Update review

The calcium paradox revisited: An artefact of great heuristic value

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1. Introduction

The 1967 paper by Zimmerman et al. [1] is a sequel to one published in the previous year [2] in which Ariaen Zimmerman and Willem Hülsmann had described an artefact produced on an isolated rat heart preparation. It is exceptional that an artefact receives so much attention and is not forgotten three decades later. The observation they made is that, when an isolated heart is perfused for 2 min with a Ca²⁺ free, otherwise normal Krebs-Henseleit buffer and then with buffer containing a physiological Ca²⁺ concentration, it rapidly deteriorates. Massive enzyme release occurs and the heart becomes pale due to myoglobin loss. The 1967 paper demonstrates that these changes are accompanied with dramatic alterations of myocardial ultrastructure, i.e. membrane disruption, myofibrillar hypercontracture and mitochondrial damage. The discoverers named this impressive artefact ‘calcium paradox’. Since the original description this phenomenon has fascinated hundreds of researchers, with the highest research activity in the 1980s. This brief review is an attempt to explain 33 years after the original description the main reasons for this long-lasting fascination. It is not intended to duplicate previous scientific reviews [3–6].

2. Why has the calcium paradox been so fascinating?

In the 1970s and 1980s the pathophysiological importance of calcium for the heart was in the centre of scientific awareness. The calcium paradox was soon regarded as a paradigm in this area of research as it became clear that repletion of the once Ca²⁺ depleted heart leads to massive Ca²⁺ influx into the myocardial cells, a phenomenon also observed in other situations of severe myocardial cell injury. In particular, it was a widely accepted hypothesis that the calcium paradox represents a paradigm for the pathomechanism of severe ischemia-reperfusion injury. In the words of Albrecht Fleckenstein [7]: “Certainly, with restoring the blood perfusion of the previous ischemic region, an unlimited Ca²⁺ supply is re-established, possibly comparable with the ‘calcium paradox’ described by Zimmerman and Hülsmann [1966].”

Another reason for the interest in the calcium paradox is that this phenomenon is of obvious relevance to the design of crystalloid cardioplegic solutions on which much work had been done since the 1960s. The opposing poles for the best strategy to achieve myocardial protection were arrest by Na⁺ and Ca²⁺ removal or arrest by K⁺ and Mg²⁺ elevation with physiological Na⁺ concentration in the perfusion medium. Prominent proponents were Hans-Jürgen Bretschneider and collaborators for the first strategy (intracellular type of solution) [8,9] and Mark Braimbridge, David Hearse and collaborators [10,11] for the second (extracellular type of solution). Among other arguments, the first strategy claimed a larger reduction in ischemic energy expenditure when Ca²⁺ is lowered in myocardial tissue, the second warned against the peril of a calcium paradox.

Fascination of the calcium paradox has continued to date. In the past decade the term has increasingly been used in studies on myocardial calcium overload caused by ‘reverse mode’ activation of the sarcolemmal Na⁺/Ca²⁺ exchanger. It is debatable if this broadened use of the term calcium paradox is not misleading as it was originally introduced for an experimental situation in which reverse-mode activation of the Na⁺/Ca²⁺ exchanger may not play a causal role.

3. The causal mechanism

In the 1967 paper Zimmerman et al. [1] described the morphological correlates of the biochemical and functional changes occurring during the calcium paradox protocol.
They reported that the ultrastructural changes during Ca\(^{2+}\) depletion are minimal, but become drastic immediately after Ca\(^{2+}\) repletion. The prevalent picture consists of hypercontracted cells or cells having lost most of their structural components. Mitochondria contain electron-dense material on which the authors rightly assume that it contains calcium precipitations. Later studies on myocardial ultrastructure have described that, during Ca\(^{2+}\) free perfusion, the intercalated discs are partly separated and part of the glycocalyx, covering the sarcolemmal surface, can be lost [12–15]. Separation of intercalated discs is now known to be due to the disassembly by cadherin complexes in macula and fascia adherens junctions. Other cell–cell contacts remain structurally intact, e.g. gap junctions. Upon Ca\(^{2+}\) removal, adjacent cells become thus incompletely mechanically uncoupled.

One of the theories on the causal mechanism of the calcium paradox regards this incomplete mechanical uncoupling during Ca\(^{2+}\) depletion as being crucial for the events occurring during subsequent Ca\(^{2+}\) repletion (Fig. 1). Ca\(^{2+}\) repletion causes Ca\(^{2+}\) influx into the cells and contractile activation. Because of the weakening of cell–cell contacts the force developed in the cardiomyocytes is transmitted to the small areas where adjacent cells retain physical contact creating at these sites excessive mechanical tension. This results in disruption of the sarcolemma. It is possible that the mechanical stability of the sarcolemma is also reduced after Ca\(^{2+}\) depletion. According to this theory, all other functional and structural features of the calcium paradox are sequels of this mechanical damage. In particular, the secondary massive Ca\(^{2+}\) influx leads to hypercontracture of the myofibrils, further disruption of the primarily affected and of adjacent cells and extrusion of the constituents from the disrupted cells. Mitochondrial structural damage and amorphous depositions are also due to this massive secondary Ca\(^{2+}\) overload.

Other theories on the pathomechanism regard an altered sarcolemmal permeability developing during Ca\(^{2+}\) depletion as being crucial for the damage provoked by subsequent re-calcification. According to these theories Ca\(^{2+}\)-free conditions increase sarcolemmal permeabilities for electrolytes and/or water [6,16,17]. This leads either to forced excessive calcium uptake or to excessive osmotic swelling upon calcium repletion. The first type of argument is based on experiments where Ca\(^{2+}\) depletion was found accompanied by Na\(^{+}\) overload and on evidence that re-admission of calcium to the extracellular media caused massive calcium influx through activation of the ‘reverse mode’ of the Na\(^{+}]/Ca\(^{2+}\) exchanger. The second type of argument is based on experiments where increased extracellular oncotic pressure and/or reduced ionic strength could attenuate cell damage during Ca\(^{2+}\) repletion.

In favour of the first theory it has been argued that (i) Na\(^{+}\) overload is not necessary to cause the Ca\(^{2+}\) repletion-induced [4,18,19] injury, (ii) inhibition of contractile activation either by pharmacological inhibition [20] or energy depletion [21] upon Ca\(^{2+}\) repletion can prevent this injury and, alternatively, contractile activation without external Ca\(^{2+}\) influx is sufficient to cause severe cell injury after Ca\(^{2+}\) depletion [22], and (iii) calcium paradox injury is not seen in isolated cardiomyocytes submitted to an analogous protocol of Ca\(^{2+}\) depletion and repletion [15,23].

In favour of the second theory speak experiments indicating a strong activation of the reverse mode of the Na\(^{+}]/Ca\(^{2+}\) exchanger. These latter findings are pronounced when Ca\(^{2+}\) depletion is combined with Mg\(^{2+}\) depletion [6,24]. It seems that in absence of both Ca\(^{2+}\) and Mg\(^{2+}\) from the extracellular milieu, L-type Ca\(^{2+}\) channels become permeable to Na\(^{+}\) and that this leads to a cellular Na\(^{+}\) overload [25]. Re-calcification causes then an abrupt reverse mode activation of the Na\(^{+}]/Ca\(^{2+}\) exchanger, leading to Ca\(^{2+}\) overload and hypercontracture, in intact myocardium as well as in isolated cardiomyocytes [26]. Mg\(^{2+}\) depletion was not part of the original protocol of the calcium paradox, however [1,2]. It has been argued therefore [4,5] that the pathomechanism initiated by combined Ca\(^{2+}\) and Mg\(^{2+}\) depletion should not be subsumed under the term calcium paradox since the latter occurs in

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**Calcium Paradox**

![Diagram showing the mechanism of the calcium paradox](https://academic.oup.com/cardiovascres/article/45/1/123/411969)

Fig. 1. Mechanism of the calcium paradox, according to the mechanical theory. Ca\(^{2+}\) depletion causes partial separation of adjacent cardiomyocytes at intercalated discs. Contractile force generated upon Ca\(^{2+}\) repletion disrupts cells at these weakened sites of cell–cell contact. Terminal Ca\(^{2+}\) overload and hypercontracture follow. Since mechanical cell–cell interaction is a key element of this pathomechanism, it does not occur in isolated cardiomyocytes.
presence of a normal extracellular Mg\(^{2+}\) concentration and without a marked Na\(^+\) overload [19,27]. Instead, Ca\(^{2+}\) and Mg\(^{2+}\) depletion represents a special cause of (Na\(^+-\)induced) Ca\(^{2+}\) overload (Fig. 3).

The beneficial effects of an elevation of oncotic pressure and reduction of ionic strength throughout Ca\(^{2+}\) depletion, by replacement of NaCl by sucrose, are difficult to interpret [16,17]. They have been taken to indicate increased sarcolemmal permeability after Ca\(^{2+}\) depletion rendering cardiomyocytes susceptible to osmotic swelling [17]. It is conceivable that cell swelling is a partial cause of sarcolemmal disruption. It has also been speculated that at low ionic strength there is less Ca\(^{2+}\) removed from cellular binding sites which are critical for membrane integrity [16].

A further observation not readily explainable with one or the other of the above theories is that the calcium paradox can be attenuated by protocols imitating ischemic preconditioning [28–30]. Activation of protein kinase C seems involved in this mode of protection against calcium paradox injury. One could speculate that preconditioning reduces membrane fragility caused by Ca\(^{2+}\) depletion, possibly via changes in the phosphorylation pattern of strategic cytoskeletal elements. It has also been shown that heat stress reduces myocardial susceptibility to Ca\(^{2+}\) paradox injury [31]. Again, the protective mechanism is not known.

4. Comparison with the pathomechanism of acute reperfusion injury

For many, the calcium paradox has been a fascinating research topic as it seems to imitate and exaggerate the mechanism of cell injury provoked in myocardium by acute reoxygenation after anoxia or ischemia. The term ‘oxygen paradox’, introduced by Hearse et al. [32] for acute lethal reperfusion injury, was explicitly chosen for this reason. In the early 1980s it has been common sense that the two paradoxes are closely related biological phenomena.

Indeed, calcium paradox injury and acute lethal reperfusion injury (Fig. 2) share a number of features, such as: (i) They both result in hypercontracted cells with disrupted membranes and a consequent loss of cell constituents and stores, whereas different protective mechanisms are involved during hypothermic Ca\(^{2+}\) depletion, since Ca\(^{2+}\) depletion per se is not accompanied by a decrease of myocardial energy stores.

Fig. 2. Mechanism of the oxygen paradox (acute lethal reperfusion injury). Oxygen depletion leads to energy loss and consecutive disturbance of cellular cation control. Cytosolic Ca\(^{2+}\) concentration rises, the sarcolemma remains physically intact. When cells are reoxygenated, oxidative energy production is re-initiated. Energy plus Ca\(^{2+}\) overload leads to excessive contractile activation. This causes hypercontracture. In tissue, but not in isolated cardiomyocytes, hypercontracture leads to cell rupture, due to the forces acting between adjacent cells, and a terminal further increase in Ca\(^{2+}\) overload.

pathomechanisms which is characterised by energy-dependent contractile hyperactivation, mutual cell disruptions and calcium overload. The similarity in temperature dependency seems, however, coincidental. The protective effect of hypothermia during myocardial ischemia/anoxia is based on a slower decrease of the high energy phosphate stores, whereas different protective mechanisms are involved during hypothermic Ca\(^{2+}\) depletion, since Ca\(^{2+}\) depletion per se is not accompanied by a decrease of myocardial energy stores.

It is also clear now that there are distinct differences between the two paradoxes, such as: (i) Ca\(^{2+}\) overload in anoxic-reoxygenated cardiomyocytes seems always preceded by cellular Na\(^+\) overload, but under the classical calcium paradox protocol Na\(^+\) overload does not occur prior to massive Ca\(^{2+}\) overload. (ii) Cardiomyocytes in tissue and in the isolated state can be rescued from the oxygen paradox by a temporary interference at the time of
The calcium paradox reoxygenation [37]. It seems not possible, however, to prevent calcium paradox injury by only temporary interventions during the early phase of re-calci¢ation. (iii) Even though the complete pictures of calcium paradox and oxygen paradox injury are both missing in isolated cardiomyocytes, there is a marked difference. Brief Ca\(^{2+}\) depletion with normal Mg\(^{2+}\) and subsequent Ca\(^{2+}\) repletion does not cause any of the typical features of the calcium paradox, such as Ca\(^{2+}\) overload, hypercontracture and cytolysis in isolated cardiomyocytes. Anoxia-reoxygenation of isolated cardiomyocytes, however, causes all typical aspects of the oxygen paradox – except one: cytolysis [38]. After prolonged exposure to simulated ischemic conditions isolated cardiomyocytes develop hypercontracture upon reoxygenation, caused by re-energisation in presence of Ca\(^{2+}\) overload. They do not become disrupted because mechanical cell–cell interactions remain absent.

5. What is left?

Most articles on the calcium paradox were published in the 1980s. Searching MEDLINE for ‘calcium paradox’, one finds 174 articles in 1980–1989, 74 in 1990–1994 and 20 in 1995–1998. These figures show that the interest in this phenomenon is declining. This is understandable considering the progress in those fields of research which stimulated initially the interest in the calcium paradox, i.e. research on cardioplegia and ischemia-reperfusion.

Bretschneider’s cardioplegic solutions which are nominally Ca\(^{2+}\)-free have been used successfully and in large numbers in the clinic, predominantly in Central Europe. These crystalloid cardioplegic solutions are applied at cold temperature (4–8°C). They also contain trace amounts of Ca\(^{2+}\), due to the production process. As the calcium paradox has a strong temperature dependence and is eliminated by trace amounts of Ca\(^{2+}\), it is understandable why the calcium paradox has not turned out as a practical risk for Bretschneider’s cardioplegia. World-wide, cardioplegic principles with near-physiological Ca\(^{2+}\) concentration are, nevertheless, used much more frequently.

Research on ischemia-reperfusion injury has profited indirectly from the work on the calcium paradox even though its pathomechanism is different. This is because analysis of the calcium paradox has led to many insights in the multiple functions of Ca\(^{2+}\) in the heart that helped also the research on ischemia-reperfusion. To avoid conceptual confusions one should draw a clear line between the calcium paradox and other conditions creating a rapid myocardial calcium overload (Fig. 3). The calcium paradox is a laboratory artefact that does not occur under any natural pathophysiological circumstance. Dangerous myocardial Ca\(^{2+}\) overload is, however, a real and everyday problem in cardiac pathophysiology as it occurs whenever myocardium becomes ischemic. One of the determinants of acute lethal reperfusion injury, i.e. the oxygen paradox, is ischemic Ca\(^{2+}\) overload and this represents a true jeopardy for ischemic myocardium upon reperfusion.

The artefact called calcium paradox has been of great heuristic value.

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References

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