Calcium signalling, pacing and rhythmic cell activity in the lymphatic system - a physiologist's paradise

D.F. van Helden, School of Biomedical Sciences, University of Newcastle, NSW 2308, Australia.

My interest in this area first arose while studying mesenteric veins in young guinea pigs. The mesentery is also replete with striking arrays of lymphatic vessels, demarcated into chambers by frequently occurring unidirectional valves. A feature that really caught my attention was that the lymphatic chambers at times exhibited spontaneous rhythmical contractions that acted as "primitive" hearts to propel lymph. This phenomenon was so intriguing that the recording microelectrodes, while intended for the small veins, often "magically" strayed to record electrical activity from the lymphatic smooth muscle.

The venous studies were being made to investigate spontaneous transient depolarizations (STDs), an activity reminiscent of transient depolarizations termed "bumps" that had been reported in photoreceptors. STDs also paralleled spontaneous transient hyperpolarisations that had been reported in neurons and smooth muscle, differing primarily in their polarity. We investigated these events, providing evidence that they were generated by spontaneous release of Ca^{2+} from intracellular Ca^{2+} stores and finding that the underlying currents, which we termed spontaneous transient inward currents (STICs), were likely to be generated by Ca^{2+}-activated Cl^{-} channels. The significance of these events took on a whole new dimension once microelectrode recordings were made in lymphatic chambers, which indicated that lymphatic smooth muscle not only exhibited STDs but that they, or summations thereof, were responsible for driving rhythmical lymphatic constrictions. This was most surprising, as prior to this pacemaking was considered to be generated by voltage-dependent channels in the cell membrane, as had been first described for heart pacemaking. The fact that Ca^{2+} stores might also act as a pacemaker introduced a very different mechanism, one operated by an intracellular pacemaker "clock" set by the release-refill cycle of Ca^{2+} stores.

The mechanism soon proved to be more widespread, having roles in other pacemaker activities such as vasomotion and gastrointestinal contractions. However, there remained a significant question, as to how the mechanism operated, given that individual Ca^{2+} release events are small and could not activate sufficient current to drive pacemaking. The key to solving this came with the realisation that stores had the capacity to interact as coupled oscillators and so coordinate their activity. A simple example of a coupled oscillator system is an array of pendulums interconnected by springs, which entrain their cycles when randomly activated. In the case of stores, coupling between stores is mediated by both diffusion of store activators between stores (*e.g.* Ca^{2+}) and by voltage coupling. The latter is mediated through transmission of membrane depolarization causing enhancement of store activators and hence store cycling. It provides some 500 fold stronger coupling between stores than does diffusion of activators. Evidence for the latter has been provided by studies on blood vessels and strips of gastric smooth muscle.

The same coupled oscillator-based mechanism can generate long-range chemical/electrical signalling within cells and cellular syncytia. The mechanism subserves a similar role as sequentially conducting action potentials, but is very different depending on Ca^{2+} stores interacting as coupled oscillators and not voltage dependent channels in the cell membrane. Importantly, while coupling between Ca^{2+} stores may lead to near synchronous local chemical/electrical signalling, substantial phase delays develop between oscillators over longer distances giving the impression of a propagating wave. We refer to such propagation as Ca^{2+} phase waves. This mechanism is likely to underlie propagation of slow waves in the gastrointestinal system and hence is of considerable importance.

Biology continues to astonish even in well-studied areas such as heart pacemaking, as it has now been shown that Ca^{2+} store-based pacemaking is also a player in the generation of heartbeats. Thus two "clocks" mediate heart pacemaking, the "classical" pacemaker in the cell membrane and the intracellular Ca^{2+} store "clock". These interact symbiotically to produce robust heart pacemaking. Interestingly, lymphatic pacemaking under certain conditions may also be mediated by both these mechanisms and hence lymphatic "hearts" may not be as primitive as previously thought. The existence of store pacemaking and Ca^{2+} phase waves provides new therapeutic directions for treatment of various pathologies (*e.g.* arrhythmias). In case of the lymphatics, targeting the mechanism may lead to better treatments of disorders such as lymphedema. On the flip side, inhibiting the lymphatic pump may also provide a new first aid treatment for snakebite, as venoms usually first transit the lymphatic system. This subject is of some significance, as snakebite remains a world health problem with ~100,000 deaths and 400,000 amputations per year. Fortunately, this approach may have merit, as we find that lymphatic transport is considerably slowed by topical application of an agent that inhibits lymphatic pacemaking.