dependent inactivation within an EF-hand motif of L-type Ca(2+) channels. *Biophys. J.* 78:1906–1920. [https://doi.org/10.1016/S0006-3495\(00\)76739-7](https://doi.org/10.1016/S0006-3495(00)76739-7)
- Pitt, G.S., R.D. Zühlke, A. Hudmon, H. Schulman, H. Reuter, and R.W. Tsien. 2001. Molecular basis of calmodulin tethering and Ca²⁺-dependent inactivation of L-type Ca²⁺ channels. *J. Biol. Chem.* 276:30794–30802. <https://doi.org/10.1074/jbc.M104959200>
- Qin, N., R. Olcese, M. Bransby, T. Lin, and L. Birnbaumer. 1999. Ca²⁺-induced inhibition of the cardiac Ca²⁺ channel depends on calmodulin. *Proc. Natl. Acad. Sci. USA*. 96:2435–2438. <https://doi.org/10.1073/pnas.96.5.2435>
- Ramos-Franco, J., Y. Aguilar-Sanchez, and A.L. Escobar. 2016. Intact Heart Loose Patch Photolysis Reveals Ionic Current Kinetics During Ventricular Action Potentials. *Circ. Res.* 118:203–215. <https://doi.org/10.1161/CIRCRESAHA.115.307399>
- Restituto, S., T. Cens, C. Barrere, S. Geib, S. Galas, M. De Waard, and P. Charnet. 2000. The [beta]2a subunit is a molecular groom for the Ca²⁺ channel inactivation gate. *J. Neurosci.* 20:9046–9052. <https://doi.org/10.1523/JNEUROSCI.20-24-09046.2000>
- Reuter, H. 1967. The dependence of slow inward current in Purkinje fibres on the extracellular calcium-concentration. *J. Physiol.* 192:479–492. <https://doi.org/10.1113/jphysiol.1967.sp008310>
- Rosow, 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 β adrenergic stimulation. *J. Mol. Cell. Cardiol.* 46:249–256. <https://doi.org/10.1016/j.yjmcc.2008.10.016>
- Said, M., C. Mundiña-Weilenmann, L. Vittone, and A. Mattiazzi. 2002. The relative relevance of phosphorylation of the Thr(17) residue of phospholamban is different at different levels of beta-adrenergic stimulation. *Pflugers Arch.* 444:801–809. <https://doi.org/10.1007/s00424-002-0885-y>
- Seifert, J., and J.E. Casida. 1986. Ca²⁺-dependent ryanodine binding site: soluble preparation from rabbit cardiac sarcoplasmic reticulum. *Biochim. Biophys. Acta.* 861:399–405. [https://doi.org/10.1016/0005-2736\(86\)90447-5](https://doi.org/10.1016/0005-2736(86)90447-5)
- Shirokov, R., G. Ferreira, J. Yi, and E. Ríos. 1998. Inactivation of gating currents of L-type calcium channels. Specific role of the $\alpha 2$ delta subunit. *J. Gen. Physiol.* 111:807–823. <https://doi.org/10.1085/jgp.111.6.807>
- Snopko, R.M., A.S. Aromolaran, K.L. Karko, J. Ramos-Franco, L.A. Blatter, and R. Mejía-Alvarez. 2007. Cell culture modifies Ca²⁺ signaling during excitation-contraction coupling in neonate cardiac myocytes. *Cell Calcium*. 41:13–25. <https://doi.org/10.1016/j.ceca.2006.04.033>
- Sperelakis, N. 1984. Hormonal and neurotransmitter regulation of Ca⁺⁺ influx through voltage-dependent slow channels in cardiac muscle membrane. *Membr. Biochem.* 5:131–166. <https://doi.org/10.3109/09687688409150275>
- Suko, J., I. Maurer-Fogy, B. Plank, O. Bertel, W. Wyskovsky, M. Hohenegger, and G. Hellmann. 1993. Phosphorylation of serine 2843 in ryanodine receptor-calcium release channel of skeletal muscle by cAMP-, cGMP- and CaM-dependent protein kinase. *Biochim. Biophys. Acta.* 1175: 193–206. [https://doi.org/10.1016/0167-4889\(93\)90023-1](https://doi.org/10.1016/0167-4889(93)90023-1)
- Teutsch, C., R.P. Kondo, D.A. Dederko, J. Chrast, K.R. Chien, and W.R. Giles. 2007. Spatial distributions of Kv4 channels and KChip2 isoforms in the murine heart based on laser capture microdissection. *Cardiovasc. Res.* 73:739–749. <https://doi.org/10.1016/j.cardiores.2006.11.034>
- Tillotson, D. 1979. Inactivation of Ca conductance dependent on entry of Ca ions in molluscan neurons. *Proc. Natl. Acad. Sci. USA*. 76:1497–1500. <https://doi.org/10.1073/pnas.76.3.1497>
- Valdivia, H.H., J.H. Kaplan, G.C. Ellis-Davies, and W.J. Lederer. 1995. Rapid adaptation of cardiac ryanodine receptors: modulation by Mg²⁺ and phosphorylation. *Science*. 267:1997–2000. <https://doi.org/10.1126/science.7701323>
- Valverde, C.A., C. Mundiña-Weilenmann, M. Reyes, E.G. Kranias, A.L. Escobar, and A. Mattiazzi. 2006. Phospholamban phosphorylation sites enhance the recovery of intracellular Ca²⁺ after perfusion arrest in isolated, perfused mouse heart. *Cardiovasc. Res.* 70:335–345. <https://doi.org/10.1016/j.cardiores.2006.01.018>
- Wang, L., and H.J. Duff. 1997. Developmental changes in transient outward current in mouse ventricle. *Circ. Res.* 81:120–127. <https://doi.org/10.1161/01.RES.81.1.120>
- Wei, S.K., H.M. Colecraft, C.D. DeMaria, B.Z. Peterson, R. Zhang, T.A. Kohout, T.B. Rogers, and D.T. Yue. 2000. Ca(2+) channel modulation by recombinant auxiliary beta subunits expressed in young adult heart cells. *Circ. Res.* 86:175–184. <https://doi.org/10.1161/01.RES.86.2.175>
- Yang, L., A. Katchman, J. Kushner, A. Kushnir, S.I. Zakharov, B.-X. Chen, Z. Shuja, P. Subramanyam, G. Liu, A. Papa, et al. 2019. Cardiac CaV1.2 channels require β subunits for β -adrenergic-mediated modulation but not trafficking. *J. Clin. Invest.* 129:647–658. <https://doi.org/10.1172/JCI123878>
- Yue, D.T., S. Herzog, and E. Marban. 1990. Beta-adrenergic stimulation of calcium channels occurs by potentiation of high-activity gating modes. *Proc. Natl. Acad. Sci. USA*. 87:753–757. <https://doi.org/10.1073/pnas.87.2.753>
- Zhang, J.F., P.T. Ellinor, R.W. Aldrich, and R.W. Tsien. 1994. Molecular determinants of voltage-dependent inactivation in calcium channels. *Nature*. 372:97–100. <https://doi.org/10.1038/372097a0>
- Zhang, J., B. Chen, X. Zhong, T. Mi, A. Guo, Q. Zhou, Z. Tan, G. Wu, A.W. Chen, M. Fill, et al. 2014. The cardiac ryanodine receptor luminal Ca²⁺ sensor governs Ca²⁺ waves, ventricular tachyarrhythmias and cardiac hypertrophy in calsequestrin-null mice. *Biochem. J.* 461:99–106. <https://doi.org/10.1042/BJ20140126>
- Zühlke, R.D., G.S. Pitt, K. Deisseroth, R.W. Tsien, and H. Reuter. 1999. Calmodulin supports both inactivation and facilitation of L-type calcium channels. *Nature*. 399:159–162. <https://doi.org/10.1038/20200>