Gene expression of stretch activated channels and mechano-electric feedback in the heart

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Summary

1. Mechanoelectric feedback (MEF) in the heart is the process by which mechanical forces on the myocardium can change its electrical properties. MEF has been demonstrated in many animal models, ranging from isolated cells, through isolated hearts to whole animals. In humans, MEF has been demonstrated directly in both the atria and the ventricles. It seems likely that MEF provides either the trigger or the substrate for some types of clinically important arrhythmias.

2. MEF may arise because of the presence of stretch sensitive (or mechano-sensitive) ion channels in the cell membrane of the cardiac myocytes. Two types have been demonstrated: a non-specific cation channel (SAC: conductance of ~25 pS) and a potassium channel with a conductance of ~100 pS. The gene coding for the SAC has not yet been identified. The gene for the potassium channel is likely to be TREK, a member of the tandem pore potassium channel gene family. We have recorded stretch sensitive potassium channels in isolated rat myocytes which have the properties of TREK channels expressed in heterologous systems.

3. It has been shown that TREK mRNA is expressed heterogeneously in the rat ventricular wall, with 17 fold more expression in endocardial compared to epicardial cells. This difference is reflected in the TREK currents recorded from endocardial and epicardial cells using whole cell patch clamp techniques, although the difference in current density was less pronounced (approx 3 fold). Consistent with this, we show here that when the ventricle was stretched by inflation of an intra-ventricular balloon in a Langendorff perfused isolated rat heart, action potential shortening was more pronounced in endocardium (30% shortening at 40 mm Hg) compared to epicardium (10% shortening at the same pressure).

4. Computer models of the mechanics of the (pig) heart show pronounced spatial variations in strain in the myocardium with large transmural differences (in the left ventricle in particular) and also large differences between the base and apex of the ventricle.

5. The importance of MEF and the non-homogeneous gene expression and strain distribution for arrhythmias is discussed.

Mechano-electric feedback in the heart

At the cellular level, the mechanisms by which electrical activation initiates and controls contractile activity (Excitation-Contraction coupling) in the heart are now reasonably well understood.[1] Similarly, at the integrative level, the spatial and temporal pattern of activation and repolarisation of the myocardium is well documented, although the mechanisms controlling this are somewhat less well understood.[2] Notably, however, most efforts to understand cardiac physiology have treated electrical activation and contractile activity as essentially separate, i.e. it is implicit in most models that although electrical activation can control contraction, there is no converse process by which mechanical forces on the myocardium can alter its electrical properties. In fact it has been known for some time that there is such a process in the heart, called mechano-electric feedback (MEF), although the mechanisms responsible for it and its implications for cardiac physiology are still a matter of investigation.

The observation that mechanical forces on the myocardium can produce electrical responses is far from novel. For example, over a century ago there were reports of cardiac rhythm disturbances and sudden death caused by non-penetrating mechanical impacts to the chest. In 1876 Nélaton reported a case of sudden death of a manual labourer due to precordial impact.[3] Post mortem examination showed no signs of internal structural damage, death presumably being due to ventricular arrhythmia. This condition became known as “Commotio cordis”, and it is thought to still be responsible for the death of perhaps >100 athletes per year in the USA, due to chest impacts from baseballs or hockey pucks.[4]

Experimental models of MEF

It is fairly easy to demonstrate MEF in animal experiments; as early as 1915 Bainbridge reported that the heart rate of anaesthetised dogs was increased when the right atrium was distended by injecting fluids into the jugular vein.[5] In the ventricle, rapid stretch can produce striking electrical disturbances, indeed, if the applied stretch is sufficiently large it can produce premature ventricular excitation[6,7] or runs of ventricular tachycardia.[8] These responses are not due to reflex loops, or other extracardiac effects, since they can be demonstrated in isolated tissues[7] and even at the level of single myocytes.[9] MEF has also been convincingly demonstrated in humans[10] and, as noted above, can be powerful enough to generate dangerous arrhythmias. For example, it has been suggested that abnormal ventricular wall movement because of regionally impaired ventricular function can trigger ectopic beats.[11,12]

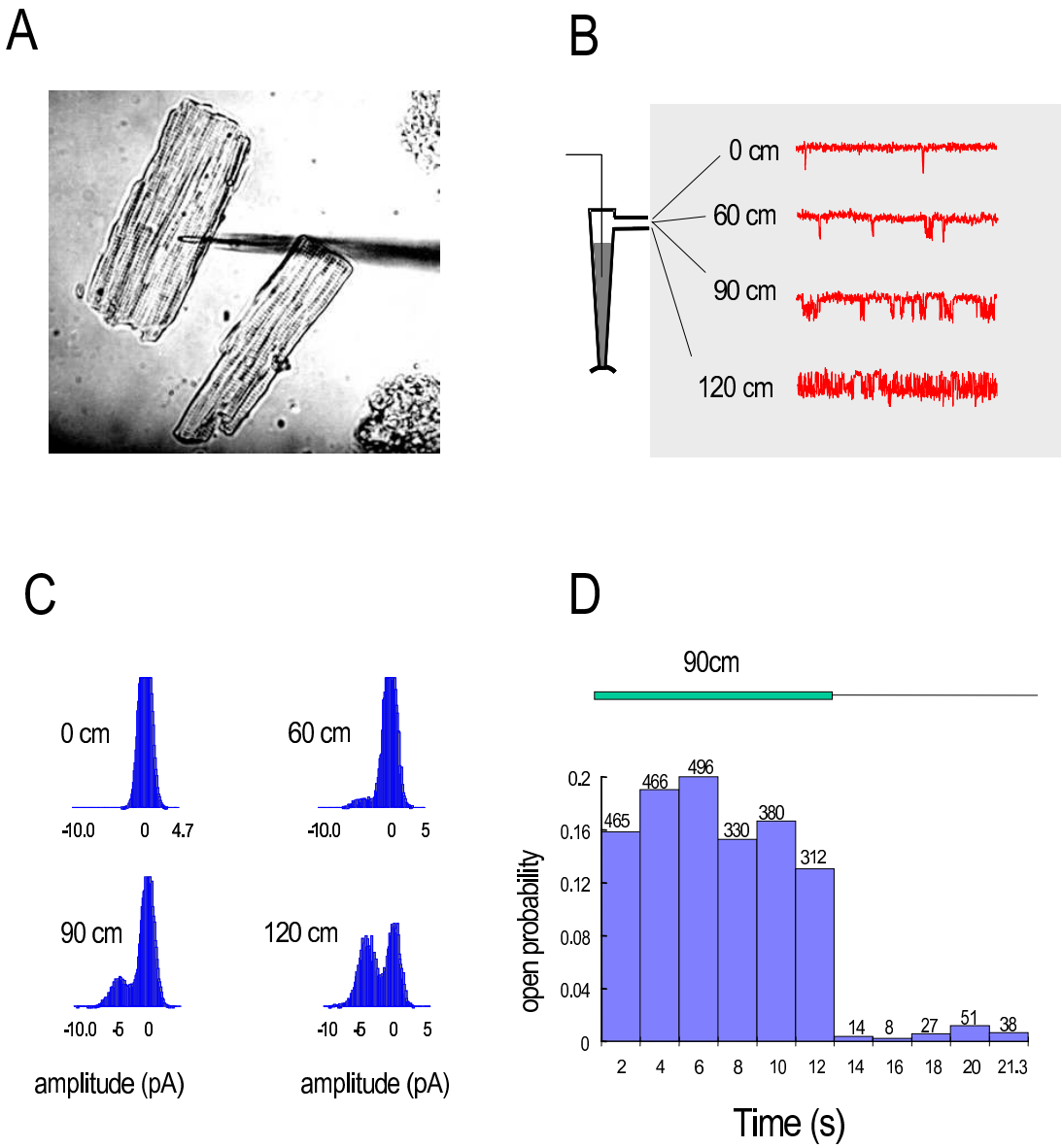

Figure 1. Properties of a stretch-activated potassium channel in rat cardiac myocytes. Panel A: *Patch clamp recording of single potassium channel activity was performed with isolated adult rat myocytes. Each of the two myocytes shown is about 150 µm in length.* ***Panel B:*** *In either cell–attached or inside out configuration, application of suction to the pipette increased the channel open probability from close to zero with no suction to between 0.7 and 0.8 with 120 cm H_2O (panel B). Holding potential was 40 mV throughout, the channel has a conductance of ~115 pS.* ***Panel C:*** *all-points amplitude histograms of recordings at different suctions, showing the progressive appearance of channel activity at slightly less than 5 pA, with no change in channel amplitude with suction.* ***Panel D:*** *Time course of the response to suction. During application of 90 cm H_2O (shown by bar above), channel open probability was maintained between 0.16 and 0.2. Po was measured in 2 s bins- the number of events measured is shown above the bars (between 300 and 500 per bin during the suction). On sudden release of suction, Po dropped to less than 0.01. Note that Po fell in much less than 2 s (number of events in first bin after release is 14).*

The role of stretch sensitive ion channels

The major mechanism by which MEF is produced in cardiac myocytes is the activation of stretch-sensitive, or mechanosensitive, ion channels. Two types of stretch sensitive channels have been described in cardiac tissues: a non-selective cation channel and several different but closely related potassium selective channels.[13,14,15]

The stretch sensitive potassium channels belong to a family called the tandem-pore channels,[16,17] which are a set of potassium channels having the signature K^+ pore sequence TXGYG or TXGFG, in the same way as the Kv family of channels, but with two such sequences on a single subunit. The subunits lack a voltage sensing sequence

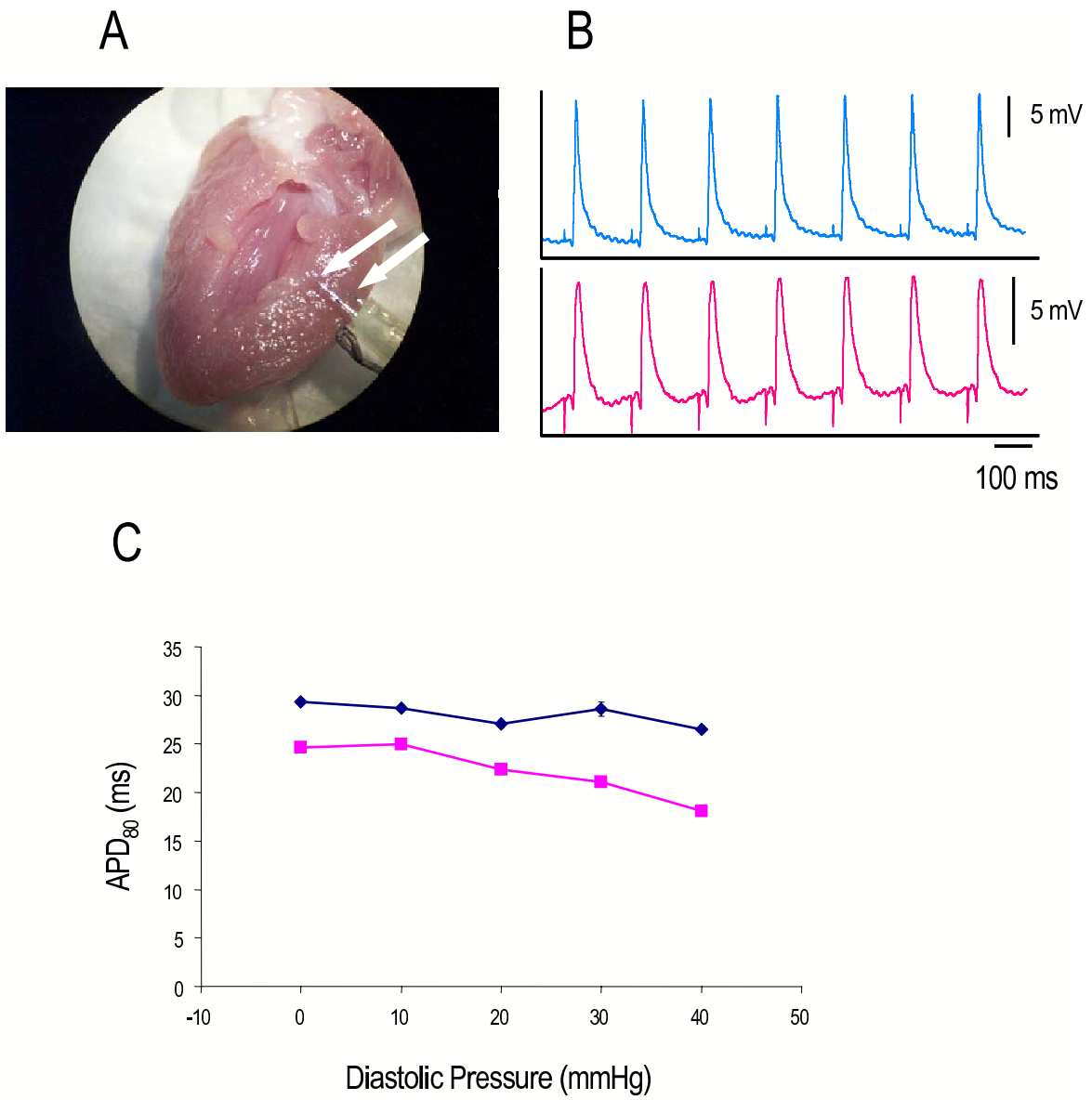

Figure 2. Simultaneous recording of sub-epicardial and sub-endocardial action potentials in isolated heart. *Monophasic action potentials were recorded with plunge electrodes from near the epicardial and endocardial surfaces of the left ventricle in isolated Langendorff perfused rat hearts.* ***Panel A:*** *A heart cut open after the experiment to show the placement of the electrodes within the left ventricular wall. Arrows show the position of the tips of the recording electrodes.* ***Panel B:*** *Simultaneous recording of sub-epicardial (lower trace) and sub-endocardial (upper trace) action potentials. The heart was paced at 5 Hz. The horizontal bar shows 100 ms, the vertical bars 5 mV.* ***Panel C:*** *Y axis: Action potential duration at 80% repolarisation (APD_{80}) for epicardial (diamonds) and endocardial (squares) monophasic action potentials recorded in an isolated rat heart from the left ventricular free wall. Intraventricular pressure was increased by inflation of a balloon to produce the diastolic pressures shown on the x axis. Error bars (± SEM) are smaller than the symbols (n=10).*

analogous to the S4 segment of Kv family of channels and hence none of the members of the family have intrinsic voltage dependence. Of the dozen or so members of the family, TREK-1, TREK-2 and TRAAK have been shown to be stretch-sensitive. Of these, TREK-1 has been found to be expressed in cardiac tissues of several species[16,18] and, when expressed in heterologous systems, TREK-1 forms a mechanosensitive potassium channel with the same characteristics as the channel recorded in isolated myocytes using patch clamp techniques.[19,20]

An example of a recording of this channel is shown in Figure 1. In isolated adult rat cardiac myocytes, we recorded a stretch activated channel in inside-out membrane patches (Figure 1). The channel could be reliably and reversibly activated by application of moderate suction to the patch clamp pipette. Channel open probability was close to zero at atmospheric pressure, rising to 0.7 or more with pressure differences of 120 cm H_2O (Figure 1B). The

channel conductance did not change with applied pressure (Figure 1C), nor did the mean open time change (data not shown). The channel responded to changes in pressure very rapidly (faster than our ability to resolve changes in Po), and showed no accommodation, hysteresis or persistence of activity (Figure 1D). With symmetrical solutions of 140 mM K^+, the channel had a conductance of 115 pS and was very selective for potassium ions (~100:1 K^+:Na^+).

The other type of stretch sensitive channel generally reported is a non-specific cation channel which has been recorded electrophysiologically in cardiac cells[21] as well as other cell types.[14] The gene coding for this channel has not been unequivocally identified, but it has recently been shown that TRPC1 channels are mechanosensitive, and, since TRPC1 is expressed in cardiac tissue, it seems likely that this is the genetic identity of the SAC in cardiac muscle.[22]

Although MEF and the channels responsible for it can be readily demonstrated experimentally, the physiological function of MEF is not clear. Partly, this has been due to the lack of specific blockers for either type of channel with which to perform experimental investigations of MEF, although a recently discovered toxin (GsMtx-4) which specifically and potently blocks SACs has been shown to be effective in preventing atrial fibrillation.[23] Unfortunately, there are as yet no comparable pharmacological tools to elucidate the role of the potassium channels TREK-1, TREK-2 and TRAAK.

The importance of MEF for arrhythmias

It has been suggested that an important role for MEF may be in controlling the dispersion of repolarisation of the action potential in the heart by integrating mechanical function and action potential morphology.[24] A large dispersion of repolarisation (that is, action potentials in different parts of the myocardium repolarising at different times) is arrhythmogenic,[25] and the pattern of repolarisation in the normal heart is such that it minimises such dispersion. This is achieved by cells in different parts of the heart having different action potential durations, thus tending to synchronise repolarisation across the ventricle wall, despite the later activation of epicardial cells.[26,27] This difference in action potential duration is largely a result of epicardial and endocardial cells having different levels of gene expression of potassium channels. For example, voltage-activated potassium channels such as Kv 4.2 and Kv LQT1 have been found to be expressed differentially across the ventricular wall,[28,29] with the expression of Kv 4.2 mRNA being more than eight times higher in epicardial muscle cells compared to papillary muscle cells.[30] Such a synchronisation of action potential repolarisation only occurs if the pattern of activation is undisturbed; in the absence of any other compensating mechanism a slowing of action potential propagation in some parts of the myocardium (for example by ischaemia), will tend to de-synchronise repolarisation. It is thought that MEF provides this compensating mechanism, since the slowing of action potential propagation produces a mechanical as well as electrical desynchronisation.[24]

MEF is not homogeneous in the ventricle

If this is indeed the role of MEF, one might expect to see a regional variation in MEF in the myocardium in the same way as for other channels. This has in fact been demonstrated. Dutetre and co-workers reported in 1972 that mechanically-induced changes in action potential duration were dissimilar in different parts of the intact left ventricle.[31] Similarly, Takagi and co-workers reported that epicardial monophasic action potential duration was shortened while endocardial monophasic action potential duration remained unaltered when canine left ventricles were subjected to stretch.[32] We have recently shown that similar differences in the electrical responses of endocardial and epicardial cells to stretch can be observed in Langendorff perfused isolated rat heart. Monophasic action potentials were recorded simultaneously from the sub-epicardial and sub-endocardial layer in rat hearts using fine needle electrodes (Figure 2A). When the intraventricular pressure was increased both action potentials shortened at all phases of repolarisation, consistent with the activation of stretch sensitive ion channels. However, the endocardial action potential shortened more than the epicardial, at all levels of repolarisation (Figure 2C). This differential shortening of the action potential with stretch would be expected to alter the dispersion of action potential repolarisation and indeed mechanically-induced disturbances in the dispersion of repolarisation have recently been reported in human.[33] This indicates that MEF is not homogeneously distributed in the ventricle. Such spatial variation in MEF in the myocardium could arise from two (not mutually exclusive) sources — either the level of expression of stretch sensitive channels could vary, or the degree of strain in the myocardium could be different in different parts of the myocardium.

Spatial distribution of stretch sensitive channels in the myocardium

As noted above, voltage dependent potassium channels have been shown to have a heterogeneous distribution and this underlies the differences observed in action potential shape in different parts of the heart.[28,34] Regional differences in the expression of the tandem pore potassium channels has not been so well studied. One study, using Northern Blot techniques, has shown that TBAK-1 and TASK-1 (non-mechanosensitive members of the tandem pore potassium channels) are not differentially expressed in epicardial and endocardial cells.[35] In contrast, we have recently shown in rat hearts that the gene expression level of TREK-1, quantified using real-time RT-PCR against GAPDH as a comparator gene, is heterogeneous between epicardial and endocardial cells. Gene expression was found to be 0.34 ± 0.14 in endocardial cells compared to 0.02 ± 0.02 in epicardial cells ($p < 0.05$). This is reflected in a different current density; whole cell chloroform-activated background potassium currents in epicardial and endocardial cells, presumed to be due to

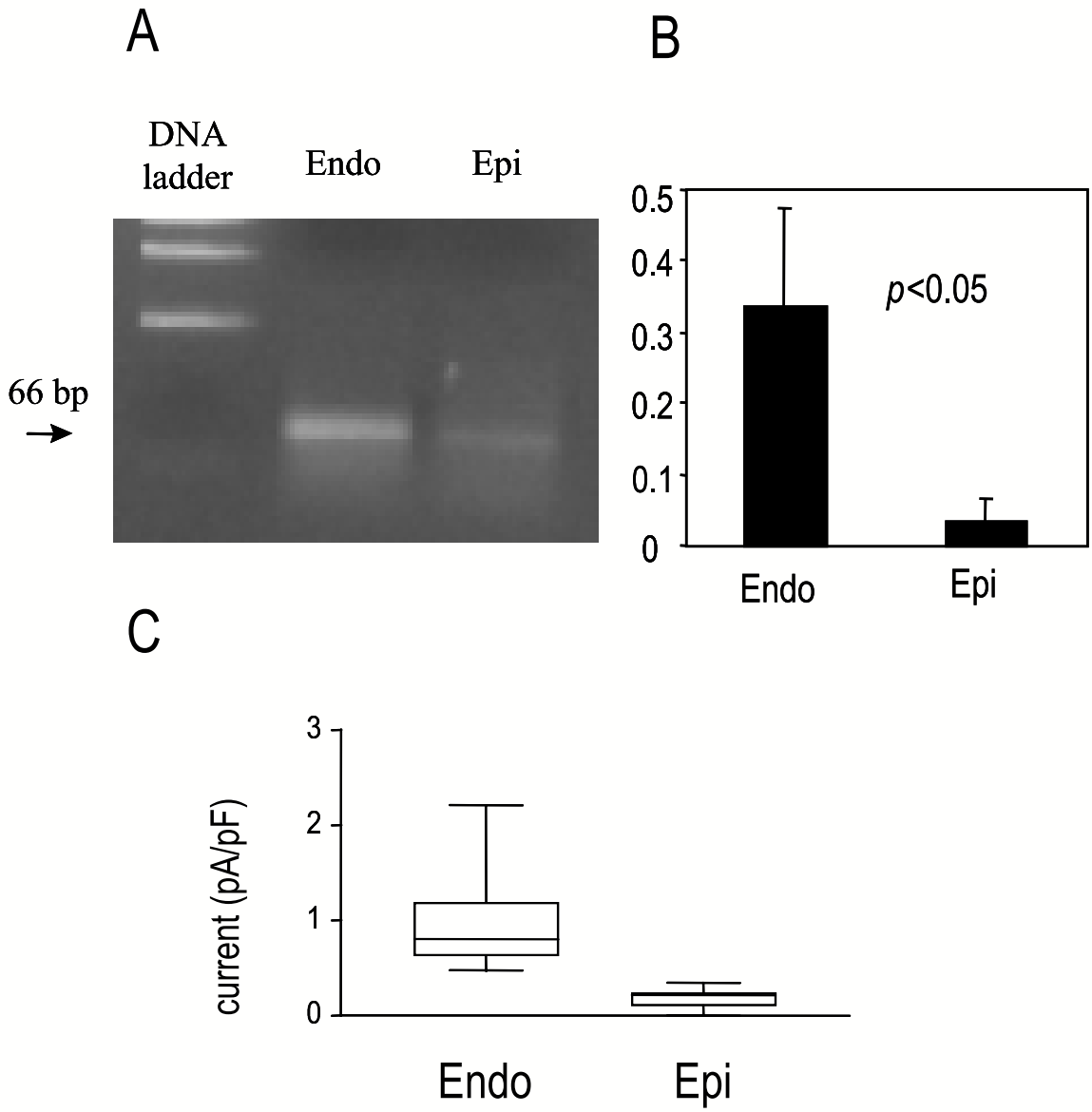

Figure 3. Panel A: *PCR results using TREK-1 primers to amplify TREK-1 mRNA in epicardial and endocardial myocytes. Batches of myocytes were isolated from epicardial or endocardial samples of rat left ventricle and subjected to PCR. Samples of the cell preparations were examined by whole-cell patch clamp recording of voltage activated potassium currents to confirm them as being epicardial or endocardial electrophysiologically before PCR. PCR showed a greater expression of TREK-1 mRNA in endocardial cells.* ***Panel B:*** *Expression level of TREK-1 quantified using real-time PCR and expressed relative to the expression of GAPDH. Bars show mean of 6 determinations, error bars are ± SEM.* ***Panel C:*** *Background potassium currents were recorded using whole-cell patch clamp in isolated myocytes. TREK-1 currents were activated by application of chloroform. The resulting currents, normalised to cell capacitance, were much larger in endocardial cells than in epicardial cells (box plots of currents; n = 6)* (redrawn from Tan JH, Liu W, Saint DA. Exp. Physiol. 2004 89(3):237-42.)

TREK-1, were 0.21 ± 0.06 pA/pF and 0.8 ± 0.27 pA/pF respectively ($p \leq 0.05$) (Figure 3).

Its not known whether the SACs are similarly heterogeneously distributed; in the absence of identification of the gene coding for this channel, it has not been possible to perform similar expression studies. The recent report that TRPC1 likely to be the SAC in heart[22] suggests the obvious corollary that studies of the distribution of TRPC1 gene expression are warranted.

Distribution of stress in the myocardium

However, there is another factor which could produce a heterogeneous distribution of MEF in the myocardium; the distribution of strain at the cellular level may not be uniform. While heart wall motion and local myocardial deformation and strain can now be estimated with

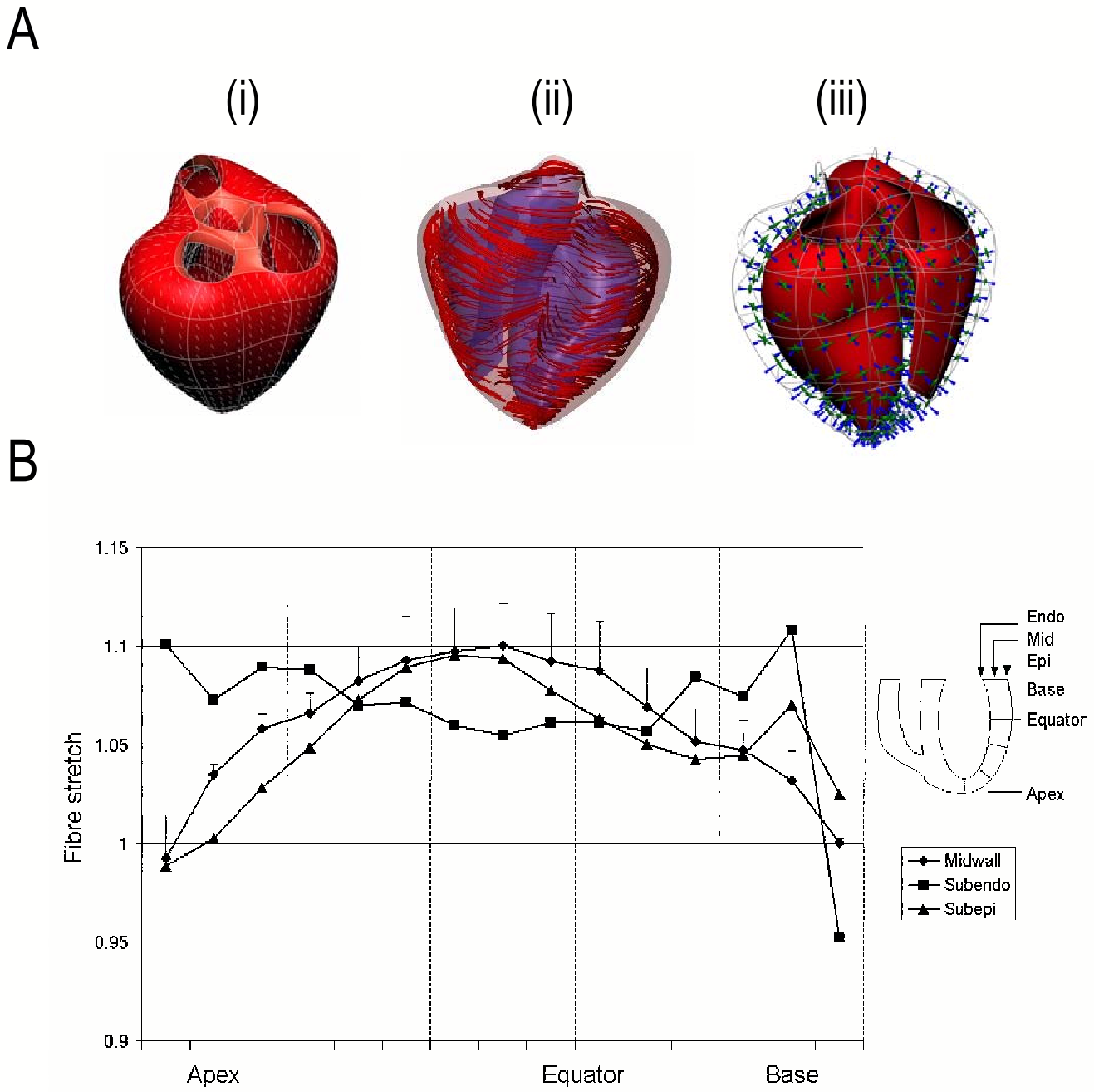

Figure 4. Panel A: *Computer models of the heart comprise: accurate representation of the geometry (i), fibre orientations in 3 dimensions through the myocardium (ii) and stress distributions (iii).* ***Panel B:*** *plot of stresses calculated in the model of pig heart at different points of the myocardium plotted from base to apex for sub-endocardial (squares), mid-myocardial (circles) and sub-epicardial (triangles) layers.*

surprising precision, regional stress cannot be measured, although computer modelling based on established continuum mechanics principles has enabled it to be estimated with some confidence.[36] The 3D geometry of right and left ventricles in dog and pig hearts, and the myocyte arrangement throughout the ventricular wall in these species has been well characterised.[37,38,39] These data have been incorporated into a detailed finite element model of cardiac anatomy, together with information about myocardial material properties.[40,41] Cellular mechanisms including membrane channel characteristics, excitation-contraction coupling and cross-bridge cycling dynamics are also included in this continuum description. This is part of a world-wide effort to model the cellular, structural, mechanical and electrical properties of the heart and it has been used widely to study the distributions of myocardial stress and deformation [42,43,44,45] and the spread of cardiac electrical activation.[46,47,48,49]

In these models, there are consistent transmural gradients of 3D LV strain, with normal strains in both systole and diastole greatest in the subendocardium and least at the epicardial surface. It has been shown that ventricular geometry, muscle fibre orientation and muscle layer organization all contribute to more uniform muscle fibre stretch in diastole (and fibre shortening during systole) than might otherwise be expected. [50,51,52,53] Nonetheless, there is significant regional variation in LV fibre strain, both transmurally and from base to apex.[54] (Figure 4). Hence,

even if the level of expression of stretch sensitive channels was identical at the cellular level in all regions of the myocardium, the different amounts of strain would produce differing electrical responses. A further complication is that the relationship between strain and activation of the channels is almost certainly non-linear.

Summary

There is now substantial experimental evidence that MEF is an important facet of cardiac physiology, although its role is not well understood. The response of the myocardium to a given strain is not uniform in the ventricle, and at least one type of stretch sensitive channel is heterogeneously expressed. However, the situation is complicated by the fact that regional forces on the myocardium are also not uniform, and our knowledge of the distribution of these forces and how this might interact with the responses at the cellular level are at present very nebulous. It seems likely that an increased understanding of MEF and its role in integrating the mechanical and electrical activity of the heart will pay dividends, since MEF has been implicated in producing the serious arrhythmias seen in many cardiac diseases. For example, as many patients with heart failure die from arrhythmia as from haemodynamic failure,[55] with similar statistics being seen in cardiac hypertrophy,[56] and it may be that these arrhythmias arise from changes in the mechanical properties of the myocardium being reflected in abnormal electrical behaviour.

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