ELSEVIER

European Journal of Pharmacology 411 (2001) 143–154

ejp

www.elsevier.nl/locate/ejphar

Nucleoside transport inhibition in ischemic myocardium results in enhanced taurine efflux

Vitas Zemgulis [a,*], Gerhard Wikström [b], Axel Henze [a], Anders Waldenström [c], Stefan Thelin [a], Gunnar Ronquist [d]

[a] *Department of Cardiothoracic Surgery, University Hospital, S-751 85 Uppsala, Sweden*
[b] *Department of Cardiology, University Hospital, S-751 85 Uppsala, Sweden*
[c] *Department of Internal Medicine, Umeå University Hospital, S-901 85, Umeå, Sweden*
[d] *Department of Clinical Chemistry, University Hospital, S-751 85 Uppsala, Sweden*

Received 15 May 2000; received in revised form 18 October 2000; accepted 31 October 2000

Abstract

We measured with the microdialysis technique energy-related metabolites in ischemic myocardium over time in an experimental pig model. Emphasis was put on the dipyridamole effect when administered in the microdialysis probe inserted in ischemic myocardium. Not only adenosine but also taurine and pyruvate concentrations were significantly higher in the microdialysate during the periods of ischemia and extracorporeal circulation with cardioplegia. The enhanced efflux of taurine in ischemic myocardium induced by dipyridamole is a new finding. A mechanistic role of taurine in the prevention of Ca^{2+} overload in ischemic myocytes is discussed. Also, taurine may have stimulatory effects on glycolysis in ischemic heart.

Keywords: Myocardial infarction; Nucleoside transport; Adenosine; Taurine; Dipyridamole

1. Introduction

The nucleoside adenosine plays a major role in the autoregulation of coronary blood flow (Wang et al., 1992; Tommasi et al., 2000). The action of adenosine and its nucleoside analogues at extracellular receptor sites is limited by either the rapid uptake into surrounding cells by a membrane-located nucleoside carrier or deamination by adenosine deaminase to inosine (Mohrman and Heller, 1990; Abd-Elfattah et al., 1998; Deussen et al., 1999). The nucleoside carrier is a membrane-bound, non-concentrative, bi-directional transporter of broad specificity (Jarvis et al., 1989; Suleiman et al., 1992). It is also possible to inhibit this carrier mechanism (Miras-Portugal et al., 1986; Mohrman and Heller, 1990; Kalsi et al., 1998; Figueredo et al., 1999), which might be expected to potentiate the effects of adenosine and other nucleosides. Adenosine has also been suggested to play a pivotal role in the preconditioning phenomenon (Finegan et al., 1993; Harrison et al., 1998; Figueredo et al., 1999). However, it is unlikely that protection is solely due to continuous presence of elevated adenosine levels (Kalsi et al., 1998; Figueredo et al., 1999). Adenosine also exerts effects on the ATP-dependent K^+ channel (K^+ ATP channel) (Abd-Elfattah et al., 1998). This channel is involved in any situation of ischemic/reperfusion injury with concomitant free radical formation (Satoh, 1996, 1998; Piper et al., 1998). Hence, cardiac K^+ ATP channel opening is induced by oxygen free radicals produced by the xanthine oxidase reaction (Obata et al., 1998). Also, nucleoside trapping before and after ischemia attenuated free radical production, reperfusion-mediated arrhythmia and ventricular dysfunction (stunning) (Abd-Elfattah et al., 1998). Dipyridamole is a well-known inhibitor of the adenosine membrane carrier (Miras-Portugal et al., 1986; Banker et al., 1991; Wang et al., 1992; Babsky et al., 1998; Kalsi et al., 1998). Accordingly, dipyridamole would reinforce the extracellular adenosine effects. However, there is evidence that dipyridamole itself may have a direct effect on the K^+ ATP channel (Abd-Elfattah et al., 1990).

When studying these intricate mechanisms, different experimental set ups have been employed with conflicting

* Corresponding author. Tel.: +46-18-6113000; fax: +46-18-6113926.
E-mail address: vitas.zemgulis@thorax.uas.lul.se (V. Zemgulis).

0014-2999/01/$ - see front matter
PII: S0014-2999(00)00844-X

results depending on the in vitro or in vivo-design. We devised a myocardial ischemia/reperfusion experimental in vivo-model with extracorporeal circulation with the explicit aim of being as close as possible to the clinical situation during open-heart surgery (Zemgulis et al., 2000). The objective of the present study was to measure with the microdialysis technique energy-related metabolites and taurine in ischemic myocardium over time in the experimental pig model. Emphasis was put on the dipyridamole effect when given in the microdialysis probe while being inserted in ischemic myocardium.

2. Material and methods

2.1. Ethical consent

Approval was obtained from the Animal Research Ethical Committee of Uppsala University (C 257/96).

2.2. Animals

The study involved experiments on 10 domestic pigs of mixed breed of Hampshire, Yorkshire and Swedish Landrace. The animals weighed 28–35 kg and were fed a standard diet.

2.3. Specification of drugs and equipment

2.3.1. Drug specification

Atropin (Atropin NM Pharma; Stockholm, Sweden), Azaperone (Stresnil; Janssen, Beerse, Belgium), Cardioplegic St. Thomas type I solution (Ringer-Acetate 1000 ml, K^+ 16 mmol, Mg^{2+} 16 mmol, procainum 1 mmol, Cl^- 49 mmol, Tribonate 10 ml), Clomethiazole edisylas (Heminevrin 8 mg/ml; Astra Södertälje, Sweden), Dipyridamole (Persantin® 5 mg/ml; Boehringer Ingelheim Int., Ingelheim, Germany), Fentanyl (Fentanyl 50 μg/ml; Pharmalink, Pharmaceuticals, Roscea, Ireland), Fenylefrin hydrochloride (Fenylefrin-hydroklorid 10 mg/ml, Apoteksbolaget, Umeå, Sweden), Fluorescein (Fluorescite® Injection 250 mg/ml; Alcon Laboratories, Texas, USA), Heparin sodium (Heparin 5000 IU/ml; Løvens Kemiske Fabrik, Danmark), Krebs–Ringer phosphate buffer (sodium phosphate, 20 mM, magnesium chloride, 5 mM and sodium chloride, 110 mM, pH 7.4), Lidocaine hydrochloride (Xylocard® 20 mg/ml; Hässle Läkemedels, Sweden), Mannitol (Mannitol Pharmacia and Upjohn; Solna, Sweden), Pancuronium bromide (Pavulon; Organon Teknika), Potassium chloride (Kaliumklorid Braun 150 mg/ml, Braun Medical, Bromma, Sweden), Ringer-acetate (Ringer-acetate; Fresenius Kabi, Sweden; Na^+ 131 mM, K^+ 4 mM, Ca^{2+} 2 mM, Mg^{2+} 1mM, Ac^- 30 mM, Cl^- 110 mM), Tiletamine/zolazepam (Zoletil®; Reading Laboratories, Carros, France), Tribonate (Tribonat®; Pharmacia and Upjohn, Solna, Sweden), Triphenyl-tetrazolium chloride (TTC, Tetrazolium Red; Sigma, St. Louis, USA), Zylazine (Rompun; Bayer, Leverkusen, Germany).

2.3.2. Equipment specification

ABL 300 and OSM 3 Hemoximeter (Radiometer, Copenhagen, Danmark), BAS Liquid Chromatography UV/VIS Detector, BAS Pump PM-48 (Bioanalytical Systems, West Lafayette, Indiana, USA), Bulk Tubings (1/4 × 1/16 and 3/8 × 3/32, Baxter Medical, Sweden), Capnomac Ultima analyzer (Datex, Helsinki, Finland), Cardiotomy reservoir with filter (BCR-2500, Bentley; Baxter), CMA/100 Microdialysis Pump, CMA/200 Autoinjector, CMA/260 Degasser, CMA/280 Fluorescense Detector, CMA/20 Microdialysis Probe (CMA/Microdialysis, Sweden), DataJet Integrator, Precision Isocratic Pumps SP 4290 and SP 8810 (Spectra-Physics, San Jose, CA, USA), Direct Digital Writer, series 7100 (Marquette Electronics), Hemochron 400 (International Technidyne, Edison, NJ), Labino UV Floodlight H135 (Labino, Sweden), Membrane Oxygenator (Univox Membrane Oxygenation Module, Bentley, Baxter, Irvine, CA or Hollow Fibre Membrane Oxygenator QUADROX HMO 1000, Jostra Medizintechnik, Hillringen, Germany), Monitor (Tram series 7010; Marquette Electronics, Milwaukee, WI), Non-pulsatile pump type PMO 10-220 (Gambro, Lund, Sweden), Prolene 3/0 and 4/0 (Ethicon, Nordestedt), Retrograde coronary sinus perfusion cannula with manual-inflating cuff and silicone body (15 Fr., Medtronic DLP), Scanners: Nikon 35 mm Film Scanner LS-2000 (Nikon, Tokyo, Japan), Image-wave scanner FBS D600 (Taiwan); Servo ventilator (Servo 900C; Siemens-Elema, Solna, Sweden), Softwares for PC: GetARef™ 4.0 (DatAid, Sweden), Scion Image (Release Beta 3b, Scion, 82 Worman's Mill Ct., USA), Sigma Plot® 5.0 (SPSS, Chicago, IL, USA), Statistica™ (Release 5.1 '97, Stat Soft®, Tulsa, OK, USA); Solid-State Coagulator with Iso-Bloc, model SSC 1 (Valleylab Boulder, CO, USA), Thermodilution Catheter CritiCath, 7F, model SP5107H (Ohmeda, Singapore), Tetrafluoroethylene (TFE) Polymer pledgets 3 × 7 mm (diminished to 3 × 4 mm, Davis + Geck Wayne, NJ, USA).

2.4. Premedication

Following an overnight fast and prior to transportation, the pigs were sedated by intramuscular injection of 40 mg azaperone.

2.5. Anaesthesia

Induction of anaesthesia started when the animals were delivered in the experimental laboratory, by intramuscular injection of 0.04 mg/kg atropine, 6 mg/kg tiletamine/zolazepam and 2.2 mg/kg zylazine. The pigs were then placed in supine position on the operating table and 5 μg/kg fentanyl was injected through a previously inserted ear vein line. A tracheostomy was performed and artificial

ventilation instituted through an indwelling 6 mm inner diameter cuffed endotracheal tube, using a Servo ventilator in volume-cycled mode. The inspiratory time was 25% and inspiratory pause was 10% of inspiratory cycle with a positive end-expiratory pressure of 4 cm H_20. The inspired fraction of oxygen was 0.4. Muscle relaxation was achieved by 0.2 mg/kg pancuronium bromide given intravenously (i.v.) and anaesthesia maintained by continuous infusion of clomethiazole edisylas at 400 mg/h, pancuronium at 2 mg/h and fentanyl at 150 μg/h. The tidal volume was 10 ml/kg and respiratory frequency adjusted to maintain an end-tidal carbon dioxide tension between 5.2 and 5.6 kPa using a Capnomac Ultima analyzer. The respirator was disconnected from the animal during extracorporeal circulation with cardioplegia. The tidal volume was reduced to 5 ml/kg throughout the reperfusion with extracorporeal circulation. Body temperature, measured with a thermodilution catheter in the main pulmonary artery, was kept constant by means of a heated mattress (Duncker et al., 1996).

2.6. Catheterization

The common carotid artery and external jugular vein were exposed through a longitudinal incision along the right aspect of the trachea. A catheter (18 gauge) was inserted into the carotid artery and the jugular vein for blood sampling and pressure recordings. A thermodilution catheter was introduced into the jugular vein and advanced to pulmonary artery wedge position for measurement of cardiac output and pressures. Urine output was recorded by suprapubic bladder drainage.

2.7. Monitoring

Arterial blood gases, hematocrit, heart rate, systemic and pulmonary arterial pressures and cardiac output were continuously measured and registered on a direct digital writer.

2.8. Coronary occlusion

Regional myocardial ischemia was produced by a snaring of the left anterior descending coronary artery, at a level corresponding to the distal third of this artery (Fig. 1). A 3–0 prolene suture tightened by tetrafluoroethylene pledget (3 × 4 mm) and secured by a plastic tube was used for this maneuver, with care taken to preserve the adjacent return coronary vein. Regional myocardial ischemia was confirmed by visible cyanosis and cessation of contraction and systolic bulging of the myocardium corresponding to the occluded portion of the coronary artery. In order to prevent ventricular fibrillation, each animal received an i.v. bolus injection of lidocaine hydrochloride, 1 mg/kg, after sternotomy and an additional 1 mg/kg before coronary occlusion.

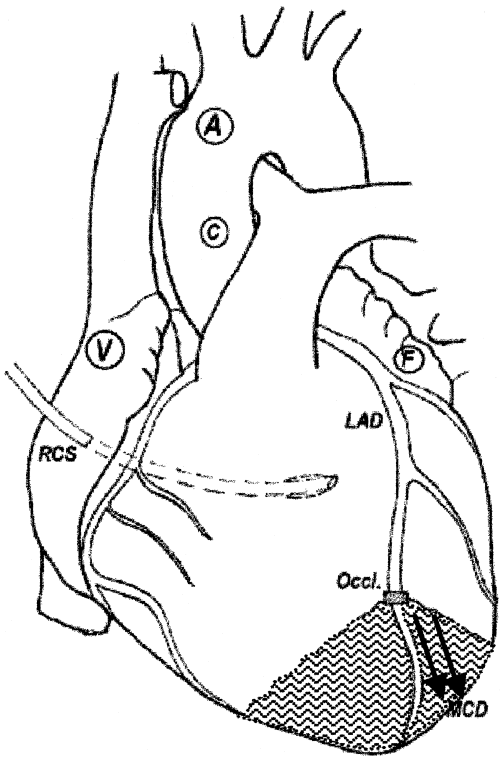

Fig. 1. View of experimental procedure. Occlusion (Occl) of the left anterior descending coronary artery (LAD) produced regional myocardial ischemia, as indicated by the shaded area at risk. Arrows correspond to tips of inserted microdialysis probes (MCD). RCS = retrograde coronary sinus cannula. Sites of aorta cannulation (A), venous return (V), cardioplegia (C) and flourescein injection (F) are shown. Further details are given in Section 2.11.

2.9. Time-schedule

A delay of 30 min was allowed following completion of animal preparation until the commencement of baseline measurements. The time course of the experiments then involved four different phases, I–IV (Fig. 2):

I. Coronary occlusion on beating heart—60 min.
II. Hypothermic (30°C) extracorporeal circulation and cardioplegia— 45 min.
III. Reperfusion and rewarming to 38°C on extracorporeal circulation— 30 min.
IV. Beating heart without support—60 min.

2.10. Extracorporeal circuit

The extracorporeal circuit consisted of a membrane oxygenator, a cardiotomy reservoir with filter, polyvinyl chloride tubings and a non-pulsatile pump.

2.11. Cardiopulmonary bypass

The pigs were connected to the extracorporeal circuit following median sternotomy and longitudinal pericar-

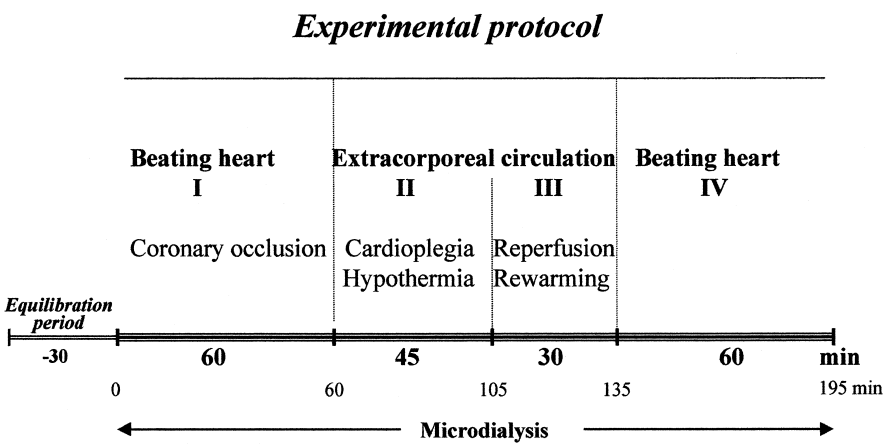

Fig. 2. Experimental protocol showing the time course of events comprising phases I–IV.

diotomy, with sodium heparin, 400 IU/kg, administered i.v. prior to cannulation. A single 28-French venous return cannula was inserted into the right atrial appendage and a 16-French cannula was introduced into the ascending aorta. Priming of the circuit was done with Ringer-acetate solution (600 ml), mannitol 15% (200 ml) and 2500 IU sodium heparin. Activated clotting time was maintained above 400 s throughout each experiment.

Cardiopulmonary bypass was conducted in a non-pulsatile fashion, at a flow rate of 64.2 ± 4.4 ml/kg/min limited by the venous return. As soon as optimal flow was established, artificial ventilation was stopped and the respirator disconnected from the endotracheal tube. Hypothermia at 30°C was induced by means of a thermal exchanger connected to the oxygenator and mean arterial pressure was maintained in the range of 40–72 (mean 52.9 ± 5.7) mm Hg, if necessary by an intravenous bolus of 50 μg epinephrine. To limit hemodilution (mean hematocrit 28.4 $\pm$ 1.2%), no further crystalloid solutions or allogenic blood were given. At the end of the bypass, all the pump prime was returned to the animal through the aortic cannula.

2.12. Cardioplegia

The aorta was cross-clamped and cardioplegic solution (4°C) was injected into the aortic root until cardiac arrest. A minimum of 15 ml/kg was given and repeated to abolish recurrent cardiac activity (total 18.3 ± 1.9 ml/kg).

2.13. Revascularisation

Revascularisation was "simulated" by removing the coronary occlusion.

2.14. Reperfusion

Artificial ventilation was re-instituted, rewarming to 38°C was started and the aortic cross-clamp removed. Hearts not resuming spontaneous contraction were electrically defibrillated.

2.15. Microdialysis technique

The microdialysis technique is based on the general principle of dialysis, though adapted to microscale under in vivo conditions (Lindroth and Mopper, 1979). Hereby, a separation of non-diffusable macromolecules from diffusable ions and low molecular weight compounds is achieved by means of a semi-permeable membrane with a molecular cut-off value of about 20 kDa. This technique permits continuous monitoring of low molecular constituents of the extracellular fluid, i.e. the interstitial fluid, with minimal perturbation of the normal cell physiologic state after the equilibration period. Since the microdialysate represents the interstitial fluid, it is possible to study the organ-related shift in metabolite and ion distribution reflecting dynamic cellular events such as different degrees of energy crisis due to ischemia. The microdialysis equipment consisted of the CMA/20 microdialysis probe with an outer diameter of 0.5 mm and a 10-mm flexible membrane. A CMA microdialysis pump was used with three syringes. Before use, all probes were checked by visual inspection for air bubbles, and before the experiments started, sterile water was infused for probe preservation and for detection of possible probe leakage.

2.15.1. Probe insertion

The probes were inserted into the myocardium at baseline according to a modified Seldinger technique (Seldinger, 1953), in order to minimize mechanical injury to the microdialysis membrane. Two probes (Fig. 1) were placed into the area at risk and administration was started immediately (one containing dipyridamole dissolved in dialysis buffer, the other plain dialysis buffer). Of 10 operated animals, the technical outcome was good in 7, which constituted the basis for the statistical analysis. The

probes in the three other experiments were not adequately positioned within the ischemic area, as judged by triphenyl-tetrazolium chloride staining (see below) and microdialysis data.

2.15.2. Microdialysis fluid

Inserted probes were perfused with a modified Krebs–Ringer phosphate buffer at a flow rate of 2 μl/min and the dead space in the tubing system was 10 μl. Dipyridamole was added in a final concentration of 99.1 μM to the buffer perfusing one of the probes in the area at risk. The dipyridamole buffer was prepared and filtered immediately before the experiment from a slightly acid dipyridamole stock solution. An equilibration period of 30 min was allowed after probe insertion until sampling in order to guarantee baseline values of interstitial fluid (Mori et al., 1998). Samples were taken at 10 min intervals.

2.15.3. Biochemical analyses

Microdialysate samples were analyzed by high performance liquid chromatography. Lactate and pyruvate were separated on a Polypore H column, 250 × 4.6 mm (particle size 10 μm). Sulphuric acid (2.5 mM) was used as eluant and the eluate was read at 214 nm on a BAS UV detector with a flow rate of 0.4 ml/min. Adenosine, inosine and hypoxanthine were separated on a Nucleosil 100 C 18 column, 100 × 4.0 mm (particle size 3 μm) by elution at a flow rate of 0.8 ml/min with a 10-mM phosphate buffer, pH 6.1, containing 8% methanol, and the eluate was read at 254 nm on the BAS UV detector. Taurine was separated after precolumn derivatization with orthophtaldialdehyde (Nucleosil 100 C 18 column, 60 × 4.0 mm) and measured by a fluorescence detector. All peaks were well separated from each other and the position of each metabolite in the chromatogram corresponded exactly with that of respective standard solutions.

2.16. Estimation of infarct size and probe position

Estimation of the infarct size was accomplished by means of planimetric comparison of myocardium, which had lost activity of the lactate dehydrogenase enzyme system with myocardium virtually devoid of direct coronary artery perfusion (Jestädt and Sandritter, 1959; Lie et al., 1975; Boor and Reynolds, 1977; Fishbein et al., 1981; Reimer et al., 1985). At the end of the experiment, the left anterior descending coronary artery was re-occluded and 2 ml fluorescein was injected into the left atrium (Fig. 1) and the heart was allowed to beat for 30 s in order to guarantee uniform dyeing of the perfused, viable myocardium. The accuracy of triphenyl-tetrazolium chloride staining is dependent upon washout of dehydrogenase enzymes from necrotic myocardium. The animals were sacrificed by an injection of potassium chloride (Close et al., 1996). The heart was rapidly excised, rinsed in saline solution, filled with agarose gel and placed in a 7.5 × 8 × 11-cm rectangular, translucent plastic box designed to accommodate the heart in longitudinal fashion with the occluded coronary artery positioned posteriorly. Then the box was filled with agarose and following stabilization of the gel the myocardium was cut into four 9 mm slices, from apex in cranial direction facing the atrioventricular valve plane.

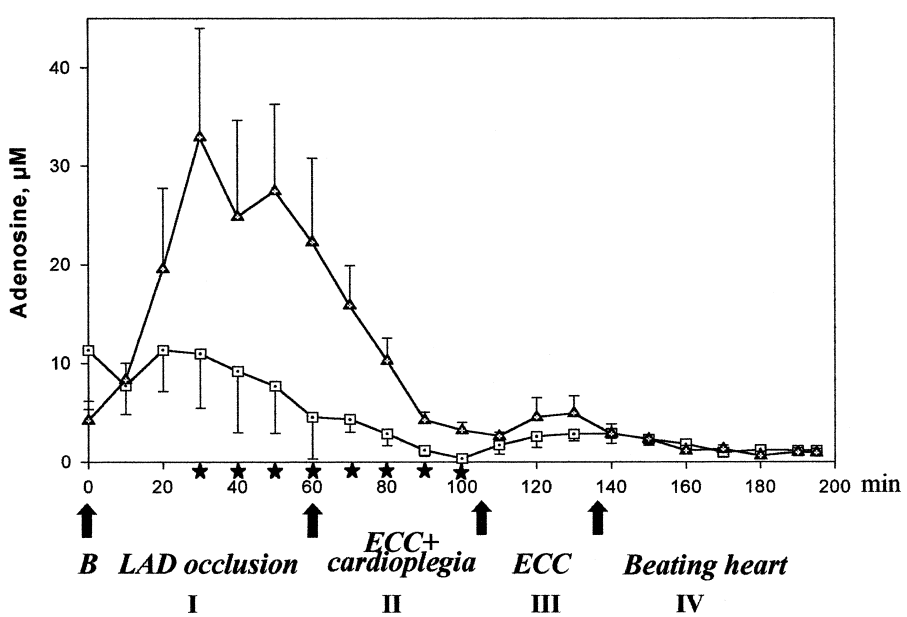

Fig. 3. Time course of changes in microdialysate *adenosine* concentration in the ischemic myocardium in a probe with (triangle) and without (dotted squares) administered dipyridamole. *X*-axis expresses time in min. Vertical bars indicate 1 S.E.M. ★ denotes a statistically significant difference ($P < 0.05$) between values at particular timing. B = baseline. LAD = left anterior descending coronary artery. ECC = extracorporeal circulation.

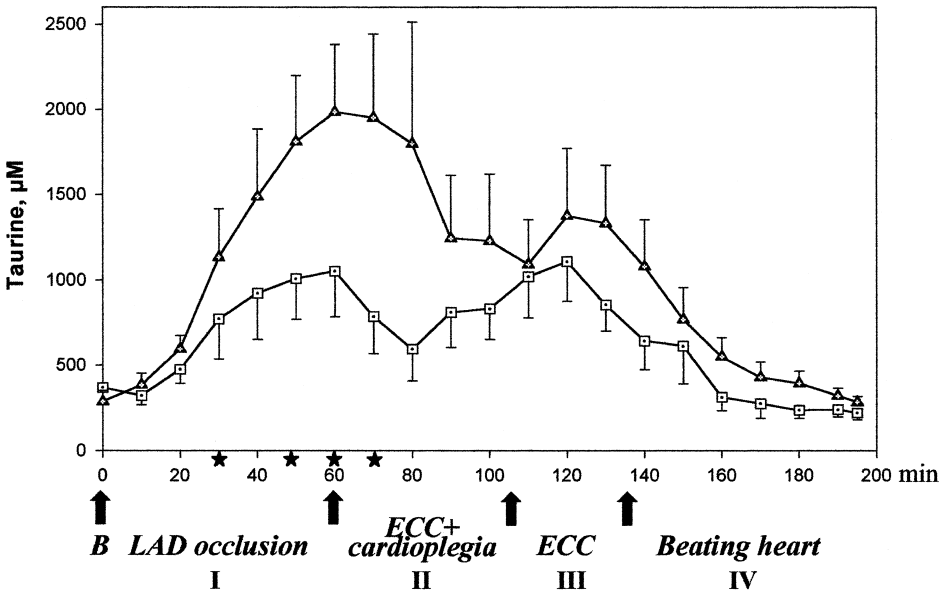

Fig. 4. Time course of changes in microdialysate *taurine* concentration in the ischemic myocardium. Symbols as in Fig. 3.

This was accomplished by retracting the short end walls of the box and pushing the heart in the agarose gel block along an underneath-positioned millimeter-scale. The surface of each slice facing the atrioventricular plane was photographed in daylight and in ultraviolet light. Delineation of viable myocardium was clearly appreciated because the area at risk was non-fluorescent in ultraviolet light. The infarcted area was visualized following 20 min immersion of the slices in triphenyl-tetrazolium chloride, 1% at 37°C. Tetrazolium dyes formed coloured precipitates in the presence of intact dehydrogenase enzyme systems. Hence, viable myocardium was delineated by a bright red appearance whereas areas of myocardial necrosis lacked dehydrogenase activity (provided the washout was satisfactory) and therefore failed to stain. Such necrotic areas were distinctly discernible and thus quantifiable. The slices were again photographed in the manner described above. The quantification of the area at risk and estimation of the infarcted area were performed according to previously described technique (Zemgulis et al., 2000). In brief, my-

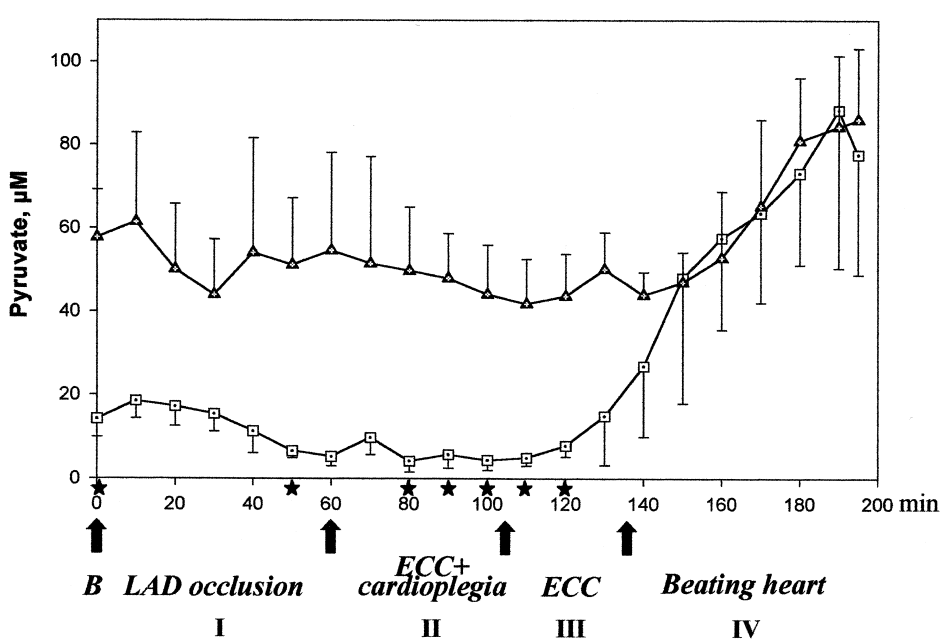

Fig. 5. Time course of changes in microdialysate *pyruvate* concentration in the ischemic myocardium. Symbols as in Fig. 3.

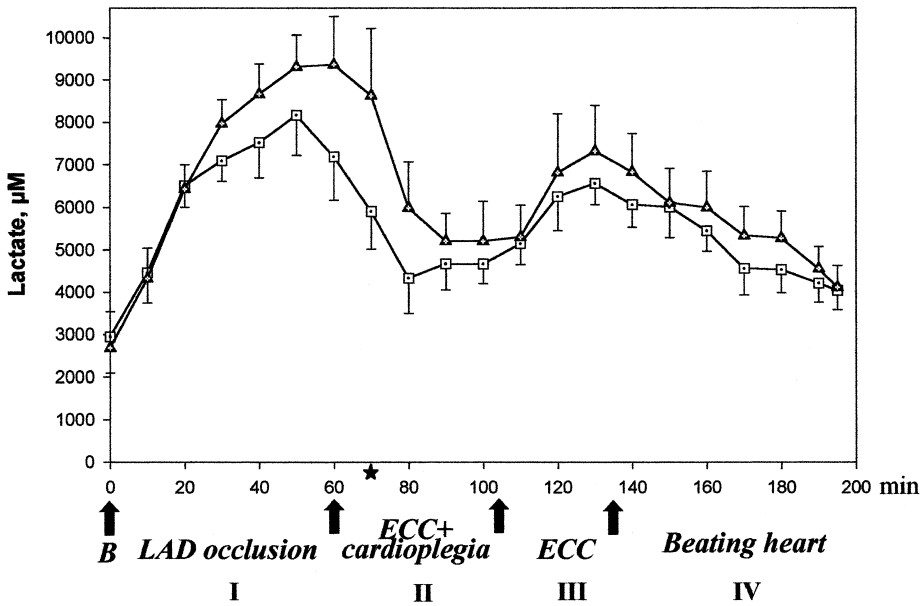

Fig. 6. Time course of changes in microdialysate *lactate* concentration in the ischemic myocardium. Symbols as in Fig. 3.

ocardial area at risk and infarct size were quantified with the modified topographical evaluation methods using transparent plastic sheets, image-wave and/or diascanner.

2.17. Statistical analysis

Data in the text, tables and figures are presented as means ± 1 S.E.M. Physiological variables were compared before and during coronary occlusion, during cardiopulmonary bypass and after extracorporeal circulation on beating heart without support. Analyses were made with Student's *t*-test for dependent samples. They were also compared with Wilcoxon matched pair test and subjected to ANOVA. A P-value < 0.05 was considered statistically significant. All data were analysed with the aid of Statistica™ for Windows.

3. Results

3.1. Microdialysis probe position

The position of the microdialysis probe channels in infarcted area was verified after triphenyl-tetrazolium chloride staining (Zemgulis et al., 2000).

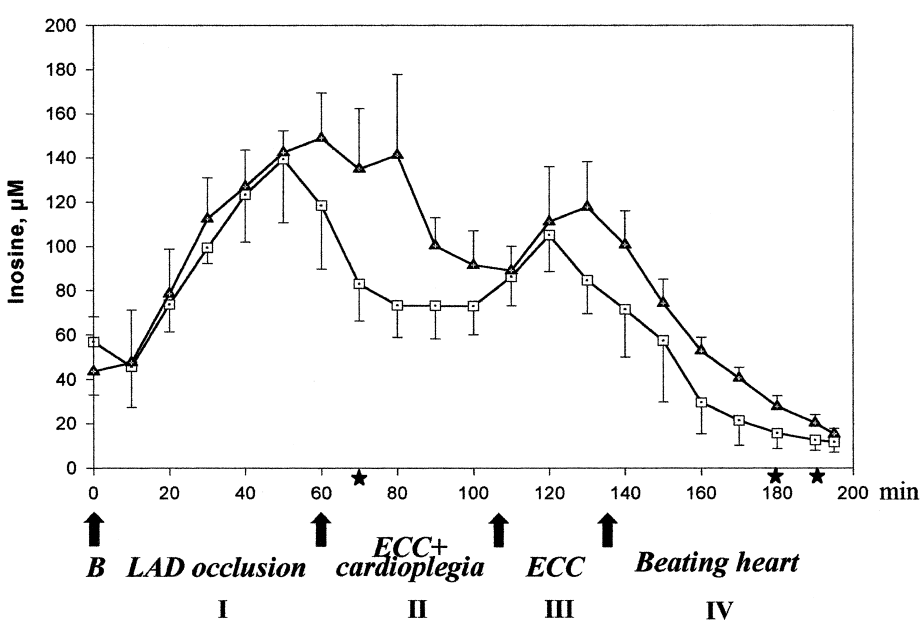

Fig. 7. Time course of changes in microdialysate *inosine* concentration in the ischemic myocardium. Symbols as in Fig. 3.

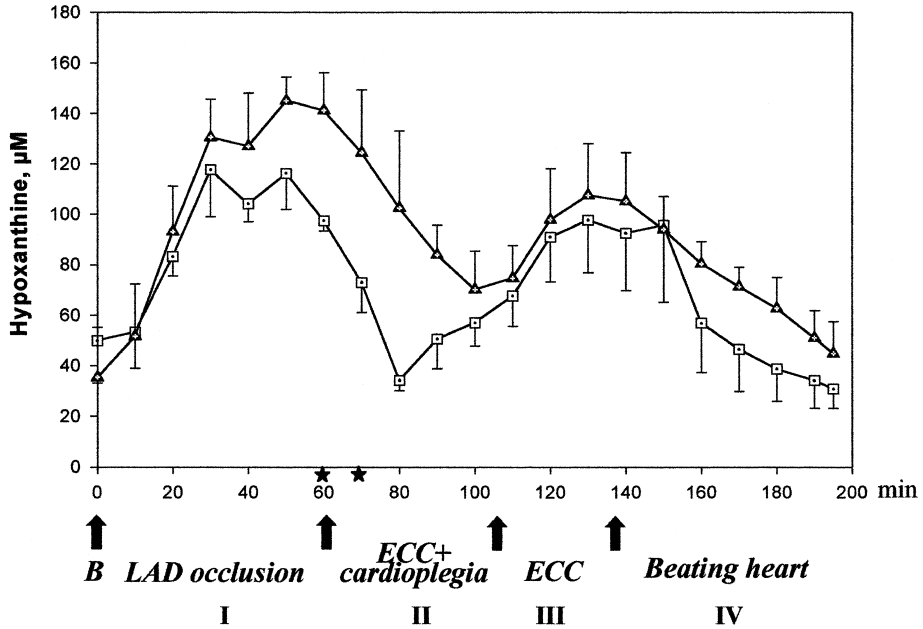

Fig. 8. Time course of changes in microdialysate *hypoxanthine* concentration in the ischemic myocardium. Symbols as in Fig. 3.

3.2. Adenosine

The two probes both monitored energy-related metabolites in ischemic myocardium, one administering dipyridamole, the other not. Adenosine microdialysate concentration of the dipyridamole probe was significantly higher during the periods of coronary occlusion and extracorporeal circulation with cardioplegia (Fig. 3, phases I–II). This was in favour of the adopted view that dipyridamole is an inhibitor of the adenosine influx into the myocardial cells. This discrepant findings between the two probes was not apparent during the last two periods of reperfusion with and without extracorporeal circulation (Fig. 3, phases III–IV).

3.3. Taurine

Taurine was found in significantly higher concentration in the microdialysate of dipyridamole probe and this was evident for the two first periods (Fig. 4, phases I–II). Typically, a distinct rise of taurine was observed after 20 min of coronary occlusion, i.e. the time period for the

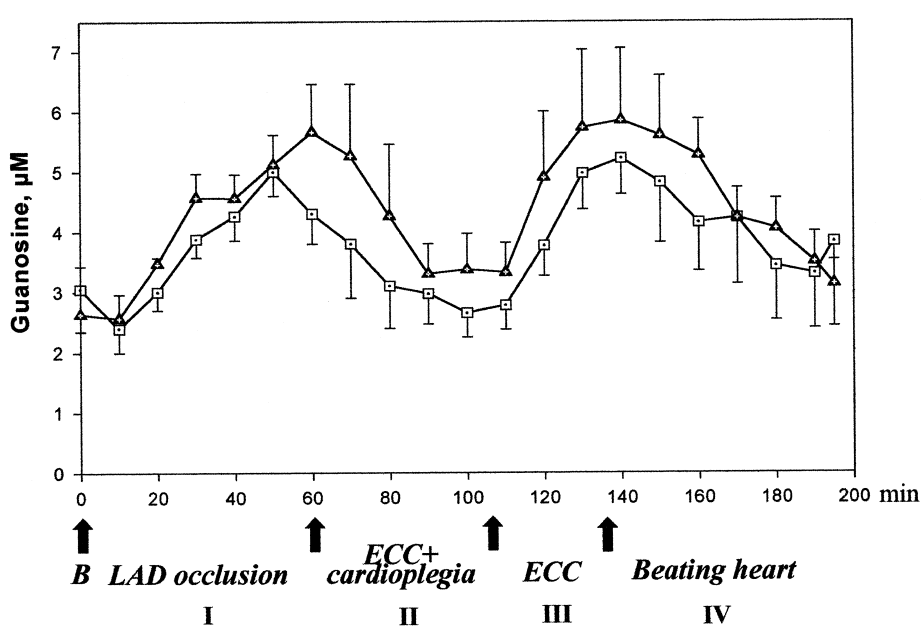

Fig. 9. Time course of changes in microdialysate *guanosine* concentration in the ischemic myocardium. Symbols as in Fig. 3.

transition from reversible to irreversible myocardial ischemic injury (Jennings et al., 1969). Most conspicuous was also the diminished discrepancy between the two probes regarding taurine during the last two periods (Fig. 4, phases III–IV), which was analogous to adenosine (Fig. 3).

3.4. Pyruvate, lactate and purine base derivatives

The level of pyruvate microdialysate concentration was distinctly higher for the dipyridamole probe in the beginning of the experiment. It means that ischemia was not a necessary prerequisite for this phenomenon. The difference between the two probes regarding pyruvate was kept steadily without no gross alterations during the first three periods (Fig. 5, phases I–III) and they did not converge until the last period (Fig. 5, phase IV).

Opposite to pyruvate, the lactate profiles of the two probes (Fig. 6) were similar with only minor discrepancies and a typical biphasic appearance was noted in agreement with a previous study under the same experimental conditions (Zemgulis et al., 2000). A similar tendency was also seen for the three purine base derivatives (Figs. 7–9). However, it should be noted that both inosine and hypoxanthine concentrations in microdialysate were significantly higher in the dipyridamole probe during extracorporeal circulation with cardioplegia (Figs. 7 and 8, phase II).

4. Discussion

The present study shows that adenosine transport can be inhibited in ischemic myocardium by dipyridamole administered at a low concentration via the microdialysis probe. This compound has been used extensively for clinical purposes, especially in the cardiovascular system. However, its effectiveness depends on the tissue under study (Plagemann and Wohlhueter, 1984; Miras-Portugal et al., 1986; Sanchez-Olea et al., 1996; Phillis et al., 1997) and its actual concentration (Banker et al., 1991; Satoh, 1996; Tommasi et al., 2000).

4.1. Dipyridamole affects adenosine and taurine microdialysate concentration in ischemic myocardium

We confirm previous studies that the interstitial levels of adenosine are increased during myocardial ischemia (Wikström et al., 1995a,b; Harrison et al., 1998; Zemgulis et al., 2000). The cellular origin of the adenosine has been under debate and the participation of *ecto* 5′-nucleotidase cannot be ruled out (Abd-Elfattah et al., 1990; Harrison et al., 1998; Kuzmin et al., 1998; Obata et al., 1998; Deussen et al., 1999). It appears, however, that most adenosine (83%) has an intracellular origin while a minor part (8–11%) is formed by *ecto* 5′-nucleotidase (Deussen et al., 1999). The increased levels of adenosine on dipyridamole administration were indicative of an inhibitory action on the adenosine cellular trapping mechanism. Taurine, although being shifted in time by about 20 min, showed a similar curve profile as adenosine under these conditions. Hence, increased levels of both adenosine and taurine were the result of the ischemic trauma and a further increase of these substances was provoked by dipyridamole. While adenosine increase by dipyridamole was expected, the corresponding increase of taurine concentration was not. This alteration of the taurine level was not merely an unrelated consequence of the surgical procedure, since taurine data from a microdialysis probe in non-ischemic myocardium did not show any increase of taurine level (data not shown). Further, one could argue that alterations in haemodynamic variables caused by dipyridamole would lead to a rise in taurine concentration. However, such was not the case since we monitored these changes by two microdialysis probes, inserted in myocardium as given in Fig. 1. Hence, the area at risk was subjected to the same alterations in haemodynamic variables, but still the taurine augmentation was only observed in the microdialysate of the probe given dipyridamole. Although the dipyridamole effect is not totally specific, since it has also a blocking effect on the Cl^- channel (Sanchez-Olea et al., 1996; Pasantes-Morales et al., 1997; Moran et al., 1997) and on hypoxanthine transport (Plagemann and Wohlhueter, 1984; Wang et al., 1992), there is hitherto no known effect by dipyridamole on the taurine transporter in myocardium.

4.2. Taurine transport systems and clinical implications

Three different taurine transport systems have been recognized: the taurine transport via specific receptors (Huxtable and Chubb, 1977; Chovan et al., 1980; Yudilevich, 1989; Lang et al., 1999); the Na^+/taurine symport system, where the sodium gradient energizes the concentrative intracellular uptake of taurine (Suleiman et al., 1992, 1997; Satoh, 1998); a third system has been suggested for the taurine efflux, the Na^+-independent diffusion mechanism of release, i.e. along its chemical gradient (Sanchez-Olea et al., 1991; Rasmusson et al., 1993; Moran et al., 1997; Phillis et al., 1997).

The Na^+/taurine symport system has been claimed to be bidirectional (Suleiman et al., 1992). Therefore, it has been proposed that Na^+/taurine may escape from the myocytes via this route (where in fact the downhill taurine chemical gradient energizes the uphill efflux of Na^+), which could be beneficial to prevent intracellular Ca^{2+} overload (Suleiman et al., 1997; Satoh, 1998, 1999; Ascione et al., 1998). Accordingly, it has been speculated that taurine exerts this positive effect in modifying susceptibility to heart cell injury due to Ca^{2+} overload in certain animal models (Ohta et al., 1986, 1988; Liu et al., 1998; Satoh, 1999). This would be accomplished by the taurine/Na^+ symport out of the myocyte followed by a Na^+/Ca^{2+} antiport to achieve a Ca^{2+} unloading of the

cell. The final result would then be an increased extracellular concentration of taurine as a consequence of the Na^+/Ca^{2+} antiport system to prevent Ca^{2+} overload. We noticed a further increase of taurine in presence of dipyridamole. The reason for this is not clear at present. The explanation for this increased concentration of taurine by dipyridamole could be either a primary effect on the taurine carrier in the sarcolemma membrane or secondary to the inhibited adenosine trapping.

As regards primary effects of dipyridamole, it is known that not only the nucleoside carrier is affected but also the glucose carrier (Abdel Aleem et al., 1991; Kalsi et al., 1998; Tommasi et al., 2000). Furthermore, some amino acids, and among them taurine, are known to be involved in cellular volume regulatory systems in which the Cl^- channel may be participating. As a matter of fact, taurine swelling-activated efflux was inhibited by dipyridamole via the Cl^- channel (Sanchez-Olea et al., 1996; Pasantes-Morales et al., 1997; Phillis et al., 1997; Moran et al., 1997).

The consequences of inhibition of the adenosine trapping by dipyridamole are complex and related to deteriorated cellular energy metabolism. Since adenosine improves mitochondrial oxidative efficiency (Babsky et al., 1998), the deprivation of intracellular adenosine results in accelerated ATP depletion (Banker et al., 1991; Harrison et al., 1998). This would lead to an exacerbated ischemic damage including an increased influx of Ca^{2+} activating the taurine/Na^+ symport system. Hence, we do not know whether the increased taurine seen in our experimental model is the result of only one or both transport systems being operative.

The metabolic effects of taurine, which is not used in ribosomal protein synthesis, are not fully known. There have been some speculations that taurine may affect the Ca^{2+}-ATPase of the sarcoplasmic reticulum (Schaffer et al., 1992) and the Na^+/K^+-ATPase of the plasma membrane itself (Mankovskaya et al., 1998). Indeed, taurine directly modulates the open probability of the K^+ ATP channel (Satoh, 1996), which influences the free radical formation (Obata and Yamanaka, 1998). Therefore, taurine may affect the sarcoplasmic reticulum Ca^{2+}-ATPase (Xu et al., 1997) and possess enzyme protective effect against myeloperoxidase systems (Gutierrez-Correa and Stoppani, 1999).

Further, a membrane derangement has been accounted for taurine in that this β-amino acid can inhibit phospholipid *N*-methyltransferase, which catalyses the conversion of membrane phosphatidylethanolamine to phosphatidylcholine (Hamaguchi et al., 1991; Punna et al., 1994), which may influence membrane microarchitecture and function. Of considerable interest is the recent proposal (Labudova et al., 1999) that taurine can upregulate a branched amino acid-transporter in the myocardial sarcolemma, which in turn may be functionally coupled to the carbohydrate-branched chain amino acid metabolic pathway. As a matter of fact, there is evidence that some of the actions of taurine may be linked to insulin and its effects on phosphofruktokinase (Lampson et al., 1983), which is the allosterically regulated enzyme of glycolysis. The net effect of taurine on the heart is therefore a stimulation of glucose utilization. This should be regarded in the context of adenosine being a stimulator of glucose uptake independent of its vasodilatory effect, thus facilitating the glycolytic flux in ischemic myocardium (Law and Raymond, 1988; Wyatt et al., 1989; Tommasi et al., 2000). We therefore anticipate that the increased pyruvate levels in presence of dipyridamole was the direct effect of an enhanced glycolytic flux but with a concomitant altered pyruvate/lactate ratio. Still, a similar tendency was discernible as regards lactate.

In summary, we have observed an enhanced efflux of taurine in presence of dipyridamole in ischemic myocardium. Taurine may have a facilitating effect on glycolysis as noticed by increased levels of pyruvate and lactate. Further studies are warranted to investigate the possible beneficial effect of dipyridamole during myocardial ischemia and reperfusion.

Acknowledgements

This study was supported by grants from the Swedish Heart and Lung foundation, the Uppsala Läns Heart and Lung foundation, Erik, Karin and Gösta Selanders foundation, Akademiska Hospital development foundation, Josef and Linnéa Carlssons foundation. The authors are indebted to Lena Nalmo and Åke Ericson for the skilful technical assistance. We also thank Drs. Lena Håkansson and Anders Ericsson for the stimulating discussions.

References

Abd-Elfattah, A.S., Jessen, M.E., Hanan, S.A., Tuchy, G., Wechsler, S., 1990. Is adenosine 5′-triphosphate derangement or free-radical-mediated injury the major cause of ventricular dysfunction during reperfusion? Circulation 82, IV341–IV350.

Abd-Elfattah, A.-S.A., Hoehner, J., Wechsler, S., 1998. Identification of nucleoside transport bindings sites in the human myocardium. Mol. Cell. Biochem. 180, 105–110.

Abdel Aleem, S., Badr, M., Frangakis, C., 1991. Stimulation of fatty acid oxidation in myocytes by phosphodiesterase inhibitors and adenosine analogues. Life Sci. 48, 97–102.

Ascione, R., Gomes, W.J., Angelini, G.D., Bryan, A.J., Suleiman, M.-S., 1998. Warm blood cardioplegia reduces the fall in the intracellular concentration of taurine in the ischemic/reperfused heart of patients undergoing aortic valve surgery. Amino Acids 15, 339–350.

Babsky, A.M., Doliba, M.M., Doliba, N.M., Osbakken, M.D., 1998. Adenosine improves cardiomyocyte respiratory efficiency. Ukr. Biokhim. Zh. 70, 73–81.

Banker, M.C., Hicks, G.L., Tibi, P., Pearce, F., 1991. Metabolic and functional cardiac impairment after reperfusion with persantine. J. Surg. Res. 51, 154–157.

Boor, P.J., Reynolds, E.S., 1977. A simple planimetric method for

determination of left ventricular mass and necrotic myocardial mass in postmortem hearts. Am. J. Clin. Pathol. 68, 387–392.
Chovan, J.P., Kulakowski, E.C., Sheakowski, S., Schaffer, S.W., 1980. Calcium regulation by the low-affinity taurine binding sites of cardiac sarcolemma. Mol. Pharmacol. 17, 295–300.
Close, B., Banister, K., Baumans, V., Bernoth, E.-M., 1996. Euthanasia of experimental animals. European commision, DGXI. Regeringskansliets offsetcentral, Stockholm.
Deussen, A., Stappert, M., Schäfer, S., Kelm, M., 1999. Quantification of extracellular and intracellular adenosine production. Understanding the transmembranous concentration gradient. Circulation 99, 2041–2047.
Duncker, D.J., Klassen, C.L., Ishibashi, Y., Herrlinger, S.H., Pavek, T.J., Bache, R.J., 1996. Effect of temperature on myocardial infarction in swine. Am. J. Physiol. 270, H1189–H1199.
Figueredo, V.M., Diamond, I., Zhou, H.-Z., Camacho, S.A., 1999. Chronic dipyridamole therapy produces sustained protection against cardiac ischemia–reperfusion injury. Am. J. Physiol. 277, H2091–H2097.
Finegan, B.A., Lopaschuk, G.D., Coulson, C.S., Clanachan, A.S., 1993. Adenosine alters glucose use during ischemia and reperfusion in isolated rat hearts. Circulation 87, 900–908.
Fishbein, M.C., Meerbaum, S., Rit, J., Lando, U., Kanmatsuse, K., Mercier, J.C., Corday, E., Ganz, W., 1981. Early phase acute myocardial infarct size quantification: validation of the triphenyl tetrazolium chloride tissue enzyme staining technique. Am. Heart J. 101, 593–600.
Gutierrez-Correa, J., Stoppani, A.O.M., 1999. Inactivation of myocardial dihydrolipoamide dehydrogenase by myeloperoxidase systems: effect of halides, nitrite and thiol compounds. Free Radical Res. 30, 105–117.
Hamaguchi, T., Azuma, J., Schaffer, S., 1991. Interaction of taurine with methionine: inhibition of myocardial phospholipid methyltransferase. J. Cardiovasc. Pharmacol. 18, 224–230.
Harrison, G.J., Willis, R.J., Headrick, J.P., 1998. Extracellular adenosine levels and cellular energy metabolism in ischemically preconditioned rat heart. Cardiovasc. Res. 40, 74–87.
Huxtable, R., Chubb, J., 1977. Adrenergic stimulation of taurine transport by the heart. Science 198, 409–411.
Jarvis, S.M., Williams, T.C., Lee, C.W., Cheeseman, C.I., 1989. Active transport of nucleosides and drugs. Biochem. Soc. Trans. 17, 448–450.
Jennings, R.B., Sommers, H.M., Herdson, P.B., Kaltenbach, J.P., 1969. Ischemic injury of myocardium. Ann. N. Y. Acad. Sci. 156, 61–78.
Jestädt, V.R., Sandritter, W., 1959. Erfahrungen mit der TTC-(= Triphenyltetrazoliumchlorid-) Reaktion fur die pathologisch-anatomische Diagnose des frischen Herzinfarktes. Z. Kreislaufforsch. 48, 802–809.
Kalsi, K.K., Smolenski, R.T., Yacoub, M.H., 1998. Effects of nucleoside transport inhibitors and adenine/ribose supply on ATP concentration and adenosine production in cardiac myocytes. Mol. Cell. Biochem. 180, 193–199.
Kuzmin, A.I., Lakomkin, V.L., Kapelko, V.I., Vassort, G., 1998. Interstitial ATP level and degradation in control and postmyocardial infarcted rats. Am. J. Physiol. 275, C766–C771.
Labudova, O., Yeghiazarjan, C., Höger, H., Lubec, G., 1999. Taurine modulates expression of transporters in rat brain and heart. Amino Acids 17, 301–313.
Lampson, W.G., Kramer, J.H., Schaffer, S.W., 1983. Potentiation of the actions of insulin by taurine. Can. J. Physiol. Pharmacol. 61, 457–463.
Lang, F., Uhlemann, A.C., Lepple-Wienhues, A., Szabo, I., Siemen, D., Nilius, B., Gulbins, E., 1999. Cell volume regulatory mechanisms in apoptosis cell death. Herz 24, 232–235.
Law, W.R., Raymond, R.M., 1988. Adenosine potentiates insulin-stimulated myocardial glucose uptake in vivo. Am. J. Physiol. 254, H970–H975.
Lie, J.T., Pairolero, P.C., Holley, K.E., Titus, J.L., 1975. Macroscopic enzyme-mapping verification of large, homogenous, experimental myocardial infarcts of predictable size and location in dogs. J. Thorac. Cardiovasc. Surg. 69, 599–605.
Lindroth, P., Mopper, K., 1979. High performance liquid chromatographic determination of subpicomole amounts of amino acids by precolumn fluorescence derivatization with *o*-phthaldialdehyde. Anal. Chem. 51, 1667–1674.
Liu, G.-X., Yang, Y.-Z., Gu, Q.-B., Liu, Y.-H., Guo, Q., 1998. Effects of taurine on L-type voltage-dependent Ca^{2+} channel in rat cardiomyocytes infected with coxsackievirus B_{31}. Chung Kuo Yao Li Hsueh Pao/Chung-Kuo Yao Li Hsueh Pao [Acta Pharmacologica Sinica] 19, 238–240.
Mankovskaya, I.M., Seredenko, M.M., Vavilova, G.L., Kharlamova, O.N., Bystryukov, V.A., 1998. Antioxidant action of taurine in acute hypoxic hypoxia. Fiziol. Zh. 44, 65–72.
Miras-Portugal, M.T., Torres, M., Rotllan, P., Aunis, D., 1986. Adenosine transport in bovine chromaffin cells in culture. J. Biol. Chem. 261, 1712–1719.
Mohrman, D.E., Heller, L.J., 1990. Transcapillary adenosine transport in isolated guinea pig and rat hearts. Am. J. Physiol. 259, H772–H783.
Moran, J., Miranda, D., Pena-Segura, C., Pasantes-Morales, H., 1997. Volume regulation in NIH/3T3 cells not expressing P-glycoprotein: II. Chloride and amino acid fluxes. Am. J. Physiol. 272, C1804–C1809.
Mori, E., Haramaki, N., Ikeda, H., Imaizumi, T., 1998. Intra-coronary administration of L-arginine aggravates myocardial stunning through production of peroxynitrite in dogs. Cardiovasc. Res. 40, 113–123.
Obata, T., Yamanaka, Y., 1998. Glibenclamide, an antagonist of ATP sensitive K channels, blocks free radical generation in the rat myocardium. Neurosci. Lett. 257, 57–59.
Obata, T., Sato, T., Yamanaka, Y., Arita, M., 1998. NO and cGMP facilitate adenosine production in rat hearts via activation of ecto-5-nucleotidase. Pfluegers Arch. 436, 984–990.
Ohta, H., Azuma, J., Onishi, S., Awata, N., Takihara, K., Kishimoto, S., 1986. Protective effect of taurine against isoprenaline-induced myocardial damage. Basic Res. Cardiol. 81, 473–481.
Ohta, H., Azuma, J., Awata, N., Hamaguchi, T., Tanaka, Y., Sawamura, A., Kishimoto, S., Sperelakis, N., 1988. Mechanism of the protective action of taurine against isoprenaline induced myocardial damage. Cardiovasc. Res. 22, 407–413.
Pasantes-Morales, H., Sanchez Olea, R., Miranda, D., Moran, J., 1997. Volume regulation in NIH/3T3 cells not expressing P-glycoprotein: I. Regulatory volume decrease. Am. J. Physiol. 272, C1798–C1803.
Phillis, J.W., Song, D., O'Regan, M.H., 1997. Inhibition by anion channel blockers of ischemia-evoked release of excitotoxic and other amino acids from rat cerebral cortex. Brain Res. 758, 9–16.
Piper, H.M., Garcia-Dorado, D., Ovize, M., 1998. A fresh look at reperfusion injury. Cardiovasc. Res. 38, 291–300.
Plagemann, P.G.W., Wohlhueter, R.M., 1984. Hypoxanthine transport in mammalian cells: cell type-specific differences in sensitivity to inhibition by dipyridamole and uridine. J. Membr. Biol. 81, 255–262.
Punna, S., Ballard, C., Hamaguchi, T., Azuma, J., Schaffer, S., 1994. Effect of taurine and methionine on sarcoplasmic reticular Ca^{2+} transport and phospholipid methyltransferase activity. J. Cardiovasc. Pharmacol. 24, 286–292.
Rasmusson, R.L., Davis, D.G., Lieberman, M., 1993. Amino acid loss during volume regulatory decrease in cultured chick heart cells. Am. J. Physiol. 264, C136–C145.
Reimer, K.A., Jennings, R.B., Cobb, F.R., Murdock, R.H., Greenfield, J.C., Becker, L.C., Bulkley, B.H., Hutchins, G.M., Schwartz, R.P., Bailey, K.R., Passamani, E.R., 1985. Animal models for protecting ischemic myocardium: results of the NHLBI cooperative study. Comparison of unconscious and conscious dog models. Circ. Res. 56, 651–665.
Sanchez-Olea, R., Moran, J., Schousboe, A., Pasantes-Morales, H., 1991. Hyposmolarity-activated fluxes of taurine in astrocytes are mediated by diffusion. Neurosci. Lett. 130, 233–236.
Sanchez-Olea, R., Morales, M., Garcia, O., Pasantes-Morales, H., 1996. Cl channel blockers inhibit the volume-activated efflux of Cl and taurine in cultured neurons. Am. J. Physiol. 270, C1703–C1708.
Satoh, H., 1996. Direct inhibition by taurine of the ATP-sensitive K^+

channel in guinea pig ventricular cardiomyocytes. Gen. Pharmacol. 27, 625–627.

Satoh, H., 1998. Cardiac actions of taurine as a modulator of the ion channels. Adv. Exp. Med. Biol. 442, 121–128.

Satoh, H., 1999. Taurine modulates IKr but not IKs in guinea-pig ventricular cardiomyocytes. Br. J. Pharmacol. 126, 87–92.

Schaffer, S.W., Punna, S., Duan, J., Hamaguchi, T., Azuma, J., 1992. Mechanism underlying physiological modulation of myocardial contraction by taurin. In: Lambardini, J.B., Schaffer, S.W., Azuma, J. (Eds.), Taurine: Nutritional Value and Mechanisms of Action. Plenum, New York, pp. 193–198.

Seldinger, S.I., 1953. Catheter replacement of the needle in percutaneus arteriography. Acta Radiol. 39, 368–376.

Suleiman, M.S., Rodrigo, G.C., Chapman, R.A., 1992. Interdependence of intracellular taurine and sodium in guinea pig heart. Cardiovasc. Res. 26, 897–905.

Suleiman, M.S., Moffatt, A.C., Dihmis, W.C., Caputo, M., Hutter, J.A., Angelini, G.D., Bryan, A.J., 1997. Effect of ischaemia 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.

Tommasi, S., Carluccio, E., Bentivoglio, M., Corea, L., Picano, E., 2000. Low-dose dipyridamole infusion acutely increases exercise capacity in angina pectoris. A double-blind placebo controlled crossover stress echocardiographic study. J. Am. Coll. Cardiol. 35, 83–88.

Wang, T., Mentzer, R.M., Van Wylen, D.G.L., 1992. Interstitial adenosine with dipyridamole: effect of adenosine receptor blockade and adenosine deaminase. Am. J. Physiol. 263, H552–H558.

Wikström, B.G., Ronquist, G., Waldenström, A., 1995a. Dynamics of myocardial metabolism in the preconditioned porcine heart studied using continuous microdialysis. Eur. Heart J. 16, 563–569.

Wikström, G., Ronquist, G., Nilsson, S., Maripu, E., Waldenström, A., 1995b. Continous monitoring of energy metabolites using microdialysis during myocardial ischaemia in the pig. Eur. Heart J. 16, 339–347.

Wyatt, D.A., Edmunds, M.C., Rubio, R., Berne, R.M., Lasley, R.D., Mentzer, R.M., 1989. Adenosine stimulates glycolytic flux in isolated perfused rat hearts by A_1-adenosine receptors. Am. J. Physiol. 257, H1952–H1957.

Xu, K.Y., Zweier, J.L., Becker, L.C., 1997. Hydroxyl radical inhibits sarcoplasmic reticulum Ca^{2+}-ATPase function by direct attack on the ATP binding site. Circ. Res. 80, 76–81.

Yudilevich, D.L., 1989. Blood-tissue transport of substrates in the heart: studies by single circulation tracer dilution. Int. J. Microcirc.: Clin. Exp. 8, 397–409.

Zemgulis, V., Ronquist, G., Bjerner, T., Henze, A., Waldenström, A., Thelin, S., Wikström, G., 2000. Energy-related metabolites during and after induced myocardial infarction with special emphasis on the reperfusion injury after ECC. Acta Physiol. Scand. (Accepted).