Boosting the reserves: Additive regulation of cardiac repolarisation.

Adam P. Hill[1]

1. Computational Cardiology, Victor Chang Cardiac Research Institute, Sydney, NSW 2226, Australia.

Since the first description of the mechanism of digitalis toxicity in this journal in 1978 (Kass *et al.*, 1978), abnormal calcium handling has been established as a key player across a spectrum of ventricular arrhythmias. Subsequent decades of research have taught us how calcium signalling is tightly coupled to cardiomyocyte membrane electrophysiology meaning any disturbance to the electrical system will unavoidably affect calcium signalling, and vice-versa. We now appreciate the role of calcium handling in the pathogenesis of a range of cardiac dysfunctions ranging from complex multifactorial degenerative diseases such as heart failure to primary arrhythmias such as Timothy Syndrome and CPVT. Similarly, adrenergic signalling is central to arrhythmogenesis in primary electrical disorders, heart failure or post-infarct. Teasing out the intricacies of the relationship between these two processes in relation to electrophysiology of the heart is therefore a key focus area for cardiovascular physiology.

The interplay between calcium and adrenergic signalling pathways in arrhythmogenesis is perhaps demonstrated most clearly in the repolarisation disorder Long QT type 1 (LQTS1), which occurs as a result of mutations in the KCNQ1 gene, that codes for the pore forming subunit of the slow delayed rectifier current (I_{Ks}). In LQTS1, reduced repolarisation reserve in the context of high adrenergic tone results in reactivation of calcium channels to cause ectopic membrane depolarisations that may initiate the ventricular tachyarrhythmia torsades de pointes. The converse of this scenario is how does I_{Ks} in the normal heart protect against the generation of ectopic beats, especially in the setting of increased adrenergic stimulation? In the current issue, Bartos *et al.* show how intracellular calcium and adrenergic stimulation individually and additively regulate the activity of I_{Ks} in rabbit cardiomyocytes (Bartos *et al.*, 2016). Historically, the first suggestion that the delayed rectifying current (I_K) might be regulated by calcium came forty years ago (McGuigan & Tsien, 1974) and this was demonstrated experimentally some years later (Tohse, 1990). Bartos *et al*. expand on this to tease out the calcium dependence of individual components of I_K (separated into slow and rapid components - I_{Ks} and I_{Kr}) in a human-relevant larger mammal model. Surprisingly, they show that rather than being dynamically regulated as $[Ca^{++}]$ rises and falls over the course of the transient, I_{Ks} is maximally activated at concentrations continuously present in the

This is an Accepted Article that has been peer-reviewed and approved for publication in the The Journal of Physiology, but has yet to undergo copy-editing and proof correction. Please cite this article as an 'Accepted Article'; doi: 10.1113/JP273940.

sub-membrane space throughout the action potential. Furthermore, they demonstrate that adrenergic stimulation, while having similar effects on the functional properties of I_{Ks}, is additive at all concentrations of calcium.

Importantly, the authors go further and incorporate these findings into their established *in silico* models of ventricular electrophysiology. In recent years, such computational approaches have flourished and provide new avenues for quantitative analysis of cellular mechanisms and understanding of disease. In relation to measuring and modelling calcium homeostasis, much of recent progress has been driven by the Bers group, including many of the additions and updates to contemporary *in silico* models that paved the way for the current study (see eg. Shannon *et al.* 2004; Grandi *et al.* 2010). However, a key factor that has been largely absent from many existing models is an adequate description of the complex interplay between calcium handling, sympathetic tone and membrane electrophysiology – including major components of repolarisation reserve such as I_{Ks}. The findings by Bartos *et al.* begin to fill this important knowledge gap and will no doubt help realise the potential of *in silico* electrophysiology for interrogating mechanism and ultimately risk stratification in disease.

What then are the implications of the Bartos study for our understanding of disease? The authors suggest that under conditions of calcium overload – such as in the failing heart – calcium regulation of I_{Ks} might be disrupted to contribute to the occurrence of lethal arrhythmias. This is certainly an important area for research as up to half of deaths in heart failure patients occur suddenly (Ørn & Dickstein, 2002). Typically, in heart failure there is elevated diastolic calcium with reduced or attenuated peak of the transient. Even so, since peak sub-membrane calcium concentrations are almost an order of magnitude higher than that shown to be required for maximal activation of I_{Ks}, it is unlikely even in the failing heart that sub-membrane calcium is lowered sufficiently to reduce I_{Ks} activity during the action potential. However, the findings of Bartos *et al.* offer several other potential routes to pathogenesis. First, they show that the effect of adrenergic stimulation in activating I_{Ks} is completely additive, over and above the effect of calcium. In the failing heart, β-adrenergic signal transduction can be dramatically reduced as a result of receptor desensitisation as well as changes in enzyme expression (Bristow, 1993). This would significantly reduce the I_{Ks} contribution to repolarisation, regardless of the calcium concentration and particularly at high heart rates. A second, more subtle possibility raised by the authors relates to the kinetics of I_{Ks} activation. In addition to changes in the baseline and amplitude of the calcium transient, those from failing hearts also have a slower rising phase which could result in slower I_{Ks} activation and hence reduced repolarisation in phase 2 of the action potential. Whilst in the present study the authors were not able to accurately measure this, it poses an interesting question for future work to explore the kinetics of I_{Ks} activation in relation to dynamic changes in calcium. The Bartos paper therefore presents several important findings around quantification of the regulation of I_{Ks} by calcium and adrenergic stimulation and updates their *in silico* ventricular model to reflect them – thus filling an important knowledge gap in computational electrophysiology. The study also sets the scene for future work, perhaps in tissue from human failing hearts, that would help further tease out the subtleties of regulation of the repolarisation reserve by both calcium and adrenergic signalling.

Bartos D, Morotti S, Ginsburg K, Grandi E & Bers DM (2016). Quantitative analysis of the Ca^{2+}-dependent regulation of delayed rectifier K^+ current I_{Ks} in rabbit ventricular myocytes . *J Physiol (Lond)*1–35.

Bristow MR (1993). Changes in myocardial and vascular receptors in heart failure. *J Am Coll Cardiol*; DOI: 10.1016/0735-1097(93)90465-D.

Kass RS, Lederer WJ, Tsien RW & Weingart R (1978). Role of calcium ions in transient inward currents and aftercontractions induced by strophanthidin in cardiac Purkinje fibres. *J Physiol (Lond)* **281,** 187–208.

McGuigan J & Tsien RW (1974). Some limitations of the double sucrose gap, and its use in a study of the slow outward current in mammalian ventricular muscle. With an Appendix. *J Physiol (Lond)*.

Tohse N (1990). Calcium-sensitive delayed rectifier potassium current in guinea pig ventricular cells. *Am J Physiol* **258,** H1200–H1207.

Ørn S & Dickstein K (2002). How do heart failure patients die? *European Heart Journal Supplements*.