L-Arginine administration prevents reperfusion-induced cardiomyocyte hypercontracture and reduces infarct size in the pig

Ferran Padilla, David Garcia-Dorado*, Luis Agulló, Javier Inserte, Amaya Paniagua, Sonia Mirabet, José A. Barrabés, Marisol Ruiz-Meana, Jordi Soler-Soler

Department of Cardiology, Hospital General Universitario Vall d’Hebron, Pg. Vall d’Hebron 119-129, Barcelona 08035, Spain

Received 12 October 1999; accepted 9 February 2000

Abstract

Objective: Stimulation of cGMP synthesis protects cardiomyocytes against reoxygenation-induced hypercontracture. The purpose of this study was to determine whether L-arginine supplementation has a protective effect against reperfusion-induced hypercontracture and necrosis in the intact animal.

Methods: Twenty-four Large-White pigs were randomized to receive either 100 mg/kg of L-arginine IV or vehicle 10 min before 48 min of coronary occlusion and 2 h of reperfusion. Hemodynamic variables, coronary blood flow and myocardial segment length changes (piezoelectric crystals) were monitored. Postmortem studies included quantification of myocardium at risk (in vivo fluorescein), infarct size (triphenyltetrazolium reaction), myocardial myeloperoxidase activity and histological analysis. Systemic, coronary vein, and myocardial cGMP concentration were measured in additional animals.

Results: Administration of L-arginine had no significant effect in hemodynamics or coronary blood flow. During reperfusion, myocardial cGMP content was reduced in the LAD as compared to control myocardium (P<0.02). L-Arginine increased myocardial cGMP content and caused a transient increase in plasma cGMP concentration during the initial minutes of reperfusion (P<0.02). The reduction in end-diastolic segment length induced by reperfusion, reflecting hypercontracture, was less pronounced in the L-arginine group (P<0.02). Infarct size was smaller in pigs receiving L-arginine (47.9±7.2% of the area at risk) than in controls (62.9±4.9%, P=0.047). There were no differences between groups in leukocyte accumulation in reperfused myocardium (P=0.80). Conclusion: L-Arginine supplementation reduces myocardial necrosis secondary to in situ ischemia–reperfusion by a direct protective effect against myocyte hypercontracture. © 2000 Published by Elsevier Science B.V. All rights reserved.

Keywords: Coronary disease; Infarction; Ischemia; Necrosis; Nitric oxide; Reperfusion

1. Introduction

Myocyte hypercontracture is a major mechanism of cell death during myocardial reperfusion [1]. Hypercontracture is the result of an excessive contractile activation during the first minutes of reperfusion caused by restoration of ATP synthesis in the presence of elevated cytosolic Ca\(^{2+}\) concentration [1,2]. Transient contractile blockade during the first minutes of reperfusion, until the cell recovers Ca\(^{2+}\) control, has been consistently shown to prevent hypercontracture and to reduce necrosis induced by transient ischemia [3,4]. However, there is a lack of contractile inhibitors acceptable to be safely administered to patients.

Previous studies have shown that myocardial cGMP content is reduced during reperfusion following prolonged ischemia [5,6]. Increasing cellular cGMP concentration protects isolated cardiomyocytes from reoxygenation-induced hypercontracture [6–8]. In the isolated rat heart, increasing myocardial cGMP concentration during reperfusion has a powerful negative inotropic effect not observed in previously nonischemic myocardium, attenuates hypercontracture, and limits necrosis [7].

We have recently described that L-arginine supple-

*Corresponding author. Tel.: +34-93-489-4038; fax: +34-93-489-4032.
E-mail address: dgdorado@hg.vhebron.es (D. Garcia-Dorado)
ment limits myocardial necrosis in the isolated rat heart submitted to anoxia-reoxygenation by a cGMP-dependent mechanism [8]. Although previous studies in the isolated rat heart are in general consistent with the hypothesis that L-arginine has a protective effect against myocardial ischemia–reperfusion injury [9–11], the results of in vivo studies are controversial. When these results have been positive, they have been associated with reduced polymorphonuclear leukocyte (PMN) accumulation [12–14]. The present study tested the hypothesis that L-arginine supplementation, at doses used in patients [15], protects myocardium against necrosis induced by transient coronary occlusion by preventing cardiomyocyte hypercontracture occurring during the early phase of reperfusion. For this purpose, a pig model of transient coronary occlusion was used.

2. Methods

Animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health, and all experimental procedures were approved by the Research Commission of the Hospital General Vall d’Hebron.

Twenty-four Large White pigs (16 males) with an average age of 15 weeks (40.9 ± 1.5 kg) were premedicated with 10 mg/kg azapenone IM, anesthetized with thiopental 30 mg/kg IV, intubated and mechanically ventilated with room air. A continuous infusion of thiopental was used to maintain anesthesia. The right femoral artery was catheterized with a 5F introducer and a catheter advanced into the thoracic aorta. A midline sternotomy was performed and the pericardium was opened. The left anterior descending coronary artery (LAD) was dissected free at its midpoint and surrounded by an elastic snare. As previously described [4] 2 pairs of 1-mm diameter ultrasonic crystals were inserted into the inner third of the left ventricle wall in the LAD and circumflex territory respectively. The crystals of each pair were placed approximately 1 cm apart, and were stimulated by a System 6 microsonometer (Triton Technology, San Diego, CA, USA) and monitored with an HM 205-3 oscilloscope (Hameg Instruments, Frankfurt Main, Germany). A pressure transducer catheter (Mikro-tip, Millar Instruments, TX, USA) was advanced into the left ventricle through a small puncture in the lateral free left ventricular wall, and a transit time flow probe (T-106, Transonic Systems, NY, USA) was placed around the dissected segment of the LAD. Body core temperature was monitored and myocardial temperature in the area at risk was continuously measured by means of an intramyocardial probe introduced at a depth of 5 mm into the ventricular wall at the center of the LAD territory (Digi-Sense, Cole-Parmer Instrument, Illinois, USA).

2.1. Study protocol

Animals were randomly allocated to one of two groups of treatment. One group received 100 mg/kg of L-arginine in 100 ml infused into the femoral vein over 20 min (Harvard Syringe Infusion Pump 22, Harvard Apparatus, South Natick, MA, USA). Solution pH was fitted at 7.4 with HCl and osmolarity ranged between 300 and 350 mOsm. The other group (control) received the same volume of saline. Ten min after the infusion had been delivered, the LAD was occluded for 48 min followed by 2 h of reperfusion.

2.2. Study monitoring

Arterial pH and partial pressures of arterial O₂ and CO₂ were monitored. Ventilatory parameters were adjusted to keep pH between 7.36 and 7.44, pO₂ within 80 and 110 mmHg, and pCO₂ between 37 and 44 mmHg. An electric blanket was used to maintain temperature at 36–37°C. Haematological and biochemical determinations were performed before sternotomy and at the end of the reperfusion period. Lead II of the ECG, aortic pressure, LV pressure and coronary blood flow signals were digitized at a sampling rate of 100 Hz per channel (digitization card Tecfyn ISC-16E/CR, RC Electronics, Goleta, CA, USA) and continuously recorded (MTP95000, Astro-Med, West Warwick, RI, USA). All measurements were performed during sinus rhythm. When an animal presented any arrhythmia at a time at which hemodynamic measurements were scheduled, these measurements were performed at the closest time point in which arrhythmias were absent.

2.3. L-Arginine and cGMP concentrations

Venous blood samples (5 ml) for L-arginine and cGMP determination were withdrawn prior to infusion of L-arginine or placebo, immediately before coronary occlusion, at 15 and 45 min of occlusion, and at 120 min of reperfusion. The samples were placed in an ice-filled tray and plasma was obtained in the following 30 min. L-Arginine concentration was analyzed by reversed-phase HPLC (Pico-Tag™, Waters, MA, USA), after pre-column derivatization with phenylisothiocyanate using UV detection. cGMP concentration was measured by radioimmunoassay using acetylated [³H]cGMP as previously described [8]. Five additional animals were used to monitor cGMP concentration at the great coronary vein. These animals were submitted to the same instrumentation and study protocol and were randomly allocated to the L-arginine (n = 3) or to the control groups (n = 2). In these animals, a 5F multipurpose catheter (Cordis, Roden, The Netherlands) was advanced through the right jugular vein into the great coronary vein as close as possible to the LAD occlusion site. Sequential aortic and coronary vein blood samples were simultaneously obtained to monitor...
arteriovenous cGMP gradient in the LAD territory. Finally, myocardial cGMP content was measured in eight additional animals submitted to L-arginine or control group study protocols. In these animals, ventricular fibrillation was induced after 10 min of reperfusion by application of an epicardial DC current, and the apical third of the ventricles was sectioned with a dermatome blade and immersed in liquid nitrogen. The whole procedure lasted less than 3 s. Frozen myocardial fragments were obtained from reperfused and control myocardium, pulverized under liquid nitrogen, and homogenized using cold trichloroacetic acid at 7.5% (weight/volume). cGMP concentration was measured in the homogenates as previously described [7].

2.4. Segment length measurements

End-diastolic and end-systolic segment lengths (EDSL, ESSL), and maximal and minimal segment length were measured as previously described [4]. Systolic shortening was calculated as the ratio of the difference between EDSL and ESSL divided by EDSL. EDSL throughout the experiment was referred to its initial value before drug infusion. Amplitude of segment length change was calculated as the difference between the maximal and the minimal values of segment length throughout a cardiac cycle [16]. The reduction in this amplitude was assumed to reflect an increase in myocardial stiffness and used as an index of the development of rigor contracture [16].

2.5. Post-mortem studies

After 2 h of reperfusion, the LAD was re-occluded and 5 ml of 10% fluorescein was injected into the left atrium. The heart was excised, cooled at 4°C, and cut into 5–7 mm slices. The third slice, starting from the apex, was processed for determination of myeloperoxidase (MPO) activity. The rest of the slices were illuminated from the basal side (the apical side of the forth slice was also imaged) with ultraviolet light to outline the area at risk, and digital images were obtained (Olympus Digital Camera C-1400L, Olympus Optical, Tokyo, Japan). The slices were then incubated at 37°C for 10 min in 1% triphenyltetrazolium chloride (TTC) buffered at pH 7.4 and imaged again under white light with a reference scale. The area at risk and the area of necrosis were measured in the digitized images using commercially available software (SPSS for Windows 7.0). The homogeneity between groups was tested by two-tail Student’s t-test for independent samples. The hypothesis of a beneficial effect of L-arginine, established a priori, and based on isolated myocytes and rat heart studies was tested by one-tail Student’s t-test for mean comparisons. Differences in cGMP content between control and reperfused myocardium in treated and control animals were evaluated by means of the ANOVA test. Changes in segment length and physiologic parameters were studied by means of the MANOVA test. A critical P-value of 0.05 was used for all tests. All values are expressed as mean ± S.E.M.

2.6. Histological analysis

The second slice of each heart was fixed in 20% formaldehyde and 4-μm thick sections including the whole area at risk were obtained (Leica RM2145 microtome, Leica Instruments, Nussloch, Germany) and stained with Masson’s trichrome. The correlation between the negative reaction of TTC and the extent of contraction band necrosis was analyzed in six randomly selected hearts (three from each group) in which the extent of contraction band necrosis was quantified morphometrically. An average of 167 ± 16 digital microphotographs of adjacent optical fields (X400) were blindly obtained from each slice (DP10 Olympus microscope digital camera, resolution 1280×1024 pixels, Tokyo, Japan) according to serial lines irradiating from the center of the ventricular cavity and covering the whole area at risk, as identified in the corresponding fluorescent image. The extent of contraction band necrosis was calculated from the fraction involved in each image and compared with the extent of the area of negative TTC reaction in the myocardial slice.

2.7. Myeloperoxidase activity in myocardial tissue

MPO activity was determined in myocardial tissue as an index of PMN accumulation. Samples obtained from the area at risk and control zone of the third myocardial slice were stored at −80°C until processed for analysis. MPO activity was spectrophotometrically determined as previously described by a modification of Bradley’s method [16] (Reader 234 ATTC, SLT Labinstruments, Salzburg, Austria), and expressed in units defined as the quantity of enzyme that was able to degrade 1 μmol/l/min of peroxide at 25°C.

2.8. Statistical analysis

All measurements were performed by investigators blind to treatment allocation. Statistical analysis was performed using commercial available software (spss for Windows 7.0). The homogeneity between groups was tested by two-tail Student’s t-test for independent samples. The hypothesis of a beneficial effect of L-arginine, established a priori, and based on isolated myocytes and rat heart studies was tested by one-tail Student’s t-test for mean comparisons. Differences in cGMP content between control and reperfused myocardium in treated and control animals were evaluated by means of the ANOVA test. Changes in segment length and physiologic parameters were studied by means of the MANOVA test. A critical P-value of 0.05 was used for all tests. All values are expressed as mean ± S.E.M.

3. Results

In the series of 24 animals used for measurement of infarct size, one animal died due to intractable ventricular fibrillation and two presented reocclusion of the LAD during the reperfusion period. All the results are from the remaining 21 animals, ten in the L-arginine group, and 11
in the control group. One of the eight animals used for measurement of myocardial cGMP content developed intractable hypotension and was excluded. Results in this series are from seven animals (four treated and three controls).

3.1. Acute effects of L-arginine infusion

3.1.1. Plasma L-arginine concentration

The plasma L-arginine concentration was 81.2±11.6 μM at the beginning of the experiment without differences between groups. Infusion of L-arginine was followed by a rapid increase in its plasma concentration with values significantly higher than in the control group at the onset of coronary occlusion (1496± 62 vs. 83±3 μM, P<0.0001), at the onset of reperfusion (380±42 vs. 97±11 μM, P<0.0002), and at the end of the experiment (215±25 vs. 126±10 μM, P<0.005) (Fig. 1).

3.1.2. Hemodynamics

Changes in hemodynamic variables, regional wall function, and coronary blood flow during L-arginine infusion are summarized in Table 1. L-Arginine infusion resulted in a slight but significant increase in heart rate and coronary blood flow that persisted unchanged until the onset of coronary occlusion.

3.1.3. Plasma cGMP concentration

Plasma cGMP concentration was 37.8±7.2 pmol/ml at the beginning of the experiment, without differences between groups. Infusion of L-arginine had no effect on plasma cGMP which was similar in both groups at the onset of coronary occlusion (37.1±6.9 and 35.9±9.7 pmol/ml respectively, P=0.92).

3.2. Coronary occlusion and reperfusion

3.2.1. Hemodynamics

Changes in heart rate during coronary occlusion and reperfusion were virtually identical in both groups. The small difference in heart rate that appeared between the two groups after infusion of the solution containing the allocated treatment persisted throughout the experiment, and heart rate remained higher in the L-arginine group (114±6 vs. 98±6 at the onset of reperfusion, 114±7 vs. 95±7 after 5 min of reperfusion, and 114±6 vs. 100±4 after 2 h, P<0.05). Mean aortic pressure slightly increased in all animals (from 87.8±16.3 mmHg before coronary occlusion to 96±15 mmHg before reperfusion) and remained stable until the end of the experiment (100±15.9 mmHg), without differences between groups. Changes in LV pressure during ischemia–reperfusion were not different in both groups (Fig. 2).

3.2.2. Coronary blood flow

There were no differences between groups in coronary blood flow during reperfusion, although a non-significant trend was observed towards higher values in the L-arginine group (Fig. 3).

3.2.3. Regional wall function

Changes in EDSL and systolic shortening during ischemia and reperfusion are represented in Fig. 4. Coronary occlusion induced a rapid and marked increase in EDSL and abolition of systolic shortening identical in both groups of treatment. Reperfusion induced an immediate reduction of EDSL that was much less pronounced and
reversible in the l-arginine group than in controls (Fig. 4a). After 30 min of reflow, EDSL was 107.8±3.2% in the l-arginine group vs. 98.0±3 in controls (P=0.025), and this difference was maintained until the end of the experiment (109.0±3.5 vs. 100.3±2.1, P=0.043). There was no significant recovery of contractile function during reperfusion, and systolic shortening remained close to 0% and nearly identical in the two groups until the end of the experiment. However, during the initial 5 min of reperfusion there was a significantly more negative value in the l-arginine group reflecting more severe dyskinesia (Fig. 4b).

Changes in the amplitude of segment length change observed during coronary occlusion were similar in both groups. After 5 min of ischemia, the change in segment length during the cardiac cycle in the ischemic area was assumed to be passive. All subsequent measurements of the amplitude of passive segment length change were normalized as a percentage of the value measured after 5 min of ischemia. In all animals, the normalized amplitude of segment length change remained stable during the first 3.2.4. Arrhythmias

Three animals in the l-arginine group, and three in the control group presented ventricular fibrillation after 22.3±2.1 and 26.7±3.8 min of coronary occlusion respec-
3.2.5. cGMP concentration

Plasma cGMP concentration was similar in both groups during ischemia. During reperfusion, however, there was a transient but significant increase in plasma cGMP in the L-arginine group compared to basal value (from 33.3±6.0 to 62.9±13.8 pmol/ml, P=0.02) (Fig. 5). In the additional five experiments in which it was measured, basal cGMP in the great cardiac vein was 27.8±6.4pmol/ml. Infusion of L-arginine was associated with a transient rise in coronary vein cGMP peaking at 10 min after the onset of reperfusion (166±21% of baseline value) that was not observed in controls (122±38%, P=NS). After 1 h of reperfusion, cGMP concentration at the great coronary vein was 93±18% of basal value in the L-arginine group and 64±19% in controls. Arteriovenous difference in cGMP concentration, undetectable at baseline (−1±1 pmol/ml), showed a small increase immediately after infusion of L-arginine (7±3 pmol/ml immediately before coronary occlusion, vs. 2±4 pmol/ml in controls). Arteriovenous difference after 1 min of reperfusion was 3±1 pmol/ml in the L-arginine group and −1±3 pmol/ml in controls, and after 1 h of reperfusion, the differences were 2±2 and −4±5 pmol/ml respectively. In animals allocated to 10 min of reperfusion, myocardial cGMP content was reduced in reperfused as compared to control myocardium, and was consistently higher in animals receiving L-arginine (Table 2).

3.2.6. Area at risk and infarct size

There were no differences between the L-arginine and control groups in the mass of myocardium at risk (18.1±1.6 and 19.0±2.1 g respectively, P=0.76). However, the mass of myocardial necrosis was smaller in animals receiving L-arginine than in controls. Infarct size, defined as the percent of myocardium at risk developing necrosis, was significantly smaller in the L-arginine group (Fig. 6).

3.2.7. Histology

All infarcts were almost exclusively composed of contraction band necrosis. In the six hearts in which quantitative histology was performed, contraction band necrosis involved 36±11% of the area under examination, and TTC reaction detected necrosis in 51±9% of this area, with a close correlation between both measurements (r=0.82, P<0.05).

3.2.8. Myeloperoxidase activity

Myocardial MPO activity in control myocardium was 0.46±0.08 U/g. In reperfused myocardium, MPO content was significantly higher without differences between L-arginine and controls groups (1.17±0.26 and 1.1±0.12 U/g respectively, P=0.8).

3.2.9. Blood chemistry and hematological determinations

There were no significant changes in hematocrit, platelet count, glucose, potassium, sodium or urea plasma concentrations throughout the experiment in any of the groups.
4. Discussion

This study demonstrates that L-arginine supplementation, at doses used in patients, reduces myocardial necrosis secondary to transient coronary occlusion by mechanisms independent of changes in aortic blood pressure, coronary blood flow or myocardial PMN accumulation. Treatment with L-arginine did not modify indexes of the progression of ischemic injury, as the time of onset of rigor contrac- ture or time to peak electrical unsta- bility, but was associated with attenuated myocardial segment shrinkage, an index of reperfusion-induced hypercontracture [17], and with reduced extent of contraction band necrosis, the pathological correlate of hypercontracture. These results are consistent with previous observations indicating that L-arginine sup- plementation protects cardiomyocytes against reperfusion-induced hypercontracture.

4.1. NO kinetics in reperfused myocardium

Endothelial cells are the main source of NO in myocar- dial tissue under normal conditions. Endothelial NO is essential for normal cardiac perfusion and function. NO, through stimulation of cGMP synthesis in target cells, inhibits the adhesion of platelets and leukocytes to the endothelial surface, relaxes adjacent smooth muscle cells, and modulates cardiomyocyte contraction [18,19]. However, excessive NO concentration may be harmful either by direct reaction with cell proteins or DNA, or through highly reactive species produced by the reaction of NO with O$_3^-$ [20]. The effects of ischemia–reperfusion on myocardial NO concentration have not been completely elucidated. Clearly, after several hours of reperfusion, the expression of the inducible isof orm of NOS (iNOS) results in production of large amounts of NO [21,22]. However, most studies suggest that NO availability is reduced in myocardium reperfused after prolonged ischemia [8,12,23–25]. The contributions of reduced synthesis and increased rate of reaction with O$_3^-$ to this reduction have not been established. A large number of studies have shown that administration of NO donors and manoeuvres increasing myocardial NO availability during early reperfusion improve post-ischemic functional recovery [14,26,27], and reduce cell necrosis in reperfused myocardium [8,12,13,25].

L-Arginine is the only known substrate for NO synthesis in vivo. Under normal conditions the concentration of L-arginine in endothelial cells is well over the level necessary to saturate NOS. However, there is ample evidence that increasing plasma L-arginine concentration enhances NO synthesis [28,29], a phenomenon termed ‘L-arginine paradox’. During ischemia and reperfusion the increase in NO synthesis induced by L-arginine administration is easier to understand. Under these conditions, reduced ATP-dependent L-arginine transport or regenera- tion from L-citrulline may impair NO synthesis [10,30]. On the other hand, L-arginine deprivation may switch NOS activity to the production of O$_3^-$ instead of NO, and this may be the main source of O$_3^-$ during the first few min of reperfusion [10]. Administration of L-arginine may at- tenuate both phenomena and increase NO availability [10].

4.2. Previous studies

Previous observations in the isolated rat heart perfused with crystalline, arginine-free media have shown that addition of L-arginine improves functional recovery after transient ischemia [9,24,25] and reduces reperfusion ar- rhythmias [31]. The effect of L-arginine during in vivo ischemia–reperfusion has been less investigated, and has yielded more controversial results, probably due, to a large extent, to differences in models (hypoxia, sublethal isch- emia, prolonged ischemia), doses and time of L-arginine administration, and end-points analyzed. L-Arginine was found to adversely influence postschismic or posthypoxic functional recovery in the pig and dog models [23,32]. Systemic administration of L-arginine after the onset of ischemia failed to reduce infarct size in pigs submitted to 45 min of coronary occlusion and reperfusion [33]. In contrast, intracoronary infusion of L-arginine during reperfusion reduced myocardial PMN accumulation and necrosis in dogs submitted to 60 min of coronary occlusion and 270 min of reperfusion [14]. A reduction in infarct size and myocardial MPO content was found in cats submitted to 60 min of coronary occlusion and reperfusion [10]. Administration of L-arginine may at- tenuate both phenomena and increase NO availability [10].

4.3. Mechanism of protection

The results of the present study support the hypothesis that the increase in NO availability and cGMP synthesis caused by L-arginine protects cardiomyocytes against reperfusion-induced hypercontracture. It has been shown that cGMP concentration is reduced in reperfused or reoxygenated myocardium [5,8], and that this reduction is attenuated or reversed by the administration of L-arginine [5,8,9]. In the present study, animals receiving L-arginine showed a significant increase in great coronary vein and arterial cGMP concentration during the early phase of reperfusion. Our results do not allow the determination of...
the possible contribution of extra-myocardial sources of cGMP release to the observed increase in arterial cGMP concentration. However, the results of measurement of myocardial cGMP content in seven additional animals are fully consistent with our working hypothesis. Measurement of myocardial cGMP content had to be performed in an additional series of experiments since this measurement and quantification of infarct size cannot be performed in the same animal. cGMP has an inhibitory effect on cardiomyocyte contractility [18,19]. This effect is slight under normal conditions but may be potent during the first minutes of reperfusion [7]. In the present study, a significantly more severe dyskinesia was observed in the L-arginine group during the early phase of reperfusion. The negative inotropic effect of cGMP appears to be due to a desensitization of contractile myofilaments to Ca$^{2+}$ [18,19], although a role of reduced Ca$^{2+}$ availability has not been excluded [35]. The ability of stimulation of cGMP synthesis in preventing reoxygenation or reperfusion-induced hypercontracture could be thus explained by its inhibitory effect on contractility.

It cannot be excluded that other actions of L-arginine could have contributed to its protective effect against myocardial necrosis secondary to ischemia–reperfusion. Increased NO production and reduced oxidative stress have been found to attenuate reoxygenation-induced sarcolemmal fragility [36], a mechanism contributing to sarcolemmal rupture and cell death during myocardial reperfusion [37]. cGMP reduces gap junction conductance, an effect that could interfere with propagation of hypercontracture [38]. Finally, administration of L-arginine may slow the progression of ischemic injury. In particular, its vasodilatory action could result in reduced hemodynamic load and lower oxygen requirements. However, the slight (but statistically significant) hemodynamic actions of L-arginine observed in this study were in the opposite direction, and pressure–rate product was slightly higher in treated animals than in controls. Moreover, the indexes of progression of ischemic injury, as the onset of ischemic rigor contracture or of electric instability, were not delayed in animals receiving L-arginine as compared to controls. Overall, the results of the present study strongly suggest that the observed protective effect of L-arginine against myocardial necrosis is mediated, at least in part, by a direct effect on cardiomyocytes.

### 4.4. Implications

The present study support previous observations indicating that manoeuvres aimed at increasing cGMP in reperfused myocardium may have a protective effect against hypercontracture and necrosis. On the other hand, many studies have demonstrated that L-arginine supplementation improves endothelial function in hypercholesterolemic animals [39] and in patients with a variety of conditions [28,29]. The present study suggests that L-arginine supple-

### References


