Nuclear Calcium Transients: *Hermes Propylaios* in the Heart

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“...O cunning guide, son of Maia, I fear that you might steal my lyre and my curved bow. For you hold the office from Zeus to establish deeds of exchange among men throughout the fertile earth. But if you would suffer to swear the great oath of the gods for me, either by nodding your head or by the mighty water of the Styx, you would accomplish everything pleasing to my own heart...” (Homeric Hymns)

In Greek Mythology, Hermes (Greek: Ἑρμῆς), son of Zeus and the pleiad Maia, was perhaps the most complex deity of transitions and boundaries, contemporarily simplified as messenger god that moved freely between the higher worlds at Olympus, our world with human mortals and the grim underworld of the deceased. As second youngest of the Olympian gods and symbolizing Hermes' function as divine messenger, he is often sculptured as a youngster with winged sandals, winged cap, and holding his symbolic herald's staff, the Greek kerykeion, consisting of two snakes wrapped around a winged staff. Hermes received multiple epithets including Propylaios, freely translated as "at the gateway", reflecting a deeper philosophical significance of Hermes.

As with all imaginary Greek deities, Hermes’ actions do not describe a uniformly positive character. Tales rather tell a complex, three-layered personality, ranging from a truly uplifting spirit as would be expected from his divine roots, to a character with an innocent yet cunning nature, but at times he also displayed unexpected evil. With these three faces, Hermes likely symbolized how our Greek ancestors segmented the universe, consisting of a noble and divine upper world physically located at Mount Olympus; planet Earth with all its living creatures including mankind; and finally, the dark side of disease, suffering and death, represented by the underworld.

Cytoplasmic calcium and excitation contraction coupling
The multi-faceted roles that calcium plays in the heart may well bear analogy to the mythological character Hermes. First and foremost, for the maintenance of cardiac contraction, there may be no more important ion than calcium. During each heartbeat, a small leak of calcium through voltage-dependent L-type calcium channels (VDLC) triggers massive calcium release from the sarcoplasmatic reticulum (SR) through SR bound ryanodine receptors (RyR2) into the cytoplasm of heart muscle cells, which triggers muscle contraction in a process referred to as excitation-contraction coupling.1 Cardiac relaxation is initiated by the concerted action of the sodium/calcium exchanger and muscle-specific SR calcium ATPase-2 (SERCA2), which re-sequesters calcium into the internal calcium storage pool, allowing for the next quantal release of calcium. On top of this elaborate system of dedicated calcium release channels and calcium pumps, the β-adrenergic receptor system controls the magnitude of the calcium transient by activating protein kinase A, a kinase that by virtue of its direct phosphorylation of the calcium release channels and calcium pumps allows for intracellular calcium transients with higher amplitude and faster decay.

Heart failure is characterized by disturbed excitation-contraction coupling, systolic and diastolic abnormal contractile properties, fueling speculations that correcting the calcium transient may form a rationale to recover cardiac contractility. Indeed, the cornerstone of contemporary heart failure pharmacotherapy is β-adrenergic blockade2,3 and at least one rationale for its therapeutic benefit is suppression of the hyperadrenergic state and restore normal RyR2 calcium release channel function in patients with heart failure.4 On top of that, at least one new therapeutic strategy aimed to normalize calcium transients in heart failure has now reached clinical stages where viral-mediated gene therapy is directed to increase SERCA2a expression to improve SR Ca^{2+} reuptake.5
Nucleoplasmic calcium transients

Secondly, each cytoplasmic calcium transient also elicits nucleoplasmic calcium transients, which is a far less studied rhythmic phenomenon that also takes place simultaneously during cardiac contraction.\(^6\) Potentially, alterations in the kinetics or amplitude of nucleoplasmic calcium transients may have functional ramifications, because this pool of calcium represents the prime candidate to influence calcium-dependent gene regulatory signaling events in a phenomenon referred to as excitation-transcription coupling.\(^7\) Indeed, a vast body of literature indicates that calcium/calmodulin acts as an important second messenger in the heart downstream of growth factors, adrenergic signaling and mechanical stretch that have the ability to trigger hypertrophic growth of the myocardium.\(^8\) Immediate recipient signals for calcium as second messenger are the calcium/calmodulin-dependent phosphatase calcineurin,\(^9\) which enables translocation of the transcription factor NFAT to the nucleus and in turn activates transcriptional pathways that reprogram cardiac gene expression either alone or by activating additional hypertrophic transcription factors including GATA4, MEF2 or Hand2.\(^9,10\)

Alternatively, calcium/calmodulin-dependent protein kinase (CaMK) signaling relieves histone-deacetylase (HDAC)-dependent repression of MEF2 transcriptional activity and facilitates the induction of MEF2-responsive gene expression.\(^11\)

Nucleoplasmic calcium transients obey distinct boundaries, involve different partners, and play by slightly different rules than their cytoplasmic counterparts. First, the main store for nucleoplasmic calcium transients is formed by the nuclear envelope. As such, the nuclear envelope not only contributes to nuclear structure and insulation from the surrounding cytoplasm, but also serves as the main reservoir for bidirectional transport of calcium ions and allows bidirectional transport of macromolecular complexes. The balance between release and
storage of calcium in the nuclear envelope is regulated by calcium release channels, including the RyR2 and inositol 1,4,5-trisphosphate receptor (IP3R), Ca\(^{2+}\)-buffering proteins, and calcium pumps (SERCA2) that refill the nuclear envelope with calcium,\(^{12}\) reminiscent to the sarcoplasmatic reticulum storage control.

Prior characterizations of the nucleoplasmic calcium transient established that generation of the systolic calcium peak likely involves both a passive and an active component. The passive component is probably mediated by cytoplasmic calcium diffusion through nuclear pore complexes to increase nuclear calcium concentration, since the amplitude of the nuclear calcium transient is proportional to the amplitude of the cytoplasmic calcium transient.\(^{13}\) However, several lines of evidence also indicate that the nucleus is capable of actively storing and releasing calcium ions, since individually studied myocytes displayed prominently higher nucleoplasmic diastolic calcium concentration and higher nucleoplasmic systolic calcium peaks.\(^{13}\) This observation is in line with the fact that endothelin-1 can induce an independent increase in nuclear calcium without changing cytosolic calcium transients, and that this form of calcium release originates from perinuclear stores.\(^{14}\)

Stimulation of heart rate frequency increases systolic nuclear calcium in parallel with cytoplasmic systolic calcium, but diastolic nuclear calcium rises substantially higher than their cytoplasmic counterparts, demonstrating a slower decay of the nucleoplasmic calcium transient and a much higher buildup of nuclear calcium when diastole is shortened.\(^{13}\) Whether this frequency-dependent physiological maneuver has implications for excitation-transcription coupling has remained unclear to date. In conclusion, prior studies support the idea that calcium release from the nuclear envelope at least in part plays an active role in nuclear calcium transient generation and fed speculations that nucleoplasmic transients may possibly alter in and
contribute to pathophysiological processes.

**Nucleoplasmic calcium transients and excitation-transcription coupling**

In this issue of *Circulation*, a novel report from the group of Pieske and coworkers now adds valuable new information to the pathophysiology of nuclear calcium homeostasis. Ljubojevic *et al.* used a cross-species and multidisciplinary approach to systematically compare the structural and functional changes of the nuclear envelope and nuclear calcium handling in myocytes derived from the healthy myocardium, the early hypertrophic phase and late stage heart failure.

In short, early during hypertrophy, the authors observed an increased nuclear size and less frequent intrusions (invaginations) of the nuclear envelope into the nuclear lumen.

Accompanying these ultrastructural changes, a reduced expression of the calcium release channel RyR2 and the calcium pump SERCA2, versus an increase in IP3R, and a differential localization of these various calcium transporters at the nuclear envelope were observed. Most importantly, nuclear calcium transients displayed diminished amplitudes, lengthening of nuclear transient duration and increased diastolic calcium levels, reminiscent to cytoplasmic calcium transients from failing myocytes. Finally, the ultrastructural changes of the envelope and alterations in nucleoplasmic transients were accompanied by a higher activation status of CAMKII and concomitant nuclear export of HDAC4, suggesting a functional contribution of the altered nucleoplasmic transient in excitation-transcription coupling. These data therefore provide a plausible explanation for the mode of activation of calcium/calmodulin-induced gene regulatory systems in the earliest phases of heart failure.

As with all breakthrough studies, these new observations also provoke a myriad of new questions that will stimulate the exploration of new experimental horizons. One less obvious question would start with asking the rationale for the mere existence of nuclear transients in the
healthy heart. Likely, our lack of understanding this phenomenon must be related to a fine-tuned comprehension of the genomic architecture that may require calcium-dependent processes for normal nuclear function. As similar findings have been observed in other organs besides the heart, it is expected that our field will witness the birth of new disciplines and technologies, including nuclear electrophysiology and optimized fluorescent reporter systems. Other questions are whether the tubular invaginations of the nuclear envelope resemble sarcolemmal T-tubuli, allowing juxtaposition of as of yet to be discovered critical calcium release channels and fine-tuned control of nuclear calcium, and what mechanism provokes the observed reduction of nuclear pore intrusions. On a more molecular level, readily testable hypotheses that now arise are whether the various calcium release channels and pumps also undergo post-translational modifications similar to their counterparts at the sarcolemma and SR, and if so, at which amino acid sites, controlled by which balance of kinases/phosphatases and what functional ramifications these biochemical changes may provoke. A particularly intriguing aspect of the current study is the observation that with higher heart rate frequency, nuclear diastolic calcium concentration increased to a much higher extent by a slower decay than cytoplasmic transients. It is tempting to speculate that the frequency dependent rise in diastolic nuclear calcium is causally related to the activation of pro-hypertrophic gene regulatory cascades and translates to the often elevated heart rates of heart failure patients. Indeed, the SHIFT trial data demonstrate a beneficial effect of lowering heart rate by ivabradine in patients with advanced systolic heart failure, both in terms of attenuated LV remodeling and less frequent hospitalizations. To more firmly establish whether tachycardia is causally related to the earlier phases of cardiac remodeling, animal models of heart failure can be placed on heart rate lowering medication and simultaneously monitor nuclear calcium transients, cardiac remodeling and disease symptoms.
Notwithstanding these as of yet unanswered questions, the current study is in support of the premise that the nuclear ultrastructure is causally related with alterations in nuclear calcium transient generation and the pathophysiological phenomenon of excitation-transcription coupling. The multi-faceted functions of the seemingly simple ion calcium transversing physical boundaries and communicating at the level of the sarcomere, nuclear integrity and excitation-transcription coupling are not unlike mythological Hermes, influencing the divine sarcomeric contraction, homeostatic nuclear calcium transients in life and igniting myocardial disease and death.

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