Biology of the Heart Muscle

The Scientific Basis of Medicine

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University of London Audio-Visual Centre, 1977.

Produced by David R Clark.

Made for British Postgraduate Medical Federation.

Colour

Duration: 00:30:13:04

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<**Professor PC Harris to camera**>

Modern cardiac medicine and surgery have developed largely because of our knowledge of haemodynamics and the chemical properties of the heart. But as the solution of the mechanical problems of heart disease become practical possibilities, so it becomes increasingly evident that the health of the heart muscle tissue itself is very often the deciding factor between life and death.

Let's take, for instance, the case of myocardial hypertrophy, that built-in function of the heart muscle cell which saves more lives each year than all our pills and all our cardiac operations. What do we know about its mechanisms, or, how can we tell when, during the course of its development, the heart becomes irreversibly damaged? Or, to take another example, how can we protect the heart against the ill effects of ischemia? What can the surgeon do when the heart fails to come off the pump? Or how do the antiarrhythmic drugs act?

Such new problems require new techniques and new ideas, and while the ideas which underlay our solutions of the mechanical problems of the heart depended on classical physics and gross anatomy, the techniques which are required by our present problems depend on biochemistry and ultrastructure.

As a background to all this, in this talk, I hope to outline the structure and the function of the myocardial cell. It will be illustrated by electron micrographs and practical demonstrations which have kindly been provided by the members of the team of the Myocardial Research Labs of the Cardiothoracic Institute at the National Heart Hospital, and it gives me great pleasure to thank all my friends there for their kind advice and help.

As in all biological problems it is impossible to distinguish, to separate between the structure of the heart and its function. And we shall consider the structure and function of the myocardial cell under four headings.

<**Harris over table listing four main headings relating to the structure and function of the myocardial cell**>

The mechanism of contraction; the supply of energy; the process of excitation; and excitation-contraction coupling. Let’s begin by considering the mechanism of contraction.

<**Harris over electron micrographs and an animated diagram highlighting the contractile structures of the cardiac cell**>

The contractile structures in the cardiac cell are the thick and thin filaments. These are orientated longitudinally in the cell and are arranged in alternating arrays. The thick filaments are composed of myosin and comprise the darker staining band in the middle of the field. Thin filaments, here shown diagrammatically in orange, are composed mainly of actin but also carry the regulatory proteins, tropomyosin and the troponins. They arise from each side of a Z band. This muscle is fixed in contraction.

Relaxation and contraction consist of the movement of the thin filaments between the thick filaments.

The filaments are grouped in fascicles, the myofibrils, which extend the length of the cell and comprise many alternating arrays of thin and thick filaments. Here you see a low-powered electron micrograph showing the width of the whole cell, including its nucleus. The Z bands of the different myofibrils are approximately in register across the cell and it's this which gives the striated appearance under the light microscope.

Here is a high-powered transverse section of a portion of a cell. Now the thick filaments have been cut across and you can just see the thin filaments between them.

Now we shall turn to the provision of energy.

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<**Harris over animated diagram highlighting provision of energy for heart muscle contraction**>

The mechanism of contraction requires energy. And this is provided by the breakdown of adenosine triphosphate, or ATP. The hydrolysis of ATP results in the formation of adenosine diphosphate, ADP, and inorganic phosphate together with the liberation of energy. The process is carried out by an enzyme, myofibrillar ATPase, which specifically links the liberation of energy, ATP, to the function of mechanical contraction. But, of course, the myofibril couldn't go on indefinitely using up the cell's ATP unless there was some mechanism to reconstitute it. This function is mainly carried out by the mitochondria which lie in close proximity to the myofibrils.

<**Harris over electron micrographs of mitochondria, then back to last diagram**>

Mitochondria are rounded or sausage-shaped bodies. They are composed of an outer membrane which surrounds the cell, and an inner membrane which is highly

folded. The ATP-producing enzyme systems are located in the inner membrane and the effect of the folding is to increase the surface area of the membrane and thus its capacity to form ATP.

The cardiac cell is abundantly supplied with mitochondria, which you can see here as the dark staining bodies between the myofibrils.

The reformation of ATP from ADP and inorganic phosphate requires energy. You don't get something for nothing. And this energy, in turn, is derived from the oxidation of metabolites.

<**Harris over graph detailing the function metabolites of the human heart, then diagram showing the removal of hydrogen from metabolic intermediaries**>

This diagram shows the arteriovenous differences of metabolites in the human heart, expressed as their oxygen equivalents. Free fatty acids are the most important source of oxidative energy for the heart, followed closely by glucose. Lactate is utilised only to a small degree, and pyruvate in negligible amounts.

The pathways of oxidation of free fatty acids and glucose are complex. But fortunately, we need only concern ourselves with one process which recurs throughout their catabolic pathways.

The process of removal of hydrogen. Hydrogen is removed from the metabolic intermediaries by a number of different dehydrogenase enzymes, and this whole catabolic system can be looked upon simply as a device to produce hydrogen. The hydrogen, which is removed, is then burned to water and it is the energy liberated by the combustion of hydrogen which is utilised to reform ATP.

<**Harris to camera, then over graphs and diagrams showing how the body utilises hydrogen for energy**>

I was brought up to believe that the energy of life was provided by the burning of food stuffs to carbon dioxide and water. This isn't strictly true, it's only the energy provided by the burning of hydrogen to water which is utilised for biological purposes. From this point of view, the production of carbon dioxide is simply a by-product, the flame of life is a hydrogen flame.

But if you mix hydrogen and oxygen together, and apply a lighted match, the result is an explosion and the energy released is far too violent to be made use of biologically. So, instead, the mitochondrion brings the two elements together, gradually, along a chain of intermediaries – the respiratory chain.

As each link in the chain becomes reduced, it oxidises the preceding link and a small quantity of energy is liberated. At three links in the chain, this energy is used to form ATP. In this way, the consumption of 1 atom of oxygen gives rise to 3 molecules of ATP.

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The phosphorylation of ADP to ATP is an integral part of the mechanism in the 3 relevant links in the respiratory chain. So much so that those links will not function in the absence of ADP. Under normal conditions, there is an abundant supply of hydrogen and a sufficient supply of oxygen. What controls the function of the respiratory chain is, therefore, the supply of ADP.

<**Harris over electron micrograph of mitochondria, then film of experiment using mitochondrial suspension**>

We can show this on a preparation of mitochondria, isolated from the heart. By homogenising the heart and subjecting it to ultracentrifugation at the appropriate rates, a preparation of pure mitochondria can be harvested.

In this experiment, the mitochondrial suspension has been oxygenated and placed in a temperature-controlled chamber in which an oxygen electrode records the pO2

continuously. If the mitochondria respire, they take up oxygen and the pO2 falls. Here the mitochondria are provided with oxygen and substrate to provide hydrogen but they are not provided with ADP, so there is very little consumption of oxygen. Here we add ADP to the mitochondrial suspension. This is immediately followed by an abrupt fall in pO2, indicating that the presence of ADP has caused the respiratory chain to function and oxygen to be consumed.

When all the ADP has been used up, the rates of respiration of the mitochondria returns to its initial low level. In this way, there is a beautifully adjusted control mechanism which supplies ATP automatically in proportion to its rates of utilisation. As the myofibril contracts, it hydrolyses ATP to ADP, utilising the energy produced, but at the same time, the production of ADP causes the mitochondria to respire and reform ATP.

Under conditions of hypoxia, this control mechanism fails, because the respiratory chain cannot function. The concentration of ATP falls and the energy requiring processes of the cells suffer.

Now we shall turn to the process of excitation.

<**Harris over electron micrograph and animated diagram showing function of myocardial cell**>

Each myocardial cell is surrounded by a cell membrane. It used to be thought that the myocardium was a syncytium, but we now can see, under the electron microscope, that there is a complete separation of individual cells at the intercalated discs. In certain portions of the disc, the two adjacent cell membranes are closely applied to each other to form a nexus and it is thought that this is where excitation is transmitted from one cell to the next.

The cell wall membrane has an organised structure of lipid and protein. It isn't simply an inert barrier but possesses active enzyme functions. Important among these is the activity of the sodium potassium exchange pump. The pump actively expels sodium

from the cell and pools in potassium. Because of the activity of the pump, the concentration of potassium is high inside the cell, while the concentration of sodium is low.

Outside the cell, the concentration of potassium is low, while that of sodium is high. The permeability of the membrane varies greatly between the different ions. Among the cations, the membrane is fairly permeable to potassium but not to sodium or calcium. Among the anions, it is fairly permeable to chloride but not to the organic acids and negatively charged proteins inside the cell. The combination of an active sodium-potassium exchange pump and different permeabilities of different ions leads to a resting potential difference between the outside and inside of the cell so that the inside is electro-negative by about 90mV with respect to the outside.

When the wave of excitation reaches the cell, it causes a change in the permeability characteristics of the cell wall membrane which gives rise to the action potential.

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<**Harris over animated electrical tracing of action potential of cardiac muscle, to camera in between**>

The action potential of cardiac muscle is not a simple spike but is of relatively long duration. The permeability properties of the cell wall membrane change in a regular sequence during the inscription of the action potential. The initial sharp reduction in the transmembrane potential is due to the large inward movement of sodium ions. As we have seen, these positively charged ions are present in high concentration outside the cell so that they pour into the cell along both the concentration gradient and an electrical gradient. This is followed by a less pronounced but more prolonged inward movement of calcium ions. Finally, potassium ions move out of the cell, thus restoring the resting transmembrane potential.

Several drugs affect the action potential and the movement of ions. Quinidine, for instance, slows the initial rate of rise, due to the inward movement of sodium ions.

Beta stimulants increase the inward movement of calcium ions, while beta blockers prevent this action. Verapamil decreases the inward movement of calcium.

By the end of the action potential, the cell has gained sodium ions and lost potassium ions. So it is the job of the sodium-potassium exchange pump to extrude the sodium and retrieve the potassium during the resting phase. The pump has to move ions against their resting concentration gradients and this requires energy. Just as the energy required for the action of the myofibrils is provided by the breakdown of ATP, so is the energy required for the action of the sodium potassium pump. A specific sodium potassium ATPase is located in the cell wall membrane and links the energy liberated by the hydrolysis of ATP to the activity of the pump.

<**Harris over diagram plotting the activity of sodium potassium against ouabain**>

This is where the cellular action of cardiac glycosides appears to occur. The glycosides inhibit the activity of sodium potassium ATPase and thereby the function of the sodium potassium pump. This diagram plots the activity of sodium potassium ATPase on the vertical axis against rising concentrations of ouabain on the horizontal axis. As the concentration of ouabain increases, the activity of the ATPase is inhibited.

<**Harris over electrical reading, electron micrograph and diagrams illustrating the link between excitation and contraction**>

And now we have to consider the link between excitation and contraction. The up-stroke of the action potential is followed almost immediately by the onset of contraction, shown here on the lower tracing. The rapidity of the link between excitation and contraction is facilitated by the existence of a system of transverse tubules which pass inwards at right angles from the surface of the cell at the level of each Z band. In this way, the wave of excitation reaches rapidly deep into the interior of the cell.

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<**Harris shown seated next to previous diagram, then electron micrographs of sarcoplasmic reticulum**>

The chemical mediator between excitation and contraction appears to be the calcium ion. In addition to the transverse tubules, each myofibril is surrounded by an irregular network of longitudinal tubules, or the sarcoplasmic reticulum.

Here, we see the sarcoplasmic reticulum as the section of the electron microscope grazes the surface of a myofibril. Specialised endings of the sarcoplasmic reticulum occur in relation to the T tubules and the surface of the cell. This electron micrograph shows such an ending, lying in close apposition to a T tubule. Here you see a transverse tubule, cut in cross-section. On either side of it are endings of sarcoplasmic reticulum located between the mitochondria and the tubule itself. Sarcoplasmic reticulum has the property of actively and avidly accumulating calcium ions. This property requires energy in order to function and it isn't surprising that the energy is, once again, supplied by the hydrolysis of ATP.

We can show this on preparations of sarcoplasmic reticulum isolated from the heart. This fraction has been isolated in a similar way to the mitochondrial fraction we saw earlier. Notice how the membranes of the sarcoplasmic reticulum have been broken and formed into vesicles.

<**Harris, seated, to camera. Then over film showing spectrophotometer tracing of calcium ions**>

In order to study the uptake of calcium by such a preparation, we suspend it in a solution of calcium ions, to which has been added the calcium ion-sensitive pigment, murexide. In this way, through a sensitive spectrophotometer, we can follow changes in the concentration of calcium ions.

Here you see a recording of the calcium ion concentration of the solution into which the sarcoplasmic reticulum has been suspended. Now we shall add some ATP and stir it. You can see when the ATP has been added on the recording because opening the cuvette housing lets in the light and sends the pen off the paper. Now you will see the concentration of calcium ions begin to change. An upward movement of the pen means a decrease in the concentration of calcium ions in the suspending medium and, therefore, an uptake of calcium by the sarcoplasmic reticulum.

A little while later on, when all the ATP has been used up, the uptake of calcium ion flattens out again.

<**Harris, seated, refers to earlier diagram**>

In the cell, the uptake of calcium by the sarcoplasmic reticulum is a combination of such active transport and physical binding. It is so powerful that during relaxation the calcium ion concentration in the sarcoplasm falls to somewhere in the region of 10^{-7} molar. But it seems that when the wave of excitation passes along the membrane and down the T tubules, it comes into apposition with the specialised terminals of the sarcoplasmic reticulum, and when this happens it causes the sarcoplasmic reticulum to release its calcium so that the concentration of calcium ions in the vicinity of the myofibrils suddenly rises to somewhere in the region of 10^{-5} molar. And it is this sudden increase in the concentration of calcium which causes the myofibril to contract.

<**Harris over filmed experiment showing changes in calcium ion concentration**>

We can demonstrate the effect of such a change in calcium ion concentration on the myofibrils by removing the cell wall membrane by the action of chemicals. In this way the myofibrils are bathed directly by the liquid surrounding the muscle and one can change the calcium ion concentration of the bathing liquid at will.

Here is such a skinned muscle preparation. It is a tiny fragment of papillary muscle, about 3mm long, held between two hooks. One hook is attached to a transducer to measure the tension. At the moment, the skinned muscle is bathed in a buffered solution at a calcium ion concentration of 10 to the minus 8 molar. The pen recorder shows that the muscle is in a state of relaxation. Now the solution is changed from 10^{-8} molar to 10^{-5} molar, by changing the flask from which the pump perfusing the muscle is being supplied. The increased calcium ion concentration results in a contraction of the myofibrils. You can imagine that this represents, in slow motion, what happens when the excitatory wave reaches the endings of the sarcoplasmic reticulum and causes the release of calcium ions. Although, as we have seen earlier, there is a certain small inward movement of calcium across the cell wall during excitation, it is the release of calcium by the sarcoplasmic reticulum which seems to be the important cause of the increase in the cytoplasmic concentration of calcium during systole.

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<**Harris to camera, then back to previous film**>

A little later on, you can see that the myofibrils have reached a new, steady state of contraction, the degree of which is determined by the concentration of calcium ions.

Now we shall return the muscle to a 10^{-8} molar calcium ion concentration. Once again the muscle relaxes and you can imagine that this represents in slow motion what happens when the sarcoplasmic reticulum starts taking up calcium again after the action potential is finished.

If we jump a little space of time, we can now see that the muscle has returned to its pre-existing relaxed state.

<**Harris over table relating to actions of myosin and actin**>

Contraction of the myofibril, caused by the sliding movement of the thick and thin filaments is due to the interaction of the myosin of the thick filaments and the actin of the thin filaments. In the resting stage, when the calcium ion concentration is low, the myosin and actin are kept apart by the obstructive action of the regulatory proteins – tropomyosin[1] and the troponins, which are situated on the thin filaments. When, following excitation, the cytoplasmic calcium ion concentration rises to 10^{-5} molar, the obstructive action of the regulatory proteins becomes inhibited. This allows myosin to interact with actin and the process of contraction occurs.

<**Harris over graph showing myofibrillar ATPase and concentration of calcium ions**>

Finally, as another example of the wonderfully adjusted co-ordination of the cell, we find that the ATPase activity of they myofibrils is also sensitive to the concentration of calcium ions. This graph plots myofibrillar ATPase activity on the vertical axis, against the concentration of calcium ions on the horizontal axis. Notice how the ATPase activity increases specifically between 10^{-7} and 10^{-5} molar calcium, the two concentrations thought to occur in relaxation and contraction.

In this way, calcium ions not only switch on the interaction between myosin and actin but also activate, at the same time, the mechanism for providing the energy for contraction.

<**Harris, seated, to camera**>

And so we've come full circle. Contraction of the myofibrils, provision of energy by the mitochondria, excitation across the cell wall membrane, release of calcium by the sarcoplasmic reticulum initiating contraction. From time to time we've hinted at clinical implications. There hasn't been time to take these implications further but I would like to end by saying how, in my everyday clinical practice, I find the understanding generated by such studies increasingly helpful and relevant.

[1] Appears on the diagram as topomyosin.

<End credits>