Commentary

Targeting late \( I_{\text{CaL}} \) to close the window to ventricular arrhythmias

Luis A. Gonano and Alicia Mattiazzi

"...basic science...provides the essential raw material for translation and continues to represent humanity’s best hope to meet a wide range of public health challenges..." – Ferric C. Fang and Arturo Casadevall (Fang and Casadevall, 2010).

Triggered activity and EADs

Triggered arrhythmias originate from aberrant cell membrane depolarizations that occur during or after completion of the cardiac action potential (AP). Although the phenomenon was recognized early and associated with cardiac arrhythmias in recordings relying on monophasic APs (Segers, 1941; Bozler, 1943), the concept of triggered activity was coined several decades later by Paul Cranefield (Cranefield, 1975) who, incidentally, served as editor-in-chief of this journal for more than 25 yr. This new term aimed to differentiate the slow membrane depolarization that depends on a previous AP (triggered activity) from automaticity, a slow membrane depolarization with different properties like the rhythmicity and spontaneity and independence on a preceding AP. Cranefield also originated the term “afterdepolarizations” for this triggered activity and described what are now traditionally known as early afterdepolarizations (EADs) “...that appears before the membrane potential has returned to the level it had at the beginning of the upstroke of the action potential,” and delayed afterdepolarizations (DADs), which occur “...after repolarization is complete i.e., after the membrane potential has returned to the level seen before the action potential” (Cranefield, 1975, 1977).

EADs present as aberrant early voltage membrane oscillations that interrupt or retard repolarization during phase 2 and/or 3 of the cardiac APs (see Fig. 1). They occur preferentially (though not exclusively) during bradycardia under conditions of reduced repolarization reserve, and are associated with prolongation of the cardiac AP (Weiss et al., 2010).

Despite their early recognition, the underlying mechanism of afterdepolarizations, particularly those of EADs, remained largely unknown for many years and are still a matter of debate (Zhao et al., 2012; Kurata et al., 2020). This is in part because of the difficulty of dissecting the possible influence of the different variables that interact at the plateau of the AP (membrane voltage, different ion currents, and intracellular Ca\(^{2+}\) cycling) on EADs generation. This issue is of crucial clinical importance given that EADs have the potential not only for the initiation (triggering), but also for the perpetuation of ventricular arrhythmias in several syndromes by providing the substrate for reentrant mechanisms (Weiss et al., 2010; Shimizu and Horie, 2011; de Lange et al., 2012; Shimizu, 2013; Wit and Boyden, 2007; Colman et al., 2017).

One of the first insights into the underlying mechanisms of EADs was provided by Marbán et al. (1986). By using the Ca\(^{2+}\) blocker nitrendipine and the (at that time recently discovered) Ca\(^{2+}\) agonist Bay K 8644, their experiments strongly suggested that EADs arise from Ca\(^{2+}\) entry through sarclemmal L-type Ca\(^{2+}\) channels (LTCC; Marban et al., 1986). A few years later, January and Riddle showed that EADs could be elicited only over a narrow range of takeoff potentials, the same voltage range at which recovery from inactivation and activation of Ca\(^{2+}\) channels occurs (January et al., 1988; January and Riddle, 1989). These experiments significantly extended the comprehension of the origin and mechanisms of EADs, providing evidence that linked them to the “Ca\(^{2+}\) window current” for the first time.

Besides L-type Ca\(^{2+}\) current (\( I_{\text{CaL}} \)), several inward currents have been associated with the origin of EADs, like the late Na\(^{+}\) current (Attwell et al., 1978; January et al., 1991; Maltsev et al., 1998) and the Na\(^{+}\)-Ca\(^{2+}\) exchanger current (Volders et al., 1997; Choi et al., 2002). Although we will not discuss these mechanisms here, experimental evidence supports that these mechanisms may also play a role in EAD generation. For instance, it has been shown that EADs may appear under conditions of SR Ca\(^{2+}\) overload that favor spontaneous SR Ca\(^{2+}\) release. Similar to the mechanism involved in DAD production, spontaneous Ca\(^{2+}\) leak elicited membrane potential depolarizations via Ca\(^{2+}\)-sensitive currents (primarily the Na\(^{+}\)-Ca\(^{2+}\) exchanger) that may fire EADs (Volders et al., 1997; Choi et al., 2002). Indeed, the generation of...
EADs in phase 3 of the cardiac AP has been tightly associated with this mechanism (Volders et al., 2000; Zhao et al., 2012).

The Ca\(^{2+}\) window current and the late Ca\(^{2+}\) current

Early experimental evidence indicated that the steady-state activation and inactivation parameters of the \(I_{\text{CaL}}\) voltage overlap, resulting in a window current at plateau potentials (Brown et al., 1984; Josephson et al., 1984; McDonald et al., 1986; Cohen and Lederer, 1987). Within this voltage window, a fraction of LTCC remains available for activation, producing a persistent Ca\(^{2+}\) current. The Ca\(^{2+}\) window current and the late Ca\(^{2+}\) current

This window current (or pedestal, which reflects the incomplete inactivation of the channel (Cohen and Lederer, 1987).

In the present issue, Angelini et al. (2021) provide evidence that EAD-induced arrhythmias may be suppressed by targeting the late Ca\(^{2+}\) current. Based on early theoretical work (Tran et al., 2009; Qu and Chung, 2012) and by testing the effect of changes in specific gating properties by dynamic clamp, the authors previously emphasized the role of the Ca\(^{2+}\) window current on EAD generation. They concluded that EAD formation was affected mainly by a depolarizing shift of the half-activation potential, a hyperpolarizing shift of the half-inactivation potential, and a reduction of the noninactivating pedestal current, the last being the most reliable strategy (Madhvani et al., 2015).

These findings have therapeutic implications, namely that novel agents which alter the voltage dependence and/or kinetics of \(I_{\text{CaL}}\) could be developed to suppress EAD-mediated arrhythmias without adversely depressing excitation–contraction coupling.

In the present work, the authors went a step further and tested this possibility in different preparations, ranging from a human CaV1.2 channel clone to rabbit ventricular myocytes and ex vivo rat and rabbit perfused hearts, by using a pharmacological approach (roscovitine) to selectively target late \(I_{\text{CaL}}\) (Angelini et al., 2021).

They found that roscovitine, originally developed as an anticancer agent, reduced the late \(I_{\text{CaL}}\), i.e., it decreases the pedestal component of the LTCC steady-state inactivation, suppresses EADs and Ca\(^{2+}\) transients that EAD originates (early after Ca\(^{2+}\) transients), induced by oxidative stress and hypokalemia in isolated myocytes, and either prevents or suppresses ventricular tachycardia/fibrillation in ex vivo rabbit and rat hearts subjected to hypokalemia and/or oxidative stress, without altering Ca\(^{2+}\) transients or cell shortening. As stated above, several compounds that decrease \(I_{\text{CaL}}\) window area, like Ca\(^{2+}\) channel blockers, may either diminish or suppress EADs (Cohen and Lederer, 1987). Calmodulin and Ca-calmodulin–dependent protein kinase (CaMKII) inhibitors were also able to suppress EADs that originate in oxidative stress or chronic atrioventricular blocks (Xie et al., 2009; Qi et al., 2009). However, the relevant finding of this work (which also provides its translational strength) is the demonstration that it is possible to avoid EADs
just by lowering $I_{\text{Cal}}$, pedestal, without the need of suppressing peak $I_{\text{Cal}}$, as with traditional Ca$^{2+}$ channel blockers. The main consequence of this finding is that it is possible to abolish EADs with no negative impact on excitation–contraction coupling and inotropy (Fig. 1).

Mechanistically, the authors demonstrate that the above-mentioned effects rely on the binding of R-roscovitine to the extracellular side of LTCC given that intracellular application of the drug was unable to prevent EADs. This finding is in agreement with previously reported data indicating that LTCCs contain two extracellularly exposed roscovitine binding sites. One of these sites is stereo selective (sensitive to R-roscovitine) and mediates slowed-LTCC activation, while the other is stereo insensitive and allows pharmacological "open state voltage-dependent inactivation" of LTCC (Yarotskyy et al., 2010).

Unfortunately, roscovitine has several targets other than LTCC. Indeed, roscovitine, as a multikinae inhibitor, could affect multiple cell functions. For instance, it has been shown to block hERG (Ganapathi et al., 2009; Cernuda et al., 2019) and Ito channels (Buraei et al., 2007). The authors acknowledge these limitations and clearly state that this work constitutes more a proof of concept than a direct clinical tool. This necessary proof of concept also represents a challenge and motivation for future studies on the search of new late $I_{\text{Cal}}$ inhibitors to prevent EADs triggered arrhythmias without affecting inotropy.

This latter aspect, i.e., the impact of late $I_{\text{Cal}}$, inhibition on Ca$^{2+}$ transients and contractility, should be carefully considered in future studies. While the present results suggest that the lack of effect on the peak of $I_{\text{Cal}}$ leads to a preservation of Ca$^{2+}$ transient amplitude, we could expect that reducing total Ca$^{2+}$ entry through $I_{\text{Cal}}$ would impact Ca$^{2+}$ content and Ca$^{2+}$ release (Trafford et al., 2001; Fig. 1). These actions may in turn affect the activity of calmodulin and Ca$^{2+}$-dependent kinases, like CaMKII, known to regulate Ca$^{2+}$ current inactivation. Therefore, the inotropic and intracellular Ca$^{2+}$-handling impact of late $I_{\text{Cal}}$ inhibition deserves further study considering its impact on different species/models and on a long-term basis. Moreover, it is known that $I_{\text{Cal}}$, inactivation curves are shaped by voltage and Ca$^{2+}$-dependent effects (Lee et al., 1985; Findlay, 2002; Findlay, 2004).

However, the relative role of these factors on late Ca$^{2+}$ current is far for being clear. Does roscovitine affect voltage-dependent inactivation, as might be expected according to the extracellular action of the drug and the extracellularly exposed roscovitine binding sites of LTCC (Yarotskyy et al., 2010), or can it also affect Ca$^{2+}$-dependent inactivation? Knowledge of the relative effects of Ca$^{2+}$- and voltage-dependent inactivation on late $I_{\text{Cal}}$, is a future necessary step for the search of new drugs that prevent EADs.

A second aspect that deserves additional research is whether reduction of $I_{\text{Cal}}$, is applicable to treat EADs of different origins. In this and previous works (Madhvani et al., 2015) the authors succeeded in suppress H$_2$O$_2$ and hypokalemia-induced EADs. However, and as previously mentioned, EADs that appeared in phase 3 of the AP may have a great influence of SR Ca$^{2+}$ release. Would they respond to drugs that inhibit late $I_{\text{Cal}}$? What about EADs-associated DADs (Xie et al., 2009)?

Interestingly, a recent study showed that widening or narrowing the $I_{\text{Cal}}$ window generated or abolished EADs, respectively, in human and rabbit atrium myocytes, and that this maneuver was proposed as a potential therapy for atrial EADs. In these myocytes, however, pedestals were not detected (Kettlewell et al., 2019). Could late $I_{\text{Cal}}$, inhibitors be useful in this case?

In summary, although much more work needs to be performed, the present results reveal the emergence of a new class of antiarrhythmic drugs that, unlike Ca$^{2+}$ channel blockers, may suppress EADs without significantly affecting cardiac inotropism.

Acknowledgments
David A. Eisner served as editor.

The assistance of Maria Ines Vera in preparing artwork is gratefully acknowledged.

This study was supported by grants from the National Research Council, Argentina (PIF #0305) to A. Mattiazz and from Fondo para la Investigación Científica y Tecnológica (FONCYT; PICT 2018-02204) to L.A. Gonano. The authors declare no competing financial interests.

References
Angelini, M., A. Pezhouman, N. Savalli, M.G. Chang, F. Steccanella, K. Scantlon, G. Calmettes, M. Ottolia, A. Pantazi, H.S. Karagueuzian, et al. 2021. Suppression of ventricular arrhythmias by targeting late L-type Ca$^{2+}$ current. J. Gen. Physiol. 153:e202102584. https://doi.org/10.1085/jgp.202102584
Antoons, G., P.G. Volders, T. Stankovicova, V. Bito, M. Stengl, M.A. Vos, and K.R. Sipido. 2007. Window Ca$^{2+}$ current and its modulation by Ca$^{2+}$ release in hypertrophied cardiac myocytes from dogs with chronic atrioventricular block. J. Physiol. 579:147–160. https://doi.org/10.1113/jphysiol.2006.124222
Attwell, D., I. Cohen, D. Eisner, M. Ohba, and C. Ojadi. 1979. The steady state TTX-sensitive (“window”) sodium current in cardiac Purkinje fibres. Pflugers Arch. 379:137–142. https://doi.org/10.1007/BF00586939
Bouler, E. 1943. The initiation of impulses in cardiac muscle. Am. J. Physiol. 138: 273–282.
Brown, H.F., J. Kimura, D. Noble, S.J. Noble, and A. Taupignon. 1984. The slow inward current, i{i}, in the rabbit sino-atrial node investigated by voltage clamp and computer simulation. Proc. R. Soc. Lond. B Biol. Sci. 222:305–328. https://doi.org/10.1098/rspb.1984.0066
Buraei, Z., G. Schofield, and K.S. Elmslie. 2007. Roscovitine differentially affects CaV2 and Kv channels by binding to the open state. Neuropharmacology 52:883–894. https://doi.org/10.1016/j.neuropharm.2006.10.006
Cernuda, B., C.T. Fernandes, S.M. Allam, M. Orzillo, G. Suppa, Z. Chia Chang, D. Athanasopoulos, and Z. Buraei. 2019. The molecular determinants of R-roscovitine block of hERG channels. PLoS One. 14:e0217733. https://doi.org/10.1371/journal.pone.0217733
Choi, B.-R., F. Burton, and G. Salama. 2002. Cytosolic Ca$^{2+}$ triggers early afterdepolarizations and Torsade de Pointes in rabbit hearts with type 2 long QT syndrome. J. Physiol. 543:615–631. https://doi.org/10.1113/jphysiol.2002.024570
Cohen, N.M., and W.J. Lederer. 1987. Calcium current in isolated neonatal rat ventricular myocytes. J. Physiol. 391:169–191. https://doi.org/10.1113/jphysiol.1987.sp016732
Colman, M.A., H. Ni, B. Liang, N. Schmitt, and H. Zhang. 2017. In silico assessment of genetic variation in KCNAs reveals multiple mechanisms of human atrial arrhythmogenesis. PLOS Comput. Biol. 13:e1005587. https://doi.org/10.1371/journal.pcbi.1005587
Choi, B.R., F. Burton, and G. Salama. 2002. Cytosolic Ca$^{2+}$ triggers early afterdepolarizations and Torsade de Pointes in rabbit hearts with type 2 long QT syndrome. J. Physiol. 543:615–631. https://doi.org/10.1113/jphysiol.2002.024570
de Lange, E., Y. Xie, and Z. Qu. 2012. Synchronization of early afterdepolarizations and arrhythmogenesis in heterogeneous cardiac tissue models. Biophys. J. 103:365–375. https://doi.org/10.1016/j.bpj.2012.06.007
Crane, P.F. 1975. The Conduction of the Cardiac Impulse: The Slow Response and Cardiac Arrhythmias. Futura, Mount Kisco, NY.

Crane, P.F. 1977. Action potentials, afterpotentials, and arrhythmias. Circ Res. 41:415–423. https://doi.org/10.1161/01.res.41.4.415

Fang, F.C., and A. Casadevall. 2010. Lost in translation—basic science in the era of translational research. Infect. Immun. 78:563–566. https://doi.org/10.1128/IAI.01318-09

Findlay, I. 2002. Voltage- and cation-dependent inactivation of L-type Ca2+ channel currents in guinea-pig ventricular myocytes. J. Physiol. 541: 731–740. https://doi.org/10.1111/j.1424-9094.2002.01979.x

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

Ganapathi, S.B., M. Kester, and K.S. Elmslie. 2009. State-dependent block of HERG potassium channels by R-roscovitine: implications for cancer therapy. Am. J. Physiol. Cell Physiol. 296:C701-C710. https://doi.org/10.1152/ajpcell.00633.2008

January, C.T., V. Chau, and J.C. Makielski. 1991. Triggered activity in the heart: cellular mechanisms of early after-depolarizations. Eur. Heart J. 12(Suppl F):4–9. https://doi.org/10.1093/eurheartj/12.suppl_F.4

January, C.T., and J.M. Riddle. 1989. Early afterdepolarizations: mechanism of induction and block. A role for L-type Ca2+ current. Circ. Res. 64: 977–990. https://doi.org/10.1161/01.RES.64.5.97

January, C.T., J.M. Riddle, and J.J. Salata. 1988. A model for early afterdepolarizations: induction with the Ca2+ channel agonist Bay K 8644. Circ. Res. 62:563–571. https://doi.org/10.1161/01.RES.62.3.563

Josephson, I.R., J. Sanchez-Chapula, and A.M. Brown. 1984. A comparison of calcium currents in rat and guinea pig single ventricular cells. Circ. Res. 54:144–156. https://doi.org/10.1161/01.RES.54.2.144

Kettlewell, S., P. Saxena, J. Dempster, M.A. Colman, R.C. Myles, G.L. Smith, and A.J. Workman. 2019. Dynamic clamping human and rabbit atrial calcium current: narrowing ICaL window abolishes early afterdepolarizations. J. Physiol. 597:3619–3638. https://doi.org/10.1113/JP277827

Kurata, Y., K. Tsumoto, K. Hayashi, I. Hisatome, Y. Kuda, and M. Tanida. 2020. Multiple dynamical mechanisms of phase-2 early afterdepolarizations in a human ventricular myocyte model: Involvement of spontaneous SR Ca2+ release. Front. Physiol. 10:1545. https://doi.org/10.3389/fphys.2019.01545

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

Madhvari, R.V., M. Angelini, Y. Xie, A. Pantazis, S. Suriany, N.P. Borgstrom, A. Garfinzé, Z. Qu, J.N. Weiss, and R. Olcese. 2015. Targeting the late component of the cardiac L-type Ca2+ current to suppress early afterdepolarizations. J Gen. Physiol. 145:395–404. https://doi.org/10.1085/jgp.20141288

Maltsev, V.A., H.N. Sabbah, R.S. Higgins, N. Silverman, M. Lesch, and A.I. Undrovinas. 1998. Novel, ultralow inactivating sodium current in human ventricular cardiomyocytes. Circulation. 98:2545–2552. https://doi.org/10.1161/01.CIR.98.23.2545

Marban, E., S.W. Robinson, and W.G. Wier. 1986. Mechanisms of arrhythmogenic delayed and early afterdepolarizations in ferret ventricular muscle. J. Clin. Invest. 78:1185–1192. https://doi.org/10.1172/JCI112701

McDonald, T.F., A. Cavalli, W. Trautwein, and D. Pelzer. 1986. Voltage-dependent properties of macroscopic and elementary calcium channel currents in guinea pig ventricular myocytes. Pflügers Arch. 406:437–448. https://doi.org/10.1007/BF00583365

Qi, X., Y.H. Yeh, D. Chartier, L. Xiao, Y. Tsuji, B.J. Bundel, I. Kodama, and S. Nattel. 2009. The calcium/calmodulin/kinase system and arrhythmogenic afterdepolarizations in bradycardia-related acquired long-QT syndrome. Circ. Arrhythm. Electrophysiol. 2:295–304. https://doi.org/10.1161/CIRCEP.108.818654

Qu, Z., and D. Chung. 2012. Mechanisms and determinants of ultralong action potential duration and slow rate-dependence in cardiac myocytes. PLoS One. 7:e43587. https://doi.org/10.1371/journal.pone.0043587

Segers, M. 1941. Le role des potentiels tardifs du coeur. Mem. Acad. R Med. Belg. 11–30.

Shimizu, W. 2013. Update of diagnosis and management of inherited cardiac arrhythmias. Circ. J. 77:2867–2872. https://doi.org/10.1253/circj.CJ-13-1217

Shimizu, W., and M. Horie. 2011. Phenotypic manifestations of mutations in genes encoding subunits of cardiac potassium channels. Circ. Res. 109:97–109. https://doi.org/10.1161/CIRCRESAHA.110.224600

Trafford, A.W., M.E. Diaz, and D.A. Eisner. 2001. Coordinated control of cell Ca2+ loading and triggered release from the sarcoplasmic reticulum underlies the rapid inotropic response to increased L-type Ca2+ current. Circ. Res. 88:195–201. https://doi.org/10.1161/01.RES.88.2.195

Tran, D.X., D. Sato, A. Yochelis, J.N. Weiss, A. Gardinkel, and Z. Qu. 2009. Bifurcation and chaos in a model of cardiac early afterdepolarizations. Phys. Rev. Lett. 102:258103. https://doi.org/10.1103/PhysRevLett.102.258103

Volders, P.G., A. Kulcmar, M.A. Bos, K.R. Sipido, H.J. Wellens, R. Lazzara, and B. Szabo. 1997. Similarities between early and delayed afterdepolarizations induced by isoproterenol in canine ventricular myocytes. Cardiovasc. Res. 34:348–359. https://doi.org/10.1016/S0003-6633(96)00270-2

Volders, P.G., M.A. Bos, B. Szabo, K.R. Sipido, S.H. de Groot, A.P. Gorgels, H.J. Wellens, and R. Lazzara. 2000. Progress in the understanding of cardiac early afterdepolarizations and torsades de points: time to revise current concepts. Cardiovasc. Res. 46:376–392. https://doi.org/10.1016/S0003-6633(00)00022-5

Weiss, J.N., A. Gardinkel, H.S. Karageuzian, P.S. Chen, and Z. Qu. 2010. Early afterdepolarizations and cardiac arrhythmias. Heart Rhythm. 7: 1891–1899. https://doi.org/10.1542/hjrhm.2010.09.017

Wit, A.L., and P.A. Boyden. 2007. Triggered activity and atrial fibrillation. Heart Rhythm. 4(3, Suppl):S17–S23. https://doi.org/10.1542/hjrhm.2006.12.021

Xie, L.H., F. Chen, H.S. Karageuzian, and J.N. Weiss. 2009. Oxidative-stress-induced afterdepolarizations and calmodulin kinase II signaling. Circ. Res. 104:79–86. https://doi.org/10.1161/CIRCRESAHA.108.183475

Yarotskyy, V., G. Gao, L. Du, S.B. Ganapathi, B.Z. Peterson, and K.S. Elmslie. 2010. Roscovitine binds to novel L-channel (CaV1.2) sites that separately affect activation and inactivation. J. Biol. Chem. 285:43–53. https://doi.org/10.1074/jbc.M109.076448

Zhao, Z., H. Wen, N. Fefelova, C. Allen, A. Baba, T. Matsuda, and L.H. Xie. 2012. Revisiting the ionic mechanisms of early afterdepolarizations in cardiac myocytes: Predominant by Ca waves or Ca currents? Am. J. Physiol Heart Circ. Physiol. 302:H1636–H1644. https://doi.org/10.1152/ajpheart.00742.2011