Cardiopulmonary Resuscitation

Pharmacological Interventions for Augmentation of Cerebral Blood Flow

BY

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Abstract


Cardiac arrest results in immediate interruption of blood flow. The primary goal of cardiopulmonary resuscitation (CPR) is to re-establish blood flow and hence oxygen delivery to the vital organs. This thesis describes different pharmacological interventions aimed at increasing cerebral blood flow during CPR and after restoration of spontaneous circulation (ROSC).

In a porcine model of cardiac arrest, continuous infusion of adrenaline generated higher cortical cerebral blood flow during CPR as compared to bolus administration of adrenaline. While bolus doses resulted in temporary peaks in cerebral blood flow, continuous infusion led to a sustained increase in this flow.

Administration of vasopressin resulted in higher cortical cerebral blood flow and a lower cerebral oxygen extraction ratio as compared to continuous infusion of adrenaline during CPR. In addition, vasopressin generated higher coronary perfusion pressure during CPR and increased the likelihood of achieving ROSC.

Parameters of coagulation and inflammation were measured after successful resuscitation from cardiac arrest. Immediately after ROSC, thrombin-antithrombin complex, a marker of thrombin generation, was elevated and eicosanoid levels were increased, indicating activation of coagulation and inflammation after ROSC. The thrombin generation was accompanied by a reduction in antithrombin. In addition, there was substantial haemoconcentration in the initial period after ROSC.

By administration of antithrombin during CPR, supraphysiological levels of antithrombin were achieved. However, antithrombin administration did not increase cerebral circulation or reduce reperfusion injury, as measured by cortical cerebral blood flow, cerebral oxygen extraction and levels of eicosanoids, after ROSC.

In a clinical study, the adrenaline dose interval was found to be longer than recommended in the majority of cases of cardiac arrest. Thus, the adherence to recommended guidelines regarding the adrenaline dose interval seems to be poor.

Keywords: Adrenaline (epinephrine), Cardiac arrest, Cardiopulmonary resuscitation, Cerebral blood flow, Guidelines, Post-resuscitation period, Vasopressin

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"Juni natt blir aldrig av,
liknar mest en daglig dag.
Slöjikt lyfter sig dess skymning
och bärs bort på fjusa hav."

J Martinsson
List of Papers

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

I  Increased cortical cerebral blood flow by continuous infusion of epinephrine during experimental cardiopulmonary resuscitation.  
Johansson J, Gedeborg R, Basu S, Rubertsson S  

II  Vasopressin versus continuous adrenaline during experimental cardiopulmonary resuscitation.  
Johansson J, Gedeborg R, Rubertsson S  

III  Adrenaline administration during cardiopulmonary resuscitation – poor adherence to clinical guidelines.  
Acta Anaesthesiologica Scandinavia. Accepted for publication.

IV  Antithrombin reduction after experimental cardiopulmonary resuscitation.  

V  Antithrombin administration during experimental cardiopulmonary resuscitation.  
# Abbreviations

<table>
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<tr>
<th>Abbreviation</th>
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<tr>
<td>ACLS</td>
<td>Advanced cardiac life support</td>
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<td>AT</td>
<td>Antithrombin</td>
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<td>CPR</td>
<td>Cardiopulmonary resuscitation</td>
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<tr>
<td>i.v.</td>
<td>Intravenous, intravenously</td>
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<td>ROSC</td>
<td>Restoration of spontaneous circulation</td>
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<td>TAT</td>
<td>Thrombin-antithrombin complex</td>
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<td>VF</td>
<td>Ventricular fibrillation</td>
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<td>VT</td>
<td>Ventricular tachycardia</td>
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<td>15-keto-DH-PGF$_{2\alpha}$</td>
<td>15-keto-dihydro-prostaglandin F$_{2\alpha}$</td>
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Introduction

Cardiac arrest results in immediate interruption of blood flow to vital organs. The primary goal of cardiopulmonary resuscitation (CPR) is to re-establish blood flow, and hence oxygen delivery, to the heart and brain. Despite the advances in CPR during the last decades, the outcome after cardiac arrest remains unsatisfactory [1]. Even if spontaneous circulation is initially restored, the early mortality and morbidity are high, as a result of ischaemic injury to the heart and brain.

The cerebral injury that occurs during and after cardiac arrest is believed to evolve during three different phases: first, the non-intervention period from cardiac arrest to the initiation of CPR, with no circulation; secondly, the resuscitation period with suboptimal oxygen delivery to the brain; and thirdly, the reperfusion phase (after the restoration of spontaneous circulation (ROSC)), with haemodynamic disturbances, impaired cerebral autoregulation, an inflammatory response and activation of the coagulation cascade. In order to improve the outcome after cardiac arrest, it is of vital importance to shorten the time interval from arrest to ROSC. In addition, it is most certainly of great importance to provide for sufficient oxygen delivery to vital organs during the resuscitation and reperfusion phases. For this reason, a spectrum of pharmacological and mechanical interventions has been evaluated over the years, with the aim of improving the circulation during CPR and after ROSC.

This thesis mainly focuses on pharmacological interventions during CPR and their effects on the cerebral circulation and haemodynamics during and immediately after the resuscitation. Aspects of haemoconcentration and activation of coagulation and inflammation during the initial post-resuscitation period are also considered.
Background

Cerebral blood flow under normal conditions and during CPR

The brain needs oxygen and glucose to maintain cellular integrity and to perform electrophysiological activities. After seconds of normothermic cardiac arrest, brain oxygen is depleted and consciousness is lost. Additionally, after a few minutes the energy stores are emptied. Thus, constant and adequate oxygen and energy supply to the brain is essential in order to maintain cerebral function.

Under normal conditions, the brain receives about 15% of normal cardiac output. Despite changes in blood pressure, cerebral blood flow is kept relatively constant by virtue of continuous changes in cerebral vascular resistance (autoregulation). However, in situations such as CPR, when the blood pressure is below the lower limit of autoregulation, cerebral blood flow is pressure-dependent and varies linearly with cerebral perfusion pressure.

During CPR, external chest compressions generate about 10-40% of normal cerebral blood flow [2]. This blood flow is insufficient to provide adequate oxygen and energy supply to the brain. Only a short period of low-flow conditions can be tolerated without permanent brain damage. Pharmacological interventions during CPR are therefore most likely required in order to optimise cerebral blood flow and thus prevent ischaemic injury.

Cerebral blood flow after cardiac arrest

After resuscitation from cardiac arrest, cerebral blood flow is impaired and autoregulation is compromised [3-5]. Immediately after ROSC there is a short period of multifocal no-reflow, a phenomenon first described by Ames et al. [6]. Factors contributing to this disorder include post-ischaemic hypotension, increased blood viscosity, activation of coagulation, leucocyte-endothelial interactions and a reduction in the calibre of small vessels due to compression by swollen cells [6-9]. The severity of no-reflow is believed to depend mainly on the duration of ischaemia [10-13]. After the period of no-reflow, brief transient cerebral hyperaemia is followed by protracted global and multifocal hypoperfusion. The no-reflow phenomenon and the delayed hypoperfusion phase are believed to have a great impact on the neurological
outcome. Neurological recovery after resuscitation from cerebral ischaemia has been shown to depend on the magnitude of post-ischaemic reperfusion [14].

Pharmacological interventions to increase vital organ blood flow during CPR
Administration of vasoactive drugs during CPR has been practised for just over one hundred years [15]. Such drugs can improve cerebral blood flow and oxygen delivery. In addition, vasoactive drugs can increase coronary perfusion pressure and thereby the likelihood of achieving ROSC [16]. At present, two vasoactive drugs are recommended for use during CPR: adrenaline and vasopressin.

Adrenaline
The concept of using adrenaline during cardiac arrest was first introduced by Crile and Dolley in the beginning of the 20th century [15]. In the 1960s Redding and Pearson further investigated the role of adrenaline and other vasoactive drugs during CPR [17,18]. On the basis of their results, adrenaline has since been the vasoactive drug of choice in the treatment of patients with cardiac arrest.

General remarks
The pharmacological effects of adrenaline are mediated by adrenergic receptors. Adrenaline has both α- and β-adrenergic effects. The main effect of α-stimulation is an increase in vascular resistance, which increases the central blood volume, raises the arterial pressure and thereby improves the vital organ blood flow. In experimental studies of CPR, adrenaline administration has been shown to increase cerebral and myocardial blood flow [19,20] and improve the chances of achieving ROSC [21]. Thus, the α-adrenergic agonist effects are considered to be mainly beneficial. The β-adrenergic actions of adrenaline are inotropic and chronotropic and may not be exclusively beneficial. In a state of cardiac arrest, β-adrenergic stimulation causes an increase in myocardial oxygen demand [22]. It may also result in severe tachycardia, arrhythmias and myocardial dysfunction after ROSC [23,24].

The optimal dose
In early experimental studies of cardiac arrest in dogs, adrenaline was successfully injected intracardially at a dose of 1 mg [17]. When these results were converted into clinical recommendations, it was assumed that intravenous (i.v.) administration would be as effective as the intracardiac route.
Consequently, the standard-dose for i.v. administration became 0.5-1 mg without adjustment for body weight [25].

In the 1980s this clinical recommendation of 0.5-1 mg was questioned and several studies were conducted to evaluate the effects of higher adrenaline doses. Experimental studies showed that high-dose adrenaline was more effective than standard-dose adrenaline in that it generated higher cerebral and myocardial blood flow [26,27] and higher coronary perfusion pressure [28]. As a consequence of the promising experimental studies of high-dose adrenaline, the clinical recommendations of the American Heart Association and the European Resuscitation Council were adjusted in 1992. According to these guidelines, it was recommended that the adrenaline dose be increased to either escalating doses (1, 3, 5 mg), an intermediate dose (5 mg) or high-dose (0.1 mg/kg) if a 1 mg dose had been ineffective [29,30].

Later in the 1990s, several clinical studies were conducted to compare the standard-dose to high-dose adrenaline. No improvement in the outcome was observed when high-dose adrenaline was administered [31-33]. Furthermore, high plasma levels of adrenaline during CPR were found to be associated with unsuccessful resuscitation [34], indicating that excessive adrenergic stimulation might have adverse effects. In addition, further experimental data indicated detrimental effects of high-dose adrenaline on the brain and heart, resulting in a worse outcome [35-37]. Consequently, in the subsequent international guidelines of the year 2000, the recommendation concerning use of high-dose adrenaline was adjusted, stating that it was “acceptable but not recommended” [38]. Thus, the currently recommended adrenaline dose for administration during CPR is still 1 mg, but whether this is the optimal dose remains a matter of controversy.

The optimal route and mode of administration
Adrenaline should preferably be administered i.v., but under specific circumstances intratracheal and even intracardiac administration can be indicated [38].

It is recommended that adrenaline be administered intermittently during CPR, but the optimal dose interval has been investigated to a far lesser extent than the optimal dose. However, the question of the dose interval is probably of major importance, since adrenaline has a very short duration of action. Irrespective of whether standard- or high-dose adrenaline is given, the cerebral blood flow peaks after one minute and after approximately two minutes the effect has disappeared [39]. The clinically relevant duration of action is probably in fact only about one minute. Initially, the recommended interval between adrenaline doses was 5 minutes [25]. In the guidelines of 1992 the recommendation was adjusted to administration every 2-3 minutes [29,30]. However, in the international CPR guidelines of the year 2000 the chosen dose interval was 3-5 minutes [38]. A possible explanation for the
extension of the dose interval was a fear of adverse effects of excessive adrenaline administration.

In view of the short duration of action of adrenaline, continuous infusion might be a more appropriate way to administer this drug during CPR. This alternative is also briefly mentioned in the ACLS (Advanced cardiac life support) guidelines of 2000 [38]. However, no referential support from the literature on adrenaline infusion during CPR is given in these guidelines and the recommended infusion rate of “1 µg/min” is somewhat confusing. The intended infusion rate is most certainly 1 µg/kg/min. The effects of continuous administration of adrenaline during CPR were evaluated in an earlier experimental dose-response study [40], and 10 µg/kg/min seemed to be the most beneficial dosage in terms of vital organ blood flow. However, when the guidelines of 2000 were formulated, no direct comparisons between continuous and intermittent administration of adrenaline had been reported.

! Adrenaline has a very short duration of action and the recommended dose interval of 3-5 minutes might therefore be too long.

* In study I the effects of continuously administered adrenaline as compared with those of intermittent bolus administration during CPR were evaluated.

**Vasopressin**

It was initially observed that patients with cardiac arrest had extremely high circulating levels of vasopressin and that elevated levels of endogenous vasopressin correlated to a better outcome after cardiac resuscitation [34,41]. On account of these observations, a series of experimental studies on the effects of vasopressin administration during CPR were conducted in the 1990s.

**General remarks**

Vasopressin is a naturally occurring hormone, which under normal physiological conditions has antidiuretic actions. In high pharmacological doses, vasopressin acts as a potent peripheral vasoconstrictor. It is a non-adrenergic vasoconstrictor, which acts by direct stimulation of smooth muscle V1 receptors. During the last decade, vasopressin has been found to have promising vasoconstrictor effects, with increased vital organ blood flow, during CPR [42-45]. In addition to increasing the cerebral perfusion pressure, it has also been suggested that vasopressin affects the cerebral blood flow by dilating major cerebral arteries [46,47]. Also, a possibly important characteristic of vasopressin is that the haemodynamic response in severe acidosis remains intact, while the adrenergic pressor response is blunted [48,49]. It is therefore possible that vasopressin may be especially beneficial in prolonged CPR. In
addition, the absence of a β-adrenergic effect is probably beneficial regarding myocardial oxygen consumption. However, there is evidence that vasopressin may have adverse effects. In contrast to adrenaline, vasopressin has a relatively long duration of action [38,50], which may be detrimental during the early post-resuscitation period. It has been postulated that residual intense peripheral vasoconstrictive effects can cause a decline in myocardial contractility and a decreased cardiac index after ROSC [51].

The optimal dose and route of administration

Different doses of vasopressin have been tested in experimental studies of cardiac arrest and the optimal dose appears to be in the range of 0.4-0.8 U/kg. Vasopressin has mostly been tested as a single bolus dose, which seems appropriate in relation to its relatively long duration of action [38,50]. In the latest ACLS International Guidelines [38], vasopressin was introduced as an alternative to adrenaline for ventricular fibrillation (VF) and pulseless ventricular tachycardia (VT) (“acceptable, not harmful, supported by fair evidence”). The recommended dose is 40 U and it should be administered as a single dose only. If there is no response to vasopressin 5-10 minutes after the injection, it is considered acceptable to resume adrenaline 1 mg every 3-5 minutes. In the experimental setting, the results of use of a combination of vasopressin and adrenaline are conflicting [48,52]. However, in a clinical setting this combination was recently found to be effective [53].

Vasopressin should preferably be administered i.v., but experimental studies have shown that intraosseous and intratracheal routes are reliable alternatives for vasopressin administration if i.v. access is not available [54,55].

Adrenaline versus vasopressin in experimental studies

Bolus administration of adrenaline has been compared with vasopressin in several experimental studies. It has been demonstrated that vasopressin administration results in higher cerebral and myocardial blood flow and higher coronary perfusion pressure as compared to intermittent administration of adrenaline [42-45]. In many of these studies, however, the blood flow was measured with the microsphere technique, which only allows spot observations of blood flow. The selected time points for observation were 90 s and 5 minutes after the drug administration. Because of the differences in pharmacodynamic properties, adrenaline will have little or no effect at these time points, while vasopressin will still be at its peak of action. To circumvent the major differences in duration of action between the two drugs, a comparison between continuous administration of adrenaline and bolus administration of vasopressin might be necessary. Furthermore, continuous measurement of cerebral blood flow during the experiment would be essential to avoid the problem with spot observation.
Adrenaline and vasopressin in clinical studies

The use of adrenaline during CPR has wide clinical acceptance, although no clinical study has provided convincing evidence that adrenaline actually improves the outcome after cardiac arrest [56]. In fact, there are studies that have even indicated a worse outcome after the use of adrenaline [57,58]. Thus, there seems to be a discrepancy between the beneficial effects of adrenaline achieved in experimental studies and the lack of improved outcome in clinical studies. This discrepancy has several possible explanations. First, caution needs to be observed when interpreting these clinical results, since these studies were not designed to test whether adrenaline administration during CPR is beneficial. No prospective placebo-controlled trial of the possible benefits of adrenaline during CPR has been completed. Attempts have been made, but because of ethical dilemmas and the participating physicians’ strong belief in adrenaline, almost half of the patients enrolled received standard adrenaline treatment even though they were not supposed to according to the study protocol [59]. Another reason for the above discrepancy is that while experimental studies have focused on vital organ blood flow and ability to achieve ROSC, clinical studies have mostly had survival, discharge from hospital and neurological outcome as primary endpoints. Furthermore, in the experimental setting adrenaline is often administered under optimal conditions, i.e. into a central venous line, after a relatively short duration of no-flow or low-flow conditions and during excellent cardiac massage. The circumstances in the clinical setting of CPR can be quite the opposite. Finally, and maybe most importantly, in the clinical situation of cardiac arrest one must consider whether adrenaline is in fact administered according to the guidelines. It has previously been demonstrated that in general, physicians are relatively non-compliant with ACLS guidelines during cardiac arrest [60]. The question whether or not there is compliance to the recommended dose interval of adrenaline has not been investigated. Bearing the short duration of action in mind, the actual dose interval in routine practice could be of great importance when evaluating the effects of adrenaline during CPR.
Since vasopressin usage during CPR has only a short history, the experience from its use in clinical practice is still limited. In 1997 and 2001, two relatively small randomised controlled studies comparing vasopressin and adrenaline in cardiac arrest were presented [61,62]. The results of use of vasopressin in these studies were rather discouraging. Although vasopressin was somewhat superior to adrenaline in restoring spontaneous circulation, there were no differences in ultimate endpoints such as neurological outcome and hospital discharge. In a recent large, randomised controlled multicentre study, the effects of vasopressin and adrenaline in out-of-hospital cardiac arrest were investigated. No difference was found between vasopressin and adrenaline in the management of VF and pulseless electrical activity, but vasopressin was superior to adrenaline in patients with asystole [53].

Coagulation and inflammation after cardiac arrest

In situations such as trauma, disseminated intravascular coagulation and multiple organ failure, the coagulation cascade and the inflammatory system are activated [63]. There are several links between coagulation and inflammation. The inflammatory response results in a systemic activation of the clotting cascade, and activation of coagulation can promote inflammation [64,65]. This vicious circle also seems to take place after cardiac arrest.

The occurrence of stagnant blood flow, endothelial cell damage and increased levels of catecholamines during and after cardiac arrest causes activation of the coagulation system [66]. Intravital microscopy has shown the presence of microthrombi in cerebral vessels as early as 5 to 10 minutes after cardiac arrest [67]. In clinical studies of cardiac arrest, marked activation of blood coagulation and fibrin formation after resuscitation has been demonstrated [68-70]. Thus, there seems to be an imbalance between coagulation, anticoagulation and fibrinolysis after cardiac arrest.

When ROSC is achieved, the cerebral ischaemic tissue is reperfused with oxygenated blood, which is the obvious goal of resuscitation. However, reperfusion of the post-ischaemic tissue may induce potentially harmful pathophysiological reactions, which can contribute to the cerebral injury [9]. This
phenomenon has been referred to as reperfusion injury, in which inflammatory cascades have been proposed to be key issues [71].

During reperfusion after cardiac arrest there is marked activation of leucocytes [72]. This activation is believed to play an important role in the development of tissue damage and microcirculatory reperfusion disorders after ischaemia and reperfusion [9,72-75]. Leucocytes may worsen ischaemic damage by several mechanisms, including occlusion of the microvasculature, release of cytotoxic enzymes, increased cytokine release and generation of oxygen free radicals [76]. In fact, cardiac arrest is followed by a “sepsis-like” syndrome with increased markers of inflammation which correlate to a poor outcome [77-79]. Thus, contradictorily, although reperfusion is essential for tissue survival, it may also exacerbate further injury.

**Thrombin**

Thrombin is the essential enzyme product of the coagulation cascade. It activates factors V, VIII, XI and XIII, aggregates platelets, catalyses conversion of fibrinogen to fibrin, and inhibits fibrinolysis, and following cerebral ischaemia it constricts arteries [80]. Thrombin is also believed to have proinflammatory functions; both by having an important role in reperfusion-induced leucocyte recruitment (rolling and adhesion) and in increasing microvascular permeability [80,81]. Furthermore, thrombin generation is most certainly an important event in the development of an atherosclerotic plaque, in which both inflammation and coagulation are involved [82]. In addition, thrombin is generated [83], and is associated with an adverse outcome [84], in the ischaemic event of unstable coronary artery disease.

**Antithrombin**

Antithrombin (AT) is the most important inhibitor of the coagulation system, acting at many levels of the coagulation cascade. Most importantly, AT directly inhibits activated thrombin through the formation of the thrombin-antithrombin complex (TAT). In addition to its anticoagulant properties, AT has anti-inflammatory activity [81,85-87], mainly by stimulating the production of prostacyclin in endothelial cells.

AT is produced in the liver and has a half-life of about 3 days. In situations with acute AT consumption, the half-life can be reduced to a few hours. It is well known that in serious trauma, shock and infection, severe AT deficiency occurs as a consequence of excessive consumption. In these conditions, low levels of AT have correlated to high mortality [88]. It is uncertain how large the decrease in AT has to be to have a severe effect on coagulation and inflammation. However, even a reduction of AT within the normal range is accompanied by an increase in thrombin generation [89]. Since thrombin appears to increase in ischaemia/reperfusion [83], there is
reason to believe that AT is consumed in cardiac arrest, with an AT deficiency as a consequence.

| ! Coagulation and inflammation are activated after cardiac arrest. |
| ☀ In study IV the levels of antithrombin and thrombin-antithrombin complex after cardiac arrest were determined. |

Pharmacological interventions to counteract coagulation and inflammation after ROSC

Different studies have been conducted to evaluate the use of thrombolysis during and after CPR, with the aim of counteracting the activated coagulation system. In both experimental and clinical studies thrombolytic therapy has been shown to improve microcirculatory reperfusion [8,90]. However, the major drawback of the use of thrombolytics during or shortly after CPR has been the potential risk of severe bleeding complications. But some authors believe that the risk of bleeding may have been exaggerated and that thrombolytic therapy might be beneficial during and after CPR [91].

Pharmacological interventions aimed at achieving neuroprotection against reperfusion injury have been evaluated. The use of drugs to inhibit leucocytes during ischaemia and reperfusion has been shown to reduce tissue injury in experimental models of cerebral ischaemia and reperfusion [74]. Also, antioxidant therapy has been shown to improve the functional outcome after cardiac arrest [92]. In clinical studies, however, attempts at pharmacological neuroprotection have had rather discouraging results [93].

AT treatment in the setting of cardiac arrest has never been investigated, but could hypothetically be beneficial from two perspectives: first to counteract the activated coagulation system and secondly to reduce the inflammatory response. AT administration has been found to be effective in several experimental sepsis models [87], but has failed to improve the outcome significantly in clinical sepsis [94,95]. In experimental models of non-septic diseases with a systemic inflammatory response syndrome, such as trauma, burns, and preeclampsia, AT administration has been effective [96]. In addition, during reperfusion after focal ischaemia AT reduces leucocyte recruitment in microvessels [81], and after brain ischaemia thrombin inhibition improves cerebral blood flow [97]. Thus, through its anticoagulative and anti-inflammatory properties AT could possibly increase the cerebral microcirculation and reduce reperfusion injury after ROSC.
Activation of coagulation and inflammation during and after cardiac arrest seems to contribute to a disturbance of cerebral microcirculation and to reperfusion injury. Study V addressed the question whether administration of AT during CPR could diminish reperfusion injury and improve the cerebral microcirculation after ROSC.
Aims

The aims of the studies described in this thesis were:

1. to study the effects of continuous administration of adrenaline during CPR, as compared to bolus administration of adrenaline, on cortical cerebral blood flow and global cerebral oxygen extraction during CPR and after ROSC;

2. to study the effects of bolus administration of vasopressin during CPR, as compared to continuous infusion of adrenaline, on cortical cerebral blood flow and global cerebral oxygen extraction during CPR and after ROSC;

3. to estimate the adrenaline dose interval used during CPR in routine practice;

4. to measure plasma levels of AT and TAT complex during reperfusion after cardiac arrest;

5. to determine whether AT administration during CPR increases the cerebral microcirculation and reduces reperfusion injury after ROSC.
Materials and methods

Study design

Studies I, II and V were randomised, controlled, blinded, experimental studies. Study IV was a descriptive experimental study without a control group. The experimental studies were conducted and reported in accordance with the Utstein-style guidelines for laboratory research [98]. Study III was a descriptive clinical study.

Experimental studies of cardiac arrest (Papers I, II, IV and V)

Animals

The experimental protocols were approved by the Institutional Review Board for Animal Experimentation in Uppsala, Sweden. The studies were carried out on domestic pigs of Swedish country breed, 11-15 weeks old (Table 1).

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<th>Mean weight, kg (SD)</th>
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<td>II</td>
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<td>IV</td>
<td>10</td>
<td>24.8 (1.3)</td>
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<tr>
<td>V</td>
<td>24</td>
<td>25.6 (1.3)</td>
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The porcine model is commonly used in biomedical research. This model is particularly useful in experimental studies of CPR, since the response to myocardial ischaemia and the quantitative regional blood flow generated by closed chest cardiac massage resemble those in humans [99]. An important difference between the porcine model and the majority of cardiac arrest victims is the presence of arteriosclerosis in the latter. The studies in this thesis were conducted on young healthy animals without arteriosclerotic disease,
which is in contrast to the majority of cardiac arrest victims, who are elderly and have a history of cardiovascular disease [100].

Anaesthesia and fluid administration

Adequate anaesthesia is necessary in experimental research in CPR, not only for humane reasons but also for reduction of stress. The choice of anaesthetic regimen may have an important impact in animal resuscitation research [98]. Unfortunately, there is no perfect anaesthesia for laboratory models of CPR. In the present experimental studies, induction and maintenance of anaesthesia were kept identical to allow comparisons between studies.

Anaesthesia was induced with an intramuscular injection of tiletamine, zolazepam, xylazine and atropine. It is well documented that this combination of anaesthetics gives reliable induction with maintenance of good cardiovascular function in experimental models [101]. In addition, an i.v. bolus dose of morphine and ketamine was given. Ketamine is sympathomimetic, with marked effects on haemodynamics, and was therefore not used for maintenance of anaesthesia [102]. The anaesthetics used for induction have a relatively short duration of action. Hence they probably have no influence on the measurements made during the experiment. During the preparation the pigs were ventilated with a 70/30 mixture of N₂O/O₂. For maintenance of anaesthesia throughout the experiment, an i.v. infusion of pentobarbital, morphine and pancuronium was given. Pentobarbital causes a reduction in cerebral blood flow, but autoregulation and CO₂ reactivity are preserved [103].

To compensate for water losses, a bolus of acetated Ringer’s solution 30 ml/kg, was given at the end of the preparation. In addition, during the entire experiment an infusion of 2.5 % glucose, 18 ml/kg/h, was administered. The amount of fluid replacement was based on findings in earlier experimental studies by our research group [104,105].

Body temperature was controlled with a heating pad, aiming for a core temperature of 38°C.

Cardiopulmonary resuscitation protocol

Induction of cardiac arrest

Ventricular fibrillation was induced with an alternating current shock of 40-60 V through two subcutaneous needles. Cardiac arrest was defined as VF on the ECG and loss of arterial pulsation. Ventilation was stopped when cardiac arrest was induced.

Resuscitation protocols

The time schedule in the four experimental studies was kept identical regarding the length of the non-intervention interval, the duration of external chest
compressions, the time for first intervention with a vasoactive drug and the duration of defibrillations (Fig. 1).

**Figure 1.** Flow chart of experimental protocols in studies I, II, IV and V. A = bolus dose of adrenaline (20 μg/kg). A = bolus dose of adrenaline (20 μg/kg) if spontaneous circulation is not restored. Continuous infusion of adrenaline was administered at 10 μg/kg/min. Vasopressin was administered as a bolus dose (0.4 U/kg). AT = antithrombin (250 U/kg). NaCl = natrium citrate (vehicle for AT). ROSC = restoration of spontaneous circulation.

After a 5-minute non-intervention interval, external chest compressions were initiated with a frequency of 80/min and ventilation was resumed with 100% O₂. Volume-controlled ventilation was used, aiming for an arterial PCO₂ within the range of 5.0-5.5 kPa. After 2 minutes of CPR, pharmacological intervention was started.

**Pharmacological interventions during CPR**

In study I, the effects of intermittent bolus administration and of continuous infusion of adrenaline were investigated. The bolus group received bolus doses of adrenaline i.v. (20 μg/kg) every third minute during CPR. The continuous group received an initial bolus of adrenaline (20 μg/kg) and at the same time a continuous infusion of adrenaline (10 μg/kg/min) was started.

The doses of adrenaline used were based on the following considerations:
- 20 μg/kg is considered as the standard dose in experimental research and this dose approximately corresponds to the adult clinical guideline of 1 mg adrenaline.
Adrenaline administration every 3-5 minutes is recommended in clinical guidelines. To achieve the most beneficial effect, the shortest recommended time interval was used in this experimental study.

A previous experimental dose-response study on continuous infusion of adrenaline during CPR had shown that 10 μg/kg/min was needed to optimise vital organ blood flow.

A bolus dose of adrenaline was given in parallel with the start of the continuous infusion in order to rapidly achieve an adequate plasma concentration of adrenaline.

In **study II** the effects of bolus administration of vasopressin and continuous infusion of adrenaline were compared. The vasopressin group received a bolus of vasopressin (0.4 U/kg). The bolus dose and the infusion rate of adrenaline were the same as in the continuous group in study I.

The dose of vasopressin used was based on the following considerations:
- The vasopressin dose of 0.4 U/kg is the most commonly used dose in experimental studies.
- 0.4 U/kg of vasopressin approximates the dose recommended in clinical guidelines.

In **study IV** parameters of coagulation and inflammation after cardiac arrest were measured. In order to mimic a clinical scenario, a standard dose of adrenaline (20 μg/kg) was administered every third minute during CPR.

In **study V** the effects of AT administration during CPR were investigated. Adrenaline was given during CPR as in study IV. The treatment group received an i.v. bolus injection of 250 U/kg of AT after 7 minutes of CPR.

The dose and the timing of AT were based on the following considerations:
- The dose of AT most commonly used in experimental studies on sepsis has been 250 U/kg.
- The goal was to achieve supraphysiological levels of AT in order to achieve both anticoagulant and anti-inflammatory activity.
- AT was administered at the end of the resuscitation period in order to mimic a clinical situation of cardiac arrest in which AT treatment can be used.

**Chest compressions**

A major concern in studies of CPR is the difficulty in standardising chest compressions. In all experimental studies, a manual sternal compressor was used for external chest compressions and the investigator performing chest compressions was blinded regarding the assigned treatment. In study II, external chest compressions were initiated with a metronome-guided frequency in order to facilitate a constant compression rate. In addition, and most im-
portantly, in the same study the intrathoracic pressure generated during CPR was measured by means of an oesophageal catheter. This pressure was used as a covariate in the statistical model to eliminate this bias.

**Restoration of spontaneous circulation**

ROSC was defined as a pulsatile rhythm with a systolic blood pressure > 60 mm Hg maintained for at least 10 minutes.

**Critical care protocol**

Five minutes after ROSC, FiO₂ was reset to 0.3. If at that time the arterial pH was less than 7.20, a tris buffer mixture of 1 mmol/kg was administered and the minute ventilation was adjusted, aiming for a PCO₂ of 5.0-5.5 kPa. No other interventions were performed during the observation period. The observation period was 4 hours in studies I, IV and V, and 2 hours in study II.

**Measurements**

ECG, systemic arterial blood pressure, right atrial pressure, pulmonary artery blood pressure and temperature were monitored continuously. Cardiac output and blood gases (Papers I, II and V) were measured intermittently.

**Cortical cerebral blood flow**

Laser–Doppler flowmetry was used for continuous measurement of cortical cerebral blood flow in studies I, II and V. Laser-Doppler flowmetry analyses the Doppler shift of a laser beam scattered by moving blood cells in a small volume of tissue [106-108]. The registered signal of perfusion represents the product of the velocity and the concentration of moving blood cells within the measuring volume. Absolute perfusion values cannot be determined with the currently available laser-Doppler instruments. Cortical cerebral blood flow was recorded every fifth second and is presented as a fraction of the baseline flow level prior to induction of cardiac arrest.

The major advantage of the laser-Doppler technique is that it allows continuous measurements of blood flow in situations with non-steady-state blood flow such as CPR and during reperfusion. Furthermore, the technique is well documented and validated, also during low flow states. However, the laser-Doppler technique does have some possible disadvantages that need to be considered:

1. *The observed tissue is relatively small (approximately 1-2 mm³)*:
   - The local measurement of blood flow with laser-Doppler was therefore combined with analysis of the cerebral oxygen extraction, in order to reflect the global cerebral circulation.
   - Although the observed tissue may appear small, it will give a fair spot test of the microcirculation, since 1-2 mm³ = 1-2 *10⁹ μm³, which hosts millions of capillaries and erythrocytes.
Laser-Doppler flowmetry has been validated for measurement of blood flow in the central nervous system, and excellent correlation to blood flow in larger regions of this tissue, as measured by the microsphere technique, has been reported [109].

2. There will be artefacts in the recorded flow level due to movement, contact with large surface vessels and changes in the haematocrit:
   - To prevent movement artefacts, the probe holder and the pig’s head were firmly fixed.
   - When the probe was placed on the cortical surface, care was taken to avoid vessels of the pia mater. If extremely high values of perfusion units were observed while the probe was being placed in position, contact with large vessels was suspected and the probe was placed in a somewhat different position.
   - Haemoconcentration will increase the blood viscosity and thereby reduce the blood flow. In addition, since laser-Doppler analyses the concentration of moving blood cells, changes in the haematocrit need to be considered when interpreting the results.

3. The possible occurrence of multiple scattering, i.e. scattering of light by more than one moving blood cell, will affect the recorded blood flow level:
   - At a high blood flow velocity and with a high concentration of moving blood cells, there is an increased risk of multiple scattering. This phenomenon will lead to underestimation of the blood flow. However, under these conditions the computer program that analyses the Doppler shift uses an algorithm that calculates and compensates for the increased risk of multiple scattering.

**Coronary perfusion pressure**

The coronary perfusion pressure was calculated as the difference between the diastolic aortic and right atrial pressures measured simultaneously (Papers I, II and V). Coronary perfusion pressure correlates to myocardial blood flow [19] and predicts the possibilities of achieving successful resuscitation (ROSC) [110].

**Jugular vein oxygen saturation/ cerebral oxygen extraction**

In order to reflect the balance between global cerebral oxygen supply and consumption, blood gases in cerebral venous blood were analysed (Papers I, II and V). A catheter was inserted in the left internal jugular vein and passed cranially as far as possible towards the base of the skull. In the pig, the internal jugular vein branches into several extracranial vessels. In order to ensure that cerebral venous blood would be obtained, a correct intracranial position of the jugular catheter was confirmed by x-ray (Papers II, IV and V). The catheter was secured with a purse-string suture around the incision in the vein. Thus the vein itself was not ligated, which meant that continuous
blood flow in the vein was allowed after insertion of the catheter [111]. In
study I, jugular vein oxygen saturation was analysed, and in studies II and V
the cerebral oxygen extraction ratio was calculated.

Radioimmunoassay of 8-iso-PGF$_{2\alpha}$ and 15-keto-DH-PGF$_{2\alpha}$

The eicosanoids 8-iso-PGF$_{2\alpha}$ and 15-keto-DH-PGF$_{2\alpha}$ are well-known bio-
markers of oxidative injury and inflammatory response. It has been demon-
strated that the plasma levels of these eicosanoids after cardiac arrest corre-
late to the duration of the ischaemic insult [112]. Plasma samples from the
jugular bulb were analysed for 8-iso-PGF$_{2\alpha}$ (an indicator of oxidative injury)
in studies I, IV and V, and for 15-keto-DH-PGF$_{2\alpha}$ (an indicator of inflamma-
tory response) in studies IV and V, by radioimmunoassay (RIA) as described
elsewhere [113,114].

Antithrombin, thrombin-antithrombin complex and soluble fibrin

Thrombin-antithrombin complex is a widely used marker of thrombin gen-
eration. When fibrinogen is converted by thrombin, soluble fibrin is formed,
and this can be used as a marker of thrombin activity.

AT and soluble fibrin were determined by functional chromogenic assays.
These methods are based on the function of these enzymes. It is an advan-
tage to use this kind of technique instead of techniques based on antibodies,
since these may differ between species. TAT was determined in jugular
blood by an enzyme-linked immunosorbent assay (ELISA) and might possi-
bly therefore have been influenced by species differences in antibodies. It
has been demonstrated, however, that this technique is applicable in the pig
[115,116].

Clinical study of adrenaline use during cardiac arrest
(Paper III)

CPR data

As part of an intrahospital audit at Uppsala University Hospital, all patients
with cardiac arrest in whom CPR was attempted were registered prospec-
tively during the period from 15 January to 31 December 2000. The register
contained information such as place of onset of cardiac arrest (in-hospital or
out-of-hospital), the time interval between initiation and termination of CPR
(CPR interval) and whether or not adrenaline was administered during CPR.

In all, 107 CPR events (107 patients) were recorded in the register. The
total dose of adrenaline was retrieved retrospectively from the patient re-
cords. The following CPR events were excluded from the analysis:
limited CPR attempts due to a documented order “do not to attempt resuscitation” (DNAR)
- CPR of unknown duration
- CPR in which an unknown dose of adrenaline was administered
- CPR with a duration < 5 minutes (considered too short to allow for calculation of a dose interval).

After these exclusions, 53 patients were included in the study and in 49 of these the interval between adrenaline doses could be calculated. Prior to the calculation of the dose interval, 3 minutes was subtracted from the registered CPR interval to allow for the initial assessment, defibrillations and establishment of venous access [117]. This was done in order to reduce the risk of overestimating the dose interval. Under the assumption that 1 mg doses were administered, the dose interval was then calculated by dividing the CPR interval by the total dose of adrenaline.

**Educational status of physicians**
Training in advanced CPR in accordance with the standardised national programme, based on the recommendations by the European Resuscitation Council, was registered in the hospital’s computerised personnel administrative system. Recent education in ACLS was defined as having attended a course in ACLS within the last three years.

**Analysis and statistics**
Data are presented as mean (standard deviation) for variables normally distributed and otherwise as median (range or 25th–75th percentiles). The Shapiro-Wilk test for normality was used on all variables and if this test was significant the normal probability plot was assessed. When applicable, a residual plot was also assessed to ascertain the normal distribution of residuals. For laboratory parameters with lognormal distribution, statistical analysis was performed after logarithmic transformation of the data.

ANOVA for repeated measurements was carried out to test differences between groups and differences from baseline. If significant (p<0.05), planned comparisons were used to test differences at individual time-points between groups and compared to baseline. For haemodynamic parameters, continuously or frequently sampled, the area under the curve was calculated. Differences between groups were tested with Student’s t-test for variables normally distributed and otherwise with the Mann-Whitney U-test. Covariance analysis was used to analyse group differences. The baseline level of each variable before the interventions was tested as the covariate in the statistical model and used when appropriate (p<0.15). Fisher’s exact test was used for comparisons of proportions. When linear correlation was used,
Spearman’s rank correlation coefficient ($r$) was calculated. $P$ values <0.05 were considered significant.
Results

Bolus administration of adrenaline

In studies I, II, IV and V, bolus doses of adrenaline were administered every third minute during resuscitation. In study V, continuous measurements of both cortical cerebral blood flow and coronary perfusion pressure were performed during CPR. As illustrated in Figure 2, adrenaline clearly had a very short duration of action on both these parameters.

Figure 2. Mean levels of cortical cerebral blood flow and coronary perfusion pressure during cardiopulmonary resuscitation (CPR). The cerebral blood flow was measured by continuous laser-Doppler flowmetry and is presented as a fraction of the baseline flow level before cardiac arrest. Bolus A = bolus dose of adrenaline (20μg/kg). (Paper V, n = 24)
In study I, the effects of bolus administration of adrenaline were compared with those of continuous adrenaline infusion. As in study V, bolus administration of adrenaline generated a short peak in cortical cerebral blood flow that lasted about 1-2 minutes. The generated blood flow was lower with bolus administration of adrenaline than with continuous infusion of adrenaline.

**Continuous administration of adrenaline**

In studies I and II adrenaline was infused continuously during CPR. In both studies continuous administration caused an increase in cortical cerebral blood flow. In contrast to bolus administration of adrenaline (Paper I), continuous adrenaline infusion caused a sustained increase in cerebral blood flow that lasted throughout the resuscitation period (Fig. 3). This blood flow was higher during CPR with continuous than with intermittent adrenaline administration (p=0.009).

![Graph](image)

*Figure 3. Mean levels of cortical cerebral blood flow during cardiopulmonary resuscitation (CPR). The blood flow was measured by continuous laser-Doppler flowmetry and is presented as a fraction of the baseline flow level. Bolus A = bolus dose of adrenaline (20µg/kg). Bolus A + continuous A = bolus dose of adrenaline (20µg/kg) with simultaneous start of a continuous infusion of adrenaline (10µg/kg/min). (Paper I, n = 21)*

Although the cerebral blood flow was increased in the group receiving continuous adrenaline, this did not result in higher jugular saturation as com-
pared to bolus administration. Continuous adrenaline infusion seemed to create higher and less fluctuating coronary perfusion pressure than repeated bolus doses of adrenaline, but this difference was not significant (Fig. 4). After ROSC, there was no difference either in cortical cerebral blood flow or in cerebral oxygen extraction between the two groups.

Figure 4. Mean coronary perfusion pressure during cardiopulmonary resuscitation (CPR). This pressure was calculated as the difference between the diastolic aortic and right atrial pressures measured simultaneously. Bolus A = bolus dose of adrenaline (20 μg/kg). Bolus A + continuous A = bolus dose of adrenaline (20 μg/kg) with simultaneous start of a continuous infusion of adrenaline (10 μg/kg/min). (Paper I, n = 24)

In study II the effects of continuous infusion were compared with those of a bolus dose of vasopressin. Continuous infusion of adrenaline was found to be less effective than vasopressin in generation of cortical cerebral blood flow and coronary perfusion pressure.
Vasopressin

In study II, vasopressin was administered as a single bolus dose in the beginning of the resuscitation period and its effects were compared with those of continuous infusion of adrenaline. Vasopressin resulted in a sustained increase in cortical cerebral blood flow that lasted throughout the resuscitation period. This blood flow was higher during CPR with vasopressin than with continuous adrenaline (p<0.001) (Fig. 5). The increase in cerebral blood flow caused by vasopressin also resulted in lower cerebral oxygen extraction as compared to that following adrenaline infusion (p<0.001). Also, 6 minutes after drug administration the vasopressin-treated group had a higher arterial and jugular pH and lower PCO₂ in arterial, jugular and mixed venous blood (p<0.01).

Figure 5. Mean levels of cortical cerebral blood flow during cardiopulmonary resuscitation (CPR). The blood flow was measured continuously by laser-Doppler flowmetry and is presented as a fraction of the baseline flow level before cardiac arrest. Drug administration: In the vasopressin group, a bolus dose (0.4 U/kg) was administered. In the adrenaline group, an initial bolus dose (20µg/kg) was given simultaneously with the start of a continuous infusion of adrenaline (10µg/kg/min). (Paper II, n = 24)
Vasopressin also generated a higher coronary perfusion pressure than continuous adrenaline during CPR (p<0.001) (Fig. 6). This was due to higher diastolic aortic pressure in the vasopressin group. Successful resuscitation was achieved in 12/12 pigs in the vasopressin group, but in 5/12 in the adrenaline group (p=0.005).

Figure 6. Mean levels of coronary perfusion pressure during cardiopulmonary resuscitation (CPR). This pressure was calculated as the difference between the diastolic aortic and right atrial pressures measured simultaneously. Drug administration: In the vasopressin group, a bolus dose (0.4 U/kg) was administered. In the adrenaline group, an initial bolus dose (20 μg/kg) was given simultaneously with the start of a continuous infusion of adrenaline (10 μg/kg/min). (Paper II, n = 24)

There were no differences in cortical cerebral blood flow, cerebral oxygen extraction, cardiac output or mean arterial pressure after ROSC.
In Figure 7, the levels of jugular vein oxygen saturation in the treatment groups in studies I and II are compared. Six minutes after drug administration, this saturation was higher in the vasopressin group than in the three adrenaline groups (p<0.001).

Figure 7. Mean levels of jugular vein oxygen saturation at baseline, after 2 minutes of cardiopulmonary resuscitation (CPR) immediately before drug administration, and 6 minutes after drug administration. Drug administration: In the vasopressin group, a bolus dose (0.4 U/kg) was administered. In the continuous adrenaline groups, an initial bolus dose (20 μg/kg) was given simultaneously with the start of a continuous infusion of adrenaline (10 μg/kg/min). In the bolus adrenaline group, an adrenaline dose (20 μg/kg) was administered 7, 10 and 13 minutes after induction of cardiac arrest. Vertical bars indicate 95% confidence intervals. (Papers I and II, n = 44)
Adrenaline dose interval

In study III, data for evaluation of adrenaline dose intervals were available in 53 patients with cardiac arrest out of 107 registered. Of these 53 patients, 40 (76%) were not given adrenaline every 3-5 minutes as recommended in the current guidelines (Fig. 8). In 36 patients (68%) the adrenaline dose interval was longer than recommended and in 4 patients (8%) no adrenaline was administered. The median interval between adrenaline doses during CPR was 6.5 minutes (25th-75th percentile: 5.1-10.4).

![Figure 8. Calculated adrenaline dose interval in individual patients. White bars = shorter dose interval than recommended, light grey bars = within recommended dose interval, dark grey bars = longer dose interval than recommended, black bars = no adrenaline administration. # indicates dose interval longer than 15 minutes (16, 16, 17 and 44 minutes respectively). Median and 25th–75th percentile of calculated adrenaline dose interval in relation to recommended dose interval are indicated. (Paper III, n = 53)](image)

There was no difference in dose intervals between resuscitations led by physicians with and without recent education in ACLS. The calculated interval between adrenaline doses was longer in out-of-hospital cardiac arrest than in in-hospital cardiac arrest (p=0.01).
Coagulation and inflammation after ROSC

In study IV, coagulation parameters after CPR were determined. Immediately after ROSC there was a reduction in AT and a concomitant increase in TAT (Fig. 9). The AT concentration in jugular venous blood was lower than that at baseline at all time points except in the 5-minute sample (p<0.05). The lowest mean AT level was 79 %, which occurred 60 minutes after ROSC. The TAT level was higher than that at baseline at all time points during the first 3 hours of the observation period (p<0.05). The mean TAT level was increased almost five-fold 5 minutes after ROSC.

Similar results for AT and TAT were confirmed in the control group in study V (Fig. 9). There was a non-significant increase in the level of soluble fibrin in jugular venous blood as compared to baseline in the control group in study V.

![Figure 9](image-url)

*Figure 9.* Mean levels of antithrombin (AT) and thrombin-antithrombin complex (TAT) in jugular venous blood at baseline and after restoration of spontaneous circulation (ROSC). TAT is presented as logarithmic values. Vertical bars indicate 95 % confidence intervals. (Paper IV and control group in paper V, n = 15)
Eicosanoids were measured in studies I, IV and V (Fig. 10). The parameters determined were considered to be markers for oxidative injury (8-iso-PGF$_{2\alpha}$) and an inflammatory response (15-keto-DH-PGF$_{2\alpha}$). Both 8-iso-PGF$_{2\alpha}$ and 15-keto-DH-PGF$_{2\alpha}$ increased markedly after ROSC in all studies and remained elevated for 3-4 hours. The eicosanoid levels were not affected by the different pharmacological interventions in studies I and V.

![Graph](image)

**Figure 10.** Mean levels of 15-keto-DH-PGF$_{2\alpha}$ and 8-iso-PGF$_{2\alpha}$ in jugular venous blood at baseline and after restoration of spontaneous circulation (ROSC). Vertical bars indicate 95% confidence intervals. (Papers I, IV and V; 8-iso: n = 40, 15-keto: n = 24).

**Antithrombin administration**

In study V, the treatment group received 250 U/kg of AT at the end of the resuscitation period. The mean AT concentration achieved during the observation period was 380%. AT administration did not increase the cerebral circulation or reduce reperfusion injury, as measured by cortical cerebral blood flow, cerebral oxygen extraction and levels of eicosanoids, after ROSC. AT treatment resulted in a non-significant decrease in soluble fibrin as compared to the control group.
Haemoconcentration

The levels of haemoglobin were measured both during CPR (Papers I and II) and after ROSC (Papers I, II, IV and V). Despite continuous fluid administration throughout the experiments, there was marked haemoconcentration during CPR and initially after successful resuscitation (Fig. 11). Five minutes after ROSC, the haemoglobin concentration was 30-40% higher than at baseline in all studies. Thereafter, it gradually decreased.

Figure 11. Mean haemoglobin concentrations at baseline, during cardiopulmonary resuscitation (CPR) and after restoration of spontaneous circulation (ROSC). Vertical bars indicate 95% confidence intervals. (Papers I, II, IV and V, n = 38-82).
Discussion

Resuscitation: areas of improvement

There are several different areas to attack in the struggle to improve the outcome after CPR. This thesis deals with the following objectives: firstly, to increase vital organ blood flow during the actual resuscitation phase. Secondly, to further clarify the spectrum of different disturbances that may contribute to the cerebral injury which can occur both during CPR and after ROSC. Thirdly, to improve vital organ blood flow after ROSC and diminish reperfusion injury. Finally, to evaluate the clinical usage of adrenaline during CPR and the adherence to ACLS guidelines.

Generation of adequate vital organ blood flow during CPR

Since standard CPR cannot establish sufficient blood flow to vital organs [118], several different vasoactive pharmacological substances have been tried and evaluated during resuscitation. Despite decades of intense research in this field, the clinical guidelines regarding such drugs have remained relatively unchanged, with intermittent administration of adrenaline still considered as a gold standard. However, after promising results in experimental and clinical studies, vasopressin was introduced in the most recent guidelines as an alternative to adrenaline for VF and pulseless VT.

Adrenaline: bolus versus continuous administration

It is reasonable to assume that a constantly high cerebral blood flow during CPR would be beneficial for the neurological outcome. In studies I and V, it was demonstrated that bolus injections of adrenaline resulted in temporary peaks in cortical cerebral blood flow, followed by intervals with little or no effect. This is in accordance with an earlier report that the duration of action was short - irrespective of whether standard- or high-dose adrenaline was used [39]. Thus, the consequence of intermittent administration of adrenaline is repeated periods without the desirable effect. During the last two decades, research in the field of adrenaline use in CPR has almost exclusively con-
cerned the optimal dose with bolus administration. It might also be of importance, however, to focus on the optimal mode of administration.

Considering the apparently short duration of the effects of a bolus dose of adrenaline, the recommended dose interval appears inappropriate. Thus there are two different options for adjustment of the administration in order to match the short duration of action, namely to shorten the dose interval or to apply continuous infusion. However, a shorter dose interval could be highly impractical in the clinical setting and would certainly be difficult to adhere to, as demonstrated in study III. Hence, continuous infusion might be a more attractive solution, but this modality has not been evaluated in the clinical setting, although a case report of successful usage of continuous adrenaline during CPR was described as early as in the beginning of the 20th century [119].

Today, continuous infusion of vasoactive drugs is commonly used in anaesthesia and intensive care, and is of particular importance in the case of drugs with a short duration of action. In situations of hypotension in the critically ill, continuous infusion of adrenaline is widely practised. During CPR in experimental studies, adrenaline infusion has occasionally been used [20,40,120,121], but until now no comparisons have been made between continuous and intermittent adrenaline.

In study I, continuous infusion of adrenaline was found to generate higher and less fluctuating cortical cerebral blood flow than bolus injections of adrenaline every third minute. Even though the shortest recommended interval was used for intermittent administration, adrenaline seemed to be more effective when infused continuously. Another advantage of continuous infusion is that adequate doses will more likely be administered throughout the resuscitation period, since the dose intervals do not need to be remembered. Pumps for drug administration are easy to handle and should not cause any substantial delay in the initiation of the pharmacological intervention.

Continuous infusion of adrenaline was used in both study I and study II. Notably, the haemodynamic effects of adrenaline in study II tended to be weaker than those in study I. The experimental set-up, the adrenaline dose and the timing of the intervention were identical in these two studies. The reasons for the poor effects of adrenaline in study II can only be speculated upon. Wide inter-individual variability in catecholamine pharmacology during CPR is well established [122,123], and adrenaline doses that are adequate in some animals may be toxic in others. Also, rapid tachyphylaxis may develop, possibly as a result of desensitisation of myocardial and peripheral adrenergic receptors [124], leading to reduced effects of the drug. It is also possible that under conditions of CPR, continuous administration of adrenaline may have the unfavourable effect of triggering the development of a hyperadrenergic state. Thus, although continuous adrenaline may be more beneficial for cerebral blood flow than bolus administration, the possibility of adverse effects on the heart must be kept in mind.
Vasopressin

Vasopressin administration during CPR has been evaluated in a number of studies over the last decade. Several experimental studies have demonstrated that bolus administration of vasopressin is superior to bolus administration of adrenaline in the generation of vital organ blood flow during CPR [42-45]. We hypothesised that the superiority of vasopressin could be due to its considerably longer duration of action as compared to that of adrenaline. Bearing the differences in pharmacodynamics in mind, bolus administration of vasopressin was compared with continuous infusion of adrenaline (Paper II). Although adrenaline was administered continuously, vasopressin was considerably more effective in generating blood flow to vital organs. By continuous measurement of cerebral blood flow and coronary perfusion pressure, the sustained and substantial increase in these parameters generated by one single dose of vasopressin could be observed. The increase in cerebral blood flow caused by vasopressin during CPR was sufficiently pronounced to decrease cerebral oxygen extraction and reduce cerebral venous PCO₂. In addition, vasopressin administration generated a substantial increase in coronary perfusion pressure, and consequently successful resuscitation was achieved in all animals.

Vasopressin receptors differ between humans (arginine vasopressin) and pigs (lysine vasopressin). Exogenously administered arginine vasopressin, which was used in study II, may therefore result in a different haemodynamic response in pigs compared to that in humans. However, since humans have arginine vasopressin receptors, the circulatory effects of arginine vasopressin administered to humans may be even more pronounced than those in pigs.

Disturbances during and after CPR

Coagulation

After resuscitation from cardiac arrest, several different mechanisms contribute to a disturbed microcirculation and to reperfusion injury. One of these mechanisms, previously described, is activation of the coagulation system, which is believed to impair the cerebral microcirculation in particular [125]. Our observations of increased levels of thrombin (as reflected by TAT) and decreased levels of AT in studies IV and V further support earlier findings of an activated coagulation system during reperfusion after cardiac arrest [68-70]. However, although the TAT levels increased several-fold, there was only a moderate reduction in AT. Additionally, the generation of thrombin did not result in a significant increase in soluble fibrin, which could have been expected if the coagulation had been more powerfully activated. These findings possibly indicate that the physiological levels of AT are sufficient to
counteract the thrombin generation in this model. However, it is likely that in a situation with a longer duration of cardiac arrest and in the presence of arteriosclerosis, with a damaged and dysfunctional endothelium, more profound effects on the coagulation system would have occurred. This theory is supported by observations of increased TAT levels in patients with arteriosclerotic vascular disease [82].

Haemoconcentration
A consistent finding in all our experimental studies was the presence of substantial haemoconcentration in the initial period after ROSC. This occurred despite considerable fluid administration throughout the experiment. Five minutes after ROSC, the haemoglobin concentration was 30-40% higher than at baseline in all studies. This is in accordance with earlier reports of a reduction in plasma volume to about 60% after resuscitation from cardiac arrest [104,126].

Haemoconcentration after cerebral ischaemia in cases of cardiac arrest is believed to result from capillary leakage during the period of arrest and reperfusion [127]. The occurrence of haemoconcentration may severely interfere with the microcirculation, since the blood viscosity rises substantially with an increased haematocrit [128]. Accordingly, it has been demonstrated that volume expansion during CPR and reperfusion improves the cerebral microcirculation after ROSC [5,129]. However, volume expansion has not achieved a role in clinical CPR [38], on account of its possible negative effects on myocardial function.

Inflammation
It is widely accepted that cardiac arrest triggers an inflammatory response, and several different inflammatory mediators have been found to be elevated during reperfusion [77-79]. The thrombin generation and concomitant AT reduction observed in studies IV and V could possibly be factors contributing to this inflammatory response.

In studies I, IV and V, eicosanoids were measured for validation of oxidative injury and an inflammatory response after cardiac arrest. In all three studies, the measured eicosanoids were increased during the post-resuscitation period, providing further evidence of involvement of these disturbances after CPR. This is in accordance with previous findings in both experimental and clinical studies, indicating that oxidative injury and an inflammatory response may contribute to microcirculatory disturbances and reperfusion injury [72,74,77,78,112]. The possibly detrimental effects of the inflammatory disturbances, however, cannot be assessed from the present studies, which only confirm their occurrence after cardiac arrest.
Pharmacological interventions to increase cerebral blood flow and reduce reperfusion injury

Interventions aimed at preventing further cerebral injury after ROSC may have their site of action at several different levels. Hypertensive reperfusion, volume expansion, induced hypothermia and thrombolysis are some of the interventions that have had promising results [5,8,129,130]. In the latest international guidelines, the evidence for benefit from these interventions was considered insufficient to recommend their use in clinical practice [38]. However, since these guidelines were written, important studies have been published regarding hypothermia [131,132] and thrombolysis [90,91]. The next ACLS guidelines will most likely include a recommendation of therapeutic hypothermia for unconscious survivors of cardiac arrest and possibly also of the use of thrombolysis in association with CPR.

In study V, our intention was to counteract both coagulation and inflammation and thereby to increase the cerebral microcirculation and attenuate cerebral injury. To achieve this, AT was administered, since it possesses both anticoagulant and anti-inflammatory properties. However, in this experimental setting we found no beneficial effects of AT administration.

There are several possible explanations for the lack of effect of AT. First, the observed thrombin activation after resuscitation was accompanied by only a moderate reduction of AT, possibly indicating that the physiological levels of AT were sufficient. Secondly, in order to mimic a clinical situation of cardiac arrest, AT was administered at the end of the resuscitation period. Earlier experimental evidence indicates that AT should be administered at an early phase to be able to overcome thrombin generation and its consequences [94]. It is therefore possible that most of the microcirculatory disturbances and inflammatory reactions may already have been triggered by the time AT was given. Thirdly, the microcirculatory disturbances that could be affected by AT may play only a minor role in the disturbance of cerebral microcirculation in this model. Finally, it is unlikely that one single pharmacological intervention would inhibit the complex mechanisms that contribute to the post-resuscitation syndrome. Combinations of therapies are most certainly required to counteract the spectrum of post-resuscitation disturbances.

Adherence to ACLS guidelines

In contrast to previous ACLS guidelines, the guidelines of the year 2000 were evidence-based and can therefore be considered as prescriptive. It is clear that efforts should be made to practice CPR in accordance with these guidelines in order to improve the outcome further.

It has been demonstrated that in general, physicians are relatively non-compliant with ACLS guidelines when managing cardiac arrest [60]. In
study III it was found that adherence to ACLS guidelines was poor regarding the recommended adrenaline dose interval, an issue not studied previously. At the time of the data collection, national guidelines recommended that adrenaline should be administered every 2 to 3 minutes during CPR [30]. Only 4 out of 53 patients were treated in accordance with these recommendations.

Studies have shown that lack of adherence to guidelines is not a specific problem for ACLS, but a general problem in medicine, and several possible barriers to adherence to guidelines have been identified [133]. These include lack of outcome expectancy and lack of familiarity with current guidelines, which may contribute to non-adherence regarding adrenaline usage during CPR. In study III, the physician in charge had had recent education in ACLS in the majority of the events. However, recent education in ACLS did not markedly affect the adherence to guidelines in study III, which is in accordance with an earlier report [60]. In study III, the individual physician was only occasionally confronted with the resuscitation scenario. Thus, it is possible that despite recent training in ACLS, a lack of clinical experience may constitute an additional barrier to adherence to guidelines in a stressful situation such as cardiac arrest.

Another reason for the prolonged dose interval observed might be the absence of a strategy for how to achieve such a precise and narrow dose interval as that recommended. It has recently been demonstrated that absence of leadership and explicit task distribution among the resuscitation team members is associated with poor ACLS performance [134]. Such deficiency might possibly also contribute to failure of adrenaline administration. In order to improve the adherence regarding the adrenaline dose interval, the physician in charge could appoint one person in the resuscitation team to be responsible for the administration of adrenaline every 3 to 5 minutes during the entire resuscitation. Another solution could be to have audio prompts from the defibrillator every 3 to 5 minutes, alerting the physician to consider another dose of adrenaline.

Although several experimental studies have shown positive effects of adrenaline during CPR [19-21], these benefits have still not been demonstrated convincingly in clinical studies [56-59]. The reason for this discrepancy between experimental and clinical studies has not been thoroughly addressed. One explanation might be that adrenaline is not administered as recommended. A combination of the short duration of action of adrenaline and long dose intervals, as found in study III, may partly explain why adrenaline has failed to improve the outcome as compared to placebo in clinical studies.
ACLS guidelines: the past, present and future

Since the first publication of ACLS guidelines, adrenaline has been the vasoactive drug of choice. The search for an optimal dose of adrenaline has been extensive, but the original 1 mg dose is still recommended. Compared with the intense research regarding the optimal dose, the dose interval has been far less investigated or discussed. Despite the short duration of action of adrenaline, the recommended between-dose interval was extended in the international guidelines of the year 2000. The extension of the dose interval is not well motivated and may lack a base of evidence. In view of the findings in these studies, it seems as if the potentially beneficial effects of adrenaline during CPR are currently not being optimally utilised.

After promising results in experimental studies, vasopressin was introduced as an alternative to adrenaline for VF and pulseless VT in the guidelines of the year 2000. In clinical studies the superiority of vasopressin over adrenaline has not been completely convincing [61,62]. However, in a recent multicentre study of out-of-hospital cardiac arrest, vasopressin was shown to be more effective than adrenaline among patients with asystole [53]. This finding may widen the indications for vasopressin to include asystole in the next ACLS guidelines. In the same study, however, adrenaline was just as effective as vasopressin in resuscitation from VF and pulseless electrical activity. Thus, it seems premature to conclude that the era of adrenaline is coming to an end. In addition, there may still be ways to improve the usage of adrenaline as discussed above. To find a dose regime of adrenaline that would be as effective as vasopressin could have some clinical advantages. Adrenaline has wide clinical acceptance and has been used in cardiac arrest for over a century [12], whereas the clinical experience of vasopressin is still somewhat limited. In addition, adrenaline is far less expensive than vasopressin.

The generation of high vital organ blood flow during CPR is most certainly of great importance, but improvements in several other areas are also necessary to improve the outcome after cardiac arrest. As demonstrated in this thesis, haemoconcentration, coagulation and inflammation occur after resuscitation. These disturbances may all contribute to an impaired microcirculation, reperfusion injury and eventually a worse outcome. In the present studies, administration of AT at the end of the resuscitation period failed to improve the cerebral microcirculation. Although this finding was somewhat discouraging, anticoagulative and anti-inflammatory interventions may still be beneficial during and after resuscitation. However, it is highly unlikely that one single intervention can counteract the wide spectrum of disturbances in ischaemia and reperfusion after cardiac arrest. Future studies, and eventually clinical practice, might depend on combinations of therapies to improve the outcome after cardiac arrest.
The bridge between research and the actual treatment of the individual patient is based on two crucial components: first, guidelines have to be adequately updated, with recommendations based on current scientific evidence. Secondly, guidelines need to be accepted and adhered to in clinical practice. If one of these links fails, advances in research will be fruitless. The concept of evidence-based ACLS guidelines was a key step regarding the first issue. International consensus on the science of resuscitation has obvious advantages, but may also have possible disadvantages. A consensus tends to create a culture of rigidity, leading to delay in the introduction of new advances [135]. The second component, successful implementation of guidelines, may be a particular problem in stressful situations such as cardiac arrest. Data in this field are limited, and research in the area of how to improve adherence to guidelines seems to have been somewhat neglected. This may be an issue of great potential for improvement in the outcome after cardiac arrest. In future ACLS guidelines, aspects of adherence to recommendations should be integrated. This might promote satisfactory compliance to ACLS guidelines in the clinical setting of CPR and possibly improve the outcome.
Conclusions

- Continuously administered adrenaline generated higher cortical cerebral blood flow during CPR as compared to intermittent bolus doses of adrenaline. Thus, the currently recommended practice of bolus administration of adrenaline during CPR might not be optimal for the cerebral circulation.

- Vasopressin increased the cortical cerebral blood flow and reduced cerebral oxygen extraction during CPR to a considerably greater extent as compared to continuous infusion of adrenaline. In addition, vasopressin generated higher coronary perfusion pressure and increased the likelihood of achieving ROSC.

- In the majority of cases of adult cardiac arrest, adrenaline appeared to have been administered at longer dose intervals than are recommended. Thus, non-compliance to ACLS guidelines may contribute to the lack of beneficial effects of adrenaline in clinical outcome studies.

- After resuscitation the circulating levels of eicosanoids are increased, indicating oxidative injury and an inflammatory response.

- There is substantial haemoconcentration after resuscitation.

- AT is moderately reduced and TAT concomitantly increased following successful resuscitation after cardiac arrest. There is reason to believe that this AT reduction is a result of activation of thrombin and thereby consumption of AT.

- High-dose administration of AT during CPR does not seem to offer any beneficial effects on cerebral circulation or reperfusion injury after ROSC in this experimental setting.
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