Role of Sarcoplasmic Reticulum in the Contractile Dysfunction during Myocardial Ischaemia and Reperfusion

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Summary
In the myocardium, the sarcoplasmic reticulum (SR) plays an essential role in the regulation of cytosolic free Ca\(^{2+}\) ion concentration and, hence, in the contraction-relaxation cycle. The aim of this review is to summarize the role of the SR, particularly the main SR Ca\(^{2+}\) transport proteins, Ca\(^{2+}\)-ATPase pump and Ca\(^{2+}\) release channel (ryanodine receptor), in contractile impairment during ischaemia and reperfusion. As suggested by most studies, SR dysfunction may contribute to contractile failure during ischaemia. However, SR function is largely restored during reperfusion and minor changes are unlikely to explain the severe postischaemic contractile dysfunction.

Key words
Sarcoplasmic reticulum – Heart – Ischaemia – Reperfusion – Stunning

Introduction
Ischaemia occurs when the reduction of blood supply is so severe that the delivery of oxygen to the myocardium is inadequate for the needs of the heart. This leads to a cascade of metabolic and electrophysiological events which are initially reversible. Prolonged ischaemia leads to irreversible tissue injury, cell necrosis or myocardial infarction. The logical therapy for ischaemia is undoubtedly reperfusion. Paradoxically, while reperfusion after brief ischaemia is necessary for preventing irreversible changes, it is also accompanied by additional injury known as myocardial stunning. Stunning was first demonstrated under experimental conditions (Heyndrickx et al. 1975). However, there is a growing body of evidence showing that stunning is not just a laboratory curiosity, but a real clinical event which can potentially occur, e.g. in thrombolytic therapy, coronary angioplasty or following cardiopulmonary bypass (Vatner and Heyndrickx 1995). This review will be focused on the role which SR plays in contractile dysfunction during myocardial ischaemia and reperfusion.

Sarcoplasmic reticulum and intracellular Ca\(^{2+}\) homeostasis

The pathogenesis of acute ischaemia involves a series of responses to hypoxia, diminished delivery of substrates and accumulation of toxic waste products (Opie 1991). Although the precise mechanism of injury is not completely clear, it is generally assumed that altered intracellular Ca\(^{2+}\) homeostasis is involved. Intracellular Ca\(^{2+}\) regulates a variety of cellular functions in cardiac cells, the essential role being the regulation of the contraction-relaxation cycle. According to a generally accepted model of the contraction-relaxation cycle (for review see Feher and Fabiato 1990, Langer 1992), transsarcolemmal Ca\(^{2+}\) influx through voltage-gated Ca\(^{2+}\) channels and the Na\(^+\)/Ca\(^{2+}\) exchanger is insufficient to activate the myofilaments directly, but it triggers the release of
additional Ca\(^{2+}\) from the sarcoplasmic reticulum (SR). Ca\(^{2+}\) released from the SR binds to the regulatory protein troponin C. This, in turn, allows actin and myosin to interact. Relaxation occurs when Ca\(^{2+}\) is removed from the myoplasm. The resting level of Ca\(^{2+}\) concentration is restored by a combined action of the sarcosomelal Ca\(^{2+}\)-ATPase and Na\(^+\)/Ca\(^{2+}\) exchanger, which transport Ca\(^{2+}\) out of the cell, but also mainly by the action of the SR Ca\(^{2+}\)-ATPase pumping calcium back into the lumen of the SR. Mitochondria also transport Ca\(^{2+}\). It is assumed that this transport is related to the regulation of various intramitochondrial enzymes and not to the contraction-relaxation cycle (Carafoli 1987). Thus, contractile activity is determined by three factors: a) transsarcomemal Ca\(^{2+}\) transport, b) function of the SR and c) myofilament sensitivity/responsiveness to Ca\(^{2+}\). The aforementioned model suggests that the SR plays a pivotal role in regulating cytoplasmic Ca\(^{2+}\) concentration and also in the contraction-relaxation cycle.

The SR is a complex structure composed of three different components: 1) the longitudinal SR which is formed by a network of tubules surrounding the myofibrils, 2) the junctional SR - terminal cisternae which are in contact with transverse tubules, and 3) the corbular SR - terminal cisternae which are not in functional contact with the sarcolemma or T-tubules (Jorgensen et al. 1988).

These components differ in their protein composition and functions (Jones and Cala 1981). The major role of the longitudinal SR is to take up Ca\(^{2+}\) liberated from the myofibrils during relaxation, being rich in Ca\(^{2+}\)-ATPase and phospholamban. These proteins are also found in the corbular but not in the junctional SR. Both junctional and corbular SR, associated with Ca\(^{2+}\) storage and release, are rich in calcium binding protein calsequestrin and Ca\(^{2+}\) release channels (Jorgensen et al. 1988). Feher and Lipford (1985) assessed that approximately 43% of vesicles prepared from cardiac SR contain both Ca\(^{2+}\)-ATPase and Ca\(^{2+}\) release channel.

The sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) is made up of a single polypeptide of 100 kDa (for review see Lompré et al. 1994). This protein transports two Ca\(^{2+}\) ions per one molecule of hydrolysed ATP. The activity of Ca\(^{2+}\)-ATPase is regulated by a variety of factors such as pH, Mg\(^{2+}\), Ca\(^{2+}\), ATP, ADP and phospholamban. The unphosphorylated phospholamban binds to the Ca\(^{2+}\)-ATPase and inhibits its activity. When phosphorylated by protein kinases, phospholamban is released from Ca\(^{2+}\)-ATPase and the affinity of protein for Ca\(^{2+}\) increases (for review see Verboomen et al. 1995).

Ca\(^{2+}\)-induced Ca\(^{2+}\) release from the SR occurs through a channel which is formed from 560 kDa monomers, one of the largest proteins present in cells (for review see Feher and Fabiato 1990). The SR Ca\(^{2+}\) channel, also known as a ryanodine receptor, is activated by a plant alkaloid ryanodine in nanomolar concentrations. In micromolar concentrations, ryanodine blocks the channel (Feher and Lipford 1985). Activity of the cardiac ryanodine receptor is also affected by Ca\(^{2+}\), Mg\(^{2+}\), adenosine nucleotides and by protein phosphorylation (for review see Feher and Fabiato 1990). The role of inositol(1,4,5)trisphosphate (InsP\(_3\))-induced Ca\(^{2+}\) release from SR in cardiac muscle is considered to be too slow to play a role in the contraction-relaxation cycle (Feher and Fabiato 1990). Calsequestrin is the major Ca\(^{2+}\)-binding protein of cardiac SR. It serves as a calcium buffer in the lumen of SR reducing the gradient against which Ca\(^{2+}\)-ATPase must transport Ca\(^{2+}\). It has been proposed that calsequestrin may not only be a passive Ca\(^{2+}\) buffer, but also a regulator of Ca\(^{2+}\) release from the SR (for review see Yano and Zerain-Herzberg 1994). Alterations in function of any SR protein following ischaemia and reperfusion should have significant effects on the mechanical function of the heart.

**Function of sarcoplasmic reticulum during ischaemia**

The effect of ischaemia on SR function has been examined in several studies using predominantly homogenates or SR vesicles isolated from the ischaemic hearts. For more details on the methods used for the study of SR function, the reader is also referred to recent review of Mubagwa (1995). In spite of different ischaemic models, almost all indicate that ischaemia depresses SR function. The Ca\(^{2+}\)-uptake rate decreased progressively with increasing duration of ischaemia, the decrease was also observed after only 5 min of ischaemia (Imai et al. 1983, Feher et al. 1989, Limbruno et al. 1989, Rehr et al. 1991, Davis et al. 1992, Kaplan et al. 1992). A kinetic analysis of Ca\(^{2+}\)-uptake indicated that the depression is the result of a decrease in the maximum rate (V\(_{\text{max}}\)) but not of the altered affinity of Ca\(^{2+}\)-ATPase to Ca\(^{2+}\) (K\(_{\text{Ca}}\)). Since regulation of Ca\(^{2+}\)-ATPase activity by phospholamban affects K\(_{\text{Ca}}\), the results suggest that phospholamban is not involved in the effect of ischaemia (Kaplan et al. 1992). Altered SR function was also observed in studies which used intact SR in skinned cardiac fibres, prepared from ischaemic human myocardium (Luciani et al. 1993) and also in myocytes subjected to simulated ischaemia (Hohl et al. 1992).

Since the net Ca\(^{2+}\) uptake is determined by Ca\(^{2+}\) influx mediated by Ca\(^{2+}\)-ATPase and by Ca\(^{2+}\) efflux through the Ca\(^{2+}\) release channel, an abnormal Ca\(^{2+}\) uptake may be the result of the dysfunction of either or both fluxes. In most studies, the contribution of Ca\(^{2+}\) release channel to the net Ca\(^{2+}\) uptake was assessed indirectly using SR Ca\(^{2+}\) channel blockers (ryanodine or ruthenium red) which close the channel.
in a subpopulation of SR vesicles containing both Ca$^{2+}$-ATPase and Ca$^{2+}$ channel (Feher and Lipford 1985). The effect of ischaemia on these SR proteins has not yet been adequately clarified. Several studies have proposed that Ca$^{2+}$-ATPase function remains normal, while the Ca$^{2+}$ efflux through the Ca$^{2+}$ channel is increased (Feher et al. 1989, Davis et al. 1992). In contrast, other studies (Limbruno et al. 1989, Hohl et al. 1992) reported an ischaemia-induced decrease in both Ca$^{2+}$-ATPase activity and activity of the Ca$^{2+}$ release channel. Finally, other studies which attempted to characterize the ischaemic damage of SR suggested that depressed net Ca$^{2+}$ uptake is not due to a change of Ca$^{2+}$ channel activity but to a decrease in transport mediated by Ca$^{2+}$-ATPase (Rehr et al. 1991, Kaplan et al. 1992). The reason for the discrepancies between various studies is not clear. It is possible that these discrepancies are related, at least partly, to the different species used. We have shown that, under identical experimental conditions, ischaemia in the rat heart decreases Ca$^{2+}$-ATPase mediated Ca$^{2+}$ transport and stimulates Ca$^{2+}$ channel activity, while in the rabbit heart it decreases only Ca$^{2+}$-ATPase (Mubagwa et al. 1997). Another question, not yet clarified, is the mechanism of ischaemia-induced change in SR function. The changes observed in in vitro Ca$^{2+}$ uptake studies persisted after heart homogenization and also after the isolation of SR vesicles. Since Ca$^{2+}$ uptake measurements were performed under optimal conditions (pH, K+, Mg$^{2+}$, ATP), identical to those of the controls, the observed decrease is therefore not due to a lack of ATP or other cytoplasmic changes. However, altered pH, Mg$^{2+}$ and ATP levels during ischaemia may reduce the SR function in the intact heart to a much greater extent than that observed in vitro under optimal conditions (Hohl et al. 1992, Korge and Campbell 1995). It is unlikely that the altered SR function is due to protein degradation, because short reperfusion periods were sufficient to restore Ca$^{2+}$ uptake. This view is supported by the fact that the content of Ca$^{2+}$-ATPase did not change after 40 min of ischaemia as detected by SDS-polyacrylamide gel electrophoresis (Yoshida et al. 1990). The mechanism of the ischaemic effect on SR remains unknown. Further possible mechanisms may include changes in the redox potential and phosphorylation state of transport proteins, or modification of membrane lipids. It should also be pointed out that contractile failure during ischaemia is not solely caused by SR dysfunction, but that changes occur at all stages of excitation-contraction coupling (Hajjar and Gwathmey 1990).

Function of sarcoplasmic reticulum in stunned myocardium

Almost all studies have shown that Ca$^{2+}$ uptake decreases during ischaemia, suggesting that altered SR function might play a role in the mechanism of stunning. However, the role of SR in postischaemic contractile dysfunction has been explored with only limited success. Studies, in which reperfusion was started after prolonged ischaemia (40–60 min), have documented that ischaemia and reperfusion induced greater depression of SR function than ischaemia alone (Hohl et al. 1992). The reduction of Ca$^{2+}$-ATPase activity in the ischaemic-reperfused myocardium was associated with proteolytic degradation of the pump (Yoshida et al. 1990). However, most studies where reperfusion was started after brief ischaemic periods have shown that Ca$^{2+}$ uptake was restored almost completely or at least partly (Feher et al. 1989, Limbruno et al. 1989, Rehr et al. 1991, Davis et al. 1992, Kaplan et al. 1992). In one study (Lamers et al. 1993), Ca$^{2+}$ uptake by SR was even slightly increased. These authors have also shown that the phosphorylation state of phospholamban is unchanged in the stunned myocardium. Thus, instead of aggravation of the ischaemia-induced changes, the data showed a recovery of SR function. In another study (Krause et al. 1989), a reduction in Ca$^{2+}$ uptake rate and Ca$^{2+}$-ATPase activity in stunned myocardium was observed. This inherent defect of SR function can be potentiated by an increase in Mg$^{2+}$ concentration occurring during reperfusion (Krause and Rozanski 1991); however, the data from the ischaemic myocardium was not given. Recently, Zucchi et al. (1996) showed a decrease in SR Ca$^{2+}$ uptake in the human myocardium after reversible ischaemia and reperfusion during cardiac surgery.

The studies demonstrating a decrease in Ca$^{2+}$ uptake or only a partial recovery of SR function after brief ischaemia differ in the character and cause of this effect. Several studies showed that a decrease of Ca$^{2+}$ uptake in the reperfused myocardium is related to an increase in Ca$^{2+}$ release through Ca$^{2+}$ channels because it could be reversed by ryanodine (Feher et al. 1989, Davis et al. 1992). However, 3H-ryanodine binding studies showed that this effect is not related to changes in either the number of channels or the probability of channel opening (Wu and Feher 1995, 1996). In another study (Limbruno et al. 1989), complex modification of SR function was observed. This modification involves reduced Ca$^{2+}$-ATPase activity, and in contrast to a previous study, decreased Ca$^{2+}$ release through SR Ca$^{2+}$ channel. This is consistent with a further study showing a reduced number of SR Ca$^{2+}$ release channels after ischaemia, persisting after reperfusion (Zucchi et al. 1994).

Furthermore, recent studies have suggested that changes in SR Ca$^{2+}$ channels play a role in ischaemic preconditioning (Zucchi et al. 1995, Tani et al. 1996a,b). Finally, increased gene expression of Ca$^{2+}$-ATPase, phospholamban and calsequestrin was found, indicating a repair process in the stunned myocardium (Frass et al. 1993).
Mechanism of myocardial stunning

Stunning is generally considered as a form of reperfusion injury, but the precise mechanism of this dysfunction is not known. Several studies have shown that inadequate energy supply and mitochondrial injury are not involved in the mechanism (Flameng et al. 1990, Kaplan et al. 1992, for review see Piper et al. 1994). Contractile dysfunction persisted despite the fact that the ATP level, which had markedly decreased during ischaemia, was restored to nearly normal values. Current views, which are not necessarily mutually exclusive and may be related are:

1) disturbances in the intracellular \( \text{Ca}^{2+} \) homeostasis,
2) a decrease in myofilament responsiveness to \( \text{Ca}^{2+} \),
3) reversible injury from oxygen free radicals.

Ischaemia induces a progressive rise in cytoplasmic \( \text{Ca}^{2+} \) concentration that persists during the early stages of reperfusion. In the view of the fact that SR plays a key role in the regulation of cytoplasmic \( \text{Ca}^{2+} \), attention was focused on SR function in the stunned myocardium. However, most studies have shown that SR function is at least partly recovered during reperfusion. Changes, if observed, were minor and are unlikely to explain severe postischaemic contractile dysfunction (Silverman and Stern 1994). Moreover, several studies have shown normal or increased, but not decreased, \( \text{Ca}^{2+} \) transient (Kusuoka et al. 1990, Hofmann et al. 1993, Gao et al. 1995, Seki and MacLeod 1995). Decreased \( \text{Ca}^{2+} \) uptake could not be the cause of such an increase in the \( \text{Ca}^{2+} \) transient. However, studies using heart homogenates or isolated SR may be inappropriate to characterize SR function in stunned myocardium. Using potentiated state contractions in the intact isolated heart, Mattheussen et al. (1993) have shown that SR function is abnormal during the early period of reperfusion. Du Toit and Opie (1994) reported that inhibition of SR \( \text{Ca}^{2+} \) -ATPase in the intact heart during ischaemia and reperfusion by cyclopiazonic acid and thapsigargin attenuated the severity of reperfusion stunning. These authors hypothesized that inhibition of the \( \text{Ca}^{2+} \) -ATPase pump causes depletion of \( \text{Ca}^{2+} \) stores, and thus mitigates the intracellular \( \text{Ca}^{2+} \) transient in the reperfused heart.

Intracellular calcium is also controlled by sarcolemmal transport systems: \( \text{Na}^+ / \text{Ca}^{2+} \) exchanger and \( \text{Ca}^{2+} \) -ATPase. The concept, that the \( \text{Na}^+ / \text{Ca}^{2+} \) exchanger is involved in the mechanism of myocardial stunning is supported by the study showing the protective effect of reperfusion with high \( \text{Na}^+ \) solutions (Kusuoka et al. 1993). It is suggested that at high extracellular \( \text{Na}^+ \) concentrations, the exchanger performs \( \text{Ca}^{2+} \) extrusion from the cell and decreases the intracellular overload and stunning. Sarcolemmal \( \text{Ca}^{2+} \) -ATPase was also shown to be affected by ischaemia-reperfusion (Dixon et al., 1990). Depression of the \( \text{Ca}^{2+} \) pump as well as \( \text{Na}^+ / \text{Ca}^{2+} \) exchanger activity, observed in this study, was possibly mediated by oxygen free radicals generated during ischaemia-reperfusion. In addition, sarcolemmal \( \text{Na}^+,\text{K}^-\text{ATPase} \), which is closely linked to the activity of \( \text{Na}^+ / \text{Ca}^{2+} \) exchanger, might also contribute to altered \( \text{Ca}^{2+} \) homeostasis since its activity is markedly reduced after hypoxia or brief ischaemia (Grinwald 1992, Ziegelhoffer et al. 1993, Vrbjar et al. 1995).

An increasing body of evidence suggests that a decreased myofilament responsiveness to \( \text{Ca}^{2+} \) plays a crucial role in the pathogenesis of myocardial stunning (Marban and Kusuoka 1987). This decrease results from a transient exposure of myofilaments to high \( \text{Ca}^{2+} \). Recent studies have shown that the decrease in myofilament responsiveness occurs when perfusion is restored, supporting the idea that myocardial stunning is a reperfusion injury (Miller et al. 1996). It is suggested that a transient increase of \( [\text{Ca}^{2+}]_j \) might activate protein kinases or proteases that attack myofibrillar proteins, resulting in decreased \( \text{Ca}^{2+} \) responsiveness (Kusuoka et al. 1990). Phosphorylation of myofibrillar proteins by cAMP-dependent protein kinase (PKA) is considered to be an important mechanism of cardiac activity regulation (for review see Solaro and Van Eyk 1996). When troponin I is phosphorylated by PKA, the myofilament sensitivity to \( \text{Ca}^{2+} \) is reduced. Moreover, PKA can be activated during ischaemia (Strasser et al. 1988). However, our recent data have shown that phosphorylation of myofibrillar proteins by PKA is not altered during ischaemia or reperfusion (Kaplán et al. 1996). On the other hand, several studies suggest that myofibrillar proteins are attacked by proteases which are activated early during reperfusion (Gao et al. 1995, 1996).

An alternative explanation of mechanisms of postischaemic contractile dysfunction concerns free radical induced injury. Several studies have shown that a burst of oxygen free radicals occurs immediately after reperfusion (e.g. Bolli et al. 1988). In vitro studies demonstrated free radical-induced changes in various intracellular functions (for review see Kaul et al. 1993, Kaneko et al. 1994). It still remains to be clarified whether some of them are targets of free radical attack during reperfusion.

In conclusion, although SR dysfunction cannot be excluded from the mechanism of stunning, it is probably not the major cause of contractile dysfunction of the stunned heart. On the other hand, further studies are needed to clarify the alternative hypothesis of contractile impairment in the postischaemic heart.

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References


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