

Ryanodine receptor modulation

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The RyR2 channel complex continues to be the focus of attention for cardiovascular researchers owing to its role as a critical node in regulation of the heartbeat, and as potential target for therapeutic intervention in cardiac disease. Some of the main factors responsible for controlling RyR2 activity under physiological and disease conditions were discussed in the Session '*Ryanodine Receptor Modulation*'. Whereas Xander Wehrens gave an overview of experimental work on RyR2 modulation by posttranslational modifications, and Mike Stern presented results of theoretical studies of Ca cycling performed using mathematical modeling and computer simulations. These presentations and the following round-table discussion fostered collective contemplation of key issues and controversies pertaining to RyR2 function in health and cardiac disease such as heart failure and arrhythmias.

One of the major questions regarding the control of RyR2-mediated SR Ca release is how SR Ca release is terminated and contained on the beat-to-beat basis given the self-regenerating nature of Ca-induced Ca release. Using a theoretical approach, Mike Stern presented strong evidence that even a modest release of Ca from the SR is expected to deplete Ca in the cisternae of SR and that SR Ca release must extinguish itself as result of emptying of the SR Ca stores. This conclusion is interesting as it challenges the prevailing notion that release termination involves some special gating mechanisms such as RyR2 deactivation caused by the drop of luminal Ca that leaves a substantial Ca reserve in the SR (1,2). Studies using new genetic mouse models with targeted ablation of components of the putative luminal Ca sensor (CASQ2, HRC) combined with direct measurements of luminal Ca with low-affinity dyes is expected to provide important clues regarding the mechanistic basis of Ca release termination in the near future.

While it is conceivable that SR Ca depletion is the dominant factor behind SR Ca release termination, there is strong experimental evidence that SR Ca depletion by itself is insufficient to account for post-release refractoriness of Ca signaling, a well-documented feature of cardiac cells thought to be important for preventing abnormal Ca release in the diastolic period. Indeed, recent experimental evidence suggests that impairment of RyR2 refractoriness contributes to dysregulated Ca cycling and arrhythmogenesis in models of both genetic and acquired cardiac disease (3). To resolve this issue future theoretical studies will evaluate models of myocyte Ca cycling that incorporate RyR2 luminal Ca regulation while experimental studies will focus on defining the specific molecular mechanisms of RyR2 refractoriness (e.g. luminal Ca, phosphorylation, oxidation etc.) and their impairments in cardiac disease.

Studies over the past decade have clearly demonstrated that RyR2 activity can be strongly modulated by various types of posttranslational modifications, including S-nitrosylation, oxidation, and phosphorylation (4,5). The role of RyR2 phosphorylation has been a consistent source of interest and controversy since the seminal paper by Andy Marks and coworkers (6) reporting that increased RyR2 phosphorylation by PKA renders the channel hyperactive and 'leaky' in heart failure (HF). The main tenets of the Marks' RyR2 phosphorylation hypothesis, further supported by subsequent papers by his and other groups, are that 1) RyR2 phosphorylation by PKA at the residue serine 2808 (S2808) is increased in HF, 2) that increased PKA phosphorylation of RyR2 leads to dissociation of the FK506-binding protein 12.6 (FKBP12.6), and 3) that the activity of RyR2 in failing hearts is increased leading to abnormal SR Ca release. Each of these tenets has been challenged in other papers.

Increased phosphorylation of S2808 on RyR2 has been reported in humans with HF and various animal models ranging from mouse, rat, to dogs (7-10), although other papers did not observe increased PKA phosphorylation in a canine model of HF (11-13). Although it has

remained difficult to resolve these differences in findings, recent studies suggest that the level and site of phosphorylation might depend to some degree on the type of HF (i.e., ischemic or non-ischemic) (14). It was concluded at the meeting that these findings may explain some of the controversies in the literature, and would need to be verified in larger patient populations and large animal models. Additionally, evidence has been accumulated that RyR2 phosphorylation by CaMKII at S2814 plays a role in the physiological modulation of RyR2 (15) and can become elevated in failing hearts (5; 14). Consideration of this issue by the attendees revealed an emerging sentiment that while phosphorylation by PKA is of somewhat uncertain significance, RyR2 phosphorylation by CaMKII plays a role in both physiological and pathophysiological settings (16). The importance of ongoing discussions of these studies, sharing of animal models and reagents, and additional mechanistic studies into the signaling pathways responsible for activation of kinases are thought to be essential to move towards more consensus in this field.

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