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Mitochondria as a source of mechanical signals in cardiomyocytes

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Aims The myofibrillar and nuclear compartments in cardiomyocytes are known to be sensitive to extracellular mechanical stimuli. Recently, we have shown that alterations in the mitochondrial ionic balance in cells *in situ* are associated with considerably increased mitochondrial volume. Theoretically, this swelling of mitochondria could impose mechanical constraints on the myofibrils and nuclei in their vicinity. Thus, we studied whether modulation of mitochondrial volume in cardiomyocytes *in situ* has a mechanical effect on the myofibrillar and nuclear compartments.

Methods and results We used the measurement of passive force developed by saponin-permeabilized mouse ventricular fibres as a sensor for compression of the myofibrils. Osmotic compression induced by dextran caused an increase in passive force. Similarly, mitochondrial swelling induced by drugs that alter ionic homeostasis (alamethicin and propranolol) markedly augmented passive force (confirmed by confocal microscopy). Diazoxide, a mitochondrial ATP-sensitive potassium channel opener known to cause moderate mitochondrial swelling, also increased passive force (by $28 \pm 5\%$ at 10% stretch, $P < 0.01$). This effect was completely blocked by 5-hydroxydecanoate (5-HD), a putative specific inhibitor of these channels. Mitochondrial swelling induced by alamethicin and propranolol led to significant nuclear deformation, which was visualized by confocal microscopy. Furthermore, diazoxide decreased nuclear volume, calculated using three-dimensional reconstructed images, in a 5-HD-dependent manner by $12 \pm 2\%$ ($P < 0.05$). This corresponds to an increase in intracellular pressure of 2.1 ± 0.3 kPa.

Conclusion This study is the first to demonstrate that mitochondria are able to generate internal pressure, which can mechanically affect the morphological and functional properties of intracellular organelles.

Keywords Mitochondria • Mechanical signalling • Mitochondrial swelling • Myofibrils • Nucleus

1. Introduction

The cardiomyocyte contains an array of very densely packed organelles, as a consequence of which the volume of free, unoccupied cytosol is rather limited. Specialized cellular functions are highly organized within structural and functional compartments, such as mitochondria, sarcoplasmic reticulum (SR), and myofibrils. A close spatial localization of these compartments and molecular crowding of the cytosol provide favourable conditions for direct functional interactions to occur between the compartments. For example, internal junctions exist between SR and T-tubules that allow functional interactions between dihydropyridine and ryanodine receptors, which play a central role in excitation–contraction coupling.[1] Structural contacts between the SR and mitochondria are involved in the control of Ca^{2+} homeostasis.[2,3] Similarly, mitochondria cluster in the vicinity of the nucleus, resulting in the formation of contacts between these organelles that enable the energy requirements for nuclear function to be met.[4] These energy requirements, which are high, dictate that the distance of energy transfer between the mitochondrial membrane and the nuclear envelope must be as short as the cellular architecture will allow. A few years ago, we[5] and others[6] demonstrated a direct adenine nucleotide channelling between the mitochondria and cell ATPases due to the close proximity of mitochondria to the SR and myofibrils. Importantly, such channelling is very sensitive to cell architecture perturbations, indicating that contacts between the different organelles are a prerequisite for energy transfer by this mechanism.[7,8]

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Much recent work suggests that the crosstalk between cell organelles has not only a biochemical but also a mechanical nature (for review, see Ingber[9]). Indeed, physical forces that arise within the cell are able, via an internal molecular framework of actin microfilaments, microtubules, and intermediate filaments, to directly or indirectly modulate biochemical processes, by influencing the kinetics of protein–protein or protein–ligand binding, or by shifting chemical equilibria and molecular polymerization events. In other words, mechanical stimuli affecting 'tensegrity' (tensional integrity) might be translated into changes in biochemical activities. Identifying the cellular sources of these stimuli able to modulate the tensegrity network is thus of high importance.

Recently, we have shown that various conditions that induce mitochondrial depolarization or increase K^+ accumulation in the mitochondrial matrix (by activation of ATP- or Ca^{2+}-dependent K^+ channels as well as by inhibition of the K^+ efflux pathway via the K^+/H^+ exchanger) strongly increase Ca^{2+}-dependent contractile force in rat ventricular fibres with selectively permeabilized sarcolemma.[10] This effect seems to be unrelated to the ATP-generating activity of mitochondria or Ca^{2+} homeostasis. Osmotic compression of intracellular structures abolishes the effect of mitochondria-induced force modulation, suggesting a mechanical basis for the interaction between the organelles. We hypothesized that mitochondria are able to significantly increase their volume, thereby mechanically compressing the myofilament compartment and, in turn, leading to an augmentation of developed force. Indeed, mitochondrial depolarization and K^+ accumulation in the matrix were associated with a considerable increase in mitochondrial volume in permeabilized neurons.[11] Theoretically, such mitochondrial swelling could induce mechanical constraints on at least two types of organelle in close vicinity to the mitochondria—the myofibrils and nucleus.

Thus, the aim of the present work was to determine whether modulation of mitochondrial volume in cardiomyocytes *in situ* is able to have a mechanical impact on the myofibrillar and nuclear compartments. Bearing in mind that both compartments are sensitive to compression, such interactions might represent a novel mechanism of regulation of the cardiomyocyte via mitochondrial functional state.

2. Methods

2.1 Preparation of permeabilized fibres and cells

Three- to 6-month-old male C57BL/6 mice were anaesthetized by intraperitoneal injection of sodium thiopental, according to the recommendations of the Institutional Animal Care Committee (INSERM, Paris, France). The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Hearts were removed and rinsed in ice-cold Ca^{2+}-free Krebs solution equilibrated with 95% O_2/5% CO_2. Fibres (diameter 150–300 μm) were dissected from left ventricular papillary muscles. Specific permeabilization of the sarcolemma was achieved by incubating the fibres for 30 min in relaxing solution (basic solution at pCa 9, see below) that also contained 50 μg/mL saponin at 4°C. After skinning, fibres were maintained in relaxing solution at 4°C. Basic solution contained (in mM): *N,N*-bis[2-hydroxyethyl]-2-aminoethanesulfonic acid 60, (pH 7.1), free Mg^{2+} 1, MgATP 3.16, sodium phosphocreatine 12, K_2HPO_4 3, and taurine 20, dithiothreitol 0.5; ionic strength was adjusted to 160 mM with K methanesulfonate (total K^+ concentration was 80 mM). Free [Ca^{2+}] was buffered with 10 mM EGTA. The required [Ca^{2+}] was obtained by varying the CaK_2EGTA/K_2EGTA ratio. This 'intracellular' milieu was supplemented with mitochondrial respiratory substrates, 5 mM glutamate and 2 mM malate. Colloid osmotic pressure of the different dextran solutions was calculated according to van't Hoff's law extended by virial coefficients for dextran of 70.[12]

Individual rat ventricular myocytes were obtained as previously described[13] and maintained at 37°C.

2.2 Mechanical experiments

The protocol for measurement of passive force was as follows. Fibres were tied at both ends with a natural silk thread and mounted to a force transducer (AE 801, Aker's Microelectronics, Horton, Norway). Fibres were immersed in solution in 2.5 mL chambers arranged around a disc, which sat in a 22°C water bath with a magnetic stirrer. At the beginning of each experiment, the fibre was adjusted to the slack length (sarcomere length 1.85–1.9 μm) in basic solution in the virtual absence of Ca^{2+} (pCa 9). Then fibres were stretched in steps of 5% up to the maximal stretch of 30% above slack length. This pattern of stretches was repeated either in the same solution (control) or in the presence of drugs modulating mitochondrial volume.

2.3 Confocal microscopy

To visualize mitochondria, cardiomyocytes were loaded for 30 min at room temperature with 200 nM MitoTracker Green. Images were acquired before and after treatment with various mitochondrial modulators for 10–15 min using an LSM 510 Zeiss confocal microscope equipped with a Plan-Apochromat 63×/1.4 oil immersion objective and using the 488 nm line of an Argon laser for excitation and an LP505 nm filter for detecting emission.

For measurement of nuclear volume, the nuclei in saponin-permeabilized cardiomyocytes were stained with 5 μg/mL propidium iodide for 10 min and analysed further by confocal microscopy. Sections (256 x 256 pixels) were acquired before and after treatment with various mitochondrial modulators using a 543 nm laser line and emission was monitored using a band pass emission filter (BP 563–660 nm). Voxels were recorded at 60–120 nm lateral and 100 nm axial intervals. For each cell, the number of Z-plane images was selected so as to cover the complete vertical dimension of the nuclei (total number of Z-planes per cell was 60–140). For each series, eight to nine cells were analysed. Raw images underwent three-dimensional (3D) deconvolution and were reconstructed using the AutoDeblur and Autovisualize X software package (Media Cybernetics, MD, USA). The file names for acquired images were then encoded to avoid bias and later all images were subjected to morphometric analysis. A grid of points was superimposed on the 2D image sections after which the points that overlaid the fluorescent signal were counted. Nuclear volume was then estimated using the Cavalieri principle. In order to approximately estimate the degree of mitochondrial swelling, 3D images of mitochondria were obtained with 4–6 Z-planes.

For visualization of the nuclear membrane, cardiomyocytes were stained for 15–30 min with 1 μM BODIPY® FL glibenclamide at 37°C then visualized using the 488 nm line of an Argon laser for excitation and an LP505 nm filter for detecting emission.

2.4 Statistics

All results are expressed as means ± SEM. Different groups were compared using one-way ANOVA, or repeated measures ANOVA followed by Bonferroni's *post hoc* test. A probability value of <0.05 was considered significant.

3. Results

3.1 Passive force as a sensor of compression of the myofibrillar compartment in cardiac fibres

To estimate intracellular compression, we measured passive force developed by saponin-permeabilized fibres. The passive characteristics (passive force and stiffness) of cardiac myofilaments are known to be very sensitive to mechanical compression associated with a decrease in lattice spacing.[14,15] Furthermore, and in contrast to active tension, passive force can be measured in the absence of calcium, thus avoiding any confusing effects, this ion may have on cellular compartments such as mitochondria. Energy consumption by cellular ATPases is also much lower in the absence of Ca^{2+}, so that the ATP-generating function of the mitochondria in permeabilized fibres under these conditions is not very significant.

First of all, we tested whether passive force responds to compression of the myofibrillar compartment. *Figure 1* shows that after stretch under control conditions, there was an increase in passive force when permeabilized fibres undergo stepwise lengthening in the presence of 15% dextran (46 kPa). In contrast, there was no difference in passive tension when permeabilized fibres underwent two consecutive cycles of passive stretch under the same control conditions. These results confirm that induction of passive force by myofibrillar compression can be used as a sensor for intracellular tension.

3.2 Swelling of the mitochondrial matrix increases cardiac fibre passive force

Alamethicin is a well-known ion channel-forming peptide that induces mitochondrial swelling.[16,17] We confirmed the mitochondrial swelling effect of alamethicin in cardiomyocytes. Confocal microscopy of cardiomyocytes after incubation with alamethicin revealed considerable augmentation of mitochondrial size (*Figure 2A* and *B*, upper panels). Spaces between mitochondria disappeared and distances between mitochondrial rows became shorter. We further studied whether such mitochondrial swelling is able to change the mechanical properties of fibres. We found that incubation of permeabilized fibres with alamethicin indeed significantly increased passive force. As can be seen from *Figure 2A*, *B*, and *E*, increases in relaxed fibre length induce a much higher force when mitochondria are swollen by alamethicin.

Another way to increase mitochondrial volume is to inhibit the mitochondrial K^+/H^+ exchanger, thereby inhibiting K^+ efflux from the matrix of energized mitochondria. *Figure 2C* and *D* (upper panels) shows that propranolol, an inhibitor of the exchanger, has effects similar to those of alamethicin. The mitochondria had considerably greater volume and the space between the organelles was compressed. On the basis of the 3D images, we estimate that propranolol increased mitochondrial volume from $\sim 1.88 \pm 0.16\ \mu m^3$ ($n = 17$) to $3.04 \pm 0.17\ \mu m^3$ ($n = 17$), $P < 0.001$. Similar to alamethicin, propranolol increased passive force in permeabilized preparations at different fibre lengths (*Figure 2C*, *D*, and *F*).

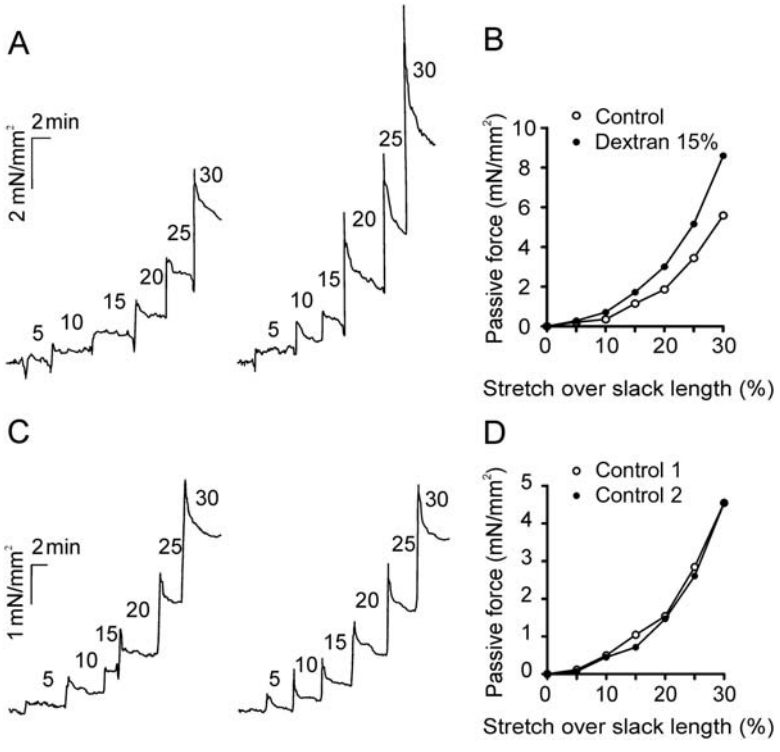

Figure 1 Effect of 15% dextran on the passive force developed by permeabilized ventricular fibres. (*A* and *C*) Traces of a typical experiment showing the increase in passive force following fibre stretch. Numbers indicate the percentage augmentation in fibre length above slack length. The first cycle of stretch in the absence of dextran was followed by a second cycle of stretch in the presence (*A*) or in the absence (*C*) of dextran. (*B* and *D*) Plots showing passive force as a function of fibre stretch for experiments is shown in (*A* and *C*), respectively.

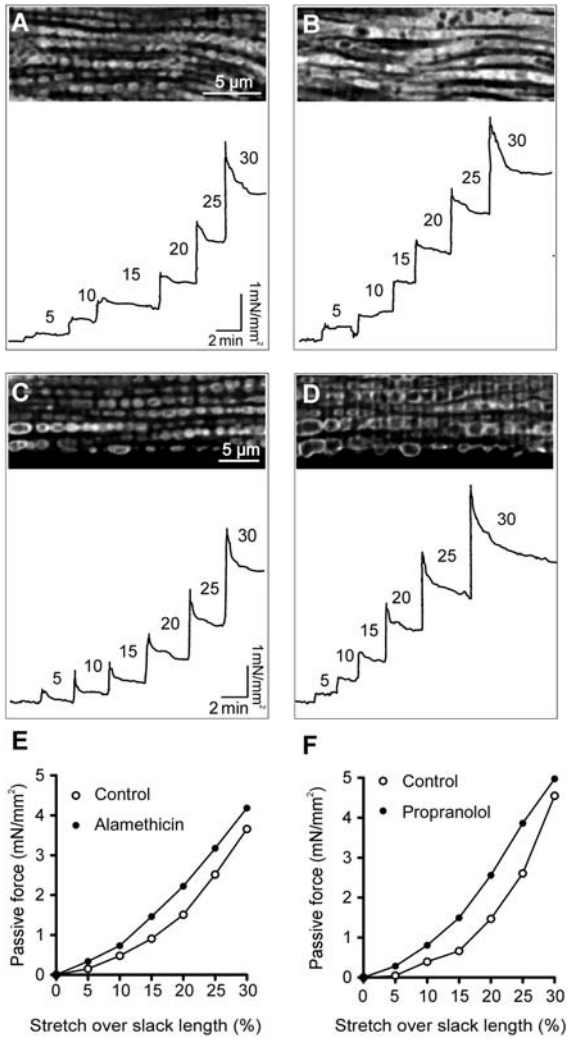

Figure 2 Effects of drugs inducing mitochondrial swelling on passive force development by permeabilized ventricular fibres. (*A*–*D*) Upper panels—confocal images of cardiomyocytes before (*A* and *C*) and after addition of 10 μg/mL alamethicin (*B*) or 1 mM propranolol (*D*). Lower panels—traces of typical experiments showing the increase in passive force following fibre stretch. Numbers indicate the percentage augmentation in fibre length above slack length. (*E* and *F*) Plots showing passive force as a function of fibre stretch in the presence of alamethicin (*E*) or propranolol (*F*).

In the next series of experiments, we studied the effects of diazoxide, a mitochondrial ATP-sensitive potassium channel opener known to cause moderate mitochondrial swelling by inducing potassium accumulation in the matrix. Diazoxide treatment increased passive force mainly at moderate lengths of stretch; at 10% stretch, force was augmented by 28 ± 5% (*Figure 3*). The solvent DMSO had no significant effect. On the other hand, 5-hydroxydecanoate (5-HD), a putative specific inhibitor of ATP-sensitive mitochondrial potassium channels, completely blocked the effect of diazoxide (*Figure 3*), confirming the role of channel opening in mediating diazoxide's effect on passive force.

Altogether, these experiments show that mitochondrial swelling has a marked impact on the mechanical properties of cardiomyocytes.

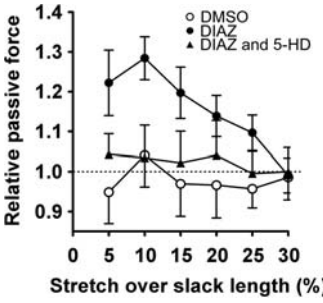

Figure 3 Effects of 200 μM diazoxide (DIAZ) or diazoxide + 150 μM 5-HD on passive force at different degrees of stretches. Fibres were stretched in steps of 5% up to the maximal stretch of 30% above slack length. This pattern of stretches was repeated in the presence of either solvent (DMSO), or diazoxide only, or diazoxide + 5-HD. The *y*-axis is the ratio of forces (second set/first set) for each stretch length. Diazoxide significantly ($P < 0.001$) increased passive force. This effect was completely removed by 5-HD ($P < 0.01$ vs. DIAZ group).

3.3 Nuclear volume as a sensor of intracellular mechanical interactions

In this series of experiments, our first aim was to estimate to what extent nuclei are sensitive to intracellular pressure. We used 70 kDa dextran, known to be too large to cross nuclear pores, to increase pressure on the nuclear envelope. *Figure 4A–C* is a 3D reconstruction of nuclei which demonstrate that when dextran concentration was increased from 0 to 6% or 15%, significant changes in nuclear morphology occurred. *Figure 4D* shows that the dextran-induced increase in osmotic pressure is inversely related to nuclear volume. These experiments further show that nuclear volume can serve as a fine sensor for detecting intracellular pressure.

3.4 Swelling of the mitochondrial matrix decreases nuclear volume

We next determined whether mitochondria can increase intracellular pressure sufficiently to alter nuclear morphology. *Figure 5A–C* shows that both alamethicin and propranolol induce significant mitochondrial swelling that completely changes the geometry of nuclei. The surface of sections through the nuclei was considerably reduced. The normal convex form of the nuclei was lost due to compression by swollen mitochondria. Three-dimensional reconstructions of these nuclei (*Figure 5D–F*) demonstrate that this is associated not only with remodelling of nuclear shape but also with nuclear compression.

Interestingly, alamethicin and propranolol induced different patterns of swelling. Under alamethicin, the mitochondrial mass looks more homogenous, sometimes without clear boundaries between individual mitochondria (*Figure 5B*). In contrast, under propranolol (*Figure 5C*), the structures of individual mitochondria are seen to be preserved. These differences probably relate to different mechanisms by which swelling is induced. Alamethicin is an ion channel-forming antibiotic, which induces large pores in the inner mitochondrial membrane. This leads to a massive entry of water into the mitochondrial matrix, resulting in the progressive loss of cristae structure and

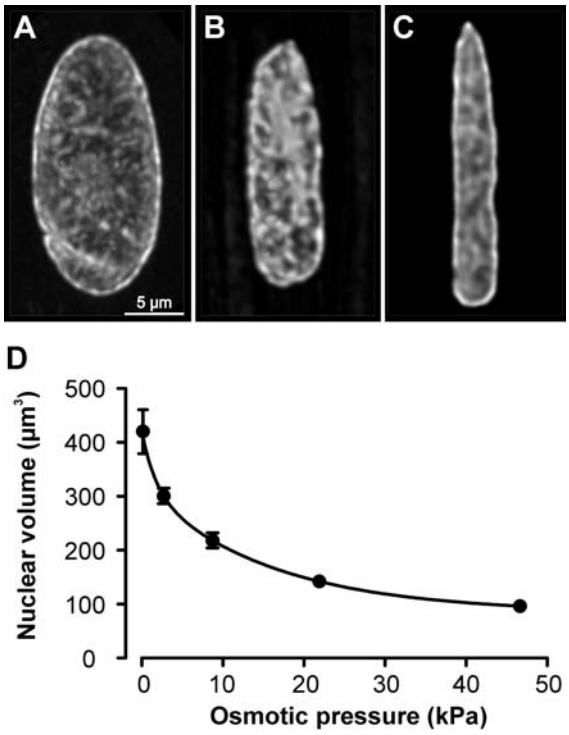

Figure 4 Osmotic pressure compresses nuclei. (*A*–*C*) Confocal images of nuclei under different osmotic pressures (*A*, without dextran; *B* and *C*, in the presence of 6 and 15% dextran, respectively). (*D*) Calculated nuclear volume as a function of osmotic pressure in the incubating medium.

a dilution of the matrix.[17] Moreover, such large-scale swelling is able to cause the inner membrane in some mitochondria to rupture, which could in turn cause the boundaries between neighbouring mitochondria to disappear. Propranolol in contrast does not form pores in the membrane but instead inhibits the K^+/H^+ antiporter, leading to progressive accumulation of potassium ions in the matrix. There is no rupture of the inner membrane and the mitochondria appear distinct and separated. Importantly, it is well known that pore formation by alamethicin depolarizes mitochondria, whereas inhibition of the K^+/H^+ antiporter does not result in inner membrane depolarization. The differences in membrane potential may also affect the pattern of MitoTracker Green distribution.

In the next series of experiments, we studied the effects of more moderate mitochondrial swelling induced by two drugs that cause potassium accumulation in the matrix, valinomycin and diazoxide. Nuclear volume was calculated using 3D reconstructed confocal images, allowing us to estimate precisely nuclear volume before and after incubation in the presence of the drugs. The pressure that induced nuclear compression was calculated using a calibration curve obtained with dextran. *Figure 6A* shows that incubation of permeabilized cardiomyocytes with the ionophore valinomycin led to a significant reduction in nuclear volume (by $12 \pm 2\%$). In order to exclude the possibility that this decrease was due to inhibition of mitochondrial ATP-generating activity, we treated permeabilized cells with bongkrekic acid, an adenine nucleotide translocator blocker that does not alter matrix volume.[10,11] Inhibition of ADP phosphorylation without mitochondrial swelling did not change the nuclear volume (*Figure 6A*). Another drug, diazoxide, had a similar compressing effect on the nuclei. Diazoxide treatment decreased the nuclear volume by $12 \pm 2\%$ (*Figure 6B*), whereas the solvent DMSO had no effect. Importantly, 5-HD, a putative specific inhibitor of ATP-sensitive mitochondrial potassium channels, completely blocked the effect of diazoxide (*Figure 6B*). *Figure 6C* and *D* shows that the pressures exerted on the nucleus in the presence of valinomycin or diazoxide are approximately 2 kPa.

Theoretically, there are two possible mechanisms by which the interior of the nuclei could have been compressed: mitochondrial swelling or increased nuclear envelope volume. To exclude the second possibility, we investigated whether mitochondrial modulators can affect the nuclear envelope. As can be seen in *Figure 6E*, diazoxide had no effect on the geometry of the nuclear envelope. This confirms that the mechanical effect on nuclear shape is mediated by the mitochondria.

4. Discussion

This study is the first to demonstrate that densely packed cells like cardiomyocytes are able to rapidly generate internal pressure, which can mechanically affect morphological as well as functional properties of intracellular organelles. The study of mechanotransduction has historically focused on how externally applied forces can affect cell signalling and function. However, a growing body of evidence suggests that forces generated internally are as important in regulating cell behaviour.[18] Here, we identify a novel source of intracellular force, the mitochondria.

Using nuclear volume and passive tension as intracellular force sensors, we have been able to show that dramatic intracellular forces are generated by swollen mitochondria in cardiomyocytes. Given the tight packing of organelles and myofibrils in cardiomyocytes, the significant volume occupied by mitochondria (≈45% of cell volume in adult mouse cardiomyocytes), and the extremely low free cytosolic volume (around 4–7% of cell volume), any increase in mitochondrial mass will automatically be at the expense of other compartments' volume. The physical constraints of cardiomyocytes also make large increases in the volume of subcellular structures impossible. For example, over-expression of PGC-1α, the master regulator of mitochondrial biogenesis, in adult mice induces only a modest increase in mitochondrial number. Nevertheless, such an increase leads to loss of sarcomeric structure and development of cardiomyopathy.[19] In contrast, in neonatal cardiomyocytes, which have loosely packed organelles, PGC-1α over-expression is able to induce a dramatic increase in mitochondrial number and size.[20]

The myofilaments and nuclei are perhaps the most likely structures to respond to increased intracellular pressure. Indeed, the myofibrillar compartment is known to be rather compressible. Osmotic compression, for example, caused by a weak increase in 500 kDa dextran concentration (in the range 0–6%, which corresponds to an augmentation of osmotic pressure by 7 kPa) decreases the cardiac myofibrillar lattice spacing by about 20%.[21] Interestingly, further increases in osmotic pressure have a much weaker compressing effect on lattice spacing. Thus, a relatively small increase in mitochondrial volume seems able to induce a relatively large reduction in lattice spacing.

The distance between thick and thin filaments is suggested to be an important determinant of active and passive tension. As myosin and actin filaments come closer together, both myofilament Ca^{2+}

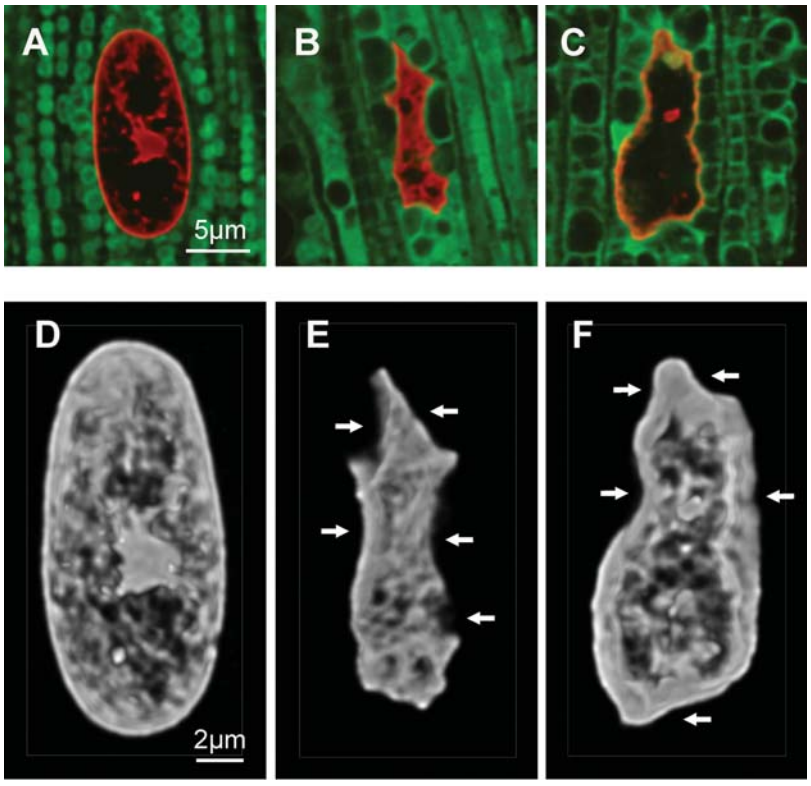

Figure 5 Mitochondrial swelling compresses nuclei. (*A*–*C*) Confocal images of cardiac fibres in control (*A*) and in the presence of 10 μg/mL alamethicin (*B*) or 500 μM propranolol (*C*). (*D*–*F*) Three-dimensional images of nuclei in control (*D*) and in the presence of alamethicin (*E*) or propranolol (*F*). Arrows indicate sites of deformation produced by swollen mitochondria.

sensitivity[21–23] and maximal Ca^{2+}-induced tension[24] increase. This could be explained by the hypothesis that the number of strong-binding cross-bridges that are formed is directly related to the proximity of the myosin heads to binding sites on actin.[25] However, compression of the myofibrillar compartment is also able to increase calcium-independent force — rigor tension[10] as well as passive tension.[14,15] In addition, the distance between protein filaments can affect the probability of weak cross-bridges forming, thus modulating the passive characteristics of cardiac muscle[14] and titin-based passive force.[26] In summary, compression of the myofibrillar compartment might be a factor modulating both contractility and passive characteristics of the myocardium. Our results suggest that relatively small increases in mitochondrial volume could induce a marked reduction in lattice spacing and a concomitant increase in passive force. Interestingly, at high stretch values, the effect of mitochondrial swelling on passive force disappears and, similarly, there is a weaker effect of high osmotic pressure on mechanical properties. It is tempting to speculate that the lattice spacing at high stretch is already decreased, so that the impact of mitochondrial swelling mitochondria is relatively weak.

One may speculate that the mechanism we describe could link mitochondrial swelling with the increased passive force observed in ischaemic myocardium. The contractile properties of myofibrils are very sensitive to rapid metabolic changes occurring in the cytosol, such as changes in pH and the concentration of inorganic phosphate and adenine nucleotides. For example, in ischaemia such metabolic changes inhibit active force generation and elevate passive force and myocardial stiffness. Also, mitochondrial volume, which is regulated by electrochemical potential-sensitive ionic flux across the inner mitochondrial membrane, seems to be increased under ischaemic conditions. As a consequence, swollen mitochondria may compress the neighbouring myofilaments, thus increasing both passive force/stiffness and active force in ischaemic myocardium without increasing energy expenditure. This could be an important adaptive mechanism under conditions in which contractile force is depressed due to the cytosolic accumulation of protons and inorganic phosphate.

Agents that cause mitochondrial swelling via non-specific permeabilization of the inner mitochondrial membrane (alamethicin)[27] or via K_{ATP} channel opening (diazoxide) (for review, see Garlid and Paucek[28]) are able to induce reactive oxygen species (ROS) production. However, the mechanical effects found in our study do not seem to involve ROS. Previously, we showed that moderately high ROS concentrations inhibit myofibrillar creatine kinase but do not alter the intrinsic properties of myofibrils such as the ability to develop rigor tension and myosin ATPase function.[29] Very high ROS concentrations produce irreversible alterations in contractility. In contrast, the mechanical effects of mitochondrial swelling appear to be completely reversible.[10] In addition, it has been shown that propranolol has a marked effect on passive force but does not affect mitochondrial ROS production.[30]

In the present work, we have also shown that mitochondria are able to mechanically compress the nucleus and to change its geometry. Such an effect is not trivial, because the nucleus is the stiffest cell

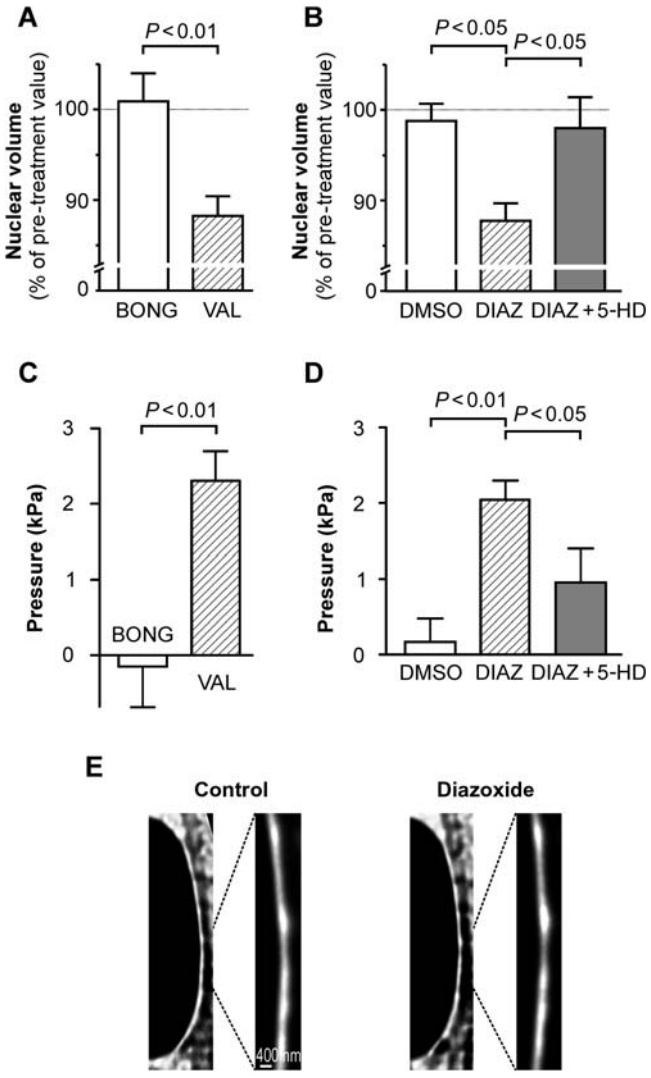

Figure 6 Valinomycin and diazoxide decrease nuclear volume. (*A*) Effect of 10 μM valinomycin on nuclear volume where 25 μM bongkrekic acid was used as a negative control. (*B*) Effect of 150 μM diazoxide and 150 μM 5-HD on nuclear volume. (*C* and *D*) Calculated pressure exerted on nuclei in the presence of valinomycin (*C*) or diazoxide (*D*). (*E*) Nuclear envelope confocal image in the presence and in the absence of diazoxide.

organelle.[31,32] It is therefore surprising that mitochondrial swelling induced by alamethicin and propranolol leads to a drastic modification of nuclear shape. Mitochondria were able to completely change the usual, oval geometry of the nuclei, thus revealing the existence of a great potential force arising from the cell interior.

Although we saw significant nuclear deformation when mitochondria were considerably swollen, even with moderate swelling induced by valinomycin and diazoxide, which was difficult to visualize using confocal microscopy, there was nuclear compression. Since the volume of the nuclear envelope was not augmented, it can be concluded that the volume of the nuclear interior was reduced as a result of mitochondrial swelling.

A question of great importance is how modulation of nuclear shape by mechanical signalling influences cell function. For many mechanotransduction events, the downstream cellular pathways of force-sensed gene transcription are well characterized (for review, see Dahl *et al.*[32]). Various mechanisms have been proposed to explain how forces that affect nuclear shape modulate gene transcription. Inside the nucleus, such forces could result in conformational changes to the DNA double helix or higher-order chromatin structure, which could then lead to changes in transcriptional activity. Force can induce remodelling and disassembly of the macromolecules, thus influencing transcriptional processes (for review, see Zlatanova and Leuba[33] and Marko and Poirier[34]). It has also been suggested that mechanical changes in nuclear shape can affect nuclear matrix proteins (NMPs) such as NMP-1 and NMP-2, which are able to regulate DNA supercoiling and interact directly with gene promoter sequences.[35,36] Force-induced conformational changes could further alter the accessibility of chromatin and genes to transcription factors. In addition to direct effects of force on DNA structure, nuclear deformation could also result in large-scale reorganization of genes within the nucleus.[32] Mechanical stress may also have an impact on nuclear transport processes as it has been shown that nuclear pore complexes function in a mechanically sensitive manner.[37]

The lamina network of the nuclear envelope seems to play an important role in transducing the mechanical signals that regulate

nuclear function. Nuclear lamins, a type of intermediate filament protein, provide structural support for the nuclear envelope and bind directly to chromatin and to chromatin-binding NMPs (see Dahl *et al.*[38] and references therein). Disruption of normal lamin organization inhibits RNA polymerase-dependent gene transcription.[39] It has been suggested that lamins and probably another nuclear envelope protein, emerin, are important mediators of strain-induced gene regulation.[40] Mutations in the genes encoding nuclear lamins cause a wide spectrum of diseases sometimes called 'laminopathies' (for review, see Worman and Bonne[41]). The pathogenesis of these diseases is still not known but altered expression of mechano-sensitive genes is thought to be involved. Mechanotransduction mediated by nuclear envelope deformation is thought to be triggered either by forces arising from the extracellular environment, or by intracellular forces arising from the cellular response to changes in extracellular matrix stiffness. Our study shows that cardiomyocytes (and therefore possibly other cell types) have an internal source of mechanical tension which is able to affect markedly nuclear volume and shape.

We have demonstrated in this work that diazoxide, an opener of mitochondrial ATP-dependent K^+ (K_{ATP}) channels, induces significant compression of nuclei. Diazoxide is well known to exert a cardioprotective action by mimicking the infarct size-limiting effect of preconditioning, i.e. by increasing the resistance to ischaemia. Such resistance needs time to be developed after diazoxide administration, and it is abolished by 5-HD, a putative blocker of K_{ATP} channels (for review, see O'Rourke[42]). The mechanism of post-ischaemic protection induced by diazoxide remains unclear, but most hypotheses propose that modulation of mitochondria by K_{ATP} channel opening is a triggering signal. Interestingly, various drugs inducing mitochondrial matrix swelling elicit cardioprotection.[42,43] It is tempting to speculate that nuclear compression caused by diazoxide-induced mitochondrial swelling might somehow modulate nuclear function, involving the activation of cellular cascade(s) that lead to increased cardiac resistance to ischaemic stress. This speculation is supported by our observation that 5-HD, which usually antagonizes the protective effect of diazoxide, is able to completely abolish diazoxide-induced nuclear compression.

It is possible that changes in mitochondrial geometry within cardiomyocytes influence not only the myofibrils and nuclei but also other organelles. Mechanical signals may displace the internal junctions between the SR and T-tubules that are responsible for functional interactions between dihydropyridine and ryanodine receptors, or they may displace the structural contacts between the SR and mitochondria that are involved in the control of Ca^{2+} homeostasis, excitation–contraction coupling, and regulation of adenine nucleotide channelling. In addition, mitochondria-induced internal mechanical signalling may act on cytoskeletal proteins such as the microfilaments, microtubules, intermediate filaments, or Z-disc-related structures that serve as stress–strain sensors (for review, see Ingber[9] and Hoshijima[44]). Also, deformation of the nuclear compartment by swollen mitochondria should have a direct mechanical effect on local cytoskeletal structures such as the tubulin or desmin filaments.

A limitation of this work is that it was performed at 22°C (as are almost all mechanical studies performed on permeabilized muscle preparations). This temperature is normally used in order to improve the stability of cells or fibres and to prevent uncontrolled force development, which could bias the results of experiments. This temperature also allowed us to compare our data with earlier results on mechanical activity that we and others have reported. However, studies on non-contracting cells have shown that at 37°C, mitochondria *in situ* can also demonstrate a marked swelling. Exposure of HeLa cells to a hypo-osmotic medium leads to a large (more than two-fold) increase in the mitochondrial diameter.[45] Similarly, at the same temperature, treatment of neurons with alamethicin or the potassium transporter valinomycin[46] or with glutamate[47] induces a marked mitochondrial remodelling leading to an increase in mitochondrial diameter.

In conclusion, we have shown that in the cardiomyocyte *in situ*, mitochondria represent a source of significant internal force production. The force they can exert is able to compress both the myofibrillar and nuclear compartments. This suggests a potential role for mitochondrial swelling in the regulation of myofilament and nuclear function by internal mechanical signalling.

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