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MINIREVIEW

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 sarcolemmal 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) transsarcolemmal 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+}, adenine 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_{max}) but not of the altered affinity of Ca^{2+}-ATPase to Ca^{2+} (K_{Ca}). Since regulation of Ca^{2+}-ATPase activity by phospholamban affects K_{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, ^{3}H-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 Ca^{2+}-homeostasis,
2) a decrease in myofilament responsiveness to Ca^{2+},
3) reversible injury from oxygen free radicals.

Ischaemia induces a progressive rise in cytoplasmic 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 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, Ca^{2+} transient (Kusuoka *et al.* 1990, Hofmann *et al.* 1993, Gao *et al.* 1995, Seki and MacLeod 1995). Decreased Ca^{2+} uptake could not be the cause of such an increase in the 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 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 Ca^{2+}-ATPase pump causes depletion of Ca^{2+} stores, and thus mitigates the intracellular Ca^{2+} transient in the reperfused heart.

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

An increasing body of evidence suggests that a decreased myofilament responsiveness to 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 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 $[Ca^{2+}]_i$ might activate protein kinases or proteases that attack myofibrillar proteins, resulting in decreased 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 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

BOLLI R., PATEL B.S., JEROUDI M.O., LAI E.K., MCCAY P.B.: Demonstration of free radical generation in "stunned" myocardium of intact dogs with the use of the spin trap alpha-phenyl N-tert-butyl nitrone. *J. Clin. Invest.* **82:** 476–485, 1988.

CARAFOLI E.: Intracellular calcium homeostasis. *Annu. Rev. Biochem.* **56:** 395–433, 1987.

DAVIS M.D., LEBOLT W., FEHER J.J.: Reversibility of the effects of normothermic global ischemia on the ryanodine-sensitive and ryanodine-insensitive calcium uptake of cardiac sarcoplasmic reticulum. *Circ. Res.* **70:** 163–171, 1992.

DIXON, I.M.C., KANEKO, M., HATA, T., PANAGIA, V., DHALLA, N.S.: Alterations in cardiac membrane Ca^{2+} transport during oxidative stress. *Mol. Cell. Biochem.* **99:** 125–133, 1990.

DU TOIT E.F., OPIE L.H.: Inhibitors of Ca^{2+} ATPase pump of sarcoplasmic reticulum attenuate reperfusion stunning in isolated rat heart. *J. Cardiovasc. Pharmacol.* **24:** 678–684, 1994.

FEHER J.J., FABIATO A.: Cardiac sarcoplasmic reticulum: calcium uptake and release. In: *Calcium and the Heart.* G.A. LANGER (ed.), Raven Press, New York, 1990, pp. 200–268.

FEHER J.J., LIPFORD G.B.: Mechanism of action of ryanodine on cardiac sarcoplasmic reticulum. *Biochim. Biophys. Acta* **813:** 77–86, 1985.

FEHER J.J., LEBOLT W.R., MANSON N.H.: Differential effect of global ischemia on the ryanodine-sensitive and ryanodine-insensitive calcium uptake of cardiac sarcoplasmic reticulum. *Circ. Res.* **65:** 1400–1408, 1989.

FLAMENG W., ANDRES J., FERDINANDE P., MATTHEUSSEN M., VAN BELLE H.: Mitochondrial function in myocardial stunning. *J. Mol. Cell. Cardiol.* **22:** 1–11, 1990.

FRASS O., SHARMA H.S., KNOLL R., DUNCKER D.J., MCFALLS E.O., VERDOUW P.D., SCHAPER W.: Enhanced gene expression of calcium regulatory proteins in stunned porcine myocardium. *Cardiovasc. Res.* **27:** 2037–2043, 1993.

GAO W.D., ATAR D., BACK P.H., MARBAN E.: Relationship between intracellular calcium and contractile force in stunned myocardium – direct evidence for decreased myofilament Ca^{2+} responsiveness and altered diastolic function in intact ventricular muscle. *Circ. Res.* **76:** 1036–1048, 1995.

GAO W.D., LIU Y.G., MELLGREN R., MARBAN E.: Intrinsic myofilament alterations underlying the decreased contractility of stunned myocardium. A consequence of Ca^{2+}-dependent proteolysis? *Circ. Res.* **78:** 455–465, 1996.

GRINWALD P.M.: Sodium pump failure in hypoxia and reoxygenation. *J. Mol. Cell. Cardiol.* **24:** 1393–1398, 1992.

HAJJAR R.J., GWATHMEY J.K.: Direct evidence of changes in myofilament responsiveness to Ca^{2+} during hypoxia and reoxygenation in myocardium. *Am. J. Physiol.* **259:** H784–H795, 1990.

HEYNDRICKX G.R., MILLARD R.W., MCRITCHIE R.J., MAROKO P.R., VATNER S.F.: Regional myocardial function and electrophysiological alterations after brief coronary occlusion in conscious dogs. *J. Clin. Invest.* **56:** 978–985, 1975.

HOFMANN P.A., MILLER W.P., MOSS R.L.: Altered calcium sensitivity of isometric tension in myocyte-sized preparations of porcine postischemic stunned myocardium. *Circ. Res.* **72:** 50–56, 1993.

HOHL C.M., GARLEB A.A., ALTSCHULD R.A.: Effect of simulated ischemia and reperfusion on the sarcoplasmic reticulum of digitonin-lysed cardiomyocytes. *Circ. Res.* **70:** 716–723, 1992.

IMAI K., WANG T., MILLARD R.W., ASHRAF M., KRANIAS E.G., ASANO G., DE GENDE A.G., NAGAO T., SOLARO R.J., SCHWARTZ A.: Ischaemia-induced changes in canine cardiac sarcoplasmic reticulum. *Cardiovasc. Res.* **17:** 696–709, 1983.

JONES L.R., CALA S.E.: Biochemical evidence for functional heterogenity of cardiac sarcoplasmic reticulum vesicles. *J. Biol. Chem.* **256:** 11809–11818, 1981.

JORGENSEN A.O., BRODERICK R., SOMLYO A.P., SOMLYO A.V.: Two structurally distinct calcium storage sites in rat cardiac sarcoplasmic reticulum: an electron microprobe analysis study. *Circ. Res.* **63:** 1060–1069, 1988.

KANEKO M., MATSUMOTO Y., HAYASHI H., KOBAYASHI A., YAMAZAKI N.: Oxygen free radicals and calcium homeostasis in the heart. *Mol. Cell. Biochem.* **139:** 91–100, 1994.

KAPLAN P., HENDRIKX M., MATTHEUSSEN M., MUBAGWA K., FLAMENG W.: Effect of ischemia and reperfusion on sarcoplasmic reticulum calcium uptake. *Circ. Res.* **71:** 1123–1130, 1992.

KAPLÁN P., MUBAGWA K., PONGO E., RAEYMAEKERS L., FLAMENG W.: Effect of ischemia and reperfusion on phosphorylation of myofibrillar proteins in rabbit myocardium. *Physiol. Res.* **45:** 33P, 1996.

KAUL N., SIVESKI-ILISKOVIC N., HILL M., SLEZAK J., SINGAL P.K.: Free radicals and the heart. *J. Pharmacol. Toxicol. Meth.* **30:** 55–67, 1993.

KORGE P., CAMPBELL K.B.: Regulation of calcium pump function in back inhibited vesicles by calcium-ATPase ligands. *Cardiovasc. Res.* **29:** 512–519, 1995.

KRAUSE S.M., ROZANSKI D.: Effect of an increase in intracellular free [Mg^{2+}] after myocardial stunning on sarcoplasmic reticulum Ca^{2+} transport. *Circulation* **84:** 1378–1383, 1991.

KRAUSE S.M., JACOBUS W.E., BECKER L.C.: Alterations in cardiac sarcoplasmic reticulum calcium transport in the postischemic "stunned" myocardium. *Circ. Res.* **65:** 526–530, 1989.

KUSUOKA H., KORETSUNE Y., CHACKO V.P., WEISFELDT M.L., MARBAN E.: Excitation-contraction coupling in post-ischemic myocardium. Does failure of activator Ca^{2+} transient underlie stunning? *Circ. Res.* **66:** 1268–1276, 1990.

KUSUOKA H., CAMILION DE HURTADO M.C., MARBAN E.: Role of sodium/calcium exchange in the mechanism of myocardial stunning: protective effect of reperfusion with high sodium solution. *J. Am. Coll. Cardiol.* **23:** 240–248, 1993.

LAMERS J.M.J., DUNCKER D.J., BEZSTAROSTI K., McFALLS E.O., SASSEN L.M.A., VERDOUW P.D.: Increased activity of the sarcoplasmic reticular calcium pump in porcine stunned myocardium. *Cardiovasc. Res.* **27:** 520–524, 1993.

LANGER G.A.: Calcium and the heart: exchange at the tissue, cell, and organelle levels. *FASEB J.* **6:** 893–902, 1992.

LIMBRUNO U., ZUCCHI R., RONCA-TESTONI S., GALBANI P., RONCA G., MARIANI M.: Sarcoplasmic reticulum function in the "stunned" myocardium. *J. Mol. Cell. Cardiol.* **21:** 1063–1072, 1989.

LOMPRÉ A.-M., ANGER M., LEVITSKY D.: Sarco(endo)plasmic reticulum pumps in the cardiovascular system: Function and gene expression. *J. Mol. Cell. Cardiol.* **26:** 1109–1121, 1994.

LUCIANI G.B., D'AGNOLO A., MAZZUCCO A., GALLUCCI V., SALVIATI G.: Effects of ischemia on sarcoplasmic reticulum and contractile myofilament activity in human myocardium. *Am. J. Physiol.* **265:** H1334–H1341, 1992.

MARBAN E., KUSUOKA H.: Maximally Ca^{2+}-activated force and myofilament Ca^{2+} sensitivity in intact mammalian hearts. *J. Gen. Physiol.* **90:** 609–623, 1987.

MATTHEUSSEN M., MUBAGWA K., RUSY B.F., VAN AKEN H., FLAMENG W.: Potentiated state contractions in isolated hearts: effects of ischemia and reperfusion. *Am. J. Physiol.* **264:** H1663–H1673, 1993.

MILLER W.P., McDONALD K.S., MOSS R.L.: Onset of reduced Ca^{2+} sensitivity of tension during stunning in porcine myocardium. *J. Mol. Cell. Cardiol.* **28:** 689–697, 1996.

MUBAGWA K.: Sarcoplasmic reticulum function during myocardial ischemia and reperfusion. *Cardiovasc. Res.* **30:** 166–175, 1995.

MUBAGWA K., KAPLAN P., FLAMENG W.: The effects of ryanodine on calcium uptake by the sarcoplasmic reticulum of ischemic and reperfused rat myocardium. *Fundam. Clin. Pharmacol* **11:** 315–321, 1997.

OPIE L.H.: Lack of oxygen: ischemia and angina. In: *The Heart Physiology and Metabolism.* L.H. OPIE (ed.), Raven Press, New York, 1991, pp. 425–450.

PIPER H.M., NOLL T., SIEGMUND B.: Mitochondrial function in the oxygen depleted and reoxygenated myocardial cell. *Cardiovasc. Res.* **28:** 1–15, 1994.

REHR R.B., FUHS B.E., HIRSCH J.I., FEHER J.J.: Effect of brief regional ischemia followed by reperfusion with or without superoxide dismutase and catalase administration on myocardial sarcoplasmic reticulum and contractile function. *Am. Heart J.* **122:** 1257–1269, 1991.

SEKI S., MACLEOD K.T.: Effect of anoxia on intracellular Ca^{2+} and contraction in isolated guinea pig cardiac myocytes. *Am. J. Physiol.* **37:** H1045–H1052, 1995.

SILVERMAN H.S, STERN M.D.: Ionic basis of ischemic cardiac injury: insight from cellular studies. *Cardiovasc. Res.* **28:** 581–597, 1994.

SOLARO R.J., VAN EYK J.: Altered interactions among thin filament proteins modulate cardiac function. *J. Mol. Cell Cardiol.* **28:** 217–230, 1996.

STRASSER R.H., KRIMMER J., MARQUETANT R.: Regulation of β-adrenergic receptors: impaired desensitization in myocardial ischemia. *J. Cardiovasc. Pharmacol.* **12:** S15–S24, 1988.

TANI M., ASAKURA Y., HASEGAWA H., SHINMURA K., EBIHARA Y., NAKAMURA Y.: Effect of brief hypoxia on reperfusion arrhythmias and release of Ca^{2+} by rat heart homogenate blocked by ryanodine. *Cardiovasc. Res.* **31:** 263–269, 1996a.

TANI M., ASAKURA Y., HASEGAWA H., SHINMURA K., EBIHARA Y., NAKAMURA Y.: Effect of preconditioning on ryanodine-sensitive Ca^{2+} release from sarcoplasmic reticulum of rat heart. *Am. J. Physiol.* **271:** H876–H881, 1996b.

VATNER S.F., HEYNDRICKX G.R.: Ubiquity of myocardial stunning. *Basic. Res. Cardiol.* **90:** 253–256, 1995.

VERBOOMEN H., MERTENS L., EGGERMONT J., WUYTACK F., VAN DEN BOSCH L.: Modulation of SERCA2 activity: regulated splicing and interaction with phospholamban. *Biosci. Rep.* **15:** 307–315, 1995.

VRBJAR N., DŽURBA A., ZIEGELHÖFFER A.: Influence of global ischemia on the sarcolemmal ATPases in the rat heart. *Mol. Cell. Biochem.* **147:** 99–103, 1995.

WU Q. Y., FEHER J.J.: Effect of ischemia and ischemia-reperfusion on ryanodine binding and Ca^{2+} uptake of cardiac sarcoplasmic reticulum. *J. Mol. Cell. Cardiol.* **27:** 1965–1975, 1995.

WU Q.Y., FEHER J.J.: Ryanodine perfusion decreases cardiac mechanical function without affecting homogenate sarcoplasmic reticulum Ca^{2+} uptake: comparison with the stunned heart. *J. Mol. Cell Cardiol.* **28:** 943–955, 1996.

YANO K., ZARAIN-HERZBERG A.: Sarcoplasmic reticulum calsequestrins: structural and functional properties. *Mol. Cell. Biochem.* **135:** 61–70, 1994.

YOSHIDA Y., SHIGA T., IMAI S.: Degradation of sarcoplasmic reticulum calcium-pumping ATPase in ischemic-reperfused myocardium: role of calcium-activated neutral protease. *Basic Res. Cardiol.* **85:** 495–507, 1990.

ZIEGELHÖFFER A., GRÜNERMEL J., DŽURBA A., PROCHÁZKA J., KOLÁŘ F., VRBJAR N., PELOUCH V., OŠŤÁDAL B., SZEKERES L.: Sarcolemmal cation transport systems in rat hearts acclimatized to high hypoxia. Influence of 7-oxo-prostacyclin. In: *Heart Function in Health and Disease.* B. OŠŤÁDAL, N.S. DHALLA (eds.), Kluwer Academic Publishers, Norwell, 1993, pp. 219–228.

ZUCCHI R., RONCA-TESTONI S., YU G., GALBANI P., RONCA G., MARIANI M.: Effect of ischemia and reperfusion on cardiac ryanodine receptors-sarcoplasmic reticulum Ca^{2+} channels. *Circ. Res.* **74:** 271–280, 1994.

ZUCCHI R., RONCA-TESTONI S., YU G., GALBANI P., RONCA G., MARIANI M.: Postischemic changes in cardiac sarcoplasmic reticulum Ca^{2+} channels. A possible mechanism of ischemic preconditioning. *Circ. Res.* **76:** 1049–1056, 1995.

ZUCCHI R., RONCA-TESTONI S., DINAPOLI P., YU G. Y., GALLINA S., BOSCO G., RONCA G., CALIFIORE A.M., MARIANI M., BARSOTTI A.: Sarcoplasmic reticulum calcium uptake in human myocardium subjected to ischemia and reperfusion during cardiac surgery. *J. Mol. Cell. Cardiol.* **28:** 1693–1701, 1996.

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