Section of Anesthesiology and Intensive Care Medicine,
Department of Physiology and Pharmacology
Karolinska Institutet, Stockholm, Sweden

# Cardiac function in experimental septic and non-septic conditions

## with special reference to the endothelin system

David Konrad

M.D.


Karolinska Institutet

Stockholm 2006

Printed by Larserics Digital Print AB
Published by:

ISBN 91-7140-984-X

*"How strange is the lot of us mortals! Each of us here for a brief sojourn;*
*for what purpose we know not, though sometimes sense it.*
*But we know from daily life that we exist for other people first of all*
*for whose smiles and well-being our own happiness depends."*

*Albert Einstein*

To all my kin, past and present
with special reference to my family
Jeanette, Isak, Hannah and Max

# Abstract

Myocardial depression in sepsis has been intensely investigated for the past 30 years and still poses a considerable issue in the intensive care units. Both systolic and diastolic dysfunction is implicated, correlating with severity of illness and mortality.

The importance of the endothelin (ET) system in normal physiology has been extensively studied. ET is a powerful vasoconstrictive peptide, has cardio-regulatory effects and is released from the endothelium. In sepsis, ET levels are dramatically increased and correlate to severity of disease. Thus, ET has been proposed as a mediator of myocardial depression in sepsis. Another mechanism proposed for cardiac dysfunction is decreased myofilament sensitivity for calcium ($Ca^{2+}$). Levosimendan, a $Ca^{2+}$ sensitizing agent, has been used successfully in patients with severe heart failure and decreased myocardial $Ca^{2+}$ sensitivity has been shown in septic conditions.

The major aims of this thesis was to, in a porcine model *in vivo,* investigate cardiovascular effects of levosimendan in experimental sepsis, to characterize the influence of the ET system on cardiac performance in the normal and septic heart. Also, to assess myocardial function in endotoxemia and effects of intervention in the ET system by load-independent analysis of the left ventricular pressure-volume relation (LVPVR) by conductance volumetry.

Endotoxin administration was associated with major cardiovascular effects, pulmonary hypertension and increased systemic oxygen extraction. Levosimendan attenuated all these effects and also evoked vasodilation which improved splanchnic blood flow.

Tezosentan, a dual ($ET_A$/$ET_B$) ET receptor antagonist, was administered in established, high-volume resuscitated endotoxemia and resulted in increased cardiac index, stroke volume index and LV end-diastolic volume by counteracting pulmonary hypertension and improving LV compliance. However, these effects were dose-dependent. When increasing the dose of tezosentan ten-fold, the initial beneficial response was not sustained. Exaggerated vasodilation and severely low coronary perfusion pressure is a possible mechanism for the increased mortality seen with the higher dose of tezosentan.

Effects of ET receptor activation in the healthy heart were evaluated by LVPVR following intra-coronary infusion of endothelin-1 (ET-1) and sarafotoxin 6c (S6c, $ET_B$ receptor agonist). ET-1 caused a dose-related increase and S6c a decrease in systolic function, possibly mediated via $ET_A$ receptors. Both ET-1 and S6c caused similar impairment in diastolic function, mediated via $ET_B$ receptors.

By the same methodology, effects of tezosentan were studied in endotoxemia, where endotoxin caused marred relaxation but not systolic dysfunction. Tezosentan caused impairment in parameters of systolic performance but significantly improved diastolic relaxation.

We conclude that, in endotoxemia, intervention with levosimendan is beneficial, markedly improving cardiovascular function and regional blood flow. Therefore, levosimendan is a promising drug in septic states and should be evaluated for specific cardiac effects and used in future clinical trials. Furthermore, the ET system contributes significantly to cardiac performance in both healthy and acute experimental septic conditions. Diastolic dysfunction and pulmonary hypertension are suitable targets for ET receptor antagonism. Bearing in mind the dose issue and the possible negative inotropic effects, antagonizing the much up-regulated ET system in septic patients may well be meaningful to explore in the clinical setting.

*Keywords: sepsis, cardiac, endothelin, endotoxin, levosimendan, contractility, diastolic.*

# List of publications

I. Oldner A, Konrad D, Wanecek M, Rudehill A, Rossi P and Weitzberg E.
**Effects of levosimendan, a novel inotropic calcium-sensitizing drug, in experimental septic shock**
*Crit Care Med. (29) 2185-93, 2001*

II. Konrad D, Oldner A, Rossi P, Wanecek M, Rudehill A, and Weitzberg E
**Differentiated and dose-related cardiovascular effects of a dual endothelin receptor antagonist in endotoxin shock**
*Crit Care Med 32: 1192-99, 2004.*

III. Konrad D, Oldner A, Wanecek M, Rudehill A, Weitzberg E, Biber B, Johansson G, Häggmark S and Haney M
**Positive inotropic and negative lusitropic effects of endothelin receptor agonism in vivo**
*Am J Physiol Heart Circ Physiol 289(4): H1702-9, 2005*

IV. Konrad D, Haney M, Johansson G, Wanecek M, Weitzberg E and Oldner A
**Cardiac effects of endothelin receptor antagonism in endotoxemic pigs**
*Submitted September 2006*

# Contents

# List of abbreviations

| | |
|---|---|
| ATP | Adenosine triphopshate |
| cAMP | Cyclic adenosine monophosphate |
| CI | Cardiac index |
| CO | Cardiac output |
| CVP | Central venous pressure |
| dP/dTmax | Maximum derivative of left ventricular pressure |
| dP/dTmin | Minimum derivative of left ventricular pressure |
| ECE | Endothelin converting enzyme |
| Ees | End-systolic elastance |
| EF | Ejection fraction |
| ET | Endothelin |
| $ET_A$ | Endothelin receptor type A |
| $ET_B$ | Endothelin receptor type B |
| ET-1 | Endothelin-1 |
| LPS | Lipopolysaccharide (endotoxin) |
| LV | Left ventricle |
| LVEDAI | Left ventricular end-diastolic area index |
| LVEDV | Left ventricular end-diastolic volume |
| LVESV | Left ventricular end-systolic volume |
| LVP | Left ventricular pressure |
| LVPVR | Left ventricular pressure-volume relation |
| LVSWI | Left ventricular stroke work index |
| MAP | Mean arterial blood pressure |
| MPAP | Mean pulmonary artery pressure |
| NO | Nitric oxide |
| PCWP | Pulmonary capillary wedge pressure |
| PRSW | Preload recruitable stroke work |
| PVR | Pulmonary vascular resistance |
| PWRmax | Maximum instantaneous pressure-flow product |
| SV | Stroke volume |
| SVI | Stroke volume index |
| SVR | Systemic vascular resistance |
| SW | Stroke work |
| S6c | Sarafotoxin 6c, $ET_B$ receptor agonist |
| Tau | Time constant for pressure decay |
| $t_{1/2}$ | Half time for pressure decay |

# Introduction

*"Employ your time in improving yourself by other men's writings,*
*So that you shall gain easily what others have labored hard for"*
*Socrates*

## Sepsis

Hippocrates himself, some 2500 years ago, recognized fever as a major symptom of infection. He also realized the prognostic implications of localized versus systemic infections. The word sepsis derives from Greek, originally denoting decay because the sepsis syndrome was associated with decaying tissues. Today, sepsis is associated with infection rather than tissue decay and is often used as equivalent of bacteremia (bacteria in blood). The first scientist to isolate bacteria from patients was Louis Pasteur who in 1879-80 found bacteria in blood from patients with puerperal septicaemia. 12 years later, Richard Pfeiffer made the observation that the bacteria *Vibrio cholerae* (causing cholera) released two types of toxins[1]. The first toxin was detectable when the bacteria were alive but the other only when they had been killed. He hypothesized that the latter, more potent toxin, was stored within the bacteria thus coining the term *endotoxin*. In subsequent studies he found that inoculating heat-killed *V. cholerae* bacteria in guinea pigs caused the animals to die. Pfeiffer concluded that the heat-stable protein was a part of the insoluble bacterial cell wall and identified endotoxins from other bacterial species as well. Other investigators in Europe reported similar findings e.g. Centanni[2] who found that extracts from gram-negative bacilli had the ability to induce fever, hence calling them *pyrotoxins*. The structure and chemical nature of endotoxin was then pursued over the following decades. Boivin *et al*[3] reported that lipopolysaccharide (LPS), the most abundant lipid in the gram-negative bacterial cell wall, was the active component.

## Epidemiology

The yearly incidence of sepsis has been increasing by 9% each year and amounts to 50-95 cases per 100000 people per year[4]. In the USA, sepsis accounts for 2% of hospital admissions and there have been similar reports of incidence in Europe[5]. Between 11 and 21% of all ICU admissions are due to severe sepsis[6,7] and calculated annual overall incidence of severe sepsis in adult people range from 0.8-3.0 per 1000[6,8]. Overall mortality seem to decrease but the number of sepsis-related deaths is increasing[4,5]. Patients with septic shock have a high risk of death compared with other

critically ill patients[5]. Predisposing risk factors to septic shock include age, cancer, immunodeficiency states and genetic factors[5,9-11]. 10 % of all intensive care admissions are due to septic shock and carries a mortality rate of 60-70%[4,5].

## Classification and causes

Sepsis is a disease of many faces and not until 1991, when Bone *et al*[12] suggested a universal terminology, was there a consensus regarding definitions (*table 1*). These definitions were modified in 2002[13] and are now widely accepted. Signs and symptoms of patients with sepsis are diverse and these definitions are attempts to aid clinicians in diagnosing sepsis

Infection is the hallmark of sepsis and 80% of such patients have infections in the chest, urogenital system, abdomen or bloodstream[4-6,9]. Gram-negative bacteria account for 25-40%, gram-positive for 25-50% and polymicrobial infections 25%[6,7]. Sepsis due to fungi or multi-resistant bacteria is becoming more common whereas viruses and parasites only account for 2-4% of the sepsis cases.

**Table 1.** Simplified from Bone *et al* 1992[12]

| ***Diagnostic criteria for sepsis*** | |
|---|---|
| Sepsis | Infection (confirmed or suspected) and one of the following:<br>Temperature >38.3 or <36 $^{o}$C<br>Heart rate >90<br>Tachypnea<br>Leukocytes >12 or <4 $10^{3}$ $mL^{-1}$ |
| Severe sepsis | As sepsis and signs of organ dysfunction or hypoperfusion |
| Septic shock | As severe sepsis and persistent hypotension (systolic blood pressure <90 or mean arterial blood pressure < 70 mmHg in spite of volume administration |

# Septic myocardial depression

Indeed, sepsis is a devastating condition with prognosis deteriorating as more organ systems become affected. Cardiovascular instability and collapse contributes considerably to organ dysfunction by decreasing oxygen delivery to tissues with a higher than normal oxygen demand. The effect of myocardial dysfunction in a state of sepsis can therefore be detrimental. To better understand the concept of myocardial depression in sepsis, a brief recapitulation of cardiac terminology and physiology ensues.

## Cardiac physiology

### *Functional anatomy*

The heart can be looked upon as two parallel pumps, a right and left, each consisting of an atrium and a ventricle. These four chambers consist of three parts: the *endocardium*, which is a thin inner layer of endothelial cells lining the cavities of the chambers and are thus in direct contact with the blood; the *myocardium*, which mainly consist of muscle tissue; and the *epicardium*, which is the inner layer of the *pericardium*, a fibrous sheath of mesothelial cells that encase the heart. Cardiac muscle shares some characteristics of both skeletal and smooth muscle, yet it is unique in morphology. The *sarcomere* (*figure 1*) is the smallest unit of the contractile complex and consists of myosin (thick) and actin (thin) molecule threads called filaments. A third filament, titin, is a big, elastic protein that provides structural integrity as well as passive tension to the stretched muscle[14]. The sarcomeres are separated end-to-end by Z-lines which serves as anchors and titin connects this anchor to the sarcomere. The contractile apparatus, the actin-myosin complex, form sliding crossbridges that convert stored energy (adenosine triphosphate, ATP) into force and motion[15].

### *Physiology of contraction and relaxation*

In order to contract cardiac muscle, actin and myosin must interact. Actin is arranged in polymers in a double helical structure. Within the sulcus of this helix lies tropomysin which forms a complex with troponin[16] (*figure 1*). Troponin itself consists of three different components: *troponin T* that binds to tropomysin; *troponin I* that inhibits the reaction of actin to myosin and *troponin C* that binds $Ca^{2+}$. The binding of $Ca^{2+}$ leads to a conformational change of the myosin head that moves on actin and contraction ensues[17]. At the time of activation, the sarcomere length is somehow sensed and adjustments made to manage contractile force. This length-force relationship (the Starling mechanism, below) could be due to that the stretch of the muscle determines the number of crossbridges available for contraction.

Relaxation is less well understood but follows detachment of the myosin head from actin. This may be caused by an altered binding state for $Ca^{2+}$ on troponin C. The elastic properties of titin may assist in pulling the filament back[18].

### *The role of calcium*

Upon depolarization, $Ca^{2+}$ channels open (L-type), leading to a slow inward flux of $Ca^{2+}$ (*figure 1*). The increase in cytosolic $Ca^{2+}$ leads to opening of calcium-dependent $Ca^{2+}$channels (the ryanodine receptor) in the sarcoplasmic reticulum (SR) which causes a rapid, additional increase in $Ca^{2+}$ concentration[19]. $Ca^{2+}$ binds to troponin C, where magnesium ($Mg^{2+}$) is an important cofactor, enabling the conformational change of myosin (above). Increased pH, i.e. alkalinization, in the myocyte will increase the

sensitivity of $Ca^{2+}$ binding to troponin C thus facilitating contraction. This is one of the proposed mechanisms for the suggested positive inotropic effects of endothelin-1 (ET-1)[20] (below). The importance of calcium sensitivity clinically is highlighted by the fact that the calcium sensitizing agent levosimendan is increasingly used for treating severe congestive heart failure[21]. Besides sensitizing the troponin C for calcium, levosimendan also activates $K_{ATP}$-channels, resulting in vasodilation.

Reaccumulation of $Ca^{2+}$ back into the sarcoplasma is accomplished by a $Ca^{2+}$/ $Mg^{2+}$-ATPase pump, an energy consuming process. This pump can be augmented by cAMP (cyclic adenosine monophosphate) dependent phosphorylation of an associated sarcoplasmic protein, phospholamban. In fact, in the healthy heart, β-adrenergic stimulation increases cAMP and phosphorylation of phospholamban and thus increases activity of the $Ca^{2+}$/ $Mg^{2+}$ pump, shortening relaxation time[22]. In heart failure these β-adrenergic mechanisms are impaired, contributing to both systolic and diastolic dysfunction[23].

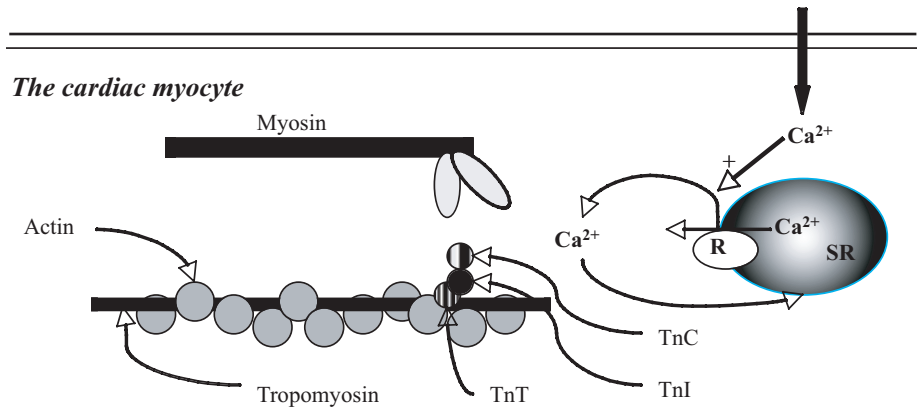


**Figure 1.** *Illustration of the intracellular events of the cardiac myocyte following depolarization. Upon depolarization, $Ca^{2+}$ channels open, leading to a slow inward flux of $Ca^{2+}$. The increase in cytosolic $Ca^{2+}$ leads to opening of calcium-dependent $Ca^{2+}$ channels (the ryanodine receptor, R) in the sarcoplasmic reticulum (SR) which causes a rapid, additional increase in $Ca^{2+}$ concentration. Actin is arranged in polymers in a double helical structure. Within the sulcus of this helix lies tropomoysin which forms a complex with the troponins. Troponin T (TnT) binds to tropomyosin, troponin I (TnI) inhibits the reaction of actin to myosin and troponin C (TnC) binds $Ca^{2+}$. The binding of $Ca^{2+}$ leads to a conformational change of the myosin head that moves on actin and contraction ensues. Relaxation follows detachment of the myosin heads from actin which may be due to an altered binding state for $Ca^{2+}$ on TnC. Reaccumulation of $Ca^{2+}$ back into the SR is an energy consuming process.*

## *Contractility*

The term contractility is elusive and rather difficult to grasp. One definition states that contractility is the intrinsic contractile ability of the heart independent of changes in the other codeterminants of cardiac output (CO), or simplified: what the cardiac muscle can do unaffected by external factors. Unfortunately (for scientists), cardiac muscle is always under the influence of its environment *in vivo*, making contractility difficult to measure. Nevertheless, one can try to control or account for these external factors in order to investigate effects on myocardial function where they matter, i.e. in the body. In this sense it may be wise to distinguish contractility from performance. Whereas contractility reflects the intrinsic conditions of the myocardium irrespective of external factors, cardiac performance is what the cardiac muscle does, given the effect of both external and internal factors, at the time of contraction.

There are several determinants of contractility (*figure 2*). The *length-force* relationship, Starling mechanism, which means that increased length (preload) will generate increased force (up to a point). The *force-frequency* relationship means that the higher the frequency (heart rate) the higher the force. This effect, known as the Bowditch effect, is believed to be due to increased intracellular $Ca^{2+}$ concentration[24]. Cardiac myocytes also have some sort of chemical memory of the preceding beat, the *force-interval* relationship[25]. The Anrep effect denotes the increase in contractile force seen in response to a sudden increase in afterload, a *force-afterload* effect, whereas the Gregg effect denotes the increased contractile response to high intracoronary pressures[26]. Naturally, receptor mediated stimulation affects contractility as well, e.g. with the autonomic nerve system and specifically adrenergic influences on heart function being recognized for many years.

β-adrenergic receptor activation results in increased heart rate and contractility (or rather, performance) via intracellular increase in cAMP. Noradrenaline, released from post ganglionic sympathetic nerve fibers, is the main neurotransmitter. The increase in cAMP act on certain protein kinases which ultimately lead to an increase in intracellular $Ca^{2+}$. The parasympathetic system less densely innervates the ventricles but still influence contractility. Acetylcholine is released and decreases the intracellular levels of cAMP, either by inhibiting release of noradrenaline, or by increasing cyclic guanosine monophosphate (cGMP) upon binding to muscarinic receptors which in turn stimulates cAMP breakdown.

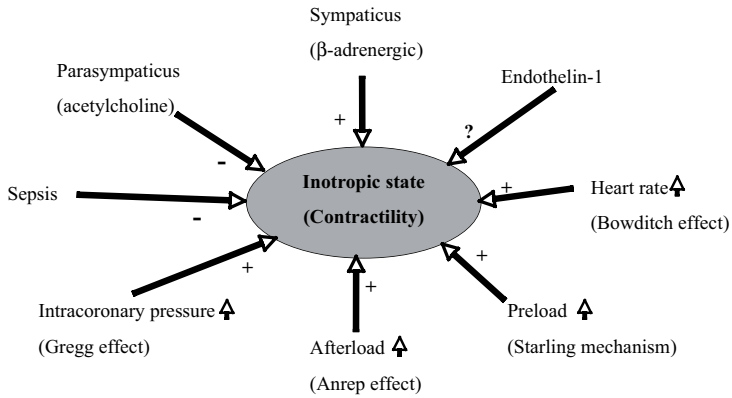


**Figure 2.** *Factors that influence the contractile state of the myocardium*

## *Relaxation*

Diastole, or relaxation, can and does influence cardiac performance significantly, as illustrated by the current discussion of the role of diastolic dysfunction in heart failure. Relaxation is an active, energy-consuming process and the velocity of muscle relengthening has a clear relation to the original fiber length as well as pre- and afterload[27]. In the intact heart, diastole can be divided into four phases: 1, *isovolumic relaxation*, which follows directly after end-systole and displays a rapid drop in chamber pressure with no, or little, change in volume; 2, *rapid ventricular filling*, where ventricular volume, but not pressure, increases; 3, *slow ventricular filling* (or diastasis) where there is an increase in both pressure and volume and 4, *atrial systole* where pressure rises more than volume. In spite of the nomenclature, there could be some volume change during isovolumic relaxation due to the fact that the ventricle can change shape. The sudden increase in myocardial perfusion that occurs during diastole could, through an increase in myocardial turgor, possibly lead to an increase in chamber stiffness. Also, higher afterload will affect onset of isovolumic relaxation[28] and its progress. Ventricular filling takes place mainly during phase 2 and 3 (about 80%) whereas atrial contraction contributes 5-15% normally. In diseased states, the relative contribution to ventricular filling by atrial contraction can markedly increase. Filling of the ventricles are very closely related to heart rate, driving pressure from the atrium, pressure gradient across the atrio-ventricular valves but also to the contractile and relaxation state of the ventricle[29].

As mentioned above, sympathetic nerve system activation not only increases contractile performance but also shortens relaxation time by stimulating the reaccumulation of $Ca^{2+}$ into the sarcoplasma in the healthy heart, and desensitization of β-adrenergic receptor agonist effect is well described in heart failure.

### *Endothelium and cardiac function*

For the last 25 years, the endothelium has emerged as an essential component of the cardiovascular system. The discovery of the fundamental role for endothelium regarding vasomotor tone in 1980 by Furchgott *et al*[30] was crowned with the Nobel Prize in 1998, together with Ignarro and Murad (for the discovery of nitric oxide, NO). We know now that the endothelium plays an important role in cardiac growth, rythmicity, metabolism and contractile performance[31]. This section focuses on the endocardial endothelial cells as well as the endothelial cells of the myocardial capillaries, both of which lay in close proximity to cardiomyocytes. For further details, please see the comprehensive review by Dirk Brutsaert[31].

With respect to autocrine or paracrine signaling, cardiac endothelial cells produce NO, ET-1, prostacycline ($PGI_2$) and convert angiotensin I to angiotensin II. NO has been widely investigated regarding its role in myocardial performance and both positive and negative findings regarding contractility and relaxation have been reported. At low concentrations, NO seems to demonstrate positive inotropic effects *in vitro*[32], but higher NO levels lead to a negative inotropic response[33]. Type II NO synthase, which produce high amounts of NO, is induced in sepsis by endotoxin and by a number of cytokines including TNF-$\alpha$ and IL-1$\beta$. In fact, these cytokines have been proposed to exert negative inotropic effect in sepsis via NO.

## History of myocardial depression in sepsis

Myocardial depression in shock was first described by Carl Wiggers[34] who in 1947 used a hemorrhagic model. The fact that the heart is affected in a negative way in many septic patients has also been known for quite some time. The first reports on myocardial depression came in the 1960s with the finding that some septic patients displayed low CO, thin, thready pulse and cold, clammy skin[35,36]. Such patients were initially believed to have heart failure in contrast to patients with high CO, warm skin and bounding pulse. Contrarily, Wilson *et al*[37] showed that high or normal CO and low systemic vascular resistance (SVR) is a feature of human septic shock and also showed that administration of endotoxin to humans resulted in a similar picture. This opinion was not broadly accepted until the mid 1970s when pulmonary artery catheters became widely used. These catheters provided a better estimate of left ventricular preload using pulmonary capillary wedge pressure (PCWP) instead of central venous pressure (CVP) as a measure of left ventricular end-diastolic volume (LVEDV). By then it became clear that adequately resuscitated septic shock patients typically displayed high CO and low SVR[38,39]. Survivors were found to respond to fluid challenges by increasing CI and LV stroke work index (LVSWI) to a greater extent than non-survivors. However, in both these groups, some patients deteriorated in CI and LVSWI in response to fluid administration and this was interpreted as myocardial dysfunction[40]. Thus, despite

the fact that patients had high CO upon presentation, septic myocardial depression was nonetheless present. MacLean *et al* were among the first to argue that myocardial depression remained an issue in sepsis because metabolic demand exceeded myocardial performance[35].

## Human studies and results

The findings by MacLean and colleagues was supported much later when first Calvin *et al* and later Parker *et al*, showed that resuscitated septic patients had an increase in LVEDV, i.e. a dilation of the LV and a reduction in left ventricular ejection fraction (LVEF)[41,42]. Interestingly, survivors had a more pronounced reduction in LVEF, due to greater increase in LVEDV, which resolved within 7 to 10 days. This meant that septic myocardial depression could be reversible. Non-survivors maintained the initial LVEF until death, and thus appeared to be unable to dilate their LV. These findings implicate diastolic dysfunction as a predictor of survival of septic shock.

By the increasing use of echocardiography, several investigators could show that patients with sepsis and septic shock indeed had diastolic dysfunction including reduced rapidity of ventricular filling and reduced left ventricular relaxation[43-45]. These investigators also found more severe abnormalities in non-surviving patients including impaired systolic function[46]. Systolic dysfunction has also been shown using pulmonary artery catheter derived data. In 1977, Weisel *et al* reported that the ability to respond to a fluid challenge by an increase in LVSWI correlated to survival[40] and Ognibene *et al* demonstrated inability of septic shock patients to increase LVSWI in response to fluid as compared with sepsis without shock or trauma patients[47].

The early notion that myocardial depression was due to hypoperfusion or global myocardial ischemia has been proven faulty[48-50]. Cunnion *et al*[51] investigated septic shock patients (by means of coronary sinus thermodilution catheter) with evidence of myocardial depression and found no signs of hypoperfusion, no net myocardial lactate production but rather increased myocardial oxygen availability. These findings were confirmed by Dhainuat *et al*[52] as well as in numerous animal investigations of adequately resuscitated septic shock[53-56]. The possibility of microcirculatory abnormalities, such as shunting, has also been investigated with hitherto no evidence of altered myocardial oxygen metabolism or lack of high-energy phosphates[57-59]. However, elevations of cardiac troponin I is often found in septic shock patients and correlate with cardiac dysfunction[60,61]. Whether this finding is due to toxic effects of endotoxin, cytopathic dysoxia or some other mechanism is still unclear.

## Animal studies and results

A number of animal models of myocardial depression in sepsis have been developed in different species. It is noteworthy that none of these exactly mimic human sepsis.

Each model has its own limitations and advantages but all have contributed in some way to unravel the intriguing puzzle of cardiac dysfunction in sepsis.

In isolated cardiomyocytes as well as in papillary muscles, endotoxin administration has been associated with impaired contractility[62-65]. In Langendorff preparations (isolated, denervated hearts) from small rodents both systolic and diastolic dysfunction have been described in response to endotoxin (LPS)[66-69]. These results are not necessarily reproduced using *in vivo* models. In such intact models, both neurohormonal activity and circulating substances could modify the negative effects on cardiac performance. Cell-cell interactions are also vital in regulating the contractile state of the myocardium[31]. Cardiac loading conditions change substantially by LPS or other modes of inflicting sepsis, making interpretation of cardiac effects difficult. When load-independent assessments of cardiac performance are used, such as the left ventricular pressure-volume relation (LVPVR), results differ[54,70-75]. It would appear that compensatory mechanisms can initially mask depressed cardiac function but at later stages, cardiac depression is evident[73].

## Current opinions

Many mechanisms responsible for evoking or contributing to myocardial depression in models of sepsis have been proposed, some of which are mentioned here (*table 2*).

The search for a *myocardial depressant substance* has been intense and basic characteristics determined. It is a heat-stable protein between 10 and 25 kD in size. The existence of such a substance is supported by the fact that ultrafiltrate from septic patients evoke dysfunction of rat cardiomyocytes[76]. Many cytokines fit such characteristics and much attention has been drawn to TNF-$\alpha$ and IL-1$\beta$. Both these cytokines, as well as endotoxin, can induce contractile dysfunction[77]. A plausible mechanism is, via several intracellular steps, induction of nuclear factor kappa B (NF$\kappa$B), a pro-inflammatory factor leading to increased production of cytokines in the cardiomyocyte[78]. By intervening in these steps towards activation of NF$\kappa$B, cardiac dysfunction is ameliorated or abolished in some septic models[79].

As discussed before, *NO* has substantial influence on myocardial performance. NO is also induced in sepsis and by endotoxin[80] and higher levels of NO may impair contractility. A mitochondrial version of NO synthase has been described and may inhibit certain complexes of the respiratory chain[81]. Another proposed mechanism for myocardial depression in sepsis is *cytopathic dysoxia*, i.e. an uncoupling of oxidative phosphorylation leading to relative shortage of ATP thus impairing cardiac performance[82]. The role of calcium in myocardial function has been discussed above and *decreased myofilament sensitivity for* $Ca^{2+}$ is also suggested to mediate septic myocardial depression[83,84]. *In vitro* studies have shown decreased sensitivity for $Ca^{2+}$ and impaired contractility in myocytes from endotoxic hearts. Interestingly, this effect could concentration-dependently be reversed by a calcium-sensitizing agent[63]. Also,

inhibition of the ryanodine receptor (impairing $Ca^{2+}$ release from the SR) has been shown in septic pigs[85] as well as impairment of the reuptake of $Ca^{2+}$ by the SR[86]. Several other mechanisms for inducing myocardial depression in sepsis have also been proposed (*table 2*). Thus, considering the many hypotheses and the data to support them, it is unlikely that only one of these is solely responsible for myocardial depression in sepsis, but rather that this condition is the result of several interacting mechanisms.

**Table 2.**

| ***Proposed mechanisms for myocardial depression in sepsis*** | |
|---|---|
| Myocardial depressant substance(s) | Decreased expression of heat shock proteins |
| Cytokines (TNF-α, IL-1 β) | Cytopathic dysoxia |
| Calcium desensitization | Activation of toll-like receptor 4 |
| Endothelin | Apoptosis |
| Nitric oxide | Increased expression of adhesion molecules (ICAM-1, VCAM-1) |

# The endothelin system

The initial description of the ET system by Yanagisawa *et al* 1988[87] has led to intense research regarding this family of peptides. The ETs are involved in many physiological and pathophysiological processes and pharmaceuticals intervening in the ET system are today in clinical use.

## Biosynthesis and cellular release of ET-1

The ET system is comprised of three isopeptides: ET-1, -2 and -3. The biologically most important isoform in human cardiovascular physiology and pathophysiology seem to be ET-1[88] which also is the focus of this thesis. ET-1 is primarily synthesized as a precursor, preproET-1, which gene is located on the human chromosome 6[89]. PreproET-1 consists of 212 amino acids and is cleaved by endopeptidases[90] to yield bigET-1, a 38-amino acid structure which possesses biological activity although 140 times less potent than ET-1[91]. In turn, bigET-1 is cleaved, intra- or extracellularly, by membrane-bound metalloproteases called endothelin converting enzymes (ECEs)[92] to ET-1 which has the length of 21 amino acids (*figure 3 and 8*).

ET-1 is continuously released from endothelial cells, contributing to vasomotor tone, but is also stored intracellularly and released upon stimulation[93]. Such stimulatory factors on production and release of ET-1 include endotoxin, ET-1 itself, angiotensin II[94], catecholamines, thrombin, growth factors, cytokines, free radicals, hypoxia and shear stress (*figure 3*). Inhibitory factors include NO, atrial natriuretic peptide, heparin

and prostaglandins $E_2$ and $I_2$[95]. The release of ET-1 is mainly to the abluminal side[96,97] making ET-1 foremost an autocrine and paracrine mediator rather than endocrine. However, in conditions with high circulating levels of ET-1, such as sepsis, this peptide may also act in an endocrine fashion[98]. Thus plasma levels only reflect a minor portion of ET-1 released and may not be a true estimate of ET-1 activity but rather a surplus of interstitial and tissue-bound ET-1. Nevertheless, plasma levels of ET-1 do correlate with severity of disease, such as congestive heart failure[99] and can also have prognostic and diagnostic values[100]. Clearance of circulating ET-1 is quite rapid with a plasma half-life of about one minute[101], whereas effects such as vasoconstriction are sustained for one hour[102,103]. The major site for ET-1 clearance is the pulmonary circulation[104] (below).

## ET receptors and signal transduction

To date three different receptors for ET-1 has been identified, $ET_A$, $ET_B$ and $ET_C$ (the $ET_C$ receptor has not been cloned from humans)[105,106]. The two ET receptors found in humans, $ET_A$ and $ET_B$ are highly conserved across mammalian species (85-90%)[95]. Both $ET_A$ and $ET_B$ receptors are found on smooth muscle cells where they mediate vasoconstriction. The $ET_B$ receptor is also found on endothelial cells[107] mediating vasodilation by release of NO or prostacycline[108]. If administered exogenously, ET-1 causes a transient vasodilation (due to stimulation of endothelial $ET_B$ receptors) followed by sustained vasoconstriction ($ET_A$ and smooth muscle $ET_B$ receptors)[87]. The net effect of ET-1 is dependent on the local receptor population at different vascular beds[109] but vasoconstrictor effects are generally more pronounced in veins than arteries[110]. Several specific ET receptor antagonists have been developed over the years which have aided researchers in investigation the role of the ET system[111].

$ET_A$ and $ET_B$ receptors belong to the G protein-coupled family (*figure 3*). Activation of the $ET_A$ receptor leads to activation of phosphoinositide-specific phospholipase C (PLC) which in turn activates two "second messengers": diacylglycerol (DAG, membrane-bound) and phosphatidylinostol ($IP_3$, soluble). $IP_3$ rapidly increases intracellular calcium by mobilization from the sarcoplasmic reticulum[112]. DAG, together with calcium, activates protein kinase C which stimulates the sodium/hydrogen ($Na^+$/$H^+$) exchanger[113,114], leading to intracellular alkalinization. PKC also triggers a negative feed-back on calcium signaling[115,116]. The increase in cytosolic $Na^+$ may also reverse the normal direction of the sarcoplasmic $Ca^{2+}$/$Na^+$ exchanger[117,118] (*figure 3*), thus further increasing cytosolic $Ca^{2+}$. The latter effect may further promote contraction of the myofilaments and thus cause vasoconstriction in smooth muscle cells or contraction of the cardiomyocyte. The intracellular effects of $ET_B$ receptor activation are quite similar to those of activating $ET_A$ but the endothelial $ET_B$ receptor can also activate phospholipase $A_2$ with ensuing release of prostacycline. Thus, agonist binding of the endothelial $ET_B$ receptor can mediate vasodilation by release of NO and prostacycline.

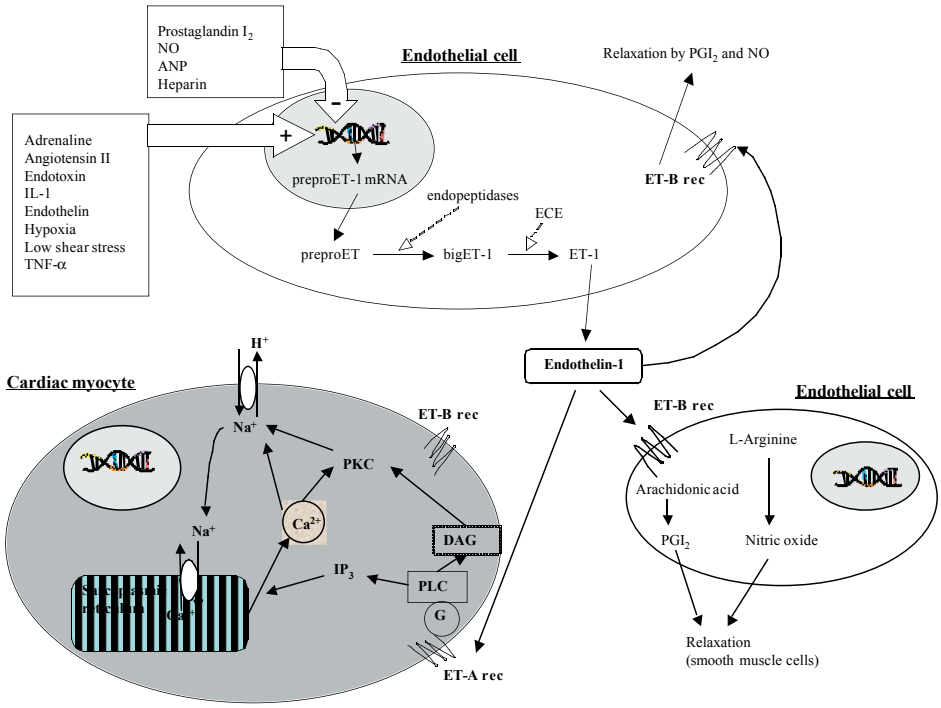


**Figure 3.** ***Synthesis and intracellular effects of endothelin-1 (ET-1).***
*Upper: ET-1 is synthesized as a precursor, preproET-1, which gene is located on the human chromosome 6. PreproET-1 consists of 212 amino acids and is cleaved in the cytoplasm by endopeptidases to bigET-1, a 38-amino acid structure which possesses biological activity, although less potent than ET-1. In turn, bigET-1 is cleaved by endothelin converting enzymes (ECE) to ET-1 which has the length of 21 amino acids. Stimulatory and inhibitory factors on production and release of ET-1 are shown in the figure.*
*Left: Endothelin A ($ET_A$) and B ($ET_B$) receptors are coupled to G proteins. Activation of the $ET_A$ receptor leads to activation of phosphoinositide-specific phospholipase C (PLC) which in turn activates two "second messengers": diacylglycerol (DAG, membrane-bound) and phosphatidylinostol ($IP_3$, soluble). $IP_3$ rapidly increases intracellular calcium ($Ca^{2+}$) by mobilization from the sarcoplasmic reticulum. DAG, together with calcium, activates protein kinase C (PKC) which stimulates the sodium/hydrogen ($Na^+/H^+$) exchanger, leading to intracellular alkalinization. PKC also triggers a negative feed-back on calcium signalling by ET-1 (not shown). The increase in cytosolic $Na^+$ may also activate the $Ca^{2+}/Na^+$ exchanger leading to additional increase in cytosolic $Ca^{2+}$ that may further promote contraction of the myofilaments and thus cause vasoconstriction in smooth muscle cells or contraction of the cardiomyocyte.*
*Right: The intracellular effects of $ET_B$ receptor activation are shown for the endothelial cell, leading to production and release of prostacycline ($PGI_2$) and nitric oxide (NO).*

Both $ET_A$ and $ET_B$ receptors are internalized following ligand binding but whereas the $ET_A$ receptors are recycled to the surface, the $ET_B$ receptors are degraded in lysozymes[119] thereby clearing circulating ET from plasma. Recycling of $ET_A$ receptor may explain the long-lasting effect of $ET_A$ activation (more receptors available on the cell surface) relative to the short acting effects of $ET_B$ receptors (require *de novo* synthesis) regarding vasoconstriction.

## ET and sepsis

The involvement of the ET system in sepsis was first described by Pittet *et al*[100] and Weitzberg *et al*[120] who found a five-fold increase of ET-1 in septic patients compared with healthy volunteers. The highest plasma levels measured of ET-1 comes from septic subjects[121]. ET-1 has also been found to correlate positively to severity of sepsis[100] and mortality[122] as well as negatively to cardiac index (CI)[100].

As mentioned earlier, endotoxin is a powerful stimulator of ET-1 expression and secretion[123] and increased amounts of pre-pro ET-1 mRNA are found in many models of sepsis. Other cytokines, such as tumor necrosis factor α (TNF-α) and interleukin-1β (IL-1β), are elevated in human sepsis[124] and are also known to induce ET-1 production. In clinical studies of sepsis, ET-1 has also been associated with pulmonary hypertension and renal dysfunction[100,125].

A number of studies performed in various animal models of sepsis have investigated effects of ET-1. Endotoxin-induced pulmonary hypertension is, in part, ET dependent and is associated with an increase in extra-vascular lung water and deterioration in gas exchange. Such effects are effectively abolished by ET receptor antagonism[126]. Splanchnic perfusion disturbances are commonly encountered in sepsis[127] and ET-1 causes splanchnic vasoconstriction[128]. Oldner *et al* demonstrated increased oxygen delivery to the gut as well as improvement in mucosal pHi (intracellular pH assessed by tonometry) by ET receptor antagonism in porcine endotoxemia[129]. Arterial plasma levels of ET-1 has also been shown to correlate negatively with mucosal pHi[130]. Apart from the intestines, ET is also a powerful regulator of hepatic blood flow during septic conditions. Endotoxin has been shown to increase hepatic vascular resistance six-fold and this effect was abolished by antagonizing both $ET_A$ and $ET_B$ receptors, i.e. dual ET receptor antagonism[130]. Renal function and perfusion is also under the influence of ET-1 which causes reduction of renal blood flow[128,131]. The effects in septic settings of ET antagonism differs regarding renal effects where some have shown improved renal blood flow and urine output[132] while others have not[133]. In human sepsis, bigET-1 have been shown to negatively correlate to creatinine clearance, a measure of renal function[125].

## ET and cardiac function

There is a bewildering array of reports regarding the effects of ET on cardiac function with, at times, contrasting results. The inotropic responses vary depending on whether single myocytes, multi-cellular preparations, whole heart or *in vivo* models are used. Most studies have been performed *in vitro* and have the advantage of looking at very specific effects of ET-1, contributing valuable insights in cellular response. However, cardiac function is a result of complex interactions between the myocardium and endothelium, with constant cell-to-cell feed-back mechanisms in response to load, neuro-hormonal stimulation and beat-to beat adaptation. Thus, what is true for the isolated myocyte may not necessarily hold true for the *in vivo* situation. Alas, *in vivo* studies of cardiac performance and ET-1 using load-independent methods are scarce.

Both $ET_A$ and $ET_B$ receptors are found in the human heart[117]. The cardiomyocytes express mainly, but not exclusively, $ET_A$ receptors whereas endothelial cells and fibroblasts express $ET_B$ receptors[134,135]. Regarding expression of ET receptors, the porcine and human hearts are very similar[136].

### *Endothelin and contractility*

ET has been reported to induce both positive and negative inotropy *in vitro*[137-140]. In human myocardium, ET-1 has been shown to exert positive inotropic effects[141] which seem to be mediated by $ET_A$ receptors. When antagonizing $ET_A$ receptors by an intra-coronary infusion in healthy humans, there were clear signs of impaired systolic performance suggesting that ET-1, via $ET_A$ receptors has a tonic, positive inotropic effect without any effect on relaxation[142].

However, the effects on cardiac performance *in vivo* may be masked by the vasoconstrictive effects of ET-1. Systemic administration of exogenous ET-1 (i.e. intravenous infusion) result in a transient drop in vascular resistance due to stimulation of endothelial $ET_B$ receptors with subsequent NO release, followed by a sustained increase in SVR (activation of $ET_A$ receptors, above). This results in an increase in cardiac pre- and afterload and a decrease in CO commonly interpreted as a negative inotropic effect[143]. Reductions in coronary perfusion *per se* by exogenous ET-1 may impair cardiac performance due to ischemia[144].

Several mechanisms for the effect on contractility by ET-1 have been proposed. Binding to the $ET_A$ receptor leads to an increase in cytosolic $Ca^{2+}$ which promotes contractility (*figure 3*). Alkalinization of the cytoplasm via activation of the Na/H exchanger enhances $Ca^{2+}$ sensitivity of troponin C which, in turn, increases contractility. Also, ET-1 has been reported to interact with endogenous noradrenaline, modulating contractility and efficiency through cross-talk among different neuro-hormonal signals[145].

Interestingly, in pathological conditions, ET-1 has been shown to induce negative inotropic effects. When infusing an ET receptor antagonist intra-coronary to patients

with congestive heart failure (CHF), MacCarthy *et al*[142] found opposite results compared with healthy subjects. In CHF patients, measures of contractile performance improved, suggesting negative effects of ET-1, whereas in patients without evidence of CHF, contractile performance was impaired. An almost identical pattern has been described in human and pig cardiomyocytes from healthy vs. CHF subjects[141,146]. One hypothesis is that, in the initial development of CHF, ET-1 contributes to contractile function by adaptive up-regulation but, as CHF progresses, the adaptive response become maladaptive due to altered receptor expression or sensitivity to ET-1 and vascular effects dominate. In summary, many but not all report positive effects of ET-1 *in vitro* but *in vivo* findings are less clear and may depend on healthy versus diseased cardiac conditions.

### *Endothelin and relaxation*

The effects of ET-1 and myocardial relaxation is less well studied. In isolated muscle preparations ET-1 was associated with improved relaxation due to $ET_A$ receptor activation[147] whereas in human myocytes, ET-1 impaired relaxation[148]. In a septic model in rats, bigET-1 infusion caused marred relaxation. ET-1 infusion in healthy volunteers was associated with impaired relaxation although this study may have been flawed by alterations in cardiac load and the use of intravenous, exogenous ET-1[143]. Regarding stiffness, ET-1 has been shown to improve distensibilty in a model of acute cardiac overload[149,150]. Thus the literature is far from clear regarding the lusitropic effects of the ET system.

## Calcium, ET and septic myocardial depression

The increasing awareness of dysfunctional cardiac calcium handling in CHF poses the highly interesting question whether calcium desensitization is also of importance in septic states. In addition, there seem to be indications that calcium sensitizing agents may affect the ET system.

Despite the fact that our group and others repeatedly have found beneficial hemodynamic and regional perfusion effects by antagonizing the ET system in experimental sepsis, there is still no conclusive evidence regarding the specific cardiac effects of ET. Moreover, ET-1 has been shown to have opposing effects in healthy versus diseased myocardium.

For these reasons we wanted to study the effects of a novel calcium sensitizing drug in an experimental septic model and to investigate possible effects of this drug on the ET system. We also wanted to further investigate the specific cardiac effects of ET receptor activity in both healthy and septic hearts *in vivo* with improved control regarding extra-cardiac influences on performance.

*"The heart has its reasons of which reason know nothing"*
*Blaise Pascal*

# Aims

The aims of this thesis was to investigate aspects of cardiovascular dysfunction in models of sepsis with particular emphasis on calcium desensitization, the endothelin system and also to further characterize the functional properties of the endothelin system in normal, *in vivo* cardiac physiology.

The specific aims were:

- To evaluate a new $Ca^{2+}$ sensitizer, levosimendan, in a model of experimental sepsis with regard to cardiovascular and regional effects.
- To investigate possible connections between levosimendan and the ET system in experimental sepsis.
- To evaluate cardiac performance and perfusion effects of endotoxemia.
- To study different degrees of ET receptor antagonism in endotoxin shock and especially effects on cardiac performance.
- To investigate load-independent cardiac effects of ET receptor stimulation by exogenous ET receptor agonists with respect to both systolic and diastolic performance.
- To measure and evaluate load-independent cardiac effects of acute endotoxin administration *in vivo.*
- To explore the effects of ET receptor antagonism in acute endotoxemia by load-independent assessment of myocardial systolic and diastolic performance.

# Materials and methods

For a detailed account of materials and methods, the reader is referred to the individual papers.

## The porcine model

*"I like pigs. Dogs look up on us. Cats look down on us. Pigs treat us as equals."*
*Winston Churchill*

Sepsis is a very complex and dynamic syndrome, and somewhat heterogeneous in its clinical presentation. A wide variety of models have been developed in different species using diverse approaches to simulate human sepsis in order to assess organ-specific pathophysiology. The choice of model has obvious impact on results depending on species, fluid administration, type of sepsis-inducing insult, administration of endotoxin (bolus versus continuous), acute versus chronic experiments, anesthesia and so forth. For this thesis, the porcine model of continuous endotoxemia was employed in three of the studies.

There are several benefits in using pigs as a model of human sepsis. They have similar sensitivity for endotoxin and tissue antigenicity to humans[151]. Their size allows for extensive instrumentation, serial measurements and repeated blood samples. There is a marked resemblance between pig and human cardiac anatomy, physiology, ET system and coronary circulation which makes the pig heart attractive for research[136,152] and hearts from pigs are even being considered for xenotransplantation[153].

## Endotoxin

Endotoxin (LPS) is a part of the gram negative bacterial cell wall, levels of which are detectable in human sepsis[154]. Continuous LPS administration does not mimic the gradual release of bacteria into the bloodstream of septic patients but the physiologic response does resemble sepsis. When administered to healthy volunteers there was a very similar cardiovascular response compared with septic shock[155]. Bolus administration of LPS or intravenous infusion of live pathogens most often causes a rapid onset of cardiovascular instability and a state of hypodynamic shock that carries high mortality. Various cecal ligation and puncture models have been used that are good models of abdominal infection and sepsis, though sepsis development in this type of model often takes time, even days. Thus, a stepwise increase in LPS from *E.Coli*

was used in papers I, II and IV, reaching maximum infusion rates within 45 minutes. This rate was then kept for the entire experiment except for paper I in which endotoxin infusion was stopped after three hours.

In papers I and II, the endotoxin dose used was 20 $\mu g \cdot kg^{-1} \cdot h^{-1}$ whereas in paper IV 0.25 $\mu g \cdot kg^{-1} \cdot h^{-1}$. This reduction in administered endotoxin was due to an increase in LPS potency in new batches. The resulting dose used in paper IV caused similar effects as papers I and II (*table 3*).

# Anesthesia and surgical preparation

All animals used were fasted overnight with free access to water. On the morning of the experiment premedication was administered (ketamine 20 $mg \cdot kg^{-1}$ and atropine 25 $\mu g \cdot kg^{-1}$ in papers I and II; ketamine 10 $mg \cdot kg^{-1}$, atropine 50 $\mu g \cdot kg^{-1}$ and azaperone 4 $mg \cdot kg^{-1}$ in papers III and IV) prior to transport to the laboratory. An intravenous cannula was placed in a marginal ear vein and anesthesia induced by pentobarbithal 12 $mg \cdot kg^{-1}$. Anaesthesia was maintained by continuous infusions of, in paper I: pentobarbithal 3-6 $mg \cdot kg^{-1} \cdot h^{-1}$ and fentanyl 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$; in paper II: midazolam 0.3-0.4 $mg \cdot kg^{-1} \cdot h^{-1}$ and fentanyl 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$; papers III and IV: pentobarbital 5 $mg \cdot kg^{-1} \cdot h^{-1}$, midazolam 0.3 $mg \cdot kg^{-1} \cdot h^{-1}$ and fentanyl 20 $\mu g \cdot kg^{-1} \cdot h^{-1}$. Muscle relaxants were administered only in papers I and II as pancuronium bromide 0.5 $mg \cdot kg^{-1} \cdot h^{-1}$. Prior to administration of the muscle relaxant, anesthetic depth was evaluated by pain stimuli to the fore hoof. A tracheotomy was performed and the animals mechanically normoventilated. Body temperature was maintained at 38-39 $^{o}C$ by heating pads. For details regarding surgical preparation and catheterization, please see the material and methods section in each paper of this thesis.

# Biochemical analyses

## Endothelin-1

ET-1 levels in plasma were analyzed in papers I-IV by radioimmunoassay as described by Hemsén *et al*[156]. The cross-reactivity for bigET-1 was 0.03%, for ET-2 27% and for ET-3 8%.

## Troponin I

In papers II and IV plasma levels of troponin I were assessed using commercially available assays: in paper II the Axsym microparticle enzyme immunoassay (AxSYM, Abbott, Chicago, IL, USA) and paper IV two-position immunoenzymatic assay (Beckman Coulter Inc., Fullerton, CA, USA)

# Cardiac performance

Assessing intrinsic cardiac effects *in vivo* is challenging since the heart has a unique ability to adapt to alterations in load, pressure and frequency in a very short time frame in addition to having a chemical memory of the preceding beat. The heart *in vivo* is also under the influence of neuro-hormonal control. However, studies of the *in vivo* heart can provide extensive information of cardiac performance given its environmental influences and confounders may be minimized by comparing groups of individuals. Assessing the relation between heart function and an experiment intervention requires a stable cardiovascular model as the 'platform'. In this case, the animals were well anesthetized and resting, so that potential effects on the autonomic nervous system (and heart) by stimulation from factors not related to the protocol were minimized.

## Pulmonary artery catheter derived parameters

In papers I and II, cardiovascular performance was assessed by derived parameters from the pulmonary artery catheter (PAC). Such parameters include CO, stroke volume (SV) and left ventricular stroke work calculated according to standard formulas. These parameters are load-dependent and can be influenced by vascular, as well as cardiac effects. In order to interpret such findings one needs to take pre- and afterload into account.

## Echocardiography

In paper II, assessments of ventricular volumes were made by means of two-dimensional echocardiography. This method has previously been shown to be a better estimate of left ventricular preload than conventional invasive monitoring[157]. Following left-sided thoracotomy, a transducer probe (V510B, Acuson, Mountain View, CA, USA) was placed directly upon the epicardium. The probe was placed to get a short axis view of the LV at the mid-papillary level. A loop of 4-6 beats was recorded and end-diastolic/end-systolic areas were calculated (Acuson 128 echocardiograph) after manually outlining the endocardial surface. Fractional area of contraction (%) was calculated as:

$$\frac{(\text{LVEDAI}-\text{LVESAI})}{\text{LVEDAI}} \times 100$$

where LVEDAI is left ventricular end-diastolic area index and LVESAI is left ventricular end-systolic area index. In addition, right ventricular end-diastolic diameter was measured at the widest portion of the right ventricle, a non-validated estimate of right-sided preload.

## Left ventricular pressure-volume relations

In papers III and IV, cardiac performance was assessed by analyzing the pressure-volume relationship of the left ventricle *in vivo*. The continuous volume measuring method employed was based on conductance, where a number of electrodes produce an electric field in the surrounding tissue (in this case within the LV) and sense the electrical field conducting effects of the blood (*figure 4*). The sensed signal is then calibrated for absolute volume by assessment of blood conductivity for electricity, for signal representing surrounding tissue that is not blood and calibrating signal magnitude relative to a second measurement of volume, in this case stroke volume measured by thermodilution[158,159]. These calibrations were made immediately before each measurement. The conductance catheter used contains multiple electrodes which provide volume assessments in five intra-ventricular segments. Intra-ventricular pressure was measured by tip manometry with the sensor place in the conductance catheter in a mid-ventricular position. In this way, pressure as well as volume could be captured simultaneously and continuously. The conductance catheter was advanced via the carotid artery through the aortic valve into the LV and optimal position checked and confirmed with fluoroscopy before and after experiments.

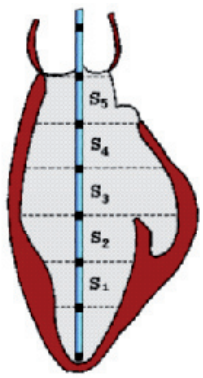


**Figure 4.** *Schematic of the left ventricle (long axis) with the conductance catheter in place. The separate segments each continuously measure volume in the corresponding segment of the left ventricle as described in the text. Continuous left ventricular pressure monitoring (not shown) is simultaneously performed in the mid-ventricle position.*

### *Systolic function*

In order to analyze systolic function *in vivo* by means of LVPVR and analysis of the relation of systolic performance to load (i.e. the Starling relation) one must experimentally alter preload conditions in a controlled fashion and collect pressure-volume data during consecutive heart cycles. This alteration of preload was done by transiently occluding the inferior vena cava (IVCO), creating a progressive decline in preload (*figure 5*). To accomplish this, a balloon occlusion catheter used was placed via a jugular vein to a position where the inferior vena cava inflow to the right atrium could be partially or completely occluded by balloon inflation, and this placement was

also assisted by fluoroscopy. The IVCO maneuver was always done during brief apnea to avoid respirator induced alterations of venous return. Six to eight consecutive heart beats were then analysed and end-systolic pressure-volume points were plotted and least squares regression was performed. The slope of this regression line is the *end-systolic elastance* (Ees). Using the same heart cycles, stroke work (SW, area within a pressure-volume loop) was plotted against end-diastolic volume (EDV) and the regression line was plotted in the same manner. This correlation slope is named *preload recruitable stroke work* (PRSW). Other indices of systolic function used were $dP/dT_{max}$/EDV, i.e. the first derivative of pressure increase during systole related to EDV[160] and *preload indexed maximal power* ($PWR_{max}$/EDV), i.e. the instantaneous maximal product of LV volume change and pressure during systole related to EDV[161].

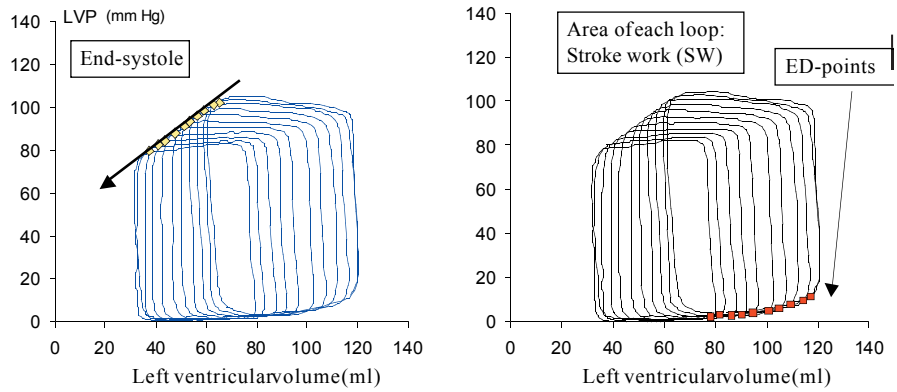


**Figure 5.** *Variations in preload to determine parameters of systolic performance. Increasing reduction in preload after inferior vena cava occlusion is depicted in this figure. The end-systolic points (left) are linear within these ranges of preload alteration. The slope of this line, end-systolic elastance (Ees), corresponds well to myocardial systolic function. In the same heart cycles, the area of each loop represents stroke work (right). These are plotted against the end-diastolic points (ED) and the slope of the relation of SW to ED is the preload-recruitable stroke work (PRSW).*

## *Diastolic function and efficiency*

Diastolic function was assessed using pressure-volume relations during passive filling (*figure 6*). End-diastolic pressure and filling (LVEDP/LVEDV) denotes stiffness whereas the time constant tau ($\tau$) for pressure decay and the half-time for pressure decay ($t_{1/2}$) from $dP/dT_{min}$ both are measurements of isovolumic relaxation[162].

Mechanical efficiency was assessed as the proportion of SW to PVA (pressure-volume area = total potential energy) without preload alteration. In paper III, the relation between PVA and single-beat myocardial oxygen consumption ($PVA/MVO_2$) was also assessed as a parameter for myocardial efficiency[163].

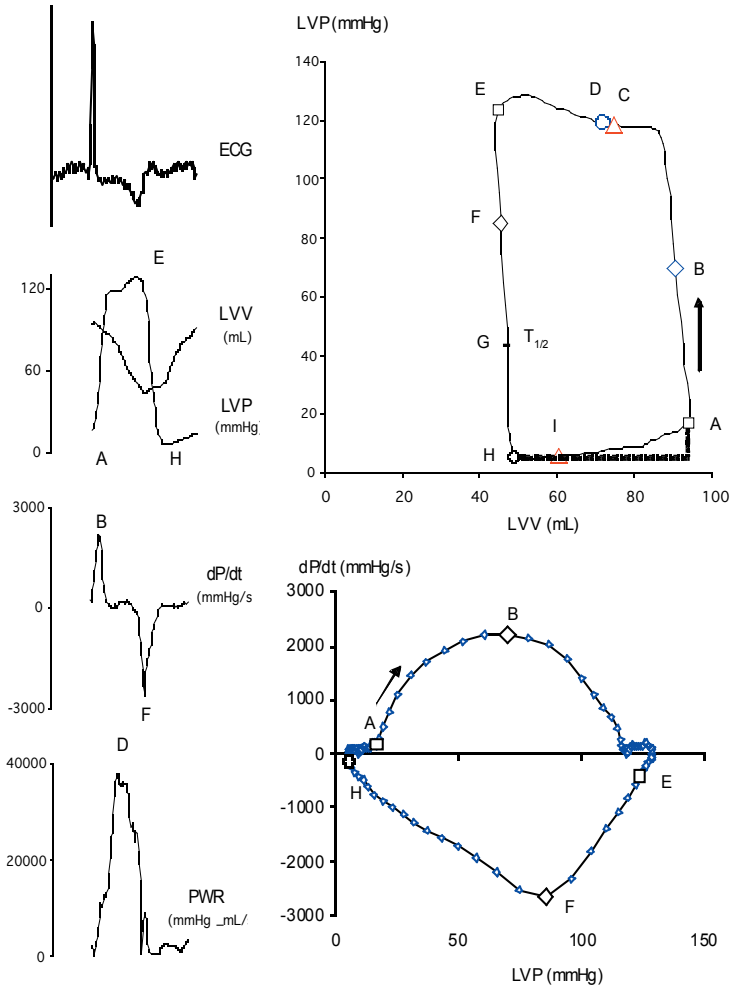


**Figure 6.** *Demonstration of a representative pressure-volume cycle (upper right), with measurement points in the cycle noted for end-diastole (A), dP/dtmax (B) and dP/dtmin (F), dV/dtmin (C) and dV/dtmax (I), $PWR_{max}$ - maximal instantaneous power (D), end-systole (E), $t_{1/2}$ - point for pressure reduction half-time from dP/dtmin (G), and the point between end-isovolumic relaxation and start-filling (H). On the panels to left, some of these functions are displayed over time for the same heart cycle with the same aspects of the heart cycle noted for A, B, C, D, E, F, H, and I. The bottom right panel demonstrates change in pressure during the entire heart cycle, where logarithmic decay of pressure shows a linear phase in this format between F and H, where $t_{1/2}$ and tau are measured.*
*Courtesy of Ass. Prof. Michael Haney and Göran Johansson, Dept Perioperative and Surgical Sciences, Umeå University, Umeå, Sweden.*

## Myocardial and regional blood flow

In papers II-IV myocardial blood flow was measured by two different principles. In paper II, following left-sided thoracotomy, an ultrasonic flow probe (Transonic Systems, Ithaca, NY, USA) was placed around the left anterior descending artery (LAD) and flow continuously recorded. The same principle was used for measurements of portal and renal blood flow in paper I. The retrograde thermodilution technique was used for determining myocardial blood flow in papers III and IV by placing a dual thermistor-tipped coronary sinus catheter (Webster, CA, USA) into the great cardiac vein using fluoroscopic guidance. Blood flow was intermittently recorded just prior to each measurement of cardiac performance.

Assessment of intestinal mucosal pH (pHi) was made in paper I by means of ileal tonometry. In brief, a saline filled balloon catheter was inserted in the ileum and partial pressure of $CO_2$ ($pCO_2$) was measured from the balloon aspirate[133]. This partial pressure is believed to reflect mucosal $pCO_2$ and, through Henderson-Hasselbalch transformation, mucosal pH (pHi).

## Drugs

### *Levosimendan*

In paper I, levosimendan (*figure 7*) was administered prior to start of endotoxin infusion. Levosimendan, a pyridazinone-dinitrile derevative, is a calcium sensitizer and exert its effect by binding to troponin C[164]. This binding is strong in the presence of high $Ca^{2+}$ concentrations (in systole) and less avid when cytosolic $Ca^{2+}$ decreases (in diastole)[165]. The binding of levosimendan stabilizes the calcium-induced conformational change of troponin C thereby changing the actin-myosin crossbridge kinetics and improve contractility. Levosimendan also causes vasodilation by activating $K_{ATP}$ channels[166]. In addition, levosimendan has been reported to block the release of ET-1[167].

Levosimendan is rapidly distributed in humans with a distribution half-life of 0.1-0.26 hours[168]. About 97% is protein bound and elimination half-life is approximately one hour. One of the metabolites of levosimendan, following reduction and acetylation, is biologically active with a longer half-life. This metabolite, OR-1896, may prolong the hemodynamic effects of levosimendan[169].

### *Tezosentan*

The dual ET receptor antagonist tezosentan (*figure 7*) (Actelion, Allschwil, Switzerland) was used in papers II and IV. Tezosentan competitively and specifically antagonizes both $ET_A$ and $ET_B$ receptors in a 30:1 ratio[170]. It is relatively short-acting and water soluble, designed for acute parenteral use. Tezosentan has a distribution

half-life of 0.1 hours and elimination half-life of three hours in humans[171]. There are no known active metabolites of tezosentan. In paper II, tezosentan was dissolved in sodium chloride and infused two hours after start of endotoxin. One group received tezosentan as a bolus-infusion of 1 mg·kg$^{-1}$ in 10 minutes followed by a continuous infusion of 1 mg·kg$^{-1}$·h$^{-1}$ for the remainder of the experiment. The other group received tezosentan of 10 mg, both as bolus and continuous infusion in the same manner.

In paper IV intervention with tezosentan (bolus-infusion 1 mg·kg$^{-1}$ in 10 minutes followed by a continuous infusion of 1 mg·kg$^{-1}$·h$^{-1}$) was made three hours after start of endotoxin. The reason for this was allowing a longer time frame for endotoxin induced effects on the myocardium.

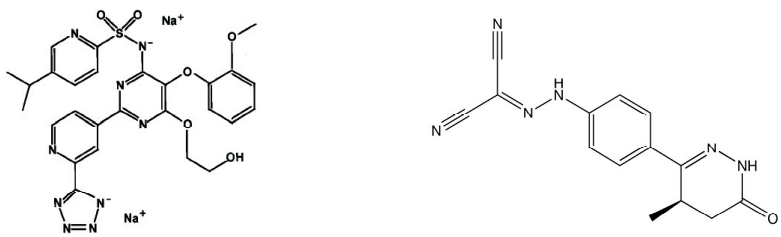


**Figure 7.** *Molecular structure of tezosentan, a dual endothelin receptor antagonist (left), and levosimendan, a calcium sensitizing compound (right).*

## *Endothelin-1*

In paper III intra-coronary ET-1 (*figure 8*) (American peptide Co., Sunnyvale, CA, USA) was infused in incremental doses from 1, 2, 4 and 8 pmol·kg$^{-1}$·min$^{-1}$. Infusions were made through a guiding catheter (Guidant) placed with the tip in the left main coronary artery and position checked with fluoroscopy. Infusions were made for 30 minutes and during the last 10 minute period of the infusion, measurements were made. Infusion of ET-1 was also made intravenously with a higher dose of 10 pmol·kg$^{-1}$·min$^{-1}$.

*Sarafotoxin*

The sarafotoxin is naturally occurring as snake venom from the *atractaspis engaddensis* (also known as burrowing asp, notorious for causing the death of Cleopatra) and its name originates from the Hebrew name: "Saraf EnGedi". In paper III we used sarafotoxin 6c (S6c, American peptide Co., Sunnyvale, CA, USA) which is a synthetic 21 amino-acid peptide (*figure 8*) and a highly specific functional $ET_B$ receptor agonist[172]. Infusions were made intracoronary as described above with 1 and 2 $pmol \cdot kg^{-1} \cdot min^{-1}$.

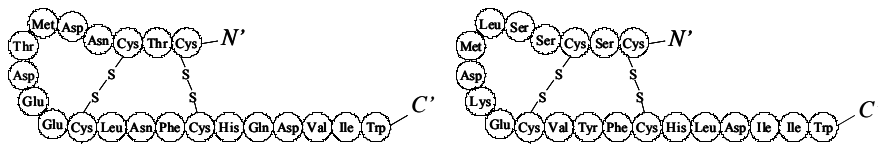


**Figure 8.** *Amino-acid sequences of endothelin-1, endogenous $ET_A$ and $ET_B$ receptor agonist (left) and sarafotoxin 6c, exogenous $ET_B$ receptor agonist (right).*

# Results and comments

*"You can observe a lot just by watching".*
*Yogi Berra*

## Effects of endotoxin

Endotoxin invariably caused marked effects in both the systemic and pulmonary circulation *(table 3*). This was associated with increases in plasma levels of ET-1 like immuno-reactivity (ET-1 LI) ranging from a 2- to 4-fold increase. In paper I a clear shock response was seen with an increase in heart rate (HR), decrease in CI, stroke volume index (SVI), mean arterial blood pressure (MAP), systemic vascular resistance index (SVRI), portal and renal blood flow as well as mixed venous oxygen saturation ($SvO_2$). In paper II, the response was primarily hyperdynamic due to a more aggressive resuscitation protocol: CI, HR and coronary blood flow was initially increased whereas SVI, MAP, SVRI and $SvO_2$ decreased. Again, in paper IV, the response to endotoxin was hypodynamic: decrease in CI, SVI, MAP and $SvO_2$ with an increase in HR and SVRI. In all animals receiving endotoxin, a marked and sustained pulmonary hypertension was seen, commonly noted in porcine endotoxemia.

One of the main reasons for the differences in endotoxin-mediated effects is fluid administration. In papers I and IV we used Ringers' solution of 20 $mL \cdot kg^{-1} \cdot h^{-1}$ whereas in paper II the animals received 30 $mL \cdot kg^{-1} \cdot h^{-1}$ of which 15 $mL \cdot kg^{-1} \cdot h^{-1}$ of Ringers' solution and 15 $mL \cdot kg^{-1} \cdot h^{-1}$ of dextrane. There was also a difference in dose of endotoxin. In papers I and II the pigs received 20 $\mu g \cdot kg^{-1} \cdot h^{-1}$ and in paper IV 0.25 $\mu g \cdot kg^{-1} \cdot h^{-1}$. Regardless of dose of endotoxin, an increase in plasma levels of troponin I was evident, analyzed in papers II and IV. This finding was not accompanied by reductions in myocardial blood flow.

| | CI | HR | MAP | SVRI | MPAP | $SvO_2$ |
|---|---|---|---|---|---|---|
| Paper I | ↓ | ↑ | ↓ | ↓ | ↑ | ↓ |
| Paper II | ↑ | ↑ | ↓ | ↓ | ↑ | ↓ |
| Paper IV | ↓ | ↑ | ↓ | ↑ | ↑ | ↓ |

**Table 3.** *A summary of effects of endotoxin administration on selected parameters of general hemodynamics from papers I, II and IV. CI: cardiac index, HR: heart rate, MAP: mean arterial pressure, SVRI: systemic vascular resistance index, MPAP: mean pulmonary artery pressure, SvO2: mixed venous oxygen saturation*

# Effects of levosimendan (paper I)

Under basal conditions, levosimendan evoked a vasodilatory response seen as decreases in MAP, SVRI, pulmonary vascular resistance (PVRI) and gut vascular resistance and increases in HR and pulmonary shunt (possibly by interfering with hypoxic pulmonary vasoconstriction). There was a tendency for an increase in CI by levosimendan at baseline. The dose chosen for levosimendan (200 $\mu g \cdot kg^{-1} \cdot h^{-1}$) was based on a dose-response series in three animals.

During endotoxemia, levosimendan attenuated the endotoxin-induced impairment in CI, systemic oxygen delivery and $SvO_2$ and increased oxygen consumption compared with endotoxin controls (*figure 9*). No further increase in HR or decrease in MAP was seen. Levosimendan also counteracted the pulmonary hypertension but with no further effects on arterial oxygenation or pulmonary shunt. Splanchnic perfusion was improved by levosimendan seen as increases in portal blood flow, gut oxygen delivery and consumption. No positive effects were seen regarding intestinal mucosal pHi.

Levosimendan did not cause any significant effects on arterial ET-1 plasma levels.

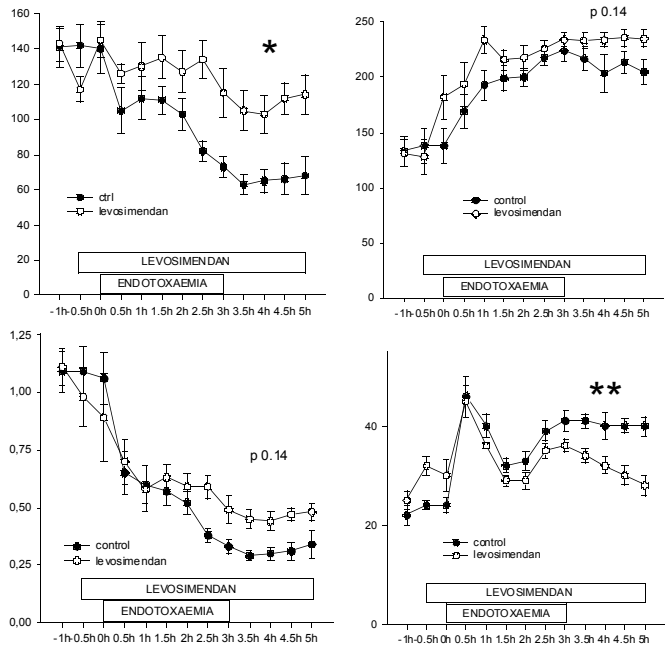


**Figure 9.** *Effects of levosimendan, a calcium sensitizer, in endotoxemia compared with endotoxin controls. Levosimendan (open circles, n=6) infusion was started 30 minutes prior to start of endotoxin infusion (-0.5h). Levosimendan was continued throughout the experiment and compared with endotoxin controls (black circles, n=8). Endotoxin was terminated after 3 hours (3h) in both groups. Data are presented as mean (SEM). *=p< .05, **=p< .01. Top left: CI (cardiac index); top right: HR (heart rate); bottom left: SVI (stroke volume index); bottom right: MPAP (mean pulmonary artery pressure).*

# Effects of tezosentan (papers II and IV)

The overall effects of tezosentan administration in established endotoxin shock were differentiated and related to dose. In paper II, tezosentan (lower dose) caused a rapid and sustained increase in CI and SVI without a concomitant change in HR or MAP compared with controls (*figure 10*). Pulmonary hypertension was attenuated and pulmonary as well as systemic vascular resistances decreased. $SvO_2$ was improved and oxygen delivery and consumption increased. In contrast, effects of a higher dose of tezosentan were ultimately detrimental. Although the initial response to the higher dose was beneficial, and similar to the lower dose of tezosentan, deterioration in CI, SVI, oxygen delivery and consumption and SvO2 was evident 1.5 hours after start of tezosentan. A sudden elevation of PCWP was seen at this time (*figure 11*) although effects on MPAP were sustained, indicating a sudden rise in LV end-diastolic pressure. MAP was also reduced leading to a decrease in coronary perfusion pressure (CPP). The higher dose of tezosentan was also associated with a higher mortality rate (50% vs. 0% for tezosentan low dose compared with 20% for controls).

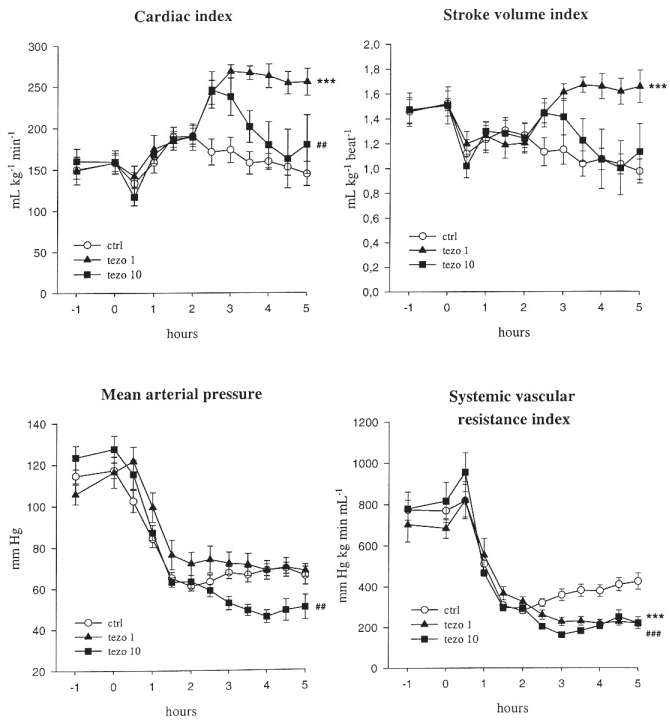


**Figure 10.** *Effects of tezosentan, a dual ET receptor antagonist, in endotoxemia. Endotoxin infusion was started at 0 hours and continued until end of experiment in all groups. Tezosentan was infused at 1 mg·kg$^{-1}$·h$^{-1}$ (tezo1, n=7, black triangles), at 10 mg·kg$^{-1}$·h$^{-1}$ (tezo10, n=10, black squares) starting after 2 hours of endotoxin infusion and both groups compared with endotoxin controls (n=20, open circles). Data are presented as mean (SEM). ***=p< .001 for tezo1, ##=p< .01 and ###=p< .001 for tezo10.*

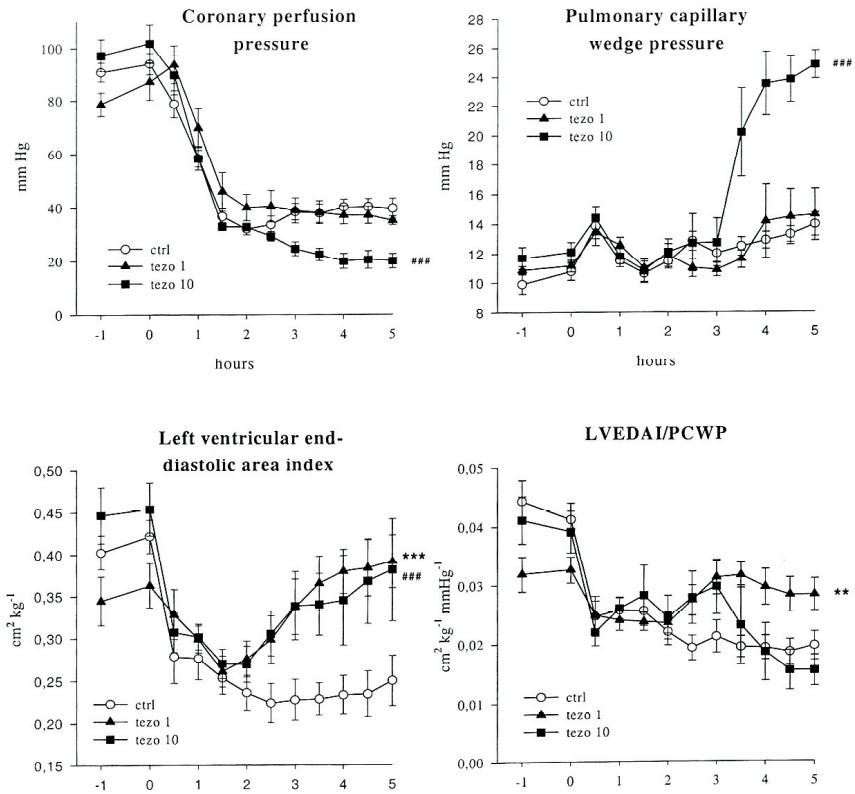


**Figure 11.** *Effects of tezosentan, a dual ET receptor antagonist, in endotoxemia. Endotoxin infusion was started at 0 hours and continued until end of experiment in all groups. Tezosentan was infused at 1 mg·kg$^{-1}$·h$^{-1}$ (tezo1, n=7, black triangles), at 10 mg·kg$^{-1}$·h$^{-1}$ (tezo10, n=10, black squares) starting after 2 hours of endotoxin infusion and both groups compared with endotoxin controls (n=20, open circles). LVEDAI/PCWP= left ventricular compliance (left ventricular end-diastolic area/pulmonary capillary wedge pressure). Data are presented as mean (SEM). **=p< .01, ***=p< .001 for tezo1, and ###=p< .001 for tezo10.*

Thus, in paper IV, the lower dose of tezosentan was used in the same manner as above and was equally beneficial regarding general hemodynamics as in paper II. CI and SVI increased and the same vasodilatory response occurred with decreases in MAP, SVRI and abolishment of pulmonary hypertension (*figure 12*). There was a strong tendency for an increase in $SvO_2$ (p=0.051) compared with endotoxin controls.

In both studies, tezosentan caused further increases in plasma levels of ET-1 due to antagonism of $ET_B$ receptors (below in discussion). There was a dose-related effect in paper II where the higher dose of tezosentan caused higher levels of ET-1.

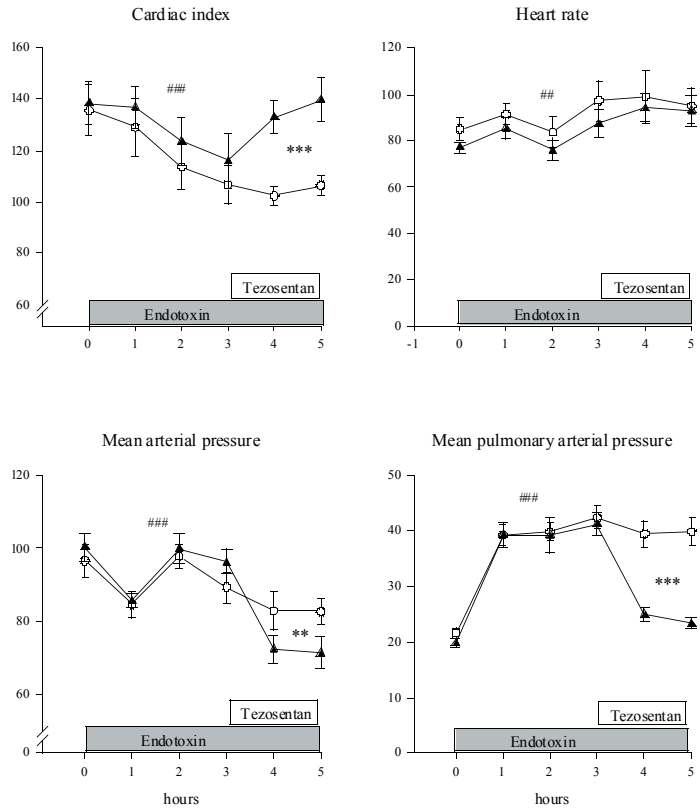


**Figure 12.** *General hemodynamics were studied following endotoxin administration for five hours. After three hours of endotoxemia, tezosentan (1 mg·kg$^{-1}$·h$^{-1}$) was started (n=8, black triangles) and compared with animals receiving endotoxin alone (controls, n=8, open circles). Data are presented as mean (SEM). Effects of endotoxin prior to intervention are displayed as: ##=p <.01, ###=p < .001. Significant differences between groups post intervention are displayed as: ** =p< .01 and *** = p< .001.*

# Effects of exogenous ET-1 and sarafotoxin (paper III)

In the experimental protocol for paper III we used incremental doses of exogenous ET-1 ($ET_A$ and $ET_B$ receptor agonist) and S6c ($ET_B$ receptor agonist) to evaluate the cardiac effects (next section, below). Infusions were made in the left main coronary artery and for ET-1 also intravenously. Regarding intracoronary administration of ET–1 there were no discernible effects on HR, CO or SV regardless of dose. MAP and SVR increased with the two higher doses and MPAP with the highest dose. CVP was increased by every dose of ET-1. Although statistically significant all these changes were minor. Contrarily, when infused intravenously, ET-1 caused more pronounced hemodynamic effects. HR, CO and SV decreased and CVP, MAP and SVR increased. No effects on the pulmonary circulation were seen.

The intracoronary infusion of S6c was associated with decreases in CO and SV whereas HR and MAP remained unchanged. CVP increased (both doses) as did SVR (higher dose) but pulmonary circulation was unaffected.

## The ET system and cardiac performance

To evaluate the regulatory role by the ET system on cardiac physiology, ET-1 ($ET_A$ and $ET_B$ receptor agonist) and S6c ($ET_B$ receptor agonist) were administered in healthy, anesthetized pigs (paper III). Cardiac performance was assessed by LVPVR using conductance volumetry (see materials and methods) and myocardial blood flow measured by thermodilution. Intracoronary applied ET-1 caused improvements in parameters of contractile status (*figure 13*): Ees, PRSW and $dP/dT_{max}$/EDV in a seemingly dose-related fashion. $PWR_{max}$/EDV was unaltered by ET-1. However, when infused intravenously, ET-1 did not have any effect on these parameters of systolic function. The administration of S6c intracoronary caused negative effects on systolic function (*figure 13*): no changes in Ees or PRSW but seemingly dose-related impairments in $PWR_{max}$/EDV and $dP/dT_{max}$/EDV.

Diastolic function parameters were also affected by ET receptor stimulation. Isovolumic relaxation time was assessed as $\tau$ (tau) and $t_{1/2}$ (pressure half-time). ET-1 and S6c both caused prolongation (=impairment) of isovolumic relaxation regardless of administration site (*figure 14*). All doses of ET-1, except the lowest, and all doses of S6c had this effect. Neither ET-1 nor S6c had any evident effects on LV stiffness.

There have been previous reports of ET-1 mediated coronary vasoconstriction[144], thus great cardiac vein blood flow was evaluated. ET-1 did not cause any changes in myocardial blood flow independent of route of administration. A concomitant increase in myocardial oxygen consumption was seen which is expected when improving contractile function. On the other hand, S6c in both doses was associated with decreased coronary blood flow, myocardial oxygen delivery and consumption.

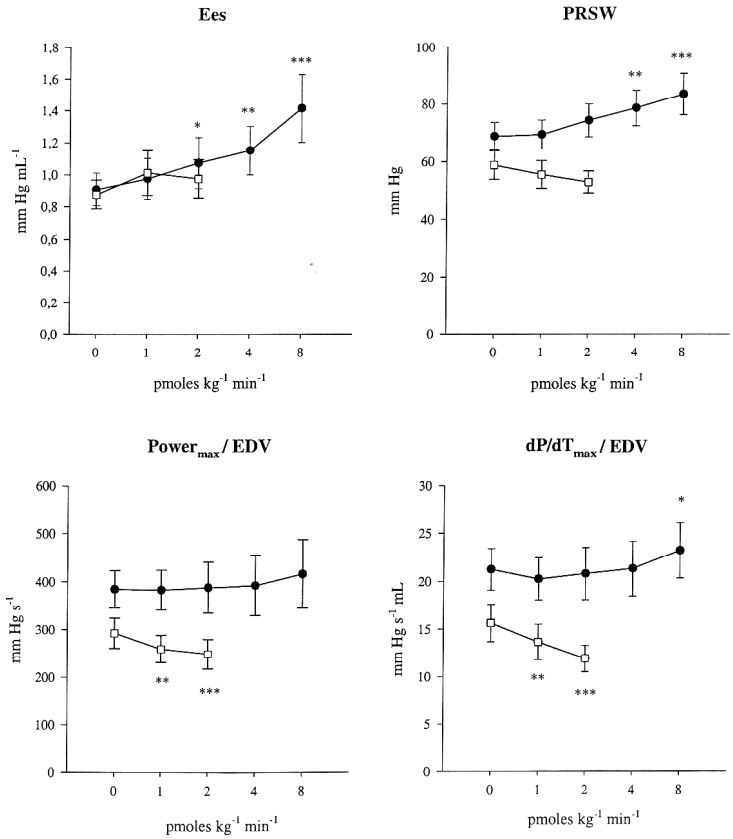


**Figure 13.** *Myocardial contractile function parameters: end-systolic elastance (Ees), preload-recruitable stroke work (PRSW), power$_{max}$ / end-diastolic volume (Power$_{max}$/ EDV) and dP/dT$_{max}$/EDV were studied following intra-coronary endothelin-1 (ET-1, black circles) infusion in nine pigs in increasing doses from 1 to 2 to 4 to 8 pmol·kg$^{-1}$·min$^{-1}$. After a recovery period, sarafotoxin 6c (S6c, white squares) was infused in 10 pigs in the same manner with doses of 1 and 2 pmol·kg$^{-1}$·min$^{-1}$. Data are presented as mean (SEM). Each dose was compared to its corresponding baseline value and significant differences displayed as: * = p < .05, ** = p < .01 and *** = p < .001.*

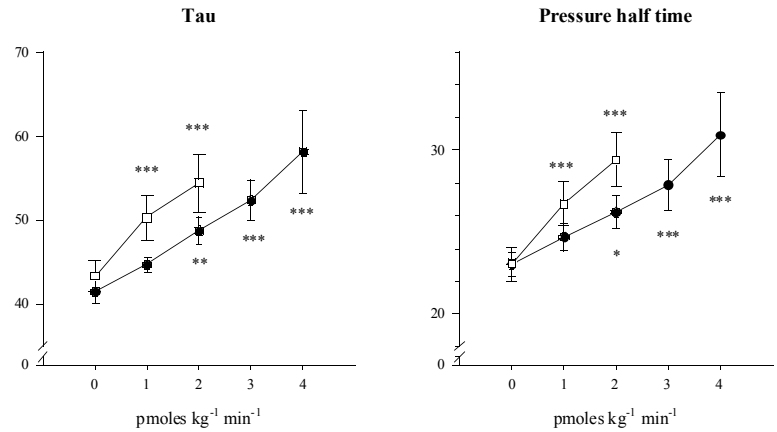


**Figure 14.** *Isovolumic relaxation parameters: tau and pressure half-time were studied following intra-coronary endothelin-1 (ET-1, black circles) infusion in nine pigs in increasing doses from 1 to 2 to 4 to 8 pmol·kg$^{-1}$·min$^{-1}$. After a recovery period, sarafotoxin 6c (S6c, white squares) was infused in 10 pigs in the same manner with doses of 1 and 2 pmol·kg$^{-1}$·min$^{-1}$. Data are presented as mean (SEM). Each dose was compared to its corresponding baseline value and significant differences displayed as: * = p < .05, ** = p < .01 and *** = p < .001.*

# The ET system and cardiac dysfunction in endotoxemia

The issue of myocardial dysfunction in endotoxemia is addressed in papers II and IV. In order to appreciate cardiac effects both pressure and volume need to be taken into account. In paper II left ventricular volume (LVEDAI) was assessed by means of two-dimensional echocardiography as described above. A rapid drop in LVEDAI occurred after endotoxin administration (*figure 11*) and onset of pulmonary hypertension. This effect was totally abolished by tezosentan, both doses. This resulted in an improved LV compliance in the lower dose since PCWP (the surrogate parameter for LV end-diastolic pressure) was unchanged (compliance= LVEDAI/PCWP). Contrarily, PCWP rose suddenly in the higher tezosentan group yielding LV compliance comparable to controls.

There were no differences in HR between either group which otherwise could be an explanation for the differences in CI. Preload was obviously lower in the controls as indicated by differences in LVEDAI (preload=end-diastolic volume or stretch). Afterload did not differ between the groups of lower dose of tezosentan and controls as evidenced by the same MAP, but MAP was significantly lower in the higher tezosentan group. The ejection fraction, or fractional area of contraction, did not differ between groups at any time point. In order to further characterize the effects on systolic performance, relative changes in left ventricular stroke work index (derived from PAC

data) was plotted against relative changes in preload (LVEDAI) thus constructing a Starling relation (*figure 15*). From this a deterioration of systolic function is suggested for controls and the higher dose group of tezosentan, seen as down- and rightward shifts in the left ventricular performance-preload relation. As for tezosentan lower dose, an up- and rightward shift is seen which may represent just another point of the same Starling curve, i.e. contractile status is unchanged during an observation with increased preload. Although indicative of positive effects on myocardial systolic and diastolic properties by tezosentan treatment, the methods used were still load-dependent.

In paper IV, cardiac performance was investigated by analysis of the LVPVR. Endotoxin infusion did not cause detectable effects on parameters of systolic function (*figure 16*): Ees, PRSW, $PWR_{max}$/EDV and $dP/dT_{max}$/EDV. Tezosentan infusion was associated with impairment in these parameters (not statistically significant for PRSW, p=0.067) suggesting a deterioration of myocardial contractile status. On the other hand, endotoxin caused impairment in isovolumic relaxation and mechanical efficiency (seen as $\tau$ and SW/PVA respectively) with no changes seen in LV stiffness (LVEDP/LVEDV). However, an improvement in diastolic function was seen both regarding isovolumic relaxation (tau and $t_{1/2}$) and LV stiffness by tezosentan administration (*figure 17*).

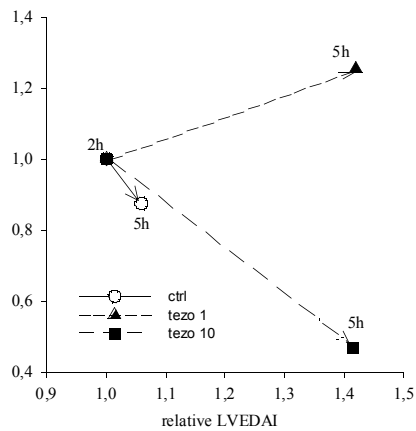


**Figure 15.** *Starling relations regarding effects of tezosentan, a dual ET receptor antagonist, in endotoxemia. Endotoxin infusion was started at 0 hours and continued until end of experiment in all groups. Tezosentan was infused at 1 mg·kg$^{-1}$·h$^{-1}$ (tezo1, n=7, black triangles), at 10 mg·kg$^{-1}$·h$^{-1}$ (tezo10, n=10, black squares) starting after 2 hours of endotoxin infusion and both groups compared with endotoxin controls (n=20, open circles). Relative changes in LVSWI (left ventricular stroke work index) and relative changes in LVEDAI (left ventricular end-diastolic area index) were plotted from time of start of intervention (2 hours) till end of experiment (5 hours).*

# Discussion

*"Do not be daunted by the enormity of the world's grief. Do justly, now. Love mercy, now. Walk humbly, now. You are not obligated to complete the work, but neither are you free to abandon it."*

*Talmud*

There is now overwhelming amount of data to support the concept of myocardial depression in sepsis. Deterioration of myocardial function in septic patients is often evident and correlates to morbidity and mortality. Although clinically relevant, it is not yet clear by which means this depression occurs and, accordingly, how to treat it. A number of mechanisms have been proposed but no single mediator can as yet explain this phenomenon. Rather, the intense host-defense reaction seen in sepsis is likely to trigger a cascade of pro- and anti-inflammatory agents that results in impaired cardiac function. Within the context of this complex and dynamic clinical syndrome, we have investigated myocardial events to try to evaluate the biological relevance of the ET system.

Despite the fact that our group and others have shown positive effects regarding CO, regional perfusion and even survival by antagonizing the ET system in experimental sepsis, it has not been clear whether these effects directly have improved myocardial function or whether the beneficial effects seen are secondary to prominent vasodilation, altering loading conditions. In fact, the effect of the ET system in normal cardiac physiology has not been clear either with results differing among models and species. The development of a new class of drugs, calcium sensitizers, has been proven beneficial in severe heart failure and calcium desensitization is one proposed mechanism for septic myocardial depression. The results of this thesis have included some new findings concerning calcium sensitivity in experimental sepsis and the ET system in regulating myocardial performance both in septic and non-septic states.

## Levosimendan in endotoxemia

The rationale for investigating the effects of levosimendan in endotoxemia is that calcium desensitization is a proposed mechanism for the development of myocardial dysfunction in sepsis. Furthermore, interaction between levosimendan and ET system has been suggested[173]. Paper I of this thesis is actually the very first publication on levosimendan in septic conditions. Regarding CHF and levosimendan there are numerous publications and ongoing clinical trials. In a study of 146 CHF patients

with New York Heart Classification of III and IV, levosimendan administration caused significant reduction in ET-1 plasma levels[173]. In other reports of severe CHF, plasma levels of ET-1 are elevated and correlates to severity of disease[99,174] as well as constitute a strong, independent predictor of death in such patients[175]. Consequently, we hypothesized that, in septic conditions, levosimendan would enhance cardiovascular performance by increasing myocardial $Ca^{2+}$ sensitivity and possibly by decreasing plasma ET-1 levels.

Levosimendan was administered before endotoxin as the pharmacokinetic properties in pigs were unknown and, in humans, the beneficial effects of levosimendan may not be apparent for some time[176]. Thus, in this short term experiment, pretreatment would allow characterization of baseline effects as well as time to evaluate effects in endotoxemia. The baseline effects of levosimendan included vasodilation, tachycardia and a strong trend towards increased CI. Besides its $Ca^{2+}$ sensitizing effect, levosimendan is also known to be a vasodilator by activating $K_{ATP}$ channels in vascular smooth muscle[177,178] and possibly by blocking ET-1 release[167].

Levosimendan attenuated the endotoxin induced deterioration in CI, $SvO_2$, oxygen delivery, pulmonary hypertension and enhanced oxygen consumption (*figure 9*). In addition, gut oxygen delivery was also improved by levosimendan. With respect to cardiac performance, the increase in CI was not due to differences in heart rate but appeared to be mediated by an increase in SV ($p=0.14$, *figure 9*) in the face of lower MAP. No parameters of LVEDV were used but PCWP, a surrogate measure of LV end-diastolic pressure, was not affected by levosimendan as was the case with LVSWI (not shown). Thus, one cannot draw conclusions regarding specific cardiac effects.

The hypothesis of levosimendan interacting with the ET system in endotoxemia could not be confirmed. There were no differences regarding ET-1 plasma levels between controls and levosimendan treated animals. The issue of $Ca^{2+}$ desensitization as a major component in myocardial depression in sepsis is likely but the use of levosimendan in septic patients still remains to be further investigated. Some reports show positive effects on endotoxin mediated depression in contractility by $Ca^{2+}$ sensitizers *in vitro*[63,179] and also *in vivo*[180]. In the first clinical study of levosimendan in septic shock patients, levosimendan treatment was indeed beneficial and associated with increases in CI, SVI, LVSWI and EF compared with dobutamine, a β-adrenergic receptor agonist[181]. As suggested by one author: "results should be confirmed with the pressure-volume loop technique that may even further explore myocardial diastolic parameters as relaxation and ventricular compliance"[180]. Still, levosimendan is a very promising drug in sepsis.

# The ET system in normal cardiac physiology

The results of paper III implies that ET-1 plays a role in regulating the contractile force of the left ventricle. This is well in line with MacCarthy *et al*[142] who infused an $ET_A$ receptor antagonist in humans which led to a decrease in LV performance, suggesting that $ET_A$ receptor activation has a tonic positive contractile effect. This is also in accordance with our findings since only ET-1, not the $ET_B$ selective agonist S6c, evoked a positive effect on cardiac performance (*figure 13*). Instead, S6c was associated with marred contractile function. Other studies support the positive effect of ET-1 via the $ET_A$ receptor as well[131,182,183]. Goldberg *et al*[148], among others, have shown positive contractile effects on human myocytes that are $ET_A$ dependent and mediated via activation of protein kinase C and stimulation of the Na/H exchanger. This mechanism will render an increase in intracellular pH which is known to sensitize troponin C for $Ca^{2+}$[184] although ET-1 has recently been shown to improve contractility in the absence of intracellular alkalinization[185].

Also regarding diastole did ET receptor agonism exert notable effects. Both ET-1 and S6c induced impairment in isovolumic relaxation (*figure 14*) in a seemingly dose-related fashion. This would indicate rather strongly that activation of $ET_B$ receptors is mainly responsible for this effect. In a study of human myocytes, ET-1 caused impairment in time to 50% relaxation, slightly reduced by adding an $ET_A$ receptor antagonist but still displaying significantly impaired relaxation, which suggests that the $ET_B$ receptor is also involved[148].

There are reports that ET-1 is preferentially secreted abluminally from the endothelial cells[96,97] directed to the underlying smooth muscle cells. Infusing ET-1 intravasally may not represent the physiological route of ET-1 mediated effects. In order to achieve discernible effects, doses of ET-1 used rendered high plasma levels of ET-1, exceeding pathophysiological range. However, in pathophysiological processes plasma levels may only indirectly reflect local tissue activity. In studies of human CHF, heart tissue levels of ET-1 are very high[141,186] suggesting that the increased plasma levels of ET-1 merely reflects the increased tissue levels. To our knowledge, no studies have investigated effects of exogenous ET-1 and the ensuing tissue concentrations. In order to achieve relevant tissue levels of ET-1 it is suggested that high concentrations of exogenous ET-1 is needed. Also, systemic effects were quite small even in the highest intracoronary dose.

# The ET system and the septic myocardium

There have been several reports where the supportive role of ET-1 regarding systolic cardiac function in normal physiology is changed in pathological conditions. When investigating the effects of ET receptor antagonism in patients with dilated

cardiomyopathy these patients improved their contractile performance[142]. Very similar effects have also been reported in porcine myocytes[146], whereas in healthy hearts, ET-1 seem to have a basal positive effect on cardiac performance. In congestive heart failure, ET-1 plasma levels are also elevated. The specific cardiac effects of ET receptor activity under septic conditions *in vivo* have not been thoroughly studied.

## Systolic function

The results of paper II would indirectly suggest a positive inotropic effect (*figure 15*) or rather that the negative effects of endotoxin on cardiac performance are counteracted by dual ET receptor antagonism. This is supported by the fact that CI and SVI was increased by tezosentan with no difference in HR or MAP which means that frequency or afterload differences cannot explain the difference between tezosentan and controls. However, preload was different as evidenced by LVEDAI (*figure 11*) and, as mentioned in the introduction section, preload is a major determinant of contractility.

To address the issue of preload-effects as responsible for the beneficial effects by dual ET receptor antagonism, we needed to use preload independent parameters of systolic function. Hence the method of analyzing LVPVR by means of conductance volumetry (see below). By inflating a balloon in the inferior vena cava (IVCO), the resulting preload alteration could be used to analyze systolic function independent of preload (*figure 5*). In paper IV, this was done in circumstances similar to paper II, intervening with tezosentan in established endotoxemia. Again there were no differences in HR whereas MAP was slightly decreased by tezosentan. Interestingly, using load-independent parameters of systolic function, tezosentan impaired contractile performance (*figure 16*). This finding stresses the importance of controlling preload when striving to assess systolic function *in vivo*. It should be noted that, in our model, systolic function was not depressed at three hours (*figure 16*) so the deterioration in systolic performance by tezosentan strongly suggest that in acute endotoxemia, the ET system aids in upholding cardiac systolic performance.

Based on the results mentioned above one would be inclined to antagonize specifically the $ET_B$ receptor since there is some evidence of the positive inotropic effect via $ET_A$ receptor activation. However, selective $ET_B$ receptor antagonism may be fatal in experimental septic conditions[130,187]. This phenomenon could be explained by unopposed $ET_A$ receptor stimulation in combination with diminished clearance of circulating ET-1, resulting in massive vasoconstriction, sudden increase in cardiac afterload and death. Within our group, we have also shown that specifically antagonizing $ET_A$ receptors[188] did not cause positive cardiovascular effects as compared with dual ET receptor agonism[189]. Thus, the dual ET receptor antagonist tezosentan was used.

There is an ongoing debate as whether cardiac systolic function is depressed or

not in acute, septic conditions. Even when using load-independent techniques, results are diverging. Some authors report similar findings as ours[70,71], some increased[72,73] and some depressed contractile function[54,74,75]. Intriguingly, Ishihara *et al*[73] reported biphasic, time-dependent changes in LV systolic performance in awake pigs receiving continuous infusion of endotoxin for 24 hours. They reported an initial increase in Ees in the first hours followed by a significant sustained decrease in Ees after seven hours and onward. Studies in humans of myocardial depression are invariably not done within the first few hours of sepsis debut but investigators often find depressed systolic function upon presentation in the ICU and days thereafter[45,47]. Our findings, within the limited time frame under which they are conducted, do not rule out the possibility that ET-1 may play a significant role in the clinical presentation of depressed systolic function at a later stage of sepsis. Interestingly, ET plasma levels have been shown to remain elevated in up to 28 days after onset of severe sepsis[125].

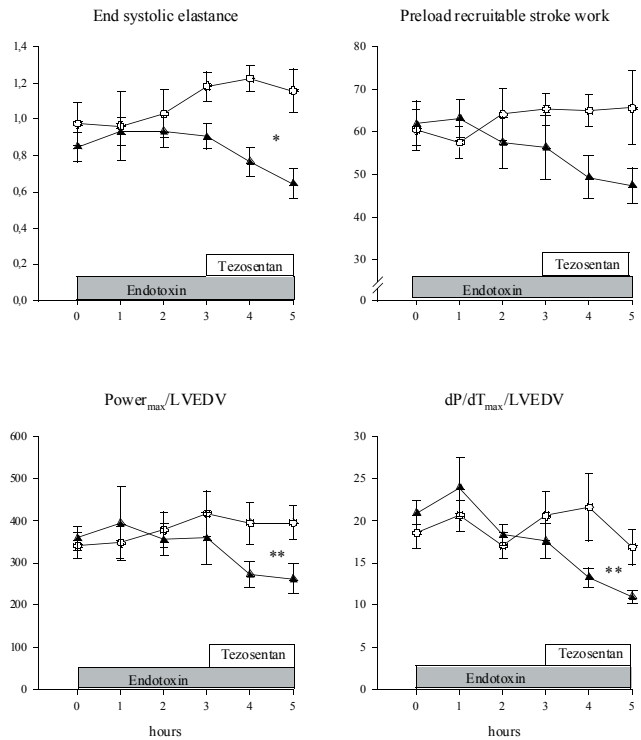


**Figure 16**. *Myocardial contractile function parameters: end-systolic elastance (Ees), preload-recruitable stroke work (PRSW), power$_{max}$ / end-diastolic volume (Power$_{max}$/EDV) and dP/dT$_{max}$/EDV were studied following endotoxin administration for five hours. After three hours of endotoxemia, tezosentan (1 mg·kg$^{-1}$·h$^{-1}$) was started (n=8, black triangles) and compared with animals receiving endotoxin alone (controls, n=8, open circles). Data are presented as mean (SEM). Differences between groups post intervention are displayed as: * = p< .05 and ** = p< .01.*

## Diastolic function

In the field of congestive heart failure there has been increasing evidence of diastolic preceding systolic dysfunction[190]. The ET system has also been implicated in diastolic dysfunction. When administering ET-1 to healthy volunteers, Kiely *et al* found impairment in LV relaxation using echocardiography[143]. There is also increasing evidence of diastolic dysfunction in sepsis[44]. In vasopressor dependent septic shock patients, diastolic dysfunction is present and suggested to precede onset of systolic dysfunction[45]. Furthermore, in experimental models of sepsis there are several reports of diastolic abnormalities[74,75]. In septic rats, isovolumic relaxation, seen as prolongation of tau, was negatively affected and further so when adding bigET-1[191].

Our findings are indeed in accordance with these investigators. In paper II, diastolic compliance was impaired by endotoxin but improved upon administration of tezosentan in the lower dose (*figure 11*). The initial response to the higher dose of tezosentan was equally beneficial in this regard but was not sustained. The latter finding is likely due to a very low coronary perfusion pressure attained by severe vasodilation which, together with increased LV pressure, reduced myocardial perfusion to inadequate levels.

The beneficial effects on LV compliance by tezosentan, lower dose, was reproduced in paper IV, and here displayed as a decrease in LV stiffness (*figure 17*). In addition, parameters of isovolumic relaxation (tau) was impaired by endotoxin but improved by tezosentan. This effect may well have contributed to the positive effects seen in papers II and IV regarding CO. Improved diastolic function following ET receptor antagonism has also been shown *in vitro* in both human and rabbit cardiomyocytes[148,192].

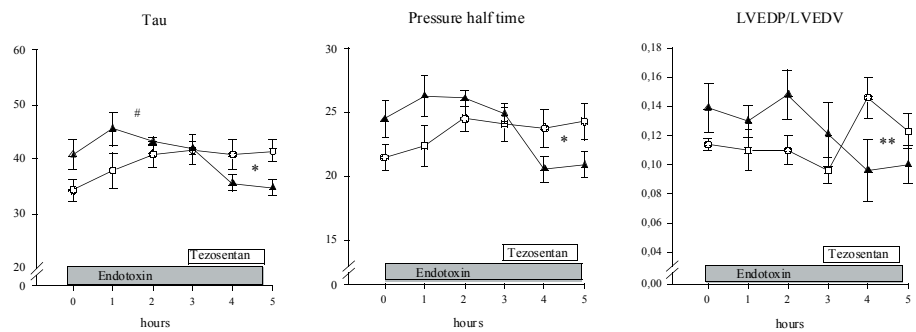


**Figure 17**. *Isovolumic relaxation parameters: tau and pressure half-time as well as left ventricular stiffness were studied following endotoxin administration for five hours. After three hours of endotoxemia, tezosentan (1 mg·kg$^{-1}$·h$^{-1}$) was started (n=8, black triangles) and compared with animals receiving endotoxin alone (controls, n=8, open circles). Data are presented as mean (SEM). Effects of endotoxin prior to intervention are displayed as: #=p< .05. Differences between groups post intervention are displayed as: * = p< .05 and ** = p< .01.*

## Dysoxia

Global cardiac hypoperfusion has been ruled out as a factor in myocardial depression in sepsis[51,52]. Even so, cardiac troponin is elevated in sepsis and correlates to morbidity and LV dysfunction[60,61,193]. Our results are in conformity with these reports. Neither in paper II nor IV were endotoxemia associated with decreased coronary blood flow. Still, troponin I levels were increased in both papers. This effect by endotoxin is not clear but possibilities include direct toxic effects by endotoxin, regional micro-hypoperfusion[194] not detected by our method or mitochondrial disturbances[195] (cytopathic dysoxia). In fact, ET has been shown to cause both mitochondrial dysfunction as well as microcirculatory disturbances[196,197]. Interestingly, tezosentan, lower dose in paper II, was associated with significant less troponin I release than controls.

# Vascular effects of ET receptor antagonism

The vasodilatory effects of ET receptor antagonism promoted the increase in CI seen in both paper II and IV, rather than inotropic effects (above). Vasodilation ensued as a result of smooth muscle ET receptor antagonism. This effect was most clearly seen in the pulmonary circulation (*figure 12*) thus facilitating LV filling as evidenced by increased LVEDAI or LVEDV. The first, shorter peak in mean pulmonary artery pressure (MPAP) is largely due to thromboxane $A_2$ release whereas the second, sustained peak is ET dependent[198]. The beneficial effects on PVRI in endotoxemia by tezosentan has recently been shown to be dependent on antagonizing pulmonary venous $ET_B$ receptors leading to significant decrease of pulmonary capillary pressure[199] and reduced extra-vascular lung water[126].

Oxygen delivery and consumption as well as $SvO_2$ were increased by tezosentan in paper II. There was a strong tendency to improvement in $SvO_2$ also in paper IV ($p=.051$). This effect is interpreted as generally improved perfusion by ET receptor antagonism, e.g. splanchnic blood flow as previously shown by Oldner *et al*[130].

# Functional assessment of cardiac performance in vivo

The word contractility has frequently been used to describe systolic performance. As mentioned in the introduction section, describing results which reflect contractility status should be reserved for studies where as many myocardial conditions as possible can be assessed and/or experimentally controlled. For *in vivo* studies of the ET system on myocardial effects in this thesis, heart performance has been measured, together with as many of the relevant factors that can influence myocardial performance as

possible. *In vivo* assessment of cardiac performance can be highly accurate, though this has meant a high degree of invasive measurement. While much work to produce *in vitro* findings are useful, *in vivo* testing is always necessary in order to assess clinical relevance. Concerning heart function, one always needs to assess ventricular performance findings within the context of the external influences to the heart.

In the experimental setting, some of the external influences can be easily controlled and measured. Resting state, temperature, electrolyte environment etc. can be controlled. As far as evaluating ventricular loading conditions, creating pressure-volume relations *in vivo* can be accomplished in many ways. Sonographic crystals can be inserted surgically and together with a micro-manometer used for describing LVPVR. This allows measurement of cardiac performance in awake animals. The method used in papers III and IV, conductance volumetry, is perhaps less invasive but, for acute experiments, requires anesthesia. This method has been shown accurate within physiological HR and blood pressure[200]. In order to assess cardiac performance, preload alterations are made, in our case by inflating a balloon in the inferior vena cava (*figure 5*). Thus preload independent parameters of performance can be determined. Sudden changes in afterload (MAP) can also influence the results, however this was not the case in papers III and IV. In the latter study, concentration of hemoglobin varied significantly which was controlled for by calibrating for blood conductivity before each measurement. This was also done for volume signal and surrounding tissue[201].

# Summary and future perspectives

The concept of myocardial depression in sepsis has now been established for more than 30 years. Knowledge is increasing but therapeutic strategies are wanting. In this thesis we have evaluated the hemodynamic response of levosimendan, a calcium sensitizer. The resulting beneficial cardiovascular and regional perfusion effects are promising and large clinical, randomized trials are warranted. Preceding these, levosimendan should be evaluated in clinically relevant models of sepsis with load independent assessment of cardiac performance.

We have also investigated effects of the ET system in anesthetized pigs. ET-1 seems to have a dual cardio-regulatory role: positive effects on myocardial contractile performance, probably mediated via $ET_A$ receptors, and negative lusitropic effects that are $ET_B$ receptor mediated. When antagonizing these receptors with tezosentan in endotoxemia, general hemodynamic parameters are much improved, both in hyper- and hypodynamic situations. Diastolic function parameters were also improved by tezosentan. However, by assessing cardiac performance by LVPVR *in vivo*, a negative inotropic effect of dual ET receptor antagonism was unraveled. Thus, the early activation of the ET system in endotoxemia (and possibly clinical sepsis) may serve to uphold the

contractile performance of the left ventricle. It is possible that this adaptation becomes maladaptive in later stages of sepsis, resembling the effect of ET in congestive heart failure. This of course needs to be evaluated thoroughly.

These results raise some major issues. If ET system activation is beneficial regarding cardiac systolic function, why would you antagonize such an effect? Also, as shown in paper II where higher dose of tezosentan was detrimental with increased mortality, is a drug with such narrow therapeutic interval appropriate for treating patients? The questions posed come down to timing and dosage. Also, and perhaps most pertinent, if ET receptor antagonists in sepsis are associated with decreased contractile performance, is that a reasonable price to pay for much improved general hemodynamics and perfusion?, Cardiac diastolic dysfunction, pulmonary hypertension as well as splanchnic hypoperfusion are targets suitable for ET receptor antagonism. Perhaps alternative routes of administration for ET receptor antagonists should be tried, minimizing systemic side-effects. Also, it may well be that other dual ET receptor antagonists could have a more beneficial receptor antagonizing profile, targeting the $ET_B$ receptor to a higher degree than tezosentan.

These issues and more remain to be addressed in the future. The ET system is a major pathophysiological mediator in sepsis and it will be worthwhile to investigate, with the goal of finding an effective treatment of septic patients through drugs acting on the ET receptors.

# Conclusions

- Levosimendan, a calcium sensitizing agent, improves cardiovascular and regional function in experimental sepsis.
- Levosimendan does not appear to mediate its positive effects in experimental sepsis via the endothelin system.
- Exogenous ET-1 causes a dose-related increase in myocardial contractile performance, probably mediated through $ET_A$ receptors.
- $ET_B$ receptor activation by exogenous ET-1 and S6c impairs diastolic function.
- Signs of depressed cardiac performance are seen in high-volume resuscitated endotoxemia.
- Acute endotoxemia evokes left ventricular diastolic but not systolic dysfunction, assessed by load-independent pressure-volume relations *in vivo*.
- Endothelin receptor activity supports myocardial contractile function in acute endotoxemia.
- Endothelin receptor activity impairs left ventricular relaxation and compliance as well as general hemodynamics in acute endotoxemia.
- Higher dose of tezosentan, a dual ET receptor antagonist, is detrimental in endotoxemia, possibly due to exaggerated vasodilation, leading to critically low coronary perfusion pressure.

# Acknowledgements

*"Medical scientists are nice people, but you should not let them treat you"*
*August Bier*

I would like to extend my profound gratefulness to the colleagues, past and present, of the Department of Anesthesiology and Intensive Care Medicine, Karolinska, Solna, for being part of an outstanding scientific environment and a continuous source of inspiration. Especially, I wish to thank:

My tutor and mentor in science as well as in the Intensive Care Unit, associate Professor **Anders Oldner**, for your everlasting support, faith, encouragement, dedication and the remarkable ability to see hope where none is to be found. Also, for truly making me hooked on critical care during the legendary glory days, the summer of -99.

My tutor, Professor **Eddie Weitzberg**, for accepting my as a doctoral student in this amazing group of scientists among which the air is filled with humanity. For your never-ending ideas and suggestions to keep the christmas tree well decorated and for calling to make sure "Is it alive?"

My tutor, associate Professor **Michael Haney,** for putting pressure and volume in close relation to my heart. Also, for welcoming me to your extraordinary lab in Umeå with incredible hospitality and warmth.

My tutor, **Michael Wanecek**, for generous support, ideas, criticism and positive attitude. Also for not making me alone kicking paper baskets.

My comrade in arms, in shining blunt scissors, **Patrik Rossi**, for sharing magnificent moments of success as well as dark ones of despair, in addition to your thoughts in fruitful discussions of life, work and science.

Associate Professor **Anders Rudehill**, scientist emeritus extraordinarius, for your constant willingness to help out, your impeccable ability for spotting weaknesses, both in texts and protocols and for all the "kanellängder" you got us over the years.

Head of research at the Karolinska University Hospital and former head of our department, Professor **Sten Lindahl** for continuously and successfully creating improvements for scientists and supporting academia at the department. Also, for hiring me and believing in me as a resident, encouraging my first steps in science.

Head of the department of Anestesiology and Intensive Care Medicine at Karolinska University Hospital, Solna, Professor **Claes Frostell** for your ongoing efforts to improve our department, both in research and clinically, and for your support of my scientific and clinical development.

Head of research at the department of Anesthesiology and Intensive Care Medicine at Karolinska University Hospital, Solna, Professor **Lars I Eriksson**, for your endless enthusiasm in creating an excellent atmosphere for doctoral students at the department. Also, for aiding in tactical navigation in the process of putting forward this thesis.

Head of the Intensive Care Unit at Karolinska University Hospital, Solna, associate Professor **Claes-Roland Martling**, for enormous support and faith in me, coaching both clinical and tactical aspects of critical care.

Former head of the department of Anesthesiology and Intensive Care Medicine at Karolinska University Hospital, Solna, associate Professor **Lars Irestedt** for contributing to and developing the magnificent, unique atmosphere at the department.

Professor **Björn Biber**, former head of the department of Perioperative and Surgical Sciences, Umeå University, and co-author. For creating a fantastic scientific environment at the lab and for welcoming me to conduct my experiments with you.

Co-author **Göran Johansson**, Perioperative and Surgical Sciences, Umeå University, for invaluable contribution to this thesis in many ways and for meticulous evaluation of results as well as for your contagious positive attitude.

Co-author **Sören Häggmark**, Perioperative and Surgical Sciences, Umeå University, for excellent technical assistance in catheterization and your cheerful spirit. Also for introducing me to "bäver-gel".

Biomedical scientists **Margareta Stensdotter**, for superb technical assistance in porcine surgical preparation and for analysing ET-1, and **Pernilla Abrahamsson** for valuable aid in the Umeå lab.

Head of the animal department at the Department of Physiology and Pharmacology **Lennart Henriksson** for invaluable assistance and care for our four-legged friends.

Professor **Kjell Alving**, Professor **Jon Lundberg** and biomedical scientist **Carina Nihlén** at the Department of Physiology and Pharmacology for help and support.

**Marcus&Marcus,** my oldest friends and companions. For always being by my side and in my mind since the times of tennis, chocolate cake and video-making. For your friendship that still flourish between us and our families.

**The "buds**", all of you, for sharing comradeship and friendship since my childhood in medicine and for your unmistakable appetite for life.

My parents, **Kerstin** and **Peter**, for all the love conveyed, care provided and support supplied as well as your generosity and never-ending belief in me.

My grandparents, **Marika** and **Ernö**, **Elsa** and **Torsten.** For all your love and support and, although not present in life, you are present in mind. **Kalle** too, and **Johan** is still as loving and generous as before.

My siblings **Gabriel**, for all the good times and fight times we had growing up, **Ebba,** for your tremendous support of our family and **Erland,** for your love and affection. **Kati**, my aunt, custodian of family history, for your profound love and care.

My parents-in-law, **Britt-Marie** and **Leif**, for unconditional, invaluable support and help with everything, more than I can account for.

My pride and my joy are my beloved children **Isak**, **Hannah** and **Max**. Your mere presence makes my world meaningful, your laughter my heart light. Your ability to unveil my shortcomings, your clever insightful remarks still astonishes me and encourages me to improve. Since the minute you were born, I love you, always.

And, to conclude, the person most important to me, my beloved wife **Jeanette**. For your great patience with me, regarding the process of this thesis but mainly regarding everything else, I thank you. I cannot say how happy I am and how lucky I consider myself to be to have met you and even more, for your love. Of whole my heart, I love you, I admire you and I hope you will always join me on the subway. To my place, it runs all life.

# References

*"The wisdom of the wise, and the experiences of ages, may be preserved by quotations"*

*Isaac D'Israeli*


1. Pfeiffer: Untersuchungen über das Choleragift. *Ztschr. f. Hyg.* 1891-92, 11:393-412.
2. Centanni: Untersuchungen uber das Infektionsfieber - das Fiebergift der Bakterien. *Dtsch Med Wochenschr* 1894, 20:148.
3. Boivin, A. and Messrobeanu, L.: Recherches sur les antigenes somatiques et sur les endotoxines des bacteries. Considerations generales et expose des techniques utilisee. *Rev. Immunol. (Paris)* 1935(1):553-569.
4. Martin, G. S., Mannino, D. M., Eaton, S.*, et al.*: The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003, 348(16):1546-54.
5. Annane, D., Aegerter, P., Jars-Guincestre, M. C.*, et al.*: Current epidemiology of septic shock: the CUB-Rea Network. *Am J Respir Crit Care Med* 2003, 168(2):165-72.
6. Finfer, S., Bellomo, R., Lipman, J.*, et al.*: Adult-population incidence of severe sepsis in Australian and New Zealand intensive care units. *Intensive Care Med* 2004, 30(4):589-96.
7. Guidet, B., Aegerter, P., Gauzit, R.*, et al.*: Incidence and impact of organ dysfunctions associated with sepsis. *Chest* 2005, 127(3):942-51.
8. Angus, D. C., Linde-Zwirble, W. T., Lidicker, J.*, et al.*: Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med* 2001, 29(7):1303-10.
9. Alberti, C., Brun-Buisson, C., Burchardi, H.*, et al.*: Epidemiology of sepsis and infection in ICU patients from an international multicentre cohort study. *Intensive Care Med* 2002, 28(2):108-21.
10. Hubacek, J. A., Stuber, F., Frohlich, D.*, et al.*: Gene variants of the bactericidal/ permeability increasing protein and lipopolysaccharide binding protein in sepsis patients: gender-specific genetic predisposition to sepsis. *Crit Care Med* 2001, 29(3):557-61.
11. Lin, M. T. and Albertson, T. E.: Genomic polymorphisms in sepsis. *Crit Care Med* 2004, 32(2):569-79.
12. Bone, R. C., Balk, R. A., Cerra, F. B.*, et al.*: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992, 101(6):1644-55.
13. Levy, M. M., Fink, M. P., Marshall, J. C.*, et al.*: 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med* 2003, 31(4):1250-6.
14. Labeit, S., Kolmerer, B. and Linke, W. A.: The giant protein titin. Emerging roles in physiology and pathophysiology. *Circ Res* 1997, 80(2):290-4.

15. Langer, G.: The Myocardium, 2nd edn. San Diego, CA: Academic Press; 1997.
16. Solaro, R. J. and Rarick, H. M.: Troponin and tropomyosin: proteins that switch on and tune in the activity of cardiac myofilaments. *Circ Res* 1998, 83(5):471-80.
17. Rayment, I., Holden, H. M., Whittaker, M.*, et al.*: Structure of the actin-myosin complex and its implications for muscle contraction. *Science* 1993, 261(5117):58-65.
18. Helmes, M., Trombitas, K. and Granzier, H.: Titin develops restoring force in rat cardiac myocytes. *Circ Res* 1996, 79(3):619-26.
19. Wrzosek, A.: Regulation of Ca2+ release from internal stores in cardiac and skeletal muscles. *Acta Biochim Pol* 2000, 47(3):705-23.
20. Winegrad, S.: Endothelial cell regulation of contractility of the heart. *Annu Rev Physiol* 1997, 59:505-25.
21. Lehtonen, L.: Levosimendan: a calcium-sensitizing agent for the treatment of patients with decompensated heart failure. *Curr Heart Fail Rep* 2004, 1(3):136-44.
22. Davis, B. A., Edes, I., Gupta, R. C.*, et al.*: The role of phospholamban in the regulation of calcium transport by cardiac sarcoplasmic reticulum. *Mol Cell Biochem* 1990, 99(2):83-8.
23. Ross, J., Jr., Miura, T., Kambayashi, M.*, et al.*: Adrenergic control of the force-frequency relation. *Circulation* 1995, 92(8):2327-32.
24. Koch-Weser, J. and Blinks, J. R.: The Influence of the Interval between Beats on Myocardial Contractility. *Pharmacol Rev* 1963, 15:601-52.
25. Anderson, P. A., Manring, A., Serwer, G. A.*, et al.*: The force-interval relationship of the left ventricle. *Circulation* 1979, 60(2):334-48.
26. Schouten, V. J., Allaart, C. P. and Westerhof, N.: Effect of perfusion pressure on force of contraction in thin papillary muscles and trabeculae from rat heart. *J Physiol* 1992, 451:585-604.
27. Brutsaert, D. L. and Sys, S. U.: Relaxation and diastole of the heart. *Physiol Rev* 1989, 69(4):1228-315.
28. Gaasch, W. H., Blaustein, A. S., Andrias, C. W.*, et al.*: Myocardial relaxation. II. Hemodynamic determinants of rate of left ventricular isovolumic pressure decline. *Am J Physiol* 1980, 239(1):H1-6.
29. Cheng, C. P., Freeman, G. L., Santamore, W. P.*, et al.*: Effect of loading conditions, contractile state, and heart rate on early diastolic left ventricular filling in conscious dogs. *Circ Res* 1990, 66(3):814-23.
30. Furchgott, R. F. and Zawadzki, J. V.: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980, 288(5789):373-6.
31. Brutsaert, D. L.: Cardiac endothelial-myocardial signaling: its role in cardiac growth, contractile performance, and rhythmicity. *Physiol Rev* 2003, 83(1):59-115.
32. Kojda, G., Kottenberg, K., Nix, P.*, et al.*: Low increase in cGMP induced by organic nitrates and nitrovasodilators improves contractile response of rat ventricular myocytes. *Circ Res* 1996, 78(1):91-101.
33. Mohan, P., Brutsaert, D. L., Paulus, W. J.*, et al.*: Myocardial contractile response to nitric oxide and cGMP. *Circulation* 1996, 93(6):1223-9.
34. Wiggers, C. J.: Myocardial Depression in Shock - a Survey of Cardiodynamic Studies. *American Heart Journal* 1947, 33(5):633-650.
35. MacLean, L. D., Mulligan, W. G., McLean, A. P.*, et al.*: Patterns of septic shock in man--a detailed study of 56 patients. *Ann Surg* 1967, 166(4):543-62.

36. Clowes, G. H., Jr., Vucinic, M. and Weidner, M. G.: Circulatory and metabolic alterations associated with survival or death in peritonitis: clinical analysis of 25 cases. *Ann Surg* 1966, 163(6):866-85.
37. Wilson, R. F., Thal, A. P., Kindling, P. H.*, et al.*: Hemodynamic Measurements in Septic Shock. *Archives of Surgery* 1965, 91(1):121-&.
38. Winslow, E. J., Loeb, H. S., Rahimtoola, S. H.*, et al.*: Hemodynamic studies and results of therapy in 50 patients with bacteremic shock. *Am J Med* 1973, 54(4):421-32.
39. Wilson, R. F., Sibbald, W. J. and Jaanimagi, J. L.: Hemodynamic effects of dopamine in critically ill septic patients. *J Surg Res* 1976, 20(3):163-72.
40. Weisel, R. D., Vito, L., Dennis, R. C.*, et al.*: Myocardial depression during sepsis. *Am J Surg* 1977, 133(4):512-21.
41. Calvin, J. E., Driedger, A. A. and Sibbald, W. J.: An assessment of myocardial function in human sepsis utilizing ECG gated cardiac scintigraphy. *Chest* 1981, 80(5):579-86.
42. Parker, M. M., Shelhamer, J. H., Bacharach, S. L.*, et al.*: Profound but reversible myocardial depression in patients with septic shock. *Ann Intern Med* 1984, 100(4):483-90.
43. Munt, B., Jue, J., Gin, K.*, et al.*: Diastolic filling in human severe sepsis: an echocardiographic study. *Crit Care Med* 1998, 26(11):1829-33.
44. Jafri, S. M., Lavine, S., Field, B. E.*, et al.*: Left ventricular diastolic function in sepsis. *Crit Care Med* 1990, 18(7):709-14.
45. Poelaert, J., Declerck, C., Vogelaers, D.*, et al.*: Left ventricular systolic and diastolic function in septic shock. *Intensive Care Med* 1997, 23(5):553-60.
46. Monsalve, F., Rucabado, L., Salvador, A.*, et al.*: Myocardial depression in septic shock caused by meningococcal infection. *Crit Care Med* 1984, 12(12):1021-3.
47. Ognibene, F. P., Parker, M. M., Natanson, C.*, et al.*: Depressed left ventricular performance. Response to volume infusion in patients with sepsis and septic shock. *Chest* 1988, 93(5):903-10.
48. Elkins, R. C., McCurdy, J. R., Brown, P. P.*, et al.*: Effects of coronary perfusion pressure on myocardial performance during endotoxin shock. *Surg Gynecol Obstet* 1973, 137(6):991-6.
49. Hinshaw, L. B., Archer, L. T., Spitzer, J. J.*, et al.*: Effects of coronary hypotension and endotoxin on myocardial performance. *Am J Physiol* 1974, 227(5):1051-7.
50. Schneider, A. J., Teule, G. J., Groeneveld, A. B.*, et al.*: Biventricular performance during volume loading in patients with early septic shock, with emphasis on the right ventricle: a combined hemodynamic and radionuclide study. *Am Heart J* 1988, 116 (1 Pt 1):103-12.
51. Cunnion, R. E., Schaer, G. L., Parker, M. M.*, et al.*: The coronary circulation in human septic shock. *Circulation* 1986, 73(4):637-44.
52. Dhainaut, J. F., Huyghebaert, M. F., Monsallier, J. F.*, et al.*: Coronary hemodynamics and myocardial metabolism of lactate, free fatty acids, glucose, and ketones in patients with septic shock. *Circulation* 1987, 75(3):533-41.
53. Lee, K. J., Dziuban, S. W., Jr., van der Zee, H.*, et al.*: Cardiac function and coronary flow in chronic endotoxemic pigs. *Proc Soc Exp Biol Med* 1988, 189(2):245-52.
54. Lee, K., van der Zee, H., Dziuban, S. W., Jr.*, et al.*: Left ventricular function during chronic endotoxemia in swine. *Am J Physiol* 1988, 254(2 Pt 2):H324-30.
55. Goldfarb, R. D., Glock, D., Kumar, A.*, et al.*: A porcine model of peritonitis and bacteremia simulates human septic shock. *Shock* 1996, 6(6):442-51.

56. Konrad, D., Oldner, A., Rossi, P.*, et al.*: Differentiated and dose-related cardiovascular effects of a dual endothelin receptor antagonist in endotoxin shock. *Crit Care Med* 2004, 32(5):1192-9.
57. Solomon, M. A., Correa, R., Alexander, H. R.*, et al.*: Myocardial energy metabolism and morphology in a canine model of sepsis. *Am J Physiol* 1994, 266(2 Pt 2):H757-68.
58. Hotchkiss, R. S., Rust, R. S., Dence, C. S.*, et al.*: Evaluation of the role of cellular hypoxia in sepsis by the hypoxic marker [18F]fluoromisonidazole. *Am J Physiol* 1991, 261(4 Pt 2):R965-72.
59. Hotchkiss, R. S. and Karl, I. E.: Reevaluation of the role of cellular hypoxia and bioenergetic failure in sepsis. *Jama* 1992, 267(11):1503-10.
60. Ammann, P., Fehr, T., Minder, E. I.*, et al.*: Elevation of troponin I in sepsis and septic shock. *Intensive Care Med* 2001, 27(6):965-9.
61. ver Elst, K. M., Spapen, H. D., Nguyen, D. N.*, et al.*: Cardiac troponins I and T are biological markers of left ventricular dysfunction in septic shock. *Clin Chem* 2000, 46(5):650-7.
62. Parker, J. L. and Adams, H. R.: Development of myocardial dysfunction in endotoxin shock. *Am J Physiol* 1985, 248(6 Pt 2):H818-26.
63. Ming, M. J., Hu, D., Chen, H. S.*, et al.*: Effect of MCI-154, a calcium sensitizer, on calcium sensitivity of myocardial fibers in endotoxic shock rats. *Shock* 2000, 14(6):652-6.
64. Yasuda, S. and Lew, W. Y.: Angiotensin II exacerbates lipopolysaccharide-induced contractile depression in rabbit cardiac myocytes. *Am J Physiol* 1999, 276(5 Pt 2): H1442-9.
65. Mei, J. M., Hu, D. Y., Chen, H. S.*, et al.*: Effect of MCI-154, a calcium sensitizer, on calcium sensitivity of myocardial contractile system in endotoxemic rats. *Acta Pharmacol Sin* 2000, 21(9):830-4.
66. Nemoto, S., Vallejo, J. G., Knuefermann, P.*, et al.*: Escherichia coli LPS-induced LV dysfunction: role of toll-like receptor-4 in the adult heart. *Am J Physiol Heart Circ Physiol* 2002, 282(6):H2316-23.
67. Raeburn, C. D., Calkins, C. M., Zimmerman, M. A.*, et al.*: ICAM-1 and VCAM-1 mediate endotoxemic myocardial dysfunction independent of neutrophil accumulation. *Am J Physiol Regul Integr Comp Physiol* 2002, 283(2):R477-86.
68. Braun-Dullaeus, R. C., Dietrich, S., Schoaff, M. J.*, et al.*: Protective effect of 3-deazaadenosine in a rat model of lipopolysaccharide-induced myocardial dysfunction. *Shock* 2003, 19(3):245-51.
69. Garner, L. B., Willis, M. S., Carlson, D. L.*, et al.*: Macrophage migration inhibitory factor is a cardiac-derived myocardial depressant factor. *Am J Physiol Heart Circ Physiol* 2003, 285(6):H2500-9.
70. Aghajani, E., Korvald, C., Nordhaug, D.*, et al.*: E. Coli sepsis induces profound mechanoenergetic inefficiency in the porcine left ventricle. *Shock* 2004, 21(2):103-9.
71. Pinsky, M. R. and Rico, P.: Cardiac contractility is not depressed in early canine endotoxic shock. *Am J Respir Crit Care Med* 2000, 161(4 Pt 1):1087-93.
72. Constable, P. D.: Acute endotoxemia increases left ventricular contractility and diastolic stiffness in calves. *Shock* 1999, 12(5):391-401.
73. Ishihara, S., Ward, J. A., Tasaki, O.*, et al.*: Biphasic changes in left ventricular function during hyperdynamic endotoxemia. *Am J Physiol* 1999, 276(5 Pt 2):R1516-24.

74. Abel, F. L., Krosl, P. and Redl, H.: Beat-to-beat evaluation of cardiac function in low-dose endotoxemia using a conscious sheep model. *Shock* 2001, 16(5):368-72.
75. Werner, H. A., Herbertson, M. J. and Walley, K. R.: Amrinone increases ventricular contractility and diastolic compliance in endotoxemia. *Am J Respir Crit Care Med* 1995, 152(2):496-503.
76. Hoffmann, J. N., Werdan, K., Hartl, W. H.*, et al.*: Hemofiltrate from patients with severe sepsis and depressed left ventricular contractility contains cardiotoxic compounds. *Shock* 1999, 12(3):174-80.
77. Kumar, A., Haery, C. and Parrillo, J. E.: Myocardial dysfunction in septic shock. *Crit Care Clin* 2000, 16(2):251-87.
78. Knuefermann, P., Nemoto, S., Misra, A.*, et al.*: CD14-deficient mice are protected against lipopolysaccharide-induced cardiac inflammation and left ventricular dysfunction. *Circulation* 2002, 106(20):2608-15.
79. Levy, R. J. and Deutschman, C. S.: Evaluating myocardial depression in sepsis. *Shock* 2004, 22(1):1-10.
80. Khadour, F. H., Panas, D., Ferdinandy, P.*, et al.*: Enhanced NO and superoxide generation in dysfunctional hearts from endotoxemic rats. *Am J Physiol Heart Circ Physiol* 2002, 283(3):H1108-15.
81. Elfering, S. L., Sarkela, T. M. and Giulivi, C.: Biochemistry of mitochondrial nitric-oxide synthase. *J Biol Chem* 2002, 277(41):38079-86.
82. Levy, R. J., Vijayasarathy, C., Raj, N. R.*, et al.*: Competitive and noncompetitive inhibition of myocardial cytochrome C oxidase in sepsis. *Shock* 2004, 21(2):110-4.
83. Tavernier, B., Mebazaa, A., Mateo, P.*, et al.*: Phosphorylation-dependent alteration in myofilament ca2+ sensitivity but normal mitochondrial function in septic heart. *Am J Respir Crit Care Med* 2001, 163(2):362-7.
84. Rabuel, C. and Mebazaa, A.: Septic shock: a heart story since the 1960s. *Intensive Care Med* 2006, 32(6):799-807.
85. Patel, D., Duke, K., Light, R. B.*, et al.*: Impaired sarcoplasmic calcium release inhibits myocardial contraction in experimental sepsis. *J Crit Care* 2000, 15(2):64-72.
86. Wu, L. L., Ji, Y., Dong, L. W.*, et al.*: Calcium uptake by sarcoplasmic reticulum is impaired during the hypodynamic phase of sepsis in the rat heart. *Shock* 2001, 15(1):49-55.
87. Yanagisawa, M., Inoue, A., Ishikawa, T.*, et al.*: Primary structure, synthesis, and biological activity of rat endothelin, an endothelium-derived vasoconstrictor peptide. *Proc Natl Acad Sci U S A* 1988, 85(18):6964-7.
88. Motte, S., McEntee, K. and Naeije, R.: Endothelin receptor antagonists. *Pharmacol Ther* 2006, 110(3):386-414.
89. Inoue, A., Yanagisawa, M., Takuwa, Y.*, et al.*: The human preproendothelin-1 gene. Complete nucleotide sequence and regulation of expression. *J Biol Chem* 1989, 264(25):14954-9.
90. Laporte, S., Denault, J. B., D'Orleans-Juste, P.*, et al.*: Presence of furin mRNA in cultured bovine endothelial cells and possible involvement of furin in the processing of the endothelin precursor. *J Cardiovasc Pharmacol* 1993, 22 Suppl 8:S7-10.
91. Rubanyi, G. M. and Polokoff, M. A.: Endothelins: molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. *Pharmacol Rev* 1994, 46(3):325-415.

92. Takahashi, M., Matsushita, Y., Iijima, Y., *et al.*: Purification and characterization of endothelin-converting enzyme from rat lung. *J Biol Chem* 1993, 268(28):21394-8.
93. Russell, F. D., Skepper, J. N. and Davenport, A. P.: Human endothelial cell storage granules: a novel intracellular site for isoforms of the endothelin-converting enzyme. *Circ Res* 1998, 83(3):314-21.
94. Emori, T., Hirata, Y., Ohta, K., *et al.*: Cellular mechanism of endothelin-1 release by angiotensin and vasopressin. *Hypertension* 1991, 18(2):165-70.
95. Levin, E. R.: Endothelins. *N Engl J Med* 1995, 333(6):356-63.
96. Wagner, O. F., Christ, G., Wojta, J., *et al.*: Polar secretion of endothelin-1 by cultured endothelial cells. *J Biol Chem* 1992, 267(23):16066-8.
97. Yoshimoto, S., Ishizaki, Y., Sasaki, T., *et al.*: Effect of carbon dioxide and oxygen on endothelin production by cultured porcine cerebral endothelial cells. *Stroke* 1991, 22(3):378-83.
98. Ahlborg, G., Weitzberg, E. and Lundberg, J. M.: Circulating endothelin-1 reduces splanchnic and renal blood flow and splanchnic glucose production in humans. *J Appl Physiol* 1995, 79(1):141-5.
99. Wei, C. M., Lerman, A., Rodeheffer, R. J., *et al.*: Endothelin in human congestive heart failure. *Circulation* 1994, 89(4):1580-6.
100. Pittet, J. F., Morel, D. R., Hemsen, A., *et al.*: Elevated plasma endothelin-1 concentrations are associated with the severity of illness in patients with sepsis. *Ann Surg* 1991, 213(3):261-4.
101. Pernow, J., Hemsen, A. and Lundberg, J. M.: Increased plasma levels of endothelin-like immunoreactivity during endotoxin administration in the pig. *Acta Physiol Scand* 1989, 137(2):317-8.
102. Sirvio, M. L., Metsarinne, K., Saijonmaa, O., *et al.*: Tissue distribution and half-life of 125I-endothelin in the rat: importance of pulmonary clearance. *Biochem Biophys Res Commun* 1990, 167(3):1191-5.
103. Vierhapper, H., Wagner, O., Nowotny, P., *et al.*: Effect of endothelin-1 in man. *Circulation* 1990, 81(4):1415-8.
104. Dupuis, J., Stewart, D. J., Cernacek, P., *et al.*: Human pulmonary circulation is an important site for both clearance and production of endothelin-1. *Circulation* 1996, 94(7):1578-84.
105. Karne, S., Jayawickreme, C. K. and Lerner, M. R.: Cloning and characterization of an endothelin-3 specific receptor (ETC receptor) from Xenopus laevis dermal melanophores. *J Biol Chem* 1993, 268(25):19126-33.
106. Kumar, C., Mwangi, V., Nuthulaganti, P., *et al.*: Cloning and characterization of a novel endothelin receptor from Xenopus heart. *J Biol Chem* 1994, 269(18):13414-20.
107. Sakurai, T., Yanagisawa, M., Takuwa, Y., *et al.*: Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. *Nature* 1990, 348(6303):732-5.
108. de Nucci, G., Thomas, R., D'Orleans-Juste, P., *et al.*: Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc Natl Acad Sci U S A* 1988, 85(24):9797-800.
109. Lodge, N. J., Zhang, R., Halaka, N. N., *et al.*: Functional role of endothelin ETA and ETB receptors in venous and arterial smooth muscle. *Eur J Pharmacol* 1995, 287(3):279-85.

110. Cocks, T. M., Faulkner, N. L., Sudhir, K.*, et al.*: Reactivity of endothelin-1 on human and canine large veins compared with large arteries in vitro. *Eur J Pharmacol* 1989, 171(1):17-24.
111. Battistini, B., Berthiaume, N., Kelland, N. F.*, et al.*: Profile of past and current clinical trials involving endothelin receptor antagonists: the novel "-sentan" class of drug. *Exp Biol Med (Maywood)* 2006, 231(6):653-95.
112. Neylon, C. B.: Vascular biology of endothelin signal transduction. *Clin Exp Pharmacol Physiol* 1999, 26(2):149-53.
113. Koh, E., Morimoto, S., Kim, S.*, et al.*: Endothelin stimulates Na+/H+ exchange in vascular smooth muscle cells. *Biochem Int* 1990, 20(2):375-80.
114. Lonchampt, M. O., Pinelis, S., Goulin, J.*, et al.*: Proliferation and Na+/H+ exchange activation by endothelin in vascular smooth muscle cells. *Am J Hypertens* 1991, 4(9):776-9.
115. Clerk, A., Bogoyevitch, M. A., Anderson, M. B.*, et al.*: Differential activation of protein kinase C isoforms by endothelin-1 and phenylephrine and subsequent stimulation of p42 and p44 mitogen-activated protein kinases in ventricular myocytes cultured from neonatal rat hearts. *J Biol Chem* 1994, 269(52):32848-57.
116. Simonson, M. S., Wang, Y. and Herman, W. H.: Ca2+ channels mediate protein tyrosine kinase activation by endothelin-1. *Am J Physiol* 1996, 270(5 Pt 2):F790-7.
117. Russell, F. D. and Molenaar, P.: The human heart endothelin system: ET-1 synthesis, storage, release and effect. *Trends Pharmacol Sci* 2000, 21(9):353-9.
118. Endoh, M., Fujita, S., Yang, H. T.*, et al.*: Endothelin: receptor subtypes, signal transduction, regulation of Ca2+ transients and contractility in rabbit ventricular myocardium. *Life Sci* 1998, 62(17-18):1485-9.
119. Bremnes, T., Paasche, J. D., Mehlum, A.*, et al.*: Regulation and intracellular trafficking pathways of the endothelin receptors. *J Biol Chem* 2000, 275(23):17596-604.
120. Weitzberg, E., Lundberg, J. M. and Rudehill, A.: Elevated plasma levels of endothelin in patients with sepsis syndrome. *Circ Shock* 1991, 33(4):222-7.
121. Battistini, B., Forget, M. A. and Laight, D.: Potential roles for endothelins in systemic inflammatory response syndrome with a particular relationship to cytokines. *Shock* 1996, 5(3):167-83.
122. Brauner, J. S., Rohde, L. E. and Clausell, N.: Circulating endothelin-1 and tumor necrosis factor-alpha: early predictors of mortality in patients with septic shock. *Intensive Care Med* 2000, 26(3):305-13.
123. Kaddoura, S., Curzen, N. P., Evans, T. W.*, et al.*: Tissue expression of endothelin-1 mRNA in endotoxaemia. *Biochem Biophys Res Commun* 1996, 218(3):641-7.
124. Kumar, A., Krieger, A., Symeoneides, S.*, et al.*: Myocardial dysfunction in septic shock: Part II. Role of cytokines and nitric oxide. *J Cardiothorac Vasc Anesth* 2001, 15(4):485-511.
125. Tschaikowsky, K., Sagner, S., Lehnert, N.*, et al.*: Endothelin in septic patients: effects on cardiovascular and renal function and its relationship to proinflammatory cytokines. *Crit Care Med* 2000, 28(6):1854-60.
126. Rossi, P., Wanecek, M., Konrad, D.*, et al.*: Tezosentan counteracts endotoxin-induced pulmonary edema and improves gas exchange. *Shock* 2004, 21(6):543-8.
127. Humer, M. F., Phang, P. T., Friesen, B. P.*, et al.*: Heterogeneity of gut capillary transit times and impaired gut oxygen extraction in endotoxemic pigs. *J Appl Physiol* 1996, 81(2):895-904.

128. Weitzberg, E., Ahlborg, G. and Lundberg, J. M.: Long-lasting vasoconstriction and efficient regional extraction of endothelin-1 in human splanchnic and renal tissues. *Biochem Biophys Res Commun* 1991, 180(3):1298-303.
129. Oldner, A.: Splancnhic organ homeostasis in experimental endotoxin shock, with special reference to the endothelin and renin-angiotensin systems. *Thesis*. Stockholm: Karolinska Institutet; 1999.
130. Oldner, A., Wanecek, M., Weitzberg, E.*, et al.*: Differentiated effects on splanchnic homeostasis by selective and non- selective endothelin receptor antagonism in porcine endotoxaemia. *Br J Pharmacol* 1999, 127(8):1793-804.
131. Cirino, M., Martin, E., Yano, M.*, et al.*: Cardiovascular and renal actions of the endothelin(B) receptor in pigs. *J Cardiovasc Pharmacol* 1997, 29(6):704-12.
132. Mitaka, C., Hirata, Y., Yokoyama, K.*, et al.*: Improvement of renal dysfunction in dogs with endotoxemia by a nonselective endothelin receptor antagonist. *Crit Care Med* 1999, 27(1):146-53.
133. Oldner, A., Wanecek, M., Goiny, M.*, et al.*: The endothelin receptor antagonist bosentan restores gut oxygen delivery and reverses intestinal mucosal acidosis in porcine endotoxin shock. *Gut* 1998, 42(5):696-702.
134. Molenaar, P., O'Reilly, G., Sharkey, A.*, et al.*: Characterization and localization of endothelin receptor subtypes in the human atrioventricular conducting system and myocardium. *Circ Res* 1993, 72(3):526-38.
135. Modesti, P. A., Vanni, S., Paniccia, R.*, et al.*: Characterization of endothelin-1 receptor subtypes in isolated human cardiomyocytes. *J Cardiovasc Pharmacol* 1999, 34(3):333-9.
136. Modesti, P. A., Vanni, S., Paniccia, R.*, et al.*: Endothelin receptors in adult human and swine isolated ventricular cardiomyocytes. *Biochem Pharmacol* 1999, 58(2):369-74.
137. Li, K., Stewart, D. J. and Rouleau, J. L.: Myocardial contractile actions of endothelin-1 in rat and rabbit papillary muscles. Role of endocardial endothelium. *Circ Res* 1991, 69(2):301-12.
138. McClellan, G., Weisberg, A., Rose, D.*, et al.*: Endothelial cell storage and release of endothelin as a cardioregulatory mechanism. *Circ Res* 1994, 75(1):85-96.
139. Mebazaa, A., Mayoux, E., Maeda, K.*, et al.*: Paracrine effects of endocardial endothelial cells on myocyte contraction mediated via endothelin. *Am J Physiol* 1993, 265(5 Pt 2): H1841-6.
140. Izumi, M., Miyamoto, S., Hori, M.*, et al.*: Negative inotropic effect of endothelin-1 in the mouse right ventricle. *Eur J Pharmacol* 2000, 396(2-3):109-17.
141. Pieske, B., Beyermann, B., Breu, V.*, et al.*: Functional effects of endothelin and regulation of endothelin receptors in isolated human nonfailing and failing myocardium. *Circulation* 1999, 99(14):1802-9.
142. MacCarthy, P. A., Grocott-Mason, R., Prendergast, B. D.*, et al.*: Contrasting inotropic effects of endogenous endothelin in the normal and failing human heart: studies with an intracoronary ET(A) receptor antagonist. *Circulation* 2000, 101(2):142-7.
143. Kiely, D. G., Cargill, R. I., Struthers, A. D.*, et al.*: Cardiopulmonary effects of endothelin-1 in man. *Cardiovasc Res* 1997, 33(2):378-86.
144. Ezra, D., Goldstein, R. E., Czaja, J. F.*, et al.*: Lethal ischemia due to intracoronary endothelin in pigs. *Am J Physiol* 1989, 257(1 Pt 2):H339-43.

145. Chu, L., Takahashi, R., Norota, I.*, et al.*: Signal transduction and Ca2+ signaling in contractile regulation induced by crosstalk between endothelin-1 and norepinephrine in dog ventricular myocardium. *Circ Res* 2003, 92(9):1024-32.
146. Thomas, P. B., Liu, E. C., Webb, M. L.*, et al.*: Exogenous effects and endogenous production of endothelin in cardiac myocytes: potential significance in heart failure. *Am J Physiol* 1996, 271(6 Pt 2):H2629-37.
147. Leite-Moreira, A. F., Rocha-Sousa, A., Henriques-Coelho, T.*, et al.*: [The effects of endothelin-1 on myocardial function]. *Rev Port Cardiol* 2000, 19(1):79-83.
148. Goldberg, A. T., Bond, B. R., Mukherjee, R.*, et al.*: Endothelin receptor pathway in human left ventricular myocytes: relation to contractility. *Ann Thorac Surg* 2000, 69(3):711-5; discussion 716.
149. Leite-Moreira, A. F., Bras-Silva, C., Pedrosa, C. A.*, et al.*: ET-1 increases distensibility of acutely loaded myocardium: a novel ETA and Na+/H+ exchanger-mediated effect. *Am J Physiol Heart Circ Physiol* 2003, 284(4):H1332-9.
150. Brunner, F., Bras-Silva, C., Cerdeira, A. S.*, et al.*: Cardiovascular endothelins: essential regulators of cardiovascular homeostasis. *Pharmacol Ther* 2006, 111(2):508-31.
151. Goldfarb, R. D., Dellinger, R. P. and Parrillo, J. E.: Porcine Models of Severe Sepsis: Emphasis on Porcine Peritonitis. *Shock* 2005, 24 Suppl 1:75-81.
152. Swindle, M. M., Smith, A. C. and Hepburn, B. J.: Swine as models in experimental surgery. *J Invest Surg* 1988, 1(1):65-79.
153. Cozzi, E. and White, D. J.: The generation of transgenic pigs as potential organ donors for humans. *Nat Med* 1995, 1(9):964-6.
154. Bengsch, S., Boos, K. S., Nagel, D.*, et al.*: Extracorporeal plasma treatment for the removal of endotoxin in patients with sepsis: clinical results of a pilot study. *Shock* 2005, 23(6):494-500.
155. Suffredini, A. F., Fromm, R. E., Parker, M. M.*, et al.*: The cardiovascular response of normal humans to the administration of endotoxin. *N Engl J Med* 1989, 321(5):280-7.
156. Hemsen, A.: Biochemical and functional characterization of endothelin peptides with special reference to vascular effects. *Acta Physiol Scand Suppl* 1991, 602:1-61.
157. Thys, D. M., Hillel, Z., Goldman, M. E.*, et al.*: A comparison of hemodynamic indices derived by invasive monitoring and two-dimensional echocardiography. *Anesthesiology* 1987, 67(5):630-4.
158. Baan, J., Jong, T. T., Kerkhof, P. L.*, et al.*: Continuous stroke volume and cardiac output from intra-ventricular dimensions obtained with impedance catheter. *Cardiovasc Res* 1981, 15(6):328-34.
159. Baan, J., van der Velde, E. T., de Bruin, H. G.*, et al.*: Continuous measurement of left ventricular volume in animals and humans by conductance catheter. *Circulation* 1984, 70(5):812-23.
160. Little, W. C.: The left ventricular dP/dtmax-end-diastolic volume relation in closed-chest dogs. *Circ Res* 1985, 56(6):808-15.
161. Kass, D. A. and Beyar, R.: Evaluation of contractile state by maximal ventricular power divided by the square of end-diastolic volume. *Circulation* 1991, 84(4):1698-708.
162. Mirsky, I.: Assessment of diastolic function: suggested methods and future considerations. *Circulation* 1984, 69(4):836-41.
163. Suga, H.: Ventricular energetics. *Physiol Rev* 1990, 70(2):247-77.

164. Levijoki, J., Pollesello, P., Kaivola, J., *et al.*: Further evidence for the cardiac troponin C mediated calcium sensitization by levosimendan: structure-response and binding analysis with analogs of levosimendan. *J Mol Cell Cardiol* 2000, 32(3):479-91.
165. Haikala, H., Nissinen, E., Etemadzadeh, E., *et al.*: Troponin C-mediated calcium sensitization induced by levosimendan does not impair relaxation. *J Cardiovasc Pharmacol* 1995, 25(5):794-801.
166. Yokoshiki, H., Katsube, Y., Sunagawa, M., *et al.*: The novel calcium sensitizer levosimendan activates the ATP-sensitive K+ channel in rat ventricular cells. *J Pharmacol Exp Ther* 1997, 283(1):375-83.
167. Gruhn, N., Nielsen-Kudsk, J. E., Theilgaard, S., *et al.*: Coronary vasorelaxant effect of levosimendan, a new inodilator with calcium-sensitizing properties. *J Cardiovasc Pharmacol* 1998, 31(5):741-9.
168. Sandell, E. P., Hayha, M., Antila, S., *et al.*: Pharmacokinetics of levosimendan in healthy volunteers and patients with congestive heart failure. *J Cardiovasc Pharmacol* 1995, 26(Suppl 1):S57-62.
169. Antila, S., Huuskonen, H., Nevalainen, T., *et al.*: Site dependent bioavailability and metabolism of levosimendan in dogs. *Eur J Pharm Sci* 1999, 9(1):85-91.
170. Clozel, M., Ramuz, H., Clozel, J. P., *et al.*: Pharmacology of tezosentan, new endothelin receptor antagonist designed for parenteral use. *J Pharmacol Exp Ther* 1999, 290(2):840-6.
171. Dingemanse, J., Clozel, M. and van Giersbergen, P. L.: Entry-into-humans study with tezosentan, an intravenous dual endothelin receptor antagonist. *J Cardiovasc Pharmacol* 2002, 39(6):795-802.
172. Sokolovsky, M.: Endothelins and sarafotoxins: receptor heterogeneity. *Int J Biochem* 1994, 26(3):335-40.
173. Nicklas, J., Monsur, J. and Bleske, B.: Effects of intravenous levosimendan on plasma neurohormone levels in patients with heart failure: relation to hemodynamic response. *Am J Cardiol* 1999, 83(12, Supplement 2):12-15.
174. Kiowski, W., Sutsch, G., Hunziker, P., *et al.*: Evidence for endothelin-1-mediated vasoconstriction in severe chronic heart failure. *Lancet* 1995, 346(8977):732-6.
175. Pacher, R., Stanek, B., Hulsmann, M., *et al.*: Prognostic impact of big endothelin-1 plasma concentrations compared with invasive hemodynamic evaluation in severe heart failure. *J Am Coll Cardiol* 1996, 27(3):633-41.
176. Noto, A., Giacomini, M., Palandi, A., *et al.*: Levosimendan in septic cardiac failure. *Intensive Care Med* 2005, 31(1):164-5.
177. Pataricza, J., Hohn, J., Petri, A., *et al.*: Comparison of the vasorelaxing effect of cromakalim and the new inodilator, levosimendan, in human isolated portal vein. *J Pharm Pharmacol* 2000, 52(2):213-7.
178. Kaheinen, P., Pollesello, P., Levijoki, J., *et al.*: Levosimendan increases diastolic coronary flow in isolated guinea-pig heart by opening ATP-sensitive potassium channels. *J Cardiovasc Pharmacol* 2001, 37(4):367-74.
179. Behrends, M. and Peters, J.: The calcium sensitizer levosimendan attenuates endotoxin-evoked myocardial dysfunction in isolated guinea pig hearts. *Intensive Care Med* 2003, 29(10):1802-7.
180. Faivre, V., Kaskos, H., Callebert, J., *et al.*: Cardiac and renal effects of levosimendan, arginine vasopressin, and norepinephrine in lipopolysaccharide-treated rabbits. *Anesthesiology* 2005, 103(3):514-21.

181. Morelli, A., De Castro, S., Teboul, J. L.*, et al.*: Effects of levosimendan on systemic and regional hemodynamics in septic myocardial depression. *Intensive Care Med* 2005, 31(5):638-44.
182. Kelso, E. J., McDermott, B. J., Silke, B.*, et al.*: Endothelin(A) receptor subtype mediates endothelin-induced contractility in left ventricular cardiomyocytes isolated from rabbit myocardium. *J Pharmacol Exp Ther* 2000, 294(3):1047-52.
183. Leite-Moreira, A. F., Bras-Silva, C. and Pedrosa, C.: Myocardial effects of ETB receptor stimulation. *Rev Port Cardiol* 2002, 21(9):1009-16.
184. Kramer, B. K., Smith, T. W. and Kelly, R. A.: Endothelin and increased contractility in adult rat ventricular myocytes. Role of intracellular alkalosis induced by activation of the protein kinase C-dependent Na(+)-H+ exchanger. *Circ Res* 1991, 68(1):269-79.
185. Kang, M. and Walker, J. W.: Endothelin-1 and PKC induce positive inotropy without affecting pHi in ventricular myocytes. *Exp Biol Med (Maywood)* 2006, 231(6):865-70.
186. Ry, S. D., Andreassi, M. G., Clerico, A.*, et al.*: Endothelin-1, endothelin-1 receptors and cardiac natriuretic peptides in failing human heart. *Life Sci* 2001, 68(24):2715-30.
187. Wanecek, M., Oldner, A., Sundin, P.*, et al.*: Effects on haemodynamics by selective endothelin ET(B) receptor and combined endothelin ET(A)/ET(B) receptor antagonism during endotoxin shock. *Eur J Pharmacol* 1999, 386(2-3):235-45.
188. Wanecek, M., Oldner, A., Rudehill, A.*, et al.*: Endothelin(A)-receptor antagonism attenuates pulmonary hypertension in porcine endotoxin shock. *Eur Respir J* 1999, 13(1):145-51.
189. Wanecek, M., Weitzberg, E., Alving, K.*, et al.*: Effects of the endothelin receptor antagonist bosentan on cardiac performance during porcine endotoxin shock. *Acta Anaesthesiol Scand* 2001, 45(10):1262-70.
190. Fifer, M. A., Bourdillon, P. D. and Lorell, B. H.: Altered left ventricular diastolic properties during pacing-induced angina in patients with aortic stenosis. *Circulation* 1986, 74(4):675-83.
191. Brahmbhatt, S., Gupta, A. and Sharma, A. C.: Bigendothelin-1 (1-21) fragment during early sepsis modulates tau, p38-MAPK phosphorylation and nitric oxide synthase activation. *Mol Cell Biochem* 2005, 271(1-2):225-37.
192. Mebazaa, A., De Keulenaer, G. W., Paqueron, X.*, et al.*: Activation of cardiac endothelium as a compensatory component in endotoxin-induced cardiomyopathy: role of endothelin, prostaglandins, and nitric oxide. *Circulation* 2001, 104(25):3137-44.
193. Fernandes, C. J., Jr., Akamine, N. and Knobel, E.: Cardiac troponin: a new serum marker of myocardial injury in sepsis. *Intensive Care Med* 1999, 25(10):1165-8.
194. Ince, C. and Sinaasappel, M.: Microcirculatory oxygenation and shunting in sepsis and shock. *Crit Care Med* 1999, 27(7):1369-77.
195. Fink, M. P.: Cytopathic hypoxia. Is oxygen use impaired in sepsis as a result of an acquired intrinsic derangement in cellular respiration? *Crit Care Clin* 2002, 18(1):165-75.
196. Maxwell, L., Pratt, K. and Gavin, J.: Endothelin-3-induced microvascular incompetence and mitochondrial damage in rat myocardium. *Clin Exp Pharmacol Physiol* 1992, 19(10):683-8.
197. Maxwell, L., Harrison, W. R. and Gavin, J. B.: Endothelin antagonists diminish postischemic microvascular incompetence and necrosis in the heart. *Microvasc Res* 2000, 59(2):204-12.

198. Wanecek, M., Oldner, A., Rudehill, A.*, et al.*: Cardiopulmonary dysfunction during porcine endotoxin shock is effectively counteracted by the endothelin receptor antagonist bosentan. *Shock* 1997, 7(5):364-70.
199. Rossi, P.: The role of the endothelin system in experimental acute lung injury, with special reference to the formation of extra-vascular lung water. *Thesis.* Stockholm: Karolinska Institutet; 2006.
200. Kass, D. A., Beyar, R., Lankford, E.*, et al.*: Influence of contractile state on curvilinearity of in situ end-systolic pressure-volume relations. *Circulation* 1989, 79(1):167-78.
201. Steendijk, P., Van der Velde, E. T. and Baan, J.: Left ventricular stroke volume by single and dual excitation of conductance catheter in dogs. *Am J Physiol* 1993, 264(6 Pt 2):H2198-207.