Effect of acidic reperfusion on prolongation of intracellular acidosis and myocardial salvage

Javier Inserte, Ignasi Barba, Víctor Hernando, Arancha Abellán, Marisol Ruiz-Meana, Antonio Rodríguez-Sinovas, and David García-Dorado*

Servicio de Cardiología, Hospital Universitari Vall d’Hebron, Pg. Vall d’Hebron 119–129, Barcelona 08035, Spain

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Aims It has been proposed that intracellular acidosis may be the basis of the cardioprotection of different interventions, including postconditioning. However, contradictory reports exist on the effects of acidic reperfusion on myocardial salvage. Here we characterized the effect of lowering the pH of the reperfusion media (pHo) on intracellular pH (pHi) and cell death.

Methods and results The effect of acidic perfusion on reperfusion injury was studied in isolated rat hearts submitted to 40 min of ischaemia and 30 min of reperfusion, and its effect on the Na\(^+/\)Ca\(^2+\)-exchanger (NCX) was analysed in isolated myocytes. pHi and phosphocreatine (PCr) were monitored by nuclear magnetic resonance spectroscopy. Lowering pHo to 6.4 during the initial 3 min of reperfusion delayed pHi normalization, improved PCr recovery, and markedly reduced (P < 0.001) lactate dehydrogenase release and infarct size (tetrazolium reaction). This cardioprotection was attenuated as pHo was increased, and was lost at pHo 7.0. Extending acidic reperfusion to the first 15 or 30 min of reflow did not result in further delay of pHi normalization and abolished the protection afforded by the initial 3 min of acidic reperfusion unless the Na\(^+/\)H\(^+\)-exchanger (NHE) blocker cariporide was added to the acidic perfusate and HCO\(_3^−\) substituted for N-[2-hydroxyethyl]piperazine-N\(^0\)-[2-ethanesulphonic acid].

In experiments performed in fura-2-loaded myocytes exposed to low Na\(^+\) buffer adjusted to pH 6.4, the lower Ca\(^2+\) uptake indicated an inhibitory effect of acidosis on NCX.

Conclusion Acidic reperfusion for 3 min delays normalization of pHi and enhances myocardial salvage, but extending it beyond this period fails to further delay pHi recovery. This is probably due to persisting NHE and Na\(^+/\)HCO\(_3^−\)-cotransporter activities, and it is detrimental, possibly through prolonged NCX inhibition.

KEYWORDS

Postconditioning; Hypercontracture; Reperfusion therapy; Mitochondrial permeability transition; Gap junctions; Calpain

1. Introduction

Early reperfusion of ischaemic myocardium is the best strategy to limit infarct size.\(^1\) However, reperfusion itself induces additional injury that reduces myocardial salvage.\(^2,3\) Several pharmacological strategies applied at the onset of myocardial reperfusion have been shown to limit cell death in experimental studies, but translation to patients with acute myocardial infarction has been so far virtually null.\(^4,5\) This has not been the case with ischaemic postconditioning, a different approach in which protection is achieved by means of brief periods of ischaemia during the initial minutes of reperfusion.\(^6\) Recently, different studies have reported that postconditioning could be an efficient cardioprotective intervention in patients with acute myocardial infarction submitted to primary percutaneous coronary interventions.\(^7−9\) The mediators of postconditioning protection have not yet been elucidated, but it has been suggested that prolongation of acidosis during reperfusion is determinant for the protective effects of postconditioning.\(^2,10,11\) A delay in intracellular pH (pHi) recovery at reperfusion may be protective by preventing contractile activation and development of high mechanical stress caused by hypercontracture in reoxygenated cardiomyocytes while the cells re-establish Na\(^+\) and Ca\(^2+\) control.\(^12\) Moreover, acidosis inhibits other mechanisms that have been consistently been shown to be involved in reperfusion injury, such as mitochondrial permeability transition (MPT) pore opening\(^13,14\) and activation of Ca\(^2+\)-dependent proteases, e.g. calpain,\(^15\) all of them factors that could contribute to explain this paradoxical worsening of injury after pH correction. Therefore, acidic reperfusion appears to be a potential protective strategy with options for being translated to a clinical setting.
Early experimental evidence demonstrated that transient reoxygenation with acidic solutions could protect isolated myocytes and papillary muscle against cell death.\textsuperscript{14,16–18} However, the situation is less clear in intact heart. Most of the studies were performed in isolated hearts subjected to short periods of ischaemia in which the main end point analysed was not lethal injury. In these studies, acidic reperfusion has been shown to reduce the incidence of ventricular fibrillation\textsuperscript{19,20} and to attenuate myocardial stunning.\textsuperscript{21,22} Other studies, however, have failed to show a protective effect of acidic reperfusion. Prolongation of acidosis for 15 min has been described to have no beneficial effect on functional recovery after 15 min of ischaemia or on protein release when ischaemia was extended to 60 min,\textsuperscript{23} and in a working rat heart model subjected to cardioplegic ischaemia, prolonged acidosis (longer than 3 min) exacerbated reperfusion injury.\textsuperscript{24}

The aim of this study was to determine the effect of perfusion with an acidic buffer during early myocardial reperfusion on pH\textsubscript{i} and cell death and to identify the factors modulating these effects. This information is essential for further research aimed at the therapeutic application of acidic reperfusion.

2. Methods

The experimental procedures conformed with the Guide for the Care and Use of Laboratory Animals published by the United States National Institute of Health (NIH Publication No. 85–23, revised 1996), and were approved by the Research Commission on Ethics of the Hospital Vall d’Hebron.

2.1 Isolated perfused rat heart preparation

Male Sprague-Dawley rats (300–350 g) were anaesthetized with sodium pentobarbital (100 mg/kg). Hearts were removed, mounted into a Langendorff apparatus and perfused with a modified Krebs-Henseleit bicarbonate buffer (KHB, in mM: NaCl 140, NaHCO\textsubscript{3} 24, KCl 2.7, KH\textsubscript{2}PO\textsubscript{4} 0.4, MgSO\textsubscript{4} 1, CaCl\textsubscript{2} 1.8, and glucose 11) equilibrated with 95% O\textsubscript{2} and 5% CO\textsubscript{2} and maintained at 37°C as previously described.\textsuperscript{25} Flow rate was initially set to produce a perfusion pressure of 60 mmHg and was held constant thereafter. Left ventricle (LV) pressure was monitored through the use of a water-filled latex balloon inserted into the LV and inflated to obtain an end-diastolic pressure (LVEDP) between 6 and 8 mmHg. LV developed pressure (LVdP/τ) was calculated as the difference between LV systolic pressure and LVEDP. Perfusion pressure was continuously recorded using a pressure transducer connected to the perfusion line.

2.2 Experimental protocol

In a first set of experiments, the effect of prolongation of acidosis during initial reperfusion against cell death was tested. Hearts were stabilized for 30 min and then subjected to no-flow global ischaemia for 40 min followed by 30 min of reperfusion. Hearts were perfused with KHB at either pH 7.4 (control group, n = 10) or KHB adjusted to pH 6.4 (n = 7), 6.7 (n = 6) and 7.0 (n = 6) for the first 3 min of reperfusion. The pH of the acidic solution was adjusted by lowering bicarbonate concentration and increasing sodium chloride to keep osmolality constant while maintaining the same gassing mixture containing 5% CO\textsubscript{2}.\textsuperscript{20} In a second set of experiments, the effect of the duration of the acidic perfusion on reperfusion injury was analysed in two additional groups of hearts perfused with KHB adjusted to pH 6.4 for the first 15 min (n = 6) or 30 min (n = 5) of reperfusion. In two additional groups of hearts the activities of Na\textsuperscript{+}/HCO\textsubscript{3} cotransporter (NBC) and Na\textsuperscript{+}/H\textsuperscript{+} exchanger (NHE) were blocked during the first 15 min of reperfusion by using NaHCO\textsubscript{3}-free HEPES buffer adjusted to pH 7.4 (n = 6) or pH 6.4 (n = 7) and containing 10 μM cariporide.

2.3 Quantification of cell death

Lactate dehydrogenase (LDH) activity was spectrophotometrically measured in the coronary effluent throughout the reperfusion period. After reperfusion hearts were cut into four slices and incubated at 37°C for 10 min in 1% triphenyltetrazolium chloride (TTC) (pH 7.4) and imaged under white light to outline the area of necrosis as previously described.\textsuperscript{25}

2.4 Nuclear magnetic resonance spectroscopy

pH\textsubscript{i}, phosphocreatine (PCr) and adenosine triphosphate (ATP) kinetics were measured by \textsuperscript{31}P-NMR(nuclear magnetic resonance) in hearts perfused after 40 min of ischaemia with KHB free of phosphate at pH 7.4 or pH 6.4 during the first 3 min or 15 min of reperfusion. In two additional groups, hearts were reperfused for 15 min with bicarbonate-free HEPES buffer adjusted to pH 7.4 or at pH 6.4 and containing 10 μM cariporide. Spectroscopy was performed on a Bruker Avance 400 spectrometer equipped with a 20-mm probe tuned to \textsuperscript{31}P. Spectra consisted in the accumulation of 50 scans with a delay of 0.6 s between pulses that lasted for 30 s. The PCr peak was integrated with the software provided by the manufacturer, and pH\textsubscript{i} was measured by the chemical shift of the inorganic phosphate peak relative to the PCr peak.\textsuperscript{26} In order to circumvent the difficulties in the measurement of ATP caused by the low signal-to-noise ratio during reperfusion, [ATP] was calculated from the average of four consecutive spectra (corresponding to 2 min of acquisition). The γ-ATP peak was measured in the averaged spectra.

2.5 Studies in fura-2-loaded cardiomyocytes

Freshly isolated rat cardiac myocytes were obtained from adult rat hearts (300 g Sprague-Dawley male rats) by a collagenase perfusion as previously described\textsuperscript{27} and plated on laminin-precoated glass bottom culture dishes. For intracellular [Ca\textsuperscript{2+}] measurements, cells were loaded 30 min at 37°C with 2.5 μM of the acetoxymethyl ester of fura-2 (Molecular Probes, USA) in control solution (containing in mM: NaCl 140, KCl 3.6, MgSO\textsubscript{4} 1.2, CaCl\textsubscript{2} 1, HEPES 20, and glucose 5, at pH 7.4), washed twice and postincubated for 10 min in the same solution. To stimulate the reverse mode of Na\textsuperscript{+/Ca\textsuperscript{2+}}-exchanger (NCX) and induce Ca\textsuperscript{2+} overload, NaCl was reduced to 70 mM, Ca\textsuperscript{2+} was increased to 2 mM and mannitol 2.2% (v/v) was added to maintain normo-osmolarity (312 mOsm). Thapsigargin 2 μM and ryanodine 10 μM were added during the induction of cytosolic Ca\textsuperscript{2+} overload to prevent sarcoplasmic reticulum Ca\textsuperscript{2+} uptake and release. The effect of acidosis on cytosolic [Ca\textsuperscript{2+}] was induced by lowering extracellular pH to 6.4.
Cytosolic \([\text{Ca}^{2+}]\) was monitored throughout 10 min under both pH 7.4 and pH 6.4 using a ratiofluorescence imaging system (QuantiCell 900, Visitech, UK). Cells were alternately excited at 340 and 380 nm, with a bandwidth of 15 nm, by means of a fast speed monochromator. Emitted light was collected by an air-cooled intensified digital camera, and 340/380 ratios were calculated for each pixel at 1-s intervals from background-subtracted signal intensities in pairs of images consecutively obtained at the two wavelengths. Colour-coded 340/380 ratio images were generated and further calibrated at both pH values to correct for pH-dependent changes in fura-2 affinity.27

2.6 Statistical analysis

Data analyses were performed using SPSS for Windows (v. 10). Results are expressed as mean ± SEM. Differences between groups were assessed by means of simple one-way analysis of variance (ANOVA). Changes with time were assessed by multiple ANOVA. If significant differences were observed, a least significant square test was applied as a post hoc test. \(P < 0.05\) was considered to be statistically significant.

3. Results

3.1 Effects of acidic reperfusion on myocardial injury

In control hearts subjected to 40 min of ischaemia, LVEDP and LVdP were 7.2 ± 1.6 and 99.8 ± 4.0 mmHg, respectively, at the end of the equilibration period. At this time perfusion pressure was 59.3 ± 3.6 mmHg and coronary flow 12.4 ± 1.3 mL/min. No-flow ischaemia resulted in cessation of contractile activity and in a steep increase in LVEDP with a peak of 73.7 ± 10.9 mmHg 13.1 ± 0.4 min after the onset of ischaemia. No differences among groups were observed during equilibration and ischaemic periods.

During reperfusion, hearts perfused with buffer adjusted to pH 6.4 for the first 3 min showed delayed (5.5 ± 0.3 min vs. 3.3 ± 0.2 min in control group, \(P < 0.001\); Figure 1A) and attenuated (62.6 ± 5.8 mmHg vs. 115.1 ± 6.0 in control group, \(P < 0.001\); Figure 1B) hypercontracture as well as improved contractile recovery (25.2 ± 4.6% of basal values after 30 min of reperfusion vs. 5.6 ± 1.5% of basal values in control group, \(P < 0.001\); Figure 1C), reduced increase in perfusion pressure (99.9 ± 5.6 mmHg vs. 117.9 ± 3.7 mmHg, \(P < 0.001\)), less LDH release (42%, \(P = 0.001\); Figure 1D), and infarct size measured by TTC (30%, \(P = 0.001\); Figure 1E).

Figure 1  Influence of perfusion with different pH values during the first 3 min of reperfusion on: (A) the time to development of hypercontracture (in min); (B) magnitude of hypercontracture (mmHg); (C) left ventricle developed pressure recovery (measured after 30 min of reperfusion and expressed as percentage respect to basal values); (D) time course of lactate dehydrogenase (LDH) release during reperfusion; (E) total LDH released; (F) infarct size measured by TTC and expressed as percentage of ventricular mass (*\(P < 0.05\) vs. group reperfused at pH 7.4; data are mean ± SEM).
Figure 1D and E), and smaller infarcts (38%, P = 0.012; Figure 1F). Reduction of infarct size by acidosis was further confirmed in two additional groups, control (n = 4) and pH 6.4 perfused hearts (n = 4), in which the reperfusion period was extended to 1 h (49.7 ± 5.4% in control group vs. 26.2 ± 3.6% in hearts reperfused at pH 6.4, P = 0.011). The protective effects afforded by 3 min of acidic perfusion were progressively smaller as the pH of the perfusate was increased. Protection was maximal at pH 6.4 and was lost at pH 7.0 (Figures 1 and 2).

3.2 Time dependence of the effects of acidic reperfusion

Prolongation of perfusion with KHB buffer adjusted to pH 6.4 for 15 or 30 min of reperfusion did not induce further delay in hypercontracture (Figure 2A and D) and was associated with loss of the protective effects on contractile recovery (Figure 2C), hypercontracture (Figure 2B and D), perfusion pressure (Figure 2E), LDH release (Figure 3A and B), or infarct size (Figure 3C) observed when acidic perfusion was limited to the first 3 min of reperfusion. Reperfusion with bicarbonate-free HEPES buffer adjusted to pH 6.4 and containing cariporide markedly delayed hypercontracture and was still protective. When HEPES buffer was adjusted to pH 7.4 the cardioprotective effects on hypercontracture and cell death were attenuated but still statistically significant (Figures 2 and 3).

3.3 Effect of acidic reperfusion on intracellular pH and energetic recovery

pHi was 7.01 ± 0.02 under normoxic conditions without differences between groups. Ischaemia induced a progressive pHi reduction that reached its maximum (6.43) after 20 min of zero flow. Reperfusion after 40 min of ischaemia resulted in recovery of pHi within 1.83 ± 0.17 min of reperfusion. In hearts perfused with KHB at 6.4 for the first 3 min of reperfusion the onset of pHi recovery was delayed and recovery was complete (pH 7.02 ± 0.03 at 4.33 ± 0.25 min after reperfusion).
of reperfusion, Figure 4). Prolongation of acidic perfusion to the first 15 min of reperfusion did not induce further delay in pHi recovery, except when acidic perfusion was performed with bicarbonate-free HEPES buffer containing cariporide, but even in this case pHi returned to normal values after 8.3 min of reperfusion. Perfusion with HEPES buffer containing cariporide and adjusted to pH 7.4 induced a slight but significant delay in pHi recovery (2.70 ± 0.12, P = 0.002).

The concentrations of PCR and ATP in control hearts were 75.8 ± 4.5 μmol PCR/g dry weight and 30.46 ± 0.41 μmol ATP/g dry weight, respectively. No differences in these values were observed among groups during equilibration, or in the kinetics of PCR and ATP depletion during ischaemia. Upon reperfusion, PCR levels quickly recovered to 74.57 ± 9.65% of its basal value within a maximum of 2 min after reperfusion onset in control hearts, but then abruptly fell to 42.22 ± 1.98%. In hearts reperfused with acidic buffer at pH 6.4, PCR recovered to levels similar to those in the control group after 2 min of reperfusion, but the subsequent decrease was significantly attenuated. When acidic perfusion was restricted to the first 3 min of reperfusion, the initial high levels of PCR were maintained even after normalization of the pH of the perfusate. However, prolongation of acidosis beyond the initial 3 min of reperfusion resulted in a progressive reduction of PCR that eventually reached values similar to those observed in the control group. In hearts
perfused for 15 min with HEPES buffer adjusted to pH 6.4 containing cariporide, energetic recovery was similar to that observed after only 3 min of acidic reperfusion while reperfusion with HEPES buffer adjusted to pH 7.4 only resulted in a slight (1 min) delay in PCr reduction with respect to the control group (Figure 5B). In the control group, [ATP] recovered to a maximum of 22.64 ± 2.61% at minute 4 of reperfusion. Acidic reperfusion and inhibition of NHE and NBC with HEPES buffer adjusted to pH 6.4 containing cariporide showed the best recovery of ATP after 30 min of reperfusion (Figure 5C).

3.4 Effect of acidosis on low sodium-induced Ca²⁺ overload

Exposure of myocytes to low extracellular [Na⁺] (70 mM) resulted in rapid cytosolic Ca²⁺ overload in the presence of 2 mM extracellular Ca²⁺ and pH 7.4. However, lowering extracellular pH to 6.4 significantly reduced the degree of cytosolic Ca²⁺ overload under the same conditions (Figure 6).

4. Discussion

The results of our study provide for the first time direct evidence, by using NMR measurements of pH, that in the isolated rat heart there is a critical period of time for acidic reperfusion to be protective. Acidic perfusion during the first 3 min of reperfusion delays normalization of pH, improves energetic and functional recovery of cardiomyocytes and attenuates hypercontracture and cell death. These protective effects are dependent on the level of acidosis, being maximal at pH 6.4. Prolongation of extracellular acidosis beyond the first 3 min does not result in further prolongation of intracellular acidosis, due to NBC and NHE activity, and abolishes the protection afforded by
the initial 3 min of acidic reperfusion, possibly in relation to persistent NCX inhibition.

4.1 Protective effects of short periods of perfusion with acidic buffer during reperfusion

Several mechanisms could lead to the pronounced effect of pH in the development of hypercontracture in reperfused cardiomyocytes. First, prolongation of intracellular acidosis inhibits myofibrillar machinery by reducing the sensitivity of myofibrils to Ca\(^{2+}\) and prevents the development of hypercontracture during the initial minute of reperfusion when Ca\(^{2+}\) homeostasis has not been yet restored. This effect is similar to the infarct-sparing effect observed with the transient blockade of actin-myosin interaction with 2,3-butanedione monoxime during initial reperfusion. Secondly, rapid washout of extracellular H\(^+\) upon reperfusion of ischaemic myocardium creates a transmembrane H\(^+\) gradient resulting in Na\(^+\) influx coupled to H\(^+\) extrusion via NHE and NBC. Influx of Na\(^+\) increases the intracellular Na\(^+\) concentration, and the loss of the Na\(^+\) gradient, in turn, favours the reverse mode of NCX and enhances Ca\(^{2+}\) overload. In addition, several studies, including the present one, propose that acidosis could prevent Ca\(^{2+}\) entry by inhibition of NCX. Other studies described that acidosis also decreases cytosolic Ca\(^{2+}\) entry via a Ca\(^{2+}\) inward current and Ca\(^{2+}\) release from the sarcoplasmic reticulum. Supporting this effect on cationic homeostasis, it has been demonstrated that prolongation of extracellular acidosis during the early phase of reoxygenation reduces intracellular Na\(^+\) and Ca\(^{2+}\) overload. Thirdly, acidosis has been suggested to inhibit calpain activity and could thus attenuate Ca\(^{2+}\)-dependent proteolytic injury occurring during reperfusion. Recently, it has been proposed that calpain activation contributes to reperfusion-induced cell death not only by increasing membrane fragility but also by favouring Ca\(^{2+}\) entry as consequence of attenuated intracellular Na\(^+\) recovery due to calpain degradation of the proteins that stabilize Na\(^+/\)K\(^+\)-ATPase to the membrane-cytoskeleton complex. Finally, evidence has accumulated in recent years suggesting that the MPT pore is an important player in reperfusion injury. During reperfusion rapid normalization of pH may allow MPT triggered by ROS and mitochondrial Ca\(^{2+}\) overload. Therefore, prolongation of intracellular acidosis with acidic reperfusion is expected to prevent MPT.

4.2 Loss of protection with prolonged acidic perfusion during reperfusion

Protection against cell death induced by 3 min of acidic perfusion was lost when the acidic perfusion was extended to 15 min. After 5.5 min of reperfusion hearts developed hypercontracture, increased coronary resistance, and LDH release, and at the end of reperfusion, infarct size was similar to that obtained in control hearts. Continuation of acidosis for 30 min did not result in further injury. These results are in agreement with the lack of protection previously observed in hearts reperfused with an acidic buffer for 15 min. Our \(^{31}\)P-NMR data on pH could help explain the time-dependent reversion of the protective effects of acidosis. Although it is generally assumed that acidic reperfusion prolongs intracellular acidosis as long as extracellular acidosis is present, in our study this was only true for the first 3 min of reperfusion. Beyond this time hearts rapidly normalized pH despite extracellular acidosis, without significant time differences with respect to hearts in which acidic reperfusion was restricted to the first 3 min. The prolongation of pH observed in hearts reperfused with a bicarbonate-free HEPES buffer containing the NHE inhibitor cariporide demonstrates that the normalization of pH while extracellular acidosis is maintained is a consequence of the Na\(^+\) influx-dependent mechanisms of pH correction. The eventual normalization of pH despite acidic reperfusion with bicarbonate-free HEPES buffer containing cariporide indicates that other mechanisms of correction of acidosis such as washout of metabolite (lactate and CO\(_2\)) may play an important role during this phase. The slight although significant delay in pH observed in the group perfused with HEPES buffer adjusted to pH 7.4 further supports this notion. The recovery of myocardial pH despite prolonged acidic reperfusion could explain its failure in producing additional protection. However, the reasons for the loss of protection associated with prolonged extracellular acidosis are not clear. It has been suggested that prolonged acidosis could inhibit essential metabolic pathways and impair energetic recovery. However, we observed a fast and complete functional recovery in normoxic hearts perfused with acidic
buffer for 30 min upon normalization of extracellular pH (data not shown), which makes unlikely the possibility that acidosis causes irreversible deleterious effects. Alternatively, the detrimental consequences of prolonged acidosis could be explained by an inhibitory effect on NCX. We have previously shown that inhibition of NCX has time-dependent consequences on reperfusion-induced cell death. 30 Although NCX inhibition is markedly protective during the first minutes of reperfusion by preventing additional Ca\(^{2+}\) entry via reverse mode NCX activity, persistent inhibition of NCX beyond the first 4 min of reperfusion impairs recovery of Ca\(^{2+}\) homeostasis in myocytes that have survived initial reperfusion and is detrimental. 30 Although we assume the limitations of our model of isolated myocytes for extrapolation of results to intact heart due to the absence of electrical stimulation and contraction, and the non-physiological low Na\(^{+}\) concentration used to favour reverse mode NCX (as described in previous studies), the attenuated [Ca\(^{2+}\)]\(_{i}\) gain observed in myocytes in which the sarcoplasmic reticulum has been blocked when exposed to low [Na\(^{+}\)] buffer at low pH is consistent with an inhibitory effect of acidosis on NCX activity.

In contrast to our results, reperfusion with blood equilibrated at pH 6.8 for 30 min in a model of coronary artery occlusion in dogs, a more clinically relevant model, resulted in a reduction of infarct size. 31 While the reasons for this apparent discrepancy are not known, there are large differences between both experimental models and the methodological conditions used for acidic perfusion. In any case, this observation does not limit the potential clinical relevance of our observation indicating that limiting the time of acidic reperfusion could result in stronger protection.

In conclusion, our study supports the hypothesis that prolongation of intracellular acidosis during initial reperfusion protects against ischaemia/reperfusion-induced hypercontracture and cell death. Acidic reperfusion can prolong intracellular acidosis only for the first few minutes of reflow, but beyond that time, it fails to maintain intracellular acidification due to persistent NHE and NBC activity and is detrimental, probably due to impaired NCX-mediated Ca\(^{2+}\) efflux. Despite the limitations inherent to the isolated rat model that preclude direct translation to the clinical setting, these results further support the development of therapeutic strategies aimed against reperfusion injury based on prolongation of intracellular acidosis.

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