Cardiac effects of non-adrenergic inotropic drugs

Clinical and experimental studies

Birger Axelsson

Department of Surgical and Perioperative Sciences
Anesthesiology and Intensive Care
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From the Department of Surgical and Perioperative Sciences
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Birger Axelsson

Fakultetsopponent:
Professor Sven-Erik Ricksten,
Anestesiologi och Intensivvård, Inst. för kliniska vetenskaper
Sahlgrenska akademin, Göteborgs universitet.

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Cover illustration
"Weighing of the Heart" from chapter 125 of "the Book of the Dead",
a famous papyrus from the 19th Dynasty of the New Kingdom
in ancient Egypt, dated around 1250 BC.

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Eftertanke

Tage Danielsson ur ”Tankar från roten” (1974)

Jag tror på tvivlet.
Tvivlet är all kunskaps grund
och all förändrings motor.
Tvivlet är till yttermera visso trons förutsättning.
Den som tror utan att först tvivla
är en jublande dumskalle och en klingande cymbal.
Och den som tror utan att samtidigt tvivla
är en föga mindre jublande dumskalle
och en föga svagare klingande cymbal.
Tron kan försätta berg
men tvivlet kan sätta tillbaks dem igen.

Second thoughts

Tage Danielsson from “Thoughts from the root” (1974)

I believe in doubt.
Doubt is the basis of all knowledge
and the engine of all change.
Doubt is, to make doubly sure, the prerequisite of faith.
He who believes without first doubting
is an exulting blockhead and a tinkling cymbal.
And he who believes without at the same time doubting
is hardly less of an exulting blockhead
and hardly less of a tinkling cymbal.
Faith can remove mountains
but doubt can put them back in place again.

I wish to dedicate this book to my family.

Abstract

Background: Myocardial failure and dysfunction is not uncommon during critical illness and following cardiac surgery. For optimal treatment, a better understanding of the effects of inotropic drugs is needed. In this thesis, two non-adrenergic mediated inotropes, milrinone and levosimendan were studied in different models of myocardial dysfunction. The study aims were to assess the following: the effects of milrinone on blood flow in coronary artery bypass grafts during CABG surgery; the effects of milrinone on left ventricular diastolic function during post-ischaemic myocardial dysfunction; whether milrinone or levosimendan are protective or injurious during acute myocardial ischaemia, and if levosimendan potentiates myocardial function when added to milrinone in an experimental model of post-ischaemic (stunned) myocardium.

Material and Methods: In Study I, 44 patients undergoing coronary artery bypass surgery (CABG) were included as subjects. Milrinone or saline was administered in a single dose during cardio-pulmonary bypass (CPB) and coronary graft flow measurements were recorded after 10 and 30 min following CPB. In Study II; 24 patients undergoing CABG had estimations of peak ventricular filling rates made before and after CPB with administration of milrinone or saline in a single dose during CPB, performed by assessment of the rate of change in diastolic cross-sectional left ventricular area. In Study III, energy-metabolic effects of milrinone and levosimendan were measured in an anaesthetized porcine model during 45 minutes of regional myocardial ischemia. Microdialysis sampling of metabolites of local ischemic metabolism allowed assessment of glycolytic activity and the degree of myocardial calcium overload. In Study IV, in a porcine model of post-ischaemic myocardial stunning, ventricular pressure-volume relationships were analyzed when milrinone or a combination of milrinone and levosimendan were given together.

Results: In Study I, there was a clear increase in non-sequential saphenous vein graft blood flow with milrinone at 10 minutes (64.5 ± 37.4 compared to placebo 43.6 ± 25.7 ml/min (mean ± SD).). A decreasing but still measureable flow increase was seen for milrinone at 30 minutes. In Study II, an increase in early left ventricular filling rate (ventricular cross-sectional area rate of change, dA/dt) was seen in the milrinone treated group. Pre-bypass milrinone group dA/dt 22.0 ± 9.5 changed to post-bypass values dA/dt 27.8 ± 11.5 cm^2/sec). Placebo group pre-bypass dA/dt was 21.0 ± 8.7 and post-bypass 17.1 ± 7.1 cm^2/sec. A milrinone effect was demonstrated in an adjusted regression model ($p = 0.001$). In Study III, neither milrinone nor levosimendan led to a change in energy-metabolic activity during ischemia as reflected by interstitial glucose, pyruvate, lactate or glycerol. Neither drug exacerbated the relative myocardial calcium overload during ischemia. In Study IV, milrinone improved active relaxation (tau) in post-ischemic stunned myocardium, but did not markedly improve systolic function by preload recruitable stroke work. Levosimendan added to milrinone showed minimal effect on active relaxation but a positive effect on systolic function in combination with milrinone.

Conclusions: We conclude that milrinone treatment leads to an increase in blood flow in newly implanted coronary saphenous vein grafts, and improved ventricular relaxation post-cardiopulmonary bypass. Neither milrinone nor levosimendan, in this porcine model, negatively influence myocardial energy metabolism or calcium overload during acute ischaemia. Addition of levosimendan to milrinone treatment during post-ischaemic ventricular dysfunction may provide additive inotropic effects on systolic function but probably not for active relaxation.

Original papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I Mikael Arbeus, Birger Axelsson, Örjan Friberg, Anders Magnuson, Lennart Bodin, Jan Hultman.
Milrinone Increases Flow in Coronary Artery Bypass Grafts After Cardiopulmonary Bypass: A Prospective, Randomized, Double-Blind, Placebo-Controlled Study
J Cardiothorac Vasc Anesth. 2009 Feb;23(1):48-53..

II Birger Axelsson, Mikael Arbeus, Anders Magnuson, Jan Hultman.
Milrinone Improves Diastolic Function in Coronary Artery Bypass Surgery as Assessed by Acoustic Quantification and Peak Filling Rate: A Prospective Randomized Study
J Cardiothorac Vasc Anesth. 2010 Apr;24(2):244-9.

III Birger Axelsson, Göran Johansson, Pernilla Abrahamsson, Anil Gupta, Hans Tydèn, Patrick Wouters, Michael Haney.
Milrinone and levosimendan during porcine myocardial ischemia - no effects on calcium overload and metabolism.
Acta Anaesthesiologica Scandinavica, 2013 Mar 20. doi: 10.1111/aas.12095. (Epub ahead of print)

IV Birger Axelsson, Sören Häggmark, Staffan Svenmarker, Göran Johansson, Anil Gupta, Hans Tydén, Patrick Wouters, Michael Haney.
Systolic and diastolic effects of milrinone and levosimendan in porcine post-ischemic myocardial dysfunction
Manuscript

Bokomslag / Cover illustration

Fram och baksidan av omslaget för denna avhandling visar på den berömda scenen "Vägningen av hjärtat" från det 125:e kapitlet ur "De Dödas Bok", en känd papyrus från den 19:e dynastin, Nya Riket, forna Egypten, daterad till ca 1250 före Kr.
Min avhandling tar upp flera aspekter av att mäta hjärtats egenskaper i några olika situationer och omslaget visar på att det även ansågs vara viktigt för cirka 3260 år sedan även om mätmetoderna och situationen var något annorlunda.
Hjärtat skulle i det forna Egypten vägas mot guden Maat eller mot hennes symbol, dvs en strutsfjäder. Om hjärtat hade samlat på sig synd under levnaden kunde det inte balansera mot den lätta fjädern och personen skulle därför nekas vidare färd i livet efter detta. Ani, vitklädd (på vänster sida av det bakre omslaget) gör sin entre inför utvärderingen. Anubis har börjat evaluera hjärtat genom att väga det mot fjädern i åsyn av Ani och Ani´s själ, dvs fågeln med människohuvudet. Thoth, visdomens Gud (på höger sida av främre omslaget) står redo att skriva ner en rapport av hjärtutvärderingen och bredvid honom sitter Ammit. Det är ett monster bestående av en hybrid dvs en kombination av en krokodil, ett lejon och en flodhäst som är redo att äta upp Ani och Ani´s hjärta om det inte går igenom vägningsprocessen dvs om hjärtat skulle väga tyngre än fjädern. Tolv gudar agerar som vittnen och sitter ovanför på rad i domsalen eller i Maat´s hall. Originalpapyrusen kan ses på British Museum i London, England.

The front and back cover of this thesis shows the vignette of the "Weighing of the Heart" from chapter 125 of "the Book of the Dead", a famous papyrus from the 19th Dynasty of the New Kingdom in ancient Egypt, dated around 1250 BC.
My thesis is about measuring and evaluating qualities and characteristics of the heart and from the cover of this thesis this seems to have been important even around 3260 years ago though the measuring methods and the reason for measuring were of course a bit different. To find out if the deceased person was worthy to enter the afterlife world his or her heart had to be evaluated by weighing in balance with the goddess Maat or her symbol, an ostrich feather. If the heart had been heavy due to a lot of lifetime sin during it could not be balanced against the light feather, a symbol of goodness and justice and the person would be denied an afterlife. Ani, dressed in white (on the left of the back cover) is entering the trial scene. Anubis checks the balance of Ani´s heart together with the "soul" of Ani, the human headed small bird. Thoth, the God of Wisdom, stands ready to report the result (on the right of the front cover) and beside him sits the hybrid monster Ammit who is part crocodile, lion and hippopotamus. Ammit would eat Ani and Ani´s heart if it is not balanced with the feather meaning that the heart should not be heavier than the feather to pass. Twelve gods act as withnesses, sitting above the judgement area, the Hall of Maat.
The original papyrus can be seen in British Museum, London, UK..

Contents

Abbreviations

AQ	Acoustic quantification
ACE	Angiotensin-converting enzyme
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
ARB	Angiotensin receptor blocking
CABG	Coronary artery bypass grafting
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CI	Cardiac index
CO	Cardiac output
CPB	Cardiopulmonary bypass
CS	Coronary sinus
ECMO	Extracorporeal membrane oxygenation
EF	Ejection fraction
FAC	Fractional area change
FS	Fractional shortening
IABP	Intraaortic balloon pump
LITA	Left internal thoracic artery
LVEDP	Left ventricular end-diastolic pressure
LVEF	Left ventricular ejection fraction
M	Moles per litre
MAP	Mean arterial pressure
NCX	Sodium calcium exchanger
PCI	Percutaneous coronary intervention
PDE	Phosphodiesterase
PDEI	Phosphodiesterase inhibitor
PDEIII (=PDE3)	Phosphodiesterase type three
PHT	Pressure half time
PKA	cAMP-dependent protein kinase
RyR	Ryanodine receptor
SD	Standard deviation
SERCA	Smooth endoplasmic reticulum calcium ATPase
SR	Sarcoplasmic reticulum
SVG	Saphenous vein graft
SPSS	Statistical package for the social sciences
STATA	Statistical software package
Tau	Time constant of pressure decay
TEE	Transoesophageal echocardiography
TTE	Transthoracic echocardiography
U	Units

Prologue

My interest in cardiac physiology and inotropic drugs began in the early 1980's when I first encountered patients in cardiogenic shock due to extensive myocardial infarction in coronary care units. Management strategies for acute myocardial infarction have changed and improved dramatically since then. Most patients with acute symptoms of coronary artery syndrome are today treated early and aggressively with emergency percutaneous coronary interventions (PCI), and sometimes with coronary artery bypass surgery, when there is a threatening myocardial infarction. Since PCI units in many hospitals are now open 24 hours a day, this has led to a dramatic reduction in the incidence of heart failure as a complication of acute myocardial infarction.

Today, the majority of patients who need acute inotropic drug support are not admitted to cardiology wards, but are often managed in intensive care units or in operating theatres. Sometimes they undergo major operations, including cardiac surgery. Anaesthesiologists often have to take care of older and sicker patients who are scheduled for extensive cardiovascular or cancer surgery. These patients often have pre-existing coronary artery disease or valvular dysfunction or both, and have to undergo this type of surgery despite limited vital organ capacity, reduced circulatory reserve in situations of stress, bleeding, and perioperative myocardial ischaemia or infarction. Pharmacological therapy with inotropic drug support may be needed during critical illness or to support heart function when trying to separate a patient from cardiopulmonary bypass machine, or when trying to wean the patient from other forms of mechanical ventricular assist devices, including intra-aortic balloon pump (IABP) and arterio-venous extracorporeal membrane oxygenation (ECMO) systems.

In this thesis, there are investigations of two non-adrenergic mediated heart-supporting drugs: milrinone and levosimendan. The mechanism of action of milrinone is phosphodiesterase III/(IV) inhibition, while levosimendan acts primarily via a calcium-sensitizing mechanism, though it also exhibits some phosphodiesterase inhibition at higher doses. These drugs have often been used when patients are already treated with beta-blocking agents, or in situations where the beta-receptor system has undergone down-regulation.

It has been rewarding for me, to have been able to study new aspects of these non-adrenergic inotropic drugs in my thesis.

Introduction

"I was like a boy playing on the sea-shore, and diverting myself now and then finding a smoother pebble or a prettier shell than ordinary, whilst the great ocean of truth lay all undiscovered before me."

Sir Isaac Newton (1643-1727)

Historical perspective

Scientific progress in a specific field can often be characterized by paradigm shifts in understanding why things happen[1]. Although Hippocrates described heart failure in his written observations as early as during the 6th century BC, he knew of no treatment. Aelius or Claudius Galenus (Galen) (129-199) first described the anatomy of the heart and circulatory system and later William Harvey (1578-1657) demonstrated the heart to be a pump in a cardiovascular circuit. In 1785, William Withering (1741-1799) published a description of the first oral pharmacological treatment of heart failure in his description of digitalis. When Carl J Wiggers (1883-1963) examined patients with heart dysfunction using cardiac catheterization, he helped initiate another paradigm shift, when he showed that heart function could be assessed by direct observation of ventricular pressures and flows. Until the mid 1970s, no drug was used specifically for treating heart failure except digitalis, though other drugs such as diuretics and nitroglycerine were used with the aim of assisting the circulation in other ways.

The subtypes of adrenergic receptors were described by Ahlquist[2] in 1948 and an understanding of different cardiac and vascular effects of different adrenergic substances was presented. The beta-receptor was shown to mediate chronotropy (increase in heart rate) and vasodilatation while the alfa-receptor mediated vasoconstriction. Starting in the 1960s, evidence has been available concerning sympathetic nerve system over-activity during heart failure[3-6], making the use of external adrenergic substances to improve heart function less logical. Synthetic beta-adrenergic agonists were designed and tested, as the different characteristics and subgroups of adrenergic receptor systems were studied.

In 1975, dobutamine was developed[7] from isoproterenol since this was the prototypical strong beta-1 and beta-2 agonist, and where differential effects of beta-1 and 2 agonism were explored. Beta-2 adrenergic agonism was understood then as being largely vasodilatory in effect, and beta-1 agonism was thought to mostly promote chronotropy (heart rate), inotropy (contractile force) and possibly even lusitropy (active relaxation). Adrenergic receptor agonism was one cellular input, although many others were recognized, including muscarinic receptor systems (opposing beta adrenergic in effect), multiple other receptor systems and local biophysical and ionic factors. In trying to explore the optimal inotropic drugs for heart failure therapy, the goal was to optimize the effects that enhanced cardiac contractility, though with minimal or no other undesirable effects, such as

increased chronotropy. Tachycardia can be detrimental to the failing heart and also excessive veno-dilation (potentially reducing venous return to the heart and not necessarily improving the circulation). It was found that dobutamine caused moderate beta-1 agonism, including increasing heart rate in a dose-related manner, which was undesirable, an unclear effect on myocardial irritability, which was considered to be an important factor since a large number of deaths in heart failure patients were related to dysrythymia, and dose-related beta-2 vasodilatory effects. This latter may be favourable during heart failure, but not if it is excessive. Dobutamine was compared to digitalis and found to have greater inotropic effect in animals[8]. Due to the reduction in afterload (beta-2 agonist vasodilating effect), caused by dobutamine, it seemed reasonable to treat patients in acute heart failure with dobutamine rather than increasing doses of dopamine or epinephrine which cause pronounced vasoconstriction that is detrimental to the failing heart.

In the early 1980's, patients in cardiogenic shock following myocardial infarctions were often treated in coronary care units with the vasodilator and tranquilizer drug chlorpromazine (Hibernal®) 1-2 mg/hour in order to reduce afterload with careful monitoring of the blood pressure. They were also treated with nitroglycerine and diuretics in order to reduce preload and thereby to reduce the risk of pulmonary oedema. If the blood pressure dropped, dopamine or dobutamine (or a combination of these two drugs) was used in order to increase perfusion pressure while using signs and symptoms of brain and kidney function as indicators of effect.

Subsequently, down-regulation of the beta-receptor system in heart failure was described[9, 10]. When beta-receptor blocking agents became established in the routine management of ischaemic heart disease, it seemed logical to consider treatment of acute heart failure with the newly developed and described phosphodiesterase III inhibitors, and first amrinone and then milrinone became available in the late 1980s[11, 12].

The search for new inotropic drugs has continued. Evidence was presented that the long-term treatment of chronic heart failure with PDE III inhibiting drugs increased mortality[13]. It was thought that intravenous milrinone may be deleterious in ischaemic heart failure but neutral or beneficial in non-ischaemic cardiomyopathy[14]. In 2000, a drug with another inotropic mechanism (a calcium-sensitizer) was introduced and the drug was called levosimendan[15].

Further steps in the development of inotropic substances and therapy can be expected, as there have been major advances in the understanding of the molecular biology of ventricular dysfunction. Even non-drug interventions may at some point be possible, related to myocardial cell engineering system with genetically based interventions to induce cell reprogramming[16, 17].

ATP in the normal, ischaemic and failing heart

Adenosine triphosphate (ATP) is the universal currency of chemical energy within the cell. The ATP molecule contains high-energy phosphate bonds, and when the substance is hydrolysed to ADP and even further to AMP, energy is released. At any given time, the amount of ATP in the human heart (250g) has been estimated to be in the order of around 0.7g. If the synthesis of ATP suddenly stopped, the readily available ATP and ADP would be just enough for less than one-minute of energy consumption by the heart[18]. During heart failure, ATP levels observed with NMR spectroscopy appear to be reduced to about 25% of the normal resting level[19]. The ATP, ADP and AMP do not leave the cell in their original conformation, since these molecules are all heavily polarized. During myocardial ischaemia, there is a further degradation of AMP to adenosine, inositol and hypoxanthine, and ischaemia leads to net purine depletion from the cell, some amount of purine is even utilized as an energy substrate[20, 21]. The normal heart replenishes its pool of ATP approximately 10 000 times a day, and a utilization of ATP 0.07g/s means that the human heart uses around 6 kilograms of ATP each day[18].

The role of cAMP

Cyclic AMP (cAMP) is a cyclic nucleotide, functioning as a second messenger in the heart. The inner layer of the cell membrane produces cAMP by the enzyme adenylyl cyclase (also called adenyl or adenylate cyclase). This cAMP is produced when the beta-adrenergic receptor is activated and proteins within the cell wall (G proteins) then activate adenylyl cyclase forming cAMP from ATP. cAMP is ideal as a second messenger since it is rapidly generated (from ATP) and is then able to phosphorylate a large number of systems within the cell[22]. In local, compartmentalized, areas of the cell, often called “hot spots”, the messenger can rapidly be inactivated by various phosphodiesterases. There appears to be a ‘closed loop’ that regulates cAMP production during chronic heart failure[23].

The role of calcium

The calcium ion is involved in many cell-signalling processes, both between cells and within cells, from oocyte fertilisation and all the way to cell apoptosis (cell suicide)[24]. Calcium can be found as a signalling ion almost everywhere in plants and animals (including bacteria), and in other higher species. In humans, calcium ion is involved in the regulation of membrane potential for all cells with excitable membranes, and therefore it is important for the function of many types of cells including vascular smooth muscle and cardiomyocytes. Calcium is the major link from membrane excitation (depolarization) to myofilament contraction[25]. Membrane-bound energy-dependent ion pumps regulate calcium levels at rest. Normal extracellular concentration of calcium is approximately 2×10^{-3} M, which is a thousand times greater than in the cell cytosol at rest. In the

cytoplasm of the cardiac myocyte, the calcium concentration oscillates between 0.2 $\times \cdot 10^{-6}$ M during diastole up to 50 times higher (10^{-5} M) during systole[26]. During cardiac myocyte depolarization, the L-type (long-opening) calcium channel opens as a second inward current of the action potential. Potassium out-flux is the first fast current during an action potential, along with sodium influx. The calcium ion inflow initiates calcium-induced calcium release (CICR) from the sarcoplasmic reticulum (SR). The L-type calcium channel can be phosphorylated by protein kinase A in order to further increase the duration of the opening state. The SR is the main storage site of intracellular calcium. The L-type calcium channel is positioned close to the ryanodine receptor in the SR, where calcium is released into the sarcoplasm (cytoplasm).

Calcium is also the key intracellular messenger for initiating the movements of the contractile proteins. In order to be able to inactivate the contractile proteins at the end of systole, calcium must be pumped back into the SR or, alternatively, outside the cell or inside the mitochondria. The most important process is calcium re-uptake into the SR, and this is triggered by the SERCA (Smooth endoplasmic reticulum calcium ATPase) pump. An inhibitory protein, phospholamban, regulates this process. Phosphorylation of this protein increases the ability of the SERCA pump to clear the sarcoplasm of calcium, which is the biochemical parallel to an increased lusitropic function. Modulation of the function of the sarcoplasmic reticulum and the calcium transport has therefore been proposed to be a factor for ischaemia/reperfusion and cardio-protection[27-31]. The second most important mechanism for ending systole is through calcium expulsion outside the cell via the sodium-calcium exchanger (NCX) and this pump has also been studied during ischaemia[32]. To a lesser extent, calcium can also be pumped outside the cell by the sarcolemmal calcium ATPase pump and inside the mitochondria by the mitochondrial calcium uniport.

The contractile proteins in the heart

More than half of the myocardial cell volume consists of contractile proteins: actin (thin filament) and myosin (thick filament), and other components within the thin filament including tropomyosin, troponin C, troponin I and troponin T. Actin and myosin convert chemical energy from ATP into cell shortening and mechanical work. In the heart, the myocytes are arranged end-to-end so that coordinated cell shortening leads to pressure generation in the heart chambers. There are also other proteins in the heart, including the extremely large protein titin and myosin-binding protein C both of which can influence the forces that develop during the cardiac cycle, which we can observe as ventricular mechanical events in pressure-volume relations. Muscle cell fibres are cross-striated due to packed myofibrils lying within the cells. At short intervals of around 2 μm, there are invaginations of the cell membrane into the myofibrils called the T-tubuli, where the calcium ion pumps of the L-type are most likely situated. The sarcomere is the functional contractile unit, and its length in diastole is approximately 2.2 μm when the pressure within in the cardiac cavity is about 12 mm Hg. If the sarcomere length is less than 1.5 μm, no active tension can be developed. The sarcomere cannot be distended further than 2.6 μm. In experiments when the filling/distending pressure in the heart is extremely high, at a non-physiological level of 100 mm Hg, the average length of the sarcomeres does not exceed 2.3μm [33]. Frank-Starling´s relationship of the myocardium suggests that the greater the stretch of the contractile elements in pre-systole, the greater the performance generated in systole. This can be explained by recognizing that stretch can reduce ineffective overlap of the structures in the sarcomere[34], and also that stretch increases the fraction of cross-bridges activated by a given amount of calcium, in other words, an increased sensitivity to calcium.

Systolic function

Each day the heart performs approximately 100 000 systoles and diastoles, and pumps approximately 10 tons (10 000 000 ml) of blood out to the rest of the body[35]. Ejection of blood has been assumed to be predominantly a function of the heart's systolic performance, but we also recognize that the heart is a servo pump, and in health it pumps forward whatever blood reaches it. Therefore, filling of the heart, venous return, is generally the limiting factor in the circulation, if the heart contracts in order to empty itself and eject blood. However, ventricular dysfunction can occur as a result of a myocardial insult or injury, often related to coronary artery disease and myocardial infarction. When the heart´s pumping function is limited, blood (venous return) will accumulate behind a failing pump, leading to symptoms of congestion, pulmonary oedema or generalized oedema, depending on whether left or right ventricular failure occurs. The adaptation phase following acute injury is described as "early mechanical remodelling", where the

heart has intrinsic qualities of adaptation to new mechanical stresses and constraints. If myocardial injury or overloading continues more chronically, then "the adverse remodelling" can result in a dilated, eccentrically hypertrophied and mechanically ineffective left ventricle. In chronically reduced myocardial performance associated with heart failure, there is a constellation of local and distant hormonal responses that are attempts to mobilize circulatory resources for compensation, including release of angiotensin II, aldosterone and vasopressin in order to supplement both diastolic and systolic load. A prominent aspect of this is compensatory neurogenic and angiotensin II-mediated vasoconstriction, which increases afterload. These increases in load may support the circulation for a short period, but in the long term are maladaptive[36].

When echocardiography is employed to assess heart function, the most common clinically used index of systolic function is ejection fraction (EF) or its two- and even one-dimensional parallels, the fractional area change (FAC) or fractional shortening (FS). These indices of left ventricular emptying can be calculated from volumes (V), areas (A) or diameters (D) in end-diastole and end-systole, with their lower limit of normal values as follows:

$$EF = \frac{EDV - ESV}{EDV} \qquad (>50\%)^{37}$$

$$FAC = \frac{EDA - ESA}{EDA} \qquad (>35\%)^{38}$$

$$FS = \frac{EDD - ESD}{EDD} \qquad (>25\%)^{37}$$

Predictors of use of inotropic substances during separation from cardiopulmonary bypass include depressed systolic function with EF<35% and affected wall motion[39]. Drugs that reduce afterload and/or preload can affect both EF and FAC as these are load-dependent measurements of systolic function.

General tactics to support the failing circulation involve three general steps. First, optimize the degree of ventricular pre-systolic filling (preload) in order to be able to assess ventricular contractile function. Then, if the circulation is not adequate despite optimal preload, adjust systolic loading conditions (afterload). If blood pressure is high (or on the high side of normal) then reduce it in order to help the heart eject more blood during systole. If contractile function is inadequate despite optimal loading conditions, provide support by treating with an inotropic drug. In some special situations, if afterload is very low, it will need to be

increased using a vasopressor support as an early treatment in order to guarantee vital organ perfusion. Using drugs with combined inotropic and afterload reducing effects makes the last two steps as one. In order to improve contractile function, one needs to think in depth about the concept of contractility[40]. It can be described as the myocardium's intrinsic capacity to generate force during systole, achieved from a given initial fibre length.

The concept of contractility or intrinsic contractile capacity and the 'Frank-Starling relationship', and especially its descending performance limb after exceeding optimal loading, has been the subject of some controversy. In 1938, Liljestrand *et al* showed that the normal heart did not increase in size sufficiently to account for the observed increase in cardiac output, during exercise [41]. Sarnoff et al later described a set or family of ventricular curves to show how the same heart can respond in different states of contractility[42]. Contractility is assessed using load-independent demonstration of systolic performance, which is quite difficult to study *in vivo* in humans since it is difficult to control cardiac load, and also requires highly invasive measurements.

One way to generate load-independent measures of contractility is to use pressure-volume analysis and plot specific points in the heart cycle for load (stretch, or volume) and performance (pressure or other parameter) for each beat. Then describe a relationship for these points over a series of beats where there has been a subtle progressive change in load from beat to beat, though without a disturbance in contractile status. This relationship is a quantification of (relative) contractile status that can be compared in serial measures. For example, load can be marked in beats for end-diastolic or end-systolic volumes, and performance can be described as dP/dt max, stroke work or maximal instantaneous power. Loading conditions can be reduced beat by beat in consecutive heart cycles by a number of different means, most simply by transiently limiting venous return to the heart. A snare around the inferior vena cava or an occluding intravascular balloon is a common method. A family of heart cycle pressure-volume loops is generated where contractile function is static but load is slightly different between beats. Preload recruitable stroke work (PRSW), a relation based on regression of stroke work and end-diastolic volume for each beat where the slope and position of the regression line is noted, can also be determined. The steeper the slope, the greater is the contractility status[43].

Another relatively load-independent measure of contractility is end-systolic ventricular elastance (Ees), which is derived from the end-systolic pressure-volume relationship (linear regression of end-systolic pressure-volume points for a series of heart cycles) where a slope and volume intersect are reported[44]. During controlled load alterations where the end-systolic volumes become lower, the pressure-volume relationship is curvilinear. The end-systolic pressure-volume relation was described for a single beat, where the tangent line to end-systolic pressure-volume beat intersects with the zero volume, and a slope was reported[45, 46].

Diastolic function

A very early manifestation of myocardial ischaemia is diastolic dysfunction[47-49]. Hypertension, increased age and coronary artery disease alter diastole by impairing early relaxation and increasing myocardial diastolic stiffness[50, 51]. Often, a heart with diastolic dysfunction or diastolic heart failure can maintain a normal EF. Diastolic dysfunction can be observed at an early stage of a more global injury process in the heart, and regional relaxation disturbances are a very early sign of regional myocardial ischaemia and have been studied experimentally as well as in patients undergoing cardiac surgery including cardiac transplant surgery[52-58]. Diastolic dysfunction with symptoms predicts heart failure and increased mortality later in life[59, 60]. In its most severe clinical manifestation, diastolic dysfunction can lead to a large increase in left ventricular filling pressure with consequent pulmonary oedema in spite of a normal EF. This is not an uncommon cause of inadequate initial circulation during weaning from cardiopulmonary bypass during heart surgery, even with normal systolic function[61, 62]. Increased left ventricular end-diastolic pressure is a predictor of mortality in cardiac surgery patients independent of systolic function measured by EF[63].

The low sensitivity of non-invasive techniques in determining diastolic disturbances has led to proposals for invasive measurements as a standard diagnostic method[64, 65]. Echocardiography for assessing diastolic heart failure is a subject of some controversy even among experts[66].

Early and active relaxation

Relaxation is the process whereby the myocardium returns to an unstressed length and resting state at the end of systole. Intraventricular pressure decreases with no change in volume since filling has not started; this is called the isovolumetric relaxation phase. In order for the ventricular filling to start, pressure needs to fall below the level of left atrial pressure since a pressure gradient is needed for flow to occur between the chambers. On a cellular level, relaxation is initiated by calcium transition into the SR by the SERCA pump and to the outside of the cell by NCX. In the normal heart, this energy-dependent active relaxation is completed by the time that the rapid filling period begins. Only when relaxation is much delayed and/or if there is a short diastolic period due to tachycardia does active relaxation occur during the middle portion of the diastolic period, and may then interfere with diastolic filling.

Invasive methods for evaluation of active relaxation include the measurement of the time constant of isovolumic pressure decay (tau). In order to resolve small changes in pressure change rate, a high fidelity rapid response pressure sensor is used. When assuming a zero pressure asymptote we assume that the pressure of the LV does indeed go down to zero if diastole was infinite and never was interrupted by a systole. The following equation is then used to calculate tau:

$$P(t) = P_0 e^{-\frac{t}{tau}}$$

where P(t) expresses the actual pressure in the left ventricle as a function of the (variable) time, t. P_0 is the pressure at time zero, that is, when dP/dt has its most negative value (dP/dt_{min}). Normally, a mono-exponential curve will adequately describe LV pressure decay[67]. Systolic function can interact with the diastolic function in this isovolumetric relaxation phase through compression and recoil of elastic elements in the myocardial wall. This can influence relaxation rates in a way that is not related to calcium handling.

Early and late ventricular filling

When LV pressure falls during relaxation to the pressure level of the left atrium, and ventricular pressure continues to decrease, the mitral valve opens and blood flows into the left ventricle from the atrium. In a normal heart, the pressure in the left ventricle can fall below zero during isovolumetric relaxation. When ventricular pressure falls at the same time as the ventricular volume increases due to inflow from the atrium, one can say that 'suction' has contributed to ventricular filling. This suction promotes filling especially during exercise and circulatory stress (high cardiac performance in critical illness). The first ventricular filling phase corresponds to the trans-mitral E-wave (E for early). Normally the LV fills to about 70-80% of its pre-systolic volume during this phase, and the late filling due to the atrial contraction contributes to the rest of LV filling. However, during diastolic dysfunction, the ventricular filling contributed by the atrial contraction becomes much more important.

A common way to assess diastolic function using echocardiography is to further analyse the velocities of trans-mitral flow from the left atrium into the left ventricle. A ratio of the early (E) passive flow phase and the late (A) atrial flow phase provides an indication of ventricular relaxation and compliance (E/A ratio). Pulmonary vein velocities are also affected by ventricular compliance. Tissue velocities assessed with ultrasound during diastole reflect the same phenomenon. One disadvantage of E/A ratios is that a quotient of velocities indicates nothing about absolute velocities or volumes. One can assess filling with a peak-filling rate estimation using echocardiographic chamber 2-dimensional area changes (a surrogate for ventricular volume) during diastole with the help of automatic endocardial border detection. Two-dimensional ventricular areas have been validated as an estimate of LV volumes in some settings, and their measurement has been facilitated by using different types of automated tools[68-70]. Peak filling rate (PFR) measured by 2-D echo as maximal area change over time or by radioventriculography or 3-D echo as maximal volume change over time, can further be corrected by end diastolic area or volume (depending on method) using the equations below.

$$PFR_{Area} = \frac{\left[\frac{dA}{dt}\right]_{\max}}{EDA}$$

$$PFR_{Volume} = \frac{\left[\frac{dV}{dt}\right]_{\max}}{EDV}$$

Normal values for peak filling rate (PFR) in healthy humans are 36±11 cm^2/s in women, and 43±17 cm^2/s in men. The corresponding normal values for corrected PFR are reported to be within 2.0-4.5 EDV/s or 3.6±1.1 EDA/s for women and 3.8±1.4 EDA/s for men[71-73].

End-diastolic stiffness

Stiffness is a characteristic of the heart and other hollow organs, where some amount of distending pressure is present in association with the distending volume. Stiffness for a single time point in this context is the inverse of compliance. It is the amount of pressure that is required to achieve a fixed amount of distending volume, although the relation of pressure to distending volume can be quite complex over a range of volumes. From the perspective of the individual cardiac myocyte, it is the sarcomere or myofibril stretch around the entire heart chamber by a pressure or force that tries to pull them apart (a volume inside the ventricle under pressure) that determines the degree of stiffness. The heart muscle in diastole is not resting completely. An absolute change in diastolic LV pressure relative to diastolic volume can be expressed as dP/dV. Instantaneous stiffness changes, as the volume of the left chamber is changed, a fact that has been a challenge when reporting stiffness. In the past, stiffness could be expressed as dP/dV for a given volume, but now it is more often expressed as a stiffness coefficient or constant in a mono-exponential expression where the pressure in the left ventricle is a function of volume during diastole (compare with tau above) and P_0 is the pressure at zero volume.

The equation can be expressed as:

$$P(v) = P_0 e^{(stiffness_{const})v}$$

Mono-exponential curve fitting could be applied both during one heart-beat, but better during multiple beats with different preloads using an inflated balloon in the vena cava. End-diastolic P/V points from multiple related and consecutive beats over a controlled preload reduction are typically used, and an exponential curve is fitted to these points.

A stiffness-constant can be generated from the equation for this complex diastolic relation of pressure to volume over a range of loading conditions. Left ventricular distensibility describes an altered position of the pressure volume (P/V) relations of the left ventricle but not an altered slope or a different stiffness constant. A decreased distensibility can be seen as just a upward displacement of the PV relation where stiffness is a change in stiffness constant. Pericardial changes as during tamponade as well as pure right ventricular distension gives less distensibility to the left ventricle, but true diastolic dysfunction or failure seems to change both distensibility and stiffness[74].

Stiffness has previously been proposed to be mostly a passive process related to the properties to the extracellular matrix in the cytoskeleton of the myocardium. Many proteins compose this matrix, different collagens, proteoglycans and titin. Cross-linkings and glycations of these proteins plays a key role in myocardial stiffness[75]. Titin in the heart, is the largest protein in the body, by molecular weight[76]. It has been observed in isoforms that have different degrees of stiffness, and short-term phosphorylation of titin can alter its stiffness properties. End-diastolic stiffness in this way can be altered rapidly due to energy depletion, as in acute ischaemia[77].

Pressure-volume relationship of the heart

The shortening of heart cells in systole creates pressure which, when exerted on intraventricular blood volume, leads to flow (and work) once there is a pressure gradient between the ventricle and the arterial system. While the Wiggers diagram presents a time aspect for changes in heart chamber pressures and volumes, these can be plotted against each other to demonstrate a pressure-volume loop for a heart cycle, which is advantageous since pressure and volume are two different aspects of myocardial cell conditions and performance. Frank presented a "Druck – Volum" diagram as early as 1899 for the excised beating frog heart under a variety of conditions[78].

For analysis of pressure-volume relations, a high fidelity pressure recording system is needed to resolve very rapid pressure changes in the left ventricle. Assessment of ventricular volume is performed by assessment of the electrical conductance in surrounding tissues of an electrical field generated by the catheter

electrodes[46, 79-81]. Pressure-volume data are collected as a series of point measurements at brief intervals (as short as 2-5 ms between measurements, depending on which pressure-derived rapidly-changing measurement is of most interest). Another aspect of pressure-volume analysis is that it lends itself very well to analyse families of consecutive heart-beats in order to generate a relationship between the continuous beats. For some characteristics of the heart, it is necessary to look at this type of multi-beat sequence in order to examine intrinsic characteristics of the ventricle that are independent of load (stretch or pressure).

Ventricular catheters have been constructed containing electrodes for generating and sensing conductance signals of ventricular volumes. These catheters can also have a miniaturized and embedded rapid-response pressure sensor, a tip manometer. Pressures, pressure changes over time (dP/dt), volumes, volume change over time (flow), pressure x volume (work), or pressure/volume (endsystolic elastance or enddiastolic stiffness) are easily measured at all points in the heart cycle. In addition, controlled preload reduction can be used to measure load independent indices of systolic function (preload recruitable stroke work; PRSW) and to evaluate the end-diastolic period/phase by the multiple beat created stiffness constant using EDPVR (end diastolic pressure volume relationship)[82].

Creating pressure volume curves using non invasive methods like echocardiography together with a high fidelity pressure catheter, instead of a conductance catheter, has been done both in animals[83, 84] (together with validations of the different volume recordings) and in humans[85].

Different methods for left ventricular volume measurement

Volume is a three dimensional (3-D) quantity and if only length and/or areas are measured in some selected regions of the heart, approximations would be made, and formulas then used to estimate total left ventricular volume. Two-dimensional (2-D) transthoracic (TTE) and transoesophageal echocardiography (TEE) are frequently used clinically today, and ventricular lengths and areas can be easily and accurately measured[86]. There have been comparisons between assessments of systolic function measured by TEE in a single mid-papillary area plane, which is a 2-D technique using fractional area change from diastole to systole (FAC) (see systolic function above) and radionuclide angiography (RNA) that measures EF by comparing relative amounts of indicator in blood during diastole and systole. A reasonably strong correlation between these two methods has been demonstrated[87]. There is a practice in perioperative echocardiography (TEE) using a single (mid-ventricular) plane as an indicator, or surrogate, for left ventricular volume measurements[88].

Conductance volumetry[80] is considered to be a reference method for measuring heart volumes *in vivo*. Intra-cardiac ECG as well as ventricular pressure and volume can be measured by the same catheter, amplifier and data capture systems. These raw volumes, pressures and ECG data can be isolated for each specific

measurement sequence, where they can be then further analysed in an off-line non-commercial analysis software designed for that purpose.

Theoretical modelling of the function of the human heart

"Since the mathematicians have invaded the theory of relativity, I do not understand it myself anymore."
Albert Einstein (1879-1955)

The heart cycle has been traditionally divided into four different components: systolic isovolumetric contraction, systolic ejection, diastolic isovolumetric relaxation and diastolic filling (both early and late). The pattern for the pressure-volume relationship during each of these phases of the cardiac cycle differs from the other three phases. Mathematical models have been presented to describe LV pressure as a function of time or a function of volume. But there are also theoretical models of looking at pressure varying with both time and volume[89]; such a theory needs a two-variable function p(t,v) and it may (must) be visualized and expressed in a 3-D diagram.

The equation to calculate LV pressure from both time and volume is:

$$p(t,v) = a(v-b)^2 + (cv-d)\left[\frac{1-e^{-\left(\frac{t}{tau_c}\right)^{\alpha}}}{1-e^{-\left(\frac{t_p}{tau_c}\right)^{\alpha}}}\right]\left[\frac{e^{-\left(\frac{t-t_b}{tau_r}\right)^{\alpha}}}{e^{-\left(\frac{t_p-t_b}{tau_r}\right)^{\alpha}}}\right]$$

The equation is included here just to show some of the complexities in making a theoretical model of such a structure like the beating heart, in this example by using nine (constant) quantities ($a,b,c,d,t_b,t_p,tau_c,tau_r$ and α) and two (variable) quantities (t,v). Here, a is a measure of diastolic ventricular elastance, b corresponds to the diastolic volume at zero pressure, c and d are volume dependent and volume independent components of the developed pressure. Tau is a time constant, which gives characteristics during contraction tau_c and during relaxation tau_r, t_b is a time constant derived from t_p, the two different values of tau and α. Finally, α is a measure of the overall rate of onset of these systolic and diastolic processes.

Using a mathematical model like the above allows measurement relations during actual observations, where derived functions can be compared for different conditions[90]. Models are needed in order to choose a method of mathematical analysis of simple pressure or volume observations to describe these complex

cardiac events. Computerized models and even super-computers are now used to simulate cardiac conditions. "Alya Red", a computational heart, has recently been developed to simulate the human heart at the Barcelona super-computing centre (BSC)[91]. Many different constants (intrinsic characteristic responses) have been determined which are relevant for each human heart chamber[89]. More advanced mathematics can used to describe the interaction of the heart and the circulation. The performance of the heart is very intimately related to the vascular conditions that provide input (venous system) and form the output (arterial system). Blood flow can be assessed by the use of vector field multivariable calculus and the Navier-Stokes equations, which describe behaviour of incompressible viscous fluid (equations not included here)[92].

Vasodilators in acute heart failure

A well-recognized physiological goal with vasodilating agents is to facilitate ventricular ejection (increase stroke volume) when the systolic function is poor[93, 94]. According to large retrospective studies, many patients with acute heart failure present with preserved or increased blood pressure[95, 96]. Several intravenous drugs such as nitroglycerine, nitroprusside and nesiritide have been used to reduce afterload during heart failure in these circumstances[93]. However, it is still not clear how to achieve optimal vasodilator treatment[97, 98]. In contrast, patients in acute heart failure and hypotension may become severely hypotensive following inotropic vasodilator therapy, leading to severe hypo-perfusion of vital organs. In these patients, contributing factors to hypotension, such as aggressive diuretic therapy, should be explored and treated[99].

Current clinical settings for inotropic therapy

The current standard for treatment of chronic heart failure includes afterload reduction with angiotensin-converting enzyme (ACE)-inhibitors or angiotensin receptor blocking (ARB) agents, as well as diuretics. Beta-adrenergic blocking agents are also used. They do not interfere primarily with the heart, but rather reduce the effect on the heart of the neuro-hormonal storm that could lead to even further damage to the heart, unless controlled[37]. These management strategies are largely directed towards reducing or preventing ventricular overload where the body's generic response to inadequate ventricular function is to first try and increase ventricular filling by hormonal or neuro-hormonal means. The scientific and clinical evidence for the management of *chronic heart failure* is strong, where relief of symptoms can be carefully titrated over time. On the other hand, management of *acute heart failure* is perhaps more complex, and often requires acute potent interventions to achieve hemodynamic stabilization. Therefore, there is less scientific and clinical evidence for the management of acute heart failure.

The majority (70%) of patients with acute heart failure are patients who already have chronic heart failure that becomes acutely exacerbated[100]. The definition,

classification and epidemiology of acute heart failure, have been revised[100-102]. The European guidelines for the treatment of heart failure suggest that intravenous inotropic support is reserved for those patients in whom a reduction in cardiac output compromises vital organ perfusion[37]. Since cardiac output is seldom measured when new symptoms occur, an "educated guess" of this is often made based on blood pressure and signs of hypoperfusion. Clinical recommendations have been published regarding management of perioperative heart failure in cardiac surgery[103]. Acute heart failure with severe symptoms sometimes first requires emergency, life-saving interventions, such as i.v. epinephrine injections that may be used to counteract both severe bradycardia and extreme hypotension. When there is time to further evaluate the different components of the circulation, there may be a pharmacological rationale to use non-adrenergic inotropic drugs such as milrinone or levosimendan, particularly in patients who are already being treated with beta-receptor blockers, and in those with hypotension and hypoperfusion, sometimes together with vasoconstrictors[37].

Inotropic alternatives

Short-acting adrenergic agonists that are less potent than adrenaline, such as dobutamine, are often used to treat moderate, but sometimes even life threatening ventricular dysfunction[104]. The effects of adrenergic agonism using dobutamine in the setting of acute heart failure is quite complex, and to some degree unpredictable. While increased cardiac output and perfusion might be needed for the circulation, these drugs (dobutamine or adrenaline) increase myocardial oxygen consumption[105], which may have a negative effect in the failing or ischaemic myocardium with unwarranted metabolic and other side effects (like inducing myocardial infarctions)[106-110]. Treating patients using beta-receptor agonist drugs in those who are chronically treated with beta-adrenergic receptor blockers can be pharmacologically and physiologically awkward. In general, withdrawal of beta-blockers cannot be recommended, even in worsening chronic heart failure as beta-receptor blocking agents have shown to be very important in ischaemic heart disease and heart failure[111-114]. Perioperative beta-blocker therapy is associated with a reduced risk of in-hospital death among high-risk patients undergoing major non-cardiac surgery[115]. One study in carvedilol-treated patients has shown no increase in cardiac index using dobutamine unless very high doses (15-20 μg/kg/min) were used. The effect of milrinone was not altered by carvedilol[116]. In patients with advanced heart failure, particularly those already treated with beta-receptor blocking agents, levosimendan treatment is thought to achieve greater hemodynamic and neuro-hormonal stability than dobutamine[117].

The beta-receptor system can be down-regulated in heart failure, and this has been noted to occur even after a relatively short period of time, as during cardiopulmonary bypass[118]. Ischaemia can potentially result from inotropic treatment, and although adrenergic substances normally have positive lusitropic

effects, diastolic function seems more susceptible to ischaemia than systolic function[48]. In this setting, treatment with milrinone or levosimendan rather than a beta-receptor agonist is to be preferred in patients already on beta-receptor treatment, when inotropic support is needed[119-121].

Phosphodiesterase inhibiting drugs

Both cAMP and cGMP are broken down intracellulary by a large family of enzymes (phosphodiesterase, PDE)[122]. Inhibition of these enzymes leads to intracellular accumulation of cAMP and cGMP. Eleven families of phosphodiesterases have been identified so far, and these differ regarding affinity for cAMP and cGMP but also with respect to cellular expressions in different organs and their intracellular localization[123]. The presence of one cyclic nucleotide can also influence the intracellular concentrations of the other. For example, PDE3 has a higher affinity for cAMP, but as it also has some affinity for cGMP, it can act as a cGMP inhibited cAMP phosphodiesterase. Five PDE enzyme families have been found in the myocardial cells of humans, PDE1-5. About 90 % of the total PDE activity in the heart is represented by PDE1 and PDE3. About 69% of the PDE that metabolizes cAMP can be found in the cytosol, according to one study[124]. The rest is bound to membranes, as on the surface of the sarcoplasmic reticulum (SR) and to mitochondria membranes. PDE3 can be found in at least 4 isoenzymes, and PDE4 also important for the heart, found in 9 isoforms.

Amrinone, enoximone and milrinone are examples of selective inhibitors of PDE3 [125-127]. They augment protein kinase A (PKA) activity, thereby increasing both the first calcium signal and trigger "calcium-induced-calcium-release" (CICR), and even several other later calcium events in the cardiac cycle. PDE3 inhibitors can increase the amount of calcium release (from SR) via ryanodine receptor-sensitive processes[128], but also stimulate calcium storage in the SR via SERCA and active outward pumping of calcium out of the cell. Some PDE3 inhibitors seem also to have calcium-sensitizing effects on the myofilaments, which is true for pimobendan but not for milrinone[129, 130]. Instead, some of the contractile effects of milrinone analogues can be a result of blockade of adenosine receptors[131].

The advantages of using PDE3 inhibitors to treat acute heart failure, at least from a theoretical standpoint, may have to do with the uncoupling of adenylyl cyclase towards the beta-receptor due to G protein over-expression in heart failure. Lower basal cAMP values are found in the failing heart as opposed to the non-failing human myocardium[132]. In these circumstances, milrinone can inhibit the degradation of cAMP in the cell. Also, in case of on-going beta-receptor blocker treatment in patients, the inotropic action of a PDE3 inhibiting drug lies distally to the beta-receptor, is an advantage.

A major part of the cardiac effects of PDE inhibitors are not due to a global increase in cAMP, but rather to local elevation of cAMP levels in microdomains,

within a limit of 1 μm, from structures regulating the calcium transport, especially the SR. Muscle selective kinase A anchoring proteins mAKAP link the protein kinase A to the target[133, 134].

Acute administration of PDE3 inhibitors, including weaning from cardiopulmonary bypass during cardiac surgery, has been shown to exert beneficial hemodynamic effects on cardiac output, stroke volume and filling pressures, without an increase in heart rate that can be seen with catecholamines[135-148]. The pharmacokinetic aspect of milrinone administrated during cardiac surgery has been evaluated[149, 150]. Additionally, symptomatic relief has also been found in patients with acute heart failure[151]. Studies have shown improvement in symptoms without increasing myocardial oxygen consumption (when measured) in humans[152-159].

Long-term or chronic treatment of heart failure with oral preparations of milrinone and enoximone, have not improved survival, perhaps even caused harm, in chronic heart failure[13, 160, 161]. Similar mixed effects for milrinone have been reported during treatment of acute cardiac failure (OPTIME-CHF trial), though the literature has not been conclusive[162-165], which is one rationale for our present studies. Studies in animals have shown beneficial effects of intravenous milrinone in the ischaemia-reperfusion setting, suggesting that there might be a cardioprotective effect of the PDE3 inhibitor milrinone[166-168]. There is evidence in humans that administration of milrinone can reduce myocardial ischemia and myocardial infarctions in patients undergoing on-pump coronary artery bypass surgery[169].

Calcium sensitizing drugs

Levosimendan is an intravenous agent with calcium-sensitizing effects on the contractile filaments in the myocardium as it binds to troponin C, thus stabilizing the formation, and prolonging the systolic actin-myosin interaction[170-182]. In higher doses, with blood concentrations above 1 μM, additive effects on PDE3 inhibition can contribute to its cardiac effect[183]. The drug has been reported not to increase myocardial oxygen consumption [184, 185]. Although levosimendan has elimination half-time of only 1-1.5h, it has an active (inotropic) metabolite OR 1896, which has a half-life of 75-80h[183, 186]. Levosimendan is highly protein-bound in the plasma (98%), but not its metabolite OR-1896 (40%).

Levosimendan has been shown to increase cardiac output and decrease filling pressures in patients in acute heart failure[187, 188] and to improve contractility in post-ischaemic myocardium in patients with myocardial infarctions, as well as in heart failure treated with percutaneous coronary interventions[189]. Established beta-receptor blocking treatment may be associated with augmented beneficial hemodynamic effects with levosimendan. In the LIDO trial, levosimendan was shown to improve survival compared to dobutamine[187]. However, this was not confirmed in the more recent trial, SURVIVE[190]. A meta-analysis regarding levosimendan and mortality after coronary revascularisation[191] suggests a reduced

mortality by the drug, but there have also been arguments against[192] a pooled estimate when many important differences exists in the included studies.

In theory, pure calcium sensitizers can worsen diastolic function since relaxation becomes delayed. Conversely, the mild phosphodiesterase-inhibiting effect of levosimendan may explain the fact that levosimendan does not appear to worsen lusitropy[183]. Another reason for not impairing diastolic function is the calcium-dependent calcium sensitizing effect of the drug. Therefore, when lower calcium concentrations are present intracellularly, as during diastole, there may be a less sensitizing effect of levosimendan. Some studies also suggest that levosimendan has a positive lusitropic effect and thereby causes active relaxation of the myocardium during diastole[193, 194]. Isovolumic relaxation time (IVRT) has been used as a measure of diastolic function in a study of patients with aortic stenosis undergoing aortic valve replacement[194]. Other authors have proposed that IVRT´(IVRT prime; that is IVRT measured by tissue Doppler echocardiography) could better serve as a surrogate of left ventricular relaxation than IVRT[195].

Levosimendan activates ATP-dependent potassium channels in vascular endothelium, leading to vasodilatation, but also in other organs including the kidneys and the brain. These effects have been postulated to maintain closure of the mitochondrial permeability pore and might protect organs from ischaemia/reperfusion injury[186].

Metabolic support and hormons affecting the heart

Substances with cardiac effects not acting through beta-receptor agonism, include the endogenous substance glucagon, glucose-insulin-potassium (GIK), glutamate, pyruvate and thyroid hormone. Not all of these have been systematically studied in randomized, blind trials with complete hemodynamic evaluation, making it difficult to prospectively evaluate their effects on systolic and diastolic dysfunction[35, 196]. Nevertheless, some of these substances can be of value in correcting a metabolically depleted heart[197-199]. Pyruvate has been shown to have beneficial hemodynamic effects, but restriction to its intracoronary administration for any clinical benefit limits its use[200].

Recent developments in inotropic therapy

Currently used intravenous inotropic substances have so far not been demonstrated to improve survival. Short-term beneficial effects including hemodynamic improvement and relief of symptoms have been shown[201]. The search for new treatment options and new substances, including combinations of existing drugs will continue. Some interesting new drugs have been tested recently, where an attempt to combine features of positive inotropic and lusitropic actions without increasing myocardial oxygen consumption has been made[202]. For instance, istaroxime inhibits Na-K ATPase while increasing calcium via NCX and also increasing the function of SERCA-enhancing calcium uptake into the SR.

Myosin activators, such as omecamtiv mecarbil, have been tested in humans, and appear to increase systolic function and decrease heart rate, although there remain some concerns about diastolic function, as with calcium sensitizers. However, in the limited reporting of experience in humans, there seems to be no deleterious effect on exercise tolerance. In the CUPID trial[203], introducing the gene for SERCA by intracoronary injections of an adenoviral-vector carrying gene has been used for the management of cardiac failure. This type of approach may be a future option in patients with severe heart failure undergoing coronary angiography or similar interventions where direct intra-coronary administration of drugs, stemcells or genes can be performed.

Nitroxyl gas (HNO), has been described to have both positive inotropic and lusitropic effects, together with vasodilating properties, and its effects seem to be independent of the cAMP/PKA or cGMP/PKG systems. Intravenous or per oral donors of HNO are promising, and clinical studies have recently been started. Other substances like ryanodine receptor stabilizers may act by reducing calcium ion leak from the SR.

Aims

I. To measure and analyse the effects of milrinone on blood flow in coronary artery bypass grafts during CABG surgery.

II. To measure and analyse the effects of milrinone on left ventricular diastolic function during post-ischaemic myocardial dysfunction.

III. To determine if milrinone or levosimendan are protective or injurious during acute myocardial ischaemia in a porcine model.

IV. To determine if adding levosimendan to an existing milrinone effect leads to further improvement in myocardial function, in a porcine model of post-ischaemic stunned myocardium.

Materials and Methods

"If you really wish to learn, then you must mount the machine, and become acquainted with its tricks, by actual trial"
Wilbur Wright (1867–1912)

Study I

This was a prospective randomized, double blind, placebo-controlled study in 44 patients undergoing coronary artery bypass surgery at the University Hospital in Örebro.

Subjects

Forty-four patients were recruited (consecutively) from a surgical population at a single institution at the Örebro University Hospital. Inclusion criteria was stable coronary artery disease with an EF ≥ 30% in patients undergoing elective (isolated) coronary artery bypass surgery with cardiopulmonary bypass. Exclusion criteria were significant valvular disease, atrial fibrillation or EF < 30%. All subjects that were randomized into the study completed the protocol and were included in the statistical analysis.

Study protocol

All subjects were managed in a standardized way with venous and arterial lines before the induction of general anaesthesia: a central venous multilumen catheter and a TEE probe were inserted after induction. Anaesthesia was induced with thiopental, fentanyl and pancuronium and maintained with a mixture of isoflurane/oxygen/air and supplemental bolus doses of fentanyl. Surgery was performed using cardiopulmonary bypass with moderate hypothermia. Both anterograde and retrograde cold blood cardioplegia were administered in all patients in order to achieve cardiac arrest and myocardial preservation. The saphenous vein grafts were manually dissected and distended. Three senior members of the Department of Cardiac-Thoracic Surgery performed the surgical procedures. No patient needed any catecholamine injection or infusion for circulatory support during the study period. The mean arterial pressure was maintained above 50 mm Hg using phenylephrine.

The study drugs (milrinone or placebo) were given over a 15 min period with start immediately after de-clamping of the aorta. Left ventricular end diastolic pressure measurements were made 10 min after termination of cardiopulmonary bypass using a fluid filled catheter. Flow measurements (see below) were performed at 10 and 30 min after termination of cardiopulmonary bypass.

Randomization and blinding

The patients were randomized into two groups of 22 each by opening of a

sealed envelope outside the operating room during the bypass period. Nurses who were not involved in the study or the clinical care of the patient prepared the study drugs (milrinone or placebo, both clear liquids). The investigators were blinded as regards randomization and study drugs.

Study drugs and their administration

Milrinone was administered as a single bolus infusion 50μg/kg, into the cardiopulmonary bypass system, starting at the release of the aortic cross clamp. The dose was diluted in 0.9% NaCl, to a total volume of 20mL, and it was given over a 15 min period using of a syringe pump with constant flow. In the placebo group, an equivalent volume of 0.9% NaCl was given at the same rate.

Flow measurements

A transit time flow meter with 4 mm probes (Transonic System Inc, Ithaca, NY, USA) was used specifically for the study purpose to measure the graft blood flow in all patients. Before and during the study, it was calibrated by the Department of Biomedical Engineering at Örebro University Hospital. This method of flow measurement has been validated in a similar context by other authors[204]. Flows in all coronary graft were measured at 10 min and 30 min after termination of cardiopulmonary bypass. In case of sequential grafts, flow measurements were done at the proximal part of the graft. In a conduit with flowing blood, some basic assumptions can be made:

Resistance = Mean pressure difference/Mean flow
However, according to the resistance equation, the following is calculated.
Resistance = $[8 \times \eta \times L] / [\pi (\text{radius})^4]$ where η is the blood viscosity and L the length of the conduit.
Taken together, the equation for flow in conduits is as follows[205]:

$$Flow = \frac{\left[\pi \left(Pressure_{gradient}\right) r^4\right]}{8\eta L}$$

Pressure measurements

Systolic, diastolic and mean arterial pressures as well as the left ventricular end diastolic pressure (LVEDP) were measured continuously. The latter was measured 10 min after termination of cardiopulmonary bypass using a fluid filled catheter in the left ventricle.

Study II

This was a prospective, randomized, double blind, placebo-controlled study in 24 patients undergoing CABG surgery at the University Hospital in Örebro.

Subjects

The subjects in **Study II** were a subset of the first 24 consecutive patients also included in **Study I**.

Study protocol. TEE and diastolic function

The study protocol was identical to that in **Study I**, with the addition of echocardiographic evaluation of the left ventricular diastolic function. This was done by the one of the authors (BA), with TEE using a Sonos 2500 echo machine (Philips, Andover, MA, USA) equipped with AQ (acoustic quantification)[206]. Automated border detection systems has been compared to standard techniques and successfully used in many different studies[72, 207-211]. The mid-papillary view of the left ventricular short axis[88] was evaluated. The border between myocardium and blood was detected by the software used in the edge detecting system (AQ). The short axis area of the left ventricle was continuously stored in a computer using an analogue-digital signal conversion card (Keithley Metrabyte, DAS 1602, Keithley Instruments Inc Cleveland, OH, USA).

Close attention was paid to set a region of interest around the short axis view of the left ventricle and to adjust overall gain, time gain and lateral gain controls as well as compress levels. This was done to improve actual myocardial/blood border detection with the AQ software, to try to eliminate false borders[206]. The area was derived by an endocardial border detection software in the echo machine. The area curves together with the corresponding left ventricular pressure measurement were stored together in a non-commercial software (visual Basic) on a standard PC. The frame rate given by the echo system was around 40 frames/s at the chosen depth of the echo-sector; 10-12 cm. Diastolic function was evaluated as peak-filling rate i.e. maximal area change over time. The normalized peak-filling rate, or the peak-filling rate divided by end diastolic area, was measured from the same heart beat where the rate was calculated. Sets of at least 3 different LV areas were collected just before going on cardiopulmonary bypass and 10 min after termination. LV area (filling) was varied by titrated transfusions from the cardiopulmonary bypass machine. Peak-filling rate dA/dt_{max} and normalized for area $(dA/dt_{max})/EDA$[212, 213] were calculated off-line by one investigator (JH), who did not participate in the operating theatre data collection.

A pilot study was performed before including patients using a pressure generator in a bench experiment, together with the same length of fluid filled pressure recording system that was later to be used in patients. One of the authors (BA) observed that pressure signal from the 'ventricle' was delayed by the catheter/transducer by approximately 25 ms. It was also confirmed that the area

and pressure were recorded with a frame rate of 40 Hz. Test measures using manual traced area showed good correlation between the area recorded by the automated system and a manually traced one, excluding the papillary muscles.

Study III-IV

The animal model and animal handling

Concerning the animal models and ethical considerations, all animals in both **Study III-IV** were treated in line with the 'Humane Care and Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals' (1996), from the National Academy of Sciences' Institute for Laboratory Animal Research, USA[214]. A large animal model was chosen to investigate how drugs affect and influence local myocardial ischaemic insult/infarction (**Study III**) and also to study global post-ischaemic stunning (**Study IV**) in the hope that data interpretation could possibly be extended to humans. There were several reasons why this acute experimental porcine model was chosen in order to study responses to acute myocardial injury. First, to assess and analyse acute injury mechanisms[215] and protective or injurious interventions, an integrated model is advantageous. An integrated model it one where the vital organs are intact and the body's homeostatic reflexes and systemic responses to insult or injury are fully functioning. While there may be cellular responses that can be assessed in cell cultures, it is possible that these responses are not the most relevant ones in a complex organ with many types of cells and responses to injury, and therapeutic interventions. Similarly, an organ can be influenced by a whole array of neuro-humoral or inflammatory mediators released from other organs during an acute injury.

Responses to acute injury can sometimes be measured in patients in the course of conventional, standardized and acceptable treatment. However, it is not possible and acceptable to injure human subjects in order to study their response to injury. In **Study III-IV**, responses were evaluated after carefully titrated myocardial injury and highly invasive measurement techniques were used. Such studies cannot be performed in humans and large animals are therefore acceptable by the standards of our modern research ethics review committees. Pigs are probably the best available model since their anatomy and particularly their coronary circulation is very similar to that of healthy humans, with no or minimal collateral circulation between the main coronary arteries. Our studies were acute experiments, where healthy juvenile pigs were raised for research purposes, anaesthetized for the experiment and then euthanized at the end of the experiment on the same day. In this way, there was minimal or no suffering for the animals related to our studies.

Preparation

Pigs for **Study III-IV** were prepared in a standardized manner, during the initial phase. The differences in preparations between the experimental animal

studies were that **Study III** was an open chest model with local myocardial injury while **Study IV** was a closed chest model with a global myocardial injury. All animals were premedicated with weight-adjusted dosages of ketamine, xylaxine and atropine. This premedication was chosen in order to minimize circulatory depression when combined with the general anaesthetic. A venous catheter was placed in an ear vein, and anaesthesia was induced with penthobarbital. A tracheostomy was performed by surgical cutdown. Anaesthesia was maintained with infusions of fentanyl, pentobarbital and midazolam, related to bodyweight. The animals were not given muscle relaxants, and were therefore free to move if inadequately anaesthetized when stimulated by surgical or experimental events, allowing us to adjust and deepen the anaesthetic dose. Such movements were not seen in a single animal during all experiments probably due to deep and adequate anaesthesia. Arterial line, central venous line (Arrow-Howe's multi-lumen, Vingmed, Järfälla, Sweden) and introducers for Swan-Ganz (PA catheter) and coronary sinus catheters were placed through surgical venous cut-downs in the neck. Optimal catheter positions were confirmed using fluoroscopy. Hemodynamic signals were recorded continuously (AcqKnowledge®, Biopac, Goleta, California, USA) and stored in a computer in digital format. Cardiac output was measured using the pulmonary artery catheter and thermodilution technique, injecting a measured cold saline bolus at end-expiration, with an automatic injector device and recording a mean of three measurements.

Controlled local myocardial ischaemia/infarction

In **Study III**, a median sternotomy was performed in order to allow placement of a coronary artery snare (second diagonal branch of the left anterior descending artery). Microdialysis catheters were placed and secured with sampling membranes in the region of expected ischaemia.

In **Study IV**, a balloon-tipped 9F (Fogarty type) catheter was placed with the tip in the inferior vena cava at the junction with the right atrium, in order to be able to perform transient restriction of venous return to the heart, facilitating recording of a series of consecutive heart beats with a controlled alteration in preload.

In order to validate models of global ventricular post-ischaemic stunning in our laboratory, pilot studies were conducted. For the first pilots, a traditional cardiopulmonary bypass model was used with a sternotomy and venous and arterial cannulation. A period of 10-15 minutes of warm ventricular fibrillation was tested with full flow assisted by the centrifugal pump system as the intervention was warm ischaemic time before resuscitation. This first injury method was abandoned despite adequate LV stunning since the right ventricular dysfunction that also occurred provided an unstable platform for post-ischaemic study of left ventricular function. A more selective way of inducing LV injury was used based on transient left main coronary artery occlusion and controlled global LV ischaemia. This model had been validated in another institution using an open thorax and a snare

around the left main coronary artery in pigs[216-219]. Although we assessed this model, we ultimately chose a closed chest model using a balloon tipped catheter placed in the left main coronary artery. The resulting effect on MAP of these transient occlusions of the left main stem of the coronary artery is showed in the figure below. A closed chest model is preferable (though not necessary) when using left ventricular conductance volumetry.

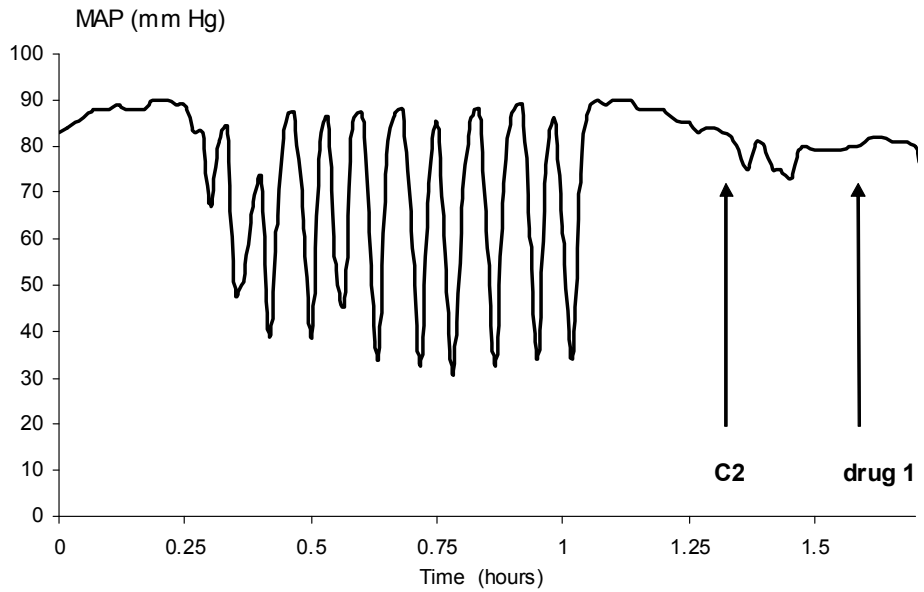


Figure 1. Ischaemic/reperfusion closed chest pig injury model. Effects on MAP when 11 transient balloon occlusions, are made in the left main stem of the coronary artery, first two times 1 min, then nine times 2 min. Control measurement before ischaemia at time zero. C2 measurement after ischaemia. Drug 1, start milrinone or placebo. Registration from an animal included in **Study IV**.

Microdialysis

Microdialysis catheters were used in **Study III** to facilitate sampling of very localized myocardial interstitial fluid, which reflect the state of local substrate availability and the production of metabolic by-product. Microdialysis sampling is based on the principle of delivering a physiological perfusate to a membrane exchange area that is very small, but allows effective exchange of solutes and small molecular weight substances (20-100 kDaltons depending on the specific catheter). Substances in the interstitial fluid pass through the membrane along a concentration gradient, and are 'captured' in the dialysate. This dialysate is collected and analysed. Equilibration between the interstitial fluid substrate concentrations and the microdialysate is never complete. However, with the substances which we measured using this specific microdialysis catheter and membrane pore size, 'recovery' was quite high and assessment of the cellular and interstitial conditions would be quite reliable[220-222].

Three microdialysis catheters were placed and secured with the sampling membranes in the substance of the LV wall (CMA 20, CMA, Solna, Sweden). Two of these were placed in the area of the ischaemic zone and one was placed in a non-ischaemic zone, to serve as a control measurement. An 'equilibration' period was observed for at least 60 minutes after placement of the catheter and before microdialysis sample collection was started. The microperfusate that was used was modified Krebs-Ringer phosphate buffer (Fresenius Kabi, Halden, Norway), and $^{45}Ca^{2+}$ (1 μCi/mL) was added to the microperfusate in all three probes. The pump flow in the microdialysis system was 2 μL/min. This flow rate was chosen to try to balance good recovery with necessary microdialysate volumes needed for analysis. Microdialysis samples were collected at 15 minutes intervals, providing a planned volume in each vial (at each interval) of 30μL. A 15-minute interval was chosen to allow microdialysate volumes needed for analysis, which match the timing of the interventions in the experimental protocol. Shorter collection intervals are possible, but were not necessary here. A 15 min interval meant that the sample represents a single averaged value during this period, although brief higher or lower levels for the measured substances could have occurred interstitially[221-223].

A CMA 600 was used for analysis of microdialysis samples (glucose, lactate, pyruvate and glycerol) in **Study III**. The radio-marker $^{45}Ca^{2+}$ was analysed using high performance liquid chromatography[224] and measurements were performed for samples in the microperfusate and microdialysate, in order to measure $^{45}Ca^{2+}$ 'recovery'. In this context, 'recovery' reflected relative amounts of the radio-marked calcium which was taken up into cells[225]. The microdialysis system and all microdialysis sample vials were handled and analysed in accordance with previous reports in plastic vials, which were immediately covered with airtight caps[226, 227]. Microdialysis pyruvate observations from the heart have however been somewhat difficult to interpret[228], as well as lactate/pyruvate ratios in identifying myocardial ischaemia.

*CS catheter measurements (**Study III-IV**)*

Coronary blood flow and myocardial oxygen consumption were measured using a 7F, 2-thermistor, coronary sinus catheter (CCS-7 U-90A; Webster Labs, Altadena, California, USA), which was placed with the tip in the great cardiac vein. Thermodilution measurements of coronary sinus blood flow were accomplished using a Wheatstone bridge (CBA-210, Webster Lab. Inc.) and a multi-channel signal amplifier (Gould TA 5000 polygraph recorder, Valley View, OH, USA)[229]. Some special anatomical difficulties are involved in using CS catheter measurements in pigs. The azygos or hemiazygos vein enters the coronary venous system from above (see figure below) and the CS catheter has to be placed further into the venous system in order to obtain blood samples with a higher oxygen extraction (which truly represents oxygen consumption by the heart).

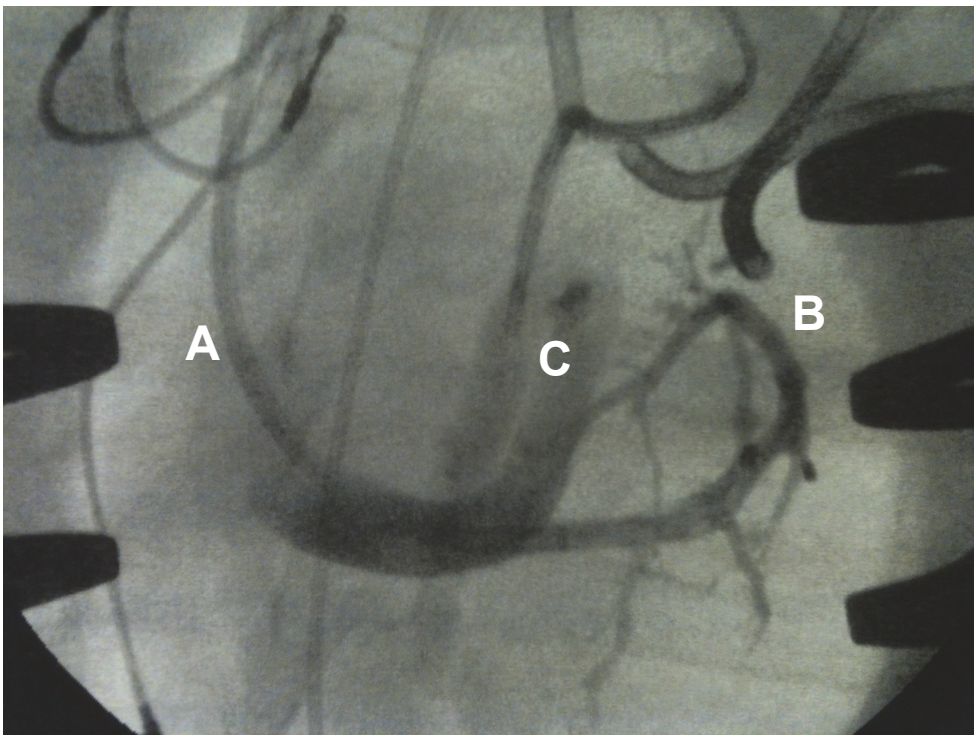


Figure 2. This fluoroscopic image is a post-mortem view from a pig experiment showing radiographic contrast in the coronary venous system injected via a coronary sinus catheter (A), demonstrating the great cardiac vein (B) and the azygos vein (C).

In addition to the procedures described above, the following methods were used specifically for the animals included in **Study IV.**

Controlled global myocardial stunning

Coronary angiography was performed to identify the coronary anatomy using an arterial introducer catheter placed by cut-down in a carotid artery. A balloon-tipped 'septostomy' catheter was used for transient controlled occlusion of the left main coronary artery (SPT002, 9.5 mm. Numed, Hopkinton, New York, USA). By repeated brief inflation and deflation of this catheter (two initial 1-min of occlusion followed by nine periods of 2-min occlusions with reperfusion 1-2 min between), for a total cumulative ischaemic period of 20 minutes, the left main-stem coronary artery occlusion was accomplished in order to establish a global ischaemic-reperfusion injury and left ventricular post-ischaemic stunning[216, 217]. Coronary occlusions for 1-min in humans have shown to result in late stunning and to produce a cumulative LV dysfunction when repeated[230].

Conductance catheter measurements and calibration

A combined conductance volumetry/tip manometry LV pigtail catheter was placed with the soft pig-tailed tip in the LV apex, allowing a stable position over the course of the experiment. The catheter was introduced through an 8.5-Fr sheath placed in the carotid artery, and further advanced to a position in the long axis with its tip in the apex of the left ventricle, using fluoroscopic guidance and also based on optimal volume signal[80, 231]. The particular catheter that we used has 12 electrodes placed 8 mm apart. Two pair of electrodes at each end of the catheter (all of which are placed inside the LV) are used to create an electric field surrounding the catheter. The field is spherical around the catheter, and is designed to incorporate the ventricular blood volume (and a little more). The conductivity of this electrical potential or field is used by different pairs of electrodes to generate signals from five segments along the length of the catheter. Each segment is recorded separately so that in case any one segment does not produce values that match the physiological LV chamber e.g. a segment at the end can find itself in the aorta and outside of the LV during systole and ventricular shortening, it can be excluded from the analysis. The conductance signal is calibrated for maximal and minimal ventricular volume difference (or stroke volume) using a measurement of stroke volume from an independent method, in our **Study IV**, the thermodilution method[44].

After the volumes had been calibrated, including the volume offset from zero, they were further analysed at each point in the experimental protocol first for a single apnoeic beat (averaged from a combination of several beats), and then for a series of consecutive beats where a control load alteration was present over the course of 6-10 beats. These single and multi-beat sequences allowed calculation of a number of aspects of ventricular performance together with the prevailing load (volume or pressure) for that beat, or beats.

The instantaneous time varying volume V(t) as a function of time (t) measured by the catheter can be displayed by the equation:

$$V(t) = \left(\frac{1}{\alpha}\right)\left(L^2\rho\right)\left(G(t) - G^p\right) = \left(\frac{1}{\alpha}\right)\left(L^2\rho G(t)\right) - V_c$$

where α is a dimensionless slope factor depending on the ratio between stroke volume measured by the thermodilution (or from conductance independent) method, to volume evaluated by the uncorrected conductance method. This latter volume is close, but often smaller than the one obtained from thermodilution. Rho (ρ) is the specific resistivity (inverse of the conductivity, sigma (σ)) of the blood measured by taking blood samples in a measuring cuvette during calibration. L is the electrode distance, G^p is the segmental parallel conductance and V_c is the correction volume that takes into account the parallel conductance from an error in calculating the volume of blood outside the left ventricle, often in the myocardium

and the right ventricle. Parallel conductance was determined for volume signal calibration using injection of 3 mL of 10% hypertonic saline into the distal lumen of the pulmonary artery catheter, before each conductance catheter parameter collection[232-237]. A signal conditioning-amplifier (Leycom Sigma 5DF, CD, Leycom, Zoetermeer, The Netherlands) was used in dual-field mode to generate volume signals. Volume and pressure data were recorded at 250 Hz.

To allow recording of consecutive heart cycles with controlled alteration of end-diastolic volumes, a (Fogarty type) 7.5 Fr balloon occlusion catheter (Vascular Technologies, Solna, Sweden) was placed with the tip in the inferior vena cava at the junction with the right atrium guided by fluoroscopy. Ventricular pressure and volume data were recorded during normal and restricted venous return, enabled by the injection of 2-3 mL saline into the balloon-tipped catheter, leading to transient partial obstruction of vena cava flow during apnoea as single-beat estimations of pressure-volume data cannot predict left ventricular contractility[238].

Left Ventricular systolic Stroke Work (LVSW), maximal left ventricular power (PWR max), maximal pressure increase over time (dp/dt_{max}), preload recruitable stroke work (PRSW)[43, 45, 46, 239, 240] as well as diastolic pressure half time (PHT), time constant of left ventricular pressure decay during isovolumetric relaxation (tau), end-diastolic pressure and volume were recorded or calculated together with end-diastolic stiffness (ED P/V).

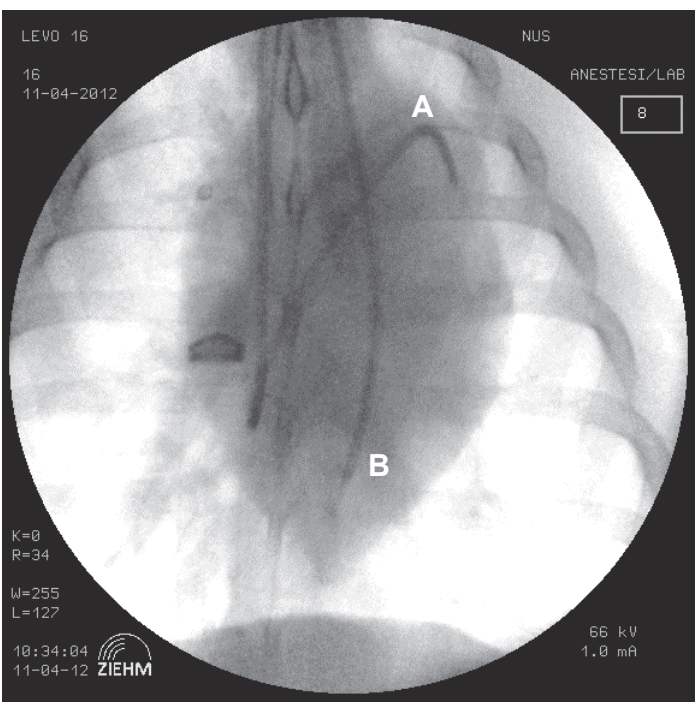


Figure 3.

Multiple catheters are seen in the heart placed from the neck vessels, including a balloon tipped catheter into the inferior vena cava (not visible in the image below, non radio-opaque), a pulmonary artery catheter for measurement of cardiac output (A), and a left-ventricular pig-tail combined conductance and pressure catheter with the tip in the left ventricular apex (B).

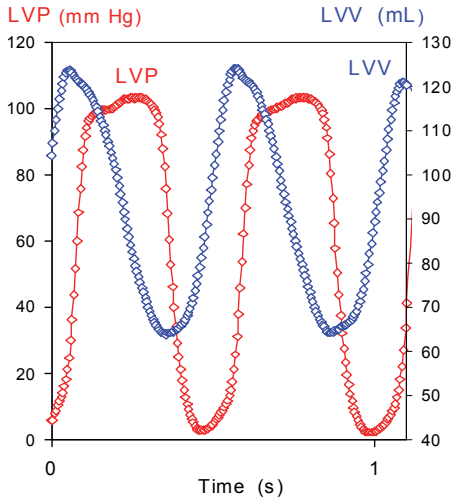
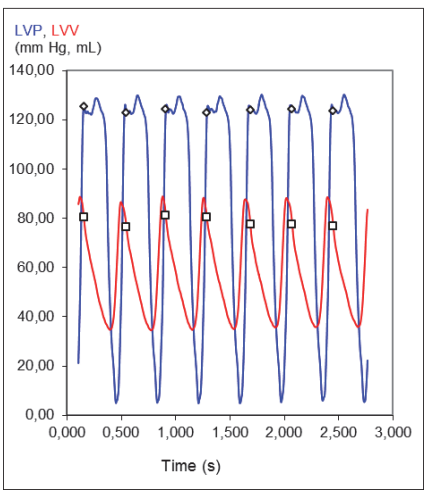


Figure 4. Left ventricular pressure (LVP) and left ventricular volume (LVV) signals measured at a sampling frequence of 250 Hz.



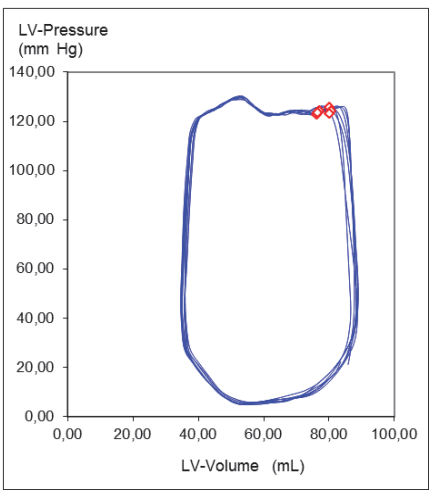


Figure 5. Several beats during apnoea (left), can be combined to a 'fusion' beat (right) for an averaged single beat analysis with specific points identifiable in the pressure-volume plane (no time factor presented in this plane).

Pressure and volume signals are collected simultaneously, and they are edited for appropriate sequences for further analysis. When analyzing pressure and volume results, one can isolate specific aspects of the heart cycle for derivations of systolic or diastolic function. These are derivations from distinct individual pressure and volume points, though to simplify, lines are connected between these points in illustrations. Further derivations can be made, including changes in pressure or changes in volume over time. Changes in pressure over time have a maximal positive value during isovolumetric contraction and a maximally negative value during isovolumetric relaxation.

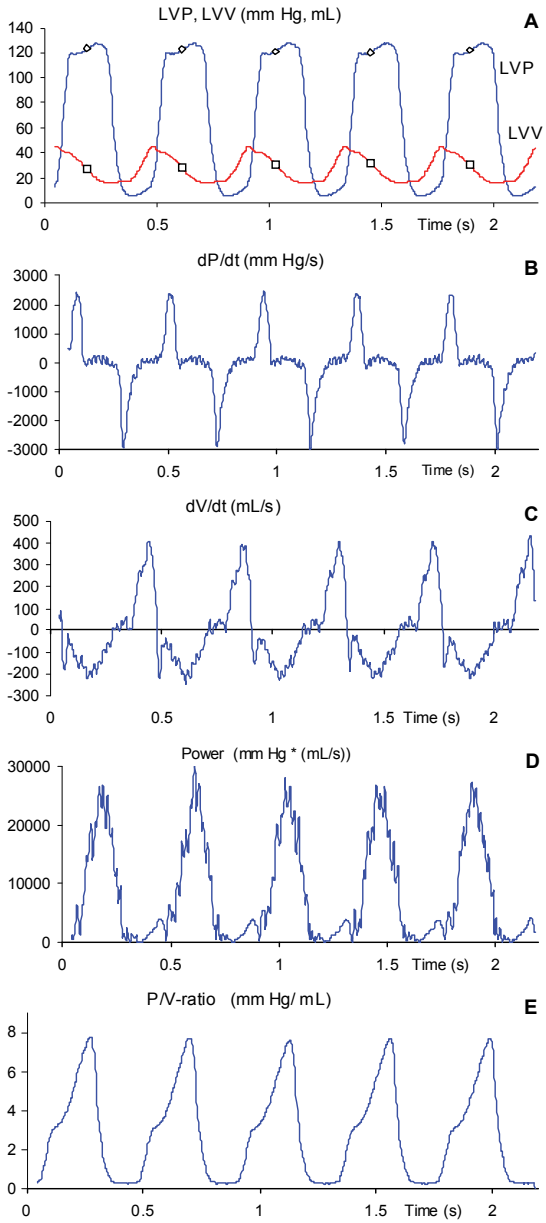


Figure 6. Pressure volume data from one of the animals. Left ventricular pressure and volume (A), dP/dt (B), dV/dt =flow, (C), Power (D) and Pressure/Volume ratio (E). Work or power can be calculated from pressure, volume and flow.

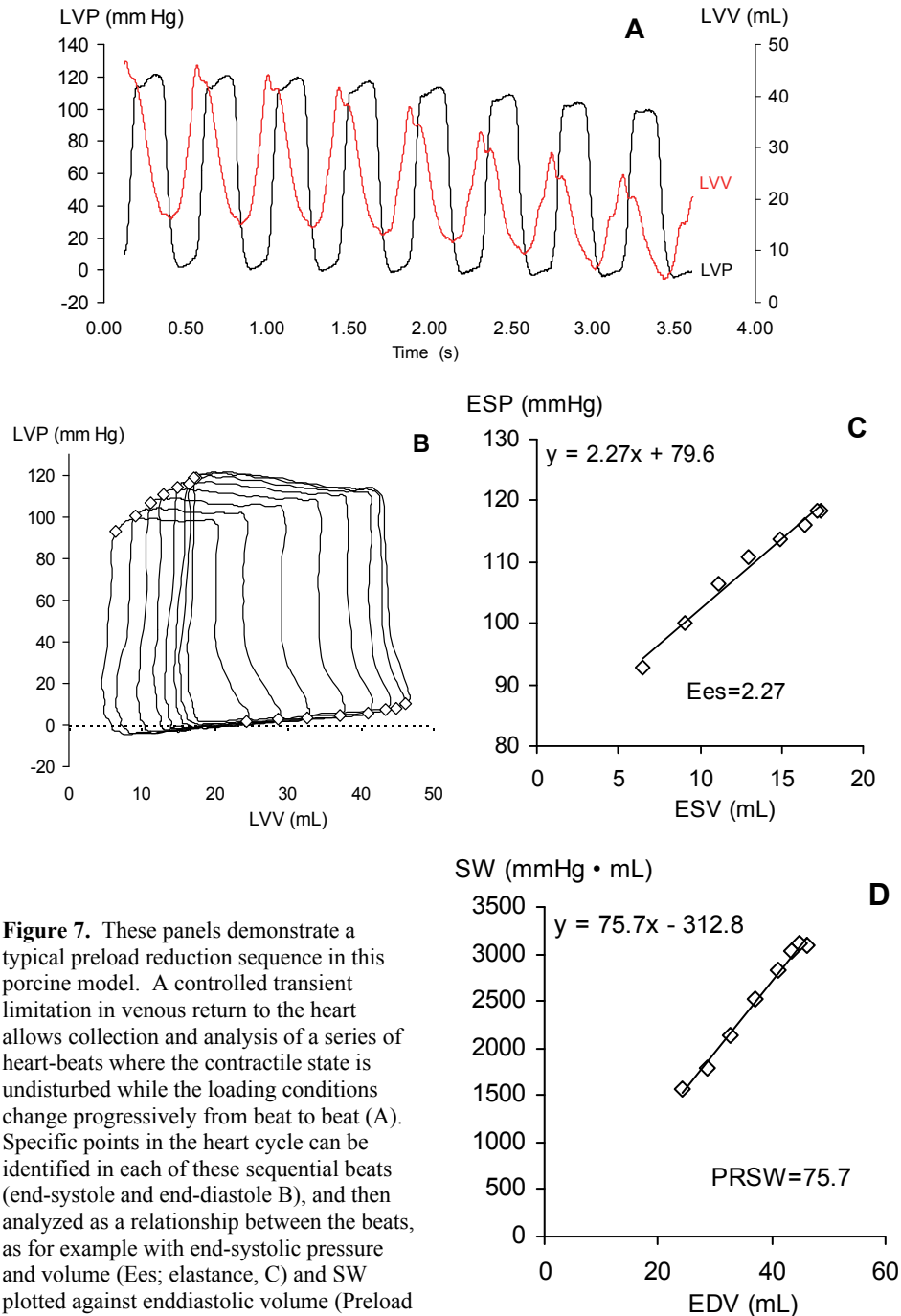


Figure 7. These panels demonstrate a typical preload reduction sequence in this porcine model. A controlled transient limitation in venous return to the heart allows collection and analysis of a series of heart-beats where the contractile state is undisturbed while the loading conditions change progressively from beat to beat (A). Specific points in the heart cycle can be identified in each of these sequential beats (end-systole and end-diastole B), and then analyzed as a relationship between the beats, as for example with end-systolic pressure and volume (Ees; elastance, C) and SW plotted against enddiastolic volume (Preload recruitable stroke work; PRSW, D)

Study III

This was an experimental large animal prospective, randomized, placebo controlled, non-blinded study. The word "placebo" is used here in an animal-experimentation context, and implies that the placebo infusions were identical to the "treatment" infusions but without an active drug i.e. infusion of 5% dextrose were administered at the same rate as in the treatment arm of the study.

Study IV

The design of the study was a prospective non-randomized, placebo controlled, non-blinded study. Pigs for **Study IV** were prepared in a standardized way described in **Study III-IV** above, but without a sternotomy.

Statistical analysis

"There are things which seem incredible to most men who have not studied Mathematics".
Archimedes of Syracus (287-212 B. C. E)

Before **Study I** was started, we retrospectively analysed graft flow in 100 coronary artery bypass surgery patients, some of whom had been given milrinone treatment, and some not. This allowed calculation of sample size ($\geq$ 40 patients) and a plan for adequate number of observations for the study.

The analysis of the differences in flow characteristics between the milrinone and the placebo group was done with help of a multiple regression model that allowed for repeated measurements at two different time periods on the same graft. A cluster analysis looking at differences in graft flow between patients as well differences within the same patient was made. The regression analysis was performed when all grafts were included as well as stratified for venous and artery grafts. The Student's t-test was used to compare differences in haemodynamics between the groups in **Study I**.

In **Study II** a multiple regression model was used. Pre CPB values of diastolic function were used to explain the post CPB values in each drug treatment group, milrinone or placebo.

In **Study III-IV**, a Kruskal-Wallis one-way analysis of variance by ranks was used. If this showed statistical significance, then a post-hoc test, the Mann-Whitney U-test was performed. Friedman's ANOVA by ranks test was used to compare the same 3 points in time within each group (**Study III**). If this test showed statistical significance, we performed a post-hoc Wilcoxon´s signed-rank test. A Mann-Whitney U-test was used to identify differences between groups at specific points in the protocol for the main variables. A p value < 0.05 was considered to be statistically significant.

Results

"Not everything that counts can be counted and not everything that can be counted counts." (Sign hanging in Einstein's office at Princeton)

In **Study I**, 44 patients undergoing coronary artery bypass grafting with comparable pre- and perioperative data were studied. The main finding was that there was a significant increase in saphenous vein graft blood flow at 10 and 30 minutes post bypass in patients receiving a single milrinone bolus of 50μg/kg, given in the cardiopulmonary bypass circuit, compared to patients receiving placebo

Table 1. Flow Rate (mL/min) of 272 Measurements on 136 Grafts in 44 Patients. (*Paper I, table 5*)

	Milrinone		Placebo	
Graft Type	10 min Mean (SD), n	30 min Mean (SD), n	10 min Mean (SD), n	30 min Mean (SD), n
Venous nonsequential	64.5 (37.4), n = 44	54.8 (29.9), n = 44	43.6 (25.7), n = 37	35.3 (22.4), n = 37
Venous nonsequential (to right coronary artery)	71.5 (44.6), n = 16	51.2 (24.2), n = 16	42.8 (30.7), n = 20	34.4 (28.4), n = 20
Venous nonsequential (to left coronary artery)	60.4 (32.8), n = 28	56.8 (32.9), n = 28	44.5 (18.9), n = 17	36.2 (13.1), n = 17
Venous sequential	120.0 (34.8), n = 5	100.0 (25.5), n = 5	73.4 (37.2), n = 14	64.1 (28.6), n = 14
Left internal thoracic artery	48.5 (31.5), n = 22	53.3 (39.7), n = 22	38.4 (22.8), n = 14	34.6 (21.3), n = 14
All	63.4 (39.1), n = 71	57.5 (34.6), n = 71	48.9 (30.5), n = 65	41.3 (26.2), n = 65

Table 2. Differences in Flow (All Grafts) Stratified for Treatment, Time, and Type of Graft. (*Paper II, table 6*)

	Difference in Flow (mL/min)		
Contrast in Stratification Parameter	Mean	*p* Value	95% CI
Milrinone versus placebo	20.5	<0.001	(9.0-32.0)
10 minutes versus 30 minutes	6.7	0.034	(0.5-12.9)

NOTE. Estimated differences are supplemented with *p* values for testing the hypothesis of no effect of the stratification variables and also with 95% confidence intervals. All values are obtained from a multivariate regression model allowing for more than 1 graft per subject and more than 1 observation per graft. Adjusted by graft type (venous nonsequential, venous sequential, and arterial grafts). Goodness-of-fit: $R^2 = 0.20$.

Patients in the milrinone group had significantly lower mean arterial blood pressure at 10 minutes after weaning from cardiopulmonary bypass, compared to the placebo group, and despite this they had higher graft flows. The mean (SD) MAP for the milrinone group was 63(7) at 10 min compared to the placebo group; 70(11) (p=0.012). The same result was seen when measuring the total pressure gradient in the grafts (MAP-left ventricular diastolic pressure (LVEDP)) in the subset of 24 patients (**Study II**). The total pressure gradient in the milrinone group

was 38(9), compared to 49(11) in the placebo group (p=0.014). The FAC (fractional area change (%)) increased in those patients treated with milrinone compared to the placebo group. FAC (mean (SD) in the milrinone group was 43(14) before CPB and 46(16) 10 min after separation compared to the placebo group; 43(10) before and 36(10), 10 min after CPB. The difference between groups was 10(4-17) (mean (95% CI), (p=0.002).

In **Study II**, the main finding was a significantly increase in early ventricular filling rate measured as ventricular cross-sectional area rate of change, dA/dt (table 3). In an adjusted regression model, milrinone was found to have a significantly greater effect on diastolic function compared to placebo (p = 0.001). The same result was seen when the normalized (to preload) peak-filling rate (dA/dt)/EDA was analysed (Table 2). The subjects had similar preoperative characteristics, including indicators of systolic function by (EF) and also diastolic function measured before bypass using dA/dt (and dA/dt normalized to EDA; (dA/dt)/EDA.

Table 3. Descriptive statistics and results from linear regression of dA/dt and (dA/dt)/EDA. (*Paper II, table 2*)

	Pre-CPB		Post-CPB		Adjusted Model	
	Milrinone n = 12 Mean (SD)	Placebo n = 12 Mean (SD)	Milrinone n = 12 Mean (SD)	Placebo n = 12 Mean (SD)	Milrinone–Placebo Mean Difference (95% CI)	*p*
dA/dt	22.0 (9.5)	21.0 (8.7)	27.8 (11.5)	17.1 (7.1)	10.0* (4.5-15.4)	0.001
(dA/dt)/EDA	2.0 (0.7)	2.0 (0.9)	2.4 (0.8)	1.7 (0.8)	0.7† (0.2-1.2)	0.013

Abbreviations: CPB, cardiopulmonary bypass; EDA, end-diastolic area.
*Adjusted by dA/dt measured pre-CPB. Unit is cm^2/s.
†Adjusted by (dA/dt)/EDA measured pre-CPB. Unit is 1/s.

In **Study II**, there were different numbers of diabetic patients in each group and even differences in the aortic clamp times between the groups. These factors have been included in the multiple regression model.

In **Study III**, neither milrinone nor levosimendan by themselves led to an increased myocardial release of glycolytic products. During regional myocardial ischaemia, neither drug worsened or improved energy metabolic conditions as reflected by interstitial glucose, pyruvate, lactate or glycerol. Additionally, neither drug exacerbated the relative myocardial calcium overload during this ischaemic period. The ischaemic intervention was clearly identified in the microdialysis results. The pre-ischaemia levels for the substances measured by the microdialysis system gave no suggestion that either of the test drugs/inotropes had an effect on glycolysis before ischaemia.

The concentration of glucose, lactate, glycerol and $^{45}Ca^{2+}$ recovery during microdialysis are shown in figure 8.

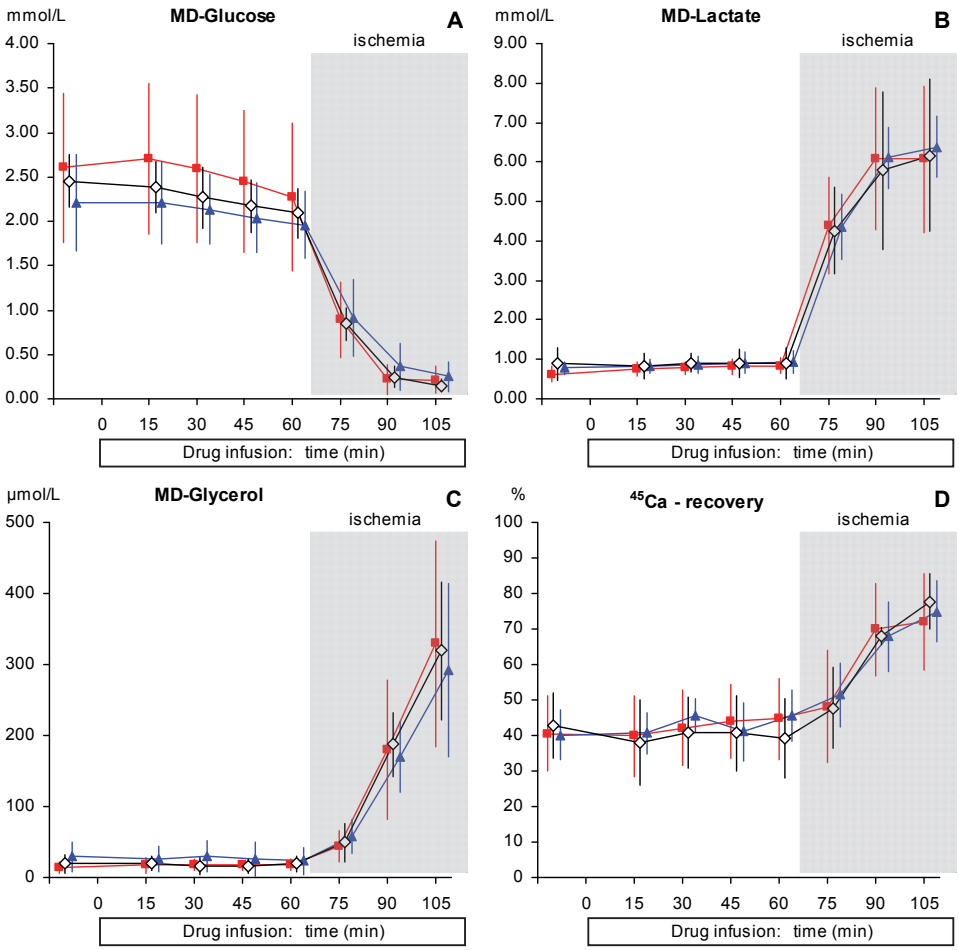


Figure 8. Ischemic microdialysis probe. Microdialysis data for Glucose (panel A), Lactate (panel B), Glycerol (panel C) and $^{45}Ca^{2+}$-recovery (panel D). Levosimendan group (filled squares, n=7), Milrinone group (filled triangles, n=7), Placebo group (open diamonds, n=6). Data are presented as mean ±95% confidence intervals.

In **Study IV**, post-ischaemia myocardial stunning was achieved, as shown by pressure-volume loops, figure 9. This was a validation for this new model, which included closed chest and balloon intravascular occlusions of the left main stem of the coronary artery, figure 10.

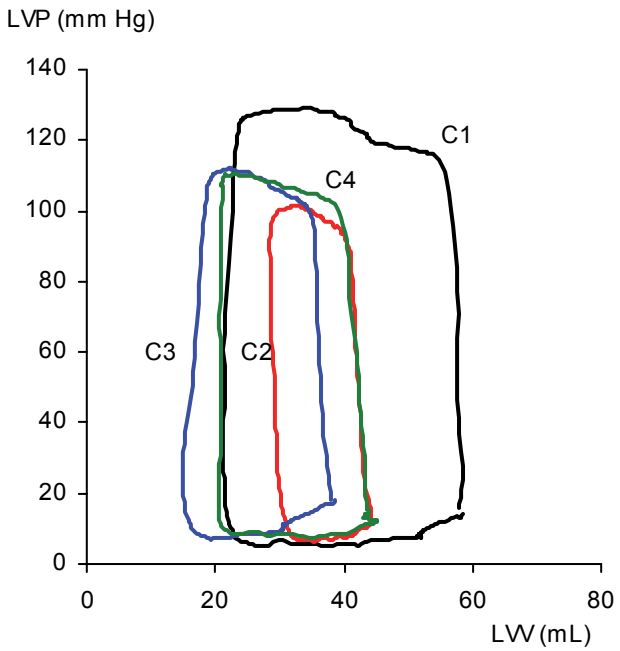


Figure 9. Pressure volume loops in a single animal collected in **Study IV**, C1 is collected before the injury, C2 after, C3 refers to a pressure volume data collected after 1 hour of milrinone treatment and C4 are a pressure volume loop collected after 2 hours of milrinone and during the second hour, additionally levosimendan. (This figure can bee seen colour in the eletronic version of this thesis)

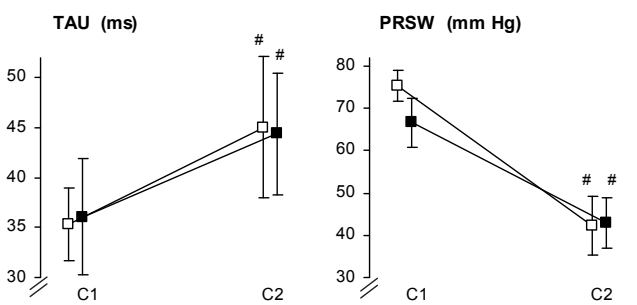


Figure 10. Tau (left panel) and PRSW (right panel) at baseline (C1) and after ischemic insults (C2) for control group (open square, n=5) and drug group (filled square, n=7).
Data are presented as mean ± 95% confidence intervals. # = $p<0.05$ using Wilcoxon Signed Rank within group test vs. C1.

Milrinone improved active relaxation in post-ischaemic stunned myocardium, as measured by tau (C3 between groups), but showed only a tendency to improve systolic function by preload recruitable stroke work (C3 vs. C2 within group) p = 0.06). Addition of levosimendan (to milrinone) did not appear to have an additional effect on diastolic function (C4 between groups) but showed a positive effect on systolic function (C4 between groups), (figure below).

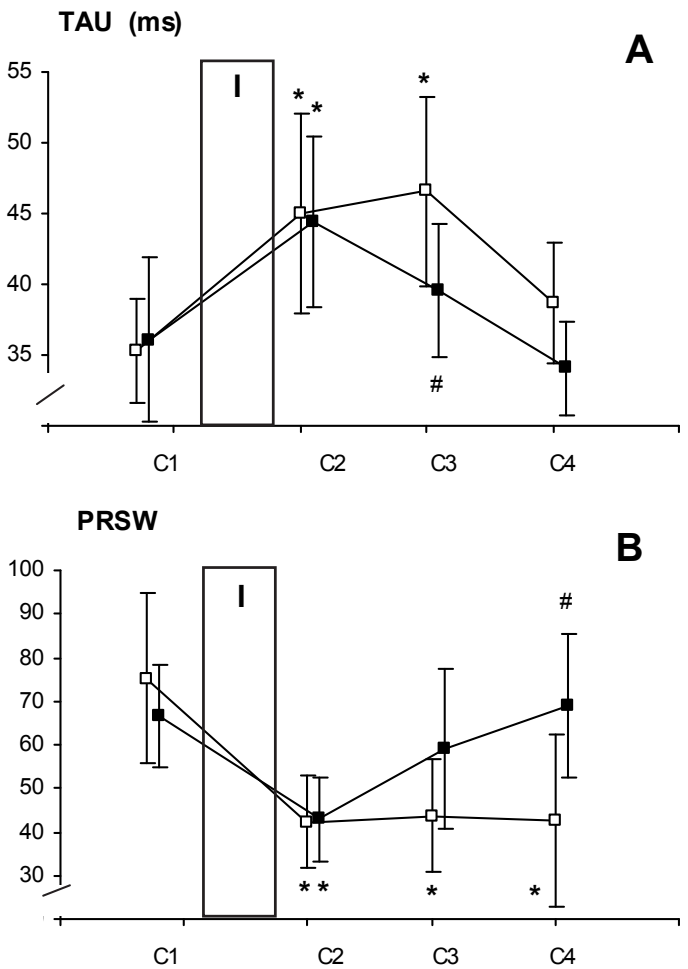


Figure 11. Tau (panel A) and PRSW (preload recruitable stroke work, panel B) at C1 (before ischemia), C2 (after ischemia), C3 (after 60 min infusion of Milrinone and at C4 (after another 60 min infusion of both milrinone and levosimendan. The rectangle (I) represents the ischemic period. Data are presented as mean ± 95% confidence intervals. Wilcoxons Signed Rank test was used within groups. * $p<0.05$ vs. C1. # $p<0.05$ using Mann-Whitney U-test between groups.

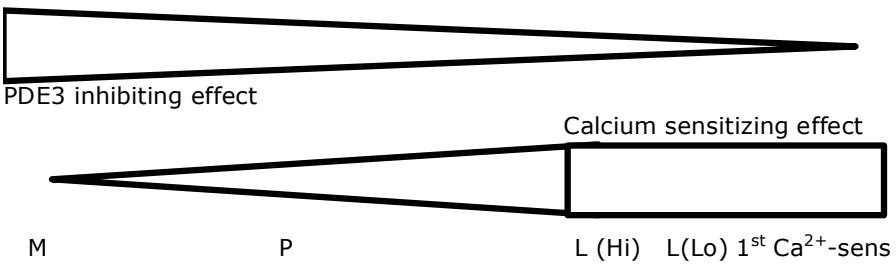
Discussion

"When one tugs at a single thing in nature, he finds it attached to the rest of the world."
John Muir, (1838-1914)

When this doctoral thesis was being planned, I set a goal of trying to study, learn and understand previous unknown effects of inotropic substances. The aim of this thesis was to try to understand the important cardiac effects of non-adrenergic inotropes that could be used to advantage together with beta-adrenergic blocking drugs. The effect of non-adrenergic inotropes on substrate delivery and utilization in the ischaemic myocardium was studied. In addition we studied ventricular performance and intrinsic myocardial contractile and lusitropic effects in different clinical and experimental situations. This was done by employing clinically accessible methods used in daily practice in a research situation, as well as the best possible reference methods used in a laboratory specifically equipped for cardiac studies in large animals. The relevant parameters have consequently been studied in details, and the findings allow us to interpret possible mechanisms of action and cardiac effects of these two drugs, a PDE3 inhibitor, milrinone and a calcium sensitizer, levosimendan.

Similarities between PDE3 inhibitors and calcium sensitizers

Many substances have both phosphodiesterase inhibiting effects but also additive calcium sensitizing properties. Levosimendan has a molecular structure similar to PDE inhibitors[186]. Examples of other drugs with mixed effects are pimobendan, a PDE inhibiting substance with calcium sensitizing effects, similar to levosimendan, which may be considered to be a calcium sensitizer with some PDE inhibiting effects. In the case of levosimendan, these properties become apparent at different doses. Although the mechanisms of action of milrinone and levosimendan (and also other members of these two drug families) are thought to be different at clinically relevant doses, they also have some common haemodynamic effects. For instance, the effects of these drugs on PDE3 inhibition and calcium sensitization vary over a range of doses, and could be visualized in the figure below. Milrinone seems to be at one end of the spectrum with PDE3 inhibiting effect, while levosimendan in low doses at the other end, has mostly calcium sensitizing effect.



M:Milrinone. P:Pimobendan. L:Levosimendan
L(Hi):Levosimendan high dose. L(Lo):Levosimendan low dose
1st Ca^{2+}-sens: First generation of calcium sensitizing drugs

PDE3 effect can be increased by hyperactivity of parathyroid gland[241]. When PDE3 inhibition is prevented by parathormone in an experimental model using calcium sensitizers, levosimendan and ORG 30029, it has been shown that calcium sensitisation itself impairs diastolic relaxation[242]. The possible interactions between milrinone and levosimendan may include: additive effects of calcium-sensitization on the myofilaments, a weak PDE3 inhibiting effect of levosimendan to a stronger PDE3 inhibitory effect of milrinone as well as some PDE4 inhibitory effect of milrinone. There may also be additive vasodilating effects of these drugs on the systemic, pulmonary and coronary circulation, especially due to the fact that these are mediated via different mechanism.

Coronary graft blood flow and diastolic function.

Study I shows that milrinone bolus treatment acutely increases flow in the saphenous vein grafts, which has not previously been observed[243]. Blood flow in the internal mammary artery has however been studied using milrinone as well as when using a combination of nitroglycerine and milrinone[244]. Milrinone seems to increase the flow in the artery more than nitroglycerine and the combination of both drugs (milrinone + nitroglycerine) was not superior to milrinone alone. Milrinone also seems superior to nitroglycerine to increase the flow in the grafted internal mammary artery when alfa-adrenergic stimuli (phenylephrine) was administrated[245, 246]. Some believe that vein grafts are just passive conduits[247] while others authors that they have a capacity to respond by smooth muscle contraction quite quickly after implantation[248]. This could be due to the effective revascularization of the ischaemic myocardium and a decrease in left ventricular diastolic pressure which in turn leads to an increase in coronary perfusion pressure gradient encouraging blood flow in the saphenous grafts. The studies included in this thesis were not designed to identify possible mechanisms. The results of intraoperative graft flow measurements during coronary artery bypass surgery have

been shown to predict in-hospital outcomes [249], when the endpoint was an in-hospital composite outcome for adverse cardiac events. The overall in-hospital mortality was however not related to intraoperative graft flow measurements in that study. The increase in coronary graft blood flow with milrinone, may be even more advantageous in patients with low graft flow (measured without the drug) if they were identified perioperatively after bypass by flow measurements. A prospective study is needed to test if milrinone increases blood flow in a subgroup of patients with low graft flow. It is well recognized that early low graft flow has a strong association with long-term poor patency and also early graft thrombosis[250-253]. Therefore, post-operative milrinone treatment may be associated with a lower incidence of early graft thrombosis. However, since post-bypass graft flow is not always routinely measured, it remains uncertain which patients have a low graft flow and may benefit from postoperative milrinone infusion. Our studies cannot answer this question but it would be interesting to investigate the incidence of early graft thrombosis related to graft flow and possible interventions. Consequently, we believe that graft flow should be measured routinely.

In lower-extremity peripherial vascular surgery it seems that milrinone does not increase saphenous vein graft flow[254] the opposite as in our observations using the vein as a conduit in CABG surgery. These differences in result may be due to the effect of milrinone on the diastolic function of the heart.

Diastolic function and milrinone

The effect of milrinone on diastolic function (early filling) was assessed in **Study II**, and milrinone treatment was associated with improved diastolic function, when used during coronary artery bypass surgery[255]. Concerning active relaxation, milrinone was shown in **Study IV** to also improve load-independent diastolic function represented by tau, where there was diastolic dysfunction present due to the experimental myocardial stunning. These results are in agreement to the known diastolic effects of milrinone, which have been described both clinically and experimentally as increasing the SERCA function in the SR by pumping calcium back into the SR during diastole[256-261].

Study II and **Study IV** confirm milrinone as a lusitropic substance in the setting of acute ischaemic injury. But there are also some authors that have not been able to confirm lusitropic effects of milrinone in patients undergoing CABG surgery using echocardiography[262]. The reasons for these differences are difficult to understand but are likely due to the differences in the measuring techniques.

Diastolic function and the combination milrinone + levosimendan

Addition of levosimendan to the stunned porcine left ventricle, which has already been pre-treated with milrinone, showed that levosimendan does not further accelerate the rate of recovery of diastolic function. Spontaneous recovery of tau was noted in the non-inotrope treated control group in the stunned

myocardium, which occurred at approximately the same rate. This result is in line with the hypotheses that levosimendan has its main effects on sensitizing the myofilaments to calcium in the excitation-contraction process, and not on active relaxation and diastole.

Systolic load-dependent effects of milrinone and levosimendan

Both milrinone and levosimendan have vasodilating properties. The mechanisms for this vasodilatory effect involve different complex signalling pathways. The vasodilatory effects of milrinone are due to an increase in intracellular cAMP level, even in the smooth muscle, leading to PKA activation of Ca^{2+}-ATPase pumps, which reduces cytosolic calcium level. Other mechanisms of action for milrinone leading to vasodilatation are phosphorylation of K^{+}-channels causing closure of voltage-sensitive-L-type Ca^{2+}-channels and probably most important, PKA-mediated inhibition of myosin light chain kinase which in turn results of less formation of myosin-actin crossbridges. In this way milrinone acts by reducing the sensitivity of calcium in the smooth musculature[263]. The vasodilatory effect of levosimendan appears to be primarily due to stimulation of ATP dependent K channels[183]. These vasodilating effects are important in bringing about ventricular unloading. When the heart is overloaded and has significant contractile dysfunction, reducing pre-systolic loading generally leads to improved systolic function. In **Study II**, we studied this using an ejection parameter, FAC, following cardiac surgery and after revascularization. These patients were not in overt heart failure with over-distended left ventricles, but might have had some degree of post-ischaemic (post-cardioplegia) ventricular dysfunction where systolic unloading allowed for better ventricular performance.

The systolic load-independent effects of milrinone and levosimendan

The load-independent systolic effects of milrinone were assessed in **Study IV** by pressure-volume analysis to allow measurement of preload recruitable stroke work in porcine hearts that had a significant amount of post-ischaemic systolic dysfunction or 'stunning'. A trend for a positive inotropic effect measured as PRSW was shown, although this did not reach statistical significance ($p=0.06$). However, this study was not designed to investigate the effect of milrinone over time or in different doses, but rather to examine the additional effects of levosimendan when milrinone had already been administered to an injured heart. The combination of milrinone and levosimendan led to a significant improvement in PRSW. It is possible that there is an additional effect when combining these two inotropes. Clinically, this has relevance since both drugs are considered to be weak to medium-strong inotropes. Although mild inotropic treatment may be a favourable way to support a patient with acute left ventricular dysfunction we have sometimes seen that despite treatment with milrinone (or another single inotropic drug), the patient still remains in a state of circulatory insufficiency despite

treatment with one inotrope, and the clinician must then consider additional support with another or second inotropic agent or even mechanical support[37, 264]. Studies of ventricular trabeculae from patients with end-stage heart failure suggests preserved lusitropic but diminished inotropic effects of cAMP dependent agents in advanced heart failure[265]. The results of **Study IV** would indicate that in this situation, adding levosimendan may give additional inotropic circulatory support. It is important, however, to consider the risk in combining inotropes since one might be exposing patients to additional side effects of drugs and potential negative interactions without any beneficial effects on the failing heart.

Metabolic effects of milrinone or levosimendan

Concerning acute ischaemia/reperfusion injury, some investigators have found protective effects for milrinone[166, 167, 266] and levosimendan[266-268] in experimental studies while no benefits or even harmful effects have been reported in large trials including patients with coronary artery disease[269]. It was decided to examine if these inotropes were protective or injurious when given in combination with acute ischaemia in **Study III**. It may be thought that inotropic drugs that increase myocardial oxygen consumption are likely to extend injury during acute ischemia and infarction, while those drugs that reduce myocardial oxygen consumption could reduce (or at least not worsen) myocardial injury. Although milrinone and levosimendan are not thought to have major effect on myocardial oxygen consumption when given in normal doses[155, 156, 176], it was still not clear if they would affect myocardial metabolism when given during acute myocardial ischaemia. These potential metabolic effects could likely be dose-dependent for the inotrope drug used.

In **Study III,** we could not identify any effects of milrinone or levosimendan compared to placebo on myocardial oxygen consumption, glycolysis or radioactive $^{45}Ca^{2+}$-recovery during ischaemia. This was an unexpected finding. However, we studied only a single dose (bolus + infusion) of these drugs and it is possible that when given in higher doses, there may be other effects of milrinone and levosimendan on myocardial oxygen consumption and substrate release. It is also possible that beneficial effects are seen only during reperfusion, a phase that we did not specific examine and this can be considered a limitation of our study. Our study design was limited to the immediate ischaemic period, and this obviously does not allow assessment of many other possible effects of the study drugs on the longer-term effects of post-ischaemic recovery, anti-inflammation or cell death.

Clinical implications

The clinical decision for the choice of a specific inotropic drug to treat general perioperative or intensive care patients with ventricular dysfunction varies. Often, these clinical treatment decisions are guided largely by institutional and hospital culture or by individual preferences. Clearly, more scientific evidence concerning

benefits and harm for use of inotropic drugs in this context is needed. Traditionally, beta-adrenergic inotropic drugs have been selected when there is an obvious need of pharmacological support of systolic and/or diastolic function. One of the most important reasons for choosing milrinone or levosimendan is that they do not seem to increase the myocardial oxygen consumption, which is in contrast to classical adrenergic agonist drugs. A good clinical therapy is also one that does not cause harm and, in certain situations where increasing myocardial oxygen consumption is detrimental to the heart, the choice of non-adrenergic inotropes may be warranted. Another common clinical scenario is a patient needing temporary inotropic support who is being chronically treated with beta-blockers. We did not examine the effects of milrinone and levosimendan together with beta-blockers in our animal studies but this remains an interesting situation, which should be addressed in future studies.

The clinical indications for non-beta adrenergic inotropes are perioperative myocardial dysfunction, commonly following cardiac surgery, acute heart failure following cardiac disease, for example myocardial infarction, and in patients with ventricular dysfunction following a critical illness. We did not see any increase in myocardial oxygen consumption with either milrinone or levosimendan in our studies. In a clinical study, levosimendan was shown to neither improve nor worsen mortality in cardiogenic shock patients, with ST elevation myocardial infarctions[270]. The results from this clinical study are in line with our results from **Study III**[271], that levosimendan (and also milrinone and placebo) did not seem to affect the metabolism in the experimentally infarcted zone.

Before inotropic drugs are administered, it is important that cardiac filling status should have been optimized. Although milrinone and possibly levosimendan have lusitropic effect[194, 265, 272, 273], when used together they do not seem to have any additional lusitropic effect. However, we did not find any evidence to suggest that the lusitropic effect worsened when combining these drugs, in **Study IV**.

The clinical importance of these studies is that they present evidence for milrinone as an effective first line therapy for diastolic disturbances if a non-adrenergic mediated drug is warranted. On the other hand, if systolic dysfunction is present, in addition the vasodilatory effect of these drugs is an advantage for the reduction in afterload. In severe cases of ventricular dysfunction, with both diastolic and systolic disturbances, our results provide some, although weak, support for the use of a combination of both milrinone and levosimendan, though in these studies there was no comparison to other inotropes.

Methodological considerations

Cardiac volume measurement in general

A new bedside non-invasive possibility to measure ventricular volume may soon be widely available: 3-D echocardiography[274, 275]. Advanced computerized tomography may also provide good structural resolution of the healthy heart's anatomy as well as following previous myocardial infarctions in patients and large animal models[276-278]. Compared to nuclear magnetic resonance imaging, 3-D echocardiography seems to accurately measure LV EF in patients with heart failure, but at the expense of underestimation of increased (>120mL/m^2)[279] left ventricular end-diastolic volumes. On the other hand 3-D echo seems to be more accurate than multislice CT in a phantom model with known volumes[280].

In a comparison between different modalities of echocardiography in the cardiac surgical setting, volume assessment by Simpson's method derived from 2-D echocardiography showed a small bias against the 3-D method. There was no clear advantage to implement 3-D in terms of volume and EF assessment[281]. The authors looked further for possible advantages by creating more true 2-D images from full volume 3-D echo, but this has, at least in this study, not led to an important improvement in assessing LV morphology and function[282].

Automated endocardial border detection and rapid temporal resolution are necessary in order to estimate ventricular volume changes during the course of a heart cycle. Volumetric assessment with 2-D and 3-D echo has been described as fast and accurate quantifications of volumes and global function with minimal user input, but has not come into routine clinical practice[283, 284]. Combining the assessment of volume measurements by 3-D together with its ability to measure myocardial systolic and diastolic deformation and strain, has increased accuracy of measurements of diastolic dysfunction[285, 286].

Starting 20 years ago, ventricular volume approximation from 2-D echocardiography using automatic systems for border detection together with pressure recordings to obtain pressure volume analysis was used to assess ventricular systolic function both in animals and in patients with severe cardiac failure undergoing partial left ventriculotomy, known as the Batista operation. Comparisons between pressure-area and pressure- volume loops have been made and these show a good correlation[287, 288]. Volume measurements using conductance volumetry together with high fidelity LV pressure measurements have been used not only in the experimental situation but also in centres with active cardiac surgical program for left ventricular failure where a complex surgical method had to be evaluated even with the risk of a catheter in the left ventricle[289-292].

There has been work in Sweden to design and test a light and very thin (0.36mm in diameter) ventricular volume-pressure measurement system which might be better tolerated by patients[293], though clinical applications of this type of

methodology with available commercial systems are still highly invasive and quite expensive considering the single use nature of these cardiac catheters in humans.

Advantages and disadvantages of the clinical study design

In evaluation of the effects of a pharmacological agent on graft blood flow in a clinical setting, one should bear in mind that many factors can influence the results of graft flow measurements. First, cardiac output is a major determinant. If the increase in cardiac graft flow is paralleled by increased cardiac work, one can assume that resistances in the myocardial small vessels have decreased to allow more flow. The graft is a conduit, which carries across a pressure head. If there is an increase in flow without any corresponding increase in regional metabolic activity, then this vasodilatory drug effect leads to what is called luxury perfusion.

In **Study I**, the same treatment, either milrinone or placebo, was allocated to all patients, regardless of initial graft flow. Drug treatment was initiated before the bypass graft was completed. In hindsight, it might have been more informative to perform the allocation and start treatment with study drug only after the graft was in place, and graft flow had been measured. This might allow control of interaction of pre-existing high or low saphenous vein graft flow rates when assessing drug effects. This would however, have required more time in the operating room, since it takes some time for milrinone loading to take place.

Our results in **Study I** showing greater graft flow in patients treated with milrinone as compared to placebo are interesting. It has been shown that the early graft flow is an important factor for early postoperative cardiac morbidity[249]. However, one cannot draw a conclusion that increasing graft flow would consequently lead to reduced morbidity. The number of subjects was small in these two clinical studies (**Study I-II**). We did not specifically include patients with pre-existing severe left ventricular diastolic dysfunction. Each subject served as his/her own control during routine CABG surgery (**Study II)**. Furthermore, we did not use cardiac output measurements in any of the clinical studies (**Study I-II**). In **Study I**, there was a significant difference in the number of patients receiving LITA grafts between the groups, making it more difficult to compare arterial graft flows between milrinone treated and saline treated patients.

Advantages and disadvantages of the large animal experimental models vs human models and study design

There are several advantages of using large animal models to study a clinical injury or vital organ dysfunction with the possibility of full control of important parameters, which could otherwise potentially introduce bias. In an acute animal model, it is ethically acceptable to induce severe injury to any organ in the interest of studying a meaningful medical scientific question, as long as the animal is anaesthetized and does not suffer. In these short-term experiments, the animal was anaesthetized before surgical preparation was done, and we performed euthanasia

as soon as the experimental data gathering was completed, without interrupting anaesthesia. Using a controlled human model for local and global myocardial ischaemia and injury is obviously not possible. Studies of transient myocardial ischaemia in humans are possible where ischaemia occurs at a predictable time and in a known way, as during percutaneous coronary intervention. However, even in these situations, it is not possible to do additional highly invasive measurements on patients. All these factors combine to make large animal acute models a necessary way to study different forms of acute human injury and disease.

Pigs were chosen for the model in the last two studies because they are uniquely suited as a model of human heart anatomy and function. They have a coronary anatomy that is similar to human anatomy, where there is little established collateral circulation in healthy animals. Left ventricular chamber anatomy is also quite similar, such that the same human ventricular measuring systems (pressure-volume sensing catheters, pulmonary artery catheters, etc.) can be employed. Pigs are not particularly good as a species for chronic human heart diseases but such models exist[294]. Similar to humans, pigs develop collateral coronary circulation, but when studying local ischaemic conditions, as in **Study III**, we do not have a reference method for identifying possible collateral circulation that can influence local metabolic conditions during coronary occlusion. However, despite some limitations, the large mature animal model (not newborn[295]) is very suitable for the purposes of these investigations (**Study III-IV**).

We chose a 45 min period of coronary occlusion in **Study III**. Longer period of occlusion (up to 80 min) have been used in pigs, giving a more complete myocardial infarction[296].

In **Study III** there were statistical comparisons between the three study groups in contrast to **Study IV** where we used the injured animal as its own control. The reason for this was that we were afraid of not being able to produce the exact amount of injury in **Study IV** due to the more difficult and complex injury model that was being studied. However, we also believed that a group without drug treatment was needed to assess the injury itself, over time. Ketamine and opioids might affect preconditioning[297]. Other racemic forms of ketamine could have been used but, on the other hand, the same anaesthetic regime was used in all animals. We used fentanyl in all animal studies, which has not been shown to have a cardioprotective effect like morphine[298].

Conclusions

I. Milrinone treatment leads to higher blood flow in newly implanted coronary saphenous vein grafts, as compared to placebo treatment.

II. Milrinone improves both isovolumetric ventricular relaxation as well as early filling during diastole, which might be a contributing factor to graft flow improvement as well as ventricular performance.

III. Neither milrinone nor levosimendan in common clinical dosages negatively influence myocardial energy metabolism or calcium overload during acute ischaemia, in a porcine model.

IV. Milrinone and levosimendan do not appear to be protective in terms of energy preservation or improvement in glycolytic conditions during myocardial ischaemia.

V. An additive levosimendan inotropic effect, to that of milrinone, in post-ischaemic ventricular dysfunction was not conclusively demonstrated, but cannot be ruled out.

VI. Milrinone and levosimendan in combination do not appear to improve active relaxation more than milrinone does by itself, in this model of post-ischaemic ventricular stunning.

Future directions

Future research in this area that would be of great interest includes testing the effect of both milrinone and levosimendan on saphenous vein graft flow, specifically in grafts that are being harvested with surrounding tissue, as many centres do today, similar to the method used in left internal thoracic arterial grafts[299-302]. Milrinone and other vasoactive drugs might have other different effects on these "fatty vein grafts" than on skeletonised saphenous vein grafts as the endothelium function has been shown to be better preserved with this new technique.

Graft patency has not been evaluated in our studies but it would be interesting to look at early coronary graft occlusion and its correlation to early graft flow. Furthermore, it would be interesting to study if drugs that can increase early graft flow might also increase patency in the early post coronary bypass surgery period when platelet inhibition therapy could not be instituted. Such studies could be started in the post bypass period including only those patients with low graft flows, which might be more prone to occlusion. Major advances have occurred in the development of new echocardiography technology, specifically in the evaluation of myocardial deformation such as strain, strain rate with or without the use of speckle tracking, tissue Doppler and 3-D[303]. The use of a broad spectrum of new echocardiography methods, together with further randomized studies in patients treated with different non-adrenergic inotropic drugs in the area of both acute heart failure, myocardial stunning and during cardiac surgery would definitely shed further light in this research field. To be able to use pressure and volume analysis using 3-D echo in the clinical setting would most certainly also be useful to better understand the dynamic cardiac function.

New inotropic drugs are in the pipe-line, since both milrinone and levosimendan have not fulfilled all expectations to be more useful for long-term use when inotropic support is sometimes needed for patients in the intensive care after cardiac surgery. Several other new drugs, especially those in the area of non-adrenergic substances, show promise and are of interest, like istaroxime[304, 305]. Translational studies with this drug, with methods similar to those used in my research are needed using the porcine heart model together with methods like conductance catheters and microdialysis, before proceeding to clinical research. It would then be important to test this drug and even milrinone and levosimendan, in clinical situations when both systolic and diastolic function or both are severely depressed, as during high-risk cardiac surgery, not just in the low- and moderate-risk patients as in our clinical studies.

The ideal inotropic substance has certainly not yet been found.

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Anders Skåål with family and my brother Gunnar with his family

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Populärvetenskaplig sammanfattning på svenska

Bakgrund:
Hjärtsvikt eller rubbningar i hjärtats mekaniska funktion är inte ovanliga särskilt i samband med svår kritisk sjukdom hos redan hjärtsjuka och i samband med hjärtkirurgi. Förståelsen av hjärtstärkande (inotropa) läkemedel är viktig i dessa situationer och denna avhandling behandlar hjärteffekter av två icke-adrenergt verkande intravenösa inotropa läkemedel. Dessa är milrinone och levosimendan och de kommer från olika klasser av läkemedel; fosfodiesterashämmare respektive calciumsensitiserare. Gemensamt för båda medlen är att de tillåter samtidig beta receptor blockerande läkemedelsbehandling då deras olika verkningsmekanismer ej medieras via beta receptorn. Flera aspekter av dessa två läkemedels hjärteffekter är okända. De vetenskapliga frågeställningar som intresserade oss särskilt är hur milrinone påverkar de nyinsatta vengraften i samband med kranskärlskirurgi; hur milrinone påverkar den del av hjärtfunktionen som sker under avslappningsfasen (diastole); om milrinone och levosimendan är skyddande eller skadliga för hjärtat i de fall de måste användas i samband med syrgasbrist i hjärtat (ischemi) eller vid manifest hjärtinfarkt. Dessutom ville vi undersöka om levosimendan kan addera ytterligare hjärtstärkande effekt till ett experimentellt försvagat hjärta som redan erhåller behandling med milrinone.

Material och Metoder:

I Studie I undersöktes 44 patienter som genomgick kranskärlskirurgi. Milrinone eller koksalt gavs i en bolusdos i hjärtlungmaskinen. Efter avslut av hjärtlungmaskin användningen mättes flödet i de nyinsatta kärlen (graften) till hjärtat utan att vi var medvetna huruvida aktivt läkemedel (milrinone) eller placebo (koksalt) hade givits. Graftflödena mättes efter 10 och 30 minuter efter hjärtlungmaskinanvändningen då bröstkorgen fortfarande var öppen. I **Studie II** tittade vi på 24 patienter i samband med kranskärlskirurgi och undersökte deras diastoliska hjärtfunktion med hjälp av ekokardiografi från matstrupen. Fyllnadshastigheten mättes både före själva hjärtkirurgin (men efter nedsövning) samt efter avslutad hjärtlungmaskinperiod. Vi studerade i likhet med **studie I** effekten av milrinone alternativt som jämförelse koksalt på den diastoliska hjärtfunktionen när dessa läkemedel administrerades under hjärtlung-maskinperioden dvs emellan de både mättillfällena. I **Studie III** övergick vi till experimentella djurstudier på gris då grishjärtat mycket liknar det mänskliga hjärtat. Ämnesomsättningen i hjärtat mättes med mikrodialys när endera milrinone, levosimendan eller en sockerlösning gavs. De sövda djuren utsattes för en experimentell hjärtinfarkt och den lokala mikrodialysmetoden mätte såväl förändringar i hjärtats infarktområde som utanför. Vi använde oss av en radioaktiv calciummetod för att särskilt studera hur calcium (som en viktig signalsubstans i hjärtat) påverkades av de givna läkemedlen. I **Studie IV** ville vi på en sövd grismodell inducera en global hjärtskada via en upprepad uppblåsning av en

ballong i det vänstra kranskärlets huvudstam. Detta ger en skada som kan motsvara den man får efter förlängd hjärtkirurgi eller vid svår och upprepad kärlkramp. Vi använde oss av tryck-volym mätningar i hjärtat för att dels utvärdera skadans grad och dessutom effekterna av milrinone på denna hjärtskada. I tillägg till milrinone användes nu också levosimendan och undersökningarna studerade också de hjärteffekter man erhöll när detta läkemedel adderades. Metoder som speglar hjärtat egen inneboende kraft såväl systoliskt som diastoliskt användes och detta är särskilt viktigt vid studier av läkemedel och övriga behandlingar som också påverkar belastningen på hjärtat via fyllnad respektive perifert kärlmotstånd.

Resultat:

I **Studie I** kunde vi se en tydlig ökning av blodflödet i de nyinsatta vengraften till hjärtat hos de patienter som gått milrinone jämfört med dem som fått koksalt. (64.5 ± 37.4 jämfört med 43.6 ± 25.7 ml/min, Medelvärde ± SD) I **Studie II** såg vi att den diastoliska funktionen (den tidiga fyllnaden av vänster kammare) var signifikant förbättrad hos de patienter som erhållit milrinone under hjärtlungmaskinperioden jämfört med dem som fått koksalt. Måttet på fyllnaden var i milrinonegruppen 22.0 ± 9.5 före hjärtlungmaskin och ökade till 27.8 ± 11.5 (Medelvärde ± SD i cm^2/s). För koksaltgruppen hade patientgruppen samma mått på diastolisk funktion före hjärtlungmaskin men sjönk istället ngt efter hjärtlungmaskin. 21.0 ± 8.7 till 17.1 ± 7.1 cm^2/s (Medelvärde ± SD). I en statistisk regressionsmodell där vi tagit hänsyn till "före-värdet" visade vi på en milrinone effekt som gav ökad tidig fyllnad av den vänstra hjärtkammaren i diastole med hög statistisk signifikans ($p = 0,001$). I **Studie III**, hade varken milrinone eller levosimendan i sig någon effekt på hjärtats metabolism mätt med mikrodialys direkt från vänster kammaren. Under regional hjärtinfarkt kunde inget av läkemedlen visa på någon skillnad i metabolismen i det drabbade området dvs inget av läkemedlen vare sig förbättrade eller försämrade den energi-metabola situationen i myokardiet mätt med lokalt glukos, pyruvat, laktat eller glycerol. Inte heller kunde någon effekt ses på omsättningen av radioaktivt calcium som tecken effekter på lokala energikrävande jonpumpar, I **Studie IV** kunde vi se att milrinone förbättrade den aktiva relaxationen av hjärtmuskeln (mätt med en parameter kallad tau beräknat med kateter inne i vänster kammare) under diastole i vår globala hjärt-skademodell. Den systoliska funktionen oberoende av hjärtats belastning visade endast en tendens ($p=0,06$) till förbättring med milrinone mätt med så kallat preload recruitable stroke work. Vid tillägg av levosimendan till de milrinone behandlade djuren kunde man inte se någon ytterligare förbättring eller försämring i diastolisk funktion men även djuren i kontrollgruppen började sin spontana återhämtning i diastolisk funktion. Systolisk dysfunktion bibehölls över tid i vår skademodell och kombinationsbehandling med milrinone och levosimendan är effektivt jämfört med placebo men studiedesignen kunde ej detektera tilläggseffekter av levosimendan även om sådana ej kan uteslutas.

Konklusion:
Vi har visat att milrinone behandling leder till ett ökat blodflöde i nyinsatta vengraft i samband med kranskärlskirurgi. Vi har också sett att milrinone förbättrar avslappningsfasen (diastole) i hjärtat efter hjärtkirurgi; om medlet ges i hjärtlungmaskinen. Det ökade blodflödet i graften kan endera vara orsak till den förbättrade diastoliska funktionen alternativt kan den förbättrade diastoliska funktionen (via effektivare trycksänkning i hjärtmuskeln) ge förutsättningar för ett bättre graftflöde. Vi har vidare sett att varken milrinone eller levosimendan givet i vanliga kliniska doser (jämfört med sockerlösning) verkar påverka energi metabola parametrar i ett experimentellt infarcerat område i ett grishjärta. Således tycks milrinone och levosimendan varken vara skyddande eller skadligt för ämnesomsättningen i själva hjärtat under en akut hjärtischemi. Ur den synvinkeln bör dessa läkemedel kunna användas utan metabol försämring vid behov av hjärtstärkande behandling vid svår syrgasbrist i hjärtat eller hjärtinfarkt. Slutligen ger milrinone behandling till ett post ischemiskt skadat grishjärta med globalt nedsatt funktion en förmåga till förbättring av den diastoliska funktionen och kombinationen milrinone och levosimendan är signifikant bättre jämfört med en placebogrupp. Direkta tilläggseffekter av levosimendan på systolisk funktion kunde ej fastställas även om sådana ej kan uteslutas. Flera studier behövs inom detta område när det gäller kombinationsbehandling av hjärtstärkande läkemedel

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Department of Surgical and Perioperative Sciences
Anesthesiology and Intensive Care
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