Clock systems, coupled node signaling and pacemaker function: experimental and computational perspective

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The sinoatrial node (SAN) is the primary pacemaker that controls the heart rate under normal conditions. The SAN consists of hundreds to thousands (depending on the mammal species) of pacemaker cells that can beat spontaneously even without any neural stimulation. A system of two coupled clock control the pacemaker cell function: the surface membrane clock (M clock), an ensemble of sarcolemmal electrogenic molecules, and the Ca^{2+} clock (the sarcoplasmic reticulum (SR)). The two clocks are coupled through Ca^{2+} signaling: local Ca^{2+} releases (LCRs) activate an inward $Na+/Ca^{2+}$ exchange (NCX) current and other Ca^{2+} dependent mechanisms that prompt the M clock to generate an action potential (AP). Ca^{2+} not only directly couples the clocks, but also does so indirectly through the phosphorylation cascade: Ca^{2+} activates calmodulin-adenylyl cyclase (AC)-dependent protein kinase A (PKA) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). These phosphorylation signaling cascades act on the same Ca^{2+} (ryanodine receptors and phospholamban) and M-clock proteins (L type Ca^{2+} channels and K^{+} channels). The increase in phosphorylation activity leads to amplification of the LCR Ca^{2+} signal and therefore to an increase in the AP firing rate. Thus, both Ca^{2+} cycling and phosphorylation activity levels indicate the degree of clock coupling: when the signal is further amplified, so are the activity levels of the membrane and SR Ca^{2+} molecules, and thus the AP firing rate is higher as well (and vice versa when the node signals are de-amplified).

Recently changes in Ca^{2+} and phosphorylation signaling were associated with different cardiac diseases, the majority of which were also associated with bradycardic or tachycardiac heart rate. To understand the mechanisms that lead to heart dysfunction, the dynamics of Ca^{2+} and phosphorylation signaling must be measured under different physiological and pathophysiological conditions. To achieve this, cultured cells that can be genetically manipulated and/or virally infected to introduce probes to measure these signals are necessary. However, culturing rabbit pacemaker cells leads to a loss of their physiological spontaneous AP firing rate and deterioration of signaling that determine the pacemaker cell function.

We designed a new culture method that sustains the pacemaker cell AP firing rate along with biochemical signaling that maintains its function. Rabbit pacemaker cells (isolated from anaesthetized animals) were maintained in culture for 48h in a medium enriched with a myofilament contraction inhibitor, either BDM (2,3-Butanedione 2-monoxime) or blebbistatin. The area of the cells was maintained at the same level as fresh cells with either BDM or blebbistatin. Importantly, the cells could be successfully infected with a GFP adenovirus using either culture conditions. However, blebbistatin maintains the AP firing rate and Ca^{2+} transient characteristics closer to fresh level than BDM. Action potential parameters, Ca^{2+} transients, and local Ca^{2+} spark parameters were similar in the cultured cells with blebbistatin and in fresh cells. Moreover, culturing cells with blebbistatin maintain the phosphorylation activity of both phospholamban and ryanodine intact.

In parallel to experimental methods, computational modeling is used to describe phenomena that cannot or hard to quantify experimentally. We used our computational model to provide insight why eliminating contraction during culture preserves pacemaker function. Our computational model predicts that (i) preservation of energy by eliminating contraction helps to conserve the pacemaker function in culture (ii) maintaining pH during culture helps to conserve the pacemaker function in culture, (iii) the side effects of BDM reduce its effectiveness compared to blebbistatin. Thus, eliminating contraction by blebbistatin during the culture period preserves rabbit pacemaker cell functions.