TRPC6 CHANNELS AS A DRIVER OF CARDIAC ARRHYTHMIAS

Andrew Trafford, Katharine Dibb, David Eisner, David Hutchings, Olivia Johnstone, Yatong Li, Hieu Nguyen, Barbara Niort

Unit of Cardiac Physiology, Division of Cardiovascular Science, University of Manchester, Manchester M13 9NT, United Kingdom.

Andrew.W.Trafford@manchester.ac.uk

Cardiac arrhythmias are a significant cause of cardiovascular morbidity and mortality and the link between perturbed intracellular calcium homeostasis and cardiac arrhythmias is long-established. For example, overloading of the intracellular calcium store, the sarcoplasmic reticulum (SR), or alterations in the ability of the SR to retain calcium leads to the spontaneous release of calcium from the SR into the cytoplasm in the form of calcium sparks and calcium waves. Some of this cytosolic calcium is then extruded from the cell by the electrogenic sodium-calcium exchanger generating an inward, depolarising, membrane current and triggered arrhythmias. Whilst this mechanism is firmly established, a key question remains regarding how calcium enters the cell to allow the SR to reach the critical threshold calcium content at which spontaneous arrhythmogenic calcium release occurs. Previous work from our group [1] had suggested that a mechanism distinct from the L-type calcium channel and reverse mode sodium calcium exchange supported a background calcium entry into cardiac myocytes. However, the lack of available pharmacological and molecular tools at the time of this original study did not allow us to identify the cause of this background calcium influx. More recently, it was suggested that canonical transient receptor potential (TRPC) channels [2] may mediate a background calcium influx in mouse ventricular myocytes. Notably, TRPC6 channels are inhibited by protein kinase G (PKG) dependent phosphorylation [3]. Given these considerations, the first aim present study was designed to identify the nature of the background calcium influx that *a priori* is required to maintain spontaneous calcium release from the SR and give rise to calcium dependent triggered arrhythmias. The second aim of the study was then to determine if the antiarrhythmic effect of acute phosphodiesterase 5 inhibition, and thus augmented PKG activity, we have recently reported [4] is, at least in part, due to an effect on the source of background calcium influx potentially occurring via TRPC6 channels. Experiments were performed in isolated sheep ventricular myocytes and Langendorff perfused mouse hearts and we used, respectively, high external calcium concentrations and programmed electrial stimulation protocols to induce arrhythmic behaviour. In isolated cell experiments we found that background calcium influx was exclusively carried by TRPC6 channels as inhibition of L-type calcium channels, sodium calcium exchange and TRPC1,4,5 channels had no effect on calcium overload induced calcium wave formation or background calcium influx. At the whole heart level, catecholamine exposure and programmed electrical stimulation reliably induced arrhythmias and arrhythmia scores were statistically attenuated by manoeuvres leading to an increase in PKG activity. Additionally, on exposure to the PDE5 inhibitor sildenafil, the frequency of calcium sparks was reduced in isolated myocytes. Together, these results indicate a specific role for TRPC6 mediated background calcium entry in the genesis of calcium dependent arrhythmias in ventricular myocytes. Additionally, our data demonstrates that targeting PKG activity also attenuates calcium dependent arrhythmias which is at least conceptually consistent with the role of PKG dependent inhibition of TRPC6 channel activity.

Acknowledgements: This work was supported by research grants from The British Heart Foundation and Medical Research Council.