Cardiac outflow of amino acids and purines during myocardial ischemia and reperfusion

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Bäckström, Tobias, Michel Goiny, Ulf Lockowandt, Jan Liska, and Anders Franco-Cereceda. Cardiac outflow of amino acids and purines during myocardial ischemia and reperfusion. J Appl Physiol 94: 1122–1128, 2003. First published November 8, 2002; 10.1152/japplphysiol.00138.2002.—A novel application of microdialysis was studied, in which myocardial outflow of amino acids and purines was monitored by intravascular microdialysis in the myocardial venous outflow during ischemia and reperfusion. Microdialysis catheters were introduced into the great cardiac vein, pulmonary artery, and external jugular vein in 20 anesthetized pigs. The left anterior descending artery was occluded in four groups of pigs for 0, 10, 15, and 60 min. Ischemia was followed by 120 min of reperfusion. Microdialysis samples were analyzed for taurine, aspartate, glutamate, hypoxanthine, inosine, and guanosine. Myocardial infarction developed when ischemia exceeded 10 min. Taurine, aspartate, inosine, and guanosine increased early in the great cardiac vein during ischemia. We found the outflow patterns of amino acids and purines to be graded in response to different lengths of ischemia. In this study we have demonstrated a graded outflow of amino acids and purines in response to ischemia and a positive correlation between infarct size and myocardial outflow of amino acids and purines. This could be of value in a clinical setting to quantify the extent of myocardial damage.

intravascular microdialysis; coronary disease; cardiovascular surgery

Coronary artery disease is one of the leading causes of death in Europe and the United States, causing ~500,000 deaths annually in the United States (14). To treat patients with established coronary artery disease, coronary artery bypass grafting has become a routine method during the last decades. Some of the patients undergoing coronary artery bypass grafting are at risk of developing myocardial dysfunction in the immediate postoperative phase, commonly caused by myocardial ischemia. The routinely used methods to monitor the metabolic state of the myocardium postoperatively are electrocardiogram (ECG) and blood studies of myocardial enzymes. Enzyme studies have been found unreliable because of frequent unspecific elevation caused by the surgical trauma, transient ischemia during aortic cross-clamping, cardiopulmonary bypass, and retransfusion of mediastinal shed blood (8, 9, 13, 18). It has also been shown that traditional ECG criteria of myocardial infarction have to be interpreted cautiously in the setting of open heart surgery (4, 17).

One possible way of increasing the reliability and the temporal resolution of the postoperative monitoring is by using a microdialysis catheter to study the metabolic changes in the extracellular fluid in the coronary sinus. In this way we actually monitor the venous outflow from the heart. The principle of microdialysis, in which a tubular dialysis membrane is introduced into an organ of interest, is to mimic the function of a capillary blood vessel. The tube is perfused with Ringer solution that equilibrates with the fluid outside the tube by diffusion in both directions. The perfusion fluid can then be sampled and analyzed (19).

The technique of microdialysis has been used to study myocardial ischemia in a number of studies, and the common way is to place the microdialysis catheters within the myocardium (7, 20, 21). To develop the technique of microdialysis to be more applicable in the clinical setting of postoperative monitoring of patients subjected to open heart surgery, we have developed the technique of intravascular microdialysis in which the microdialysis catheter is placed in the great cardiac vein (GCV) of the heart. In a clinical setting this would be a great advantage compared with intramural microdialysis because of the fact that it is not possible to tell where myocardial ischemia will develop during the postoperative course. The measurement of the amino acids taurine, glutamate, and aspartate in the extracellular fluid of the myocardium has been shown to be a good marker of energy depletion and the following inability to withhold the intracellular-to-extracellular concentration ratio (7, 15, 16). Also an increased release of purine metabolites has been shown during myocardial ischemia and reperfusion (6, 20, 21).

We have in earlier studies characterized the myocardial metabolic response to different time frames of ischemia and reperfusion (1, 11). The main objective of this study was to further characterize the myocardial

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metabolic response to ischemia and reperfusion with regard to amino acids and purines. Thus the study does not deal with the relation of the metabolites studied in the GCV in relation to levels within the myocardial tissue.

Here we report that intravasal microdialysis is a reliable and useful tool to monitor cardiac efflux of taurine and aspartate as well as inosine and guanosine from the ischemic myocardium. We also found a positive correlation between the total outflow of each of the measured substances and infarct size.

METHODS

Acceptance for this study was obtained from the local ethics committee. All animals in this study received humane care according to the guidelines set forth by the "Guide for the Care and Use of Laboratory Animals" published by the U.S. National Institutes of Health (NIH publication 85-23, revised 1996).

Animal Preparation

Swedish farm pigs (31–37 kg, n = 20) of either sex were fasted overnight and given premedication with intramuscular ketamine hydrochloride (20 mg/kg; Parke Davis, Morris Plains, NJ) and atropine sulfate (0.04 mg/kg; NM Pharma, Stockholm, Sweden). After the induction of anesthesia with intravenous pentobarbital sodium (15 mg/kg; ACO, Umeå, Sweden), an endotracheal tube was inserted orally, and the ventilation was controlled with a volume-regulated ventilator (Siemens-Elema 900 servo ventilator, Stockholm, Sweden). Blood gases were monitored throughout the experiment, and the ventilator was adjusted to maintain normal arterial blood gases. A continuous infusion of fentanyl (10 μg·kg⁻¹·h⁻¹; Janssen Pharmaceutica, Beerse, Belgium) and midazolam (100 μg·kg⁻¹·h⁻¹) was used to maintain anesthesia. A continuous infusion of pancuronium bromide (100 μg·kg⁻¹·h⁻¹; Organon Teknika, Boxtel, The Netherlands) produced necessary skeletal muscle relaxation. Body temperature was kept at 38.0–39.0°C by means of a heating pad. Ringer acetate solution (200–250 ml/h) was given intravenously throughout the experiments. Catheters were inserted into the left femoral artery for measurements of blood pressure and heart rate and in the left femoral vein for the administration of drugs and fluids. Hemodynamic measurements were done, and ECG was monitored and recorded continuously (pressure transducer PVB, Triplus 6023; pressure monitor Hewlett-Packard 78342A; pressure recorder Gould ES 100).

The heart was exposed through a left-side thoracotomy with transection of the sternum. This incision provided excellent access to the left anterior descending artery (LAD). A vessel loop was passed around the LAD, at a position from which approximately the distal two-thirds of the artery would be occluded by tightening the snare. LAD blood flow was measured by using an ultrasonic flow probe model PA 100021 connected to a CM 1000 flow meter (CardioMed AS, Oslo, Norway). The probe was placed around the artery just proximal to the snare.

Microdialysis

The microdialysis equipment comprised a CMA/65 microdialysis catheter with an outer diameter of 0.5 mm and a 10-mm-long flexible membrane (CMA Microdialysis, Stockholm, Sweden). The molecular cutoff point for the dialysis membrane was 20 kDa. The probe was perfused with a modified Ringer solution (147 mmol/l Na⁺, 4 mmol/l K⁺, 2.3 mmol/l Ca²⁺, and 156 mmol/l Cl⁻). The perfusate was infused by a CMA 106 microdialysis pump (CMA Microdialysis) at a speed of 2.0 μl/min. The dead space within the tubing system distal to the catheter was 3 μl. Samples of the perfusate were collected and contained in microvials (CMA Microdialysis). The microvials were frozen at −20°C overnight, packed in dry ice, and transported to the analysis laboratory.

Chemical Analysis

The samples were diluted 10-fold with distilled water and automatically injected with refrigerated microsamplers (CMA 200; CMA Microdialysis) into high-precision liquid chromatography systems with ultraviolet detection at a wavelength of 260 nm for hypoxanthine, inosine, and guanosine or with fluorescence detection of ~495 nm after precolumn derivatization with o-phthalaldehyde for aspartate, glutamate, and taurine. The concentrations were calculated with SP 4290 integrators (Spectra Physics, San Jose, CA) against freshly prepared standards.

Study Protocol

After the animal preparation was completed, microdialysis catheters were inserted into the GCV parallel to the LAD to obtain sampling from the myocardium, into the pulmonary artery (PA) to monitor central venous changes, and finally into the left external jugular vein (EJV) for monitoring of peripheral alterations. An equilibration period of 30 min was allowed to ensure baseline values, after which two consecutive baseline samples with 15-min intervals were collected. The pigs were randomized into four groups: group I, serving as controls; group II, 15 min occlusion of the LAD; group III, 15 min occlusion of the LAD; group IV, 60 min occlusion of the LAD. After the ischemic period, reperfusion was studied for another 120 min. In group II, the sample during ischemia was obtained after 10 min, and in group IV the first sample during ischemia was collected after 20 min and the second sample after an additional 10 min. All other samples were collected and analyzed every 15 min. The occlusion of LAD was performed by tourniquet of the snare. Complete occlusion was verified by zero flow, and myocardial ischemia was confirmed by ECG ST-segment elevation, visible regional cyanosis, and stunning.

Delineation of Ischemic Area and Myocardial Infarct Size

After 120 min of reperfusion, the LAD was reoccluded at the original site, the ascending aorta was clamped, and 1 ml/kg of 2% wt/vol Evans blue was injected into the coronary circulation to delineate the ischemic myocardium (area at risk). The heart was then arrested by an injection of potassium chloride into the coronary circulation. After death, the heart was excised and rinsed with saline at 22°C, then sliced transversely in ~1-cm slices. The area of nonstained myocardium (area at risk) on the basal surface of each slice was outlined. The slices were then incubated in a 0.8% solution of triphenyl tetrazolium chloride at 37°C for 20 min, which stains viable myocardium red. The areas of necrosis and area at risk were determined by planimetry, and the extent of necrosis was expressed as percent of the area at risk.

Statistical Evaluation

The data were analyzed by using the procedure Mixed in SAS (10). The model was set up as a repeated-measures design. Different covariance pattern models were tested, i.e.,
compounds symmetry and first-order autoregressive with and without between-subject heterogeneity. Ischemia, time, and ischemia \times time effects were fitted as fixed effects. Treatment effect was the between factor (control, ischemia 10 min, ischemia 15 min, and ischemia 60 min), and time effect was the within factor. In case of significant interaction, simple effects were examined, i.e., effects of one factor when holding the other factor fixed. To analyze the trends over time, during and after the ischemic period, we modeled the outcome variables as a polynomial function of time. Plots of data indicated that quadratic equations should be adequate for regression of the outcomes on time. Numeric values were presented as means \pm SE. A P value <0.05 was considered statistically significant. To study the correlation between total cardiac venous outflow in the GCV of each substance and infarct size, we calculated the area under the curve and tested for correlation with infarct size (GraphPad 2.01, Instat, San Diego, CA).

RESULTS

Hemodynamics

The pigs had stable circulation throughout the experiment, and there were no significant changes between group II and III vs. group I heart rate (HR), diastolic arterial pressure (DAP), systolic arterial pressure (SAP), mean arterial pressure (MAP), and rate pressure product (RPP) = MAP \times HR. In group IV, a significant decrease was observed during the ischemic period of SAP from 132 ± 9 to 96 ± 4 mmHg (P < 0.01), of MAP from 109 ± 7 to 75 ± 2 mmHg (P < 0.01), and of DAP from 81 ± 13 to 65 ± 2 mmHg (P < 0.05). However, the RPP did not change significantly (12,898 ± 2,912 mmHg\cdot beats^{-1} \cdot min^{-1} vs. 11,650 ± 1,832 mmHg\cdot beats^{-1} \cdot min^{-1}). During reperfusion, pressure values normalized in group IV. Ventricular fibrillation (VF) occurred in all pigs subjected to myocardial ischemia and was easily electroconverted with no influence on the hemodynamics after the electroconversion. VF typically developed within the first 10 min of ischemia, and the number of VF episodes varied from 1 to 9 in individual pigs. During reperfusion, VF occurred in three pigs each in groups II and III and in one pig in group IV.

Microdialysis

There were no statistically significant trends over time or differences in values between groups I–IV in concentration of substances analyzed in the dialysate from microdialysis catheters placed in the EJV or the PA (data not shown). In group I, there was no difference between concentrations analyzed in the dialysate from catheters placed in the GCV, PA, or EJV either at baseline or throughout the experiments (Figs. 1–3). Significant changes were found only between dialysate concentrations in samples obtained from the microdialysis catheters placed in the GCV.

Hypoxanthine. The preischemic mean values varied between the groups in the range of 13–19 \mu mol/l. In groups I–II there were no significant changes during ischemia compared with baseline. In group III, hypoxanthine levels in the dialysate during ischemia increased to 150% compared with baseline levels (P < 0.05), and in group IV hypoxanthine levels in the dialysate of the first sample during ischemia increased to 230% compared with baseline levels (P < 0.001; Fig. 1). In that group there was a significant (P < 0.001) change over time with a peak increase to 415% compared with baseline at 45 min of ischemia (Fig. 2). The change over time indicated a quadratic trend (P < 0.05). During the first 15 min of reperfusion, the hypoxanthine levels in group IV increased to 590% compared with baseline, and during the remaining 105 min of reperfusion the hypoxanthine concentration in groups III and IV normalized over time with a declining quadratic trend (P < 0.001 and P < 0.001, respectively; Fig. 3). The total cardiac venous outflow of hypoxanthine in the GCV was measured by calculating the area under the curve. This was then tested for correlation with infarct size and found to be statistically significant with a positive correlation (r = 0.93, P = 0.0001) between total hypoxanthine outflow and infarct size.

Inosine. The preischemic values varied between the groups in the range of 4–7 \mu mol/l. In groups I–II, there were no significant changes during ischemia compared with baseline. In groups III and IV, inosine levels in the dialysate increased to 660 and 920%, respectively, compared with baseline (P < 0.01; Fig. 1). For group IV, there was a significant (P < 0.01) change over time, with a peak increase to 2.100% at 30 min of ischemia followed by a subsequent decrease over the next 30 min of ischemia (Fig. 2). The change over time indicated a quadratic trend (P < 0.05). During the first 15 min of reperfusion in group IV there was a significant increase to 3,200% compared with baseline in the dialysate concentration of inosine. During the following 105 min of reperfusion, the inosine concentration in groups II, III, and IV normalized over time with a declining quadratic trend (P < 0.05, P < 0.001, and P < 0.001, respectively; Fig. 3). There was a significant correlation (r = 0.90, P = 0.0004) between total inosine outflow and infarct size.

Guanosine. The preischemic values varied between the groups in the range of 0.4–0.6 \mu mol/l. In groups I–II, there were no significant changes during ischemia compared with baseline. In group III the guanosine level during ischemia increased to 250% compared with baseline (P < 0.001), and in group IV the guanosine level in the dialysate of the first sample during ischemia increased to 350% compared with baseline values (P < 0.001; Fig. 1). For group IV there was a significant (P < 0.001) change over time with a peak increase to 580% at 45 min of ischemia followed by subsequent decrease over the next 15 min (Fig. 2). The change over time indicated a quadratic trend (P < 0.05). During the first 15 min of reperfusion, the guanosine levels in group IV increased to 1,250% compared with baseline, after which the guanosine levels normalized with a declining quadratic trend (P < 0.001; Fig. 3). There was a significant correlation (r = 0.75, P = 0.013) between total guanosine outflow and infarct size.
Taurine. The preischemic values varied between the groups in the range of 70–120 μmol/l. In groups I–II, there were no significant changes during ischemia compared with baseline. In groups III and IV, the taurine levels in the dialysate of the first sample during ischemia increased to 230 and 300%, respectively, compared with baseline values (P < 0.05, P < 0.001, respectively; Fig. 1). For group IV, there was a significant (P < 0.001) change over time with a peak increase to 760% at 45 min of ischemia followed by a subsequent decrease over the next 15 min (Fig. 2). During the first 15 min of reperfusion, the taurine levels in group IV increased to 1,500% compared with baseline, after which the taurine levels normalized with a declining quadratic trend (P < 0.001; Fig. 3). There was a significant correlation (r = 0.92, P = 0.0001) between total taurine outflow and infarct size.

Aspartate. The preischemic values varied between the groups in the range of 5–7.4 μmol/l. In groups I–II, there were no significant changes during ischemia compared with baseline. In group III the aspartate levels in the dialysate of the first sample during ischemia increased to 210% compared with baseline (P < 0.05) and in group IV to 210% compared with baseline values, but the changes in this group did not reach statistical significance (Fig. 1). For group IV there was change over time, with a peak increase to 240% at 30 min of ischemia followed by a subsequent decrease over the next 30 min (Fig. 2), but these changes did not reach statistical significance. During the first 15 min of reperfusion, the aspartate levels in group IV increased to 410% compared with baseline, after which the aspartate levels normalized with a declining linear trend (P < 0.001; Fig. 3). There was a significant correlation (r = 0.90, P = 0.0003) between total aspartate outflow and infarct size.

Glutamate. The preischemic values varied between the groups in the range of 180–240 μmol/l. There were no significant changes in the glutamate concentration in the first ischemic sample compared with baseline in
groups I–II or group IV (Fig. 1). In group III the glutamate levels during ischemia increased to 150% compared with baseline \( (P < 0.05) \). For group IV, there was a slight increase over time, with a peak increase of 50% at 30 min of ischemia followed by a subsequent decrease over the next 30 min (Fig. 2), but these changes did not reach statistical significance. During the first 15 min of reperfusion, the glutamate level in group III increased to 215% compared with baseline, after which the glutamate levels normalized with a declining quadratic trend \( (P < 0.001; \text{Fig. 3}) \). In group IV, the glutamate level during reperfusion normalized with a declining quadratic trend \( (P < 0.05; \text{Fig. 3}) \). There was a significant correlation \( (r = 0.74, P = 0.015) \) between total glutamate outflow and infarct size.

Infarct Size

In the four groups overall, the size of myocardial area at risk expressed as a percentage of the left ventricle was 26 ± 1% \( (n = 20) \) and did not differ between the groups. The infarction size in relation to the area at risk was 35 ± 6% in group III and 64 ± 10% in group IV; no infarction was detected in group I or group II.

DISCUSSION

The main objective of this study was to further characterize the outflow of metabolites from the myocardium in relation to ischemia and reperfusion on the basis of earlier findings of a graded outflow of energy metabolites \( (i.e., \text{lactate, pyruvate, glucose}) \) in relation to the duration of ischemia \( (1) \). In accord, here we have demonstrated a time-dependent outflow of purines and amino acids from the ischemic heart.

All metabolites analyzed showed the same pattern of release, with an increase during ischemia, reaching a plateau or a small reduction after 30 min of ischemia. During the first 15 min of reperfusion, a sharp but transient increase was noted. Significant taurine and aspartate release was only seen in animals in which myocardial infarction developed. Taurine as a marker of myocardial ischemia has been extensively studied both in humans and in experimental animals \( (1, 12, 21) \). Our results are in accord with earlier studies, with an elevation of the myocardial taurine outflow during ischemia followed by a further increase during the initial reperfusion and then normalization during the rest of reperfusion \( (7, 15, 21) \). Aspartate showed the same outflow pattern as taurine, which is in agreement with earlier studies \( (15, 16) \).

The mechanisms responsible for the myocardial outflow of these amino acids are not fully understood, but there is evidence that the mechanisms involved may be 1) swelling of the myocardial cells, which leads to activation of anion channels in the cell membrane leading to a diffusional efflux; 2) disturbances of the cell membranes by phospholipase activity, leading to diffusional efflux; and/or 3) reversal of \( \text{Na}^+ \)-dependent transporters \( (15) \). The increase of taurine levels at 15

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**Fig. 2.** Plot of means of dialysate from the great cardiac vein during baseline sampling \( (-15, 0) \), 60 min of ischemia \( (i20, i30, i45, i60) \), and reperfusion \( (R15) \) of glutamate and hypoxanthine (A), inosine and guanosine (B), and taurine and aspartate (C), for group IV \( (60 \text{ min of ischemia}) \) and group I \( (\text{no ischemia}) \). Values are means ± SE.
min of reperfusion could be interpreted as a result of a second insult at the start of reperfusion as a result of the production of oxygen-derived free radicals (21). However, in this model of total regional ischemia, a washout of metabolites, possibly in combination with reperfusion injury, seems to be the most plausible explanation. It should be emphasized, though, that the experimental setup does not allow for coronary vein flow measurement, and therefore a relation to postischemic myocardial hyperemia cannot be established.

The outflow of purines from the myocardium showed the same pattern as the outflow of amino acids, with an increase during ischemia and a further increase during the first 15 min of reperfusion. Significant outflow of inosine and guanosine was seen only in animals in which myocardial infarction developed. This is in accord with a previous study of myocardial ischemia using a cardioplegic model (21). Inosine was the predominant purine metabolite to be released from the ischemic myocardium even though hypoxanthine and

Fig. 3. Plot of means of dialysate from the great cardiac vein of hypoxanthine (A), inosine (B), guanosine (C), taurine (D), aspartate (E), and glutamate (F) for each of groups I–IV from end of ischemia (0) through 120 min of reperfusion. Values are means ± SE. First sample during reperfusion vs. last sample during ischemia. Group I, control; group II, 10 min of ischemia; group III, 15 min of ischemia; group IV, 20 min of ischemia.
guanosine showed the same pattern, which corroborates earlier findings in the extracellular fluid of the myocardium in which inosine is the predominant purine released early in ischemia and hypoxanthine is the predominant purine released late in ischemia (6, 20). After the onset of ischemia, there is a rapid decline of ATP, which is paralleled by a marked accumulation of inosine, hypoxanthine, and xanthine in the extracellular fluid (5), although we were not able to detect a shift in the release pattern by prolonging the ischemic period. In the present study, we were able to show a significant positive correlation between myocardial outflow of each of all the measured substances and infarct size. This further strengthens the use of these substances as markers of myocardial ischemia leading to infarction.

In conclusion, we have demonstrated that intravasal microdialysis is a reliable and useful monitoring tool in the setting of myocardial ischemia and reperfusion. We found release patterns of amino acids and purines that were graded in response to different time frames of ischemia and a positive correlation between infarct size and myocardial outflow of amino acids and purines. This could be of value in a clinical setting to quantify the extent of myocardial damage. The technique of intravasal microdialysis in humans with myocardial ischemia remains to be evaluated.

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