Regional fluxes of tissue plasminogen activator in porcine endotoxemia

Annette Nyberg

The Sahlgrenska Academy
AT GÖTEBORG UNIVERSITY

2007

Regional fluxes of tissue plasminogen activator in porcine endotoxemia

Annette Nyberg
Department of Anaesthesiology and Intensive Care,
Institute of Clinical Sciences, The Sahlgrenska Academy,
Göteborg University, Sweden

ISBN 978-91-628-7322-6

annette.nyberg@aniv.gu.se

All published papers are reprinted with the permission of the publisher

Printed by Intellecta Docusys AB
Göteborg, Sweden, 2007

ABSTRACT

Formation of fibrin clots in the microcirculation during severe sepsis contributes to organ failure, frequently involving the lungs and the splanchnic organs. Tissue plasminogen activator, tPA, is the key activator of intravascular fibrinolysis with plasminogen activator inhibitor type-1, PAI-1, as its main inhibitor. This thesis focuses on mesenteric, hepatic, renal and pulmonary fluxes of tPA and PAI-1 in response to infusion of endotoxin in anaesthetized, ventilated pigs as a model of experimental gram-negative sepsis. Plasma levels of the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) were analyzed to assess the host response to endotoxemia.

Endotoxemia resulted in a hypodynamic circulation that in response to resuscitation with volume and vasopressor administration developed into a hyperdynamic circulatory state. Acute lung injury, ALI, was investigated following bronchoalveolar lavage (primary ALI) and in endotoxemia (secondary ALI).
Endotoxemia acutely increased plasma tPA concentrations in all investigated vascular beds and increased mesenteric release and hepatic uptake of tPA. The hepatic uptake effectively prevented a systemic spillover of tPA from the mesenteric circulation. Hemodynamic resuscitation restored mesenteric and hepatic tPA fluxes to baseline. Sustained increases in systemic levels of tPA, notably following administration of noradrenaline, indicated contributions from other vascular regions not studied. Acute changes in mesenteric and hepatic tPA fluxes related to the dose of endotoxin but with a similar temporal pattern up to 18 hours regardless of dose. A pulmonary release of tPA was only observed in secondary ALI. No changes in renal tPA fluxes were observed throughout the studies. Levels of TNF-α correlated to concentrations and fluxes of tPA, whereas data suggested a non-concomitant relation to hepatic PAI-1 release. The molar ratio of active tPA to PAI-1 favoured anti-fibrinolysis at baseline but was reversed into a pro-fibrinolytic balance in hypodynamic endotoxemia, particularly in the mesenteric circulation. Finally, the hyperdynamic state was characterized by a marked anti-fibrinolytic balance of active tPA to PAI-1.

In conclusion, this thesis demonstrated regionally differentiated responses in plasma fluxes of both tPA and PAI-1 in response to endotoxemia. The results support TNF-α as a candidate mediator of tPA and PAI-1 release. Therapeutic strategies to enhance regional tPA fluxes and fibrinolysis in acutely septic patients warrant further investigation.

Key words: pig, endotoxin, tissue plasminogen activator, plasminogen activator inhibitor, tumor necrosis factor α

ISBN 978-91-628-7322-6

LIST OF PAPERS

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

I. Nyberg A, Seeman-Lodding H, Ahlqvist M, Fagerberg A, Jern C, Åneman A. Regionally differentiated fibrinolytic responses during volume-resuscitated acute endotoxemia in pigs.
Acta Anaesthesiol Scand 2003; 47: 1125-31.

II. Nyberg A, Fagerberg A, Ahlqvist M, Jern C, Seeman-Lodding H, Åneman A. Pulmonary net release of tissue-type plasminogen activator during porcine primary and secondary acute lung injury.
Acta Anaesthesiol Scand 2004; 48:845-50.

III. Nyberg A, Jakob S, Seeman-Lodding H, Porta F, Bracht H, Bischofberger H, Jern C, Takala J, Åneman A. Time and dose related regional kinetics of tissue-type plasminogen activator in endotoxemic pigs.
Acta Anaesthesiol Scand in press

IV. Nyberg A, Seeman-Lodding H, Declerck PJ, Fagerberg A, Jern C, Åneman A. Regional differentiation of tPA and PAI-1 kinetics in acute endotoxemia. *Manuscript*

ABBREVIATIONS

ALI	acute lung injury
AUC	area under the curve
CO	cardiac output
CVP	central venous pressure
DIC	disseminated intravascular coagulation
E coli	Escherichia coli
ELISA	enzyme-linked immunosorbent assay
ETX	endotoxin (lipopolysaccharide)
Hct	haematocrit
HR	heart rate
ICU	intensive care unit
LBP	LPS-binding protein
LPS	lipopolysaccharide (endotoxin)
MAP	mean arterial pressure
MOF	multiple organ failure
NF-κB	nuclear factor-κB
PAP	pulmonary artery pressure
PAI-1	plasminogen activator inhibitor type 1
PVR	pulmonary vascular resistance
SIRS	systemic inflammatory response syndrome
SVR	systemic vascular resistance
TNF-α	tumor necrosis factor-α
tPA	tissue plasminogen activator
Q_{HA}	hepatic arterial blood flow
Q_{PV}	portal venous blood flow
Q_{RA}	renal arterial blood flow
Q_s/Q_t	pulmonary shunt fraction

CONTENTS

ABSTRACT....5
LIST OF PAPERS....6
ABBREVIATIONS....7
INTRODUCTION....11
Sepsis....11
Historical remarks....11
Definitions and epidemiology....11
Endotoxin....12
Haemostatic balance in sepsis....12
The vascular endothelium in sepsis....13
Tissue plasminogen activator (tPA)....14
Plasminogen activator inhibitor type-1 (PAI-1)....15
Systemic vs. regional plasma levels of tPA and PAI-1....15
Hepatomesenteric tPA and PAI....16
Pulmonary tPA and PAI....16
The pig endotoxemic model to study sepsis....16
AIMS....19
MATERIALS AND METHODS....21
Animals....21
Anaesthesia....21
Surgery....22
Blood sampling and analyses....22
Analyses of tPA, PAI-1 and TNF-α....23
Calculations....23
Experimental design....24
Infusion of endotoxin....24
Hemodynamic resuscitation....25
Bronchoalveolar lavage procedure....25
Recordings and blood sampling....25
Methodological considerations....25
Statistical analyses....26
REVIEW OF RESULTS....29
Hemodynamic variables....29
Plasma concentrations of tPA (I-IV), PAI-1 (IV) and TNF-α (II-IV)....29

tPA..29
PAI-1... 32
TNF-α..32
Regional fluxes of tPA (I-IV) and PAI-1 (IV)..33
tPA..33
PAI-1... 35
Molar ratios of tPA and PAI-1 (IV)..35
DISCUSSION...37
Splanchnic and pulmonary plasma fluxes of tPA...37
Mesenteric release of tPA..38
Hepatic uptake of tPA...39
Renal plasma fluxes of tPA..40
Pulmonary plasma fluxes of tPA...40
Pulmonary plasma fluxes of tPA in acute lung injury.. 41
Time and dose effects of endotoxemia on plasma tPA fluxes.....................................43
Splanchnic and pulmonary plasma fluxes of active/total tPA and PAI-1....................44
CONCLUSIONS... 47
REFERENCES...51

INTRODUCTION

Sepsis

The term sepsis (Greek, sepo, “I rot”) has been used for 2700 years.

Historical remarks

Anton van Leeuwenhoek was the first person to see and describe bacteria, using a single-lens microscope, already in 1676 [1].
Ignaz Semmelweis hypothesized that rotten particles were the cause of puerperal fever, and dramatically reduced the mortality rate by introducing antiseptic procedures in 1847. This principle was later confirmed by Louis Pasteur who, in 1879, demonstrated that bacteria (streptococci) were indeed present in blood collected from patients with puerperal septicaemia. A decade later, Richard Pfeiffer discovered the phenomenon of bacterial lysis and devised the concept of endotoxin as a heat stable poison causing the symptoms of sepsis (1892).

In 1933, Tillett and coworkers published a report demonstrating that broth cultures of hemolytic streptococci rapidly liquefied the fibrin clot of human plasma [2]. The activating substance produced by streptococci was called streptokinase. When a similarly acting substance was observed in tissues and tissue extracts, as suggested by Fisher in 1946 and decribed by Astrup and Permin in 1947 [3], the name tissue-type plasminogen activator was introduced. Purified tPA preparations became available in the 1970s.
The presence of a tPA inhibitor in a group of patients with an impaired fibrinolytic system was described by Brakman et al in 1966 [4]. Plasminogen activator inhibitor type-1 was detected in endothelial cells in 1983 [5] and purified from endothelial cells in 1984 [6].

Definitions and epidemiology

Sepsis is defined as a state of disease with the presence of both an infectious process and a systemic inflammatory response. Severe sepsis includes organ dysfunction and septic shock is represented by acute circulatory failure with arterial hypotension despite volume resuscitation [7].
Severe sepsis and septic shock represent a clinical challenge because of its common occurrence, high associated costs of care, and significant mortality, which varies between 30-50% [8, 9]. A recent Finnish study (Finnsepsis) reported an incidence of ICU-treated severe sepsis of 0.38/1000 in the adult population [10]. This incidence is lower than that reported in international prospective studies [8, 11]. A retrospective study in Norway reported an incidence for sepsis of 1.49/1000 inhabitants [12]. The mortality in the Finnsepsis study was 15.5% in the ICU which increased to 40.9% at the one-year follow-up. The focus of infection was reported to be pulmonary in 43% of cases and intraabdominal in 32%. A vast

majority (86%) of the patients required mechanical ventilation and in 23% acute renal failure was present. Disseminated intravascular coagulation (DIC) was diagnosed in 36% of the patients, highlighting the importance of disturbed hemostasis in severe sepsis.
The reported rate of gram-negative sepsis in case of positive blood cultures varies between 30% and 50% [10, 13, 14]. Elevated levels of endotoxin are found in 70-80% of patients with severe sepsis and correlate to mortality [15, 16, 17].

Endotoxin

Endotoxin is a lipopolysaccharide (LPS) of the outer wall membrane of gram-negative bacteria. The lipid component holds the endotoxicity and the polysaccharide component holds the immunogenicity. LPS is released when bacteria undergo lysis.
Macrophages, monocytes and neutrophil granulocytes constitutively express CD14 antigen (CD14) and Toll-like receptor 4 (TLR4) on their membrane [18]. The LPS binding protein (LBP), derived from the liver, dramatically accelerates the association of LPS to CD14, thereby significantly increasing the sensitivity of cells to endotoxin [19]. Following activation of CD14, a series of intracellular events activate transcription factors. Binding to TLR4 leads to the activation of nuclear factor-κB (NF-κB) and the transcription of proinflammatory mediators, e.g. tumor necrosis factor-α (TNF-α), as well as interleukins 1, 6 and 8 [20].

Haemostatic balance in sepsis

The details of the extensive and complex interactions between the coagulation system and the inflammatory system are beyond the scope of this thesis. The reader is refereed to excellent reviews for details [21, 22, 23].

In summary, inflammation, for example initiated by endotoxemia, shifts the haemostatic balance to favour activation of coagulation and in the extreme, to trigger disseminated intravascular coagulation or thrombosis. Inflammatory mediators increase the number of thrombocytes and their reactivity, down-regulate natural anticoagulant mechanisms, initiate the coagulation system and facilitate propagation of the coagulatory response. Furthermore, fibrinolysis is impaired. In addition, clotting can increase the inflammatory response by the generation of pro-inflammatory coagulation enzymes, e.g. thrombin [22].
Activation of coagulation during sepsis is primarily driven by the tissue factor (TF) pathway. The pro-inflammatory key cytokine TNF-α induces up-regulation of TF mRNA. Expression of TF on monocytes and endothelial cells, triggers activation of coagulation. An additional source of TF might be phospholipid microparticles originating from activated monocytes [24, 25]. After binding to exposed TF, circulating Factor VII is activated. The TF/Factor VIIa complex then activates Factor X to Factor Xa, by which prothrombin is converted to thrombin. Thrombin cleaves fibrinogen into fibrin monomers and activates Factor XIII, which then covalently crosslinks fibrin monomers to form a stable clot [21, 26].

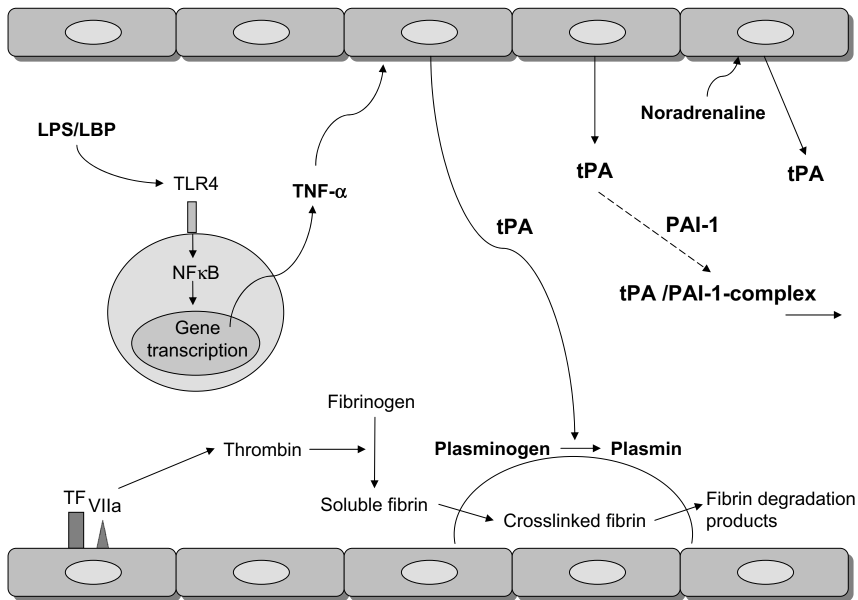

Figure 1. The basic mechanisms of the inflammatory and fibrinolytic systems studied in this thesis.

Septic patients frequently demonstrate signs of DIC with a lowered platelet count and prolongation of clotting times. Although the coagulopathy is systemic, the bleeding typically occurs in selected sites, eg. gastrointestinal mucosa, the upper airways and the urinary tract. Virchow´s classic triad of thrombosis – hypercoagulability, endothelial cell injury and reduced blood flow – is present in severe sepsis/septic shock. Ischaemic organ damage by formation of microthombi in the pre-, or more often, postcapillary circulation is a pivotal feature of multiple organ failure in sepsis [27], and has recently been adressed in several clinical trials of anticoagulant therapies [23, 28-30]. In addition to stagnant hypoxia, uncoupling of mitochondrial respiration results in cytopathic hypoxia.

The vascular endothelium in sepsis

The endothelium is a dynamic organ system *per se* with a variety of functions and is involved in the pathogenesis of several diseases [31]. The normal endothelium responds to mechanical, chemical and humoral stimuli (e.g. fluid shear stress, acidosis, hypoxia, cytokines, endotoxin, thrombin, histamine) that regulate its release of vasoactive (eg. nitric oxide, prostacyclin, endothelin-1) as well as antithrombotic and anticoagulatory substances (eg. tissue factor pathway inhibitor, heparan sulfate, protein S). In endotoxemia and sepsis, the

pathophysiological role of the endothelium is so crucial that such conditions have been suggested to be termed “endothelitis”. The endothelial cells express increased levels of surface adhesion molecules in response to e.g. LPS, TNF-α, hypoxia, acidosis and hypoglycemia. Surface adhesion molecules bind activated leucocytes that cause vasodilation, increased vascular permeability and activation of the coagulation cascade. Leucocytes produce inflammatory cytokines that impair endothelial function and constitutes a positive feedback loop between inflammation and coagulation. The normal anticoagulant properties of the endothelium are compromised and an overwhelming fibrin clot formation causes DIC, that may manifest by bleeding in case of excessive consumption of coagulation factors [32].
The endothelium is differentiated in specific organs, e.g. in the pulmonary capillaries the endothelium contains tight, impermeable intercellular junctions and in hepatic sinusoids the endothelium has intercellular gaps that allows the passage of red blood cells.

Tissue plasminogen activator (tPA)
To oppose the process of clotting, the vascular endothelium comprises several mechanisms that in a coordinated fashion forms an integrated thromboprotective programme. The main subject of this thesis is the endothelial release of tissue plasminogen activator (tPA).
The fibrin clot is degraded by the protease plasmin, that is activated by plasminogen activators such as tPA, urokinase-type PA (uPA) and a pathway involving factor XII that is incompletely understood. The key physiologic initiator of intravascular fibrinolysis is tPA. Plasmin generation is enhanced when plasminogen and tPA binds to lysine in partly degraded fibrin, thereby concentrating the fibrinolysis to the fibrin clot and enhancing the tPA activity more than hundredfold [33-35]. Urokinase-type PA takes part in the later stages of fibrin dissolution and is involved in processes like remodelling of the extracellular matrix [36, 37].

Tissue PA is a glycoprotein containing 527 amino acids. The molecular weight is between 65 and 75 kD depending on degree of glycosylation. Tissue PA is synthesised in the Golgi apparatus in the endothelial cell and is continuously released through a constitutive secretion, when transport vesicles from the Golgi apparatus fuses with the cell membrane, and through a regulated secretion from an intracellular storage pool [38, 39]. The basal plasma concentration of total tPA antigen is 5(-10) $ng \cdot ml^{-1}$. The tPA antigen represents both free, active tPA and complex-bound, inactive tPA [40]. The halflife of tPA is 3-5 minutes [41].
Considerable amounts of tPA can be released by various stimuli, e.g. thrombin, bradykinin, catecholamines, histamin, vasopressin, desmopressin, acetylcholine, factor Xa, calcium fluxes, cAMP and ADP [42-46]. Many agonists of tPA release also induce nitric oxide and prostacyclin release. Synthesis of tPA is enhanced by e.g. thrombin, histamin and short-term shear stress [47]. Ischaemia and reperfusion of endothelial cells decrease the synthesis of tPA [48].

Circulating tPA is cleared by the liver as previously been described [41, 49, 50]. Accordingly, a marked, systemic profibrinolytic state is typically reported in the anhepatic phase of liver transplantation [51]. The hepatic uptake of tPA is an active process involving two subsets of receptors: the low density lipoprotein receptor-related protein (LRP) on liver parenchymal cells and the mannose receptor on liver endothelial cells [52-54]. The importance of a third pathway for tPA clearance, involving the O-linked fucose, an unusual saccharide of tPA, remains unclear *in vivo* [55]. Active tPA is more rapidly cleared than the tPA-PAI-1 complex [41]. The total hepatic blood flow is considered important to maintain hepatic uptake of tPA in accordance with the clearance concept [56].

This thesis focuses on the regional plasma fluxes of tissue plasminogen activator (tPA) in the systemic, mesenteric, hepatic, renal and pulmonary circulations.

Plasminogen activator inhibitor type-1 (PAI-1)

The main inhibitor of tPA is plasminogen activator inhibitor type-1 (PAI-1), a 52 kD serpin containing 379 amino acids. The main origin(s) of circulating PAI-1 remains unclear, but in vitro it is synthesized by a variety of cells, e.g. endothelial cells, hepatocytes, platelets and adipocytes [57, 58]. The active form of PAI-1 is secreted and PAI-1 is not stored intracellularly . PAI-1 is an acute phase reactant [59] and plasma levels are increased after sepsis, surgery and major trauma [60].

There are three forms of PAI-1: active, latent and complex-bound. The active form is stabilised by binding to vitronectin [61]. PAI-1 spontaneously converts into a latent form [62, 63]. PAI-1 irreversibly inactivates tPA by forming a 1:1 complex [64, 65]. PAI-1 is normally in molar excess compared to tPA. The halftime of PAI-1 is 10-15 minutes. 90 % of PAI-1 is circulating in platelets and is not considered to contribute to plasma PAI-1 [66-68].

Systemic vs. regional plasma levels of tPA and PAI-1

It has long been recognized that endotoxemia induces a biphasic response characterized by an early increase of fibrinolytic activity with elevated levels of tPA, and a subsequent decrease in fibrinolysis with elevated levels of PAI-1 [69-71]. While systemic changes of pro- and antifibrinolysis in endotoxemia and sepsis have been repeatedly investigated both experimentally and clinically, very few studies exist that reports regional changes.

The perfused forearm model has been used to investigate plasma fluxes of tPA and PAI-1 in response to different stimuli and during both physiological and pathological conditions [72, 73]. Clinical and experimental data based on different sampling sites within the vascular tree have demonstrated regional divergencies in plasma tPA levels [75] but do not convey information on plasma kinetics since blood flow has not been taken into account.

Hepatomesenteric tPA and PAI

Using a multiple-organ model in pigs, the regional net release and uptake rates of tPA have been described during baseline and in response to positive end-expiratory pressure and aortic cross-clamping [76, 77]. These studies have reported a mesenteric release and a hepatic uptake of tPA, whereas no significant fluxes of tPA were found in the renal and pulmonary circulations. These studies did not assess regional PAI-1 fluxes, nor was endotoxemia or conditions of systemic inflammation investigated.
A hepatic extraction of tPA has been documented in several clinical and experimental studies, including the resulting increase in plasma tPA levels observed during the anhepatic phase of liver transplantation [78].

Pulmonary tPA and PAI

In recent years, several studies have focused attention on the coagulation cascade in the continuum of sepsis and acute lung injury (ALI) and acute respiratory distress syndrome. Activated coagulation and impaired fibrinolysis are important contributors to ALI and are regulated locally in the lung as well as being influenced by systemic coagulatory changes. Lung protective settings for mechanical ventilation have been demonstrated to attenuate activation of coagulation and to prevent inhibition of fibrinolysis by reducing PAI-1 levels. Anticoagulant therapies in ALI have been investigated using TF blockade, tissue factor pathway inhibitor [29] and recently using recombinant activated protein C [79].
Decreased levels of protein C and increased PAI-1 levels were recently reported as clinically relevant predictors of mortality in ALI/ARDS [80]. Data on transpulmonary plasma gradients for tPA and PAI-1 are sparse and contradictory. Both absence of an arteriovenous concentration gradient [81] or pulmonary flux of tPA [82] as well as a positive gradient across the right to left ventricle [83] have been reported.

The pig endotoxemic model to study sepsis

Pigs and humans generally share the same cardiovascular physiology [84, 85] and similar hemodynamic monitoring equipment used in intensive care can be used in pigs. The hemodynamic response to endotoxemia in pigs is usually biphasic with an initial hypodynamic phase followed by a later hyperdynamic phase, as opposed to the consistent hyperdynamic response observed in humans [86]. The blood volume in pigs in the 25-30 kg bodyweight range makes repeated blood sampling possible.
Obviously pigs are not humans and while pigs are young and healthy without cardiovascular disease, the typical septic patient is old with significant co-morbidities, e.g. atherosclerosis, chronic obstructive pulmonary disease, diabetes or cancer (cf. >17% prevalence in the PROWESS study, [28]). The protocol of most animal experiments is short (hours) with a single defined intervention (eg. endotoxin) and concise end-point(s), quite different to the

critically ill patient, admitted to hospital after a few days with an imprecise diagnose of infection and subjected to multiple ongoing therapies.

An infusion of endotoxin, instead of live bacteria or bacterial peritonitis, was chosen in this thesis since it provides a reproducible, simple method to study mechanisms of early sepsis that is well established in our laboratory [87, 88] and in the literature [89, 90].

AIMS

The general aim of this thesis was to gain a better understanding of early pathophysiological mechanisms of fibrinolysis in an experimental setting relevant for septic shock, with special emphasis on individual organs. To achieve this aim, regional plasma fluxes of tPA and PAI-1 were investigated during acute endotoxemia in pigs.

The specific aims were to:

- study changes in mesenteric, hepatic, renal and pulmonary fluxes of total and active tPA in hypodynamic, endotoxemic circulatory failure
- assess the effects of hemodynamic resuscitation by intravascular volume expansion on regional tPA fluxes including the effects of adding vasopressor support
- investigate the relation between the dose of endotoxin and the subsequent response in regional tPA fluxes in terms of magnitude and time course
- study changes in mesenteric, hepatic, renal and pulmonary fluxes of total and active PAI-1 in acute endotoxemia with resuscitaton
- compare pulmonary fluxes of tPA in respiratory failure induced by bronchoalveolar lavage or endotoxemia as models of primary and secondary acute lung injury
- evaluate the relation between plasma levels of TNF-α and concentrations and fluxes of tPA and PAI-1

MATERIALS AND METHODS

Animals

All studies were approved by the Ethics Committee for Animal Experiments at the University of Göteborg, Sweden, and in paper III also by the Animal Care Committee of the Canton of Bern, Switzerland. The experiments were performed in accordance with the principles set forth in the "Guide for the care and use of laboratory animals" (National Academy of Sciences, ed. 1996, ISBN 0-309-05377-3).

Study I included one group of nine landrace pigs of either gender (24-29 kg). One animal died before volume resuscitation and was not included in the final analysis.
Study II included twenty-one landrace pigs of either gender (24-35 kg) in three groups: time control experiments (CTRL, n=5), bronchoalveolar saline lavage (BAL, n=8), infusion endotoxin (ETX, n=8).
Study III included fourty-five landrace pigs of either gender (24-45 kg) in three groups: acute, high-dose endotoxin (6 hours, high ETX, n=13 including 8 animals from study I), prolonged, low-dose endotoxin (18 hours, low ETX, n=18) and time control experiments (18 hours, n=14).
Study IV included one group of eight landrace pigs of either gender (27-32 kg).

Anaesthesia

All animals were fasted overnight with free access to water. In study I and II, animals were premedicated with ketamin and azaperon intramuscularly and anaesthesia was induced with an intravenous bolus injection of α-chloralose. Anaesthesia was maintained by an infusion of α-chloralose and bolus doses of fentanyl were administered during surgery. In the high ETX animals of study III and in study IV, animals were premedicated with ketamine and midazolam intramuscularly and anasthesia was induced by intravenous thiopenthal. In the low ETX and control animals of study III, premedication was performed using ketamine and xylazin intramuscularly and midazolam and atropine was used for induction of anaesthesia. In both studies III and IV, anesthesia was maintained by a continuous infusion of thiopental and fentanyl. No muscle relaxants were used.

Animals were tracheotomised (I, II) or endotracheally intubated (III, IV) and mechanically ventilated in a volume-controlled mode. Ventilation was adjusted to normocapnia as indicated by end-tidal CO_2 levels and intermittent arterial blood gas analyses.
In studies I, II and IV, a 2.5% glucose solution was infused at a basal rate of 10 $ml^{-1} \cdot kg^{-1} \cdot h^{-1}$ that was increased to 20 $ml^{-1} \cdot kg^{-1} \cdot h^{-1}$ during surgery. In study III an infusion of saline at 8-20 $ml \cdot kg^{-1} \cdot hr^{-1}$ was administered during surgery. Core body temperature was maintained at 38-39°C using heating blankets.

Surgery

A pulmonary artery thermodilution catheter was inserted via an internal jugular vein to measure cardiac output, pulmonary arterial and occlusion pressures and to sample pulmonary arterial blood. An arterial catheter via a femoral artery (in the distal, abdominal aorta) or carotid artery was used to determine mean arterial pressure (MAP) and to sample arterial blood. A triple-lumen central venous catheter via an internal jugular vein was used to measure central venous pressure (CVP) and to administer fluid and drugs.

In studies I, II and IV, a midline laparotomy was performed. Ultrasonic transit-time flow probes were positioned around the portal vein and the hepatic artery to measured portal and hepatic arterial blood flow, respectively, giving total hepatic blood flow as the sum of both. The portal vein was catheterized to sample portal venous blood. A catheter was inserted to the hepatic vein via the femoral vein (I, IV) or the right internal jugular vein (III) to sample blood. In Bern, the superior mesenteric, one renal and the splenic arteries were fitted with flowmeters, one catheter was positioned in the mesenteric vein and a gastric and jejunal tonometer was positioned for purposes apart from this study [91]. In studies I and IV, a flow probe was positioned around the renal artery via a flank incision and retroperitoneal dissection to measure renal blood flow, and a catheter was positioned via one femoral vein in the renal vein to sample blood. The laparotomy was closed following preparation.

All blood pressures were recorded using pressure transducers positioned at heart level and connected to an AS/3 anaesthesia monitor (Göteborg) or C/5 Compact Critical Care monitor (Bern; both Datex-Ohmeda, Helsinki, Finland). Cardiac output (CO) was measured by the thermodilution technique (mean value of three measurements, cardiac output module, Datex-Ohmeda®, Helsinki, Finland). Pressure and flow data were continuously recorded using Labview software (Version 4.1, National Instruments, Austin, TX), Windaq™ 1.6 (Dataq Instruments Inc., Akron, OH, USA) or Clinisoft™ (Deio, Helsinki, Finland).

Blood sampling and analyses

Aortic (I-IV), pulmonary arterial (I-IV), portal (I, III, IV), hepatic (I, III, IV) and renal (I, IV) venous blood samples were collected simultaneously to determine tPA (I-IV), PAI-1 (IV) and TNF-α (II-IV) concentrations in conjunction with regional haemodynamic recordings and blood gas analyses. The haematocrit was measured to calculate plasma flows. The first portion of blood, corresponding to the catheter volume, was always discarded. Blood samples were collected in tubes containing 1/10 0.45 M sodium citrate buffer, pH 4.3 (Stabilyte®, Biopool AB, Umeå, Sweden) for determination of tPA and TNF-α and in tubes containing 1/10 platelet stabilizing buffer (Diatube®, Diagnostica Stago, Asnières, France) for determination of PAI-1. The acidification blocks the reaction between tPA and PAI-1. Samples

were kept on ice and plasma was separated within 20 minutes by centrifugation at 4˚C and 2000*g* for 20 minutes. Plasma aliquots were immediately frozen and stored at -70˚C.

Analyses of tPA, PAI-1 and TNF-α

Total tPA antigen was determined using an enzyme-linked immunosorbent assay (ELISA) detecting both free and complex-bound fractions with equal efficiency (TintElize® tPA, cat#1105, Biopool AB, Umeå, Sweden) [92]. Calibration was performed with a purified porcine standard [82]. Active tPA was determined by a spectrophotometric parabolic rate assay (Spectrolyse™/fibrin tPA, cat#101101, Biopool AB, Umeå, Sweden) [93]. This human assay has previously been shown by immunodepletion of porcine plasma to be specific also for porcine tPA [82]. Samples from each experimental animal were analyzed on one single microtiter plate. All samples were analyzed in duplicate. The determination of both active and total tPA makes it possible to calculate the ratio of active to complex-bound tPA. The amount of complex-bound tPA is the sum of both the active fraction (activity) and the total amount (antigen).

Plasma levels of total PAI-1 antigen (active, complex-bound and latent forms) and PAI-1 activity were determined by two monoclonal antibody-based ELISA's, both developed for the quantification of PAI-1 in pig plasma. Calibration was performed with recombinant porcine PAI-1 [94]. Samples from each experimental animal were analyzed on one single microtiter plate. All samples were analyzed in triplicate.

Plasma levels of TNF-α were determined by a commercially available ELISA for pigs (KSC3012, Biosource, California, USA). All samples were analyzed in duplicate.

Calculations

Regional net fluxes were calculated according to the Fick principle:

$$\text{net flux} = (C_{OUT} - C_{IN}) \cdot Q_p$$

The net flux is the release (positive net flux) or uptake (negative net flux) of the investigated compound, based on the inflowing (C_{IN}) and outflowing (C_{OUT}) concentrations of the compound and the plasma flow (Q_P). Regional plasma flows (Q_P) were calculated as (Q_P = Q x (100-Hct/100) using regional blood flows (Q_P) and arterial hematocrits (Hct).
The total hepatic inflow of tPA or PAI-1 was calculated using both hepatic arterial and portal venous plasma concentrations weighted to the respective plasma flows.
The total cumulative antigen and active tPA and PAI-1 net release or uptake across the mesenteric and hepatic vascular beds were calculated as the area under the curve (AUC) from baseline until the end of the study protocol (IV).

In study IV, the mesenteric and hepatic molar input and output of tPA and PAI-1 were calculated from the plasma concentrations, using the molecular weights 68000 g/mol for tPA and 52000 g/mol for PAI, and regional plasma flows. Complex-bound tPA was calculated from the corresponding measurements of total (antigen) and free (active) tPA. The difference between total and free PAI-1 represents both complex-bound and latent PAI-1.

Experimental design

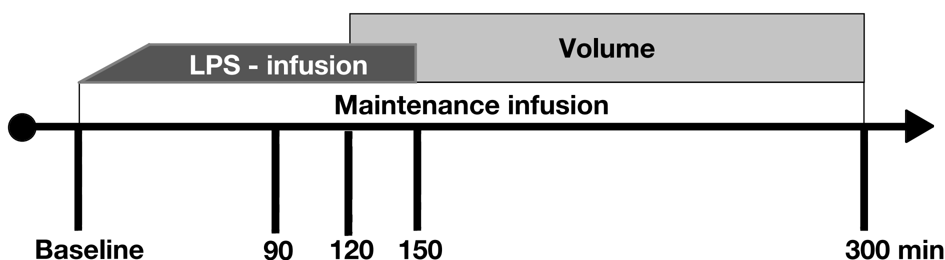

Figure 1. *The common experimental protocol used in this thesis. The infusion of endotoxin (LPS) was started at 2.5 $\mu g \cdot kg^{-1} \cdot hr^{-1}$ and doubled stepwise to 20 $\mu g \cdot kg^{-1} \cdot hr^{-1}$ during 30 minutes and then maintained for 120 minutes.*

Animals were allowed to stabilise for 30 - 60 minutes after surgery.
Baseline registrations of haemodynamic variables and blood sampling for tPA (I-IV), PAI-1 (IV), TNF-α (II-IV) and blood gas analyzes were performed in duplicate.

Infusion of endotoxin
Animals in studies I, II, III (high ETX) and IV received an infusion of *Escherichia coli* lipopolysaccharide (Serotype 0111:B4, Sigma Chemical Co, USA) starting at 2.5 $\mu g \cdot kg^{-1} \cdot hr^{-1}$ and doubled stepwise to 20 $\mu g \cdot kg^{-1} \cdot hr^{-1}$ during 30 minutes and then maintained at this rate for 120 minutes. In study III, the low ETX group received an endotoxin infusion (Escherichia coli lipopolysaccharide B0111:B4, Difco Laboratories, Detroit, MI, USA) starting at 0,4 $\mu g \cdot kg^{-1} \cdot hr^{-1}$ and increased until the mean pulmonary artery pressure reached 40 mmHg. The infusion was adjusted to maintain moderate pulmonary artery hypertension (mean PAP 25-30 mm Hg) and then held constant until the end of the experiment. The infusion was temporarily stopped if mean arterial pressure decreased below 50 mm Hg with no response to additional fluids.

Hemodynamic resuscitation

Animals were volume resuscitated at 120 minutes following the start of the endotoxin infusion with 20% albumin (200 mg/mL, Baxter Medical AB, Solna, Sweden) (I, IV) or throughout the protocol with 6% hydroxyethyl starch (Venofundin, Braun, Danderyd, Sweden) (III), and saline (Fresenius Kabi, Uppsala, Sweden) (I, III, IV). The low ETX animals in study III were volume resuscitatated with 4 % gelatin (Physiogel 4%, Braun, Emmenbrücke, Switzerland) throughout the protocol to maintain baseline pulmonary artery occlusion pressure between 5 and 8 mmHg. In study IV, a continuous infusion of noradrenaline at 5-8 $\mu g \cdot kg^{-1} \cdot hr^{-1}$ was used to restore baseline cardiac output as the primary endpoint and a mean arterial pressure above 70 mmHg as the secondary endpoint.

Bronchoalveolar lavage procedure

In study II, bronchoalveolar lavage (BAL) was performed using repeated boluses of 500 ml of isotonic saline (10-12 litres) at body temperature until the fluid exchange showed no visual signs of surfactant.

Recordings and blood sampling

In study I, haemodynamic parameters were recorded and blood sampling was performed at baseline and at 90, 150, 170, 190, 210, 250 and 300 minutes. In study II, haemodynamic registrations and blood sampling were performed at baseline and at 2 hours. In study III, registrations and blood samplings were performed at baseline at 1.5, 3, and 6 hours in all groups and after 12 and 18 hours in the low ETX and control groups. In study IV, haemodynamic registrations and blood sampling were performed at baseline and at 10, 20, 30, 50, 70, 90, 110, 120, 150, 170, 190, 210, 250 and 290 minutes.

All animals were sacrificed with an overdose of intravenous potassium chloride during deepened anaesthesia at the end of the experiments.

Methodological considerations

The multiple-organ model to investigate regional plasma fluxes of tPA and PAI-1 is well established in our research group [82]. The anaesthesia, the surgical procedures as well as the instrumentation might all affect tPA and PAI-1 kinetics. Using the animals as their own controls minimizes these possible confounding effects. Furthermore, baseline tPA and PAI-1 levels were in the same range as compared to awake, normal human subjects [73] and a minimal baseline variance was observed throughout the studies.

No control animals subjected to endotoxin infusion, but not resuscitated, were included in the thesis. This is due to the fact that un-resuscitated, endotoxemic circulatory failure carries a significant mortality (>50%, [88]) within 2 to 3 hours following start of endotoxin infusion. Assuming that the decrease in mesenteric release of tPA observed in studies I, III, and IV could be solely attributed to the resuscitation procedure, a hypothetical mean difference to

control animals would be 20 µg·min^{-1}. Given a similar standard error of the mean (7 µg·min^{-1}), 15 animals would need to be investigated to detect this extreme difference with a power of 80% at a 5% significance level. Based on a similar inherent mortality of endotoxemia as previously reported, at least 30 animals would need to be included in such a control group. Such an experimental endeavour was refrained from for obvious practical and ethical reasons.
In studies I, III and IV, restoration of baseline cardiac output was chosen as the primary resuscitation endpoint, rather than any fixed level of arterial blood pressure, since blood flow but not pressure is part of the flux equation. Albumin was chosen as resuscitation fluid in studies I and IV since devoid of any effects on tPA or PAI-1 levels *per se*. Dextrans were avoided since they interfere with hepatic tPA clearance via the mannose receptor. Starches were used in study III to comply with the experimental setup in the collaborating laboratory studying the long term endotoxemia, but have not been reported to acutely interfere with tPA or PAI-1 plasma levels.
The arteriovenous plasma concentration gradient of tPA across an organ does not take into account the intraorgan turnover of tPA and may thus provide an underestimation of true release into the plasma compartment. To overcome this, techniques based on a tracer substance, eg. radiolabelled tPA, would need to be employed to allow for the analysis of arteriovenous changes in the specific activity of the tracer. Since no radiolabelled tPA was available, this approach was not an option in the present thesis, but would be of considerable interest, particularly in the pulmonary circulation.

Statistical analyses

Data are presented as mean and standard error of the mean (SEM) (I, IV) or standard deviation (SD) (II). In study III, data are presented as mean and standard deviation for parametric data and as median and inter-quartile range for non-parametric data.
In studies I and IV, the responses to endotoxemia were evaluated by one-way ANOVA for repeated measures and the Fisher´s PLSD post-hoc test. Student´s t-test was used to test the probability that the arteriovenous gradients or calculated net release/uptake rates for tPA and PAI-1 were different from zero. In study IV, Wilcoxon signed rank test was used to test if the area under the curve for tPA and PAI-1 release were different from zero.
In study II, the ANOVA was followed by an unpaired Student´s t-test to compare the groups. The relations between TNF-α and tPA (II-IV) or PAI-1 (IV) were assessed by linear regression (II) or by within subject analysis according to Bland-Altman [95] (III-IV).
In study III, baseline values of all variables between the three groups were compared by one-way ANOVA. Differences along time during the first six hours of the protocol were assessed by ANOVA for repeated measurements using one dependent variable, one grouping factor (high ETX, low ETX, control), and one within-subject factor (time). A second ANOVA for repeated measurements was used to compare the low ETX and control groups during the 18 hour protocol. In case of a time-group interaction in one of the two ANOVAs, differences

between groups were assessed using one-way ANOVA at specific time points, and in case of a significant result, by unpaired t-tests between low ETX and control, and between high ETX and low ETX. Bonferroni correction was used for multiple comparisons.

Nonparametric tests were used to evaluate tPA kinetics, since not normally distributed (as determined by the Kolmogorov-Smirnov test): the Kruskal Wallis and the Mann Whitney *U*-test for the assessment of between-group effects and Friedman's test for within-group effects. Differences between tPA concentrations at the various sampling sites per group were also assessed with the Friedman test, and, if significant, further with Wilcoxon test (comparison between any two sampling sites).

The results were considered statistical significant at $p<0.05$ (I-IV) or lower according to the Bonferroni method (III).

Statistical analyses were performed using the Statview software package (version 5.0, SAS Institute Inc., Cary, NC) (I, II, IV) or the SPSS software package (version 12.0.1) (III).

REVIEW OF RESULTS

Hemodynamic variables

All baseline hemodynamic variables were within physiological limits [84, 96] and no significant changes were observed over time in control experiments (Table 1).

Infusion of endotoxin to a maximum 20 $\mu g \cdot kg^{-1} \cdot h^{-1}$ consistently resulted in hypodynamic, hypotensive circulatory failure within 90 minutes, characterised by an approximate reduction of CO and MAP by 50%. In parallel, pulmonary artery pressure increased in all animals and was used to adjust the infusion of endotoxin in long term animals in study III, since extreme, acute pulmonary hypertension unevitably results in the death of the animal.
During un-resuscitated endotoxemia, the decrease in Q_{PV} was associated with an increase in Q_{HA}, thus maintaining total liver blood flow, illustrating the efficacy of the hepatic arterial buffer response (HABR). In studies I and IV, Q_{RA} remained unchanged in line with the kidneys autoregulatory capacity in the present arterial pressure range. No significant hemodynamic changes were observed in low ETX animals in study III.
In study II, bronchoalveolar lavage resulted in increased pulmonary arterial pressure and pulmonary vascular resistance. The pulmonary shunt fractions increased to a similar extent in both animals subjected to endotoxin (from 8± 3 to 24± 5 %) and lavage (from 9± 4 to 19± 7 %).

Volume resuscitation successfully reached the primary endpoint to restore CO in studies I, II and IV. During resuscitated endotoxemia, both Q_{PV} and Q_{HA} returned to values not significantly different from baseline, and Q_{RA} remained unchanged.

Plasma concentrations of tPA (I-IV), PAI-1 (IV) and TNF-α (II-IV)

tPA

Regional differences in both total tPA (I, III and IV) and active tPA (IV) were found at baseline with the highest concentrations of tPA in portal venous plasma and the lowest in hepatic venous plasma (Table 2). The active fraction of tPA (IV) was highest in the portal vein (on average 50% of total tPA) and lowest in the hepatic vein (25% of total tPA) while a mean fraction around 30% was found in the aorta, pulmonary artery and renal vein.
Acute un-resuscitated endotoxemia resulted in increased plasma concentrations of tPA in all vascular beds with a peak at 90 to 120 minutes reaching systemic levels 15-fold above baseline (20-fold for active tPA, IV) with similar regional differences as observed at baseline (cf artery). The average active fraction of tPA remained highest in the portal vein (40%) and lowest in the hepatic vein (20%), while arterial and renal fractions were unchanged (30%). Notably, the active fraction of tPA in the pulmonary artery tended to increase (40%).

CO	Baseline	Unresuscitated	Resuscitated
I	4.7±0.3	2.1±0.2	7.2±0.7
II ETX	4.1±0.7	2.8±0.4	NA
II BAL	3.2±0.8	3.0±0.8	NA
III high ETX	3.6±0.3	2.9±0.1	3.8±0.4
III low ETX	3.9±0.2	4.0±0.3	4.6±0.4
IV	4.6±0.3	2.5±0.2	6.9±0.7
MAP	**Baseline**	**Unresuscitated**	**Resuscitated**
I	98±5	51±5	66±4
II ETX	98±5	62±7	NA
II BAL	98±3	92±6	NA
III high ETX	95±3	79±6	98±7
III low ETX	69±2	71±3	65±3
IV	96±5	64±6	64±4
Q_{PV}	**Baseline**	**Unresuscitated**	**Resuscitated**
I	1.37±0.24	0.64±0.08	1.03±0.19
II	NA	NA	NA
III high ETX	0.96±0.05	0.66±0.05	1.01±0.08
III low ETX	0.77±0.05	0.71±0.04	0.87±0.04
IV	1.21±0.14	0.63±0.08	0.93±0.14
Q_{HA}	**Baseline**	**Unresuscitated**	**Resuscitated**
I	0.32±0.05	0.52±0.14	0.26±0.04
II	NA	NA	NA
III high ETX	0.16±0.03	0.49±0.07	0.16±0.03
III low ETX	0.12±0.02	0.10±0.01	0.10±0.02
IV	0.29±0.06	0.65±0.16	0.27±0.03
Q_{RA}	**Baseline**	**Unresuscitated**	**Resuscitated**
I	0.17±0.02	0.15±0.04	0.14±0.02
II	NA	NA	NA
III	NA	NA	NA
IV	0.15±0.02	0.15±0.02	0.13±0.02

Table 1. Summary of hemodynamic variables in studies I-IV. All values are mean ± SEM. CO=cardiac output, MAP=mean arterial pressure, Q_{PV}=portal venous flow, Q_{HA}=hepatic arterial flow, Q_{RA}=renal arterial flow. NA = not analyzed. ETX=endotoxin, BAL=broncho-alveolar lavage. The un-resuscitated period corresponds to protocol time 90-120 minutes, and the resuscitated to 220-250 minutes.

ARTERIAL	**Baseline**	**Unresuscitated**	**Resuscitated**
I	11±2	98±12	27±2
II ETX	11±1	122±11	NA
II BAL	7.9±1	11±1	NA
III high ETX	11±1	111±8	32±4
III low ETX	9.9±1	27±5	14±2
IV	8.8±1	124±14	61±6
PULMONARY A	**Baseline**	**Unresuscitated**	**Resuscitated**
I	12±2	98±7	27±3
II ETX	10±1	84±7	NA
II BAL	7.4±1	8.5±0.1	NA
III high ETX	10±1	100±10	32±4
III low ETX	11±1	26±5	14±2
IV	10±1	129±13	62±7
PORTAL V	**Baseline**	**Unresuscitated**	**Resuscitated**
I	16±2	145±14	29±2
II	NA	NA	NA
III high ETX	14±2	145±14	35±4
III low ETX	14±1	33±6	17±3
IV	12±1	184±17	66±9
HEPATIC V	**Baseline**	**Unresuscitated**	**Resuscitated**
I	8.9±1	71±5	25±2
II	NA	NA	NA
III high ETX	7.8±1	69±6	30±4
III low ETX	8.2±1	15±2	11±2
IV	6.8±1	78±8	57±8
RENAL V	**Baseline**	**Unresuscitated**	**Resuscitated**
I	13±2	94±4	30±3
II	NA	NA	NA
III	NA	NA	NA
IV	9.5±1	115±16	61±8

Table 2. Summary of plasma concentrations of total tPA in the different vascular beds investigated in studies I-IV. All concentrations are mean ± SEM, $ng \cdot ml^{-1}$. NA=not analyzed, ETX=endotoxin, BAL=bronchoalveolar lavage. The un-resuscitated period corresponds to protocol time 90-120 minutes, and the resuscitated to 220-250 minutes.

Total tPA concentrations decreased following volume resuscitation but remained elevated at 2-fold baseline values throughout the protocol (I, III), still with the regional differences described above. The addition of noradrenaline during resuscitation was associated with a sustained increase in total tPA at levels 6-fold above baseline. Plasma concentrations of active tPA (IV) returned to baseline levels.

In study II, pulmonary and arterial concentrations of tPA increased in the ETX animals, (Table 2) whereas there were a slight, but not significant, increase in BAL animals and no changes in CTRL animals.

In study III, peak tPA concentrations were about five times higher in the high ETX group compared to the low ETX group. Peak concentrations of tPA in the low ETX group were about twice as high compared to the control group. In low ETX animals, tPA concentrations were not different from baseline and controls in the 6-18 hours period.

PAI-1

Total PAI-1 and active PAI-1 (IV) showed no significant differences in plasma concentrations among the vascular beds at baseline. Systemic total PAI-1 and active PAI-1 levels gradually increased 170 minutes after start of endotoxin infusion, during ongoing hemodynamic resuscitation, to levels about 15-fold and 30-fold, respectively, above baseline at 290 minutes.

TNF-α

The levels of TNF-α increased in all endotoxemic animals (II-IV) with a peak related to the dose of endotoxin (III) in the hypodynamic, hypotensive state, and then gradually returned towards baseline values following resuscitation. No significant changes were observed in control animals.

A contemporary correlation between peak TNF-α and tPA concentrations was demonstrated (II-IV) while late (290 min) PAI-1 levels correlated with peak TNF-α levels (110 min) (IV).

Regional fluxes of tPA (I-IV) and PAI-1 (IV)

tPA

A consistent release (positive net flux) of tPA across the mesenteric circulation and an uptake (negative net flux) of tPA across the hepatic circulation was observed (studies I, III, IV) (Table 3). Mesenteric release and hepatic uptake of tPA increased in hypodynamic, hypotensive endotoxemia and returned towards baseline during volume resuscitation.

In study III, the magnitude of mesenteric release and hepatic uptake of tPA in the high and low ETX groups related to the dose of endotoxin, but followed the same time pattern.

MESENTERIC	**Baseline**	**Unresuscitated**	**Resuscitated**
I	3.1±0.8	19±7	1.8±0.8
II	NA	NA	NA
III high ETX	2.2±0.6	24±10	1.9±1.0
III low ETX	2.1±0.3	3.1±0.6	1.9±0.5
IV	3.1±0.7	26±5	1.3±0.8
HEPATIC	**Baseline**	**Unresuscitated**	**Resuscitated**
I	-4.6±0.9	-39±8	-2.8±0.5
II	NA	NA	NA
III high ETX	-4.1±0.7	-43±7	-4.3±1
III low ETX	-3.2±0.4	-10±2	-4.1±1
IV	-4.2±0.7	-68±13	-4.6±2
PULMONARY	**Baseline**	**Unresuscitated**	**Resuscitated**
I	-5±3	5.7±40	-2.4±2
II ETX	1.4±1	98±29	NA
II BAL	0.4±1	0.3±1	NA
III high ETX	0±1	25±22	1.3±3
III low ETX	2.3±1	2.3±2	3.4±3
IV	-2.2±2	-7±9	1±7
RENAL	**Baseline**	**Unresuscitated**	**Resuscitated**
I	0.2±0.1	2.9±2	0.1±0.1
II	NA	NA	NA
III	NA	NA	NA
IV	0.1±0.03	-0.5±1	-0.1±0.1

Table 3. Summary of plasma concentrations of total tPA in the different vascular beds investigated in studies I-IV. All concentrations are mean ± SEM, $\mu g \cdot min^{-1}$. NA=not analyzed, ETX=endotoxin, BAL=bronchoalveolar lavage. The un-resuscitated period corresponds to protocol time 90-120 minutes, and the resuscitated to 220-250 minutes.

The mesenteric tPA release in both low ETX (r^2=0.58) and high ETX (r^2=0.97) groups and the hepatic tPA uptake in the high ETX group (r^2=0.99) correlated to TNF-α in un-resuscitated endotoxemia (at 90 minutes, study III).

Similar to total tPA, a consistent mesenteric release, and a hepatic uptake, of active tPA was demonstrated in study IV. Plasma fluxes of active tPA occurred in parallel to total tPA. However, the peak responses during un-resuscitated endotoxemia lasted for shorter periods of time compared to the total tPA. The peak mesenteric release of total tPA, but not active tPA, correlated with the TNF-α concentration (r^2=0.69 at 110 min). The peak hepatic uptake correlated to the peak TNF-α level (r^2=0.73 for total tPA and r^2=0.70 for active tPA at 110 min).
In study II there was a pulmonary release of tPA in endotoxemic animals while fluxes remained unchanged in lavage and control animals. This is consistent with the findings of pulmonary tPA release in the high ETX group in study III. However, no changes were observed in the low ETX and control groups and no significant pulmonary flux of tPA was observed in studies I and IV.
No significant net fluxes of tPA (I, IV) were observed in the renal vascular bed.

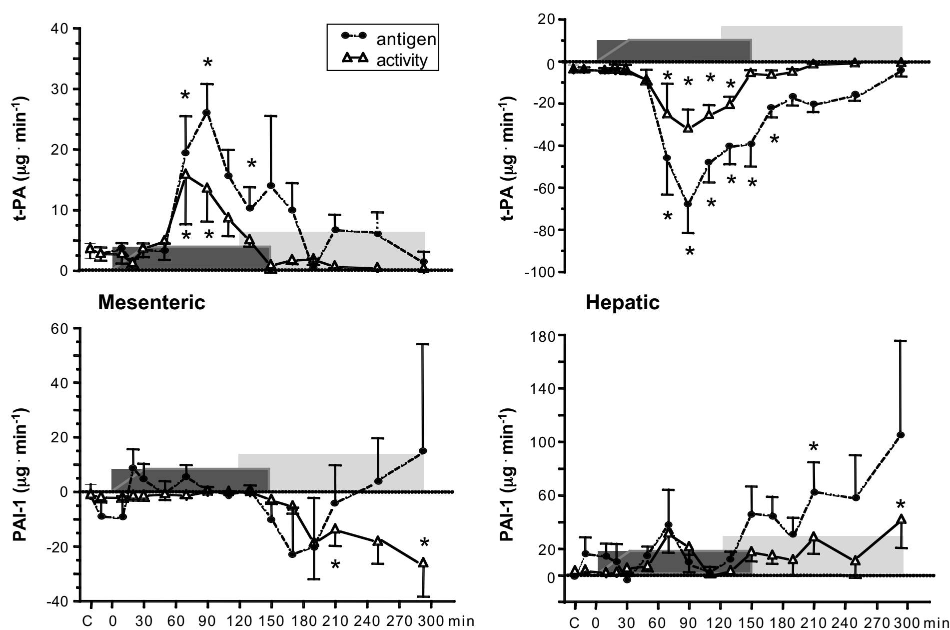

Figure 1. Fluxes of total and active tPA (top panel), and total and active PAI-1 (lower panel) in the mesenteric (left) and hepatic (right) circulations. * = $p<0.05$ by two-way ANOVA. Values are mean±SEM, n=8.

PAI-1

A late hepatic release of total PAI-1 and active PAI-1 was observed in response to endotoxemia in study IV. The hepatic release of total PAI-1 at 210 minutes correlated with the peak TNF-α levels at 110 minutes (r^2=0.59).

At the end of the protocol, a mesenteric uptake of active, but not total PAI-1, was observed along with a pulmonary uptake of total PAI-1. No correlations were found between uptake of PAI-1 and peak TNF-α levels.

No significant net fluxes of PAI-1 (IV) were observed in the renal vascular bed.

Molar ratios of tPA and PAI-1 (IV)

At baseline the mesenteric and hepatic tPA/PAI-1 molar ratio was anti-fibrinolytic. The molar excess of total PAI-1 to total tPA and active PAI-1 to active tPA in mesenteric arterial inflow, was decreased in portal venous outflow due to mesenteric net release of tPA. The subsequent hepatic uptake of tPA increased the molar excess of total PAI-1 to total tPA and active PAI-1 to tPA, respectively, in the hepatic venous outflow.

During the hypodynamic, un-resuscitated endotoxemia, the tPA/PAI-1 balance reflected a pro-fibrinolytic state with a molar excess of total tPA to total PAI-1 and active tPA to PAI-1, most evident in the portal venous outflow. The molar excess of total tPA remained in the hepatic venous outflow, although significantly attenuated, due to hepatic extraction of tPA. In contrast, hepatic extraction of active tPA resulted in a molar excess of active PAI-1 in the hepatic vein.

In hyperdynamic, resuscitated endotoxemia, the molar tPA/PAI-1 ratio was markedly anti-fibrinolytic with a substantial molar excess of total PAI-1 to total tPA and active PAI-1 to active tPA in mesenteric arterial inflow, portal venous and hepatic venous outflow.

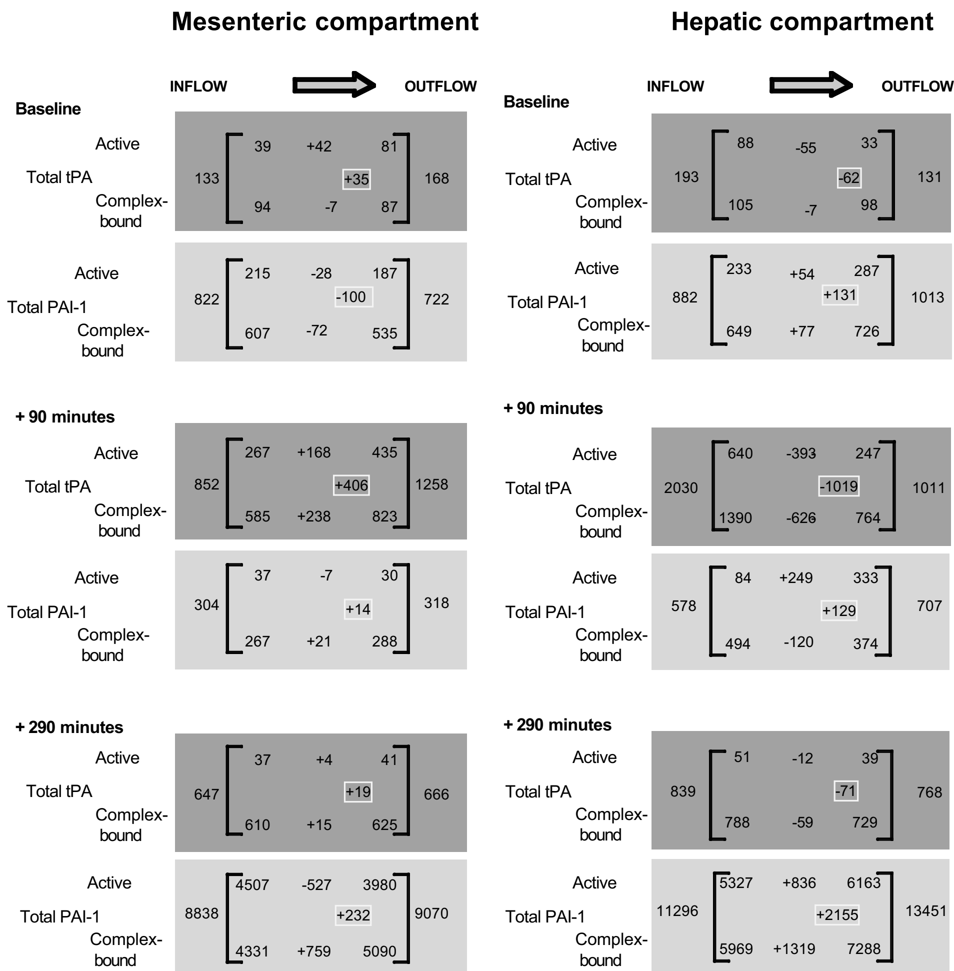

Figure 2. Local molar (pmoles) changes of tissue plasminogen activator (tPA) and plasminogen activator inhibitor 1 (PAI-1) in inflowing and outflowing plasma across the mesenteric and the hepatic vascular compartments at baseline (top panel) and at 90 (middle panel) and 290 minutes (lower panel) following administration of endotoxin. Each set of three values at brackets represent: complex-bound tPA and complex bound/latent PAI-1 (bottom value), active (top value) and total antigen (middle value) of tPA and PAI-1. The middle white box summarizes the calculated net molar release/uptake across the mesenteric and hepatic vascular compartments. All values are mean, n=8.

DISCUSSION

These studies were designed to address the basic mechanisms of early changes in key mediators of fibrinolysis in organs typically affected during sepsis and the progression to septic shock. In general, a procoagulatory hemostatic profile is observed in critically ill patients with established septic insults and this is regarded as a pivotal feature of microthrombotic ischaemic organ damage. Intravascular fibrinolysis is an essential pathway to protect against microvascular thrombotic occlusion impeding perfusion both in the physiological state and particularly during conditions with excessive formation of microthrombi. Tissue plasminogen (tPA) is the most important activator of fibrinolysis and has previously been extensively studied at the systemic level both experimentally and clinically in conditions relevant for sepsis. The actions of tPA are inhibited by serine protease inhibitors, of which plasminogen activator inhibitor type 1 (PAI-1) predominate. Similar to tPA, the majority of studies have focused on systemic arterial levels only.
The thesis demonstrates for the first time the regional differentiation of plasma fluxes of tPA and PAI-1 during acute endotoxemia in pigs, focusing on the mesenteric and hepatic circulations. The pulmonary plasma fluxes of tPA in relation to the pathogenesis of acute lung injury were also investigated and the impact of resuscitation by intravascular volume expansion and the administration of vasopressor during endotoxemic circulatory failure was highlighted. In addition, time and dose relations of endotoxemia to plasma tPA fluxes were evaluated and a compartmental model of hepatomesenteric tPA/PAI-1 molar ratios was devised.

A similar experimental setup was used in all studies, with the addition of bronchoalveolar lavage in study II. The experimental model and the regional fluxes of total tPA antigen as well as some methodological considerations are therefore discussed first, followed by a discussion of the specific results in studies II-IV.

Splanchnic and pulmonary plasma fluxes of tPA

Similar to previous studies [97-99] a marked increase in arterial plasma levels of tPA was observed in the endotoxemic, hypodynamic circulatory state. Arterial levels of tPA have been reported to correlate to the severity of sepsis and the levels in the present thesis are congruent with those found in patients with severe sepsis and septic shock [100, 101]. However, a substantial variation in plasma concentrations of tPA was demonstrated in the circulations investigated with portal venous plasma levels consistently being highest and hepatic venous levels lowest. This is in line with previous observations both in pigs and humans [75, 77, 82]. The biological significance of these differences becomes even more apparent when analyzed in terms of regional plasma fluxes of tPA, thus taking the blood flow into account. In the hypodynamic state, 20 $\mu g \cdot min^{-1}$ of tPA was released from the mesenteric organs. To put this

figure in perspective, the recommended dose following bolus administration of recombinant tPA (alteplase) in patients with acute myocardial infarction is 0.5 mg per kg body weight and hour. In the endotoxemic animals, the mesenteric organs alone released 0.05 mg per kg body weight and hour, thus one tenth of the therapeutic dose of tPA for thrombolysis in man.
It is thus obvious that some mechanism must exist to moderate the impact of this mesenteric release of tPA on the systemic circulation, otherwise a profoundly fibrinolytic state would soon develop. In fact, this thesis demonstrated that the hepatic uptake of tPA increased in parallel to the mesenteric release so that along with the 10-fold increase in preportal tPA release, hepatic uptake increased 12-fold in the hypodynamic state. A substantial mesenteric release of tPA and a concurrent hepatic uptake of tPA was uniformly demonstrated in all studies of endotoxemia (see also study III and IV).

Mesenteric release of tPA
Several stimuli act to release tPA from the vascular endothelium including rheological factors such as shear force, hypoxia, acidosis, vasoactive agents such as vasopressin and catecholamines [102], and mediators of inflammation such as tumor necrosis factor α (TNF-α) [103-104]. All these stimuli would be operative during endotoxemia and in particular in the hypodynamic circulatory state. Approximately 10% of the total amount of circulating tPA originated from the mesenteric circulation at the peak response 90 minutes following the start of endotoxin infusion. Given the vast endothelial surface in the mesenteric organs, the substantial release of tPA may not seem unexpected. The capillary surface area of the mesenteric organs receives one quarter of the normal cardiac output and represents the zone of pressure transition from the high pressure arterial inflow to the low pressure portal ouflow. Obviously, the prevention of microthrombotic circulatory obstruction is vital to preserve hemodynamic stability. Hence, a preferential, regionally increased capacity to release tPA appears physiologically logical. Furthermore, the ratio of active tPA to total tPA antigen was highest in the portal vein (see also Study IV), emphasizing the fibrinolytic capacity in the mesenteric circulation. No data exist to support that the stimuli mentioned above would exert selective effects in the mesenteric circulation. In addition to endothelial release of tPA, it has been suggested that sympathetic nerve endings might also release tPA into small artery walls and the microcirculation [105-106]. The mesenteric circulation receives a major proportion of total sympathetic outflow in both pigs and humans [107-108]. This mechanism might further contribute to the considerable mesenteric release of tPA both in the normal state and during circulatory failure with increased sympathetic activity, notably in the gastrointestinal region.
Previous studies corroborate the findings in this thesis regarding the mesenteric release of tPA. The application of positive end-expiratory pressure (PEEP) in pigs resulted in decreased portal venous flow and a marked increase in the release of both total and active tPA [77]. In a model of non-occlusive intestinal ischaemia in the pig, a tendency for the tPA/PAI-1 complex to increase during ischaemia was reported [109]. The importance of an intact mesenteric

fibrinolytic capacity is demonstrated by extensive intestinal fibrin depositions in tPA-deficient knock-out mice subjected to infusion of endotoxin [110]. In addition to its role in fibrinolysis, tPA has been demonstrated to be an important part of the protective immune response to abdominal sepsis [111]. The mesenteric release of tPA most likely involves several functions that would warrant further study, beyond the scope of this thesis.

Volume resuscitation to restore baseline cardiac output resulted in a decrease in mesenteric tPA release that returned to pre-endotoxemia levels within one hour. Notably, portal venous plasma levels of tPA remained significantly elevated at 5- to 2-fold the baseline level for the duration of the experiments. This indicates that although no significant arteriovenous tPA gradient was demonstrated to support mesenteric release of tPA, other sources of tPA outside the mesenteric organs contributed to maintain the high levels of tPA. No evidence for the renal nor the pulmonary circulations to provide this prolonged release of tPA was found (see below). The reason(s) why volume resuscitation decreased mesenteric tPA release are likely by reversal and removal of several of the stimuli for tPA release cited above. Accordingly, the results in study IV, demonstrating how TNF-α levels decreased in response to hemodynamic resuscitation, illustrate the dynamics of withdrawing a mediator of endothelial tPA release.
Neither does it seem likely that the endotoxin challenge resulted in deprivation of the total endothelial storage pool of tPA, since the time pattern was similar to the one observed in low dose, resuscited endotoxemia (see study III). Other studies in humans and experimental animals have demonstrated an early transient increase in arterial tPA levels in response to endotoxemia [69, 99]. The intravascular volume expansion may theoretically have decreased plasma levels of tPA by hemodilution. Only a minor decrease in hematocrit was observed which makes this possibility very unlikely. In addition, hemodilution would equally affect arterial and venous plasma levels and thus not affect the transorgan fluxes *per se*.

Hepatic uptake of tPA

The hepatic venous plasma concentrations of tPA were consistently lowest in all experimental series (see also studies III and IV). The hepatic uptake of tPA constituted more than 10% of the total circulating amount of tPA during the peak responses at 90 minutes following the start of endotoxin infusion. The total hepatic blood flow remained unchanged overall from baseline to hypodynamic, un-resuscitated endotoxemic circulatory failure and to the hyperdynamic, resuscitated state. The preservation of liver blood flow was the result of the hepatic arterial buffer response, resulting in a reciprocal increase in hepatic arterial blood flow when portal venous blood flow decreases [112, 113]. Despite the absence of changes in total liver perfusion, hepatic uptake of tPA varied significantly in the course of endotoxemia. This underlines the importance of the liver to maintain systemic homeostatis by moderating the impact of mesenteric tPA release in an active supply-demand way rather than a passive flow-dependent, fixed extraction mode. This is also supported by the results in study III (see below)

where a similarly effective hepatic uptake of tPA was observed in the setting of varying hepatic inflow of tPA and liver blood flow.

In strict hemodynamic terms, the liver represents a conduit of blood from a combined low pressure (portal vein) and high pressure (hepatic artery) inflow to a low pressure (hepatic vein) outflow. The liver sinusoidal capillary area receives a major proportion of resting cardiac output across a pressure gradient of approximately 5 mm Hg, and any thrombotic interruption of this flow would severely impair venous return and thus cardiac output. The lack of hepatic release of tPA may thus seem surprising and the hepatic uptake of tPA could be viewed as reducing hepatic fibrinolytic capacity. It should be noted that the arteriovenous sampling employed in the present studies does not allow the detection of simultaneous intra-organ release and uptake. The liver may thus have an upstream sinusoidal secretion of tPA, to support fibrinolysis, and a downstream sinusoidal extraction of tPA, to prevent systemic "spillover" of tPA, that would go undetected using the methodology of this thesis. However, other studies have indicated that hepatocytes are virtually devoid of tPA and more likely other systems exert anti-thrombotic actions in the hepatic microcirculation, e.g. nitric oxide and prostacyclin.

Renal plasma fluxes of tPA

This thesis provides no evidence for a significant renal release nor uptake of tPA into the plasma compartment. Renal venous plasma tPA levels were not significantly different from the arterial levels. Renal blood flow was maintained throughout the experiments in both study I and IV, consistent with effective autoregulation of renal perfusion in the arterial pressure range of the studies. A non-significant trend towards increased renal release of tPA in study I was not found in study IV. Biomarkers of endothelial dysfunction have been studied in patients developing acute renal failure (ARF) as a consequence of infectious or non-infectious systemic inflammatory response syndrome (SIRS). No significant differences in plasma tPA levels, that were within the same range as observed in the present endotoxemic model, were found in SIRS patients irrespective of origin [114]. These results are consistent with the present conclusion that changes in renal tPA kinetics are not significantly associated with the endotoxemic insult.

Pulmonary plasma fluxes of tPA

This thesis demonstrated a variable transpulmonary (pulmonary arterial to aortic arterial) plasma concentration gradient for tPA. Significant transpulmonary increases in plasma tPA levels were demonstrated in studies II and III while this increase failed to attain statistical significance in studies I and IV. Substantial amounts of active tPA and total antigen have been demonstrated in rat pulmonary tissue [74]. A constitutive expression of tPA in the endothelium of the bronchial circulation has been demonstrated that was mostly absent in the pulmonary circulation [115]. The arteriovenous sampling technique applied in this thesis does not allow

for the separation of the bronchial and pulmonary circulations, and differences in the latter may confound the results. Furthermore, an inflammatory stimulus (hyperoxia) only increased the expression of tPA protein in pulmonary vessels between 7 to 30µm in diameter [115]. An increased heterogeneity of pulmonary perfusion, as indicated by the increased venous admixture consistently observed in the studies of this thesis, may thus obscure the transpulmonary plasma tPA gradient if vessels of varying size and/or anatomical location influence the release of tPA into the plasma compartment. In previous studies in pigs, no significant step-up in pulmonary arterial to aortic plasma tPA concentration was reported [82]. The calculation of pulmonary net flux of tPA includes cardiac output as a factor. Such a large factor of flow in the equation amplify the arteriovenous concentration gradient when represented as a flux and introduces a variability to be considered in the statistical evaluation. Still, the lack of consistency in both transpulmonary concentration gradients and fluxes throughout studies I-IV indicate that pulmonary plasma kinetics of tPA warrant further investigation (see also Methodological considerations below and study II).

Pulmonary plasma fluxes of tPA in acute lung injury

Perturbations in pulmonary gas exchange are early and frequent pathological events in septic patients. Based on the severity of ventilatory failure, the condition is referred to as acute lung injury (ALI) or acute respiratory distress syndrome (ARDS). The pathogenesis of ALI is used to delineate primary ALI in case of pulmonary origin (e.g. aspiration, pneumonia, inhalation injury, contusion) and secondary ALI in case of extrapulmonary origin (sepsis, burn, pancreatitis, major surgery and massive transfusion). In this study, bronchoalveolar lavage leading to surfactant depletion was used as a model of primary ALI and infusion of endotoxin was used to establish secondary ALI.

Although a protective ventilatory strategy has been successful in reducing mortality in ALI/ ARDS, it still remains high at more than 50% [116]. Numerous trials of therapies to modulate the inflammatory response in ALI have proven ineffective. Recently, attention has been focused on changes in coagulation and fibrinolysis in ALI. Previous studies have mainly addressed alterations of coagulation in the alveolar compartment [117, 118]. This study focused on intravascular changes by assessing the pulmonary plasma flux of tPA in primary and secondary ALI. Low plasma levels of protein C and high levels of PAI-1 have been reported to be strong independent risk factors for mortality and adverse clinical outcomes in ALI/ARDS [80]. Mediators of fibrinolysis were not analyzed to expand the interpretation of this procoagulant profile. Several reports have indicated the potential of anticoagulant therapy in ALI, although the results for clinical trials remain equivocal [119]. As discussed above, high concentrations of tPA has been demonstrated in pulmonary tissue, with a distinct anatomical pattern of distribution.

Several proinflammatory cytokines including TNF-α, interleukins 1 and 6 are released early in the inflammatory response and interact with the coagulation system. In this study, TNF-α was studied to correlate the inflammatory response in ALI to plasma fluxes of tPA. Admittedly, TNF-α only represents one out of a multitude of potentially important mediators linking the inflammatory and coagulation systems. TNF-α was chosen in this study since it is generally accepted as an early cytokine [120] of inflammation that is directly linked to the actions of endotoxin [121]. TNF-α has also been demonstrated to play dual roles in the way that it both stimulates endothelial tPA release [104] and promotes thrombogenic endothelial surface properties [31].

A similar magnitude of ventilatory dysfunction as gauged by venous admixture was observed in animals subjected to lavage or endotoxemia. Pulmonary vasoconstriction and hypertension developed in both groups, in particular in endotoxemic animals. The settings for mechanical ventilation and inspired oxygen, that may both modulate the inflammatory response, were the same in both groups. However, a pulmonary release of tPA that correlated with increased TNF-α levels was only found in endotoxemic animals. The pulmonary release of tPA in endotoxemic animals can be explained by increased stimulation by TNF-α as well as an increased adrenergic, vasoconstrictor tone. Assuming that the increase in pulmonary vascular resistance is related to formation of microthrombi, as previously reported in ALI, the release of tPA in endotoxemic animals did not positively influence this process.
The limited time span in the study only allows for evaluation of acute changes and was chosen based on previous observations of the peak tPA response in endotoxemic animals (study I). It remains possible that animals subjected to lavage would develop an inflammatory response and increased tPA levels as ALI progresses with increased alveolar permeability and exposure to alveolar coagulation products. Increased levels of PAI-1, inhibiting the actions of tPA, are reported in ALI and septic patients in the first days of the disease. Thus, increased PAI-1 levels rather than changes in tPA plasma fluxes may be more important to determinate outcome in ALI [80]. As observed in study IV, plasma levels of PAI-1 do increase in endotoxemic animals. The pulmonary uptake of PAI-1 demonstrated is also consistent with increased formation of tPA/PAI-1 complexes in the lung microvasculature. Finally, pulmonary arterial levels of tPA transiently increased early in low-dose endotoxemic animals (see study III) but then returned to baseline levels for the duration of the protocol (18 hrs). These results suggest that modulating the tPA plasma levels may be a part of a very early strategy for ALI in the setting of systemic inflammation such as sepsis. Interventions aimed at the later phase when PAI-1 levels predominate would likely be clinically more relevant. Indeed, a clinical trial of protein C administration in ALI/ARDS patients is ongoing, targeting the anti-inflammatory and anti-coagulatory (mainly opposing PAI-1) properties of the drug.

As discussed above, the pulmonary flux of tPA varied between studies in this thesis. This was also noted in the individual pulmonary tPA flux during endotoxemia with four pigs demonstrating marked increases in pulmonary tPA release while moderate increases were observed in the remaining four animals. Studies in humans have demonstrated significant differences in resting steady state release rates of tPA related to genetic polymorphism for the tPA gene [122, 123]. Such genetic polymorphism may influence the tPA fluxes also in pigs, although genetic diversity is limited in the domestic landrace.
The results of the ALI experiments should principally be viewed in the context of primary and secondary ALI, providing basic insights into the marked differences in plasma tPA kinetics. The mediators of pulmonary intravascular coagulation and fibrinolysis warrant further investigation.

Time and dose effects of endotoxemia on plasma tPA fluxes
Infusion of endotoxin is a widely utilized model to study pathogenetic mechanisms of the systemic inflammatory response syndrome (SIRS) and sepsis including cardiovascular changes, cytokine release and coagulopathy. In pigs, the hemodynamic response to infusion of endotoxin is characteristically biphasic with an initial hypodynamic phase (1-3 hours) followed by a hyperdynamic phase (8-12 hours). Extended protocols are cumbersome to design due to logistical and ethical aspects and may introduce confounding factors, eg. long term anaesthesia. Consequently, most studies are designed using high doses of endotoxin to establish the multiple features of sepsis in a relatively short period. Such designs may result in an endotoxemic “intoxication” and the relevance of the observations are confined to the early phase of sepsis only. Therefore, study III was designed to comparatively investigate tPA plasma fluxes using the standard high-dose endotoxin protocol as well as an extended, 18 hours low-dose endotoxin protocol. This study was made possible in collaboration with the Experimental Surgical Laboratory of the University of Bern.

The endothelial release of tPA is characterized by an acute, rapid secretion and a slower, sustained secretion related to up-gregulation of tPA-gene expression after six to eight hours [40]. Whereas the former pathway is assessable in the standard five hours protocol of this thesis, the latter requires an extended protocol time, still definitely within the 18 hours studied. In analogy to study II, the correlation between tPA plasma fluxes and the inflammatory response was investigated by analysis of plasma TNF-α levels.

Baseline tPA levels were the same in control, low-dose and high-dose endotoxemic animals in all vascular beds investigated, and within the same range as for study I, demonstrating the reproducibility of the model taking into account that experiments were performed at two different laboratories. Infusion of endotoxin increased mesenteric release and hepatic uptake of tPA in a dose-dependent pattern that correlated to the magnitude of the inflammatory

response as reflected by TNF-α levels. No differences were found in the time pattern of these changes between animals given a low-dose or a high-dose of endotoxin. Since plasma tPA concentrations in animals given low-dose endotoxin returned to levels similar to control animals, no support was found for a delayed release of tPA tentatively associated with an up-regulation of the tPA gene expression. This is in line with previous studies, showing no up-regulation of tPA mRNA in aortic walls of endotoxemic rats [124]. It should be noted that the plasma concentrations of tPA as well as TNF-α returned to levels similar to those measured in control animals. Taken together, the correlation between the peak tPA fluxes and TNF-α, and the lack of increased tPA plasma concentrations when TNF-α was normalized, provide indirect evidence for TNF-α as a key mediator linking the tPA release to the inflammatory response. Of course, other mediators, including inflammatory cytokines not studied, cannot be excluded. The results corroborate the findings in study IV where TNF-α levels decreased concurrent to reduced release of tPA into the plasma compartment. It therefore seems plausible that TNF-α, a key cytokine in the early response to endotoxemia, rather than endotoxin per se, determines endothelial release of tPA. Administration of TNF-α to human volunteers has been reported to increase hepatosplanchnic release of tPA [97]. However, prolonged stimulation (>24 hours) of human endothelial cells with TNF-α at 10 $ng{\cdot}mL^{-1}$ has been reported to suppress tPA gene expression [125].

Mediators linked to cardiovascular control, such as circulating catecholamines, would appear to be of subordinate importance in this study of low-dose endotoxemia, since tPA fluxes still increased while a normodynamic circulation was maintained.

The results in this study indicate a therapeutic window for manipulating the tPA response early in the course of endotoxemia. Later on, this response subsides and inhibitors of tPA such as PAI-1 (see also study IV) are more likely to be relevant targets for therapeutic strategies.

Splanchnic and pulmonary plasma fluxes of active/total tPA and PAI-1

In the final study of this thesis, analyses were extended to include both tPA and its main inhibitor PAI-1. In addition, the active fractions of both tPA and PAI-1 were specifically investigated. All results were combined in a compartmental model of hepatomesenteric pro- and anti-fibrinolytic balance, based on molar ratios of tPA and PAI-1, to provide a comprehensive, conceptual model of fibrinolytic capacity in early endotoxemia.

The systemic response to endotoxemia is characterized by acutely elevated concentrations of tPA, followed by a subsequent increase in PAI-1 levels [126]. Such a biphasic pattern has been reported in humans following administration of endotoxin [69]. Clinical studies have demonstrated that organ failure in sepsis correlates with changes in plasma concentrations of tPA-PAI-1 complex [127].

The plasma concentrations as well as the regional fluxes of total tPA antigen during endotoxemia were similar to those found in studies I-III. Importantly, concentrations and fluxes of active tPA also followed the same pattern, as previously reported for tPA release in the human forearm [73]. Significant correlations between active and total tPA antigen plasma concentrations were found in all vascular beds. Regional fluxes of active and total tPA antigen also correlated in the mesenteric and hepatic vascular beds where significant changes were observed during endotoxemia. Hence, although active tPA was not analyzed in studies I-III, no evidence was found to suggest that plasma total tPA concentrations would increase without a concomitant increase in the active fraction. The average fraction of active tPA to total tPA antigen in arterial plasma was approximately 30% and remained unchanged overall in the hypodynamic phase of endotoxemia. This fraction is slightly higher compared to reports in man of 15-20% [65]. An inverse correlation was found between active tPA and total PAI-1, in line with earlier reports [73], and thus the fraction of active tPA in arterial plasma decreased towards the end of the protocol.
The plasma half-time of active tPA has been reported to be slightly shorter (3.5 minutes) compared to total tPA antigen (4.5 min) [41]. In line with this, changes in hepatic uptake of active tPA during hypodynamic endotoxemia occurred in a shorter time span compared to the total tPA antigen.
Similar to study III, hepatomesenteric fluxes of active and total tPA antigen correlated to plasma levels of TNF-α.

The addition of noradrenaline to the resuscitation protocol in study IV was made in order to increase the similarity of the experimental model to the clinical management of septic shock. While no changes were observed in the regional fluxes of tPA in study IV compared to studies I-III, arterial plasma concentrations of total tPA antigen were elevated about 3-fold towards the end of the protocol compared to studies I and III. This demonstrates the importance of adrenergic stimulation on tPA release, which must have occurred outside the mesenteric, hepatic, pulmonary and renal circulations since fluxes in these regions returned to baseline levels at this point in time. Still, the parallel increase in PAI-l levels quenched the active tPA fraction.

A release of hepatic PAI-1 was demonstrated in the late phase of the protocol. The peak TNF-α levels correlated with both plasma PAI-1 levels and hepatic PAI-1 release rates with a time lag of approximately two hours. These results are supported by earlier data indicating that TNF-α acts as an important mediator of PAI-1 gene expression in the liver [128] also during endotoxemia [99]. The significance of the within subject analysis of correlation does not, however, infer any causal relationship [95]. These results do not preclude other mediators not studied as important in the release of PAI-1. A consistent and progressive increase in systemic PAI-1 plasma levels was noted before any corresponding hepatic net release of PAI-1. This

implies increased secretion, decreased clearance or both of PAI-1 outside the organs studied potentially involving other mediators than TNF-α. Candidate sources include vascular endothelium and adipocytes [129, 130].

The conceptual model of molar hepatomesenteric input and outflow [73] demonstrate the quite dramatic changes in tPA/PAI-1 equilibrium that occur during early endotoxemia. The baseline tPA/PAI-1 balance favouring anti-fibrinolysis was reversed into a tPA/PAI-1 balance in favour of fibrinolysis during hypodynamic endotoxemia. Still, the efficient hepatic extraction of tPA moderated the 7.6-fold molar excess of active tPA to PAI-1 in the combined portal venous and hepatic arterial inflows to a 1.3-fold molar excess of active PAI-1 to tPA in the hepatic venous outflow. This clearly illustrates the importance of the liver to prevent a profoundly pro-fibrinolytic systemic state in endotoxemic circulatory failure, as also indicated in studies I and III. Finally, hemodynamic resuscitation restored mesenteric and hepatic tPA fluxes to baseline, that along with a mesenteric uptake and a hepatic release of PAI-1, resulted in a markedly anti-fibrinolytic tPA/PAI-1 balance, as typically reported in clinical studies of patients with established septic shock [21, 131].

Importantly, the dynamics of these events were impossible to discern based on analyses of systemic changes.

CONCLUSIONS

Acute, hypodynamic endotoxemia is associated with a mesenteric release and a hepatic uptake of total and active tPA, whereas renal tPA fluxes do not change. An increased pulmonary flux of tPA was observed, but significant variations in the flux during endotoxemia preclude unequivocal conclusion to be made.

Hemodynamic resuscitation by intravascular volume expansion to a normo- or hyperdynamic circulatory state restores regional plasma tPA fluxes to baseline levels. The addition of noradrenaline during resuscitation is associated with increased concentrations of tPA whereas regional fluxes of tPA do not increase.

The magnitude of the response in mesenteric and hepatic fluxes of tPA, but not the time course of changes, is related to the dose of endotoxin in the first six hours when comparing acute high-dose and extended low-dose experiments. No delayed release of tPA, tentatively related to up-regulation of tPA gene expression, occur in the following twelve hours in animals given the extended low-dose.

Resuscitated hyperdynamic endotoxemia is associated with a hepatic release of both total and active PAI-1, whereas no release occurs in the mesenteric, pulmonary or renal circulations. The molar ratios of tPA to PAI-1 in the mesenteric and hepatic plasma compartments change substantially during acute endotoxemia, particularly in terms of pro-fibrinolytic mesenteric changes in the hypodynamic phase and anti-fibrinolytic hepatic changes in the hyperdynamic phase.

Bronchoalveolar lavage as a model of primary acute lung injury is not associated with any increase in transpulmonary concentrations or fluxes of tPA, in contrast to the increased tPA levels observed in acute endotoxemia as a model of secondary acute lung injury.

Regional plasma concentrations, as well as mesenteric release and hepatic uptake of total and active tPA, correlate to arterial TNF-α levels during acute hypodynamic endotoxemia. Hepatic release of PAI-1 in hyperdynamic endotoxemia correlates to plasma levels of TNF-α in hypodynamic endotoxemia. The latter results suggest an association but no cause-effect relation can be directly inferred.

REFERENCES

1. Porter JR: Antony van Leeuwenhoek: tercentenary of his discovery of bacteria. *Bacteriol Rev* 1976, 40(2):260-269.
2. Tillett WS, Garner RL: The fibrinolytic activity of hemolytic streptococci. *J Exper Med* 1933, 58:485.
3. Astrup T, Permin PM: Fibrinolysis in the Animal Organism. *Nature* 1947, 159:681-682.
4. Brakman P, Mohler ER, Jr., Astrup T: A group of patients with impaired plasma fibrinolytic system and selective inhibition of tissue activator-induced fibrinolysis. *Scand J Haematol* 1966, 3(5):389-398.
5. Loskutoff DJ, van Mourik JA, Erickson LA, Lawrence D: Detection of an unusually stable fibrinolytic inhibitor produced by bovine endothelial cells. *Proc Natl Acad Sci U S A* 1983, 80(10):2956-2960.
6. van Mourik JA, Lawrence DA, Loskutoff DJ: Purification of an inhibitor of plasminogen activator (antiactivator) synthesized by endothelial cells. *J Biol Chem* 1984, 259(23):14914-14921.
7. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med* 2003, 31(4):1250-1256.
8. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR: Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med* 2001, 29(7):1303-1310.
9. Vincent JL, Sakr Y, Sprung CL, Ranieri VM, Reinhart K, Gerlach H, Moreno R, Carlet J, Le Gall JR, Payen D: Sepsis in European intensive care units: results of the SOAP study. *Crit Care Med* 2006, 34(2):344-353.
10. Karlsson S, Varpula M, Ruokonen E, Pettilä V, Parviainen I, Ala-Kokko TI, Kolho E, Rintala EM: Incidence, treatment, and outcome of severe sepsis in ICU-treated adults in Finland: the Finnsepsis study. *Intensive Care Med* 2007, 33(3):435-443.
11. Finfer S, Bellomo R, Lipman J, French C, Dobb G, Myburgh J: Adult-population incidence of severe sepsis in Australian and New Zealand intensive care units. *Intensive Care Med* 2004, 30(4):589-596.
12. Flaatten H: Epidemiology of sepsis in Norway in 1999. *Crit Care* 2004, 8(4):R180-184.
13. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, Suppes R, Feinstein D, Zanotti S, Taiberg L *et al*: Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med* 2006, 34(6):1589-1596.
14. Friedman G, Silva E, Vincent JL: Has the mortality of septic shock changed with time. *Crit Care Med* 1998, 26(12):2078-2086.
15. Casey LC, Balk RA, Bone RC: Plasma cytokine and endotoxin levels correlate with survival in patients with the sepsis syndrome. *Ann Intern Med* 1993, 119(8):771-778.
16. Danner RL, Elin RJ, Hosseini JM, Wesley RA, Reilly JM, Parillo JE: Endotoxemia in human septic shock. *Chest* 1991, 99(1):169-175.
17. Opal SM, Scannon PJ, Vincent JL, White M, Carroll SF, Palardy JE, Parejo NA, Pribble JP, Lemke JH: Relationship between plasma levels of lipopolysaccharide (LPS) and LPS-binding protein in patients with severe sepsis and septic shock. *J Infect Dis* 1999, 180(5):1584-1589.
18. Antal-Szalmas P, Strijp JA, Weersink AJ, Verhoef J, Van Kessel KP: Quantitation of surface CD14 on human monocytes and neutrophils. *J Leukoc Biol* 1997, 61(6):721-728.

19. Hailman E, Lichenstein HS, Wurfel MM, Miller DS, Johnson DA, Kelley M, Busse LA, Zukowski MM, Wright SD: Lipopolysaccharide (LPS)-binding protein accelerates the binding of LPS to CD14. *J Exp Med* 1994, 179(1):269-277.
20. Annane D, Bellissant E, Cavaillon JM: Septic shock. *Lancet* 2005, 365(9453):63-78.
21. Zeerleder S, Hack CE, Wuillemin WA: Disseminated intravascular coagulation in sepsis. *Chest* 2005, 128(4):2864-2875.
22. Esmon CT: The interactions between inflammation and coagulation. *Br J Haematol* 2005, 131(4):417-430.
23. Mayr FB, Jilma B: Coagulation interventions in experimental human endotoxemia. *Transl Res* 2006, 148(5):263-271.
24. Morel O, Toti F, Hugel B, Bakouboula B, Camoin-Jau L, Dignat-George F, Freyssinet JM: Procoagulant microparticles: disrupting the vascular homeostasis equation? *Arterioscler Thromb Vasc Biol* 2006, 26(12):2594-2604.
25. Nieuwland R, Berckmans RJ, McGregor S, Boing AN, Romijn FP, Westendorp RG, Hack CE, Sturk A: Cellular origin and procoagulant properties of microparticles in meningococcal sepsis. *Blood* 2000, 95(3):930-935.
26. Nesheim M: Thrombin and fibrinolysis. *Chest* 2003, 124(3 Suppl):33S-39S.
27. Hinshaw LB: Sepsis/septic shock: participation of the microcirculation: an abbreviated review. *Crit Care Med* 1996, 24(6):1072-1078.
28. Bernard GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez-Rodriguez A, Steingrub JS, Garber GE, Helterbrand JD, Ely EW *et al*: Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med* 2001, 344(10):699-709.
29. Abraham E, Reinhart K, Opal S, Demeyer I, Doig C, Rodriguez AL, Beale R, Svoboda P, Laterre PF, Simon S *et al*: Efficacy and safety of tifacogin (recombinant tissue factor pathway inhibitor) in severe sepsis: a randomized controlled trial. *Jama* 2003, 290(2):238-247.
30. Baudo F, Caimi TM, de Cataldo F, Ravizza A, Arlati S, Casella G, Carugo D, Palareti G, Legnani C, Ridolfi L *et al*: Antithrombin III (ATIII) replacement therapy in patients with sepsis and/or postsurgical complications: a controlled double-blind, randomized, multicenter study. *Intensive Care Med* 1998, 24(4):336-342.
31. Levi M, ten Cate H, van der Poll T: Endothelium: interface between coagulation and inflammation. *Crit Care Med* 2002, 30(5 Suppl):S220-224.
32. Hack CE, Zeerleder S: The endothelium in sepsis: source of and a target for inflammation. *Crit Care Med* 2001, 29(7 Suppl):S21-27.
33. Miles LA, Plow EF: Binding and activation of plasminogen on the platelet surface. *J Biol Chem* 1985, 260(7):4303-4311.
34. Miles LA, Ginsberg MH, White JG, Plow EF: Plasminogen interacts with human platelets through two distinct mechanisms. *J Clin Invest* 1986, 77(6):2001-2009.
35. Gao SW, Morser J, McLean K, Shuman MA: Differential effect of platelets on plasminogen activation by tissue plasminogen activator, urokinase, and streptokinase. *Thromb Res* 1990, 58(4):421-433.
36. Lijnen HR, Collen D: Endothelium in hemostasis and thrombosis. *Prog Cardiovasc Dis* 1997, 39(4):343-350.
37. Dano K, Andreasen PA, Grondahl-Hansen J, Kristensen P, Nielsen LS, Skriver L: Plasminogen activators, tissue degradation, and cancer. *Adv Cancer Res* 1985, 44:139-266.
38. van den Eijnden-Schrauwen Y, Kooistra T, de Vries RE, Emeis JJ: Studies on the acute release of tissue-type plasminogen activator from human endothelial cells in

vitro and in rats in vivo: evidence for a dynamic storage pool. *Blood* 1995, 85(12):3510-3517.

39. Huber D, Cramer EM, Kaufmann JE, Meda P, Masse JM, Kruithof EK, Vischer UM: Tissue-type plasminogen activator (t-PA) is stored in Weibel-Palade bodies in human endothelial cells both in vitro and in vivo. *Blood* 2002, 99(10):3637-3645.
40. Kooistra T, Schrauwen Y, Arts J, Emeis JJ: Regulation of endothelial cell t-PA synthesis and release. *Int J Hematol* 1994, 59(4):233-255.
41. Chandler WL, Alessi MC, Aillaud MF, Henderson P, Vague P, Juhan-Vague I: Clearance of tissue plasminogen activator (TPA) and TPA/plasminogen activator inhibitor type 1 (PAI-1) complex: relationship to elevated TPA antigen in patients with high PAI-1 activity levels. *Circulation* 1997, 96(3):761-768.
42. Wall U, Jern S, Tengborn L, Jern C: Evidence of a local mechanism for desmopressin-induced tissue-type plasminogen activator release in human forearm. *Blood* 1998, 91(2):529-537.
43. Jern S, Selin L, Bergbrant A, Jern C: Release of tissue-type plasminogen activator in response to muscarinic receptor stimulation in human forearm. *Thromb Haemost* 1994, 72(4):588-594.
44. Klöcking HP, Markwardt F: Pharmacological stimulation of t-PA release. *Pharmazie* 1994, 49(4):227-230.
45. Stein CM, Brown N, Vaughan DE, Lang CC, Wood AJ: Regulation of local tissue-type plasminogen activator release by endothelium-dependent and endothelium-independent agonists in human vasculature. *J Am Coll Cardiol* 1998, 32(1):117-122.
46. von Kanel R, Dimsdale JE: Effects of sympathetic activation by adrenergic infusions on hemostasis in vivo. *Eur J Haematol* 2000, 65(6):357-369.
47. Sjögren LS, Gan L, Doroudi R, Jern C, Jungersten L, Jern S: Fluid shear stress increases the intra-cellular storage pool of tissue-type plasminogen activator in intact human conduit vessels. *Thromb Haemost* 2000, 84(2):291-298.
48. Sugiki M, Omura S, Yoshida E, Itoh H, Kataoka H, Maruyama M: Downregulation of urokinase-type and tissue-type plasminogen activators in a rabbit model of renal ischemia/reperfusion. *J Biochem (Tokyo)* 2002, 132(3):501-508.
49. Nilsson T, Wallen P, Mellbring G: In vivo metabolism of human tissue-type plasminogen activator. *Scand J Haematol* 1984, 33(1):49-53.
50. Einarsson M, Smedsrød B, Pertoft H: Uptake and degradation of tissue plasminogen activator in rat liver. *Thromb Haemost* 1988, 59(3):474-479.
51. Porte RJ, Bontempo FA, Knot EA, Lewis JH, Kang YG, Starzl TE: Tissue-type-plasminogen-activator-associated fibrinolysis in orthotopic liver transplantation. *Transplant Proc* 1989, 21(3):3542.
52. Smedsrød B, Einarsson M: Clearance of tissue plasminogen activator by mannose and galactose receptors in the liver. *Thromb Haemost* 1990, 63(1):60-66.
53. Narita M, Bu G, Herz J, Schwartz AL: Two receptor systems are involved in the plasma clearance of tissue-type plasminogen activator (t-PA) in vivo. *J Clin Invest* 1995, 96(2):1164-1168.
54. Otter M, Kuiper J, van Berkel TJ, Rijken DC: Mechanisms of tissue-type plasminogen activator (tPA) clearance by the liver. *Ann N Y Acad Sci* 1992, 667:431-442.
55. Hajjar KA, Reynolds CM: alpha-Fucose-mediated binding and degradation of tissue-type plasminogen activator by HepG2 cells. *J Clin Invest* 1994, 93(2):703-710.
56. de Boer A, Kluft C, Kroon JM, Kasper FJ, Schoemaker HC, Pruis J, Breimer DD, Soons PA, Emeis JJ, Cohen AF: Liver blood flow as a major determinant of the clearance of recombinant human tissue-type plasminogen activator. *Thromb Haemost* 1992, 67(1):83-87.

57. Samad F, Yamamoto K, Loskutoff DJ: Distribution and regulation of plasminogen activator inhibitor-1 in murine adipose tissue in vivo. Induction by tumor necrosis factor-alpha and lipopolysaccharide. *J Clin Invest* 1996, 97(1):37-46.
58. Fearns C, Loskutoff DJ: Induction of plasminogen activator inhibitor 1 gene expression in murine liver by lipopolysaccharide. Cellular localization and role of endogenous tumor necrosis factor-alpha. *Am J Pathol* 1997, 150(2):579-590.
59. Juhan-Vague I, Aillaud MF, DeCock F, Philip-Joet C, Arnaud C, Serradimigni A, D C: The fast-acting inhibitor of tissue plasminogen activator is an acute phase reactant protein. *Prog Fibrinolysis* 1985, 7:146-149.
60. Kluft C, Verheijen JH, Jie AF, Rijken DC, Preston FE, Sue-Ling HM, Jespersen J, Aasen AO: The postoperative fibrinolytic shutdown: a rapidly reverting acute phase pattern for the fast-acting inhibitor of tissue-type plasminogen activator after trauma. *Scand J Clin Lab Invest* 1985, 45(7):605-610.
61. Declerck PJ, De Mol M, Alessi MC, Baudner S, Paques EP, Preissner KT, Muller-Berghaus G, Collen D: Purification and characterization of a plasminogen activator inhibitor 1 binding protein from human plasma. Identification as a multimeric form of S protein (vitronectin). *J Biol Chem* 1988, 263(30):15454-15461.
62. Boudier C, Gils A, Declerck PJ, Bieth JG: The conversion of active to latent plasminogen activator inhibitor-1 is an energetically silent event. *Biophys J* 2005, 88(4):2848-2854.
63. Levin EG, Santell L: Conversion of the active to latent plasminogen activator inhibitor from human endothelial cells. *Blood* 1987, 70(4):1090-1098.
64. Lindahl TL, Ohlsson PI, Wiman B: The mechanism of the reaction between human plasminogen-activator inhibitor 1 and tissue plasminogen activator. *Biochem J* 1990, 265(1):109-113.
65. Rijken DC: Plasminogen activators and plasminogen activator inhibitors: biochemical aspects. *Baillieres Clin Haematol* 1995, 8(2):291-312.
66. Booth NA, Simpson AJ, Croll A, Bennett B, MacGregor IR: Plasminogen activator inhibitor (PAI-1) in plasma and platelets. *Br J Haematol* 1988, 70(3):327-333.
67. Simpson AJ, Booth NA, Moore NR, Bennett B: The platelet and plasma pools of plasminogen activator inhibitor (PAI-1) vary independently in disease. *Br J Haematol* 1990, 75(4):543-548.
68. Vaughan DE, Declerck PJ, Van Houtte E, De Mol M, Collen D: Studies of recombinant plasminogen activator inhibitor-1 in rabbits. Pharmacokinetics and evidence for reactivation of latent plasminogen activator inhibitor-1 in vivo. *Circ Res* 1990, 67(5):1281-1286.
69. Suffredini AF, Harpel PC, Parrillo JE: Promotion and subsequent inhibition of plasminogen activation after administration of intravenous endotoxin to normal subjects. *N Engl J Med* 1989, 320(18):1165-1172.
70. DeLa Cadena RA, Majluf-Cruz A, Stadnicki A, Agosti JM, Colman RW, Suffredini AF: Activation of the contact and fibrinolytic systems after intravenous administration of endotoxin to normal human volunteers: correlation with the cytokine profile. *Immunopharmacology* 1996, 33(1-3):231-237.
71. Kutzsche S, Schlichting E, Aspelin T, Lyberg T: Hemodynamic changes and systemic activation of coagulation and fibrinolysis during controlled endotoxemia in pigs. *Thromb Res* 2000, 98(6):517-529.
72. Hrafnkelsdottir T, Ottosson P, Gudnason T, Samuelsson O, Jern S: Impaired endothelial release of tissue-type plasminogen activator in patients with chronic kidney disease and hypertension. *Hypertension* 2004, 44(3):300-304.

73. Hrafnkelsdottir T, Gudnason T, Wall U, Jern C, Jern S: Regulation of local availability of active tissue-type plasminogen activator in vivo in man. *J Thromb Haemost* 2004, 2(11):1960-1968.
74. Padro T, van den Hoogen CM, Emeis JJ: Distribution of tissue-type plasminogen activator (activity and antigen) in rat tissues. *Blood Coagul Fibrinolysis* 1990, 1(6):601-608.
75. Rahr HB, Sørensen JV, Larsen JF, Jensen FS, Bredahl C: Plasminogen activators and plasminogen activator inhibitor in portal blood from patients with and without gastric malignancy. *Scand J Gastroenterol* 1996, 31(2):170-174.
76. Seeman-Lodding H, Häggmark S, Jern C, Jern S, Johansson G, Winsö O, Biber B: Aortic cross-clamping influences regional net release and uptake rates of tissue-type plasminogen activator in pigs. *Acta Anaesthesiol Scand* 1997, 41(9):1114-1123.
77. Seeman-Lodding H, Häggmark S, Jern C, Jern S, Johansson G, Winsö O, Biber B: Systemic levels and preportal organ release of tissue-type plasminogen activator are enhanced by PEEP in the pig. *Acta Anaesthesiol Scand* 1999, 43(6):623-633.
78. Bakker CM, Metselaar HJ, Groenland TN, Terpstra OT, Stibbe J: Increased fibrinolysis in orthotopic but not in heterotopic liver transplantation: the role of the anhepatic phase. *Transpl Int* 1992, 5 Suppl 1:S173-174.
79. Maybauer MO, Maybauer DM, Fraser JF, Traber LD, Westphal M, Enkhbaatar P, Cox RA, Huda R, Hawkins HK, Morita N *et al*: Recombinant human activated protein C improves pulmonary function in ovine acute lung injury resulting from smoke inhalation and sepsis. *Crit Care Med* 2006, 34(9):2432-2438.
80. Ware LB, Matthay MA, Parsons PE, Thompson BT, Januzzi JL, Eisner MD: Pathogenetic and prognostic significance of altered coagulation and fibrinolysis in acute lung injury/acute respiratory distress syndrome. *Crit Care Med* 2007, 35(8):1821-1828.
81. Gough SC, Smyllie J, Sheldon T, Rice PJ, Grant PJ: The anatomical distribution of plasma fibrinolytic activity in man during cardiac catheterisation. *Thromb Haemost* 1992, 68(4):442-447.
82. Jern C, Seeman-Lodding H, Biber B, Winsö O, Jern S: An experimental multiple-organ model for the study of regional net release/uptake rates of tissue-type plasminogen activator in the intact pig. *Thromb Haemost* 1997, 78(3):1150-1156.
83. Chandler WL, Loo SC, Mornin D: Adrenergic stimulation of regional plasminogen activator release in rabbits. *Thromb Haemost* 1992, 68(5):545-549.
84. Dodds WJ, Abelseth MK: Criteria for selecting the animal to meet the research need. *Lab Anim Sci* 1980, 30(2 Pt 2):460-465.
85. Dodds WJ: The pig model for experimental research. *Fed Proc* 1982, 47:247-248.
86. Suffredini AF, Fromm RE, Parker MM, Brenner M, Kovacs JA, Wesley RA, Parrillo JE: The cardiovascular response of normal humans to the administration of endotoxin. *N Engl J Med* 1989, 321(5):280-287.
87. Treggiari MM, Romand JA, Burgener D, Suter PM, Åneman A: Effect of increasing norepinephrine dosage on regional blood flow in a porcine model of endotoxin shock. *Crit Care Med* 2002, 30(6):1334-1339.
88. Laesser M, Oi Y, Ewert S, Fändriks L, Åneman A: The angiotensin II receptor blocker candesartan improves survival and mesenteric perfusion in an acute porcine endotoxin model. *Acta Anaesthesiol Scand* 2004, 48(2):198-204.
89. Parker SJ, Watkins PE: Experimental models of gram-negative sepsis. *Br J Surg* 2001, 88(1):22-30.
90. Fink MP, Heard SO: Laboratory models of sepsis and septic shock. *J Surg Res* 1990, 49(2):186-196.

91. Porta F, Takala J, Kolarova A, Ma Y, Redaelli CA, Brander L, Bracht H, Jakob SM: Oxygen extraction in pigs subjected to low-dose infusion of endotoxin after major abdominal surgery. *Acta Anaesthesiol Scand* 2005, 49(5):627-634.
92. Rånby M, Norrman B, Wallen P: A sensitive assay for tissue plasminogen activator. *Thromb Res* 1982, 27(6):743-749.
93. Rånby M, Nguyen G, Scarabin PY, Samama M: Immunoreactivity of tissue plasminogen activator and of its inhibitor complexes. Biochemical and multicenter validation of a two site immunosorbent assay. *Thromb Haemost* 1989, 61(3):409-414.
94. Leng HM, Brouwers E, Knockaert I, Declerck PJ: Immunoassays for the quantitation of porcine PAI-1 antigen and activity in biological fluid samples. *Thromb Haemost* 2000, 84(6):1082-1086.
95. Bland JM, Altman DG: Calculating correlation coefficients with repeated observations: Part 1--Correlation within subjects. *Bmj* 1995, 310(6977):446.
96. Tumbleson ME: Swine in biomedical research. *New York, Plenum press* 1987.
97. van der Poll T, Levi M, Buller HR, van Deventer SJ, de Boer JP, Hack CE, ten Cate JW: Fibrinolytic response to tumor necrosis factor in healthy subjects. *J Exp Med* 1991, 174(3):729-732.
98. van Hinsbergh VW, Kooistra T, van den Berg EA, Princen HM, Fiers W, Emeis JJ: Tumor necrosis factor increases the production of plasminogen activator inhibitor in human endothelial cells in vitro and in rats in vivo. *Blood* 1988, 72(5):1467-1473.
99. Biemond BJ, Levi M, Ten Cate H, Van der Poll T, Büller HR, Hack CE, Ten Cate JW: Plasminogen activator and plasminogen activator inhibitor I release during experimental endotoxaemia in chimpanzees: effect of interventions in the cytokine and coagulation cascades. *Clin Sci (Lond)* 1995, 88(5):587-594.
100. Mavrommatis AC, Theodoridis T, Economou M, Kotanidou A, El Ali M, Christopoulou-Kokkinou V, Zakynthinos SG: Activation of the fibrinolytic system and utilization of the coagulation inhibitors in sepsis: comparison with severe sepsis and septic shock. *Intensive Care Med* 2001, 27(12):1853-1859.
101. Martinez MA, Pena JM, Fernandez A, Jimenez M, Juarez S, Madero R, Vazquez JJ: Time course and prognostic significance of hemostatic changes in sepsis: relation to tumor necrosis factor-alpha. *Crit Care Med* 1999, 27(7):1303-1308.
102. Menashi S, Lu H, Soria C, Legrand Y: Endothelial cell proteases: physiological role and regulation. *Baillieres Clin Haematol* 1993, 6(3):559-576.
103. Chia S, Qadan M, Newton R, Ludlam CA, Fox KA, Newby DE: Intra-arterial tumor necrosis factor-alpha impairs endothelium-dependent vasodilatation and stimulates local tissue plasminogen activator release in humans. *Arterioscler Thromb Vasc Biol* 2003, 23(4):695-701.
104. Robinson SD, Dawson P, Ludlam CA, Boon NA, Newby DE: Vascular and fibrinolytic effects of intra-arterial tumour necrosis factor-alpha in patients with coronary heart disease. *Clin Sci (Lond)* 2006, 110(3):353-360.
105. O'Rourke J, Jiang X, Hao Z, Cone RE, Hand AR: Distribution of sympathetic tissue plasminogen activator (tPA) to a distant microvasculature. *J Neurosci Res* 2005, 79(6):727-733.
106. Jiang X, Wang Y, Hand AR, Gillies C, Cone RE, Kirk J, O'Rourke J: Storage and release of tissue plasminogen activator by sympathetic axons in resistance vessel walls. *Microvasc Res* 2002, 64(3):438-447.
107. Åneman A, Eisenhofer G, Fändriks L, Friberg P: Hepatomesenteric release and removal of norepinephrine in swine. *Am J Physiol* 1995, 268(4 Pt 2):R924-930.

108. Åneman A, Eisenhofer G, Olbe L, Dalenbäck J, Nitescu P, Fändriks L, Friberg P: Sympathetic discharge to mesenteric organs and the liver. Evidence for substantial mesenteric organ norepinephrine spillover. *J Clin Invest* 1996, 97(7):1640-1646.
109. Acosta S, Nilsson TK, Bergqvist D, Björck M: Activation of fibrinolysis and coagulation in non-occlusive intestinal ischaemia in a pig model. *Blood Coagul Fibrinolysis* 2004, 15(1):69-76.
110. Carmeliet P, Schoonjans L, Kieckens L, Ream B, Degen J, Bronson R, De Vos R, van den Oord JJ, Collen D, Mulligan RC: Physiological consequences of loss of plasminogen activator gene function in mice. *Nature* 1994, 368(6470):419-424.
111. Renckens R, Roelofs JJ, Florquin S, de Vos AF, Pater JM, Lijnen HR, Carmeliet P, van 't Veer C, van der Poll T: Endogenous tissue-type plasminogen activator is protective during Escherichia coli-induced abdominal sepsis in mice. *J Immunol* 2006, 177(2):1189-1196.
112. Lautt WW, Greenway CV: Conceptual review of the hepatic vascular bed. *Hepatology* 1987, 7(5):952-963.
113. Lautt WW: Regulatory processes interacting to maintain hepatic blood flow constancy: Vascular compliance, hepatic arterial buffer response, hepatorenal reflex, liver regeneration, escape from vasoconstriction. *Hepatol Res* 2007, 37(11):891-903.
114. Garcia-Fernandez N, Montes R, Purroy A, Rocha E: Hemostatic disturbances in patients with systemic inflammatory response syndrome (SIRS) and associated acute renal failure (ARF). *Thromb Res* 2000, 100(1):19-25.
115. Levin EG, Santell L, Osborn KG: The expression of endothelial tissue plasminogen activator in vivo: a function defined by vessel size and anatomic location. *J Cell Sci* 1997, 110 (Pt 2):139-148.
116. Brun-Buisson C, Minelli C, Bertolini G, Brazzi L, Pimentel J, Lewandowski K, Bion J, Romand JA, Villar J, Thorsteinsson A *et al*: Epidemiology and outcome of acute lung injury in European intensive care units. Results from the ALIVE study. *Intensive Care Med* 2004, 30(1):51-61.
117. Idell S: Anticoagulants for acute respiratory distress syndrome: can they work? *Am J Respir Crit Care Med* 2001, 164(4):517-520.
118. Idell S: Coagulation, fibrinolysis, and fibrin deposition in acute lung injury. *Crit Care Med* 2003, 31(4 Suppl):S213-220.
119. Laterre PF, Wittebole X, Dhainaut JF: Anticoagulant therapy in acute lung injury. *Crit Care Med* 2003, 31(4 Suppl):S329-336.
120. Tracey KJ, Beutler B, Lowry SF, Merryweather J, Wolpe S, Milsark IW, Hariri RJ, Fahey TJ, 3rd, Zentella A, Albert JD *et al*: Shock and tissue injury induced by recombinant human cachectin. *Science* 1986, 234(4775):470-474.
121. Wheeler AP, Bernard GR: Treating patients with severe sepsis. *N Engl J Med* 1999, 340(3):207-214.
122. Ladenvall P, Nilsson S, Jood K, Rosengren A, Blomstrand C, Jern C: Genetic variation at the human tissue-type plasminogen activator (tPA) locus: haplotypes and analysis of association to plasma levels of tPA. *Eur J Hum Genet* 2003, 11(8):603-610.
123. Ladenvall P, Wall U, Jern S, Jern C: Identification of eight novel single-nucleotide polymorphisms at human tissue-type plasminogen activator (t-PA) locus: association with vascular t-PA release in vivo. *Thromb Haemost* 2000, 84(2):150-155.
124. Padro T, Quax PH, van den Hoogen CM, Roholl P, Verheijen JH, Emeis JJ: Tissue-type plasminogen activator and its inhibitor in rat aorta. Effect of endotoxin. *Arterioscler Thromb* 1994, 14(9):1459-1465.

125. Ulfhammer E, Larsson P, Karlsson L, Hrafnkelsdottir T, Bokarewa M, Tarkowski A, Jern S: TNF-alpha mediated suppression of tissue type plasminogen activator expression in vascular endothelial cells is NF-kappaB- and p38 MAPK-dependent. *J Thromb Haemost* 2006, 4(8):1781-1789.
126. Siebeck M, Spannagl M, Hoffmann H, Schramm W, Fritz H: Time dependent release of tissue-type plasminogen activator and plasminogen activator inhibitor into the circulation of pigs during shock. *Blood Coagul Fibrinolysis* 1991, 2(3):459-464.
127. Okabayashi K, Wada H, Ohta S, Shiku H, Nobori T, Maruyama K: Hemostatic markers and the sepsis-related organ failure assessment score in patients with disseminated intravascular coagulation in an intensive care unit. *Am J Hematol* 2004, 76(3):225-229.
128. Sawdey MS, Loskutoff DJ: Regulation of murine type 1 plasminogen activator inhibitor gene expression in vivo. Tissue specificity and induction by lipopolysaccharide, tumor necrosis factor-alpha, and transforming growth factor-beta. *J Clin Invest* 1991, 88(4):1346-1353.
129. Lupu F, Bergonzelli GE, Heim DA, Cousin E, Genton CY, Bachmann F, Kruithof EK: Localization and production of plasminogen activator inhibitor-1 in human healthy and atherosclerotic arteries. *Arterioscler Thromb* 1993, 13(7):1090-1100.
130. Lundgren CH, Brown SL, Nordt TK, Sobel BE, Fujii S: Elaboration of type-1 plasminogen activator inhibitor from adipocytes. A potential pathogenetic link between obesity and cardiovascular disease. *Circulation* 1996, 93(1):106-110.
131. ten Cate H: Pathophysiology of disseminated intravascular coagulation in sepsis. *Crit Care Med* 2000, 28(9 Suppl):S9-11.