CELLULAR MONITORING IN

OPEN HEART SURGERY

Charles Kennergren
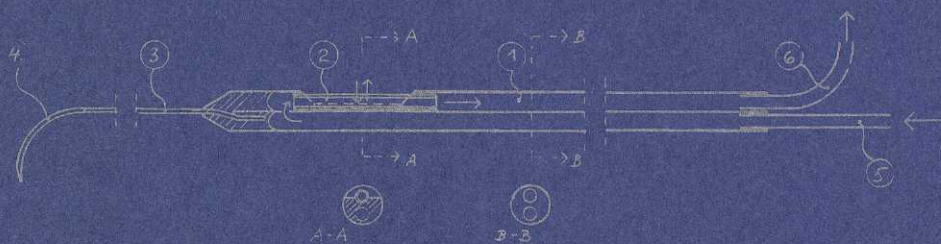
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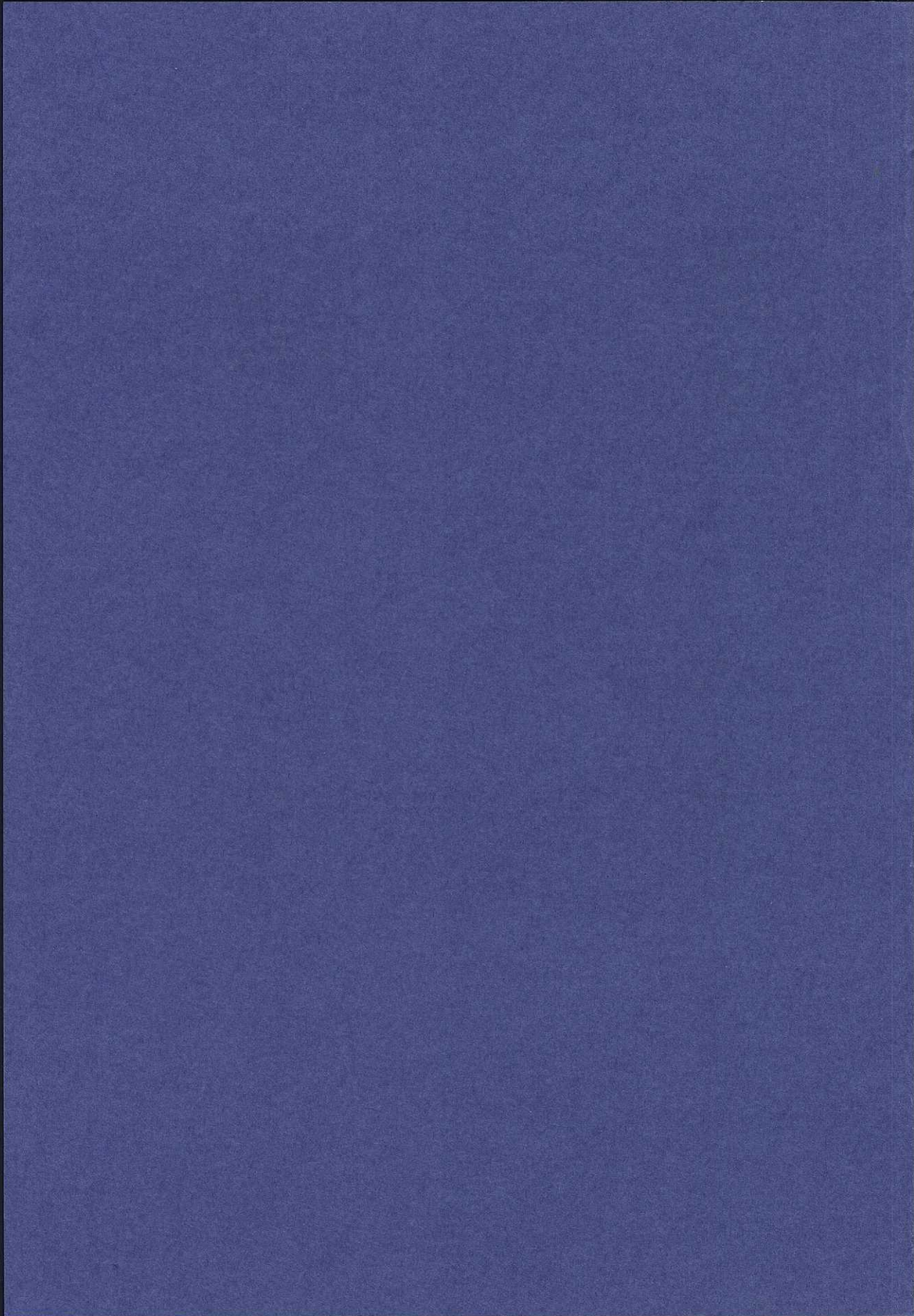
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Monitoring of markers for ischemia, free amino acids, glucose and lactate in the myocardial interstitial fluid before, during and after cardiac surgery using microdialysis technique

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen med vederbörligt tillstånd av medicinska fakulteten vid Göteborgs Universitet offentligen försvaras i aulan, Sahlgrenska Universitetssjukhuset, Göteborg, tisdagen den 6 juni 2000 kl 09.00
av

Charles Kennergren
Leg. läkare

Fakultetsopponent: Professor Urban Ungerstedt, Stockholm

Avhandlingen baseras på följande delarbeten:

I. C Kennergren, B Nyström, U Nyström, E Berglin, G Larsson, V Mantovani, P Lönnroth, A Hamberger
In situ detection of myocardial infarction in pig by measurements of aspartate aminotransferase (ASAT) activity in the interstitial fluid
Scand Cardiovasc J 31; 343-349, 1997

II. C Kennergren, V Mantovani, P Lönnroth, B Nyström, E Berglin, A Hamberger
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III. V Mantovani, C Kennergren, L Martinelli, R Gaeta, E Berglin, G Albonico, N Poggi, R Moratti, P Lönnroth, A Hamberger, M Vigano'
Intramyocardial Troponin-T monitoring with microdialysis in coronary artery by-pass surgery
Submitted

IV. C Kennergren, V Mantovani, P Lönnroth, B Nyström, E Berglin, A Hamberger
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ABSTRACT

Protection of the myocardium during open-heart surgery is a field of intense development. Numerous protocols have been proposed to minimize ischemia, since the first open-heart surgery more than 40 years ago. The evaluation is largely based on clinical outcome, since available methods lack the sensitivity required to determine the effects on myocardial oxygenation by small, but sometimes critical, methodological developments. We applied microdialysis to the myocardium with the aim to monitor the degree of ischemia from the concentrations of ASAT, troponin-T, free amino acids, glucose and lactate before, during and after cardioplegia. We also aimed to correlate postoperative events with the concentration of these markers. The myocardial interstitium of a mixed group of patients undergoing coronary artery by-pass grafting (CABG) and/or aortic valve surgery was monitored with a flexible microdialysis probe developed in our laboratory. The safety and function of the probe was first examined in an animal model for myocardial ischemia and then confirmed in man. Specific time courses were found in the interstitium for ASAT, troponin-T and several amino acids, during and after cardioplegia. Twenty and 300 times higher peak concentrations than in plasma were recorded in the interstitium for ASAT and troponin-T, respectively. The regulation of glucose and lactate was studied in the myocardial interstitium before, during and after cardioplegia in another group of patients undergoing CABG surgery. Concentrations of glucose and lactate were, to our knowledge, determined for the first time in the myocardial interstitium, with the use of internal calibration. Glucose was not critically reduced during cardioplegia, nor did lactate reach pathologically high concentrations. Even though transient elevations of marker levels coincided with postoperative clinical events in a number of patients, a larger population than was studied would be required for significant correlation. It is concluded that microdialysis sampling of the myocardial interstitial fluid is a safe procedure in clinical use. The approach has a potential for the evaluation of new technology for myocardial protection and for postoperative surveillance.

key words: cardiac surgery, myocardial ischemia, microdialysis, interstitial markers, ASAT, troponin-T, amino acids, glucose, lactate

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This thesis is based on the following original publications, which are referred to in the text by their roman numerals:

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ABBREVIATIONS

AI	arterio-interstitial
ASAT	aspartate aminotransferase
AV	arterio-venous
CABG	coronary artery bypass grafting
CK-MB	creatine kinase (heterodimer MB)
CT	computerized tomography
CX	circumflex coronary artery
ECC	extracorporeal circulation
ECG	electrocardiography
ECHO	echocardiography
EF	(left ventricular) ejection fraction
IHD	(patients with) ischemic heart disease
LAD	left anterior descending coronary artery
LD	lactate dehydrogenase
mol wt	molecular weight
MUGA	multiple uptake gated acquisition scan
NMR	nuclear magnetic resonance imaging
N-IHD	(patients with) aortic valve disease or atrial septal defect
PCA	principial component analysis
PET	positron emission tomography
TEE	transesophageal echocardiography

CONTENTS

I. INTRODUCTION

1. Background

A key issue in the development of open-heart surgery is to inflict little or no myocardial ischemic damage. Fifty years ago, Bigelow introduced the concept of whole body hypothermia and reported on recovery after 15 min of total circulatory arrest in animal experiments (Bigelow et al., 1950). Bigelow´s concept was subsequently introduced clinically (Lewis and Taufic, 1953). Shortcomings with respect to anesthesia, surgical techniques, materials and anticoagulation delayed the clinical introduction of extracorporeal circulation, even though equipment for this purpose had been developed already in the 19th century (Frey and Gruber, 1885). Before cardiopulmonary bypass was established, cross-oxygenation via the lungs of a relative had been tried to a limited extent (Warden and Lillehei, 1954). The first clinical use of a pump-oxygenator was for the closure of an atrial septal defect in 1951 (Dennis et al., 1951) and, in the following years, the field developed successfully (Gibbon 1953). In Sweden, Senning and Björk developed cardiopulmonary bypass in the early 1950s and Crafoord (Crafoord and and Senning, 1956) was the second in the world to employ the technique when removing a left atrial myxoma in 1954.

In the 1960s and early 1970s, intermittent clamping of the aorta was used in combination with moderate cardiac hypothermia. Continuous coronary perfusion with ventricular fibrillation improved oxygenation but involved a risk for air emboli and interacted with the surgery. Deep hypothermic cardiac arrest facilitates surgery but induces myocardial damage, since usually only part of the myocardium is cooled sufficiently (Kirklin and Barratt-Boyes, 1986). Despite these developments, both coronary bypass surgery (Brewer et al., 1973, Assad- Morell et al., 1975) and other open-heart surgery (Hultgren et al., 1973) involved considerable perioperative mortality and complications. Hyperosmolar cardioplegic solutions had been tried in the 1950s (Melrose et al., 1955), but were shown to be toxic to the myocardium and cause late cardiomyopathy. Cold cardioplegic solutions with high potassium content, introduced in the 1970s, were less toxic, as shown with myocardial biopsies (Braimbridge et al., 1977). This system for better protection therefore arrived timely and the development of anesthesia also contributed to the reduction in myocardial ischemia (Lell et al., 1977).

In the 1980s, most leading clinics had adopted moderate general hypothermia and intermittent cold cardioplegia, but the composition and administration of cardioplegic solutions remain a matter of argument also today. Blood containing cold and/or warm cardioplegic solutions are often used, beside crystalloid solutions (Caputo et al., 1997, Carrier et al., 1997) which are infused in the coronary arteries and/or retrogradely via the coronary sinus. Addition of free radical scavengers (Menasche et al., 1986, Dobsak et al., 1999) or similar compounds (Kjellman et al., 1997, Larrieu et al., 1987) to the solutions may further reduce myocardial ischemia and another promising approach, at least experimentally, is ischemic preconditioning of the heart (Baxter 1997, Jenkins et al., 1997). Presently available technology allows more patients to undergo bypass surgery without cardiopulmonary bypass, hypothermia or cardioplegia, but little information is available on the effects of this approach on the myocardium (Ascione et al., 1999).

Evaluation of methodologies is generally only done by clinical outcome, since a direct and accurate technique was not available for recording of myocardial metabolism.

2. Diagnosis of myocardial ischemia

2:1 Physiological and imaging techniques

Electrocardiography (ECG) diagnoses myocardial ischemia at rest and during exercise (Bjurö and Westling, 1985). ST-deviations, an ischemia-sensitive component of the ECG, reflect largely, but not entirely the concentration of potassium ions in the extracellular fluid. The degree of precordial ST deviations in early myocardial infarction is supposed to correlate with the extent of potassium accumulation and severity of myocardial ischemia (Goldman 1985), but complex geometrical factors influence the recordings. At best, early ST-deviations approximate the severity of ischemia (Opie 1998). While myocardial infarction may be diagnosed in the absence of ECG changes, a significant depression (> 2 mm) of the ST-segment during exercise is a reliable predictor of ischemic heart disease (Souhami and Moxham, 1994). However, only 2/3 of the patients with coronary artery disease have a significant ST-depression (Opie 1998). A significant Q wave (depth of 1/4 of the succeeding R-wave height or greater than 3 mV and lasting longer than 40 ms) is a reliable ECG indicator of infarction (Souhami and Moxham, 1994). The diagnostic specificity of ECG for myocardial ischemia during and after heart surgery is affected by factors such as pericardial fluid and myocardial edema, i.e. factors related to the surgical procedure (Kirklin and Barratt-Boyes, 1986).

Echocardiography (ECHO) is increasingly used during heart surgery (Click et al., 2000, Michel-Cherqui et al., 2000). The ventricular pump function is calculated from the stroke volume and cardiac output determined with echo Doppler, two-dimensional echocardiography and M-mode echocardiography. The ejection fraction (EF) of the left ventricle is calculated according to Teichholtz or Simpson (Feigenbaum 1986). During and after heart surgery, small EF changes, as well as regional changes in the motion of the cardiac wall are better measured with transesophageal echocardiography (TEE; Armstrong and Wastie, 1998). Empirically, EF as measured with ECHO may differ surprisingly from those obtained with angiography. Minor changes of heart function, especially slowly developing changes, may be better diagnosed with other methods (Schlant and Alexander, 1994).

The introduction of ultrafast computerized tomography (CT) in 1983 improved the diagnostic specificity of CT for a wide range of cardiac diseases (Pettersson et al., 1993). Ultrafast CT cine mode assesses biventricular global and regional systolic and diastolic function (Schlant and Alexander, 1994). The cine mode can also be employed to measure cardiac output, if a known amount of contrast is injected. The most important use of the cine mode is for assessment of regional and global myocardial blood flow. The sensitivity and specificity of the ultrafast CT method is probably equal to that of radionuclide methods (Brundage 1985). Both traditional and ultrafast CT are difficult to use during and directly after heart surgery, given the equipment required and the cost. Furthermore, CT lacks the sensitivity to identify minor changes in global ischemia.

Radionuclide techniques measure ventricular function (multiple uptake gated acquisition method, MUGA scan, ^{99m}Tc; Armstrong and Wastie, 1998) or document regions with

potentially reversible ischemia (^{201}Tl scan; Armstrong and Wastie, 1998). Positron emission tomography (PET) measures glucose utilization, fatty acid turnover and oxygen consumption, but may also detect coronary artery disease and assess myocardial viability (Knuuti et al., 1995, Souhami and Moxham, 1994). In spite of the advantages of radionuclide techniques, they are of limited use during and after cardiac surgery, again due to economy and logistics. In addition, time resolution, sensitivity and specificity need further investigation, if the methods shall be used for evaluation of surgical methods or for postoperative surveillance (Schlant and Alexander, 1994).

Nuclear magnetic resonance imaging (NMR) measures, with high precision, anatomical structures of the heart, especially the thickness of its wall (Armstrong and Wastie, 1998), but minor ischemic effects on the myocardium are not detected (Pettersson et al., 1993). The temporal resolution does not allow evaluation of ventricular function under stress (Schlant and Alexander, 1994). Again, the size and cost of NMR limits its use.

2:2 Biopsies

Endomyocardial biopsies are obtained fairly easily and safely and are suitable for the diagnosis of global processes, such as carcinoid heart disease, cardiac allograft rejection, amyloidosis and hemochromatosis. Focal ischemia is easily overlooked and dynamic changes are difficult to follow. The limited number of biopsies which can be taken and the time consuming tissue fixation process are drawbacks.

2:3 Sampling of blood and interstitial fluids

2:3:1 Blood

Samples from peripheral blood resolve efficiently the time course of changes for various markers, some of which have high organ specificity. Samples of blood from the coronary sinus appear to reflect, more directly, myocardial metabolism, but can only be collected for limited periods. They reflect the whole myocardium, unless a specific vein is sampled.

2:3:2 Interstitial fluid

Several techniques have been developed for sampling of the interstitial fluid of various organs. The "push-pull" technique administers and withdraws fluid at the same rate via a double lumen cannula (Gaddum 1961). Sampling of fluids over dialysis-membranes was introduced by Bito et al. (1966). Semipermeable sacs containing dextran-saline were implanted for a few hours in subcutaneous tissues, after which their contents were analyzed. Delgado et al. (1972) described the first perfused microdialysis system, a conventional push-pull cannula equipped with a permeable membrane.

In his pioneering work, Ungerstedt implanted thin tubes of microdialysis membrane into the brain for sampling of neurotransmitters (Ungerstedt and Pycock, 1974). A commercially available double lumen probe was later developed and is now available in a number of shapes (CMA Microdialysis, Stockholm, Sweden). Hamberger et al. (1985) employed a transversely implanted probe for the brain, which sampled the dorsal hippocampus. The clinical application of microdialysis has developed intensely during the last decade. Lönnroth et al. monitored glucose metabolism in human adipose tissue (Lönnroth et al., 1987) and a number

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of organs including skin, brain, skeletal and heart muscle has been investigated with microdialysis (Andersson 1995, Hamani et al., 1997, Müller et al., 1996, Habicht et al., 1998). The myocardium represents a particular challenge due to its continuous movement (Benveniste and Hüttemeyer, 1990).

3. Biochemical markers for myocardial ischemia

The concentration of marker compounds in blood plasma is essential for the diagnosis of myocardial infarction (Kattus et al., 1957, Agress 1959, Snodgrass et al., 1959). Soluble markers are released from damaged cells into the interstitium and diffuse from there into the systemic circulation. The usefulness of a marker is determined by factors such as its molecular weight, intracellular localization, rate of release/production, concentration gradients and clearance. Organ specificity obviously improves the diagnostic accuracy. The time courses in plasma differ considerably; a late peak makes early diagnosis difficult and an early peak with short duration may be missed. Extended presence of a marker in plasma facilitates late diagnosis but decreases time resolution. The distinction between for example unstable angina pectoris and myocardial infarction is often quantitative when based on marker levels. Distinction between reversible damage and necrosis may be critical for the outcome after open-heart surgery.

3:1 Enzymes

Aspartate aminotransferase (ASAT) catalyses the reaction between aspartate and alfa-keto-glutarate to form oxaloacetate and glutamate. It is found in two isoforms, mitochondrial ASAT (47 kDa) and cytoplasmatic ASAT (46 kDa). The latter predominates in plasma and has a half-life of approximately 20 h. Normally, the upper limit for ASAT in adults is 0.7 μkat/L. ASAT is not specific for the myocardium and is abundant in e.g. liver and skeletal muscle. Still, ASAT is a traditional marker for myocardial infarction, especially after cardiac surgery. In myocardial infarction, plasma ASAT starts to increase after about 12 h. A peak is reached at 24 - 36 h, and the activity then declines over 2 to 3 days. While ASAT increases 10 – 20 times in myocardial infarction and jaundice it increases more than 100 times the normal value after severe tissue damage, including acute hepatitis, crush injuries and tissue hypoxemia (Lentner 1984, Laurell 1991, Souhami and Moxham, 1994, Schlant and Alexander, 1994).

Lactate dehydrogenase (LD) is a family of enzymes that interconverts lactate and pyruvate. Five isoforms (LD_{1-5}) are found in plasma. LD_1 is a homotetramer (36 kDa, half-life approximately 100 h) and the predominant form in cardiac muscle. One or more of the other forms are found in most organs. After a slow onset, the peak in plasma is reached 2 - 3 days after a myocardial infarction. The normal upper limit for LD_1 is 0.5 – 3.0 μkat/L. LD_1 and to some extent LD_2 increases in some primary muscle disorders (Lentner 1984, Laurell 1991, Souhami and Moxham, 1994, Schlant and Alexander, 1994).

Creatine kinase (CK) catalyses the formation of phosphocreatine from ATP and creatine. In man, the enzyme consists of the M (43 kDa) and B (42 kDa) subunits and exists as the homodimers MM and BB or the heterodimer MB. The half-life of CK-MB in plasma is approximately 20 h and its upper limit is 5 - 10 μg/L, depending on the method. CK-MB is enriched in cardiac muscle (30% of total) relative to skeletal muscle (1% of total).

Consequently, elevated plasma levels are highly specific and CK-MB is considered to be "the gold standard" for the diagnosis of myocardial ischemia. CK-MB is found in plasma 3 - 9 h after presentation of pain and decreases to "normal" concentrations after 48 - 72 h. It peaks 12 - 36 h after a myocardial infarction. CK-MB also increases in plasma after extensive trauma to body muscles (Lentner 1984, Laurell 1991, Souhami and Moxham, 1994, Schlant and Alexander, 1994).

3:2 Structural proteins

Myoglobin is specific for skeletal and heart muscle and is similar to hemoglobin in many respects. Myoglobin is a peptide chain (17.5 kDa) with a half-life of 50 - 100 days in plasma. In the absence of myocardial damage, the plasma concentration is 30 - 70 µg/L for males and 25 - 45 µg/L for females. The concentrations correlate positively to body weight and muscle mass and inversely to glomerular filtration. Plasma myoglobin increases at about one hour after onset of a myocardial infarction and has a maximum at 3 - 15 h. Normal levels are approached within 2 - 3 days. The low specificity in plasma limits the clinical application of myoglobin (Lentner 1984, Laurell 1991, Souhami and Moxham, 1994, Schlant and Alexander, 1994).

Troponin T and I are myofibrillar proteins with a regulatory function in myocardial contraction. Troponin-T (38 kDa) and troponin-I (24 kDa) are both present in the thin myofibrils. In addition to this structurally bound pool, that releases troponin-T over several days following ischemia, troponin-T is present in a small cytosolic pool, that is released more rapidly. The troponins are even more sensitive markers for myocardial damage than CK-MB. The appearance in plasma starts from 3 - 9 h after onset of pain. Troponin-T remains in plasma for 6 - 14 days and troponin-I for 5 - 10 days. The specificity is very high, albeit certain unusual muscle diseases may activate the troponin-T gene in skeletal muscle. Renal insufficiency may also induce chronically increased plasma levels of troponin-T by a fully not understood mechanism. The troponins are useful for the diagnosis of myocardial infarction, especially in the period after the acute pain (Lentner 1984, Laurell 1991, Souhami and Moxham, 1994, Schlant and Alexander, 1994).
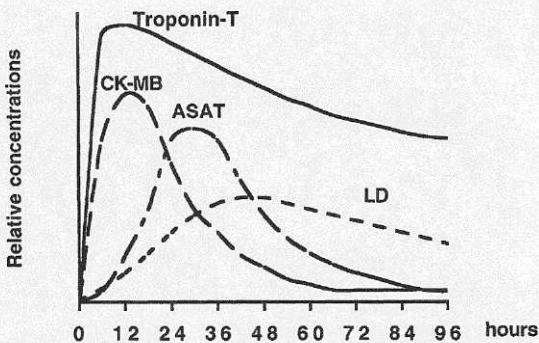

Fig. 1. Time courses and relative concentration changes of markers for ischemia in plasma: aspartate-aminotransferase (ASAT), creatine-kinase MB (CK-MB), lactate dehydrogenase (LD) and troponin-T.

II. AIM

To develop a technique for monitoring myocardial metabolism during cardiac surgery with increased sensitivity and time resolution.

Working plan:

1. To apply microdialysis for safe sampling of the myocardial interstitium in the clinical setting.

2. To monitor the myocardial interstitium with respect to:

    - ischemia triggered molecules

    - glucose and lactate

3. To correlate marker concentrations in the interstitial fluid with surgical procedures and clinical events.

III. MATERIALS AND METHODS

1. Patients

The local ethics committee approved of the studies and the patients gave their informed consent. The Radiation Safety Committee of the hospital approved of the use of isotopes in study V. Table 1 shows the clinical characteristics of the patients in papers II - V.

Table 1. Characteristics of the 45 patients included in the thesis. Seventeen patients participated in both study II and IV.

Paper	Diagnosis	N	Sex (F/M)	Age (Yr,)
II. Amino acids	IHD	9	2/7	60±3
	N-IHD	11	5/6	58±4
III. Troponin-T	IHD	7	0/7	55±4
IV. ASAT, Troponin-T	IHD	10	3/7	62±3
	N-IHD	12	5/7	59±4
V. Glucose, lactate	IHD	4	0/4	67±5
	N-IHD	9	3/6	59±5

IHD = ischemic heart disease
N-IHD = aortic valve disease or atrial septal defect

2. Microdialysis

2:1 Basic concepts

In the microdialysis approach, molecules diffuse over a semipermeable membrane in a fashion similar to that in a blood capillary. The membrane is mechanically stable and can be introduced and perfused within the myocardium in vivo. The degree of equilibration between the perfusing solution and the surrounding tissues depends on concentration gradients, flow rates, membrane area, membrane pore size and temperature (Jansson 1991). Diffusion takes place according to Avogadro´s law, i.e. each molecule diffuses over a semipermeable membrane independently of the diffusion of other molecules. The fraction of the total tissue volume that is easily diffusible is the interstitial fluid (Lindefors et al., 1989). Diffusion in tissues is considerably slower than in saline media and may also be affected by molecular interaction (Nicholson & Philips, 1981). Ultrafiltration (net flow of fluid) occurs when the

hydrostatic pressure is increased on one side of a membrane. Molecules larger than the nominal mol wt cut-off of the membrane remain largely in the tissue.

2:2 The probe

At the start of the present study, commercially available microdialysis probes did not fulfil our requirements, which were: 1. Flexibility, for use in moving organs; 2. A shape permitting transthoracic removal; 3. A membrane permeable for large marker molecules.

The probe developed in our laboratory and presently employed, has a permeable membrane tubing that is placed in the distal 15 – 20 mm of a double-lumen, non-toxic, medical-grade polyethylene (PE) tubing (outer diam 1.5 mm, see fig. 2). It has a smooth transition to an epicardial pacing wire suture (papers I and II). The two channels of the PE tubing are connected behind the sealed tip. A 9 mm section is removed from one of the channels and replaced by a 12 mm piece of semipermeable membrane. The membrane is a hydrophilic polymer containing approximately 0.1 μm pores (CPC/PE, Gambro, Lund, Sweden).
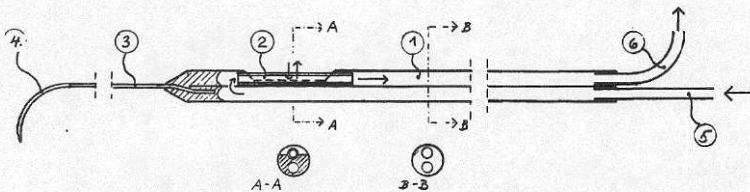

Fig. 2. Microdialysis probe: 1. Main tubing 2. Dialysis membrane 3. Epicardial pacing wire

4. Needle for insertion 5. Inlet tubing 6. Outlet tubing

Microdialysate samples left the outlet tubing 10 - 60 min after the passage of the permeable membrane depending on the length of the tubing. The outlet was connected to a vial adapter for sample collection. The vial adapter had a venting to prevent excessive pressure to build up. The probes were sterilized with ethylene oxide in the hospital under controlled temperature. The effectiveness of sterilization was checked with a biological marker.

2:3 The technique

The probes were perfused with sterile physiological NaCl (0.9%) at 2.5 μl/min using a precision pump (CMA 100, CMA, Stockholm, Sweden). Before implantation, the probes were placed in a sterile field and "primed" with medium for at least 20 min. Special care was taken to remove air bubbles from the system. Coronary vessels were identified and avoided during implantation. The probes were implanted at a depth of 2 – 3 mm, parallel to the epicardium. The probes were fixed at the desired site by curling of the cut part of the wire suture outside

the myocardium and by putting a suture around the "neck" of the probe (II). The probes were implanted as early as possible during surgery. In paper III, the probes were perfused for 105 min (range 70 – 145) before aortic cross-clamping. This time was of particular relevance, since the aim was to distinguish effects related to implantation from those, which occurred during the cardioplegic phase.

In the majority of patients, one probe was implanted in the region of the left anterior descending coronary artery (LAD) of the anterior wall, in the angle between the LAD and a diagonal branch. Another probe was placed in the lateral wall, parallel to an obtuse marginal branch of the circumflex coronary artery. In paper V, an additional probe was placed on the other side of the diagonal artery chosen. Peroperatively, 10 min samples were taken, while 60 min samples were collected postoperatively. When indicated, measurements were continued for up to five days. Sampling times were corrected for the dead-space. The probes were removed percutaneously according to the procedure for temporary pacing wires. In the first 10 patients, echocardiography was performed before and after removal of the probes to check for hemorrhages.

3. Methodological considerations

To ensure proper sampling conditions, the intraluminal pressure in the outlet tubing was varied by changing the level of the outlet, relative to that of the permeable membrane. The pressure in the outflow tubing was adjusted to approximately 25 cm H_2O in vitro when the permeable membrane and the outlet of the sampling adapter were at the same level (data not shown).

3:1 Recovery

Two types of recovery are discussed: relative recovery (concentration recovery) and absolute recovery (mass recovery) (Ungersted 1984). Relative recovery is the concentration of a compound in the dialysate in per cent of its concentration outside the probe. Absolute recovery stands for the amount recovered in the dialysate during a defined period of time ($\mu M/min$).

The relative recovery is independent of concentration changes outside the membrane, as long as the perfusion rate is constant (Ungerstedt et al., 1982). In contrast, absolute recovery varies with changes in concentrations outside the membrane.

Several factors affect relative and absolute recovery:

1. The rate of perfusion. Relative recovery correlates negatively to flow rate, whereas absolute recovery correlates positively to flow rates. Fig. 3.

2. Dialysis membrane area. Both relative and absolute recovery are positively correlated to the membrane area (Benveniste and Hüttemeyer, 1990).

3. Properties of the dialysis membrane such as mol wt cut-off and interactions of e.g. proteins with the membrane (Benveniste and Hüttemeyer, 1990).

4. Properties of the perfusion medium. Ideally, the medium should mimic the interstitial fluid. Isotonic fluids with ion concentrations, similar to that of body fluids, are frequently used.

5. Tissue pressure. A high tissue pressure results in a high relative recovery. Hence, relative recovery is higher in the myocardium than in subcutaneous tissue (Lönnroth et al., 1991). A low pressure increases the risk for a net flow of fluid.
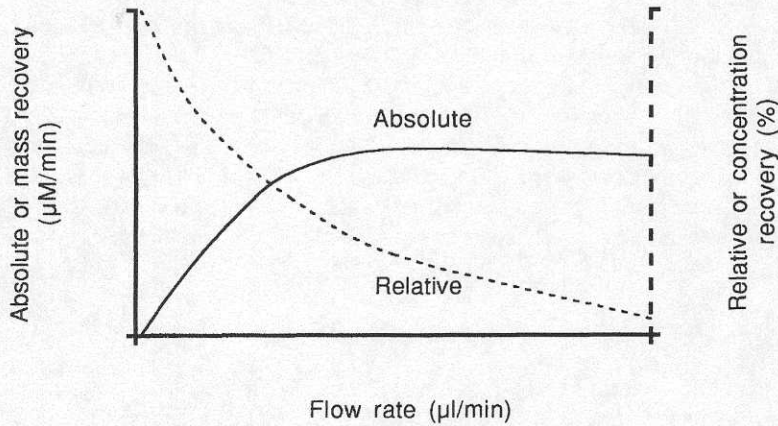

Fig. 3. Relative recovery (= concentration recovery), i.e. the concentration of a substance in the dialysate in per cent of that in the interstitial fluid surrounding the probe (dotted line) and absolute recovery (= mass recovery) in µM/min (solid line) plotted against perfusion flow rate. At zero flow rate, the relative recovery is theoretically 100%, whereas absolute recovery equals zero. When the flow rate is increased, absolute recovery increases and relative recovery decreases (after Jansson 1991).

3:2 Calibration

Recovery is often higher in vitro, consequently the use of the relative recovery obtained in vitro certainly underestimates the true interstitial concentration (Benveniste et al., 1989, 1990). Early calculations of absolute concentrations in the interstitium were based on in vitro calibrations. New and better methods were developed to avoid the error inherent to that approach:

3:2:1 Zero flow. This is based on the assumption that the concentration in the dialysate at zero flow rate equals the concentration in the interstitium, since a total equilibration is reached between the dialysate and the interstitial fluid. Variation of the flow rate for calibration purposes was first described by Jacobsson et al. (1985) and refined by Ekblom et al. (1992). The dialysate concentration of the compound of choice was measured at different flow rates. The interstitial concentration at zero flow, i.e. at 100% relative recovery, was calculated by non-linear regression analysis.

3:2:2 Zero transfer of the compound of interest. The true concentration in the interstitium equals that in the dialysate, when there is no difference in the concentration between the medium entering the probe and that leaving the probe (Lönnroth et al., 1987). The compound

of interest is included in the perfusion medium at different concentrations. It is assumed that recovery does not change during the calibration and that the concentration in the interstitium is constant.

3:2:3 Near equilibrium. A simple calibration method is to allow the microdialysate to reach almost equilibrium with the interstitium. This is achieved with a very low flow rate or a closed-loop system (Lerma et al., 1986). The small risk of excessive depletion of the interstitium is an advantage. The low time resolution is a disadvantage.

3:2:4 Internal reference. This method is based on the use of a radioactively labeled marker that is evenly distributed in all tissue compartments. An isotopic marker is added to the perfusate and the amount, which is lost to the tissue during perfusion, equals the unlabeled amount, which leaves the tissue to the dialysate (Lönnroth and Strindberg 1995, Jansson al., 1994). Care must be taken to avoid perfusate concentrations near interstitial fluid concentrations of glucose and lactate. This technique, using labeled internal references, has been validated in microdialysis measurements in adipose tissue (Lönnroth and Strindberg, 1995, skeletal muscle (Holmäng et al., 1998) and skin (Krogstad et al., 1996).

Theoretically, the interstitium may be depleted with respect to any compound, especially at high perfusion rates. Depletion of the measured substance can be limited by its addition at moderate concentration to the perfusion medium (Lönnroth et al., 1987).

3:3 Evaluation of the membrane used in papers I - V

In all studies, we used a membrane which allows the passage of the markers of interest.

Using plasma from 3 patients, relative recoveries for amino acids and ASAT were calculated. The concentrations were measured in dialysates, which were obtained from serum in vitro at 37°C, as well as directly in serum samples. The relative recovery for amino acids was 8 - 9%, in agreement with previous work, employing a probe membrane with considerably lower mol wt cut-off (Hamberger, 1984). The recovery for ASAT was considerably lower, 3% (Table 2). The concentrations of amino acids in the myocardial interstitium were then calculated assuming that relative recovery of the compounds in interstitial fluid was similar to that in serum. When using this calibration technique all amino acids, except glutamate, had an estimated interstitial concentration, which was approximately 50% of that in plasma (Table 2). Alternatively, the recovery in myocardial interstitium was approximately 50% of that in plasma. If the latter alternative is correct, the concentrations of amino acids were similar in plasma and interstitium.

TABLE 2 Recovery of amino acids and ASAT in plasma and dialysate
Concentrations in the interstitium calculated according to recovery in plasma

Amino acid	Measured concentration in plasma (nmol/ml)	Recovery (%) in plasma	Calculated concentration In interstitium (nmol/ml)
Glutamate	53	8	52
Serine	123	8	70
Glutamine	576	8	259
Glycine	205	9	92
Taurine	137	9	51
Alanine	340	9	145
Tyrosine	67	8	39
Valine	198	8	115
Phenylalanine	50	8	34
Isoleucine	55	8	31
Leucine	106	8	61
ASAT		3	

The internal calibration performed with ^{3}H-glucose and ^{14}C lactate (paper V) showed a relative recovery for glucose and lactate of approximately 30 % preoperatively and 44 % postoperatively. The interstitial concentrations of glucose and lactate were calculated accordingly.

Based on our assumption that interstitial ASAT equilibrates, or nearly equilibrates, over the membrane in the late postoperative phase, we used ASAT as an endogenous reference to estimate the interstitial concentration of ASAT (paper IV). However, this approach is dependent on a constant recovery throughout.

4. Analyses

ASAT (papers I and IV)

ASAT was used as a marker in paper IV, since there is much experience with its time course after cardiac surgery (Brandrup-Wognsen et al., 1995, McGregor et al., 1984, Ståhle et al., 1989, 1991). In the absence of ECG alterations typical of infarction, an increase of up to 3 times the upper normal limit is not associated with increased mortality after 30 days (Steuer et al., 1998). The use of ASAT for diagnosing infarction in heart surgery is facilitated since preoperative concentrations of ASAT usually are available. The patient is normally monitored

before, during and after surgery, which gives better control of other organs as sources of ASAT. Furthermore, ASAT has recently been shown to better predict one-year mortality after heart surgery than CK-MB (Steuer et al., 1998). In direct sampling of the myocardium, the non-specificity of ASAT creates little problem. The short half-life of ASAT (20 h) in plasma relative to e.g. the troponins (several days) is another advantage.

Troponin-T (papers III and IV)

Troponins have a high sensitivity both for reversible ischemic damage and infarction after heart surgery. The preoperative plasma concentration is below the range of sensitivity of the method. Troponin-T has probably the same "gold standard" status for the diagnosis of myocardial infarction as has CK-MB.

Amino acids (paper II)

Free amino acids are released from many organs in response to decreased oxygen tension, since energy dependent concentration gradients exist between the cells and the interstitium (Song et al., 1996). Amino acids are metabolized to citric acid cycle intermediates and consumed to compensate for low glucose levels (Wiesner et al., 1989). They are also synthesized via accelerated transamination (Tischler and Goldberg, 1980) and produced in proteolytic processes (Takala et al., 1980). Amino acids are sensitive markers of myocardial damage (Kimose et al., 1993, Pisarenko (a, b, c) et al., 1989). Taurine is lost from the myocardium during heart arrest (Suleiman et al., 1993), to an extent which often causes an increase of plasma taurine (Lombardini and Bricker, 1981, Cooper and Lombardini, 1981). Heart arrest in man also leads to a decrease of glutamine, glutamate and aspartate (Suleiman et al., 1993).

Lactate and glucose (papers I and V)

Glucose and lactate were monitored to gain a better understanding of the delivery and uptake of nutrients in the heart before, during and after cardioplegia. Research in this field has mainly focused on per- and post-operative situations employing plasma sampling techniques. Contradictory data have been presented regarding cardiac uptake and oxidation of glucose in the initial postoperative period (Fremes et al., 1985, Pietersen et al., 1999, Svedjeholm et al., 1991, Svensson et al., 1990 Thorelius et al., 1994). Monitoring of glucose and lactate was of special interest during cardioplegia, since the lack of coronary circulation during this phase makes it impossible to obtain plasma samples.

IV. RESULTS

1. In situ detection of myocardial infarction in pig by measurements of aspartate aminotransferase (ASAT) activity in the interstitial fluid (I)

Safety issues, mechanical properties and function were addressed in the first study of the microdialysis probe, which we had developed for myocardial use. ASAT, free amino acids and lactate were tested as markers for implantation trauma and experimental myocardial ischemia.

No safety risks were identified and the probe functioned adequately. While the implantation did not affect ASAT levels in peripheral blood or in blood from the coronary sinus, it increased ASAT in the myocardial dialysate. However, reduced levels were regained 3 h after implantation. Experimental ischemia resulted in increased ASAT, lactate and glutamate in the dialysates. The parameters were not altered in a non-ischemic region of the myocardium. The experimental ischemia increased ASAT in blood both from the periphery and the coronary sinus. However, this increase was considerably delayed.

2. Extracellular amino acids as markers of myocardial ischemia during cardioplegic heart arrest (II)

In this first study on the human myocardium, the issues of safety and function were again in focus. No safety problems developed in any of the 20 patients. The probes could be implanted almost without interference with the surgery. Free amino acids turned out to be sensitive markers for myocardial ischemia. Peaks of amino acid concentrations were recorded during and after cardioplegia, both in patients with ischemic coronary artery disease and in patients with valvular disease. Steady-state levels, or close to steady-state levels, were regained 15 – 30 h postoperatively. A first peak, most pronounced for those amino acids, which have a high concentration gradient over the cell membrane, was seen immediately after implantation of the probes. A second peak was found during cardioplegia for taurine, glutamate and aspartate, which increased by 10 – 25 times basal level. Glutamine increased ~ 5-fold, also during cardioplegia, while alanine, serine, leucine and glycine barely doubled the basal level. Moreover, this did not happen until 5 – 25 min after the end of cardioplegia. There was no apparent difference between the two regions of the heart in any of the patient groups. Amino acid levels did not correlate with aortic occlusion time. A covariation appeared possible between postoperative ischemic events and peak/basal values for certain amino acids, although not statistically significant.

3. Intramyocardial troponin-T monitoring with microdialysis in coronary artery bypass surgery (III)

The aim of this study was to distinguish temporally the effects of probe implantation and the effects of heart arrest. Consequently, the time period between implantation and heart surgery/cardioplegia had to be long enough for marker concentration to return towards basal level. The effect of probe implantation, as measured by troponin-T peak values, varied

the effects of cardioplegia could be studied without appreciable interference from the implantation trauma.

4. Long term monitoring of extracellular ASAT and troponin-T during and after cardioplegic heart arrest (IV)

ASAT and troponin-T levels were measured in plasma as well as in the myocardial interstitium during and after cardioplegia in two groups of patients, undergoing coronary artery bypass surgery (IHD) or valve surgery (N-IHD), respectively. Myocardial ASAT peaked during cardioplegia and decreased rapidly to low concentrations at 24 and 72 h, while plasma ASAT peaked 7 h after cardioplegia. The dialysate/plasma concentration ratio for ASAT was 20 during cardioplegia and 0.3 at 72 h. Little difference was detected between the levels in peripheral blood and in blood from the coronary sinus.

Troponin-T peaked in the myocardium 3 h after cardioplegia and then decreased rapidly until 10 h. Plasma troponin-T peaked approximately 2 h later, i.e. 5 – 7 h after cardioplegia. The dialysate/plasma concentration ratios for troponin-T were 300 during cardioplegia and 20 at 72 hours. Troponin-T concentrations were slightly higher in blood from the coronary sinus than in peripheral blood. Peak concentrations of ASAT and troponin-T in dialysates and plasma did not differ between patients undergoing coronary artery bypass surgery and valve surgery. Myocardial ASAT and/or troponin-T were higher (> 1 SD) in 6/6 patients, when ischemic (significant coronary artery stenosis or occlusion) regions and non-ischemic regions in the same patient were compared.

5. Myocardial extracellular glucose and lactate before, during and after cardioplegic heart arrest (V)

The concentrations of glucose and lactate and the transcapillary diffusion of glucose were measured in the myocardial interstitium in relation to cardioplegia. Myocardial glucose decreased significantly after induction of cardioplegia and remained low until one hour after cardioplegia, when it returned to the preoperative concentrations. At 25 and 35 h after cardioplegia, interstitial glucose decreased again to levels similar to those during cardioplegia. Interstitial lactate decreased after induction of cardioplegia but increased significantly before the end of the cardioplegic period. The lowest concentrations of lactate were seen at 25 and 35 h after cardioplegia.

The transcapillary membrane uptake of glucose, obtained from the arterio-venous (AV) and arterio-interstitial (AI) concentration differences, appeared to be the limiting factor for cellular glucose uptake while the heart was beating, but not during cardioplegia. Glucose did not reach critically low interstitial concentrations during cardioplegia, nor did lactate reach pathologically high concentrations in the myocardial interstitium.

6. Multivariate analysis of markers

A multivariate analysis of virtually all the data on parameters in dialysates and serum was carried out prior to the organization of the findings, which is presented in papers II and IV. The purpose was to get an unassuming picture of whether certain parameters or groups of parameters had closer relationships than others. The resulting multidimensional matrix contained 21 120 data points from 22 time periods, i.e. the values for all parameters at each

sampling time. The Principal Component Analysis technique (Wold et al., 1984, 1987) provided the analysis of the principal components of the parameters (Fig. 3a) and of time (0 h = time for declamping of the aorta, Fig. 3b). In the discriminant analysis, presented as Fig. 3c, time was made the discriminator,

The information given by the parameters analyzed in serum (S-ASAT and S-troponin-T) differed strikingly from that obtained from the dialysates (Fig. 3a). Fig 3a also demonstrates that the dialysate parameters displayed two fairly distinct groups, one containing aspartate, glutamate and taurine in addition to the marker for membrane phospholipid, phosphoethanolamine and ASAT. The other main group contained glycine, isoleucine, leucine, tyrosine, phenylalanine and valine. A similar sorting of the parameters was identified on a time course basis (paper II, Fig. 3). The results were interpreted to reflect physiologically active compounds (aspartate, etc.) and proteolysis derived amino acids (glycine, etc.), respectively. The analysis also provided a distinct time course of events. Time is presented linearly in Fig. 3c. However, this information is similar to that in Fig. 3a.
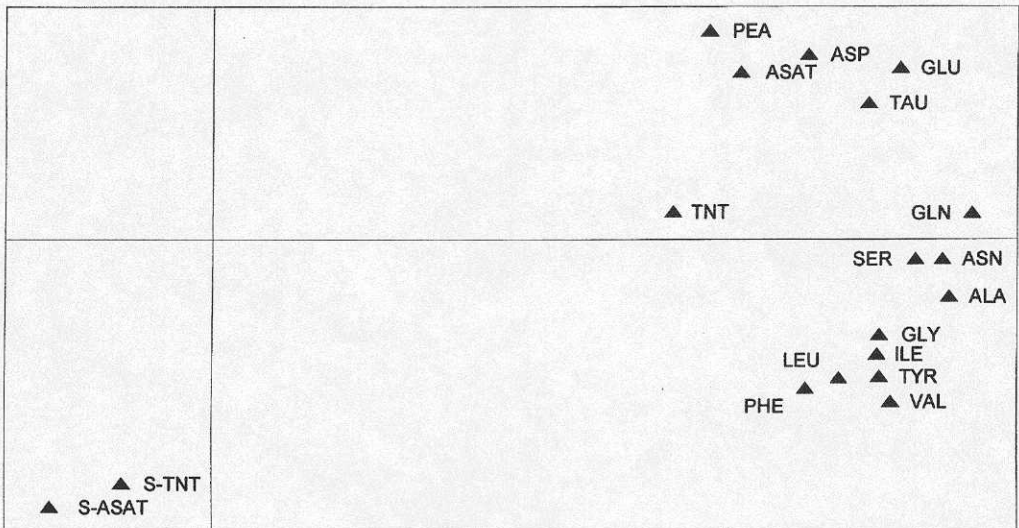

Fig. 3a. Principial component analysis of data matrix, analyses as variables. Principial component 1 versus principial component 2. ASAT (dialysate-ASAT), S-ASAT (serum-ASAT), TNT (dialysate troponin-T), S-TNT (serum troponin-T), ALA (alanine), ASP (aspartate), ASN (asparagine), GLN (glutamine), GLU (glutamate), GLY (glycine), ILE (isoleucine), LEU (leucine), PEA (phosphoetanolamine), PHE (phenylalanine), SER (serine), TAU (taurine), TYR (tyrosine), VAL (valine).
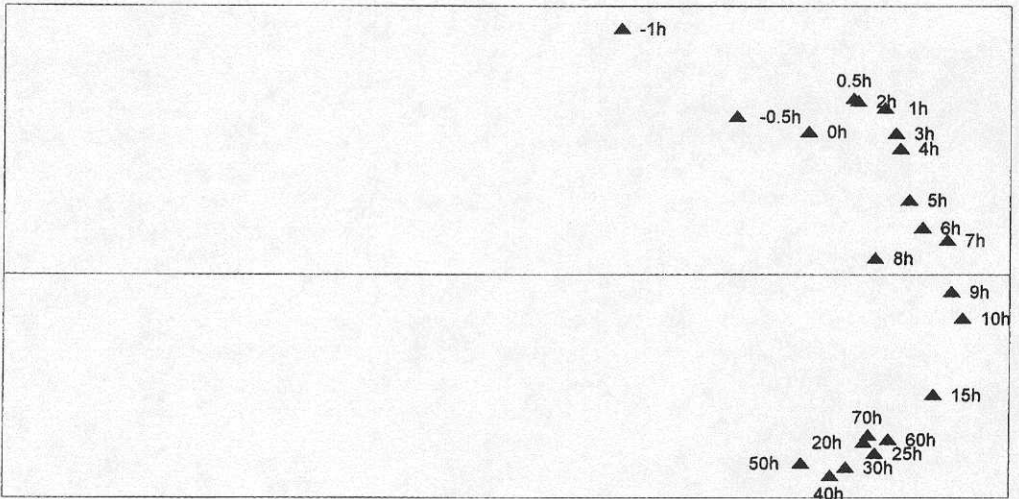
Fig. 3b. Principial component analysis of data matrix, times of sampling as variables. Principial component 1 versus principial component 2. Times after aortic declamping.
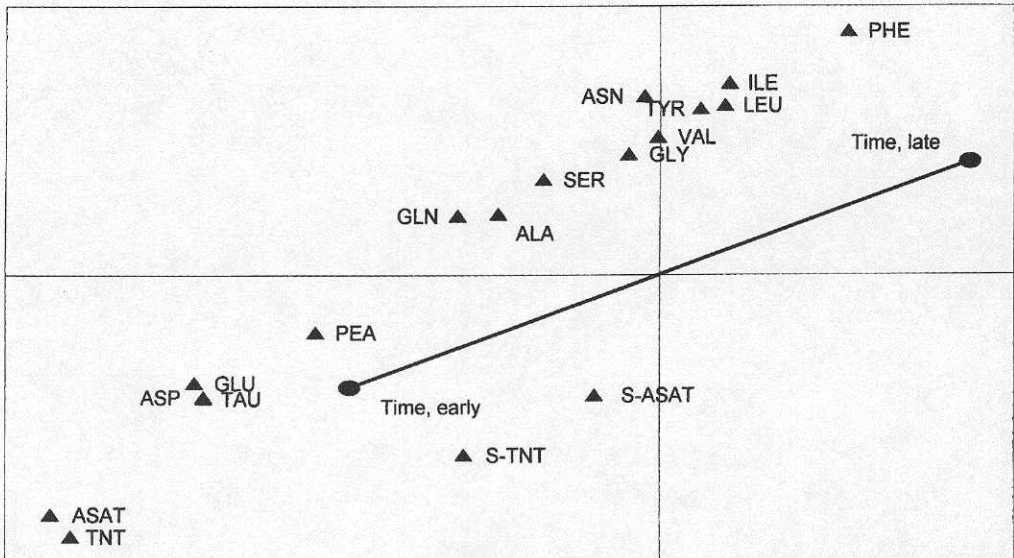

Fig. 3c. Principial component analysis of data matrix, analyses as variables. Discriminant analysis, time as discriminator. ASAT (dialysate-ASAT), S-ASAT (serum-ASAT), TNT (dialysate troponin-T), S-TNT (serum troponin-T), ALA (alanine), ASP (aspartate), ASN (asparagine), GLN (glutamine), GLU (glutamate), GLY (glycine), ILE (isoleucine), LEU (leucine), PEA (phosphoetanolamine), PHE (phenylalanine), SER (serine), TAU (taurine), TYR (tyrosine), VAL (valine).

V. DISCUSSION

1. Development of myocardial microdialysis

1:1 Safety aspects

A number of critical issues were addressed for the step from experimental work to a clinical routine for microdialysis monitoring of the myocardium. Safety aspects included construction of probes, risks for e.g. hemorrhage during or after implantation of the probes, as well as during their percutaneous removal, infections and the scenario of a probe breakdown with leakage of perfusion medium within the myocardium. The aim to monitor relatively large marker molecules for tissue damage required a permeable membrane, which is less robust and easier allows net transport of fluid than conventional dialysis membranes. These issues were addressed in a study (I), which employed an animal (pig) with a heart, the size approximating that of man. The successful avoidance of problems, which could affect the safety of the patient, encouraged us to transfer the technique to man. Further support was obtained from our knowledge that epicardial pacing leads, which are similar in size and shape to microdialysis probes, are routinely implanted and removed with few complications. Consequently, we adopted the system for anchoring pacemaker leads and could perform complication-free percutaneous probe removals after several days.

The general safety was confirmed in the first study in man (II). The risk for tissue hemorrhages was judged to be small, since ECHO monitoring showed no bleeding. The permeable membrane, although fairly fragile, was not perforated or dislocated in any of the probes, as checked with light microscopy and by perfusion after probe removal. Notably, a complete membrane perforation, even left unnoticed for 2 – 3 h, is hardly a critical challenge, since the result is a local instillation of physiological saline at 0.15 ml/h. Finally, the probes and the other microdialysis set up interfered only marginally with the main surgical procedures and the postoperative care of the patients.

1:2 Technical aspects

A requirement in microdialysis sampling is to obtain biochemical signals from the event of interest, unaltered by "noise", such as produced by tissue reactions to probe implantation. In the pig myocardium, the "noise" level was insignificant 3 hours after implantation as judged from the levels of ASAT, lactate and amino acids, neither was the implantation trauma detected by increase of any marker in the systemic circulation (I). Stable base-line levels prior to cardioplegia were rarely obtained in the clinical studies, due to ethics and practical time limits. In paper III, a report on 7 patients undergoing CABG surgery, cardioplegia was not started until ~ 2 h after probe implantation. The increase and subsequent decrease of troponin-T after implantation varied amongst the patients, most likely due to different severity of the local tissue trauma. However, the concentrations of troponin-T decreased considerably in all patients prior to the start of cardioplegia. Hence, subsequent effects on troponin-T levels were easily distinguished from the effects of implantation (III).

The role of the hydrostatic pressure for net transfer of fluid into or out of the myocardium was assessed in order to collect the same volumes of perfusate as those administered. A routine was established to achieve this goal. Net transport of fluid over the membrane could be avoided in vitro by positioning the probe and the outlet at the same horizontal level. This technique was successful for probes placed in the region of the LAD coronary artery, but probes in the region of the CX coronary artery gave occasional problems, probably since its outlet was difficult to adjust when the patient altered position. We interpreted the decreased fluid volumes to be caused by the subsequent decrease in hydrostatic pressure. Other investigators, employing membranes with large diameter pores (Benveniste and Hüttemeyer, 1990), have noted similar effects. The lack of correlation between ASAT and troponin-T in dialysates from the CX region, as compared with a significant correlation in the LAD region may be explained by these technical problems.

In cerebral tissues, a microdialysis probe drains small soluble molecules as amino acids from a tissue cylinder with a diameter of 1- 2 mm (Hamberger 1984). The volume is probably similar in the myocardium. However, while a probe integrates fairly well with cerebral tissues, it is more likely to reside in a small cavity in the myocardium, probably filled with a mixture of blood and interstitial fluid. Due to technical problems, we were unable to resolve the appearance of the implantation site with morphological methods.

Tissues may be depleted with respect to vital compounds during microdialysis (see MATERIALS AND METHODS 3:2:3). The high myocardial blood flow, the movement of the organ and looser attachment between probes and tissue may reduce the risk for depletion of the myocardium. Although depletion can be reduced by supplementation of the perfusate (Lönnroth, 1987), we had no permission from the ethics committee for any additions, except glucose and lactate. Even though neither unlabeled glucose nor unlabeled lactate was added to the perfusate in paper V, the constant recovery does not support a depletion problem. Still, the problem of depletion is probably larger with the membrane used in this thesis, since protein molecules are lost, which is not the case with most commercial probes.

The marker levels in paper I – V were given as dialysate concentrations and not as interstitial concentrations unless otherwise stated. Calculated interstitial concentrations were given for ASAT in paper I and glucose and lactate in paper V. The internal calibration performed in paper V showed a recovery for glucose and lactate of approximately 30 % preoperatively and 44 % postoperatively. A rough indication of the recovery of ASAT is given by the microdialysate/plasma concentration ratio (0.3) for ASAT at 72 h in paper IV. It seems improbable that plasma levels of ASAT at any time are higher than the interstitial levels. Thus, recovery for ASAT was less than 30%. However, direct recovery measurements in plasma suggested a recovery of 3% (Table 2 in this thesis).

1:3 Markers and sampling

ASAT was used as one of the main markers for ischemia (papers I and IV), due to extensive experience with this marker after cardiac surgery (Brandrup-Wognsen et al., 1995, McGregor et al., 1984, Ståhle et al., 1989, 1991). The background to the test battery of amino acids is the experience that they are extremely sensitive markers for brain ischemia, as studied with microdialysis (Benveniste et al. 1984, Hagberg et al., 1985). Glucose and lactate were included to obtain understanding of delivery and uptake of nutrients.

Despite its high sensitivity for myocardial ischemia, CK-MB was not used, since it is measured with a method not applicable to the pig (paper I). In addition to ASAT, we used troponin-T since we judged the risk that the large (86 kDa) CK-MB heterodimer might have an extremely low recovery and be unstable when dialyzed. Also, several reports have pointed to some false positive results with CK-MB (Katus et al., 1991, Adams et al., 1994, Mächler et al., 1994).

2. Time course of concentration changes and patient diagnosis

A mixed group of patients undergoing CABG surgery, aortic valve surgery or closure of atrial septal defects (ASD) was investigated. The first peak (following probe implantation, see above) showed a heterogenous response when compared to the second, major peak, i.e. during or shortly after the cardioplegic period. We interpret this second peak to reflect an ischemic challenge, which was induced by the surgery and cardioplegia. Its characteristics with respect to marker levels did not differ among patients having coronary artery disease and patients without this diagnose. A third peak of increased marker levels occurred 24 h and later after cardioplegia in several patients irrespective of diagnosis (paper IV). Clinical events and results with other diagnostics suggest that this peak reflects late, sometimes global, ischemic events. The main conclusion was that patients with coronary artery disease displayed small differences in marker concentrations, when compared with patients having disease involving valvular or septal defects. Consequently, the ischemia induced by cardioplegia represents a major and disturbing "noise", since it does not allow distinguishing IHD patients from N-IHD patients. We found no correlation between aortic occlusion time and postoperative marker concentrations. Hypothetically, increased levels of markers due to the longer aortic occlusion time in the N-IHD group equals an increase in markers due to ischemic disease in the IHD group.

The patients were selected (papers II – V) to minimize the risk of complications while introducing a new method. Very few complications, including perioperative infarctions, were noted. We did not have the opportunity to obtain patterns of changes typical for complications, such as myocardial infarction.

3. Amino acids

The concentrations of all amino acids increased transiently during and after cardioplegia. The range of increase was 2 - 25 times the basal level and the time range for the appearance of peak levels was approximately 60 min (paper II). Glutamate, taurine and aspartate displayed the most impressive increases (10 - 25 x basal level), which took place already 20 - 30 min after the beginning of cardioplegia. Phosphoethanolamine, a metabolite of membrane phospholipids (Buratta et al., 1998), had a similarly early, but less impressive peak (8 x basal level). Another group of amino acids, i.e. alanine, glycine, leucine and serine increased modestly (about 2 x basal level) and not until the first hour after cardioplegia.

The results in paper II confirm reports on significant loss of taurine, glutamate, glutamine and aspartate from the myocardium during cardioplegia (Suleiman et al., 1993). The loss of taurine appears to be most significant, i.e. plasma taurine is consistently increased after cardioplegia (Kragten et al., 1997, Remppis et al., 1995). Various support mixtures, including combinations of amino acids, have been tried, given either before cardioplegia or in the reperfusion phase (Pisarenko et al., 1989, Engleman et al., 1991, Thomassen 1992). The

cardioprotective action of taurine is well documented (Azari et al., 1980, Franconi et al., 1985, Kramer et al., 1981, Pasantes-Morales et al., 1985, Sawamura et al., 1983, 1986, Schaffer et al., 1987, Takihara et al., 1986, Welty et al., 1982, Wright et al., 1985). Taurine modulates the excessive accumulation of calcium ions during ischemia (Pasantes-Morales et al., 1985, Welty et al., 1982) and has a scavenging action (Wright et al., 1985), inthat it inhibits lipid peroxidation. Furthermore, pretreatment with taurine (5g) decreases the cell damage in the myocardium during reperfusion (Milei et al., 1992). Our results definitely support the use of taurine supplementation prior to cardioplegia.

4. ASAT and troponin-T

While taurine was the only amino acid, which increases in plasma during myocardial ischemia (Suleiman et al., 1993), plasma ASAT and troponin-T are part of the clinical routine in diagnosing cardiac damage. The time courses of changes in plasma ASAT and troponin-T after cardioplegia (paper IV) were in agreement with the literature (Ståhle et al., 1989, 1991, Kallner et al., 1994) i.e. peaking 5 –7 h after declamping of the aorta. In contrast, interstitial ASAT had its peak level already during cardioplegia and then decreased rapidly during the following 10 h, i. e. at which time plasma ASAT increased. The time course for interstitial troponin-T was more similar to that in plasma, the interstitial peak preceding the plasma peak by 1-2 h. The highest dialysate/plasma concentration ratio was 20 for ASAT, while it was approximately 300 for troponin-T. The true interstitial/plasma ratios were probably considerably higher, due to the low (3%) recovery of these compounds (see MATERIALS AND METHODS 3:3). Interstitial troponin-T (as plasma troponin-T) remained elevated much longer after cardioplegia than did ASAT. Alternatively, the steady-state level of interstitial troponin-T is considerably higher relative to plasma levels. The earlier "peaking" of ASAT compared to troponin-T in the interstitium may be explained by a slower release of structurally bound troponin-T than the soluble ASAT (Lentner 1984, Huseby et al., 1990). The early increase of troponin-T seen after probe implantation (paper III) may reflect the readily available, but small, pool of soluble troponin-T (Remppis et al., 1994).

Little additional information was gained from measurements in blood from the coronary sinus as compared to data from peripheral blood (paper IV). ASAT and troponin-T concentrations were similar in both throughout the period of analysis. In a comparison of marker compounds for myocardial ischemia, organ specificity has little importance when the interstitium is sampled directly. A rapid response and a short half-life are obviously of advantage. ASAT fulfills several of these requirements and a steady-state level was reached early after implantation (I), the delay of the response was short (I, IV) and the decrease after the ischemic challenge was relatively rapid (IV).

5. Glucose and lactate

In man, interstitial glucose in the myocardium was only ~ 50% of that in arterial plasma preoperatively, indicating that the entry of glucose did not balance its rate of elimination. Consequently, transcapillary transport of glucose is rate limiting for myocardial glucose uptake. This is in agreement with findings in skeletal muscle in resting subjects, in which the glucose uptake rate correlates negatively to interstitial glucose concentration (Holmäng et al., 1997, 1998). In working skeletal muscle, blood flow and capillary permeability increase to reduce the concentration difference between arterial and interstitial glucose. The interstitial glucose concentration was surprisingly low, despite the much higher blood flow in the

myocardium than in skeletal muscle, indicating that the cellular uptake of glucose in the myocardium is extremely efficient (Fremes et al., 1985, Hamada et al., 1998, Watanabe et al., 1998). In pig, the implantation of the probes did not affect dialysate glucose concentrations.

In man interstitial lactate did not differ from preoperative plasma lactate, even though lactate was lower in venous plasma than in arterial plasma. However, lactate metabolism is not only reflected by interstitial concentrations (Kammermeier and Wendtland, 1987). An interstitial concentration of lactate, higher than that in plasma, does not rule out ongoing lactate uptake and oxidation since a net myocardial release of lactate may prevail simultaneously with its uptake and oxidation (Gertz et al., 1981, Larsen et al., 1994, Wisneski et al., 1985). In pig, interstitial lactate decreased by approximately 50 % after implantation of the probes and reached a steady state after 1.5 h.

During cold cardioplegia in man, interstitial glucose decreased from approximately 3 mM/L to 1 mM/L, despite repeated administration of glucose free cold cardioplegic solution. Apparently, glucose was not totally depleted, in spite of low or abolished glucose uptake under low temperature. Since normothermic ischemia may induce increased glucose uptake by insulin independent mechanisms (Egert et al., 1997), it is interesting to note that during warm ischemia in the pig myocardium, interstitial glucose was not affected. This is in agreement with previous findings of a preserved glucose uptake in combination with a reduction of glucose oxidation during experimental ischemia in the heart (Bolukloglu et al., 1996).

Interstitial lactate also decreased initially and accumulated slowly during heart arrest, despite intermittent perfusion with cardioplegic solution. The mean interstitial lactate levels did not exceed the pre-cardioplegia levels during this period. In contrast, warm ischemia in the pig increased interstitial lactate in myocardium without delay. The effects of cold cardioplegia appear quite different from the effects of warm ischemia. In man, in spite of ischemia, the low temperature enabled the arrest of further glucose metabolism. In this situation, accumulation of lactate is probably the result of glycogen breakdown.

Interstitial glucose increased significantly immediately after cardioplegia, whereas interstitial lactate remained high. Both AI and AV differences for glucose increased significantly, indicating enhanced glucose consumption. In contrast, lactate AI was negative, as a result of net myocardial release of lactate. The elimination and oxidation of glucose, as well as of lactate, was low during the early postoperative phase, as reported previously (Svedjeholm et al., 1991). In the pig, interstitial glucose did not change postoperatively. Interstitial lactate in the pig returned to a steady-state level within 1.5 h.

At 25 and 35 h after release of the aortic cross clamp, all patients displayed very low interstitial glucose and lactate, indicating high elimination and oxidation rates, despite postprandial conditions. This is in agreement with previous studies at 4 h (Fremes et al., 1985) and 8 h (Thorelius et al., 1994) after surgery. Thus, the low interstitial levels indicate a rapid elimination of glucose and lactate from the interstitial fluid into the myocardial cells. The present data do not indicate that the poor delivery of glucose or the lactate accumulation in the myocardium potentiate the trauma induced by cold cardioplegia. In accordance, the addition of glucose and/or lactate to cardioplegic solutions does not seem to have convincing cardioprotective effects (Hearse et al., 1978, Robinson et al., 1984, Salerno and Chiong, 1980).

6. Future developments

Presently, we are evaluating possible correlations of myocardial temperature with marker levels in a group of patients undergoing valve surgery and plan to monitor patients undergoing bypass surgery without ECC as well as patients prone to complications after surgery employing ECC.

VI. CONCLUSIONS

*Microdialysis can safely be employed for sampling of the human myocardium.

*The concentrations of interstitial markers for ischemia can be estimated, during and after cardioplegia, with increased sensitivity and time resolution compared with plasma.

*The interstitial concentrations of glucose and lactate before, during and after cardioplegic heart arrest can be calculated.

*After further experience, microdialysis may be used for postoperative surveillance and for evaluation of cardioprotection.

VII. ACKNOWLEDGEMENTS

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