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COMMENTARY

Targeting late I_{CaL} to close the window to ventricular arrhythmias

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"...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 sarcolemmal 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_{CaL}), several inward currents have been associated with the origin of EADs, like the late Na^+ current (Attwell et al., 1979; 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

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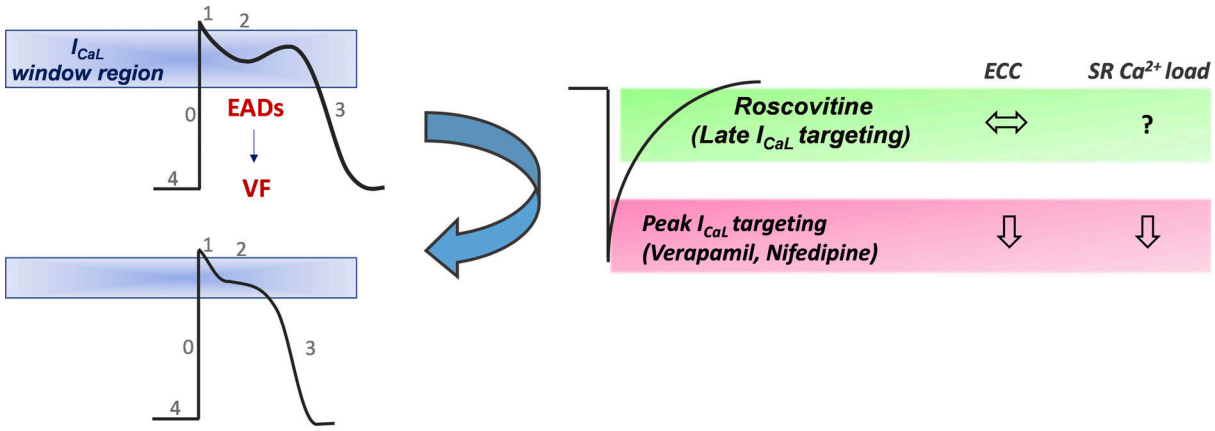

Figure 1. **Late I_{CaL} targeting narrows window current and prevents EAD/ventricular fibrillation (VF) without impairing excitation–contraction coupling (ECC).** Schematic representing EAD formation during AP and the prevention of reducing late I_{CaL} current by roscovitine. Numbers indicate phases of AP: 0 (depolarization), 1 (early repolarization), 2 (plateau), 3 (repolarization), and 4 (resting potential).

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_{CaL} volage 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. In the words of Cohen and Lederer (1987), "...the overlap of d_∞ (steady state activation) and f_∞ (steady state inactivation) represents a voltage range over which both d_∞ and f_∞ are non-zero and steady-state calcium current ('window current') will flow."

Several of the factors that contribute to set the shape and height of the Ca^{2+} window current have been experimentally manipulated to modify it. For instance, Cohen and Lederer (1987) showed that Ca^{2+} agonists and antagonists can vary the membrane potential range at which the window "opens": Bay-K 4866 shifted d_∞ and f_∞ in the hyperpolarizing direction in such a way that the magnitude of the overlap of d_∞ and f_∞ (the area of the window current) was unchanged, although the voltage range of the overlap was shifted in the hyperpolarizing direction. In contrast, D600 shifted the Ca^{2+} steady-state activation curve (d_∞) to more positive membrane potentials, reducing the overlapping window current region. In several subsequent papers, the Ca^{2+} window current was considered to play a central role in cardiac arrhythmogenesis, specifically EADs. For instance, it has been shown in cardiac myocytes with chronic atrioventricular block (cAVB) that Ca^{2+} inactivation current was shifted to more positive membrane potentials, resulting in a larger window current area in cAVB associated to EADs (Antoons et al., 2007; Qi et al., 2009). Although not specifically mentioned, the shift to the right and the larger Ca^{2+}window current observed in cAVB was associated with a higher incomplete inactivation of I_{CaL} (Antoons et al., 2007), increasing what is known as late Ca^{2+} 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_{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 Ca_V 1.2 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_{CaL} (Angelini et al., 2021).

They found that roscovitine, originally developed as an anticancer agent, reduced the late I_{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_{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_{CaL} pedestal, without the need of suppressing peak I_{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 multikinase 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_{CaL} inhibitors to prevent EADs triggered arrhythmias without affecting inotropy.

This latter aspect, i.e., the impact of late I_{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_{CaL} leads to a preservation of Ca^{2+} transient amplitude, we could expect that reducing total Ca^{2+} entry through I_{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_{CaL} inhibition deserves further study considering its impact on different species/models and on a long-term basis. Moreover, it is known that I_{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_{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_{CaL} is applicable to treat EADs of different origins. In this and previous works (Madhvani et al., 2015) the authors succeeded in suppress H_2O_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_{CaL}? What about EADs-associated DADs (Xie et al., 2009)?

Interestingly, a recent study showed that widening or narrowing the I_{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_{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.

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