Mechanisms of Acute Ischemic Contractile Failure of the Heart

Role of Intracellular Calcium

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Introduction

Ischemic heart disease is usually caused by atheroma in the coronary arteries and results in reduced or absent blood flow to cardiac cells, depriving them of oxygen and metabolic substrates and causing abnormal accumulation of ions or products of metabolism. The consequences of cardiac ischemia include reduced force production, life-threatening disturbances of cardiac rhythm, and ultimately death of the cells. Because of the high prevalence of ischemic heart disease, and its high cost in morbidity and mortality, there has been intense interest in understanding the mechanisms by which ischemia alters the normal functioning of cardiac muscle.

The effects of ischemia on contractile performance have been reviewed previously (1, 2) and are only briefly summarized here. When a beating heart is made globally ischemic, there is a rapid decline in developed pressure. This decrease in force production is apparent within 1 min and is generally complete, with little or no developed force, after 5–10 min. After 10–20 min of ischemia, a gradual rise in diastolic pressure becomes apparent (the ischemic contracture), and increases in size as ischemia progresses. If reperfusion is started before or during the early stages of ischemic contracture, eventual recovery of contractile function can be virtually complete. However, if reperfusion is delayed until the contracture is well established, muscle damage is accelerated and recovery is poor (3). In addition to reduced force production, the early minutes of ischemia are associated with electrophysiological abnormalities and arrhythmias (4). Arrhythmias also occur during reperfusion.

The early fall of force, arrhythmias, and muscle damage all contribute to acute ischemic contractile failure of the heart, which we define as impairment of cardiac function produced by a duration of ischemia within which some recovery is possible if ischemia is reversed (i.e., up to 1–2 h). Recent data, in particular the measurement of intracellular free calcium ($[Ca^{2+}]_i$) during ischemia, has given new insight into the mechanisms producing these effects. In this review we consider the following topics in the light of these new measurements: (*a*) the mechanisms causing the early rapid fall in force; (*b*) the mechanisms causing ischemic and reperfusion-induced arrhythmias; (*c*) the mechanisms responsible for ischemic and reperfusion-induced muscle damage.

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Force production by cardiac muscle

Before considering the events occurring in ischemia, it will be helpful to briefly review the factors involved in normal force production by cardiac muscle. It is now well established that cardiac muscle contracts when an action potential triggers the release of calcium (Ca^{2+}) into the cytoplasm (the Ca^{2+} transient) and that this Ca^{2+} comes principally from stores in the sarcoplasmic reticulum (SR). Experimental evidence from a variety of sources suggests that it is the entry of a small amount of Ca^{2+} from the extracellular space (the Ca^{2+} current) which triggers Ca^{2+} release from the SR (Ca^{2+}-induced Ca^{2+} release) (5). The Ca^{2+} ions then bind to troponin C on the myofibrils, allowing the interaction of actin and myosin, the splitting of ATP and the generation of force. The variations in force production observed with many interventions arise by variations in the amount of Ca^{2+} stored in the SR and subsequently released into the cytoplasm. Experiments using "skinned" muscle (in which cell membranes have been removed or permeabilized) have demonstrated the relation between the $[Ca^{2+}]_i$ and force production at the myofibrillar level (e.g., reference 6).

If the relation between $[Ca^{2+}]_i$ and force were unique, there would be no way to influence cardiac force production other than by altering systolic $[Ca^{2+}]_i$. In fact, however, the relation between $[Ca^{2+}]_i$ and force is *not* unique. Many studies with both skinned and intact heart muscle have shown that the Ca^{2+} sensitivity of the contractile proteins may also be altered (6–8). An increase in Ca^{2+} sensitivity results in greater force at a given $[Ca^{2+}]_i$, whereas a decrease in Ca^{2+}-sensitivity reduces force. There is also a third way in which force may be altered, namely by changing the maximum Ca^{2+}-activated force. Conceptually, an increase in maximum Ca^{2+}-activated force could be caused by increasing the absolute number of cross-bridges, the number of active (i.e., force generating) cross-bridges or the force produced per cross-bridge. In practice it is difficult to distinguish between changes in Ca^{2+} sensitivity and maximum Ca^{2+}-activated force unless a wide range of activating $[Ca^{2+}]$, including saturating $[Ca^{2+}]$, are studied. Also, many interventions which alter Ca^{2+} sensitivity also affect maximum Ca^{2+}-activated force.

Acute ischemic contractile failure

A. THE EARLY FALL OF FORCE

The implication of studies revealing the mechanisms of normal cardiac contraction is that the early fall of force in ischemia

could be primarily caused by (*a*) a diminished Ca^{2+} transient; (*b*) reduced Ca^{2+}-sensitivity; (*c*) reduced maximum Ca^{2+}-activated force; or (*d*) a combination of these factors. In order to distinguish between these possibilities, it is necessary to simultaneously measure $[Ca^{2+}]_i$ and force during ischemia.

Measurements of $[Ca^{2+}]_i$ in ischemia. Although methods for measuring $[Ca^{2+}]_i$ in intact heart muscle have been available for more than a decade, the difficulty of modeling ischemia in isolated tissue preparations has meant that they have been applied to ischemia only recently. Earlier studies therefore examined models such as anoxia or metabolic blockade, in which measurements of $[Ca^{2+}]_i$ are technically simpler. These models mimic certain aspects of ischemia, but they also differ from it in important respects, as shown in Table I. Because of these differences, renewed attempts have recently been made to measure $[Ca^{2+}]_i$ in ischemia. A number of different techniques and models have been used and these are now discussed.

i. Nuclear magnetic resonance (nmr). $[Ca^{2+}]_i$ can be measured in the isolated Langendorff-perfused heart using the nmr-detectable Ca^{2+} indicator 5FBAPTA (9–13). A lipid-soluble ester of 5FBAPTA is loaded into the cells by diffusion and is cleaved by endogenous esterases, releasing 5FBAPTA. Because nmr is an insensitive technique, the amount of intracellular 5FBAPTA required for an adequate Ca^{2+} signal produces significant buffering of $[Ca^{2+}]_i$ and several minutes are required for the accumulation of an adequate spectrum. Thus, initial studies obtained only a time-averaged measure of $[Ca^{2+}]_i$, in which no distinction between diastolic and systolic levels could be made. A recent refinement is to "gate" the collection of spectra into several time bins following the stimulus, allowing resolution of the Ca^{2+} transients (14). A considerable advantage of nmr over other methods of measuring $[Ca^{2+}]_i$ is that phosphorus metabolites such as ATP, creatine phosphate, and inorganic phosphate can be alternated with measurements of $[Ca^{2+}]_i$.

In experiments with Langendorff-perfused rat hearts which were made globally ischemic, time-averaged $[Ca^{2+}]_i$ over the first 6 min was *unchanged* from its control value of 600 nM. After 6 min an increase became apparent, and spectra collected from 9–15 min of ischemia showed that time-averaged $[Ca^{2+}]_i$ had increased to 3 μM. When reperfusion was initiated after 15 min of ischemia, time-averaged $[Ca^{2+}]_i$ returned to its preischemic levels. The intracellular concentration of 5FBAPTA was unchanged after ischemic exposures of up to 45 min, indicating minimal cell membrane damage with this duration of ischemia. Qualitatively similar results have been obtained using isolated ferret hearts (10, 12, 13).

ii. Fluorescent indicators. A second approach to measuring $[Ca^{2+}]_i$ in ischemia has been to load isolated Langendorff-perfused hearts with a lipid-soluble ester of the fluorescent Ca^{2+} indicator indo (15, 16). When indo-loaded rabbit hearts were subjected to global ischemia, the most striking finding was a substantial rise in both the peak systolic and end diastolic fluorescence, which was most rapid during the first 30 s and approached a plateau after 90 s (15, 16). The rise in the end diastolic fluorescence ratio was so marked that after 90 s of ischemia it equalled the peak *systolic* value before ischemia. This result may in part be due to the fact that ischemia also caused an increase in the time course of the fluorescence transient. Because the decay of the transient was prolonged and it was still declining at the next contraction, this would be expected to cause an increased end diastolic level. These effects were all rapidly reversed when ischemia was relieved after 90 s. It was also noted (16) that the effects of ischemia on the fluorescence transients were mimicked when an intracellular acidosis was induced by increasing the carbon dioxide in the superfusing solution.

iii. Aequorin. A third approach to measuring $[Ca^{2+}]_i$ in ischemia has utilized the Ca^{2+} indicator aequorin (17–19). Aequorin is a photoprotein which emits light in the presence of Ca^{2+}, and may be introduced into cells either by microinjection or by reversible membrane permeabilization procedures.

In one approach using aequorin, ischemia was simulated in isolated ferret papillary muscles by changing superfusion from Tyrode solution equilibrated with O_2/CO_2 to water-saturated N_2/CO_2 gas (17, 18). This model mimics ischemia in that oxidative phosphorylation is prevented and the extracellular space is restricted. However, one problem is that volatile products of

Table I. Comparison of Hypoxia, Metabolic Blockade, and Ischemia

	Hypoxia	Metabolic blockade	Ischemia
Oxidative phosphorylation	No	No	No
Glycolysis	Increased	No	Initially increased, later inhibited
Bulk flow past cells	Yes	Yes	No
Accumulation of ions and products of metabolism	No	No	Yes
Source of ATP	Yes	No	Yes, initially
[ATP]	Little change	Rapid fall	Gradual fall
Developed force	Slow fall to 30%, which can then be maintained	Rapid complete fall	Rapid complete fall
Contracture	No	Early, rapidly developing	Late, slowly developing
Action potentials	Little change	Rapid early failure	Gradual failure
Calcium transients	No change, or moderate increase or decrease	Rapid early failure	Initial gradual increase, later failure
Diastolic $[Ca^{2+}]_i$	Little change	Rapid early rise	Progressive increase

For further details see references 1 and 2.

metabolism, i.e., carbon dioxide, will rapidly diffuse from the preparation and the intracellular acidosis will therefore develop more slowly than in an intact heart.

When ischemia was produced using this model (Fig. 1), a small reduction in the amplitude of the Ca^{2+} transients was observed during the first 5 min. However, the major change was a large, slowly developing increase in the amplitude of the transients, which reached a maximum after 20–30 min of ischemia, and was associated with prolongation of the transients. During this period, there was also an increase in the diastolic $[Ca^{2+}]_i$. After ~ 20 min of ischemia, progressive dropout of the Ca^{2+} transients occurred, due to action potential failure, and spontaneous oscillatory increases in $[Ca^{2+}]_i$ became frequent. The effects of ischemia could be mimicked in a normally superfused preparation by adding lactic acid to the superfusate. Furthermore, it was noted that if muscles were glycogen depleted, reducing endogenous lactate production in ischemia, the ischemia-induced increase in the Ca^{2+} transients was abolished.

After ~ 1 h of ischemia, a large transient rise in $[Ca^{2+}]_i$ was often seen, after which there were no further oscillations of $[Ca^{2+}]_i$ (Fig. 1). We speculate that this phenomenon represents a terminal release of Ca^{2+} by the SR when intracellular ATP falls below a critical level. When preparations were reperfused after intermediate durations of ischemia (25–30 min), there was often a short-lived increase in the amplitude of the Ca^{2+} transients, which then returned towards control values over the course of a few minutes (17). However, when reperfusion occurred after long durations of ischemia (up to 2 h), very large increases in $[Ca^{2+}]_i$ occurred and were associated with irreversible contracture (see Fig. 1).

In another approach (19), aequorin was loaded into subepicardial cells of the left ventricle of Langendorff-perfused ferret hearts by injecting aequorin-containing solution into the extracellular space and then perfusing the heart with a low Ca^{2+} solution. When such hearts were made globally ischemic, a substantial rise in the diastolic $[Ca^{2+}]_i$ and a small increase in peak systolic $[Ca^{2+}]_i$ occurred within 2 min. These effects were reversed when reperfusion was initiated after 2–3 min of ischemia.

$[Ca^{2+}]_i$ during ischemia: comparison of methods and results. The various methods used to measure $[Ca^{2+}]_i$ give disparate results during the first few minutes of ischemia, with $[Ca^{2+}]_i$ falling, rising, or unchanged. It seems likely that the small transient fall of systolic $[Ca^{2+}]_i$ seen in the papillary muscle model (17, 18) may be an artefact related to the slower development of acidosis in this preparation unmasking the effects of early action potential shortening. As mentioned above, we also feel that the surprisingly rapid rise of systolic and diastolic $[Ca^{2+}]_i$ seen in the indo studies (15, 16) may at least in part be an artefact due to prolongation of the fluorescence transients in ischemia. A recent study using indo indicated a more gradual rise of $[Ca^{2+}]_i$ during ischemia (20). Another potential artefact whose significance requires further investigation arises from the observation that fluorescent indicators can enter endothelial cells and that agents which stimulate endothelial cells cause substantial rises in steady $[Ca^{2+}]_i$ (21). A possible artefact with nmr measurements is that the substantial Ca^{2+} buffering produced by the indicator may retard the effects of ischemia on $[Ca^{2+}]_i$ and other variables, although attempts have been made to overcome this problem by raising extracellular $[Ca^{2+}]$ (12, 13).

Taking all these factors into consideration, we feel that the most likely position is that systolic and diastolic $[Ca^{2+}]_i$ rise rather slowly during ischemia, with systolic $[Ca^{2+}]_i$ reaching a maximum after 10–30 min. After this time, progressive shortening of the action potential causes a decline in systolic $[Ca^{2+}]_i$ and eventual abolition of the Ca^{2+} transient, whereas diastolic $[Ca^{2+}]_i$ continues to rise. This scenario is also in good agreement with electrophysiological measurements, which indicate that electrical uncoupling of cells, believed to be due in part to increased $[Ca^{2+}]_i$ (22), has a similar timecourse (23, 24). Also, there is strong evidence that the gradual increase in the amplitude of the Ca^{2+} transients is driven by an acidosis (17, 25), which requires several minutes to build up (12, 13). The gradual rise in diastolic $[Ca^{2+}]_i$ later in ischemia (18) is most likely related to failure of Ca^{2+} sequestration and extrusion mechanisms due to severe ATP depletion, which takes many minutes to occur (12, 13).

In spite of the differences in detail, all the measurements of $[Ca^{2+}]_i$ agree that the early fall of force in ischemia is *not* associated with a marked reduction of the Ca^{2+} transient. Indeed, force fails in spite of a gradual *increase* in the amplitude of the transients. As mentioned in a previous section, the other main cellular mechanism likely to be the cause of the rapid fall of force is a change in the Ca^{2+} sensitivity or maximum Ca^{2+}-acti-

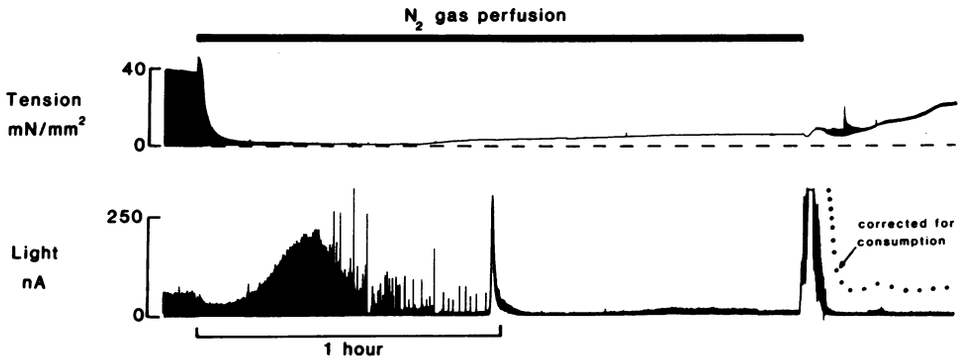

Figure 1. Records of tension (*top*) and aequorin light (a function of $[Ca^{2+}]_i$) from an isolated ferret papillary muscle exposed to N_2 gas perfusion (37°C, 1 Hz stimulation rate) (18). Simulation of ischemia produced a complex but reproducible series of changes. (*a*) Tension: force fell rapidly and completely and a slowly developing contracture began after ~ 45 min. Reperfusion after 2 h was associated with arrhythmias and irreversible contracture. (*b*) $[Ca^{2+}]_i$: calcium transients initially fell slightly, but then showed a progressive rise before gradually failing and being replaced with spontaneous oscillations of $[Ca^{2+}]_i$. After a large transient rise at ~ 1 h there were no further oscillations. A small sustained rise in diastolic $[Ca^{2+}]_i$ then occurred. Reperfusion was associated with a very large and sustained rise in $[Ca^{2+}]_i$. This large increase in $[Ca^{2+}]_i$ causes significant consumption of aequorin, reducing the magnitude of the light signal. Dotted line indicates expected light signal when corrected for aequorin consumption.

vated force of the myofibrils due to intracellular metabolite changes associated with ischemia.

Effects of metabolites on contractile function. An early hypothesis proposed that ATP depletion caused a critical reduction in the free energy available from ATP hydrolysis, but studies of ischemia show only a small ATP reduction over the period when force declines (12, 13). Another suggestion was that the decline in force is due to the acidosis caused by lactic acid accumulation, but again quantitative studies have shown that the force reduction attributable to acidosis is only a fraction of that observed in ischemia (26).

There have now been a number of systematic studies of the effects of the known metabolite changes in ischemia on the contractile functions of skinned cardiac preparations (27, 28). These have demonstrated that it is the increase in intracellular inorganic phosphate (Pi) due to phosphocreatine breakdown which has the major inhibitory effect on developed force, with a smaller contribution being made by the decrease in intracellular pH (pHi). For example, when Pi increases from 2 to 16 mM (values typical of control conditions and ischemia), the maximum Ca^{2+}-activated force decreases to ~ 50% control and the Ca^{2+} sensitivity declines by ~ 0.3 pCa units, so that at the $[Ca^{2+}]_i$ which occurs during normal contraction, developed force falls to ~ 20% of control (27). During ischemia, pH falls from a control value of ~ 7.0 to ~ 6.2 (29), and in skinned fibers this causes a reduction in maximum Ca^{2+}-activated force to ~ 70% and a 0.5-pCa unit change in Ca^{2+} sensitivity (6).

The distinction between changes in maximum Ca^{2+}-activated force and Ca^{2+} sensitivity has now been extensively documented in hypoxia, where maximum Ca^{2+}-activated force can be determined by the application of ryanodine coupled with tetanic stimulation in high extracellular $[Ca^{2+}]$ (30–32). These studies have shown that maximum Ca^{2+}-activated force falls to ~ 50%, whereas the Ca^{2+} sensitivity declines by ~ 0.2 pCa units. Furthermore, correlation of Pi and pH with maximum Ca^{2+}-activated force suggests that the depression is largely due to Pi (30). Such studies confirm that the observations made on skinned fibers also occur in the intact heart.

During ischemia such interventions are not possible, but an estimate of the contribution of metabolic changes can be made using measurements of pH and Pi from nmr experiments and the changes in Ca^{2+} responsiveness determined in skinned fibers. Fig. 2 shows the changes in $[Ca^{2+}]_i$, Pi, pHi, and developed pressure recorded in a ferret heart subject to global ischemia. Using skinned fiber data on pH (6) and Pi (27) it is possible to estimate the fractional reduction in force as ischemia progresses (for details see legend to Fig. 2). Note that this calculation predicts a shallower fall in pressure (*dotted line*) than actually observed. The effect of Pi is greater in the early period (first 5 min), whereas the effect of pH becomes progressively larger (> 5 min) as ischemia progresses. The effects of Pi and pH on force would be considerably larger if it were not for the increase in $[Ca^{2+}]_i$ during ischemia, which acts in the opposite direction. These data show that when the changes in $[Ca^{2+}]_i$ are taken into account, the known changes in metabolic factors in ischemia can account for much, but not all, of the early decline in force.

Metabolic factors are also involved in the development of ischemic contracture during long exposures to ischemia. Two main theories have been proposed to explain this phenomenon: (*a*) elevated $[Ca^{2+}]_i$ causing force production by the normal mechanism and (*b*) ATP depletion causing cross-bridges to

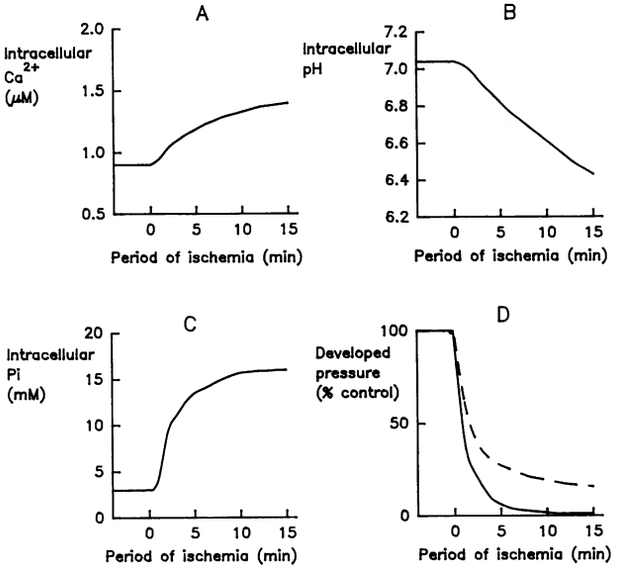

Figure 2. Changes in intracellular $[Ca^{2+}]_i$, pH, Pi, and developed pressure during global ischemia. All data are taken from experiments on ferret hearts at 30°C. (*A*) Changes in peak systolic $[Ca^{2+}]_i$ estimated from data in references 17 and 19. (*B* and *C*) show measurements of pH and Pi using nmr (29). (*D*) Solid line shows left ventricular developed pressure during ischemia (29). Dashed line in *D* is a theoretical curve calculated from the data in *A–C*, as described below. Data on the effects of pH (6) and Pi (27) obtained from experiments on skinned cardiac muscle have been used to determine how the measured changes in pH and Pi would change developed force when coupled with the observed changes in $[Ca^{2+}]_i$. As a starting point, it was assumed that under control conditions the heart was 50% maximally activated, and this defined the control $[Ca^{2+}]_i$ on the pCa-tension curves at resting pH (7.04) and Pi (3 mM). Then the change in force as a fraction of control was calculated at 1-min intervals after the onset of ischemia assuming the $[Ca^{2+}]_i$ had changed by the fractional amount shown in *A* and that the pCa/force curves had moved to those interpolated for the new pH and Pi. The fractional change in force calculated for pH and Pi were multiplied together to estimate the overall effect (27).

lock in the noncycling rigor state. Recent data obtained using nmr to measure $[Ca^{2+}]_i$ and ATP in the same preparations (13) indicates that ATP depletion correlates much better than changes in $[Ca^{2+}]_i$ with the appearance of ischemic contracture, and appears to be its primary cause.

Effects of intravascular pressure or flow on contractility. One possible reason for the unexplained component of force decline in ischemia is the hydraulic effects of ischemia on the intact heart. When cardiac muscle becomes ischemic both pressure and flow in affected blood vessels fall to low values, and these effects could influence force production by changing sarcomere length or perhaps via a flow or pressure-sensitive detector in the vessel walls. In this context, it has been shown that increases in perfusion pressure and coronary flow in the physiological range lead to unexplained increases in developed pressure and O_2 consumption (33).

A recent study (34) showed that in the physiological range of perfusion pressure, changes in pHi and Pi were small and changes in segment length could not account for the observed alterations in developed pressure when perfusion pressure was

changed. Furthermore, maximum Ca^{2+}-activated force was unaffected in this range, suggesting that changes in the Ca^{2+} transients could be involved, and a decrease in systolic $[Ca^{2+}]_i$ when perfusion pressure was lowered was in fact observed. These results suggest that there may be physiologically important modulation of myocardial $[Ca^{2+}]_i$ by flow, pressure or stretch in intact hearts.

The same laboratory has also examined the contribution of vascular collapse to ischemia (35). Their approach involved infusing the heart with microemboli (14 μm diameter) which reduce flow with little change in pressure in the larger vessels. Under their conditions (27°C), ischemia led to a fall in developed pressure to < 50% in 30 s and there was no change in action potential duration or metabolites (Pi, PCr, ATP, and pH) on this timescale. To assess the role of vascular collapse, ischemia was compared with a 14-fold reduction in flow produced by microspheres. This intervention caused identical metabolic changes to ischemia, but the fall in developed pressure was substantially slower. After 1 min, developed pressure was ~ 25% in ischemia, but 50% with microspheres. These experiments suggest a novel approach to identifying the component associated with vascular collapse, but do not give any information about the mechanism involved.

A rather different conclusion emerges from experiments on exposed pig hearts in which graded occlusion of the anterior descending coronary artery was performed (36). In these experiments, Pi doubled in a 24-s occlusion and there was a good correlation between Pi increase and reduced contractility in the ischemic region of the ventricular wall. However, during reperfusion a greater contractility for a given metabolic change was observed, suggesting that other modulators of contractility are involved, such as an increase in $[Ca^{2+}]_i$.

B. ARRHYTHMIAS

Arrhythmias during cardiac ischemia have been intensively studied, because they are a major cause of sudden death (see ref. 4 for a comprehensive review). Arrhythmias tend to occur at certain well-defined times, and of particular interest in the present context are the early arrhythmias occurring after ~ 5–30 min of ischemia and those caused by reperfusion within a few hours of the onset of ischemia. In the following sections, we consider briefly how ischemia-induced changes in membrane properties and $[Ca^{2+}]_i$ may be involved in causing these arrhythmias.

i. Ischemia-induced changes in membrane properties. As ischemia progresses, the action potential shortens, the resting potential depolarizes, the rate of rapid depolarization slows, and conduction slows (4). It is generally thought that the changes in the form of the action potential which occur during the early fall of force are too small to have much influence on force (17, 35) and this is supported by the observation that the Ca^{2+} transient at this stage is increased rather than reduced, indicating that excitation-contraction coupling is still functional.

At the electrophysiological level, ventricular tachycardias and fibrillation are thought to arise either because of triggered activity by a small focus of cells or by reentry, in which a continuous circuit of activity becomes established due to slowing of conduction and a region of unidirectional conduction (4). Whatever the initiating mechanism, in established fibrillation a continuous cycle of activity around one or more circuit becomes established.

What are the cellular changes which underlie the alterations in electrophysiology? One important factor is an increase in extracellular K^+ which arises from increased K^+ conductance (37). Part of this increase is thought to be due to the ATP-sensitive K^+ channel, because application of blockers of this channel reduces the extracellular K^+ accumulation in ischemia (38, 39). Elevated extracellular K^+ can explain most of the ischemic resting depolarization (40), which will in turn lead to partial inactivation of the Na^+ channel, thus contributing to the reduced rate of rise of the action potential, slowing of conduction and reentry type arrhythmias. It has also been shown that when one part of an isolated cardiac trabeculum is depolarized by increasing extracellular K^+, the resulting current between the normal and depolarized region can lead to automatic activity in the normal part (41).

ii. Are ischemic and reperfusion-induced arrhythmias related to $[Ca^{2+}]_i$? There is considerable evidence that ischemia-induced changes in $[Ca^{2+}]_i$ can contribute to the electrophysiological changes and to arrhythmias. An important early observation was that early ischemic arrhythmias are reduced by pretreatment with Ca^{2+} channel blockers (42). Subsequently, it was shown (43) that Ca^{2+} channel blockers exerted their protective effect by a reduction in the ischemic depolarization, most likely by reducing $[Ca^{2+}]_i$. There are several ionic mechanisms whereby an elevated $[Ca^{2+}]_i$ can lead to depolarization. It has long been recognized that the arrhythmias induced by cardiac glycosides or other interventions which increase $[Ca^{2+}]_i$ are associated with a depolarizing transient inward current. This current is thought to be activated by raised $[Ca^{2+}]_i$ (44) and can often become oscillatory due to oscillatory Ca^{2+} release from overloaded SR (45). Two possible ionic mechanisms have been identified which could carry this current: the Ca^{2+}-activated nonspecific cation channel and the Na^+/Ca^{2+} exchanger (see reference 46 for discussion).

Two further observations have strengthened the case for a role of elevated $[Ca^{2+}]_i$ in the generation of arrhythmias. First the rise in $[Ca^{2+}]_i$ discussed earlier has a timing which corresponds closely to the appearance of early ischemic arrhythmias. Furthermore reperfusion arrhythmias also occur at a time when $[Ca^{2+}]_i$ is greatly elevated and is often oscillatory. Second, agents which release Ca^{2+} from the SR and therefore prevent the oscillatory component of Ca^{2+} release, have been shown to have a substantial inhibitory effect on arrhythmias during both early ischemia and reperfusion (47). Thus, evidence for a role of elevated $[Ca^{2+}]_i$ in triggering early ischemic and reperfusion arrhythmias is substantial and suggests multiple sites at which antiarrhythmic agents might usefully be directed.

C. ISCHEMIA-INDUCED MUSCLE DAMAGE

Even relatively short durations of ischemia, where eventual functional recovery is good, may be associated with impaired contractile function, i.e., "stunned myocardium" (48). Recent work (49) indicates that this is due to reduced Ca^{2+} sensitivity and maximum Ca^{2+}-activated force in postischemic muscle, rather than to reduced Ca^{2+} transients. There is also evidence that myocardial stunning is due to the increase in $[Ca^{2+}]_i$ seen during even brief exposures to ischemia (50), possibly as a result of phosphorylation or covalent modification of the contractile proteins by intracellular Ca^{2+}-activated protein kinases and proteases (51). This suggestion is supported by the observation that the contractile dysfunction reverses over a period of

several days, consistent with the expected rate of myofibrillar protein synthesis (52). It should be noted that changes in intracellular metabolites such as pH and phosphate do not contribute to stunning, because they return to control values soon after reperfusion (49).

Longer durations of ischemia are associated not only with prolonged exposure to raised $[Ca^{2+}]_i$ during ischemia, but with very large $[Ca^{2+}]_i$ increases and/or large oscillations of $[Ca^{2+}]_i$ on reperfusion, as shown in Fig. 1. Although there is considerable evidence supporting a very large uptake of extracellular Ca^{2+} immediately after reperfusion (3, 53), the route by which Ca^{2+} enters the myoplasm is controversial. The two main theories are (*a*) that reactive oxygen radicals are formed in the myocardium on reperfusion, damage cell membranes and allow Ca^{2+} entry; and (*b*) that cell membranes initially remain essentially intact and Ca^{2+} entry is due to disrupted sarcolemmal transport mechanisms. Distinguishing between these possibilities is important, because they have different implications for optimal reperfusion strategies.

Although there is a body of data which supports the role of free radicals in reperfusion damage (54), there are methodological controversies (55) and as yet little consensus on the importance of this mechanism. Alternatively, reperfusion Ca^{2+} influx could be largely due to abnormal sarcolemmal transport (56). One possible mechanism is that Ca^{2+} enters on the Na^+/Ca^{2+} exchanger, which is far from its normal equilibrium after a period of ischemia due to a rise in the intracellular Na^+ concentration (57). Another suggested mechanism (58) is that the Na^+/H^+ exchanger is inhibited by the large extracellular acidosis in ischemia but becomes reactivated on reperfusion, causing an efflux of H^+ and an influx of Na^+, which then stimulates Ca^{2+} entry on the Na^+/Ca^{2+} exchanger. This possibility is supported by the observation that the Na^+/H^+ exchange blocker amiloride enhances recovery after ischemia (59). The idea that the sarcolemma may remain substantially intact after a period of ischemia is supported by observations that alpha adrenergic (60) or Ca^{2+} channel blockade (61, 62) may substantially reduce reperfusion Ca^{2+} uptake. Also, the observation that recovery from ischemia may be initiated with oxygen gas perfusion (17) suggests that reoxygenation in itself does not necessarily cause serious, irreversible membrane damage. Comparison of different reperfusion strategies using oxygen gas, anoxic solutions, and normal oxygenated solutions may help to establish the relative contributions of free radicals and deranged sarcolemmal transport to reperfusion damage.

The large increase in $[Ca^{2+}]_i$ on reperfusion is thought to damage cardiac muscle by essentially the same mechanisms discussed above under stunned myocardium. In addition, the large oscillations of $[Ca^{2+}]_i$ seen after intermediate durations of ischemia are likely to contribute to contractile dysfunction by contributing to the generation of arrhythmias, as discussed above.

Conclusions

Measurements of $[Ca^{2+}]_i$ in ischemia have clearly demonstrated that the early fall in force is not due to a failure of the Ca^{2+} transients, but is caused largely by the effects of intracellular metabolites on the Ca^{2+} sensitivity and maximum Ca^{2+}-activated force of the contractile proteins. However, the effects of ischemia and reperfusion on $[Ca^{2+}]_i$ do contribute to acute ischemic contractile failure via the generation of arrhythmias and the production of muscle damage.

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