“Battle of the Clocks” – Sinoatrial pacemaking through plasmalemmal ionic currents or intracellular Ca^{2+} release

M.S. Imtiaz and D.F. van Helden, The Neuroscience Group, School of Biomedical Sciences, The University of Newcastle, Callaghan, NSW 2308, Australia.

The sinoatrial node (SAN) initiates and regulates cardiac pacemaking. Traditionally, the mechanism underlying generation of pacemaking in the SAN cells (SANCs) has been considered to arise through a “clock”, termed the plasmalemma clock, which resides in the cell membrane and involves cyclical interaction of various voltage-dependent channels. Recently a rival intracellular pacemaker mechanism, termed the store clock, has been proposed to dominate cardiac pacemaking. This mechanism is proposed to arise from rhythmic Ca^{2+} release from intracellular Ca^{2+} stores which trigger or drive the plasmalemma clock. We have used experimental and modeling techniques to study the interaction between these two clocks underlying SAN pacemaking.

Tissue preparations containing the SAN and associated muscle were isolated from mouse heart freshly removed from young adult mice (age 5-8 weeks). Mice were euthanased by overexposure to the inhalation anaesthetic isoflurane (5-10% in air), a procedure approved by the Animal Care and Ethics Committee at the University of Newcastle. Tissues were loaded with a calcium indicator (Oregon Green/AM) and viewed with a high speed confocal imaging system (Perkin-Elmer Ultraview). In some cases intracellular microelectrode recordings were made simultaneously with the imaging. Numerical simulations were performed using a single SAN cell model containing two types of oscillators; 1) a plasmalemma oscillator composed of pacemaker, Na^+, K^+, and L-type Ca^{2+} currents, 2) an intracellular Ca^{2+} oscillator composed of a Ca^{2+} store with cyclical release and uptake and Ca^{2+} induced Ca^{2+} release capability. These two oscillators were linked by a Na-Ca exchanger current that transformed the Ca^{2+} oscillations into membrane potential oscillations. Thus the two clocks interacted through 1) Na-Ca exchanger and 2) Ca^{2+} entry through L-type Ca^{2+} channels. A multi-cellular model was also simulated using the single model cells now coupled by gap junctions.

The main results of our study were: 1) blockade of ryanodine receptors (RyRs) decreased pacemaker frequency but did not abolish pacemaking, thus pacemaking can continue in the absence of RyR-mediated store Ca^{2+} release; 2) action potentials continued in the presence of Ca^{2+}-free solution containing EGTA (0.1 mM) but now without associated changes in intracellular Ca^{2+}; and 3) Ca^{2+} increases were at times observed to precede action potentials during recovery from pacemaker blockade by either 2,3-buanedione monoxime (BDM) or high K^+ containing solutions. These results demonstrate that SAN pacemaking can continue in the absence of intracellular store clock but Ca^{2+} released from intracellular Ca^{2+} stores can advance the plasmalemma clock and effectively set the pacemaking frequency of the SAN (see also Lakatta *et al.*, 2003). Our modelling studies suggest that each clock is capable of dominating the other depending on the mutual frequency relationship. Importantly, in a group of gap junction connected pacemaker model cells the store clock can induce some cells to become the dominant pacemaker and *vice versa*.

We predict that in this symbiotic relationship the winner between these two clocks may vary according to various influences placed on the heart. Further studies are needed to show how these mechanisms interact to respond to such changing demands.

Lakatta EG, Maltsev VA, Bogdanov KY, Stern MD & Vinogradova TM. (2003) *Circulation Research*, **92:** e45-50.