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Short-term memory in the heart: A road map for channel trafficking required

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“Cardiac memory” refers to the phenomenon of alterations in T-wave morphology induced by a period of ventricular pacing, which persist for some time after returning to sinus rhythm.¹ To induce cardiac memory, the ventricular pacing rate only needs to achieve a modest 5% to 10% above sinus rates. Thus, different from the ventricular tachypacing-induced heart failure models, cardiac memory is not associated with pathologic changes in the heart. The degree of cardiac memory can be quantified by T-wave vector displacement (TVD). The duration of cardiac memory depends on the length of previous ventricular pacing. Dr. Rosen and his group have operationally defined short-term cardiac memory (STCM, induced by 15 minutes to 2 hours of ventricular pacing, lasting from minutes to hours) and long-term cardiac memory (LTCM, induced by 2 to 3 weeks of ventricular pacing, lasting from weeks to months).¹

The motivation to understand the molecular mechanisms and signal transduction pathways involved in LTCM is obvious. A similar phenomenon can occur in patients with implanted pacemakers who experience chronic ventricular pacing.

Alterations in T-wave morphology suggest changes in the pattern of ventricular repolarization. Therefore, when considering the molecular mechanisms underlying cardiac memory, channels contributing repolarizing currents (transient outward, I_{to} , rapid and slow components of delayed rectifier, I_{Kr} and I_{Ks}) or depolarizing currents (L-type Ca current, I_{CaL}) during ventricular action potentials are candidates. Indeed, LTCM in the canine model is associated with a preferential reduction of I_{to} and I_{Kr} in the epicardial region of the left ventricle (LV).^{1,2} In the canine heart, both I_{to} and I_{Kr} exhibit a transmural gradient (epicardial > endocardial) in the LV. A preferential decrease in epicardial I_{to} and I_{Kr} will lead to a reduction in their transmural gradient, contributing to alterations in the pattern of ventricular repolarization. LTCM is also associated with changes in the gating kinetics of I_{CaL} , which can perturb ventricular repolarization.¹ The decrease in I_{to} and I_{Kr} during LTCM is associated with a downregulation of genes encoding their pore-forming subunits (Kv4.3 and ERG, respectively). In the case of I_{to} , there is also a downregulation of the auxiliary subunit KChIP2. Experiments have begun to unveil the signaling pathways involved in I_{to} downregulation during LTCM.¹

Relative to LTCM, less is known about STCM. STCM shares the following features with LTCM: (1) it is associated with a decrease in epicardial I_{to} , and (2) it can be prevented by angiotensin-converting enzyme inhibitors, angiotensin receptor type 1 (AT1R) blockers, or I_{CaL} antagonists. However, there are no changes in Kv4.3 mRNA or in KChIP2 mRNA or protein. The mechanism for I_{to} reduction during STCM has been elusive.

Previously, the Cohen group showed that Kv4.3 (coexpressed with KChIP2) can physically associate with AT1R to form a macromolecular complex in ventricular myocytes and when coexpressed in HEK293 cells.³ Importantly, when AT1R is internalized after stimulation by angiotensin II (Ang II), it brings the associated Kv4.3 along. Internalization of Kv4.3 is correlated with a decrease in I_{to} current density.³ In this issue of *HeartRhythm*, Ozgen et al⁴ of the Rosen/Cohen group test the hypothesis that the reduction of I_{to} seen in STCM is due to an increase in KChIP2 internalization along with AT1R following stimulation by Ang II. Inspired by reports showing that internalization of surface proteins is often driven by motor proteins along microtubules,⁵ they further hypothesize that disrupting microtubules can prevent I_{to} internalization and thus prevent or reduce STCM.

Ozgen et al used a 2-hour LV pacing canine model to study STCM. They divided LV pacing dogs into 3 groups: no treatment (group 1), treatment with colchicine (microtubule disruptor, group 2), or losartan (AT1R blocker, group 3). Sham dogs (group 4) were similarly instrumented but were atrial paced. They aimed to directly link *in vivo* data (TVD, based on body surface ECG) to changes in target proteins in the heart. To achieve this aim, biopsy samples were taken from LV epicardium 1 to 2 cm away from the pacing site before and at the end of pacing, and used for biochemical experiments to quantify KChIP2, AT1R, and β -tubulin protein levels at the whole-cell level and in subcellular fractions. Finally, they used single canine epicardial myocytes to test the effects of AT1R stimulation and/or colchicine treatment on I_{to} and action potential configuration/duration.

Ozgen et al⁴ reported four major findings. First, colchicine treatment was as effective as losartan in reducing the degree of STCM, suggesting that intact microtubules were required for the manifestation of STCM. Second, although 2-hour pacing did not alter the level of KChIP2 or AT1R protein in whole-cell lysates, pacing significantly reduced both proteins in the plasma membrane (inferred from Western blotting of the light membrane fraction). Furthermore, colchicine or losartan treatment prevented pacing-induced decrease in KChIP2 and AT1R proteins at the plasma membrane. This finding indicates that LV pacing for 2 hours led to KChIP2 and AT1R internalization in a microtubule-dependent manner. Third, when comparing biopsy samples from the same hearts before and after pacing, pacing with colchicine treatment shifted β -tubulin from polymerized to nonpolymerized fractions, whereas pacing alone did not affect the level or distribution of β -tubulin. This finding confirms the efficacy and specificity of colchicine as a microtubule disruptor. Fourth, incubation of isolated canine epicardial myocytes with Ang II for 2 hours (to mimic STCM) caused a dramatic decrease in I_{to} and a prolongation of action potential duration. Both effects were prevented by simultaneous treatment with colchicine. This finding serves to correlate the biochemical data from biopsy samples with I_{to} function in ventricular myocytes. Taken together, the results fully support the investigators' hypothesis: the decrease in I_{to} during STCM can be explained by an internalization of KChIP2 with AT1R via microtubules, and disrupting micro-tubules can reduce or prevent STCM.

The study by Ozgen et al⁴ is a beautiful example of an increasing number of scenarios where changes in cellular milieu induce alterations in ion channel trafficking, which exert a major impact on cardiac cellular activity (for example, see references ⁶ and ⁷). These observations tell us that ion channels are not static proteins in the cell surface membrane. Importantly, alterations in electrical activity (electrical remodeling) cannot be entirely described by changes in gene transcription, mRNA translation, or microRNA interference. To function properly, ion channels need to be at the right place, at the right time, and in the right amount. These dynamic processes need to be regulated so that cells can properly respond to environmental cues. Failure to do so can lead to inconveniences in life, or even disastrous consequences.

Investigations of molecular mechanisms underlying ion channel trafficking and their regulation in cardiac myocytes is still a young field. It is challenging to follow protein movements through various subcellular compartments. Fortunately, cell biologists have worked out the basic itinerary of how membrane proteins traffic from the endoplasmic reticulum, through the Golgi, to reach the plasma membrane.⁸ More details about the pathways involved in endocytosis, recycling, and degradation and their regulation have been discovered.^{9,10} Tagging channel subunits with fluorescent proteins, in conjunction with live cell imaging, allows investigators to track protein movements in a real-time mode.¹¹ These techniques can be applied to cardiac myocytes using viral-based gene transfer.¹² Therefore, tools have become available and basic paths have been mapped out to enable the exploration of how ion channels move in cardiac myocytes and how their movements are regulated or modulated.

In this context, the study by Ozgen et al⁴ inspires the following questions. First, assuming that Kv4.3 is internalized along with KChIP2 and AT1R during STCM, where does this occur? Immunocytochemistry/confocal experiments suggest that Kv4 channels have 2 main addresses in ventricular myocytes, t-tubules and intercalated discs,¹³ where the channel proteins may be anchored by different mechanisms.¹⁴ The effect of Kv4.3/KChIP2 retrieval from the cell surface on the local membrane voltage during action potentials may depend on the location where the retrieval occurs: reduction of Kv4.3/KChIP2 in the t-tubule membrane may impact on excitation-contraction coupling, whereas retrieval of Kv4.3/KChIP2 from the intercalated disc area may impact on impulse conduction. Second, if Kv4.3/KChIP2 proteins are reduced at the plasma membrane but are not altered at the whole-cell level, it is reasonable to assume that within the time frame of the experiments, the channel complex has not been fully recycled back to the cell surface, nor has it been markedly degraded inside the cell. What happens to the internalized Kv4.3/KChIP2 during STCM? Do they dissociate from AT1R, and when do they recycle back to the cell surface? The answers to these questions can be incorporated into a road map for channel protein trafficking in cardiac myocytes under basal conditions and when stimulated. Such a road map will enrich our understanding of cardiac electrophysiology and its remodeling when perturbed.

Acknowledgments

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