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The importance of myocardial amino acids during ischemia and reperfusion in dilated left ventricle of patients with degenerative mitral valve disease

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Abstract

Taurine, glutamine, glutamate, aspartate, and alanine are the most abundant intracellular free amino acids in human heart. The myocardial concentration of these amino acids changes during ischemia and reperfusion due to alterations in metabolic and ionic homeostasis. We hypothesized that dilated left ventricle secondary to mitral valve disease has different levels of amino acids compared to the right ventricle and that such differences determine the extent of amino acids' changes during ischemia and reperfusion. Myocardial concentration of amino acids was measured in biopsies collected from left and right ventricles before cardioplegic arrest (Custodiol HTK) and 10 min after reperfusion in patients undergoing mitral valve surgery. The dilated left ventricle had markedly higher ($P < 0.05$) concentrations (nmol/mg wet weight) of taurine (17.0 ± 1.5 vs. 10.9 ± 1.5), glutamine (20.5 ± 2.4 vs. 12.1 ± 1.2), and glutamate (18.3 ± 2.2 vs. 11.4 ± 1.5) when compared to right ventricle. There were no differences in the basal levels of alanine or aspartate. Upon reperfusion, a significant ($P < 0.05$) fall in taurine and glutamine was seen only in the left ventricle. These changes are likely to be due to transport (taurine) and/or metabolism (glutamine). There was a marked increase in the alanine to glutamate ratio in both ventricles indicative of ischemic stress which was confirmed by global release of lactate during reperfusion. This study shows that in contrast to the right ventricle, the dilated left ventricle had remodeled to accumulate amino acids which are used during ischemia and reperfusion. Whether these changes reflect differences in degree of cardioplegic protection between the two ventricles remain to be investigated.

Keywords

Dilatation; Mitral valve surgery; Ischemia; Cardioplegia; Taurine; Glutamine; Glutamate; Alanine; Aspartate

Introduction

Cardioprotective strategies against ischemia and reperfusion are aimed at preserving intracellular metabolites and opposing disruption to ionic homeostasis. Evaluation of the efficacy of cardioplegic solutions has relied upon monitoring intracellular changes of key metabolites including amino acids [1-4]. During ischemia, anaerobic metabolism leads to the building up of lactic acid and a decrease in intracellular pH. The fall in pH triggers Na^+/H^+ exchanger to increase the intracellular Na^+ concentration which is accentuated by the reduced Na-pump activity due to a fall in ATP. Reperfusion following prolonged ischemia can lead to irreversible damage caused by Ca^{2+} loading via the $\text{Na}^+/\text{Ca}^{2+}$ exchanger and generation of reactive oxygen species [4]. In addition to causing Ca^{2+} loading, it has been shown that ischemia-induced Na^+ accumulation would also contribute to osmotic-induced cell swelling, which has been implicated as a cause of ischemia-induced sarcolemmal damage [5-7]. In response to cardiac insults/osmotic stress, heart cells release free amino acids [8] including the non-protein β -amino acid taurine [9-11]. Taurine is the main organic osmolyte in the heart [12], and its trans-sarcolemmal fluxes are dependent on the Na^+ gradient [13]. This close link between Na^+ (osmotic changes) and taurine has been supported by our study involving open heart surgery [1, 2, 11, 14-16]. These studies have demonstrated that the more the ischemically stressed the heart during cardioplegic arrest, the greater the fall of myocardial taurine following ischemia and reperfusion. We have shown that maneuvers that increase the intracellular concentration of Na^+ in isolated guinea-pig hearts trigger a fall in myocardial taurine and that this fall was due to efflux, as the amino acid could be measured in the coronary effluent [13]. This is consistent with taurine being very slowly metabolized and the finding that its concentration is raised in the blood of patients following acute myocardial infarction, unstable angina, and cardiovascular surgery [17-19]. Subsequently it was proposed that preloading the heart with taurine before a cardiac insult would improve its ability to cope by opposing changes in intracellular Na^+ and Ca^{2+} [11].

In addition to taurine, the principal free amino acids glutamine, glutamate, aspartate, and alanine also change in heart cells during cardiac insults in both experimental models and during open heart surgery [10, 11, 14]. However, unlike taurine, the changes in these amino acids involve metabolism and transport and influence the biosynthesis of several key compounds including ATP and glutathione [20].

Cardiac muscle adapts to changes in the body (e.g., substrate availability and physiologic demands) by modifying muscle function, altering the synthesis and degradation rates of specific proteins, and altering the use of substrates [21]. In hearts with mitral regurgitation, volume overload triggers structural, functional, and metabolic remodeling in the left ventricle [22]. In contrast and in the early stages of the disease, the right ventricle is not affected by these compensatory mechanisms, as it is not chronically overloaded. As a result, the cardiac pump is likely to end up having two ventricles with different metabolic states that are likely to respond differently to ischemic cardioplegic arrest. Therefore, this study was designed to investigate whether the level of amino acids in the dilated left ventricle is indeed different from the right ventricle, and whether this difference has any bearing on cellular changes in amino acids during cardioplegic arrest. In order to achieve our aim, we measured myocardial amino acids in both dilated left ventricle secondary to mitral valve regurgitation and normal right ventricle in patients with degenerative mitral valve disease at pre-ischemic level and following cardioplegic arrest and reperfusion.

Methods

We studied 12 adult patients with dilated left ventricle secondary to mitral valve regurgitation undergoing mitral valve repair/replacement surgery during 2007/2008. Dilation was confirmed when Indexed Left Ventricular End Diastolic Volume (ILVEDV) was 76 ml/m². Mean ILVEDV in this study was 95 ± 4 ml/m². The main inclusion criterion was that patients had a normal right ventricle as per the following echocardiographic criteria: volume at 2/3 of the left ventricle, right to left ventricle diameters ratio less than 0.6; triangular shape; free wall thickness less than 5–6 mm and TAPSE of more than 20 mm (TAPSE: tricuspid annular plane systolic excursion). Patients' characteristics are shown in Table 1. Exclusions included severe tricuspid regurgitation, systolic pulmonary pressure >35 mmHg, coronary disease, aortic valve disease, and type I & II diabetes mellitus. Patients had no history of arrhythmias and were in sinus rhythm. The investigation conforms to the principles outlined in the Declaration of Helsinki. Local hospital ethical approval as well as patient consent was obtained. Most of the patients (*n* = 10) were using the following medications: ACE inhibitors (e.g., enalapril) and diuretics (e.g., Frusemide).

Surgical and anesthetic techniques

A standard circuit of cardiopulmonary bypass (CPB) was used with a 40-lm filter, a Stockert roller pump, and a fiber membrane oxygenator (Dideco, Sorin, Cobe). Priming solution of the extracorporeal circuit included 1,000 ml of pH 7.4 balanced electrolyte solution, 500 ml of plasma expanding reagent (6% hydroxyethyl starch), 100 ml of 18% mannitol, and 5,000 IU of Heparin. Non-pulsatile flow was used at a rate of 2.4 l/m²/min. Systemic temperature was kept at 34°C. A left ventricular vent was inserted in all patients via the right superior pulmonary vein. Heparin IV was used at a dose of 300 UI/Kg to achieve a target ACT of 480 s.

For induction and maintenance of anesthesia, propofol infusion was used at 2–2.8 µg/ml (5.4–7.5 mg/Kg/min) combined with remifentanyl infusion at 3.5–7.60 µg/ml (0.13–0.28 µg/Kg/min.) using a Diprifusor pump. Neuro-muscular blockade was achieved with 0.6 mg/Kg of Rocuronium infused at 0.12 mg/Kg/min. Normocapnia was maintained and the lungs were ventilated with a mixture of air/O₂ = 0.5, with low flow ventilation technique. Mean arterial blood pressure of 60–70 mmHg was maintained with volume or increments of Metaraminol 0.5–1.0 mg. Following transfer to intensive care unit, sedation was maintained with propofol infusion at 1–2 µg/ml combined with remifentanyl infusion at 2.0–2.5 µg/ml.

Myocardial protection was achieved using ice-cold Custodiol (Bretschneider's HTK-solution) cardioplegia. Infusion of cardioplegia following institution of cardiopulmonary bypass and aortic cross clamping was 10.5 ± 0.5 min at a rate of 200 ml/min giving a mean total infusion volume of 2083 ± 93 ml. This resulted in a maximum drop in myocardial temperature to approximately 10°C which then gradually increases to reach around 25°C toward the end of cardioplegic arrest.

Collection of ventricular biopsies and extraction of metabolites

Transmural biopsies of the left ventricular apical or anterior-free wall and the right ventricular-free wall (3–7 mg wet weight) were taken using a Trucut needle (Baxter Healthcare Corporation, Northbrook, IL). Two biopsies were collected from each ventricle; the first biopsy was taken 5 min after institution of cardiopulmonary bypass before aortic cross-clamping (basal or pre-ischemic) and the second after 10 min of reperfusion following removal of the aortic cross-clamp. Each specimen was frozen immediately in liquid nitrogen until processing the analysis of metabolites. A research technician blind to the operative technique performed the extraction and the analyses.

Determination of amino acids in biopsy specimens

Free amino acids were measured in all biopsies collected. Amino acids were determined using HPLC according to the Pico-Tag method of Water as reported earlier [2]. Concentration of metabolites was expressed as nmol/mg wet weight.

Reperfusion injury

Markers of injury and stress were measured in blood samples collected from the radial artery. Post-operative release of creatine kinase (CK-MB) was used as a measure of myocardial injury. Plasma lactate was also measured early after reperfusion to assess the degree of metabolic ischemic stress. Elevated blood lactate concentration during cardiac surgery is an indicator of hypoxia and anaerobic glycolysis. Accumulated cardiac lactate during ischemia is normally released from heart cells immediately upon reperfusion [23].

Statistical analysis

Data were expressed as mean \pm standard error (SE). Comparison between continuous variables was made using a non-parametric test (Wilcoxon's signed rank test). All statistical analyses mentioned were performed with the aid of a computerized software package, Statview for Windows (SAS Institute Inc., Cary, NC, USA).

Results

Basal myocardial taurine and α -amino acids in left and right ventricles

The basal (pre-ischemic) intracellular concentration of amino acids in left and right ventricles is shown in Table 2. The slowly metabolizing non-protein amino acid taurine was present at a significantly higher level in the left compared to right ventricle. The same trend was also seen for the total principal protein-free α -amino acids. The difference in total protein amino acids was due to higher myocardial concentrations in left ventricle in glutamine and glutamate but not in aspartate or alanine. The total amino acids in the right ventricle were significantly less than those in the left ventricle (approximately 62% of left ventricular amino acid pool). Despite the large difference in the total free amino acid pool between the two ventricles, the percentage of individual amino acids in each ventricle remained the same. These were 28% versus 27% for taurine, 33% versus 33% for glutamine, and 30% versus 29% for glutamate in left versus right ventricle, respectively.

Changes in myocardial amino acids in left and right ventricles following cardioplegic arrest and reperfusion

There were changes in the myocardial concentration of amino acids, measured in the second biopsy (collected 10 min after reperfusion) when compared to the preischemic basal levels. These changes were different for both ventricles (Figs. 1, 2). There were no changes in the concentrations of taurine and glutamine in the right ventricle as a result of cardioplegic arrest and reperfusion (Fig. 1). However, there was a marked and significant fall in the myocardial concentration of taurine (from 17.0 ± 1.5 to 11.6 ± 1.6 nmol/mg weight, $P < 0.05$) and glutamine (from 20.5 ± 2.3 to 15.6 ± 1.6 nmol/mg weight, $P < 0.05$) in the left ventricle.

The effect of ischemic cardioplegic arrest and reperfusion on the concentration of glutamate and alanine was qualitatively similar for both ventricles, but the extent of the changes was different (Fig. 2). In both ventricles, there was a fall in glutamate and a rise in tissue alanine. However, the changes were markedly higher in the left ventricle. There were no significant changes in myocardial aspartate in both left and right ventricles.

Myocardial injury and metabolic stress during mitral valve surgery

Figure 3a shows that there is a significant rise in the blood lactate at the onset of reperfusion (release of aortic cross-clamp), which remained elevated for 30 min afterward. In order to assess the stress in individual ventricles, two markers (ratios) of metabolic stress [24, 25] were calculated. They are the alanine/glutamate and glutamine/glutamate ratios. The alanine/glutamate ratio was markedly elevated in both ventricles (Fig. 4a). However, the glutamine/glutamate ratio was significantly higher upon reperfusion in the right ventricle (Fig. 4b). Surgery was associated with significant cardiac injury as seen by significant release of CK-MB which peaks 1 h after reperfusion (Fig. 3b).

Discussion

In this study, we show a number of novel observations. First, the dilated left ventricle secondary to mitral regurgitation has markedly higher levels of taurine, glutamine, and glutamate than that of the right ventricle. Second, upon reperfusion following cardioplegic arrest, amino acids and metabolic stress in both ventricles reach similar levels. Finally, cardioplegic arrest triggers changes in the left ventricle that reflects metabolic remodeling back toward the status of the right ventricle. It is likely that these differences between the two ventricles would be associated with differences in vulnerability to ischemic cardioplegic arrest and reperfusion.

The “dilated” left ventricle has higher levels of amino acids compared to the “normal” right ventricle

Mitral regurgitation triggers a volume overload in the left ventricle that leads to cardiac remodeling, myocardial dysfunction, and eventually heart failure [26]. During the early stages of the disease (which largely applies to our study), patients remain relatively asymptomatic in New York Heart Association I or II due to physiologic compensation by left ventricle dilation and hypertrophy. Patients selected in this study had relatively “normal” right ventricle and with one exception, none had heart failure.

This study demonstrates that chronic volume overload triggers significant increase in the concentration of taurine in the left ventricle. This effect is quite marked, as taurine concentration in the left ventricle is nearly double that of the concentration in the right ventricle. Although it is extremely difficult to obtain fresh biopsy from a “normal” left ventricle, our earlier study on biopsies collected from viable myocardium of the left ventricle of patients with coronary disease (no hypertrophy or dilatation) had taurine levels at 9–10 nmol/mg wet weight [11, 14]. Such values are very similar to the levels found in the right ventricle of patients recruited for this study (Table 2). This suggests that the differences in the levels of taurine between the dilated left and normal right ventricles are due to disease progression. This conclusion is supported by study on dogs where a failing right ventricle had significantly higher taurine levels compared to the left ventricle [27].

In addition to volume overload-induced accumulation of taurine (demonstrated in this study), we have previously shown the same trend occurring in hypertrophic left ventricle due to pressure overload secondary to aortic stenosis [16]. Pressure stress has also been shown to elevate levels of vitreous taurine in a rabbit model of cataract surgery [28]. These studies indicate that mechanical stress is likely to be a key trigger of taurine accumulation. This mechanical stress could induce osmotic changes that would eventually lead to taurine accumulation. An alternative explanation could be stress (disease)-induced changes in taurine transporter activity and expression. It has been suggested that taurine accumulation due to congestive heart failure is the result of adrenergic stimulation of the taurine transporter [29].

Taurine is a β -amino acid that is very slowly metabolized and is the major osmolyte in heart cells [12]. Therefore, it can be argued that the accumulated taurine can be used to remove the excess Na^+ and help the heart to cope better with subsequent cardiac insults [13, 30] as well as having anti-apoptotic and anti-necrotic effects [31-33]. Although taurine has been implicated in the regulation of intracellular Na^+ (via the taurine transporter or by altering Na^+ channel activity [34]), this amino acid has also been linked to Ca^{2+} cycling in myocytes [34-36]. Whether taurine accumulation does alter Na^+ and Ca^{2+} concentration in dilated myocardium is not presently known.

The higher concentration of glutamine in the left ventricle will have effects on transport and metabolism [37]. Like taurine, it will influence Na^+ levels as a result of the increased Na-glutamine symport activity. Glutamine is also metabolically active and acts as a nitrogen donor for the biosynthesis of a number of compounds such as nucleotides and amino acids including glutamate [20]. In fact, the concentration of glutamate in the left compared to the right ventricle was proportionately the highest (220% higher) when compared to other amino acids. Although glutamate can be used as substrate for energy production especially during metabolic stress, it also has strong antioxidant activity in cardiomyocytes [38, 39]. Due to its cardioprotective effects, glutamate has been used to supplement cardioplegia [40-42]. The observation that alanine/glutamate ratio is lower in the left ventricle (Fig. 4a) could indicate that this ventricle has relatively less metabolic stress. However, it should be emphasized that this is a chronic effect which could also result from disease-induced changes in amino acid transporters and metabolism.

Only the left ventricle shows a marked fall in taurine and glutamine following ischemia and reperfusion

Both left and right ventricles are exposed to long duration (107 ± 7 min) of cardioplegic arrest. During this period, the heart will be exposed to ischemic/osmotic stress which will involve disruption in metabolic and ionic homeostasis. In particular, there will be accumulation of lactate which appears in the effluent following the release of the cross-clamp (Fig. 3). In addition, Na^+ will be accumulated by heart cells which will be accentuated by hypothermia [10, 11, 43]. The main routes for removing intracellular Na^+ upon reperfusion can cause injury (e.g., $\text{Na}^+/\text{Ca}^{2+}$ exchanger loads cells with Ca^{2+} , whereas Na-pump uses the much needed ATP). In addition, there are two important and fast symports that transport Na^+ with glutamine and taurine. Evidence for taurine- Na^+ symport is seen in the left ventricle as changes in tissue taurine are due to transport [30]. An efflux of taurine (and glutamine) would suggest that a significant build up in Na^+ has occurred during cardioplegic arrest and early after reperfusion. Alternatively, and a more likely explanation is that, the left ventricle has a much greater gradient for efflux than the right ventricle. The fall of taurine in the left ventricle was approximately 6 nmol/mg wet weight (Fig. 2). This would be equivalent to at least 6 mmol/l assuming 80% of the cardiac tissue is water. It has been suggested that for every time the transporter carries one molecule of taurine, it also carries between 1 and 3 Na^+ [29]. Therefore, the fall in taurine would be accompanied by at least 6 mmol/l Na^+ . This is a significant concentration; had it not been removed, it would have caused further Ca^{2+} loading via $\text{Na}^+/\text{Ca}^{2+}$ exchanger. The reason for the right ventricle not showing a significant fall in taurine is likely to be due to the unavailability of sufficient energy in the taurine gradient or that Na accumulation was not high enough to co-transport with taurine. In an earlier study [14], we provided an estimate of the Na^+ electrochemical energy across myocardial sarcolemma at approximately 14.5 kJ mol^{-1} . We proposed that if we assume a stoichiometry of 1:1, then this amount of energy would be enough to maintain a taurine gradient of (~ 10 mmol/l in tissue) and 0.04 mmol/l in plasma [14]. Clearly the elevated levels of taurine (17 nmol/mg wet weight, Table 2) in the left ventricle would have higher energy available which would be used to efflux Na^+ against its concentration

gradient. Like taurine transport, glutamine transport is Na^+ dependent, faster than other amino acids transporters, and is largely responsible for the changes in tissue glutamine during cardiac insults [37, 44]. It is evident that the dilated left ventricle is remodeled to accumulate taurine and glutamine which can be used to efflux Na^+ during ischemia and reperfusion. At the end of reperfusion, the left ventricle has similar levels of amino acids compared to the right ventricle. Whether these acute effects also alter the activity of taurine and glutamine transporters is not known, but as the two ventricles are clearly different in their response, such a possibility is unlikely within the period of cardioplegic arrest.

Ischemia and reperfusion trigger a rise in alanine and a fall in glutamate in both left and right ventricles

In contrast to the changes in taurine and glutamine, which occur only in the left ventricle, there are similar changes in glutamate and alanine during cardioplegic arrest and reperfusion in both ventricles. Glutamate is used during anaerobic energy production, as it is known to enhance anaerobic energy formation [45]. Evidence for its metabolic use is the accumulation of the byproduct alanine. In both left and right ventricles, there is a fall in glutamate and a rise in tissue alanine that is consistent with anaerobic metabolic stress. However, the fact that the fall in glutamate was higher in the left ventricle suggests more substrate-related energy production in the left ventricle. More evidence to support an apparently better left ventricle response to the cardioplegic insult is seen in the relatively lower glutamine/glutamate ratio in the left ventricle compared to the right ventricle (Fig. 4). It has been suggested that a lower ratio would reflect the generation of more energy in the cardiomyocytes in response to an increased energy demand [24]. Whether this left ventricular beneficial metabolic adaptation is also translated into reduced reperfusion injury compared to right ventricle is not presently known and requires further investigation.

Implication for myocardial protection during mitral valve surgery

In this study we have shown that the dilated left ventricle due to mitral regurgitation remodels (adapts) to improve its ability to accumulate taurine. Following cardioplegic arrest and reperfusion, the dilated ventricle (not the normal right ventricle) sustains a loss of taurine (and glutamine). Whether the loss of these amino acids confers better protection possibly by removing ischemia-induced Na^+ loading requires further study involving separate assessment of injury in both ventricles. An important factor that could have influenced the loss of amino acids is the type of cardioplegia used in this study. Custodiol HTK is an intracellular type solution with low Na^+ concentration. This is different from the more popular St. Thomas hyperkalemic extracellular type solution which has high Na^+ concentration. Clearly the Na^+ concentration gradient during cardioplegic arrest is crucial in determining the extent of amino acid transport across the sarcolemma. Therefore, a further study involving the use of a hyperkalemic extracellular type solution is needed to address this issue.

One interesting possibility arising from this study is that the disease of the left ventricle has induced an up-regulation of the taurine transporter activity and expression. If this is the case, then the left and not the right ventricle will benefit from increased availability of extracellular taurine. Therefore, it would be interesting to see whether dietary taurine supplementation to patients with mitral valve disease prior to surgery would increase taurine levels in dilated ventricle. The inclusion of taurine in the cardioplegia is not an option, as this could load heart cells with Na^+ .

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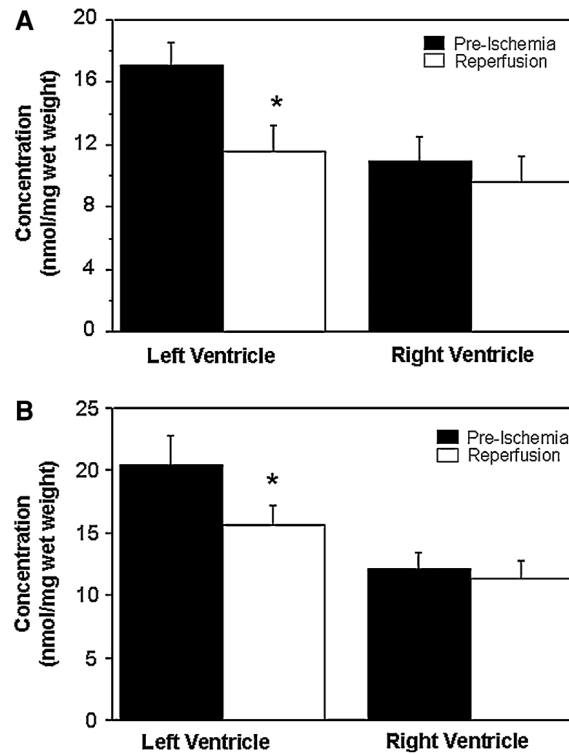


Fig. 1. Changes in myocardial taurine (a) and glutamine (b) during cardioplegic arrest and reperfusion. The concentrations of taurine and glutamine in biopsies collected from both left and right ventricles before cross-clamping the aorta (pre-ischemia) and after 10 min reperfusion. Data are shown as mean \pm SEM ($n = 12$). * P versus pre-ischemia in corresponding ventricle

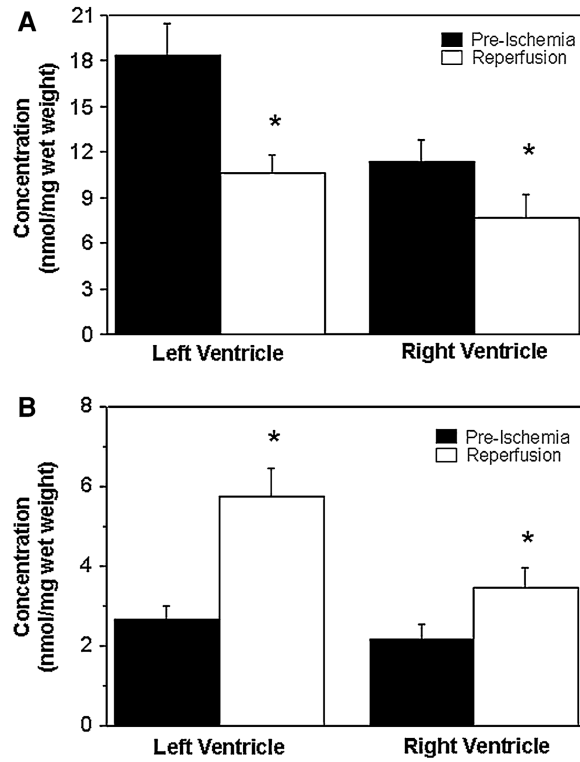


Fig. 2. Changes in myocardial glutamate (a) and alanine (b) during cardioplegic arrest and reperfusion. The concentrations of glutamate and alanine in biopsies collected from both left and right ventricles before cross-clamping the aorta (pre-ischemia) and after 10 min reperfusion. Data are shown as mean \pm SEM ($n = 12$). * P versus pre-ischemia in corresponding ventricle

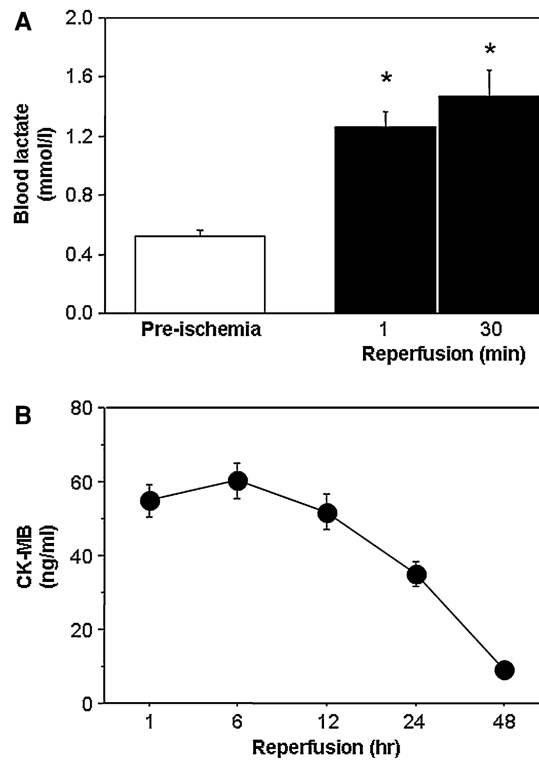


Fig. 3. Ischemic cardioplegic stress (a) and reperfusion injury (b). Lactate release following the release of cross-clamp was used to assess the metabolic ischemic stress early after reperfusion. Reperfusion injury as measured by post-operative release of creatine kinase (CK-MB). Data are shown as mean \pm SEM ($n = 12$). CK-MB values at 1, 6, 12, and 24 h were significantly higher than 48 h. * P versus pre-ischemia in the corresponding ventricle

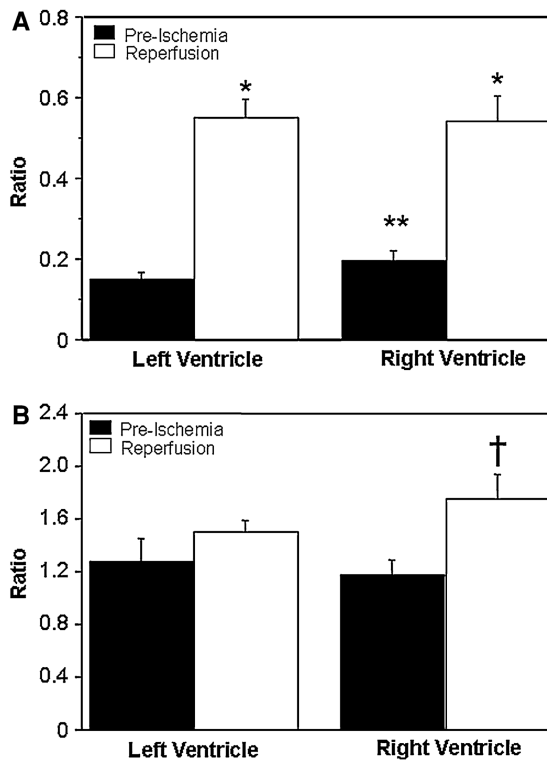


Fig. 4. Markers of myocardial metabolic stress. The alanine/glutamate (**a**) and glutamine/glutamate (**b**) ratios in left and right ventricles calculated before cross-clamping the aorta (pre-ischemia) and after 10 min reperfusion. Data are shown as mean \pm SEM ($n = 12$). * P versus pre-ischemia in the corresponding ventricle. ** P versus pre-ischemia in the left ventricle. † P versus all other values

Table 1

Patients' characteristics

Age (years)	53 ± 3
Sex (male/female)	8/4
NYHA (I/II)	5/7
Systemic hypertension (Yes/No)	5/7
Indexed left ventricular end diastolic volume (ml/m ²)	95 ± 4
Ejection fraction (%)	64 ± 2
BSA (m ²)	1.86 ± 0.05
Degree of MR (3+/4+)	8/4
Cardioplegia infusion time (min)	10.6 ± 0.4
Cardiopulmonary bypass (min)	123 ± 8
Cross-clamp time (min)	107 ± 7

Mean ± SEM

NYHA New York Heart Association, *BSA* body surface area, *MR* mitral regurgitation

Table 2

Basal myocardial amino acid concentration in left and right ventricles

Amino acid	Left ventricle (nmol/mg wet weight)	Right ventricle (nmol/mg wet weight)
Taurine	17.05 ± 1.54	10.90 ± 1.56*
Glutamine	20.49 ± 2.36	12.12 ± 1.23*
Glutamate	18.34 ± 2.17	11.35 ± 1.49*
Aspartate	2.53 ± 0.18	1.95 ± 0.32
Asparagine	0.59 ± 0.09	0.53 ± 0.08
Alanine	2.65 ± 0.35	2.18 ± 0.36
Total free amino acids	61.7 ± 4.2	39.0 ± 4.6*
Total α-amino acids	44.6 ± 3.2	28.1 ± 3.2*

Mean ± SEM

* $P < 0.05$ versus left ventricle