Electrical inhomogeneity in the human ventricle

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Cells from the midmyocardial ventricular wall (M region) have an action potential duration at 90 % of repolarization (APD_{90}) of about 400 ms if paced at 1 Hz. Action potentials are about 100 ms shorter when isolated from the endocardium or the epicardium. Such differences can also be appreciated when action potentials are recorded from a transmural tissue slice from the (normal) left ventricle from patients with cystic fibrosis as underlying disease. Therefore it seems justified to conclude that M cells exist in the human ventricle as they have been demonstrated in several other mammalian species with the dog as the most prominent example. Whether transmural repolarisation gradients suggested by these *in vitro* studies are manifest *in vivo* at physiological heart rates remains a debated issue. Two important issues play a role in this controversy. The difference in action potential duration between M cells and epi- or endocardial cells is a function of heart rate. At very low heart rate the difference can be very large, whereas at normal heart rate the difference is much smaller. Nevertheless, in man a difference in intrinsic APD_{90} of about 100 ms seems to be present within both the right and left ventricular free wall. A second important issue, however, remains. In a normal heart cells are coupled to each other by gap junctions, providing the low-resistance pathway for intercellular current flow, a prerequisite for conduction of the cardiac impulse. These gap junctions also synchronize the repolarization of the myocytes. Although cells may have substantial intrinsic electrophysiological differences, this does not necessarily imply that these intrinsic differences are overt when the cells are coupled to each other in the ventricular wall.

In the hearts of patients undergoing coronary artery surgery no midmyocardial prolonged activation-recovery intervals (ARIs) could be demonstrated. ARIs are measured in local electrograms by taking the time interval between the moment of the steepest negative deflection in the QRS interval and the moment of the steepest positive deflection in the T wave, and they are considered as the extracellular counterpart of the local transmembrane potential duration. These data allow two possible explanations: (i) in these patients, no intrinsic differences are present, or (ii) intrinsic differences are present, but are reduced by intercellular coupling. We have addressed this issue further by computer simulations of a heterogeneous strand of ventricular cells with variable coupling. In a model strand of 90 ventricular cells, the first 30 cells were endocardial cells. For these cells, the densities of I_{to}, I_{Kr}, I_{Ks} and I_{K1} (all outward currents relevant for repolarization) were reduced relative to the current densities in the model epicardial cells by 75 % for I_{to}, based on data in man, and by respectively 0, 8 and 11 % for the other three currents based on data in the dog. The next 30 cells were M cells with current densities reduced by 13, 54, and 26 % for I_{to}, I_{Ks} and I_{K1}. Finally, the last 30 cells were epicardial cells with all current densities at 100 %. By gradual increase of the intercellular coupling the dispersion in APD_{90} values had already disappeared at about 1 μS, which is less than the 'normal' value for intercellular coupling estimated at 3–12 μS. This suggests that even when M cells are prominent in human ventricle, intercellular coupling may be sufficient to abolish intrinsic differences almost completely.

It is emphasized that in *in vivo* studies in the 'model species' for M cells long action potential durations were absent in the midmyocardium, even in the presence of I_{Ks} blockers, which prolong the intrinsic action potential duration of M cells even further. Also, in porcine heart, in which transmural refractoriness was assessed, refractoriness was not longer in the midmyocardium than in the subendocardium or subepicardium.

In summary, the absence of a transmural repolarizing gradient in patients with coronary heart disease does not allow the conclusion that intrinsic differences are absent or reduced in these patients. A moderate degree of cellular coupling may effectively reduce these gradients and thus can be regarded a potent anti-arrhythmic factor. Exposure of the intrinsic heterogeneities is only possible by a high degree of electrical uncoupling. Ultimately, this is demonstrated in isolated, single myocytes.

Intracellular Ca^{2+} release sparks atrial pacemaker activity

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Electrical excitation of the mammalian heart originates from specialized pacemaker cells located in specific regions of the right atrium. The right atrium contains both primary (SA node) and latent pacemaker cells. Latent atrial pacemakers are specialized cells located outside of the SA node in specific regions of the inferior right atrium. In the event of SA node failure, latent atrial pacemaker activity can assume pacemaker control of atrial function and may generate atrial arrhythmias. In general, the electrical activity of cardiac pacemakers is determined by multiple mechanisms primarily dependent on ion channel currents within the plasma membrane. However, interventions that alter Ca^{2+} release from the sarcoplasmic reticulum (SR), alter pacemaker activity and therefore suggest a role for intracellular Ca^{2+} release in atrial pacemaker activity (Rubenstein & Lipsius, 1989; Rigg & Terrar, 1996). Therefore, the following experiments investigated the mechanisms by which intracellular Ca^{2+} release contributes to atrial pacemaker function.

Adult cats were anaesthetised with sodium pentobarbital (70 mg kg^{-1}, I.P.). Atrial pacemaker cells were isolated by Langendorff perfusion and collagenase treatment. Inhibition of intracellular SR Ca^{2+} release by ryanodine specifically depresses the last third of diastolic depolarization and markedly prolongs pacemaker cycle length (Rubenstein & Lipsius, 1989). Moreover, in spontaneously beating pacemaker cells, clamping the membrane voltage during the last third of diastolic depolarization elicits a slowly developing inward current, identified as Na–Ca exchange current (Zhou & Lipsius, 1993). Inhibition of SR Ca^{2+} release by ryanodine concomitantly inhibits the Na–Ca exchange current, diastolic depolarization and pacemaker rate. Confocal fluorescent microscopy reveals an increase in subsarcolemmal intracellular Ca^{2+} concentration due to local SR Ca^{2+} release, i.e. Ca^{2+} sparks during diastolic depolarization (Hüser *et al.* 2000). Voltage-clamp ramps within the pacemaker voltage range indicate that the diastolic Ca^{2+} release was voltage dependent and triggered at about −60 mV, a voltage too negative for activation of L-type Ca^{2+} current but compatible with activation of T-type Ca^{2+} current. In free-running pacemaker cells, nickel (Ni^{2+}; 25–50 μM), a blocker of low voltage-activated T-type Ca^{2+} current, decreases diastolic

depolarization, prolongs pacemaker cycle length and suppresses diastolic Ca^{2+} release. Ni^{2+} also suppresses low-voltage-activated Ca^{2+} release elicited by voltage-clamp ramps. Low-voltage-activated Ca^{2+} release was paralleled by a slow inward current presumably due to stimulation of Na–Ca exchange. Low-voltage-activated Ca^{2+} release was more prominent in latent atrial pacemaker cells than in SA node pacemaker cells and absent in working atrial myocytes. Gross morphology of latent atrial and SA node pacemaker cells are similar. However, latent atrial pacemaker cells exhibit a unique architecture of subsarcolemmal cisternae not seen in SA node pacemaker cells. In adjacent latent atrial pacemaker cells, subsarcolemmal cisternae are prominent in size and directly apposed to one another along the adjacent surface membranes.

We conclude that low-voltage-activated T-type Ca^{2+} current triggers subsarcolemmal Ca^{2+} sparks, which in turn, stimulates Na–Ca exchange current to depolarize the pacemaker potential towards threshold. The role of intracellular Ca^{2+} release may be more prominent in latent than SA node pacemaker activity. This mechanism therefore contributes to normal pacemaker function and may underlie atrial arrhythmias that are promoted by alterations in intracellular Ca^{2+} metabolism.

The animal procedures using in this study were in accordance with the guidelines of the Animal Care and Use Committee of Loyola University Medical Center.

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Mechanisms underlying the influence of cytosolic Ca^{2+} on pacemaking in the sino-atrial node

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Pacemaking in the sino-atrial (SA) node is thought to be determined by sequential activation and deactivation of a variety of ionic currents. Evidence has accumulated in recent years supporting the possibility that this pacemaker activity might be influenced by changes in cytosolic calcium. Calcium entry and extrusion across the surface membrane, as well as calcium release and uptake by the sarcoplasmic reticulum (SR), are expected to underlie the changes in cytosolic calcium. Detailed mechanisms by which pacemaker activity might be influenced by cytosolic calcium remain to be determined, though one possibility that has received recent support is that localised changes in calcium resembling calcium sparks may be particularly important. For example, calcium spark-like events triggered by calcium entry through T-type calcium channels and giving rise to depolarising sodium–calcium exchange current might speed the early development of the upstroke of the action potential in cat SA node cells (Hüser *et al.* 2001). In addition, the positive chronotropic effects of β-adrenoceptor agonists have been associated with a ryanodine-sensitive increase in the frequency of spark-like events during diastolic depolarisation, and to changes in the characteristics of these events in rabbit SA node cells (Vinogradova *et al.* 2002). Evidence for the presence of ryanodine receptors, and for their involvement in β-adrenoceptor-mediated increases in rate of beating in guinea-pig SA node cells, has also been reported (Rigg *et al.* 2000).

It remains possible that ionic currents other than electrogenic sodium–calcium exchange current may also be influenced by changes in cytosolic calcium. In addition, while spark-like events may well play important roles in pacemaking, more sustained and/or widespread changes in cytosolic calcium could modulate a variety of currents that contribute to pacemaker activity. The aim of this presentation is to discuss evidence gathered from guinea-pig SA node cells concerning the importance of cytosolic calcium during pacemaker activity. In some experiments, photometric techniques with indo-1 as calcium indicator were used to measure calcium changes, while in others confocal microscopy (either linescan or Nipkow disk methods) and fluo-4 fluorescence were employed. Permeabilised patch-clamp techniques (with amphotericin in the pipette solution) were consistently applied for electrical recording. Spark-like activity was detected as separable events in approximately 30 % of beating SA node cells. β-Adrenoceptor stimulation increased the frequency and amplitude of the spark-like events. Positive chronotropic effects of β-adrenoceptor stimulation were also associated with increases in the amplitude and rate of decay of whole-cell calcium transients. The I_f current activated by hyperpolarization was suppressed by loading cells with the calcium chelator BAPTA and by the calmodulin antagonist W7, though not by the calmodulin-dependent kinase inhibitor KN93. This inhibitor did, however, appear to reduce L-type calcium currents and the delayed rectifier potassium currents I_{Kr} and I_{Ks}. These delayed rectifier potassium currents were also reduced by chelating cytosolic calcium with BAPTA, by reducing SR calcium release with ryanodine and by reducing calcium entry using nifedipine. Reducing extracellular sodium led to reduced rate of beating in the first minute of exposure but to increases in the amplitude of calcium transients measured either with indo-1 photometric techniques or with fluo-4 and confocal microscopy.

These observations are interpreted as supporting the importance of cytosolic calcium in influencing pacemaker activity. It appears that the actions of calcium are complex and may involve modulation, perhaps by several different mechanisms, of many of the ionic currents contributing to pacemaker activity. While spark-like events are thought to play an important role, more sustained and/or widespread effects of cytosolic calcium may also influence pacemaker activity.

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Funny channels in the sino-atrial node

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The cardiac 'funny' (I_f) current of the sino-atrial node (SAN) is typical of pacing tissue and plays a key role in the generation and autonomic-mediated modulation of cardiac rate. Of the four isoforms of the recently cloned hyperpolarization-activated, cyclic-nucleotide-gated (HCN) channel family, three (HCN1, HCN2 and HCN4) have been found to be expressed in cardiac tissues. These HCN isoforms are heterogeneously distributed in the heart. HCN2 appears to be a predominant isoform in cardiac

muscle; in the mammalian SAN, on the other hand, where f-channels are physiologically most relevant, RNase-protection assay analysis and immunolabelling show significant expression of HCN4 and HCN1, whereas mRNA levels of HCN2 are just detectable (Shi *et al.* 1999; Moroni *et al.* 2001).

The properties of HCN channels expressed in heterologous systems are similar to those of the native I_f current and of its neuronal equivalent I_h, but substantial quantitative differences exist among isoforms. Typically, HCN1 has faster activation/deactivation kinetics, and a lower cAMP sensitivity than HCN2 and HCN4; HCN2 has in turn faster kinetics than HCN4; also, the voltage range of activation, and therefore the range where the pacemaker current is functionally important, varies among isoforms (Altomare *et al.* 2001). This is relevant to the question of the composition of native channels, since differences in the properties of native I_f/I_h currents in various tissues (DiFrancesco, 1993) may simply reflect an heterogeneous distribution of HCN isoforms.

In some cell types, however, the properties of native channels do not appear to conform to those of any of the individual isoforms locally expressed.

In the mammalian SAN, the reported activation kinetics of I_f are slower than those of heterologously expressed HCN1, and faster than those of HCN4. This leads to the idea that different HCN isoforms can co-assemble, to produce heteromers with properties intermediate between those of the individual components. Co-assembly of different HCN isoforms has indeed been demonstrated previously for HCN1 and HCN2 (Chen *et al.* 2001). HCN channels could also be modulated by auxiliary proteins. In the SAN, for example, co-transfection with the MiRP1 β-subunit has been reported to enhance expression and accelerate activation of HCN1 and HCN2 in *Xenopus* oocytes (Yu *et al.* 2001).

We used heterologous expression to understand if co-assembly of the major isoform components of HCN channels in SAN cells, HCN1 and HCN4, generates channels with the same properties as native SAN I_f channels. We investigated the kinetics and cAMP response of the current generated by cotransfected (rbHCN4 + rbHCN1) and concatenated (rbHCN4-rbHCN1, 'tandem') rabbit constructs expressed in HEK293 cells, and compared them with those of the native cardiac pacemaker current from the rabbit SAN.

We found that transfection in HEK293 cells of concatenated 'tandem' HCN1 and HCN4 isoforms generates pacemaker channels with activation kinetics approaching those of native f-channels in the SAN. However, we found no evidence for MiRP1 mediated modulation of expression and/or kinetics. Individual and cotransfected isoforms all had activation ranges more negative than the range of native f-channels, and tandem channels responded poorly to cAMP. The results are compatible with the idea that native f-channels are heteromers of HCN1 and HCN4 subunits, but only under the assumption that a 'context'-dependent mechanisms contributes to determination of the channel properties in native tissue.

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Heterogeneity and ageing of the sinoatrial node

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The sinoatrial (SA) node, the pacemaker of the heart, is heterogeneous. Functionally, the SA node can be divided into two areas, the centre and the periphery. The centre normally acts as the leading pacemaker site, whereas the periphery normally serves as the conduction pathway from the centre to the atrial muscle. The SA node is heterogeneous in many respects. (i) Protein expression: in the rabbit, whereas both the periphery and the centre express neurofilament165 but not atrial natriuretic peptide (unlike atrial muscle which expresses atrial natriuretic peptide but not neurofilament165), the periphery but not the centre expresses connexin43 (Cx43) (Dobrzynski *et al.* 2002). (ii) Electrophysiology: we have evidence of a gradient in cell type from the periphery to the centre in the rabbit in terms of action potential shape, densities of ionic currents and expression of connexins (responsible for electrical coupling) (Boyett *et al.* 2000). The possible significance of this is revealed by a mathematical model of a string of 50 SA node cells connected to a string of 50 atrial cells. In the model, the central SA node cells, with their characteristic electrophysiology and low coupling conductance, are only able to show pacemaker activity and drive the atrial cells, which are more hyperpolarized and have a high coupling conductance, when they are separated from the atrial cells by peripheral cells, which have intermediate electrophysiological characteristics and perhaps an intermediate coupling conductance. (iii) Ca^{2+} handling: for the rabbit, evidence suggests that the density of the L-type Ca^{2+} current is less in central than peripheral cells (Musa *et al.* 2002). Consistent with this, immunolabelling of Cav1.2 protein is less in central than peripheral cells (Musa *et al.* 2002). As well as a decrease in the expression of the Ca^{2+} channel, there are decreases in the expression of other Ca^{2+} handling proteins from the periphery to the centre in the rabbit: Na^+–Ca^{2+} exchanger, RYR2 and SERCA2 (Musa *et al.* 2002). There is evidence that the decrease in the density of the Ca^{2+} handling proteins from the periphery to the centre affects Ca^{2+} handling in the rabbit SA node: there is evidence that the intracellular Ca^{2+} transient is smaller and slower, the Ca^{2+} content of the sarcoplasmic reticulum (SR) is less and the contribution of the SR to the Ca^{2+} transient is less in central than peripheral cells (Lancaster *et al.* 2001, 2002). Pacing is regulated by the intracellular Ca^{2+} transient (e.g. Rigg & Terrar, 1996). Although it has been reported that the abolition of the intracellular Ca^{2+} transient by a sufficiently high concentration of ryanodine (30 μM) can abolish pacing in rabbit SA node cells (Bogdanov *et al.* 2001), we believe that the regulation is more modest: in the intact SA node of the rabbit, exposure to 30 μM ryanodine for 1 h decreased the spontaneous rate by $20 \pm 3\%$ ($n = 4$). The putative regional difference in the Ca^{2+} transient above is expected to result in a regional difference in the intracellular Ca^{2+} regulation of pacing and there is evidence of this for the rabbit SA node: 2 μM ryanodine significantly slowed pacing of putative peripheral cells by $23 \pm 8\%$ ($n = 20$), whereas it had no significant effect on the pacing of putative central cells ($n = 18$).

During ageing, there is a decline in SA node function (a decrease in spontaneous activity and a slowing of SA node conduction) and this may be related to the SA node heterogeneity. In part, it could be the result of a further loss of connexin43 (Cx43) and Ca^{2+} channels from the centre of the SA node: in the SA node centre of the guinea-pig (as in rabbit – see above) there are regions lacking immunolabelling of Cx43 and Cav1.2 proteins and these regions increased in size from 3.5 ± 0.6 mm^2 ($n = 5$) in 1 month animals to 47.7 ± 2.0 mm^2 ($n = 5$) in 38 month animals

in the case of Cx43 (Jones *et al.* 2001), and from 2.1 ± 0.1 mm^2 ($n = 4$) in 1 month animals to 18.7 ± 2.3 mm^2 ($n = 3$) in 38 month animals in the case of Cav1.2.

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Comparison of the single-cell properties of the guinea-pig and rabbit AV node

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Single-cell preparations have been used extensively to study action potential (AP) and pacemaker potential (PP) genesis in the sinoatrial (SA) and atrioventricular (AV) nodes of the heart. The location and complex morphology of the AV node has made single cell studies of this region a particular challenge. The morphological features of the AV node of the rabbit heart were well described in the 1970s (Anderson, 1972; Anderson *et al.* 1974) and, to date, data regarding the single-cell electrophysiology of the AV node have been derived exclusively from AV node cells isolated from this species. Taken collectively, the data from studies by a number of groups have shown that some electrophysiological properties of rabbit AV nodal cells resemble closely those reported for SA nodal cells from the same species, whilst other properties differ between the two regions. For example, in both SA and AV nodes, channels for inwardly rectifying K^+ current (I_{K1}) are scarce, if not entirely absent. Both cell types exhibit zero current potentials somewhat positive to the potassium equilibrium potential and cell input resistance is high, making membrane potential highly labile over the PP range. However, rabbit AV and SA nodal cells appear to differ in the delayed rectifier K^+ current subtypes present since, under similar recording conditions, SA nodal myocytes exhibit both rapid and slow components (I_{Kr} and I_{Ks}, respectively), whilst AV nodal cells exhibit only I_{Kr} (Habuchi & Giles, 1995; Sato *et al.* 2000). Also, the density of the hyperpolarisation-activated 'pacemaker' current, I_f, appears to be greater in SA than in AV nodal myocytes (Habuchi & Giles, 1995).

In order to be able to place data from rabbit AV nodal cells into a wider context, comparative data are required from one or more other species. Recently, we have isolated Ca^{2+}-tolerant single cells from the guinea-pig AV node and have begun to characterise their electrophysiological properties. Cell shapes and dimensions were comparable to those observed from rabbit AV nodal cells. Guinea-pig AV nodal cells exhibited small membrane capacitance values (~25 pF), a zero-current potential close to −40 mV and high input resistances (in excess of 1 GΩ). Whole-cell voltage-clamp experiments revealed a number of distinct current components. $I_{Ca,L}$ exhibited a similar density to that observed previously in rabbit AV node cells (Hancox & Levi, 1994) and there was evidence for I_{Na} in some cells. In contrast with the rabbit AV node, there was no evidence for a transient outward K^+ current. Delayed outward current comprised two components; these exhibited half-maximal activation voltages of −17.2 and +27.1 mV, suggesting that both I_{Kr} and I_{Ks} are present the guinea-pig AV node. At negative voltages, net inward current could be separated into three components, two of which were I_f and a background current. Surprisingly, however, a barium-sensitive inwardly rectifying current was also evident at the start of applied voltage commands, and was prominent at potentials negative to the PP range. Our experiments on guinea-pig AV nodal cells demonstrate that differences exist between rabbit and guinea-pig in AV nodal single cell electrophysiology. Further comparative studies are now required in order to determine which AV nodal cellular properties are widely preserved across species, and also to establish the functional consequences of observed differences.

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Heterogeneity of excitation–contraction coupling in pig ventricle

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The role of electrical heterogeneity in arrhythmogenesis

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Electrophysiological distinctions among the three predominant cell types that comprise the ventricular myocardium are responsible for the transmural voltage gradients that inscribe the J and T waves of the ECG. Differences in the response of epicardial, endocardial and M cells to pharmacological agents and/or pathophysiological states result in amplification of these intrinsic electrical heterogeneities, thus providing a substrate and trigger for the development of re-entrant arrhythmias, including Torsade de Pointes (TdP) commonly associated with the long QT syndrome (LQTS) and the rapid polymorphic ventricular tachycardia and ventricular fibrillation (VT/VF) encountered in patients with the Brugada syndrome.

Early repolarization of the epicardium results in abnormal abbreviation of action potential duration (APD) due to an all-or-none repolarization at the end of phase 1 of the epicardial action potential. Loss of the action potential dome in the epicardium but not endocardium gives rise to a large dispersion of repolarization across the ventricular wall, resulting in a transmural voltage gradient that manifests in the ECG as an ST segment elevation (or idiopathic J wave). Under these conditions, heterogeneous repolarization of the epicardial action potential gives rise to phase 2 re-entry, which provides an extrasystole capable of precipitating VT/VF (or rapid TdP). Experimental models displaying these phenomena show ECG characteristics similar to those of the Brugada syndrome as well as those encountered during acute ischaemia.

Transmural dispersion of repolarization is also amplified in LQTS. Disproportionate prolongation of the M cell action potential contributes to the development of long QT intervals, wide-based or notched T waves, and a large transmural dispersion of repolarization, which provides the substrate for the development of re-entrant polymorphic VT closely resembling Torsade de Pointes. An early afterdepolarization (EAD)-induced triggered beat is thought to provide the extrasystole that precipitates TdP. Experimental models of LQT1, LQT2 and LQT3 forms of LQTS suggest a protective effect of mexiletine, an agent that blocks the late sodium current, in all three genotypes. Similar studies provide the basis for the actions of β-adrenergic agonists and antagonists, showing that β-blockers are protective in LQT1 and LQT2, but may promote TdP in LQT3.

In conclusion, recent studies from a number of laboratories have demonstrated important electrical heterogeneities in ventricular myocardium. Amplification of the intrinsic heterogeneities in final repolarization give rise to the long QT syndrome, whereas amplification of those in the early phases of the action potential are thought to give rise to the Brugada syndrome. In both cases we see the development of a vulnerable window in the form of a transmural dispersion of repolarization. In the case of LQTS, re-entry is probably precipitated by an EAD-induced extrasystole, whereas in the Brugada syndrome the extrasystole is due to phase 2 re-entry. Polymorphic ventricular tachycardia develops in both. It is more rapid in the Brugada syndrome because of the reduced refractoriness and relatively slower in LQTS because of the prolonged refractoriness that attends this syndrome. These two syndromes depict paradigms of arrhythmogenesis representing a wide spectrum of mechanisms responsible for arrhythmias in structurally normal hearts. Appreciation of these mechanisms should provide us with an important stepping stone for understanding more complex mechanisms of arrhythmogenesis in structurally abnormal hearts.

Left ventricular transmural differences in E–C coupling in normal and failing hearts

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Marked differences exist in the electrical and mechanical properties of cardiac myocytes across the wall of the ventricle. These differences are thought to exist in part due to the different mechanical conditions presented to subendocardium and epicardium during the normal contraction cycle. Hypertrophy of cardiomyocytes resulting from a myocardial infarction is known to be associated with both mechanical and electrical dysfunction of the whole heart. One possible cause for these properties is the disruption of the normal transmural differences in myocyte characteristics. In this presentation I will describe a series of measurements made on tissue and cells isolated from endocardial and epicardial regions of the left ventricle of a rabbit model of left ventricular dysfunction (LVD). LVD was induced by chronic (8 week) ligation of the large circumflex branch of the left coronary artery. This model of LVD displays many of the characteristics common to the human syndrome of heart failure including marked reduction of ejection fraction, dilated end-diastolic volume and raised end-diastolic pressure (Ng *et al.* 1998). The isolated ventricle displayed a significantly lower threshold to pacing-induced ventricular fibrillation (Burton & Cobbe, 1998). I will review previous work indicating that the normal transmural differences in action potential duration are markedly changed in the model of LVD. Similarly changes in the intracellular Ca^{2+} signal in the cardiomyocytes isolated from the LVD model were not uniform across the ventricular wall (McIntosh *et al.* 2000). I will then discuss recent data on the abundance of Ca^{2+} handling proteins in endocardial and epicardial samples and discuss the relationship between these changes and the characteristics of excitation–contraction coupling in cardiomyocytes isolated from endocardial and epicardial cardiomyocytes, studied using fura-2 fluorescence measurements in voltage-clamped cells.

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Subcellular heterogeneity in cardiac ventricular myocytes

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During activation of cardiac muscle, Ca influx across the cell membrane causes local Ca release from adjacent ryanodine receptors (e.g. Wier & Balke, 1999); the global Ca transient is the sum of these independent, localised releases. This requires that Ca influx occurs adjacent to the ryanodine receptors. Immunohistochemical studies of cardiac ventricular myocytes have shown ryanodine receptors and L-type Ca channels, which provide the main trigger for Ca release, concentrated and co-localised at the t-tubule (e.g. Scriven *et al.* 2000), but the localisation of other proteins has been more controversial. Furthermore, immunolocalisation of a protein does not allow localisation of its function, which may be modulated by other factors, and quantification is difficult. We have, therefore, used osmotic shock to detubulate isolated ventricular myocytes and thus investigate the distribution of membrane currents between the t-tubule and surface membranes.

To induce detubulation, isolated rat ventricular myocytes are exposed to 1.5 M formamide for 15 min, and then resuspended in control Tyrode solution (Kawai *et al.* 1999). This results in a 30 % decrease in cell capacitance and loss of the t-tubules, visualised by staining the cell membrane with di-8-ANEPPS. However, if the cells are stained with di-8-ANEPPS before treatment with formamide, irregular fluorescence can be observed in the cell following formamide treatment, and if FITC-labelled dextran is included in the bathing solutions during detubulation, localised FITC fluorescence can be observed within the cell following detubulation. These data suggest that the t-tubules uncouple from the surface membrane and reseal within the cell during formamide treatment.

Detubulation causes an 87 % decrease in the amplitude of the L-type Ca current (I_{Ca}), with no change in its time course or voltage dependence; this agrees with immunohistochemical studies showing concentration of this channel in the t-tubules. Detubulation also causes almost complete loss of Ni-sensitive Na–Ca exchange current elicited by ramp clamps. Because immunohistochemical studies of the distribution of the Na–Ca exchanger have given inconsistent results (see Blaustein & Lederer, 1999), this was investigated further using confocal microscopy to monitor Ca efflux from ventricular myocytes, by bathing cells in a solution containing the fluorescent Ca indicator fluo-3 during the application of caffeine. In control cells, application of caffeine is accompanied by a rapid rise of

extracellular Ca, as Ca is extruded from the cell. This rise is markedly reduced following detubulation (the amplitude of the rise of intracellular [Ca] is unchanged, although its rate of decline is slowed), and the residual rise observed in detubulated cells is abolished by the Ca-ATPase inhibitor carboxyeosin. These data suggest that in rat ventricular myocytes 87 % of I_{Ca} and almost all Na–Ca exchange activity are concentrated in the t-tubules. This is consistent with the observation that in control cells, electrical stimulation results in a rapid, synchronous increase of intracellular [Ca], whereas in detubulated cells Ca initially rises at the cell periphery followed by sarcoplasmic reticulum-dependent propagation into the cell interior.

In contrast, the density of other currents – I_{Na}, I_K and I_{K1} – does not change following formamide treatment of rat ventricular myocytes, suggesting that they are more homogeneously distributed between the t-tubule and surface membranes. Steady-state current (I_{SS}) density decreases slightly following detubulation, so it may be concentrated in the t-tubules, compatible with recent work showing that TASK-1, one of the channels that might underlie I_{SS}, is found at a high concentration in the t-tubules (Jones *et al.* 2002).

It is unlikely that direct effects of formamide can account for these data, because exposure of rat atrial myocytes, which lack t-tubules, to 1.5 M formamide for 15 min has no significant effect on cell capacitance, I_{Ca}, Ca efflux during application of caffeine, the configuration of the action potential or the Ca transient.

Thus it appears that the major trans-sarcolemmal Ca flux pathways (I_{Ca} and Na–Ca exchange) are concentrated within the cardiac t-tubules, which therefore play an important and specialised role in excitation–contraction coupling in the heart.

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