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Symposium 8: Integrating cardiac function: From molecules to man

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Ion channelopathies: What have they taught us about arrhythmias and anti-arrhythmic therapy?

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The efficient pumping of blood by the heart requires the co-ordinated activity of the billions of cardiac myocytes that make up the heart. This is achieved by an electrical communication system the centrepiece of which consists of voltage-gated ion channels. Over the last decade the molecular identity of most (if not all) the voltage-gated ion channels in the heart has been elucidated. More importantly it has also been found that mutations in some of these channels (most notably the cardiac sodium channel, SCN5a, and the delayed rectifier potassium channels, KvLQT1 and HERG) result in a marked increase in the risk of lethal cardiac arrhythmias, the so-called "cardiac ion channelopathies". Determination of the mechanisms underlying the increased risk of arrhythmias in patients with these mutant channels has taught us a great deal about the molecular basis of arrhythmias. This is perhaps best illustrated in the case of loss-of-function mutations in the HERG K^+ channel and the increased risk of arrhythmias initiated by premature beats (see e.g. Lu *et al.*, 2001). Understanding the cardiac ion channelopathies has also provided insights into why so many drugs developed to be anti-arrhythmic turned out to be "pro-arrhythmic". For example most Class III anti-arrhythmics inhibit the HERG K^+ channel resulting in a "drug-induced long QT syndrome" (Vandenberg *et al.*, 2001). The big challenge now is to utilise the knowledge we have gained from understanding cardiac ion channelopathies to develop more effective anti-arrhythmic therapies.

One of the major issues that has yet to be fully addressed with respect to the role of ion channels in the genesis of cardiac arrhythmias is the heterogeneity of ion channel expression. This heterogeneity of electrical activity is most clearly illustrated by the differences in the shape and duration of cardiac action potentials recorded from cells in different regions of the heart. One consequence of this heterogeneity is that any drug that modulates ion channel activity will have different effects in different regions of the heart and by altering the delicate balance of inward and outward currents has the potential to be pro-arrhythmic. However, before we can understand the specifics of such postulated pro-arrhythmic mechanisms we need to know much more about the spatial patterns of ion channel expression in the heart and how they are affected by disease processes (see e.g. Wong *et al.*, 2000).

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Cardiac hypertrophy: comparing models and counting genes

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In the study of cardiac hypertrophy, much has been learned from polygenetic models developed by conventional selective in-breeding techniques (*i.e.* the Spontaneously Hypertensive Rat, SHR). As many cardiovascular disease states comprise a complex multigenic-dependent phenotype, it is particularly valid to use these models for investigation of the natural history of disease development and progression. However a major difficulty with these models has been that hypertrophy and hypertension are frequently coincident and identification of the genetic factors which contribute to cardiac growth independently of blood pressure has been difficult. Furthermore, the failure to co-derive genetically homogenous control strains for some models has further confounded the interpretation of data obtained from these animals.

We have recently reported the development of a novel polygenic rat strain of primary cardiac hypertrophy derived from a cross of Fisher (F344) and SHR (Harrap *et al.*, 2002). Our new Hypertrophic Heart Rat (HHR) strain exhibits cardiac and cardiomyocyte hypertrophy in the absence of hypertension. In parallel we have co-developed a Normal Heart Rat (NHR) strain with small hearts and low blood pressure as a control strain. Exploration of the cardiac growth responses in the HHR and NHR provides an opportunity to characterise the processes underlying the development of load-independent hypertrophy.

A complementary genetic approach which can be of particular value in providing insight into the mechanisms of cardiac hypertrophy is the study of mono-genetically manipulated animal models. We have investigated transgenic and gene-knockout models to explore the role of trophic and metabolic factors in inducing cardiac hypertrophy. Our studies of a transgenic cardiac-specific angiotensinogen over-expressing mouse and a cardiac-specific glucose Glut4 transporter Cre-Lox KO mouse have revealed that similar functional adaptations can be linked with quite different alterations in myocyte calcium handling in hypertrophy.

In both multigenic and unigenic models of cardiac hypertrophy we have applied candidate gene and expression profiling techniques to undertake comparative genotype-phenotype analyses. In our candidate gene investigations we have focussed on expression shifts in transporters important in modulating excitation-contraction coupling. Our genome scale ‘snapshot’ studies have suggested that regardless of the instigating genetic stimulus, the hypertrophic phenotype is associated with a major remodelling of metabolic processes.

Thus, the value of both unigenetic and polygenetic animal models in the study of cardiac hypertrophy is particularly evident when candidate gene analysis and genome-scale expression profiling techniques are used as complementary approaches.

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Modelling and imaging cardiac function during excitation-contraction coupling

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Cardiac excitation-contraction (E-C) coupling takes place in the narrow diadic cleft between the transverse-tubular membrane and the closely apposed terminal cisternae of the sarcoplasmic reticulum (SR). Within the cleft (which is only ~15nm high and some 100nm wide) large clusters of SR Ca^{2+} release channels or ryanodine receptors (RyRs) are located. It is now generally accepted that the opening of these clusters of RyRs underlies the elementary events of muscle activation called "calcium sparks", brief microscopic increases in intracellular Ca^{2+}, which can be observed in heart cells loaded with fluorescent Ca^{2+} indicators such as fluo-3.

As a result of Ca^{2+} binding reactions and indicator diffusion Ca^{2+} spark records do not provide a direct measure of the time course of Ca^{2+} release. To robustly reconstruct the underlying Ca^{2+} release time course we developed novel algorithms in which a parametric Ca^{2+} spark model is fit to experimental records. Using this approach we calculated that the peak flux amplitude is ~7-12pA suggesting that at least 15 RyRs contribute to a Ca^{2+} spark. To obtain further insight into the gating of RyRs underlying Ca^{2+} sparks we constructed a detailed Monte Carlo model of RyR gating and associated Ca^{2+} movements within the diad. In this model the movement of individual Ca^{2+} ions was traced and diffusion was implemented as a random walk. RyR gating was described by a phenomenological 4-state scheme (Stern *et al.*, 1999) that included explicit inactivation. Our calculations suggest that the geometry of the diad and the RyR cluster can significantly affect the time course of release. In our spatially explicit model we observe waves of RyR openings originating at the initial site of activation. In elongated clusters of RyRs the time course of release therefore depends on the site of wave initiation while the total amount of Ca^{2+} that is released stays nearly constant.

We also explored the effect of allosteric coupling between RyRs on the gating of large RyR clusters in the model. Allosteric coupling was implemented as a nearest neighbour interaction where transition rates of receptors in the cluster were modified based on the state of adjacent RyRs. With moderate coupling our model generated a mean Ca^{2+} release time course that was similar to that reconstructed from experimental sparks. On the other hand, strong coupling resulted in increased variability and duration of the Ca^{2+} release time course.

It has been suggested that the protein FKBP12.6 may be the molecular basis of allosteric coupling between RyRs. To test this idea we recorded sparks in the presence of FK506, a drug which removes FKBP12.6 from RyRs. Analysis of our data suggests that, although the amplitude of Ca^{2+} sparks is reduced in FK506 (as compared to control sparks), the decay time and variability of Ca^{2+} sparks is only weakly changed by 50 μM FK506 which argues against a significant role of FKBP12.6 in coupling RyR gating.

We are currently extending the model to investigate other RyR gating schemes and the effect of local SR depletion on cluster gating. Our work suggests that the combination of mathematical modelling with high resolution Ca^{2+} imaging will provide valuable insight into cardiac E-C coupling.

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Cardiac structure and electrical activation: models and measurement

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Re-entrant arrhythmia and fibrillation are three-dimensional (3D) events that involve relatively large tissue volumes and are influenced by regional variation of the electrical properties of cardiac tissue and by the complex architecture of the heart. Within this context, computer models that incorporate realistic descriptions of cardiac anatomy and the electrical properties of myocardium provide a powerful tool with which to interpret and interpolate experimental observations.

Our group has systematically measured the 3D geometry of right and left ventricles in dog and pig hearts, and has characterised myocyte orientation throughout the ventricular wall in these species. These data have been incorporated into a detailed finite element model of cardiac anatomy which has been used by ourselves and others to study normal electrical activation and re-entrant arrhythmia.

We have developed a confocal imaging technique that enables us to reconstruct the 3D organisation of cardiac myocytes and extracellular collagen matrix in relatively large tissue volumes at up to 1μm voxel resolution. Morphometric studies employing this approach confirm that ventricular myocardium is a complex hierarchy in which myocytes are arranged in discrete layers separated by cleavage planes that are relatively extensive, particularly in the left ventricular (LV) midwall.

The effect of structural discontinuity on the propagation of electrical activation has been modelled using a finite element formulation in which the electrical properties of intracellular and extracellular domains are explicitly represented. Detailed information on 3D cleavage plane organisation and muscle fibre orientation, extracted from an extended volume image of a transmural segment of rat LV myocardium, was incorporated into the model. For an ectopic midwall stimulus, the predicted spread of electrical activation was initially non-uniform and markedly affected by the discontinuous 3D arrangement of muscle layers.

The model has been validated by recording extracellular potentials at up to 36 sites within the LV free wall in sinus rhythm and during intramural pacing. *In situ* measurements were made first in an anaesthetised (Zoletil, 10mg/kg im, initially and then 2-5% halothane in oxygen), ventilated open-chest pig preparation and comparable data were then recorded with the hearts isolated and mounted in a Langendorff apparatus. Intramural transmembrane potentials were recorded adjacent to extracellular measurement sites in the isolated hearts employing a multi-channel fluorescence imaging system and a novel fibre optic probe. The results obtained are consistent with model predictions and reinforce the hypothesis that structural discontinuity may give rise to non-uniform, anisotropic propagation of electrical activation.

The significance of these observations with respect to normal activation, re-entrant arrhythmia and defibrillation will be discussed. Finally, the need for, and progress toward, development of a new generation of computer models of re-entrant arrhythmia that are anatomically realistic and incorporate accurate representations of cellular electrophysiology and include data on the spatial distribution of key transmembrane ion channels will be reviewed.