Amino Acids (2003) 24: 141–148
DOI 10.1007/s00726-002-0321-9

Amino
Acids
Printed in Austria

Preservation of amino acids during long term ischemia and subsequent reflow with supplementation of L-arginine, the nitric oxide precursor, in the rat heart

M. Desrois, M. Sciaky, C. Lan, P. J. Cozzone, and **M. Bernard**

Centre de Résonance Magnétique Biologique et Médicale (CRMBM), UMR CNRS 6612, Faculté de Médecine de Marseille, Marseille, France

Received March 3, 2002
Accepted May 16, 2002
Published online October 3, 2002;

Summary. We investigated whether L-arginine, used in heart preservation to limit endothelial damage, may influence the pool of amino acids during long term ischemia and reflow. Isolated isovolumic rat hearts (n = 23) were submitted to 8 h of hypothermic ischemia after cardioplegic arrest with the Centre de Résonance Magnétique Biologique et Médicale (CRMBM) solution with or without L-arginine (Arg and No Arg groups respectively). Hearts were freeze-clamped after ischemia (n = 11) or submitted to 60 min of reflow (n = 12) and freeze-clamped. Eight hearts were perfused aerobically for 20 min and freeze-clamped (No ischemia group). Addition of L-arginine to the CRMBM solution limited aspartate depletion and decreased lysine level at the end of ischemia. After reflow, L-arginine supplementation increased the pool of glutamate and arginine and limited the depletion of serine, asparagine, glycine and taurine. We conclude that adding L-arginine to the CRMBM cardioplegic solution during long term ischemia preserved the amino acids pool.

Keywords: L-arginine – Amino acids – Heart – Nitric oxide – Ischemia – Reperfusion

Introduction

Over the past years, L-arginine has been used as a precursor of NO to prevent endothelial damage (Szabó et al., 1998). Effects on contractility and endothelial function have been analysed. In a previous study, we have shown a protective effect on post-ischemic functional recovery of L-arginine addition to the Centre de Résonance Magnétique Biologique et Médicale (CRMBM) solution that we developed in our laboratory for long term heart preservation (Bernard et al., 1999; Desrois et al., 2000). L-arginine, besides being the precursor of nitric oxide (NO), as an amino acid is also a constituent of proteins and may participate in amino acid metabolism (Rau et al., 1979).

Importance of amino acids in ischemia-reperfusion has been shown in human hearts and in animal heart experimental models (Pisarenko et al., 1988). A loss of intracellular concentrations of taurine, glutamine (Suleiman et al., 1997b), aspartate and glutamate (Suleiman et al., 1997a) has been observed in the hearts of patients during coronary artery bypass surgery following ischemia and reperfusion and these variations have been related to the functional recovery of the heart following surgery (Pisarenko et al., 1989; Svedjeholm et al., 1996). On the other hand, it has been observed that some amino acids are able to attenuate ischemic and reperfusion injury of the heart. These protective amino acids are mainly taurine, glutamate, aspartate, glutamine, branched-chain amino-acids, methionine, cysteine and histidine (Pisarenko, 1996). The proposed mechanisms of action are closely linked to effects upon intermediary metabolism (aspartate, glutamate, BCAA (Arsenian, 1998; Schwalb et al., 1989)), ion homeostasis and myocardial performance (taurine (Zemgulis et al., 2001), histidine (Takeuchi et al., 1995), BCAA (Schwalb et al., 1989), glutamine (Khogali et al., 1998)), membrane integrity (Tau (Raschke et al., 1995), glutamine (Hinshaw et al., 1990)), scavenging of oxygen-derived free radicals (histidine, methionine, cysteine (Pisarenko, 1996)), and the biosynthesis of amino acids (glutamine

(Nelson et al., 1994)), purine (glutamine, glycine, aspartate) and pyrimidine (aspartate, glutamine) nucleotides.

To assess if L-arginine used as a NO substrate influences the pool of amino acids during ischemia and reflow, we have evaluated in the isolated perfused rat heart model the effect of providing L-arginine during a long term hypothermic ischemia followed by reflow on intracellular amino acids. Changes in phosphocreatine (PCr), adenine nucleotide and purine concentrations in tissues were also monitored as amino acids pool is linked to high energy phosphate and adenine nucleotide pool (Rau et al., 1979) which is known to be affected by ischemia and reperfusion.

Material and methods

Perfused heart preparation and functional parameters

Hearts (n = 31) were quickly removed from intraperitoneal pentobarbital anaesthetized male Sprague Dawley rats (350–400 g) and perfused at a constant temperature of 37°C and a constant pressure of 100 mmHg. The perfusion buffer was a phosphate-free Krebs-Henseleit bicarbonate buffer (pH = 7.4) which had the following composition (mM): NaCl (118), KCl (4.7), $CaCl_2$ (1.75), $MgSO_4$ (1.2), ethylenediaminetetraacetate tetrasodium (0.5), $NaHCO_3$ (25) and glucose (11) and was gassed with a mixture of 95% oxygen and 5% carbon dioxide. Left ventricular function was continuously monitored by a pressure transducer connected to a fluid filled latex balloon inserted into the left ventricle. End diastolic pressure was set at 10 mm Hg (Desrois et al., 2000). All animals received humane care in compliance with the European Convention on Animal Care. All investigations in this project were conducted under a license for animal research granted by the French Ministry of Agriculture.

Experimental protocol

(i) Isolated rat hearts (No ischemia group, n = 8) were perfused as previously described for 20 min with phosphate-free bicarbonate Krebs-Henseleit buffer, then freeze-clamped with a Wollenberger clamp precooled in liquid nitrogen. (ii) In a second control group, isolated rat hearts (No Arg group, n = 12) were perfused as previously, then arrested with the crystalloid cardioplegic CRMBM solution (Bernard et al., 1999) and submitted to 8 h of cold storage (4°C) in the same solution; 6 hearts were freeze-clamped at the end of ischemia and 6 hearts were reperfused for 60 min at 37°C before freeze-clamping. (iii) A third group of rat hearts (Arg group, n = 11) was submitted to the protocol described above but with the addition of L-arginine (L-arg, 2 mM) to the CRMBM cardioplegic solution; 5 hearts were freeze-clamped at the end of ischemia and 6 hearts were reperfused for 60 min at 37°C before freeze-clamping. The CRMBM cardioplegic solution had the following composition (mM): KCl (2), $CaCl_2$ (0.25), NaOH (120), KH_2PO_4 (2), $MgCl_2$ (13), lactobionic acid (100), raffinose (30), glutathione (3), allopurinol (1), glutamate (20), mannitol (30) and adenosine (0.5); osmolarity was 340 mOsm/L and pH was 7.4.

Biochemical assays

Analyses of amino acids, adenine nucleotides, PCr, purines, water content and CK and LDH activities were performed before ischemia (No ischemia group), after 8 h of ischemia and at the end of 60 min of reflow with (Arg group) or without (No Arg group) L-arginine in the CRMBM cardioplegic solution.

Determination of amino acid content in freeze-clamped hearts by ion-exchange high performance liquid chromatography (HPLC)

Aqueous extraction of free amino acids was conducted as described by Suleiman and Chapman (1993). Pieces (about 100 mg) of freeze-clamped hearts were immediately homogenized in a glass homogenizer (Glas Col, Terre Haute, Indiana, USA). After rinsing the Potter tube with 1 ml of water, an aliquot of the homogenate was taken for protein determination (Lowry et al., 1951). The remaining suspension was centrifuged for 10 min at 1,000 g at 4°C in a Jouan E96S refrigerated centrifuge (Jouan SA Saint-Herblain, France). Then, 0.5 ml of the supernatant was deproteinized by ultracentrifugation at 2,000 g, using Millipore ultrafiltration units and a Sorvall RC2-B refrigerated Superspeed centrifuge (Du Pont, Wilmington, DE, USA), for 60 min or more to obtain the desired volume of filtrate (200 μl). Aminoacids in the deproteinized extracts were derivatized using phenyl isothiocyanate (PITC) and a vacuum Station Waters Pico-Tag and then determined according to the Waters Pico-Tag (Millipore, Milford MA, USA) method. The phenylthiocarbamyl-derivatized aminoacids were separated by HPLC and detected at 254 nm using a 30 cm Waters Pico-Tag column, a Merck-Hitachi L-6200A pump (Merck, Darmstadt, Germany) equipped with a Rheodyne valve, a Merck-Hitachi L-7400 UV-visible detector and a regulation system of the temperature of the Waters column maintaining the temperature at 46°C. Qualitative and quantitative analyses were carried out using amino acid standards and the α-aminoadipic acid (Sigma, Poole, Dorset, UK) as an internal standard. The visible amino acids in the chromatogram were aspartate (Asp), glutamate (Glu), serine (Ser), asparagine (Asn), glycine (Gly), glutamine (Gln), taurine (Tau), histidine (His), alanine (Ala), arginine (Arg) and lysine (Lys). Citrulline (Cit), produced from L-arginine by the action of nitric oxide synthase, was not quantifiable in our experiments because it was mixed with ammonia resulting from the procedure of derivatization. The acquired data were processed using the PC integration Pack Kontron software (Kontron, Milan, Italy). Amino acids concentrations were expressed as μmoles per g of protein. Tau level was divided by 3 because of the high concentration of this compound compared to the other metabolites in the myocardial tissue.

Determination of adenine nucleotides and derivatives, PCr and purines in freeze-clamped hearts by ion-exchange high performance liquid chromatography (HPLC)

The perchloric acid extraction was adapted from Lazzarino et al. (1989). Lyophilised freeze-clamped cardiac tissues (50 to 100 mg) were immediately homogenized with ice-cold 0.6 M perchloric acid in a glass homogenizer. After 15 min, the homogenate was centrifuged for 10 min (5,000 g) at 4°C in a Sorvall RC2-B refrigerated Superspeed centrifuge. The pellet was reextracted with 0.2 ml of 0.6 M perchloric acid and the two supernatants were combined and neutralized. The neutralized tissue extracts were centrifuged as described above and the supernatants were saved for the comparative metabolite determinations. Protein calculation was carried out in the pellet dissolved into 1 ml of NaOH (1 N) according to Lowry et al. (1951) in order to express results in μmoles per g of protein. Separation of adenine nucleotide derivatives was performed by the ion-pairing reverse phase technique of Lazzarino et al. (1991). Qualitative and quantitative analyses were carried out using adenine nucleotide standards and thymidine monophosphate (Sigma, Poole, Dorset, UK) as an internal standard. The HPLC system

consisted of the same pump and detector as for the determination of amino acids. Under these chromatographic conditions, a highly resolved separation of MDA, ATP, ADP, AMP, GDP, NAD, PCr, adenosine, ascorbate, inosine, hypoxanthine, xanthine, uric acid, ADPribose, and NADP was obtained in 40 min. Total adenine nucleotides (TAN) was calculated from the sum ATP+ADP+AMP.

Analyses of CK and LDH activities and water content in freeze-clamped hearts

Portions of hearts (20 to 50 mg) were homogenized in a potassium phosphate buffer. Aliquots were removed for measurement of proteins according to the method of Lowry et al. (1951). Creatine kinase activity was measured according to Rosalki (1967) at 30°C with a Sigma kit (n°47). Lactate dehydrogenase activity was measured by the method of Bernstein and Everse (1975). Creatine kinase and lactate dehydrogenase activities were expressed in international units per mg of protein. Another portion of heart was thawed, blotted and dried in an oven for 48 h at 50°C. Water content was expressed in percent (wet weight-dry weight/wet weight). Enzymatic activities were measured with a UVIKON 930 UV-visible spectrophotometer (Kontron, Milan, Italy).

Statistical analysis

Data are expressed as means ± SEM. The effect of time within a group was evaluated with one way ANOVA. At the end of ischemia and reflow, differences between groups were analyzed with a two way ANOVA (group and time). The statistical software was the SAS/ETS system with Tukey post-hoc test and a p value of less than 0.05 was considered as significant.

Results

Data were obtained before ischemia (No ischemia group), at the end of ischemia and after reflow with (Arg group) or without (No Arg group) L-arginine in the CRMBM cardioplegic solution.

Myocardial function expressed as the rate pressure product was not significantly different before ischemia in both groups with 35480 ± 5858 mm Hg.min^{-1} for the No Arg group and 31073 ± 3615 mm Hg.min^{-1} for the Arg group. Post-ischemic functional recovery combined with a ^{31}P magnetic resonance study and a measure of the endothelial function has been detailed previously in a similar experimental protocol (Desrois et al., 2000).

Content of amino acids

A comparison of taurine and main α amino acids before ischemia and at the end of ischemia with or without L-arginine is shown in Fig. 1A. Tau content was divided by 3 because of the high concentration of this compound compared to the other metabolites in this figure and reached (μmol/g protein) 149.1 ± 7 for the No ischemia, 98 ± 8.3 for No Arg and 111 ± 11.3 for Arg groups at the end of ischemia. Ischemic arrest with the CRMBM cardioplegic solution induced a significant decrease in glutamine and taurine in both groups and aspartate in the No Arg group only. Alanine increased in the two groups. Lysine level was lower in the Arg group compared with the No Arg group. The glutamate and arginine concentrations measured at the end of ischemia contain contributions from the same amino acids present in the cardioplegic solution remaining in the extracellular space and the increase could not be quantified. Ischaemic arrest also induced a significant decrease in serine and glycine with no change in asparagine concentration in both groups.

Variations in amino acid content at the end of reflow for both groups compared to preischemic values are presented in Fig. 1B. As previously described, Tau was divided by 3 and reached (μmol/g protein) 54.2 ± 5.3 for the No Arg group and 73.2 ± 4.5 for the Arg group at the end of reflow. Decreases in aspartate, glutamine and taurine were more pronounced than at the end of ischemia in No Arg and Arg groups. Alanine returned to the preischemic level in both groups. Asparagine was significantly lower in the two groups. Serine and glycine continued to decrease in both groups. In the Arg group only, glutamate, arginine, and lysine remained higher than the preischemic value. The pool of glutamate, serine, asparagine, glycine, taurine and arginine was significantly higher in the Arg group compared to the No Arg group at the end of reflow.

Content of adenine nucleotides, PCr and purines

The comparison of adenine nucleotides, PCr and purines contents before ischemia and at the end of ischemia in No Arg and Arg groups is presented in Fig. 2A. At the end of ischemia, there were no differences between the two groups in the fall of high-energy phosphate compounds (ATP and PCr), the reduction in total adenine nucleotides (TAN) and the accumulation of end product degradation of adenine nucleotides (AMP and purines). No change in ADP was observed at the end of ischemia in both groups.

At the end of reflow (Fig. 2B), ATP and PCr increased but remained under the preischemic values, AMP and purines returned to basal values in both groups and supplementation of the CRMBM

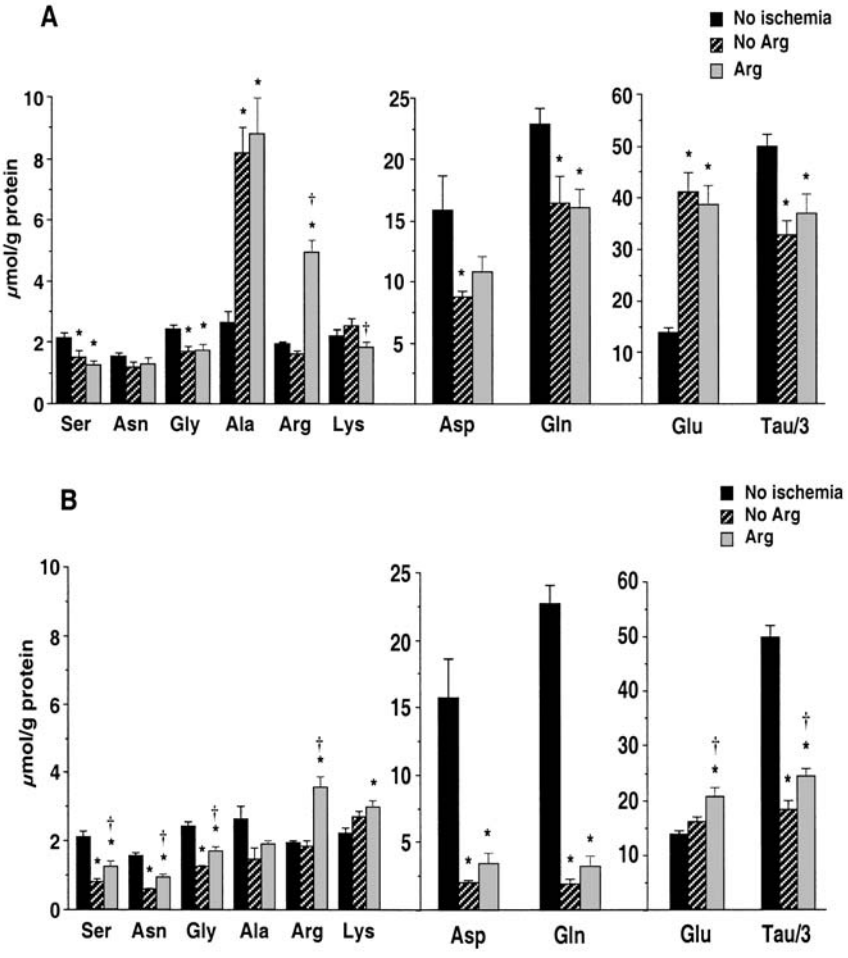

Fig. 1. **A** Content of amino acids in freeze-clamped hearts before ischemia (*No ischemia*) and at the end of 8 h of ischemia with (*Arg*) or without (*No Arg*) L-arginine. **B** Content of aminoacids in freeze-clamped hearts before ischemia and at the end of reflow in Arg and No Arg groups. Results are expressed as μmol/g protein and are means ± SEM. * vs No ischemia, $p < 0.05$. † vs Cont, $p < 0.05$. Tau concentration was expressed as divided by 3

cardioplegic solution with L-arginine did not affect the pool of metabolites.

CK and LDH activities and water content

After 60 min of reflow, a loss of CK was observed in the No Arg group (8.8 ± 0.5 IU/mg protein) compared with the preischemic value (10.3 ± 0.3 IU/mg protein) ($p = 0.019$) while no difference was observed in Arg group (9.2 ± 0.3 IU/mg protein) compared to the preischemic value ($p = 0.097$); nevertheless there was no difference between No Arg and Arg groups at the end of reflow ($p = 0.515$).

LDH activity was not significantly different in both groups (3.04 ± 0.17 IU/mg protein for the No Arg group and 3.21 ± 0.12 IU/mg protein for the Arg group) compared to the preischemic value (3.51 ± 0.22 IU/mg protein) at the end of reflow. There was no significant difference for LDH activity between the two groups at the end of reflow.

At the end of reflow, an increase in water tissue content was observed compared with the preischemic value (82.2 ± 0.04%) without significant differences between groups (85.1 ± 0.03% and 85.01 ± 0.04% for the No Arg and Arg groups, respectively).

Discussion

The cardioprotective effect of L-arginine is generally associated with the stimulation of NO production by increasing substrate availability as decreased synthesis of NO has been involved in the development of post-ischemic abnormalities of endothelial function (Engelman et al., 1995). L-arginine as an amino acid could also limit the depletion in amino acids associated to ischemia-reperfusion (Rau et al., 1979).

We show here that long term ischemic arrest with a preservation solution was associated with a fall in the intracellular concentrations of several amino acids and in particular glutamine and taurine as shown also

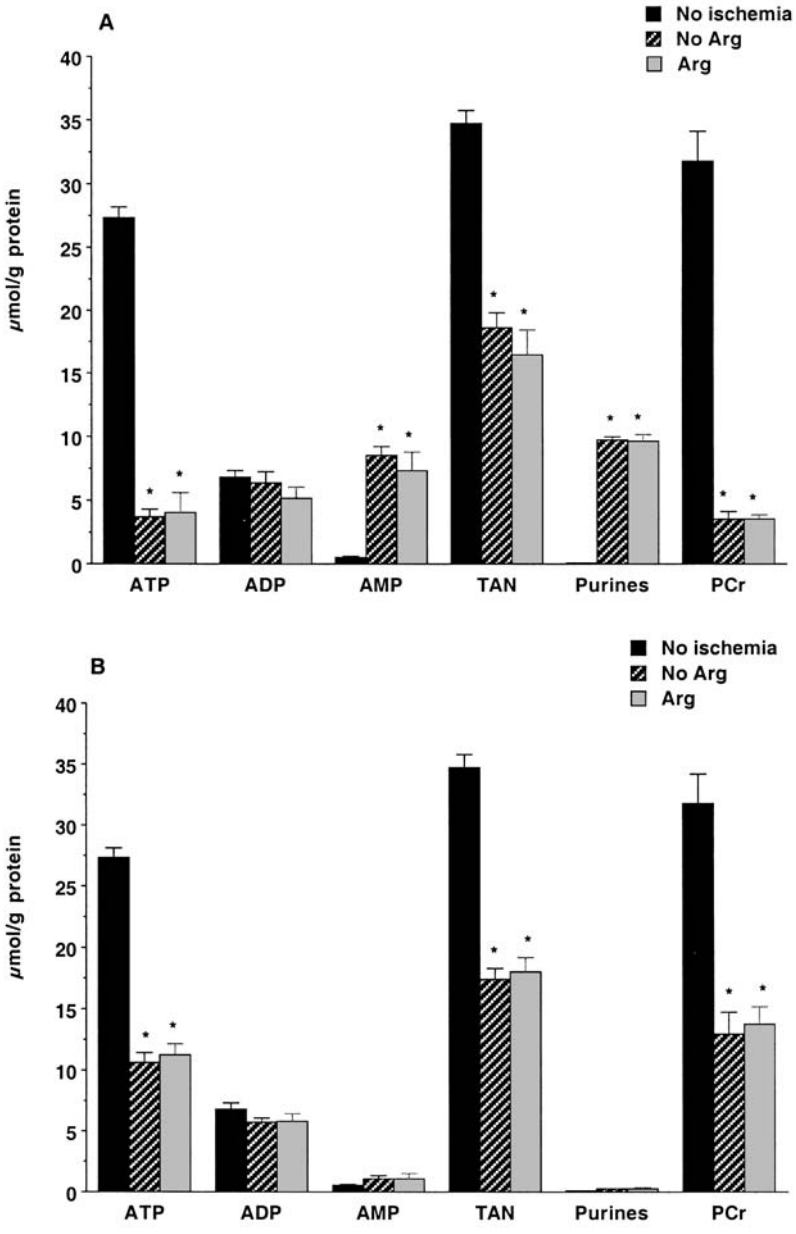

Fig. 2. **A** Content of adenine nucleotides, PCr and purines in freeze-clamped hearts before ischemia (*No ischemia*) and at the end of 8 h of ischemia with (*Arg*) or without (*No Arg*) L-arginine. **B** Content of adenine nucleotides, PCr and purines in freeze-clamped hearts before ischemia and at the end of reflow in Arg and No Arg groups. Results are expressed as μmol/g of protein and are means ± SEM. TAN, total adenine nucleotides (ATP+ ADP+AMP). * vs No ischemia, $p < 0.05$

with short ischemic times by others (Suleiman et al., 1997b). The fall in taurine has been related to transport processes (Suleiman et al., 1997b). Data from isolated myocytes and perfused guinea pig hearts were consistent with the presence of a Na^+/taurine symport which would be activated to cause efflux of sodium and taurine when the concentration of either one rises above its physiological level (Suleiman et al., 1992). Changes in the intracellular concentrations of glutamine are the results of both the metabolism and transport of this aminoacid. Glutamine may be converted to glutamate since there is a high glutaminase activity in the heart (Nelson et al., 1994). In addition, the transport of glutamine may also contribute to the observed fall in this amino acid (Rennie et al., 1996) as it exists a glutamine-Na^+ symport which is able to reverse during ischemia when Na^+ increases and then contributes to take out Na^+ and glutamine. It has been well established that during ischemia or hypoxia, there is a consumption of aspartate and glutamate (Pisarenko et al., 1987). Metabolism of both amino acids has been shown to be coupled to anaerobic energy formation in mitochondria and stimulation of anaerobic glycolysis (Pisarenko, 1996). Consistent

with these observations, the level of aspartate decreased at the end of ischemia in the No Arg group. A decrease in glutamate is not observed due to the presence of glutamate in the extracellular space as the cardioplegic and preservation solution contains this amino acid. In line with glutamate and aspartate variations, alanine increased through transaminase reactions. The loss of serine and glycine has been previously related to proteolysis (Kennergren et al., 1999).

Changes in amino acids were also observed during ischemia when L-arginine was added to the CRMBM cardioplegic solution but with some differences compared to No Arg group. To explain the protective effect of L-isomers of arginine, glutamate, ornithine and aspartate during and following anoxic and ischemic stresses, Rau et al. (1979) had suggested the involvement of L-arginine as a substrate of the transamination steps of the malate-aspartate shuttle with subsequent stimulation of anaerobic mitochondrial metabolism of glutamate and aspartate to succinate. In agreement with this hypothesis, aspartate level did not fall significantly during ischemia in the Arg group while it decreased in the No Arg group. The decrease in lysine in the Arg group is puzzling: lysine is catabolized to acetyl-CoA but it is unknown why it would proceed further in presence of L-arginine. The increase in arginine level measured at the end of ischemia contains contributions from the same amino acid present in the cardioplegic solution remaining in the extracellular space and the increase could not be quantified.

After reperfusion in the No arg group, aspartate continued to decrease compared to the end of ischemia and interestingly, glutamate level was not depleted compared to No ischemia group. Aspartate and glutamate may participate to mechanisms which facilitate the recovery of oxidative metabolism during reperfusion/reoxygenation: (i) replenishment of tricarboxylic cycle intermediates (ii) utilization of succinate formed during ischemia/hypoxia (iii) recycling of ammonia excess in glutamine and asparagine synthesis and (iv) maintenance of ionic homeostasis (Pisarenko, 1996). We had shown in a previous study that the CRMBM solution containing glutamate limited the depletion of amino acids compared to other solutions after 12 h of ischemia followed by 60 minutes of reflow (Bernard et al., 1999). We show here that after 8 h of ischemia glutamate level after reflow does not differ from the preischemic value with the native CRMBM cardioplegic solution. Alanine returned to the preischemic value as pyruvate can then be converted into acetylCoA which integrates into the TCA cycle in order to produce energy. Taurine and glutamine decreased severely at least in part because of participation of these amino acids in Na^+ extrusion (Suleiman et al., 1997b) or in regulatory mechanisms involved to reduce cell swelling (Song et al., 1998). The loss of asparagine may be the net result of changes in metabolism, transport and in rate of protein and nucleotide synthesis and degradation (Suleiman et al., 1995; Suleiman et al., 1996). The fall in asparagine has been related to Na^+ extrusion (Suleiman et al., 1995) similarly to glutamine decrease because the two amino acids can be carried on the glutamine transporter (Kilberg et al., 1980). During reflow, decrease in serine and glycine may be due to the volume regulatory mechanisms aiming to avoid cell swelling (Song et al., 1998).

As concern the Arg group after reflow, there was an increase in glutamate compared to No Arg group but also to No ischemia group and a better preservation of taurine and most of amino acids. The increase in glutamate is consistent with a contribution of L-arginine to the intermediary metabolism which should favor energy metabolism (Rau et al., 1979). Better preservation of taurine has several important implications related to its role in membrane stabilization, calcium mobilization and maintenance of normal cellular function (Zemgulis et al., 2001). In Arg group only, lysine was increased compared with preischemic value. We hypothetized that, as L-arginine and lysine share the same plasma-membrane transport mechanism called system y^+ (White, 1985), L-arginine may competitively inhibit lysine transport as it has been described in vascular smooth muscle cells (Escobales et al., 2000). More generally in Arg group, there is a higher amino acids pool which has been associated with higher functional and metabolic recovery (Khogali et al., 1998; Suleiman et al., 1997b; Svedjeholm et al., 1996). In the long term, a larger amino acids pool will favor the resynthesis of nucleotides.

The effect of L-arginine on the pool of amino acids may be due to direct action but also to indirect action related to a NO-mediated protective process. NO has various physiologic properties including vasodilatation, inhibition of platelet aggregation and neutrophil adhesion, and reduction of free-radical-mediated injury (Kelly et al., 1996). We hypothetized that NO supplementation by L-arginine provided also pro-

tection against cellular injury and limited the loss of amino acids induced by ischemia and reperfusion. As a matter of fact, a CK loss in myocardial tissue at the end of reflow was only observed in No Arg group compared with the preischemic value and shows a fall in the cellular integrity.

The rationale for giving L-arginine as a NO precursor was in particular based on the hypothesis of a local L-arginine depletion around cNOS (Huk et al., 1997). We measured here the total L-arginine pool which was not significantly depleted in the No Arg group but providing L-arginine during ischemia increased the pool in this amino acid even after 60 min of reflow.

There were no differences in the pool of adenine nucleotides and PCr with or without L-arginine in the CRMBM cardioplegic solution during ischemia and reflow. We have shown in a previous study that functional recovery was enhanced during L-arginine supplementation (Desrois et al., 2000). The fact that this was not associated with a depletion in high energy phosphates during reflow, as shown in this study, is representative by itself of a more efficient energy metabolism. Possible mechanisms involve better energy/contraction coupling and/or better recovery of oxidative phosphorylation related to improved replenishment of tricarboxylic cycle intermediates due to preservation of amino acid pool.

This study shows that providing L-arginine in the CRMBM cardioplegic solution during hypothermic ischemia preserves the aminoacid pool. In addition to NO-mediated effects, protective role of L-arginine may be due to the contribution of this amino acid to intermediary metabolism and/or to the limitation of the lost of amino acids which play an important role in metabolic activity and protein synthesis.

Acknowledgements

This work was supported by the "Ministère de la Recherche et de la Technologie" (grant n° 91 C 0959), CNRS (UMR 6612), Assistance Publique-Hôpitaux de Marseille and Programme Hospitalier de Recherche Clinique (PHRC).

References

Arsenian M (1998) Potential cardiovascular applications of glutamate, aspartate and other amino acids. Clin Cardiol 21: 620–624

Bernard M, Caus T, Sciaky M, Lan C, Cozzone PJ (1999) Optimized cardiac graft preservation: a comparative experimental study using P-31 magnetic resonance spectroscopy and biochemical analyses. J Heart Lung Transplant 18: 572–581

Bernstein LK, Everse J (1975) Determination of the isoenzyme levels of lactate dehydrogenase. Methods Enzymol 41: 47–52

Desrois M, Sciaky M, Lan C, Cozzone PJ, Bernard M (2000) L-arginine during long-term ischemia: effects on cardiac function, energetic metabolism and endothelial damage. J Heart Lung Transplant 19: 367–376

Engelman DT, Watanabe M, Engelman RM, Rousou JA, Flack JE, Deaton DW, Das DK (1995) Constitutive nitric oxide release is impaired after ischemia and reperfusion. J Thorac Cardiovasc Surg 110: 1047–1053

Escobales N, Rivera-Correa M, Altieri PI, Rodriguez JF (2000) Relationship between NO synthesis, arginine transport, and intracellular arginine levels in vascular smooth muscle cells. Amino Acids 19: 451–468

Hinshaw DB, Burger JM, Delius RE, Hyslop PA (1990) Mechanism of protection of oxidant-injured endothelial cells by glutamine. Surgery 108: 298–305

Huk I, Nanobashvili J, Neumayer C, Punz A, Mueller M, Afkhampour K, Mittlboeck M, Losert U, Polterauer P, Roth E, Patton S, Malinski T (1997) L-arginine treatment alters the kinetics of nitric oxide and superoxide release and reduces ischemia/reperfusion injury in skeletal muscle. Circulation 96: 667–675

Kelly RA, Balligand J-L, Smith TW (1996) Nitric oxide and cardiac function. Circ Res 79: 363–380

Kennergren C, Mantovani V, Lönnroth P, Nyström B, Berglin E, Hamberger A (1999) Extracellular amino acids as markers of myocardial ischemia during cardioplegic heart arrest. Cardiology 91: 31–40

Khogali SEO, Harper AA, Lyall JA, Rennie MJ (1998) Effects of L-glutamine on post-ischaemic cardiac function: protection and rescue. J Mol Cell Cardiol 30: 819–827

Kilberg MS, Handlogten ME, Christensen HN (1980) Characteristics of an amino acid transport system in rat liver for glutamine, asparagine, histidine, and closely related analogs. J Biol Chem 255: 4011–4019

Lazzarino G, Nuutinen M, Tavazzi B, Di Piero D, Giardina B (1989) A method for preparing freeze-clamped tissue samples for metabolite analyses. Anal Biochem 181: 239–241

Lazzarino G, Pierro DD, Tavazzi B, Cerroni L, Giardina B (1991) Simultaneous separation of malondialdehyde, ascorbic acid, and adenine nucleotide derivatives from biological samples by ion-pairing high-performance liquid chromatography. Anal Biochem 197: 191–196

Lowry OH, Rosebrough NJ, Farr A, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275

Nelson D, Rumsey WL, Erecinska M (1994) Glutamine catabolism by heart muscle: regulation of phosphate-activated glutaminase by ATP, citrate and chloride. Arch Biochem Biophys 314: 376–383

Pisarenko OI (1996) Mechanisms of myocardial protection by amino acids: facts and hypotheses. Clin Exp Pharmacol Physiol 23: 627–633

Pisarenko OI, Studneva IM, Khlopkov VN (1987) Metabolism of the tricarboxylic acid cycle intermediates and related amino acids in ischemic guinea pig heart. Biomed Biochim Acta 46: 141–145

Pisarenko O, Studneva I, Khlopkov V, Solomatina E, Ruuge E (1988) An assessment of anaerobic metabolism during ischemia and reperfusion in isolated guinea pig heart. Biochim Biophys Acta 934: 55–63

Pisarenko OI, Oleynikov OD, Shulzhenko VS, Studneva IM, Ryff IM, Kapelko VI (1989) Association of myocardial glutamate and aspartate pool and functional recovery of postischemic heart. Biochem Med Metab Biol 42: 105–117

Raschke P, Massoudi P, Becker BF (1995) Taurine protects the heart from neutrophil-induced reperfusion injury. Free Radic Biol Med 19: 461–471

Rau EE, Shine KI, Gervais A, Douglas AM, Amos EC (1979) Enhanced mechanical recovery of anoxic and ischemic myocardium by amino acid perfusion. Am J Physiol 236: H873–H879

Rennie MJ, Ahmed A, Khogali SE, Low SY, Hundal HS, Taylor PM (1996) Glutamine metabolism and transport in skeletal muscle and heart and their clinical relevance. J Nutr 126: 1142S–1149S

Rosalki SB (1967) An improved procedure for serum creatine phosphokinase determination. J Lab Clin Med 69: 696–705

Schwalb H, Izhar U, Yaroslavsky E, Borman JB, Uretzky G (1989) The effect of aminoacids on the ischemic heart, improvement of oxygenated crystalloid cardioplegic solution by an enriched branched chain aminoacid formulation. J Thorac Cardiovasc Surg 98: 551–556

Song D, O'Regan MH, Phillis JW (1998) Amino acid release during volume regulation by cardiac cells: cellular mechanisms. Eur J Pharmacol 341: 273–280

Suleiman M-S, Chapman RA (1993) Changes in the principal free intracellular amino acids in the Langendorff perfused guinea pig heart during arrest with calcium-free or high potassium media. Cardiovasc Res 27: 1810–1814

Suleiman M-S, Rodrigo GC, Chapman RA (1992) Interdependence of intracellular taurine and sodium in guinea pig heart. Cardiovasc Res 26: 897–905

Suleiman M-S, Wallace D, Birkett S, Angelini GD (1995) Changes in intracellular concentration of amino acids in human saphenous vein during preparation for coronary artery bypass grafting. Cardiovasc Res 30: 747–754

Suleiman M-S, Wallace D, Birkett S, Angelini GD (1996) Changes in the intracellular free amino acid pool in human saphenous vein tissue culture. Exp Physiol 81: 435–446

Suleiman M-S, Dihmis WC, Caputo M, Angelini GD, Bryan AJ (1997a) Changes in myocardial concentration of glutamate and aspartate during coronary artery surgery. Am J Physiol 272: H1063–H1069

Suleiman M-S, Moffatt AC, Dihmis WC, Caputo M, Hutter JA, Angelini GD, Bryan AJ (1997b) Effect of ischemia and reperfusion on the intracellular concentration of taurine and glutamine in the hearts of patients undergoing coronary artery surgery. Biochim Biophys Acta 1324: 223–231

Svedjeholm R, Vanhanen I, Håkanson E, Joachimsson PO, Jorfeldt L, Nilsson L (1996) Metabolic and hemodynamic effects of intravenous glutamate infusion early after coronary operations. J Thorac Cardiovasc Surg 112: 1468–1477

Szabó G, Bährle S, Bátkai S, Stumpf N, Dengler TJ, Zimmermann R, Vahl CF, Hagl S (1998) L-arginine: effect on reperfusion injury after heart transplantation. World J Surg 22: 791–798

Takeuchi K, Buenaventura P, Cao-Danh H, Glynn P, Simplaceanu E, McGowan FX, Del-Nido PJ (1995) Improved protection of the hypertrophied left ventricle by histidine-containing cardioplegia. Circulation 92: II395–II399

White M (1985) The transport of cationic amino acids across the plasma membrane of mammalian cells. Biochem Biophys Acta 822: 355–375

Zemgulis V, Wikström G, Henze A, Waldenström A, Thelin S, Ronquist G (2001) Nucleoside transport inhibition in ischemic myocardium results in enhanced taurine efflux. Eur J Pharmacol 411: 143–154

Authors' address: Monique Bernard, Ph.D., Centre de Résonance Magnétique Biologique et Médicale (CRMBM), UMR CNRS 6612, Faculté de Médecine, 27 Bd Jean Moulin, 13005 Marseille, France, Fax: 33 (4) 91 25 65 39,
E-mail: monique.bernard@medecine.univ-mrs.fr