Interventions on arterial pressure and perfusion flow rate during cardiopulmonary bypass:

Effects on global fluid shifts, cerebral metabolic and structural markers in a porcine model

Oddbjørn Haugen

"Dissertation for the degree philosophiae doctor (PhD)"

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2008

ISBN 978-82-308-0492-6

Bergen, Norway 2008

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Scientific environment

Paul Husby is professor at the Institute for Surgical Sciences, Faculty of Medicine, University of Bergen. Since 1996 he has been leading a research group engaged in experimental studies of the relationship between body temperature and fluid homeostasis. The research group is part of "Locus for Circulatory Research" which is an officially recognized research community within the Faculty of Medicine, University of Bergen.
Recently Jon-Kenneth Heltne (2002) and Marit Farstad (2006) defended their theses based on data obtained in this group.

The present study was conducted in the period from 2004 – 2006. All experiments took place in the "Vivarium" at the Faculty of Medicine and were carried out with the necessary assistance from cardiac surgeons, perfusionists and the technical staff of the laboratory. Associate professor Olav Egil Bøe, Department of Odontology, University of Bergen, has supervised the group regarding statistical processing of the data.
Paper V of the dissertation was produced in close cooperation with professor Reidar Myklebust at the Molecular Imaging Center of The Department of Biomedicine, University of Bergen.

Acknowledgements

Approaching the end of this work, I would like to thank my supervisor professor Paul Husby for his support, ideas and never failing optimism when confronted with expected and unexpected obstacles along the way. His generosity in sharing his time and experience with the group is unique and was most appreciated.

I also would like to express my gratitude to my colleague and friend Marit Farstad who, besides introducing me to the laboratory, has assisted me in the conduct of most of the experiments and participated in numerous intriguing and constructive discussions.
The contribution by my colleague Stig Hammersborg and Hege Brekke by taking care of many tedious tasks during the labor intensive experiments was also highly appreciated.
Furthermore, I would like to acknowledge the thoracic surgeons Stein Erik Rynning and Venny Kvalheim who have provided me with excellent surgery throughout theses studies.
Associate professor Olav Egil Bøe has repeatedly given us advices regarding statistical processing of the data. We are grateful for his valuable support.

The perfusionists Arve Mongstad, Else Nygreen and Finn Eliassen have helped me in this project by running the cardiopulmonary bypass machine in a most competent and consistent way. I am deeply grateful for their important contribution. I also would like to thank the laboratory staff at the Surgical Research Laboratory, Institute for Surgical Sciences, Inger Vikøyr, Gry Hilde Nilsen, Lill Andreassen, Cato Johnsen and Rune Grøvdal at the Vivarium, for their logistic and technical support, enthusiasm and always fresh coffee.

My research scholarship was based on funding from the Western Norway Health Authority. I am indebted to them for giving even “grown up” doctors the possibility to engage themselves in research activities. I also owe thanks to The Frank Mohn Foundation and the Faculty of Medicine, University of Bergen for financial support to the project. The favorable attitude from my employee, the Clinic of Surgical Services, Haukeland University Hospital, was essential for the accomplishment of this project. I would like to express my gratitude for their flexibility and good will in this period.

Finally, I want to thank Gunn, Marie, Ingrid Odella and Vebjørn for supporting me and for putting up with an absent-minded husband and father.

Introduction

On May 6. 1953 John Gibbon Jr. for the first time used cardiopulmonary bypass (CPB) successfully during cardiac surgery in a 18 year old girl with a large atrial septal defect (Gibbon, 1959). Since then the procedure has become part of the daily routine in tertiary hospitals worldwide. In 2004 646 000 CPB-procedures was performed in the US (American Heart Association, 2007) while 5163 procedures was carried out in Norway (Svennevig JL, 2005).

However, despite the huge accumulated experience with this technique, the physiological changes induced by CPB are not totally understood. Furthermore several important aspects of the practical conduct of CPB are not supported by solid scientific evidence.

The objective of the present dissertation is to contribute to a better understanding of some of the processes associated with cardiopulmonary bypass.

Abstract

Global and regional organ perfusion during cardiopulmonary bypass (CPB) depends on hemodynamic parameters as mean arterial pressure (MAP) and perfusion flow rate. These parameters may also exert influence on the load of micro-emboli delivered to the central nervous system (Sungurtekin et al., 1999), to the operating conditions (Cartwright & Mangano, 1998) and to the degree of extravascular fluid accumulation during CPB (Paper III).

The present thesis focus on some specific consequences of different MAP and perfusion flow rate values during CPB. Two particular endpoints have been addressed:

A. Net fluid balance and fluid extravasation rate during CPB (Paper I - III)
B. Cerebral biochemical changes associated with energy metabolism and ultrastructural integrity (Paper IV – V)

Paper I compares a group of animals with lowered MAP (LP-group, n=7) by use of nitroprusside and a historical control group (C-group, n=7)) with respect to fluid shifts.
Paper II compares groups with elevated MAP by norepinephrine (HP-group, n=8)) and lowered MAP by phentolamine (LP-group, n=8)), also with respect to fluid shifts.
Paper III determines fluid shifts in groups with two different CPB perfusion flow rates (LF-group, n=8 and HF-group, n=8).
Paper IV assesses cerebral biochemical markers in groups with elevated MAP by norepinephrine (HP-group, n=6) and lowered MAP by nitroprusside (LP-group, n=6).
Paper V assesses the same cerebral markers as well as mitochondrial ultrastructure by electron microscopy in animals with elevated MAP by norepinephrine (HP-group, n=8) and lowered MAP by phentolamine (LP-group, n=8).

Methods:

Young pigs aged 10-12 weeks were given general anesthesia and underwent 60 minutes of normothermic CPB (38°C) followed by 90 minutes of hypothermic CPB (28°C). Acetated Ringer's solution was given with 5 ml/kg/h i.v. and as CPB prime. Extra acetated Ringer's solution was added to the CPB venous reservoir whenever necessary, to maintain a constant level.

In paper I, II, IV and V infusions of vasoactive agents were given during the whole CPB period. MAP was kept between 60 – 80 mmHg in the animals with elevated arterial pressure and at 40 – 45 mmHg in the animals with reduced arterial pressure. The two groups of animals in paper III had CPB perfusion flow rate set to 80 ml/kg/min and 110 ml/kg/min, respectively.

Colloid osmotic pressure in plasma and interstitial fluid (wick method) was measured in addition to acid base parameters and blood chemistry. Plasma volume was determined by the carbon-monoxide method and subsequent changes were calculated based on new values of hematocrit and the measured amount of bleeding. Fluid extravasation rate was calculated as net fluid balance minus the change in plasma volume over a defined period of time.

Intracranial pressure was monitored. Cerebral glucose, lactate, pyruvate and glycerol were measured by microdialysis. After each experiment, total tissue water content was determined in relevant organs. In paper V, cerebral tissue from cortex and thalamus in two animals from each group, were examined by electron microscopy.

Results:

Paper I:

Net fluid balance was higher in the LP-group as compared with the C-group after 30 min of CPB. Fluid extravasation rate tended to be higher in the LP-group. The animals of the LP-group did have higher tissue water content in the myocardium, skin and gastrointestinal tract as compared with the control group.

Paper II:

Plasma volume was higher in the LP-group as compared with the HP-group after 60 minutes of CPB. Net fluid balance and fluid extravasation rate did not differ between the two study groups. Left myocardial tissue water content was slightly higher in the LP-group compared with the HP-group.

Paper III:

Plasma volume was higher in the HF-group compared with the LF-group after 60 minutes of CPB. During the initial phase of CPB, fluid extravasation rate was significantly higher in the HF-group. The average net fluid balance during CPB was higher and the average fluid extravasation rate tended strongly to be higher in the HF-group as compared with the LF-

group (P=0.07). Total tissue water content of the kidneys were higher in the HF-group and tended to be higher in most other organs as compared with the LF-group.

Paper IV:
Intracranial pressure increased in both groups during CPB. Intracerebral glucose decreased while lactate-pyruvate ratio and cerebral glycerol increased significantly during CPB in the LP-group as compared with pre-bypass values. The values remained stable and within normal range in the HP-group.

Paper V:
Cerebral lactate was higher in LP-group as compared with HP-group during normothermic CPB. Compared to baseline, cerebral glucose decreased and cerebral lactate, lactate-pyruvate ratio and glycerol increased in the LP-group during normothermic CPB. The values remained unchanged in the HP-group. Electron microscopy of cortical and thalamic tissue, showed a higher frequency of altered mitochondria in the LP-group as compared with the HP-group.

Conclusion:

Paper I-II suggest that different levels of MAP by use of nitroprusside, phentolamine or norepinephrine have essentially no influence on fluid extravasation rate. An impact on net fluid balance was found in paper I.

Paper III demonstrate that elevation of CPB flow rate to 110 ml/kg/min, may lead to higher positive net fluid balance and probably higher fluid extravasation rate as compared with a CPB flow rate of 80 ml/kg/min.

Plasma volume was affected by the use of these vasoactive agents and was significantly higher in the study groups receiving phentolamine as compared with norepinephrine. Indeed, plasma volume was also affected by CPB flow rate, resulting in higher values in the experimental group with higher CPB flow rate.

In paper IV and V we found that a reduction of MAP to about 40 mmHg during CPB by nitroprusside or phentolamine was associated with changes in cerebral markers of metabolism and membrane integrity compatible with cerebral ischemia and membrane degradation. Electron microscopic examination of cortical and thalamic tissue demonstrated a high frequency of mitochondrial alterations in two animals with reduced MAP by phentolamine in paper V.

Sammendrag på norsk

Blodtrykket og kroppens totale blodstrøm er to faktorer som i stor grad bestemmer hvor mye oksygen og glukose de enkelte organ får tilført. Dette gjelder generelt så vel som ved bruk av hjertelungemaskin.

Andre prosesser som for eksempel væsketransporten ut av blodårene, kan teoretisk bli påvirket av blodstrøm og blodtrykk. For å studere dette undersøkte vi unge griser i narkose som ble lagt på hjertelungemaskin i 150 minutter. Denne avhandlingen tar for seg to typer konsekvenser av at en varierer middel-blodtrykket og blodstrømmen hos forsøksdyr på hjertelungemaskin:

A. Netto væskebalanse og væskeutsivning fra blodbanens kapillærer

B. Biokjemiske og strukturelle forandringer i hjernen som påvirkes av oksygenmangel

Artikkel I : Her sammenlignet vi væskebalanse og væskeutsivning hos 7 kontrolldyr fra tidligere forsøk og 7 dyr som fikk middelblodtrykket sitt redusert til ca 40 mmHg ved bruk av nitroprussid. Vi fant noe mer positiv væskebalanse og en tendens til høyere væskelekkasje hos de dyrene som hadde fått senket middel-blodtrykket.

Artikkel II: Vi sammenlignet væskeomsetningen hos 8 dyr med hevet middelblodtrykket til 60 – 80 mmHg vha noradrenalin og 8 dyr med senket middelblodtrykk til 40 – 45 mmHg vha fentolamin. Vi fant at plasma volumet var størst hos de som hadde lavt blodtrykk. Vi fant ikke noen forskjell i væskebalansen eller væskelekkasjen ut av blodbanen mellom gruppene.

Artikkel III: Nå sammenlignet vi væskeomsetningen hos 8 dyr som hadde 80 ml/kg/min i blodstrøm med 8 dyr som hadde 110 ml/kg/min i blodstrøm på hjertelungemaskin. Vi fant at de som hadde høyest blodstrøm hadde mer positiv væskebalanse og at de i en tidlig fase også hadde større væskelekkasje ut av blodbanen enn de med lavere blodstrøm. Dessuten fant vi størst plasma volum hos de dyrene som hadde størst blodstrøm.

Artikkel IV: Her brukte vi mikrodialyse teknikk til å måle innholdet av glukose, laktat, pyruvate og glyserol i hjernen på 6 griser som hadde fått redusert middelblodtrykket til 40 mmHg vha nitroprussid og 6 griser som hadde fått hevet middelblodtrykket til 60 – 80 mmHg vha noradrenalin mens de var på hjertelungemaskin. Hos dyrene med senket blodtrykk sank

verdien av glukose mens laktat, laktat-pyruvat ratio og glyserol steg. Disse forandringene er kjent for å opptre når hjernen får utilstrekkelig blodtilførsel. Hos dyrene med høyt blodtrykk holdt verdiene seg normale.

Artikkel V: 8 dyr med redusert middelblodtrykket til 40 – 45 mmHg vha fentolamin og 8 dyr med øket middelblodtrykk til 60 – 80 mmHg vha noradrenalin, ble studert. Vi målte de samme stoffene i hjernen som beskrevet under artikkel IV. Dyrene med lavt blodtrykk hadde lavere verdier av laktat i hjernen enn de med høyt blodtrykk. Videre fant vi reduksjon i verdiene av glukose og økning i laktat, laktat-pyruvat ratio og glyserol i gruppen med lavt trykk. I tillegg tok vi ut hjernevev fra to dyr i hver gruppe og undersøkte med elektronmikroskop. Energimangel kan føre til visse karakteristiske forandringer i hjernecellenes mitokondrier med bl.a. oppsvulming. Vi fant at de dyrene som hadde fått redusert blodtrykket hadde øket andel av forandrede mitokondrier.

Konklusjoner:

- Å senke middelblodtrykket til 40 mmHg med nitroprussid, fører ikke til reduksjon i væskebalansen eller væskelekkasjen på hjertelungemaskin.
- Middelblodtrykk på 60 – 80 mmHg vha noradrenalin og 40 – 45 mmHg vha fentolamin på hjertelungemaskin gir samme væskebalanse og samme grad av væskelekkasje ut av blodbanen.
- Blodstrøm på 110 ml/kg/min på hjertelungemaskin gir mer positiv væskebalanse og høyere væskelekkasje enn blodstrøm på 80 ml/kg/min.
- Middelblodtrykk på ca 40 mmHg på hjertelungemaskin vha nitroprussid eller fentolamin fører til redusert nivå av glukose og øket nivå av laktat, laktat-pyruvat ratio og glyserol. Forandringene er forenelig med en tilstand av utilstrekkelig blodtilførsel.
- Middelarterietrykk på 40 – 45 mmHg vha fentolamin på hjertelungemaskin, kan være forbundet med skade på mitokondrier.

List of original papers

I Haugen O, Farstad M, Kvalheim V, Rynning SE, Mongstad A, Husby P. Low arterial pressure during cardiopulmonary bypass in piglets does not decrease fluid leakage. ***Acta Anaesthesiol Scand 2005; 49: 1255-1262***

II Haugen O, Farstad M, Kvalheim V, Hammersborg S, Husby P. Intraoperative fluid balance during cardiopulmonary bypass: Effects of different mean arterial pressures. ***Perfusion 2007; 22: 273-278***

III Haugen O, Farstad M, Kvalheim V, Bøe O, Husby P. Elevated flow rate during cardiopulmonary bypass is associated with fluid accumulation. ***J Thorac Cardiovasc Surg 2007; 134: 587-593***

IV Haugen O, Farstad M, Kvalheim VL, Rynning SE, Hammersborg S, Mongstad A, Husby P. Mean arterial pressure about 40 mmHg during CPB is associated with cerebral ischemia in piglets. ***Scand Cardiovasc J; 2006; 40: 54-61***

V Haugen O, Farstad M, Myklebust R, Kvalheim V, Hammersborg S, Husby P. Low perfusion pressure during CPB may induce cerebral metabolic and ultrastructural changes. ***Scand Cardiovasc J 2007; 41: 331-338***

Abbreviations

BIS	Bispectral index
BV	Blood volume
CBF	Cerebral blood flow
CFC	Capillary filtration coefficient
CO	Carbon monoxide
COPi	Colloid osmotic pressure in interstitial fluid
COPp	Colloid osmotic pressure in plasma
CPB	Cardiopulmonary bypass
CPP	Cerebral perfusion pressure
CVP	Central venous pressure
CVR	Cerebral vascular resistance
FER	Fluid extravasation rate
Hb	Hemoglobin
HbCO	Carboxyhemoglobin
Hct	Hematocrit
ICP	Intracranial pressure
Jv	Net capillary filtration
Lp	Hydraulic conductivity
MAP	Mean arterial pressure
NFB	Net fluid balance
NIRS	Near infrared spectroscopy
Pc	Capillary hydrostatic pressure
Pi	Interstitial fluid hydrostatic pressure
PV	Plasma volume
S	Capillary surface area
V_{RBC}	Erythrocyte volume
σ	Sigma = Capillary reflection coefficient

Content

Interventions on arterial pressure and perfusion flow rate during cardiopulmonary bypass: Effects on global fluid shifts, cerebral metabolic and structural markers in a porcine model

Background

A. Physiology of cardiopulmonary bypass (CPB)

Conventional CPB has proven to be a highly useful procedure by enabling the performance of advanced cardiac surgery. At the same time it imposes considerable stress and perturbation to normal human physiology.

Some aspects of the "unnatural" state of CPB will be briefly summarized:

The initiation of CPB is accompanied by an acute hemodilution and a sudden imbalance in the Starling forces, resulting in loss of fluid from the circulation to the interstitial space. The oxygen-carrying capacity of the blood is also reduced and this must be compensated for by improved microcirculation and/or a reduction in oxygen demand in order to provide adequate oxygen supply to the body.

The exposure of blood elements to the synthetic surfaces of the CPB circuit may trigger a systemic inflammatory response including activation of platelets, neutrophils and endothelial cells (Larmann &Theilmeier, 2004). The surgical trauma itself, ischemia-reperfusion, translocation of endotoxin from the gastrointestinal tract and recirculation of blood which is drained from the surgical wound may further aggravate the inflammatory response (Hess, 2005, Skrabal et al., 2006).

Moderate or severe hypothermia is often induced during cardiac surgery in order to protect the central nervous system from ischemic injury. However, hypothermia has profound impact on human physiology, with adverse effects on fluid extravasation, immune response and coagulation (Mallet, 2002).

CPB perfusion flow rate is usually kept at a level below normal cardiac output in humans. The current practice of using a CPB flow rate of 2.2 – 2.5 l/m^2/min was supported by

the observation that normothermic CPB with blood prime, normal arterial oxygen content and flow in this range, resulted in normal values for oxygen delivery and consumption (Kirklin et al., 1957, Cook, 2001).

Even the systemic arterial pressure is allowed to fall below the normal level during CPB. Autoregulatory mechanisms are assumed to preserve adequate organ perfusion (Ahonen & Salmenperä, 2004).

B. Fluid shifts during CPB

B 1. The mechanisms of microvascular fluid shifts

CPB is associated with a positive fluid balance mainly related to increased fluid extravasation from the capillaries to the interstitial space although expansion of the vascular space may also contribute (Paper II-III). The fluid shifts across the capillary membrane are basically determined by the Starling equation (Starling, 1896):

$$\mathbf{J_v = CFC\ [(P_c - P_i) - \sigma\ (COP_p - COP_i)]}$$

J_v	=	net capillary filtration rate
CFC	=	capillary filtration coefficient
		(hydraulic conductivity • capillary surface area)
P_c	=	capillary hydrostatic pressure
P_i	=	interstitial hydrostatic pressure
σ	=	capillary reflection coefficient for plasma proteins (sigma)
COP_p	=	colloid osmotic pressure in plasma
COP_i	=	colloid osmotic pressure in interstitial fluid

Mixing of the CPB prime volume with the blood of the patient leads to an acute hemodilution with reduced COP_p and consequently an enhanced transport of fluid from the capillary compartment.

The systemic inflammatory response during CPB could theoretically contribute to fluid extravasation by increasing the transcapillary forces as well as reducing σ and increasing the hydraulic conductivity (Levick, 2003). However, pre-treatment with anti-inflammatory

drugs did not reduce fluid extravasation in a recent study from our group (Farstad et al., 2004). Furthermore, we have not been able to demonstrate loss of plasma proteins from the circulation during CPB (Farstad et al., 2003).

Hypothermia has lately been identified as an independent cause of fluid extravasation during CPB (Farstad et al., 2003). The exact mechanism behind this is not established, although an early experimental work on isolated cat limb with constant flow perfusion may suggest that hypothermia may elevate P_c by an increase in postcapillary resistance (Zhang & Wolf, 1991).

The relation between the hemodynamic parameters and fluid balance during CPB are not thoroughly investigated. Both CPB perfusion flow rate and systemic arterial pressure could theoretically have an impact on transcapillary fluid shifts through mechanisms as changes in P_c or COP_p. These issues are addressed in paper I - III.

B 2. Effects of peroperative fluid accumulation

The relevance of studies on fluid shifts during CPB is based on the clinical experience that fluid accumulation and edema formation may contribute to postoperative organ dysfunction.

An association between peroperative fluid overload and postoperative adverse outcome has been demonstrated in cardiac surgery (Toraman et al., 2004), in abdominal surgery (Tambyraja et al., 2004, Itobi et al., 2006) as well as in pulmonary surgery (Møller et al., 2002). Two prospective, randomized trials demonstrated that restricted fluid administration during abdominal surgery reduced the occurrence of postoperative complications (Brandstrup et al., 2003, Nisanevich et al., 2005). In cardiac surgery with CPB, reduced fluid accumulation by ultrafiltration resulted in less respiratory, gastrointestinal and neurological postoperative complications (Luciani et al., 2001).

The effect of fluid overload on individual organ systems is less clear. Boldt demonstrated that high positive fluid balance during CPB was associated with an increase in extravascular lung water and reduction in pulmonary gas exchange (Boldt et al., 1986). Myocardial edema may impair systolic as well as diastolic function according to experimental studies (Melhorn et al., 2001). The evidence is, however, conflicting. In a recently published study of dogs undergoing CPB, myocardial edema did not affect the left ventricular function (Fisher et al., 2006).

Generalized fluid extravasation during CPB also seems to involve the brain. Magnetic resonance imaging studies have demonstrated elevated cerebral water content after CPB (Harris et al., 1998). When comparing patients referred for coronary artery bypass grafting without and with bypass, only the latter group had elevated cerebral water content after surgery (Anderson et al., 1999). Whether the cerebral swelling contributes to postoperative neurological dysfunction is not obvious, but there are clinical as well as experimental evidence that intracranial pressure may increase during CPB (Lundar et al., 1982, McDaniel et al., 1994). This in turn may reduce cerebral perfusion pressure, rendering the patient more vulnerable to systemic arterial hypotension.

C. Cerebral effects of CPB

C 1. Cerebral oxygen supply and demand during CPB

The low hematocrit level following hemodilution with crystalloid prime, results in reduced oxygen-carrying capacity of the blood. This should be balanced by either increased cerebral perfusion or reduced oxygen demand.

Most anesthetic agents have the ability to reduce cerebral metabolic rate and may also by other mechanisms exert some degree of neuroprotection (Sanders et al., 2005).

Hypothermia during CPB has been widely used to reduce cerebral metabolic rate and prevent neurological dysfunction. The relationship between temperature and metabolic rate may be expressed as Q_{10}, defined as the ratio of two metabolic rates at temperatures divided by 10 °C. The value is reported to lie between 2 and 3 in humans (Croughwell et al., 1992) corresponding to a decrease in cerebral metabolism of about 6 % per °C.

Recently increasing attention has been drawn to the fact that hypothermia inhibits the release of glutamate during and following ischemia. This seems to be another important mechanism for hypothermic neuronprotection (Shaaban et al., 2005).

Numerous clinical studies have assessed the effects of hypothermia on outcome. The results are conflicting. Apparently, modest hypothermia (35°C) provide the same cerebral protection as more profound hypothermia (Grigore et al., 2001, Shaaban et al., 2005), while only a slight degree of hyperthermia seems to have deleterious effects on the central nervous system.

C 2. Cerebral perfusion during CPB

Cerebral blood flow (CBF) is determined by Darcy's law of flow:

CBF = CPP / CVR

CPP = cerebral perfusion pressure (MAP minus intracranial pressure)

CVR = cerebrovascular resistance

The hemodilution of CPB will inevitably lead to lower viscosity and hence reduced cerebrovascular resistance according to the law of Poiseuille:

$$\mathbf{R = 8\eta L / \pi r^4}$$

R = resistance

η = viscosity

L = length of the vascular "tube"

r = radius

Cerebrovascular resistance is primarily determined by cerebral autoregulation, which can be divided into metabolic and pressure autoregulation. The latter is the ability of cerebral vessels to maintain constant blood flow above a wide range of cerebral perfusion pressure. Three important questions need to be answered:

1) Is cerebral autoregulation preserved during CPB?

2) If so, what is the lower limit for cerebral autoregulation?

3) At what level of cerebral perfusion pressure will the cerebral oxygen supply become inadequate?

The literature indicates that cerebral autoregulation is preserved during normothermic as well as hypothermic CPB, although the plateau may have a certain positive slope (Plöcl et al., 1998, Sadahiro et al., 1994, Newman et al., 1996). The lower limit of autoregulation was previously assumed to be as low as 20 – 30 mmHg during hypothermic CPB based on pooled clinical observations (Murkin et al., 1987). Later animal studies have concluded that the lower limit is about 50mmHg during normothermia as well as moderate hypothermia (Sadahiro et al., 1994). In healthy anesthetized children similar values were found (Vavilala et al., 2003).

After initiation of CPB, mean arterial pressure usually drops to values below the presumed lower limit of cerebral autoregulation and occasionally hypotension persists. Since monitoring of cerebral oxygenation is not available in most centers, management of patients with peroperative hypotension may vary considerably between surgical teams.

C 3. Neurological dysfunction after CPB

Cerebral complications are a major concern after cardiac surgery with CPB. According to the American College of Cardiology / American Hearth Association guidelines for CABG surgery, postoperative neurological deficits are divided into two types (Eagle et al., 1999):

Type 1: Major focal neurological deficits, stupor and coma

Type 2: Deterioration in intellectual function, confusion, agitation, memory deficit or seizures without evidence of focal injury

Type 1 outcome was found in 3.3 % of 416 347 patients after cardiac surgery (Hogue et al., 2001). The reported incidence of type 2 deficits varies, depending on the study design. Defined as a certain percentage or standard deviation of cognitive decline after CPB, the incidence may be 50-70 % within the first week, falling to 30 – 50 % after 6 weeks and 20 – 40 % between 6 months and a year (Newman et al., 2006).

The etiology of postoperative neurological dysfunction is not fully understood. Overt stroke is commonly attributed to episodes of macro-embolism while neurocognitive dysfunction may be caused by micro-embolism or by the systemic inflammatory response (Ahonen & Salmenperä, 2004).

The role of inadequate cerebral perfusion pressure as etiologic factor is controversial. Stockard analyzed the relation between peroperative hypotension and postoperative neurological dysfunction in 23 adult patients undergoing CPB (Stockard et al., 1973). Mean arterial pressure was recorded as a continuous curve with a horizontal line drawn at the level of 50 mmHg. The area between the line and the curve below the line was used as indicator of arterial hypotension. The study concluded that there was an association between peroperative MAP below 50 mmHg and risk of neurological injury.

The relation between cerebral perfusion pressure and energy metabolism is discussed in paper IV and V.

D. Aims of the study

D1. One objective was to study the effect of different levels of MAP and CPB perfusion flow rates on fluid balance and fluid extravasation during normothermic (38°C) and hypothermic (28°C) CPB.

These hypotheses were tested:

- Different levels of systemic arterial pressure during CPB are associated with differences in net fluid balance and fluid extravasation rate.

- Elevated flow rate during CPB leads to a more positive net fluid balance and higher fluid extravasation rate as compared with a conventional CPB flow rate.

D2. Another objective was to study the effects on cerebral energy metabolites and ultrastructure during normothermic and hypothermic CPB with two different levels of mean arterial pressure.

This hypothesis was tested:

- Mean arterial pressure of 40 – 45 mmHg during CPB is associated with cerebral ischemia and cellular injury.

Methods

E. Animals, anesthesia, surgical preparation

E1. Research animals

Immature domestic pigs, aged 12 – 14 weeks, were used (Norwegian landrace-Yorkshire hybrid). Animal handling was in accordance with guidelines given by the Norwegian Animal Research Authority. The animals were acclimatized at the facility for minimum 3 days before the experiments. Food was withdrawn 8-12 hours before anesthesia while water was available until pre-medication was given. The animals were killed with an i.v. injection of 20 ml saturated potassium chloride at the end of each experiment.

Allocation of the individual animal to study groups was performed by consecutive inclusion or by randomization (drawing notes). Due to the ethical aspects of animal research as well as the high costs, some of the animals appear in two studies. The protocols of the study groups involved were, however, identical. A chronologic overview of the animals included is disclosed below:

Paper I: 7 animals were included consecutively and compared with a historical control group (n=7).

Paper IV: 12 animals were randomly allocated to the high pressure (HP) group (n=6) and the low pressure (LP) group (n=6).

Paper V: 16 animals were assigned to a HP-group (n=8) and a LP-group (n=8). 6 animals of the HP-group were also used in paper IV.

Paper III: 16 animals were randomly assigned to the high flow (HF) group (n=8) and the low flow (LF) group (n=8).

Paper II: 16 animals were assigned to a HP-group (n=8) and a LP-group (n=8). 2 animals of the HP- group and all animals of the LP-group were also included in paper V.

E 2. Anesthesia and surgical preparation

The anesthetic protocol used in these studies was previously evaluated with standard noxious stimuli (Husby et al., 1998) and has been widely used in its original form without signs of discomfort or stress from the animals (Farstad et al., 2003, Farstad et al., 2004). Due to shivering during hypothermia, pancuronium was recently added to the protocol (Paper II-V).

The current regime includes pre-medication with 500 mg ketamine, 10 mg diazepam and 1 mg atropine given subcutaneously 30 min before start of general anesthesia.
Induction of general anesthesia is accomplished by inhalation of isoflurane in oxygen supplemented by thiopentone 5 mg/kg iv before endotracheal intubation.
Anesthesia is maintained by an iv infusion of 0.5 mg/kg/h midazolam, 7.5 µg/kg/h fentanyl, 45 µg/kg/h pancuronium and by inhalation of isoflurane 0.5 – 2.0 volume % with 50 % oxygen in air.

The surgical procedure with sternotomy and preparation of the heart for extracorporeal circulation was similar in all animals. Cannulation of aorta (18F) and the right atrium (32F)

were performed. In paper II- V the left ventricle was vented (17F) to obtain a nearly constant total body perfusion during these experiments. Accordingly, alterations in mean arterial pressure were assumed to reflect alterations in systemic vascular resistance.

Systemic arterial and venous pressures were in all studies monitored by fluid filled catheters in arteria and vena femoralis, respectively. In addition right atrial pressure was monitored in paper II-V.

In paper III, a single dose of heparin 9 mg/kg was given while in the other papers heparin 6 mg/kg was given before start of CPB with an extra 3 mg/kg added after an hour. The effect of anticoagulation was not assayed.

Surgery was usually completed after 20 – 30 minutes. All animals were then allowed 60 min of stabilization before start of bypass. Next, CPB was initiated with 60 minutes of normothermia (38°C) followed by 90 minutes of hypothermia (28ºC). 38 - 39°C is considered to be normal body temperature in domestic pigs and was consequently set as target temperature during normothermia. During cooling the water in the heat exchanger was kept at 24 °C.

F. Cardiopulmonary bypass and fluid supplementation

F 1. Cardiopulmonary bypass

The CPB circuit consisted of a filtered venous reservoir, a polypropylene hollow fiber membrane oxygenator with integrated heat exchanger and an arterial roller-pump. In paper I and IV, the Bentley SpiralOxy HSR-4000 (Baxter, USA) was used as oxygenator. This system was uncoated. Due to withdrawal from the market, the Quadrox VHK 4200 system (Jostra AG, Germany) was introduced in paper II, III and V. The new system was similar, containing a hollow fibre membrane oxygenator with an integrated heat exchanger and a venous hard shell cardiotomy reservoir. It was produced with a "non-active" coating (Safeline®).

In paper I CPB flow rate was set to 80 ml/kg/min, corresponding to 2.7 l/m^2/min in a pig weighing 30 kg. However CPB flow rate was increased to 110 ml/kg/min in the studies with high and low MAP, in order to facilitate the elevation of the mean arterial pressure with norepinephrine (Paper II, IV and V). The indexed value of 110 ml/kg/min is 3.7 l/m^2/min. In paper III the study groups had 80 and 110 ml/kg/min respectively. Flow pattern was non-pulsatile in all experiments.

In paper I the priming volume was 1000 ml. Due to introduction of left ventricular venting, the priming volume was increased to 1115 ml in paper II - V. With these priming volumes the venous reservoir of the Bentley Spiraloxy system was filled to a level of 400 ml while the reservoir of the Quadrox system was filled to 300 ml.
Acetated Ringer's solution was used as prime in all experiments.

Acid base analysis during CPB is conventionally performed either with temperature correction (pH-stat) or without (alpha-stat). In the present project acid base management was carried out according to the alpha-stat strategy.

The venous drainage during CPB was achieved by gravity through a single stage cannula in the right atrium. The height difference between the reservoir and the right atrium was kept at a fixed level of 73± 3 cm, and the drainage was secured by visual inspection and by monitoring of the right atrial pressure.

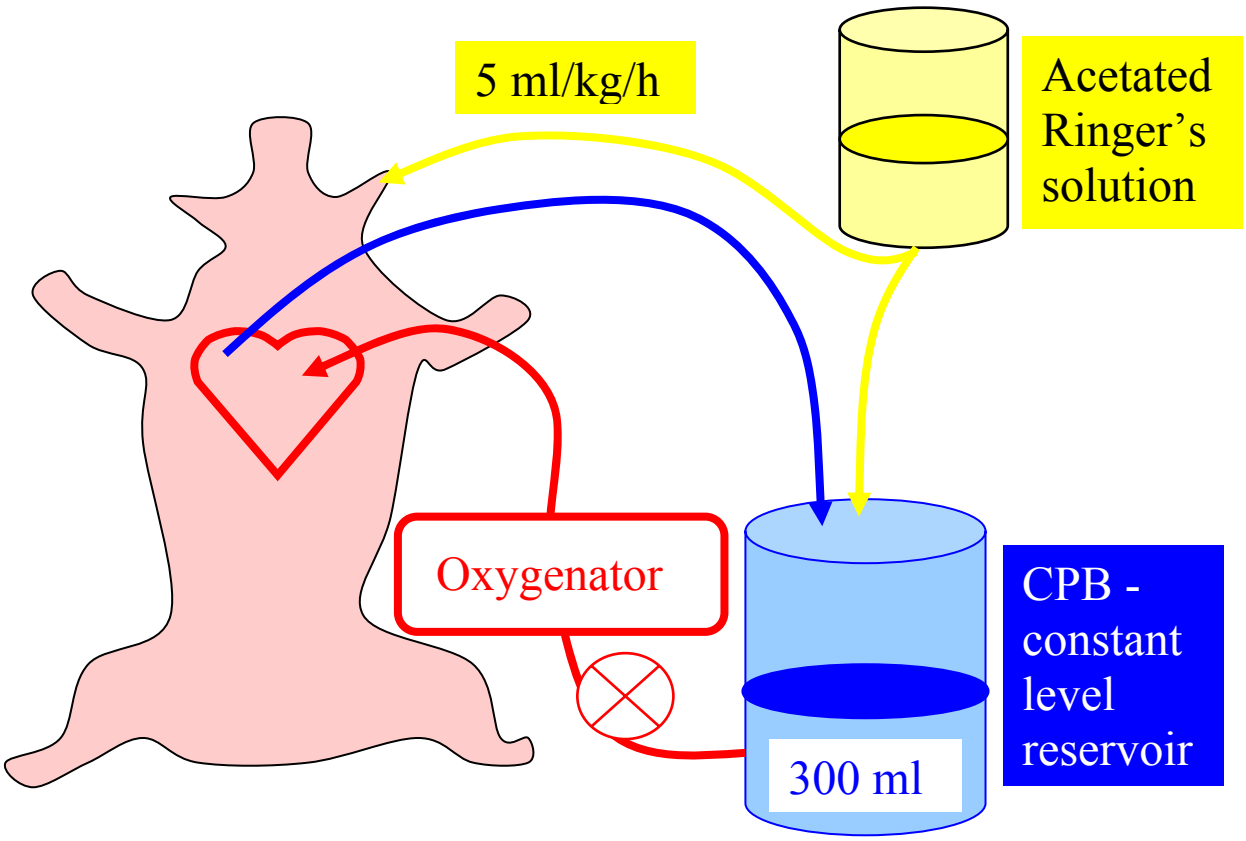

Figure 1

Sketch of the fluid supplementation protocol used in all animals during CPB

F 2. Fluid supplementation

In all animals acetated Ringer's solution was given as a continuous infusion with 5 ml/kg/h from start of anesthesia. Hemorrhage was substituted for with acetated Ringer's solution in volumes three times the blood loss. During CPB blood lost to surgical wound were returned to circulation by suction.

The fluid level of the CPB venous reservoir was carefully monitored and recorded. Whenever the fluid level fell, acetated Ringer's solution was added to restore the original level. Changes in the fluid level were assumed to reflect either fluid shift across the capillary wall or changes in the vascular capacity, provided that the venous drainage was adequate.

G. Monitoring and measurements

G 1. Determination of blood and plasma volume

In order to distinguish between fluid shifts across the capillary wall and changes in vascular capacity, we needed to measure vascular capacity.

Determination of plasma volume or blood volume may be done by various dilution techniques which have in common the administration of a tracer. The tracer should be stable and distributed evenly within the volume of interest. The final concentration may then be measured and the volume of distribution calculated. For instance, blood volume may be measured by adding radioactive labeled erythrocytes (^{51}Cr) while plasma volume can be measured by Evans blue dye.

The use of carbon monoxide (CO) as tracer for measuring erythrocyte volume was first introduced in 1882 by Grehant and Quinquard, but was not widely used before a more convenient method for measuring HbCO was established (Fogh-Andersen et al., 1987). The method was primarily developed for patients with spontaneous ventilation, but was recently adapted to patients on ventilator (Dingley et al., 1999).

Heltne et al. compared determination of plasma volume by Evans blue dye technique and by the CO-technique in a porcine model using a closed ventilator circuit. They concluded that the CO-technique was simple and safe and that the agreement between the two methods was satisfactory (Heltne et al., 2002).

In paper I - III baseline values for plasma volume in the pigs were determined by the CO-method. 30 ml of CO under atmospheric pressure was added to a closed circle breathing system 30 minutes before start of CPB. HbCO was measure before adding CO and after 10

minutes of equilibration. The following equation was used to calculate baseline plasma volume (Burge & Skinner, 1995):

1. $\mathbf{nCO = 1000 \times \left(\frac{[P_B : 101{,}333\ ] \times V\ co}{0{,}08206 \times [273 + T\ ]} \right)}$

2. $\mathbf{nHb = [\ n\ CO \times 25\ ] : \Delta HbCO}$

3. $\mathbf{V_{RBC} = [\ 644 \times Hct \times nHb\ ] : Hb}$

4. $\mathbf{BV = [V_{RBC} \times 100] : Hct \times CF}$

5. $\mathbf{PV = BV - V_{RBC}}$

nCO	=	number of mmol CO added to the breathing system
P_B	=	barometric pressure (kPa)
V_{CO}	=	volume of CO added to the breathing system (liter)
T	=	room temperature (°C)
nHb	=	number of mmol hemoglobin in the animal
Δ HbCO	=	difference in HbCO before and after CO administration
V_{RBC}	=	erythrocyte volume in the animal (milliliter)
Hct	=	hematocrit (%)
Hb	=	hemoglobin (gram per liter)
BV	=	blood volume in the animal (milliliter)
CF	=	correction factor for trapped plasma (0.96 – 0.98) (Anderson 1970)
PV	=	plasma volume in the animal (milliliter)

Every 30 minutes throughout the course of the experiment all losses of blood were recorded and new Hct values were obtained. Based on the previous Hct (Hct_1), the new Hct (Hct_2) and loss of blood during the last 30 minutes (BL_{30}), the reduction in V_{RBC} (ΔV_{RBC}) and new values for V_{RBC} and PV could be calculated as well as the change in PV (ΔPV):

ΔV_{RBC} = $[(Hct_2 + Hct_1): 2] \times BL_{30}$

New V_{RBC} = Previous V_{RBC} - ΔV_{RBC}

New PV = (New V_{RBC} x $(1-Hct_2)$):Hct_2

ΔPV = New PV – Previous PV

During CPB the calculated total PV had to be corrected for the simultaneous volume of plasma in the CPB circuit to determine the PV contained in the animal.

G 2. Calculation of fluid shifts

Net fluid balance (NFB) was calculated for every 30 minutes. The total amount of fluid added to the animal was recorded. Urinary output and plasma lost by bleeding were then subtracted to obtain net fluid balance. Fluid extravasation rate (FER) for a period of 30 minutes was calculated as follows:

FER = NFB – ΔPV

Results of FER and NFB during CPB are presented in paper I - III, using the unit of ml/kg/min.

G 3. Colloid osmotic pressures in plasma and interstitial fluid

Several methods for collection of interstitial fluid have been described and tested: the blister suction technique (Groth & Staberg, 1984, Haaverstad et al., 1996), the wick technique (Aukland & Fadnes, 1973) and more recently the technique of isolating interstitial fluid by tissue centrifugation (Wiig et al., 2003).

The method of implanting nylon wicks was introduced in 1973 (Aukland & Fadnes, 1973) and has later been evaluated in pigs (Heltne et al., 1998). The best correlation between obtained values and true interstitial colloid osmotic pressure (COPi) was found by keeping the wicks in situ for 90-120 minutes before removal.

In paper I -III interstitial fluid was sampled by the wick method from subcutaneous tissue of the lateral thorax and abdomen for determination of COPi. We used three implantation periods, each lasting 90 minutes, in order to assess the conditions before bypass, during normothermic bypass and during hypothermic bypass. By the end of each period the wicks were swiftly pulled out, submerged in mineral oil and centrifuged.

Colloid osmotic pressure in plasma (COPp) and interstitial fluid was determined by a colloid osmometer designed to accept samples of 5 µl (Aukland & Johnsen, 1974). The reference chamber was filled with acetated Ringer's solution and separated from the sample chamber by a semi- permeable membrane with cut-off level of 10 000 Dalton.

G 4. Measurement of cerebral blood flow by colored microspheres

Our laboratory has adopted the technique of measuring regional organ blood flow by infusion of colored microspheres (Kowallik et al., 1991). The same principle was previously used with radioactively labeled microspheres (Tranquilli et al., 1982), but is currently considered unacceptable in large animals as pigs, due to the inherent radiation hazard.

The method implies administration into the left atrium of a known amount of colored microspheres, sized 15 µm. All microspheres are assumed to be trapped in the microcirculation by the first passage. During CPB the microspheres are added through the arterial line of the CPB circuit. A constant rate retraction pump collects a reference blood sample from aorta, starting before administration of microspheres and continuing until all microspheres have been cleared from circulation.

Provided that the microspheres are evenly mixed within the arterial system, one can assume that there is a constant relationship between flow in an organ and the number of microspheres trapped in the same organ (Tranquilli et al., 1982):

$$\mathbf{K = Q_T / N_T = Q_R / N_R = Q_L / N_L \longrightarrow Q_L = Q_R \times N_L / N_R}$$

K	=	constant
Q_T	=	cardiac output
N_T	=	total number of microspheres administered
Q_R	=	reference blood sample flow rate
N_R	=	number of microspheres in the reference blood sample

Q_L = local organ blood flow

N_L = number of microspheres trapped in the local organ

In paper IV cerebral flow data based on these principles are presented.

G 5. Microdialysis

The idea of introducing an artificial capillary into the tissue to collect molecules from the interstitial space was first introduced in the 1970s (Delgado et al., 1972, Ungerstedt & Pycock, 1974). Microdialysis is now appreciated as a powerful research tool as well as monitoring device within the neurosurgical field.

Shortly, the technique implies that a fluid, similar to acetated Ringer's solution, is slowly perfused through the afferent and efferent part of a microdialysis catheter placed in the tissue of interest. Molecules then pass across the semi-permeable membrane of the catheter along their concentration gradient, usually to accumulate in the perfusate.

The efficacy of the microdialysis system may be expressed as the system recovery. In general, the recovery of a substance depends on the 1) length of the dialysis membrane, 2) the perfusion flow rate, 3) properties of the membrane and 4) speed of diffusion through the extracellular fluid. The term "relative recovery" refers to the concentration of a particular substance in the perfusate when it leaves the probe, expressed as a percentage of the concentration in the surrounding medium (Ungerstedt, 1991). With perfusion rate of 0.3 µl/min and a membrane length of 10 mm, relative recovery is approximately 70 % for cerebral glucose, lactate and pyruvate (Hutchinson et al., 2002). In paper IV and V we used membrane length of 20 mm, implying that we probably achieved even higher relative recovery.

The "absolute recovery" of a substance means the absolute amount collected in the perfusate per unit of time. The latter also depends on the local production or release of the substance of interest.

Numerous substances contained in the interstitial fluid, have been studied; for instance drugs and drug metabolites, energy metabolites, neurotransmitters and mediators of inflammation and cell injury, only to mention a few (Hillered et al., 2005).

Inadequate cerebral supply of glucose and oxygen lead to depletion of ATP, accumulation of NADH and conversion of pyruvate to lactate. The extracellular lactate-pyruvate ratio is a particular useful marker of cerebral ischemia since it obviates the concerns

regarding system recovery and it reflexes the intracellular redox state according to the chemical equation (Ståhl et al., 2001):

$$\textbf{Pyruvic acid} + \textbf{NADH} + \textbf{H}^+ \rightleftharpoons \textbf{Lactic acid} + \textbf{NAD}^+$$

$$\textbf{K}_{\textbf{LDH}} \times \textbf{[Lactic acid] / [Pyruvic acid]} = \textbf{[NADH]} \bullet \textbf{[H}^+\textbf{] / [NAD}^+\textbf{]}$$

Reduction in cerebral glucose level is supposed to be a sign of inadequate supply while elevation of intracerebral glycerol is considered to follow degradation of phospholipids from the cellular membranes. In paper IV and V we employed cerebral microdialysis to monitor the intracerebral levels of glucose, lactate, pyruvate and glycerol. Samples were collected and analyzed every 30 minutes. The data obtained were assessed in the light of published reference values for pigs (Reinstrup et al., 2000).

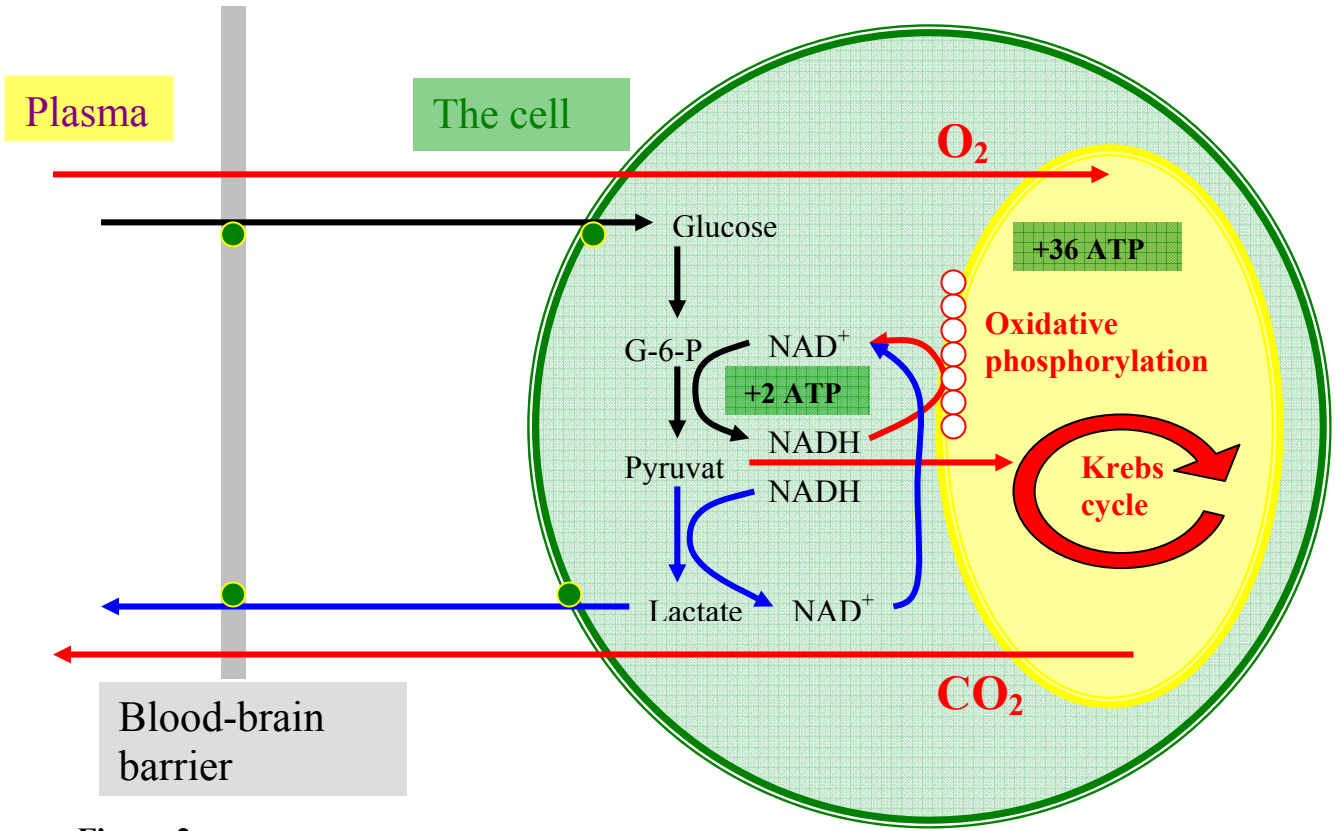

Figure 2

The major cerebral metabolic pathway. Red lines show aerobe metabolism. Blue lines show anaerobe metabolism. Black lines show the common metabolic path.

G 6. Total tissue water content

At the completion of each experiment tissue samples from the heart, lungs, liver, pancreas, kidney, stomach, intestines, thigh muscle, abdominal skin and brain were collected in triplicate, placed in pre-weighted vials, weighted again and stored in a drying chamber at 70°C. The following days the vials were weighted repeatedly until stable weight was obtained. The loss of weight was considered to represent the total water content of the tissues and was presented in the papers as the water content per weight of dried tissue. In paper I - III data from all organs are presented while in paper IV - V only cerebral water content are reported.

In paper I total tissue water content of a historical group of 13 pigs was also presented and compared with the study groups. These animals were sacrificed shortly after induction of general anesthesia and never underwent CPB. In paper IV total tissue water content in cerebral tissue from 7 of these animals were presented.

G 7. Transmission electron microscopy

The short wavelength of electrons as compared to visible light gives the electron microscope an extremely high resolution and makes it suitable for studies of sub-cellular morphology.

Morphologic changes with swelling of cerebral mitochondria have been described early after ischemic events (Yamamoto et al., 1986). A pattern of morphologic alterations have been linked to a specific biochemical process called the mitochondrial permeability transition (MPT) (Solinski et al., 2002). The same process seems to play an important role in the development of necrotic as well as apoptotic cell death (Neumar, 2000).

In paper V transmission electron microscopy was applied to study samples of cerebral tissue with respect to the integrity of mitochondria in neurons and supportive cells. Importantly the samples were collected and submerged in fixatives within 3 minutes after cardiac arrest to avoid post-mortal artifacts. Samples were taken from the frontal and parietal watershed regions in two animals from each group.

Synopsis of results

Paper I

In this study a group of animals (LP-group, n=7) with MAP lowered to 40 mmHg by use of nitroprusside and a historical control group (C-group, n=7) were compared with respect to fluid shifts during normothermic (38° C) and hypothermic (28°C) CPB.

Result: Net fluid balance was higher in the LP-group as compared with the C-group after 30 min of CPB and fluid extravasation rate tended to be higher in the LP-group. The animals of the LP-group had higher plasma volume at the end of CPB and higher tissue water content in the myocardium, skin and gastrointestinal tract as compared to the animals of the control group.

Albumin and protein masses did not change in either of the groups during CPB.

Paper II

The purpose of this study was to compare two groups of animals with respect to fluid shifts during normothermic (38° C) and hypothermic (28°C) CPB; one group with MAP elevated to 60 – 80 mmHg by norepinephrine (HP-group, n=8) and another group with MAP lowered to 40 – 45 mmHg by phentolamine (LP-group, n=8).

Result: Plasma volume was higher in the LP-group as compared with the HP-group after 60 minutes of CPB. Net fluid balance and fluid extravasation rate did not differ between the two study groups. Left myocardial tissue water content was slightly higher in the LP-group compared to the HP-group.

Paper III

Here we compared fluid shifts during normothermic (38° C) and hypothermic (28°C) CPB in groups of animals with CPB perfusion flow rate at 80 ml/kg/min (LF-group, n=8) and at 110 ml/kg/min (HF-group, n=8).

Result: Plasma volume was higher in the HF-group compared with the LF-group after 60 minutes of CPB. During the initial phase of CPB, fluid extravasation rate was significantly higher in the HF-group. The average net fluid balance during CPB was higher and the average fluid extravasation rate tended strongly to be higher in the HF-group as compared with the LF-group (P=0.07). Total tissue water content of the

kidneys were higher in the HF-group and tended to be higher in most other organs as compared with the LF-group.

Paper IV

Biochemical markers of cerebral energy metabolism and integrity were studied in groups with elevated MAP by norepinephrine (HP-group,n=6) and lowered MAP by nitroprusside (LP-group, n=6) normothermic (38° C) and hypothermic (28°C) CPB. Intracerebral pressure was monitored and microdialysis used to collect samples from the cerebral interstitium.

Result: Intracranial pressure increased in both groups during CPB. Intracerebral glucose decreased while lactate-pyruvate ratio and glycerol increased significantly during CPB in the LP-group as compared with pre-bypass values. The values remained stable and within normal range in the HP-group.

Paper V

The same cerebral metabolic markers were studies in animals with elevated MAP by norepinephrine (HP-group, n=8) and lowered MAP by phentolamine (LP-group, n=8) during normothermic (38° C) and hypothermic (28°C) CPB. In two animals from each group mitochondrial ultrastructure was studied by transmission electron microscopy.

Result: Both groups demonstrated an increase in ICP during CPB. Cerebral lactate was higher in LP-group as compared with HP-group during normothermic CPB. Compared to baseline, cerebral glucose decreased and cerebral lactate, lactate-pyruvate ratio and glycerol increased in the LP-group during normothermic CPB. The values remained unchanged in the HP-group.

Electron microscopy of cortical and thalamic tissue, showed a higher frequency of altered mitochondria in the LP-group as compared with the HP-group.

Discussion

H. The pig as research animal

Young domestic pigs are widely used in cardiovascular research. They resemble human anatomy and physiology, are easy to acquire and are inexpensive. Pigs weighting 25 – 30 kg have the same ratio between heart and body weight as humans (Hughes, 1986). From 3 months of age the central nervous circulatory regulation is mature (Verdouw et al., 1998), and sexual maturity is reached from the age of 3 -7 months (Swindle, 1998).

The physiological reference values for young pigs may differ from older ones and from humans. For instance, plasma colloid osmotic pressure in young resting pigs were found to be 17.0 ± 1.72 mmHg (Hannon et al., 1990) while in healthy young men the serum colloid osmotic pressure was 26.9 ± 4.1 mmHg (Noddeland, 1982). Farstad reported interstitial colloid osmotic pressure in 45 anesthetized pigs before start of CPB to be 8.8 ± 0.2 mmHg (Farstad et al., 2005). The corresponding value in young men is 15.8 ± 2.3 mmHg (Noddeland et al., 1982).

Despite these differences, in our opinion the monitoring of COP during the course of each experiment brings important information about the dynamic changes in transcapillary fluid shifts. Total body water content of a young pig is similar to the values of a child, 60-65 %. Cardiac output is twice the values of adult humans (Hannon et al.,1990). This is the physiological basis for applying a higher CPB flow rate in our model than what is common in clinical practice.

Two features of the cerebral arterial anatomy of pigs must be taken into account when considering studies of cerebral ischemia: 1) the internal carotid artery turns into a vascular plexus, the rete mirabile, at the cranial base 2) The cerebral arterial supply is partly provided by numerous collaterals (Haaland et al., 1995). This makes it difficult to study focal ischemia in pigs or to measure cerebral blood flow on pre-cerebral arteries.

On the other hand, pigs are excellent models for studies of global cerebral ischemia caused by hypotension or cardiac arrest (Kaakinen et al., 2006, Anttila et al., 2006), and of cerebral autoregulation under different conditions (Schmidt et al., 2002, Schmidt et al. 2003).

We therefore consider the young pig to be a suitable model in our experimental investigations on global fluid shifts and cerebral biochemical changes during CPB with hemodynamic interventions.

I. Hemodynamic parameters and fluid shifts during CPB

I 1. The relation between different levels of MAP and fluid shift

When the arterial pressure during clinical CPB is inadequate, the first measure usually is to expand the circulating volume by administration of intravenous fluid or to increase systemic resistance by use of an alpha-adrenergic agent. The addition of fluid probably would lead to a more positive peroperative fluid balance. In paper III, we addressed whether even the use of a α_1-receptors agonist would influence fluid balance.

The α_1-receptors are mainly located at the small arteries and terminal resistance arteries, but also in the venous system. They have an inherent tonic activity. The effect is mainly mediated by an increase in cytosolic [Ca^{2+}] forming a Ca^{2+} - calmodulin complex which activates the myosin light chain kinase. This in turn causes phosphorylation of the myosin heads resulting in formation of crossbridges. The relaxation is related to a reduction in cytosolic [Ca^{2+}] (Levick, 2003).

The fluid exchange across the capillary wall is determined by the factors of Starling's equation. P_c is basically unknown, but is determined by the following equation (Zhang & Wolf, 1991):

$$\mathbf{P_c = P_v + F \cdot R_v}$$

P_v = postcapillary pressure (central venous pressure)
F = blood flow
R_v = postcapillary resistance

Applying this relationship to the results of paper II, both study groups had the same F and P_v was similar in the two groups during normothermic CPB. Assuming that the relationship above is valid for a whole body model, we may infer that R_v was higher in the high pressure group due to stimulation of postcapillary α_1-receptors. Accordingly, P_c should be highest in the high pressure group during normothermic CPB.

α_1-adrenergic stimulation also affects the volume of circulation (Nette et al., 1989, Hilsted et al., 1989). In paper II we found higher plasma volume in the low pressure group as compared with the high pressure group. The resulting difference in hemodilution leads to lower values of COPp in the low pressure group. Consequently two of the Starling forces

probably differed between the groups. Their respective effects may, however, have balanced each other resulting in a similar degree of extravasation.

In paper I we found a non-significant tendency to higher fluid extravasation rate in the low pressure group. Even in this study the plasma volumes differed. Two factors may have contributed to the discrepancy between paper I and II: 1) The control group of paper I was not given any vasoactive drugs, rendering any effect via increased postcapillary resistance unlikely. 2) The low pressure group of paper I was given nitroprusside to decrease MAP. The action of this drug is mediated by nitric oxide, reducing cytosolic [Ca^{2+}] by formation of cyclic guanosine monophosphate (cGMP). cGMP, however, has the capacity of increasing capillary hydraulic conductivity (Yuan, 2002) potentially enhancing the fluid extravasation. These data does not allow any specific conclusion regarding the clinical use of nitroprusside.

I 2. The relation between CPB flow rate and fluid shift

When the clinician is confronted with an inadequate arterial pressure during CPB, an alternative to use of alpha-adrenergic agents is to increase CPB flow rate. The relationship between various levels of CPB flow rate and peroperative fluid balance was addressed in paper III.

These experiments showed that conducting CPB with two different levels of flow rate resulted in higher plasma volume in the study group with highest CPB flow rate. Consistent with these data we found a trend to lower levels of COPp in the same group presumably caused by a more extensive hemodilution.

P_c was not measured, but a theoretical argument may be made regarding its value. The following equation defines the determinants of P_c (Pappenheimer & Soto-Rivera, 1948):

$$\mathbf{P_c = (P_A + P_V R_A / R_V) / (1 + R_A/R_V)}$$

P_A = arterial pressure
P_V = venous pressure
R_A = precapillary resistance
R_V = postcapillary resistance

P_c is determined by three factors: P_A, P_V, and pre- to postcapillary resistance ratio (R_A/R_V). Systemic vascular resistance is mainly located in the precapillary part of circulation and is primarily determined by the muscular tone of arterioles.

In paper III, systemic vascular resistance was found to be lower in the high flow group as compared with the low flow group. We may therefore assume that the high flow group had lower pre- to postcapillary resistance ratio. Furthermore, there was a trend to higher P_A in the high flow group during normothermic CPB and a small tendency to higher central venous pressure. These factors may have contributed to higher P_c. Hence, the higher fluid extravasation rate in the high flow group during normothermic CPB, may have been caused by a combination of more pronounced hemodilution in this group together with a higher P_c.

The differences found in net fluid balance and fluid extravasation rate seemed to be limited to the early phase of CPB. After 30 minutes the groups demonstrated a similar degree of fluid accumulation. The data suggests that filtration gradually have been opposed by a reduction in COP_i, possibly resulting from wash-down effect on interstitial proteins. Others have demonstrated a slow increase in P_i during CPB that could also contribute to progressive reduction in fluid filtration (Rein et al., 1988).

Inadequate venous drainage may interfere with the amount of fluid added to the reservoir during CPB. Even though the venous drainage was continuously monitored by visual inspection and by measuring right atrial pressure, we can not totally ignore the possible impact of this factor on our results. We would, however, expect this factor to play an equally important role throughout the complete experiment.

J. Mean arterial pressure and cerebral pressure, flow and metabolism

J 1. Cerebral markers of metabolism

In paper IV and V we focused upon cerebral effects of different arterial pressure during CPB. Cerebral microdialysis has been used to evaluate the relation between cerebral perfusion pressure and energy metabolism in brain-injured patients (Nordström et al., 2003). In the penumbra-zone cerebral perfusion pressures below 50 mmHg was associated with an increase in lactate-pyruvate ratio while this relation was not found in the healthy hemisphere.

CPB and cerebral microdialysis has been used in animal studies on hypothermic circulatory arrest or transient global cerebral ischemia (Pokela et al., 2003, Conroy et al.,

1998). Glutamate, aspartate, dopamine, glycerol and energy metabolites were used as markers of imminent or manifest neuronal injury.

The significance of individual markers is still under debate. Lactate-pyruvate ratio is an attractive parameter owing to the close relationship to the NADH-NAD^+ ratio (Ståhl et al., 2001). Two studies with PET scan have demonstrated a positive correlation between cerebral hypoxia, in form of elevated oxygen extraction ratio, and elevated lactate-pyruvate ratio (Hutchinson et al., 2002, Enblad et al., 1996).

Cerebral ischemia may lead to degradation of membrane phospholipids by the activation of phospholipase. One of the degradation products is glycerol which is assumed to be a reliable marker of neuronal injury (Hillered et al., 2005). The relation was previously validated in a monkey model of stroke (Frykholm et al., 2001). A nine-fold, sustained increase in intracerebral glycerol was found in severe ischemic regions as judged by PET scan while in the penumbra regions a moderate increase was temporarily seen.

In paper IV and V we found that reducing MAP to 40 – 45 mmHg during CPB, lead to a significant elevation of glycerol and lactate-pyruvate ratio compared to baseline values. Lactate differed significantly between the groups in paper V.

Interestingly, a similar finding was recently published (Zoremba et al., 2007). In anesthetized dogs with cortical microdialysis, cerebral perfusion pressure was gradually reduced by infusing artificial cerebrospinal fluid into the ventricular system. The authors reported a significant increase in lactate and glycerol with cerebral perfusion pressure below 50 mmHg and an increase in lactate-pyruvate ratio when the perfusion presser dropped below 40 mmHg. These data suggest that the lower limit of autoregulation in healthy young animals is in the range of 40-50 mmHg and that the low pressure group in paper IV and V were below that limit. This conclusion is also consistent with the previous study on CPB and autoregulation in dogs (Plöcl et al., 1998)

To assess the relationship between cerebral perfusion pressure and cerebral markers in a large number of animals, we pooled the data from six studies with a total of 64 pigs included. All animals were anesthetized in accordance to the same protocol and were monitored with cerebral microdialysis. They underwent 60 minutes of normothermic and 90 minutes of hypothermic CPB. The individual studies differed with respect to fluid treatment, CPB flow rate and administration of vasoactive agents (Paper II-V, Farstad et al., 2006, Kvalheim et al., in press). Scatter plots of cerebral perfusion pressure versus cerebral levels of lactate-pyruvate ratio and glycerol are depicted below. There seems to be a deflection point at the curve corresponding to a cerebral perfusion pressure of 30 mmHg, suggesting that this

level is below the lower limit of autoregulation and that the capacity to increase oxygen extraction ratio is exhausted.

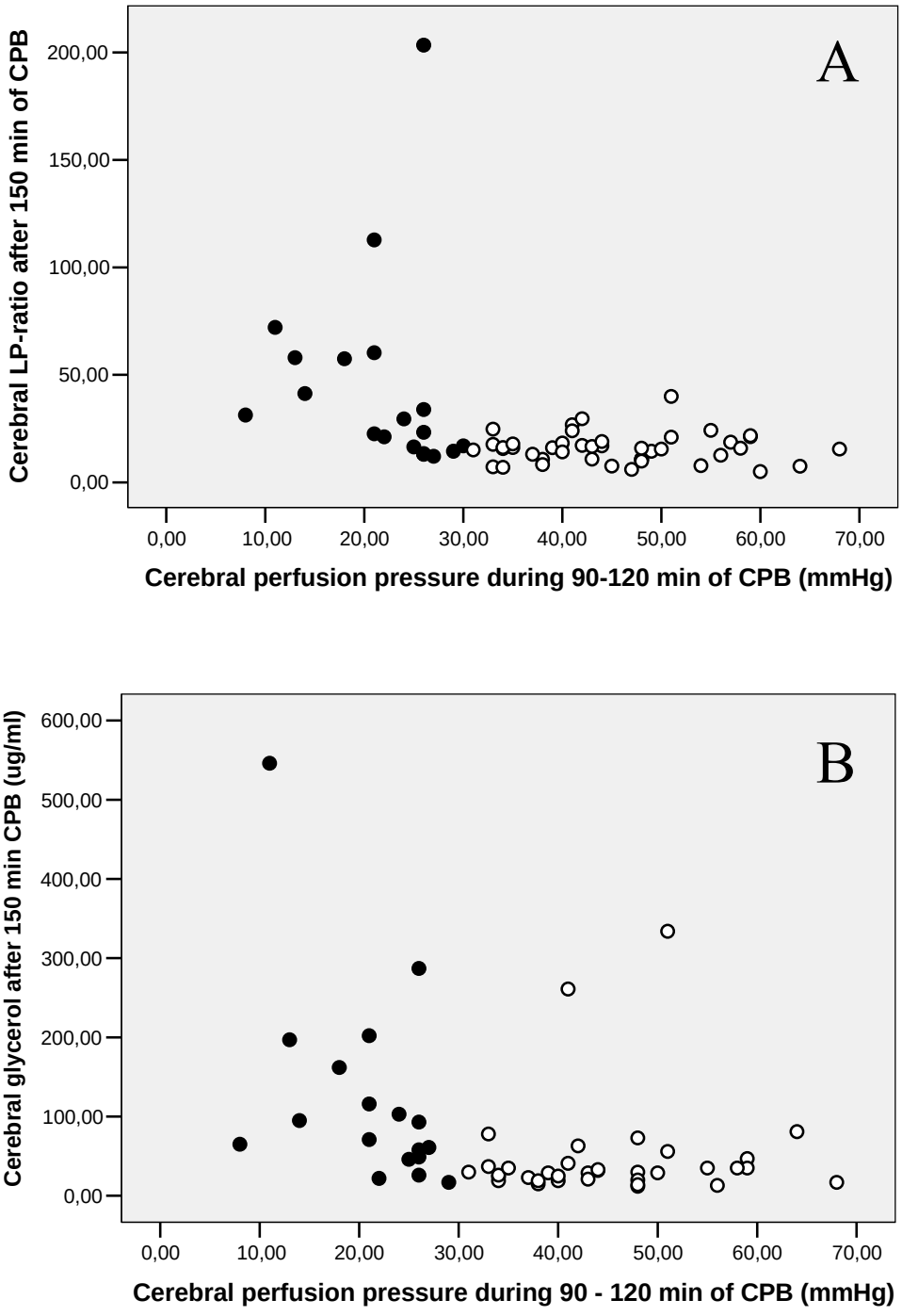

Figure 3

Cerebral lactate pyruvate ratio (A) and glycerol (B) by microdialysis against cerebral perfusion pressure (CPP) in 64 pigs after 150 minutes of CPB. Closed circles are pigs with mean CPP below 30 mmHg and open circles are pigs with mean CPP above 30 mmHg during 90 to 120 minutes of CPB.

The notion that intra-cerebral glycerol origins from membrane phospholipids is partly based on the fact that the central nervous system is nearly devoid of triglycerides. However, during conditions of disrupted blood brain barrier, glycerol from peripheral tissue, could affect the cerebral levels. Hydrolysis of triglycerides in the fat tissue is part of the metabolic stress response following alpha-adrenergic stimulation.

In neurosurgical patients, the blood brain barrier is frequently injured. Whether CPB affects the integrity of the blood brain barrier is not known and the existing data are conflicting (Cavaglia et al., 2004, Gillinov et al., 1992)

We did not measure subcutaneous glycerol levels by microdialysis except for the 5 first experiments in paper II, where we found normal values in three animals with low MAP and elevated values in two animals with high MAP. If glycerol produced in the adipose tissue, passed the blood brain barrier, we would expect elevated levels in the group with high MAP due to norepinephrine. As it turned out, this group had stable and low values of cerebral glycerol throughout the experiment.

J 2. Intracerebral pressure

Intracranial pressure was found to increase during the course of CPB in paper IV and V. Similar results were reported by others in experimental studies of CPB in dogs (Philpott et al., 1998) and swine (McDaniel et al., 1994). Intriguingly, the increase in intracranial pressure was abolished in two studies when a hyperosmolar-colloidal infusion was given during normothermic and hypothermic CPB in pigs (Farstad et al., 2006, Kvalheim et al., in press)

The blood brain barrier plays an important role in the maintenance of intracerebral water content. A dysfunction related to CPB, could render the brain more vulnerable to a drop in COPp caused by hemodilution.

Inadequate venous drainage was reported to elevate intracranial pressure in an experimental model (Plöhl et al., 1999). The impact of inadequate drainage would, however, be expected to cause the same increase in ICP in groups with and without infusion of hyperosmolar colloidal fluids.

Independently of the cause, an elevation of intracranial pressure could be deleterious by decreasing cerebral perfusion pressure.

J 3. Cerebral blood flow measurements

Blood flow measurements are essential within this field of biomedical research. In the past cerebral blood flow was measured with the ^{133}Xenon clearance technique (Friedman et al., 2000) as well as with the sagittal sinus outflow technique (Morii et al., 1986).

In paper IV, cerebral blood flow was measured with colored microspheres. No significant difference could be seen between the groups and no consistent trends were present. Unfortunately these were low quality data with wide variation within each group. The protocol of paper V originally also included the use of colored microspheres. During the processing of the tissue samples it became evident that one of the colors could not be adequately separated from the tissue. The method was therefore abandoned.

We also performed pilot experiments with cerebral laser Doppler measurements. Due to the inability of this method to provide absolute values and the sensitivity of the probes to movements and pressure against the tissue, we did not go further with the method.

Our laboratory is now about to introduce blood flow measurements by fluorescent microspheres. Published data and preliminary experience in our lab indicate that this technique may be more reliable.

K. Pharmacological considerations

K 1. Pharmacological effects on fluid shifts

The action of $ß_2$-adrenoceptor agonists is mediated by stimulating the formation of intracellular cyclic adenosine monophosphate (cAMP). In the endothelial cell, this may lead to reduced capillary permeability by enhanced formation of junctional strands in the intercellular clefts (Levick, 2003). Norepinephrine was used in paper II –V to raise the arterial blood pressure. Even though it is a nonselective α-receptor agonist, it has little effect on the $ß_2$-adrenoceptors (Westfall & Westfall, 2006). We therefore consider it unlikely that norepinephrine has interfered, by this mechanism, with the fluid shifts in the present studies.

In paper II and V we used the non-selective α-receptor blocker phentolamine to antagonize the α-adrenergic effects. This drug is unlikely to affect capillary permeability by binding to the $ß_2$-adrenoceptors either.

Cyclic guanosine monophosphate (cGMP) has the opposite effect of cAMP and may increase capillary hydraulic conductivity by activating protein kinase G and reducing the level of intracellular Ca^{2+} (Chong & Victorino, 2005). In paper I and IV, we used nitroprusside to

obtain vasodilatation, a drug that acts by releasing nitric oxide (NO) and subsequently forming cGMP.

The literature is, however, conflicting regarding the effect of NO on capillaries. Several studies in situ on isolated micro-vessels have concluded that increased NO formation leads to higher hydraulic conductivity (Meyer & Huxley, 1992, Rumbaut et al., 1995). However, another study on rats on CPB concluded that increasing NO production by L-arginine infusion resulted in reduced fluid accumulation (Wehberg et al., 1996).

The possibility of an impact of NO on the capillary permeability in paper I can not be totally ignored. This knowledge was partly the reason why we decided to conduct another similar study without the use of nitroprusside.

Isoflurane was recently reported to promote extravascular fluid accumulation in sheep (Connolly et al., 2003). Their study design did not include an alternative anesthetic protocol and the results could be generally valid for most anesthetic drugs. In case there was a specific effect of isoflurane on fluid accumulation, the impact in the present work would probably be the same in all groups.

K 2. Pharmacological effects on cerebral blood flow

Generally norepinephrine is considered not to cross the intact blood brain barrier (Kimmerly et al., 2003). In healthy volunteers infusion of norepinephrine lead to increased cerebrovascular resistance as long as MAP was elevated. When MAP was normalized by a simultaneous infusion of phentolamine, the cerebrovascular resistance also was returned to the original level (Kimmerly et al., 2003).These results were interpreted as support for the absence of local effects by norepinephrine on cerebral vasculature.

Several pathological states and perhaps even CPB, may cause a disruption of the blood brain barrier. The literature indicates that the effect of norepinephrine, even in such circumstances, predominantly is mediated by changes in systemic arterial pressure (Leone et al., 2006).

The effect of norepinephrine on cerebral autoregulation was studied in a model of sheep anesthetized with 2% isoflurane. Cerebral autoregulation was unaltered during norepinephrine infusion with a maximum dose of 40 μg/h (Myburgh et al., 2003).

Few data exist on the relation between phentolamine and cerebral autoregulation. Vlalov reported that a dose of phentolamine 1 mg/kg did not alter cerebral autoregulation in cats (Vlalov & Bacracheva, 1987). Cerebral vasodilatation by local effects of phentolamine

was suggested in the study by Kimmerly (Kimmerly et al., 2003), but the interpretation of this observation is uncertain. In paper V the animals were given phentolamine to achieve blood pressure reduction. If phentolamine, by a local effect, could reduce cerebral vascular resistance, the implication would be improved cerebral perfusion and supply of oxygen and energy substrates. However, the metabolic changes observed were more consistent with oxygen and energy depletion.

Vasodilatation by nitroprusside has been used for a long time. It appears to interfere with the cerebrovascular chemo-regulation by decreasing the sensitivity to hyperventilation. However, it does not seem to affect cerebral pressure autoregulation (Lavi et al., 2003). The literature is ambiguous with respect to its effects on cerebral blood flow during stable conditions. When positron emission tomography was used to measure cerebral perfusion in baboons, nitroprusside-induced hypotension was associated with augmented cerebral blood flow (Schuman-Bard et al., 2005). The doses of nitroprusside was in average 16±3 μg/kg/min. Studies on humans given 0.5 μg/kg/min, have not demonstrated any change in cerebral perfusion (Joshi et al., 2002, Lavi et al., 2003).

In paper I and IV we used nitroprusside in average doses of 6 to 7 μg/kg/min. The present data does not permit any conclusions as to whether cerebral resistance was lowered by local effects of nitroprusside in these low pressure groups. If so, the consequence would be an improved cerebral circulation in these groups. Instead, the results were more indicative of insufficient cerebral perfusion and supply of oxygen.

Isoflurane is a potent vasodilatator capable of impairing the cerebrovascular autoregulation in a dose-dependent manner. Clinical studies indicate that a dose of 1 MAC (minimal alveolar concentration) do not interfere with the autoregulation (Olsen et al., 1994). In pigs 1 MAC of isoflurane corresponds to an end-tidal concentration of about 2% (Holmström & Akeson, 2003). In a recent paper, cortical perfusion was assessed in pigs with isoflurane anesthesia and induced hypotension by caval block. No effect on cerebral perfusion by isoflurane 0.3 – 1.2 MAC was seen (Kimme et al., 2007).

In our anesthetic protocol isoflurane was given in the range of 0.5 – 2.0 %. Occasionally the dose was increased further to keep MAP at target level, but only as a transient measure. We therefore assume that the animals had isoflurane below or equal to 1 MAC and that cerebral autoregulation probably was not affected by it.

L. Effects of hypothermia and hemodilution

L 1. Hypothermia

Our group has previously demonstrated that hypothermia *per se* may cause enhanced fluid extravasation during CPB (Farstad et al., 2003). In the present project, we applied the same experimental design as Farstad et al. with respect to duration and temperature profile, in order to discriminate between the effects during normothermia and hypothermia.

The result (paper I-III) indicates, as previously described, an increase in fluid extravasation during hypothermia, but the pattern was less obvious. Apparently this was related to an overall higher fluid extravasation rate in these studies probably due to a more pronounced hemodilution (see below) and a higher CPB flow rate (Paper II and the HF-group of III).

L 2. Hemodilution

The study design of paper II - V, deviates from the previous papers by the introduction of a left ventricular vent. The purpose was to achieve better control of total body perfusion. As a consequence CPB circuit volume was expanded from 1000 ml to 1115 ml. The volume of dilution was the same in all study groups from start of CPB and the bearing on the results should be similar.

Hemodilution during CPB has been considered to have beneficial effects on microcirculation. However, in clinical practice a high degree of hemodilution has been associated with an unfavorable outcome (Ranucci et al., 2006, Habib et al., 2003, Karkouti et al., 2005) probably related to reduction in total body oxygen supply. In paper IV and V hematocrit was reduced to 19.8-22.3 % at the end of normothermic CPB and 17.8-18.6 % at the end of hypothermic CPB. The pattern of biochemical changes seen in these low pressure animals were interpreted as most likely the result of inadequate cerebral perfusion. However, the reduction in oxygen carrying capacity could render these animals more vulnerable to low cerebral perfusion pressure.

M. Relevance for neurological dysfunction in man

Strokes after cardiac surgery are frequently located to the cerebral watershed areas which are found in the junction between two or three arterial territories (Rovira et al., 2005). These regions are considered to be particularly vulnerable to global hypoperfusion, for instance by severe carotid artery disease or arterial hypotension (Momjian-Mayor &Baron, 2005). When 98 patients with stroke after cardiac surgery were examined by MRI with diffusion-weighted imaging, watershed-infarcts were found bilaterally in 48% and unilaterally in 68% of the patients (Gottesman et al., 2006). In the general stroke population, the incidence of watershed-infarcts was 2-5 % (Gottesmann et al., 2006). Neuronal injury in the frontal watershed area has also been demonstrated in piglets after CPB with deep hypothermic circulatory arrest (Aharon et al. 2004). Based on these facts, we isolated cerebral tissue from the watershed areas for electron microscopy in paper V.

Several clinical studies have assessed the relation between low systemic arterial pressure during CPB and postoperative neurological dysfunction. Gold compared patients with MAP of 50-60 mmHg and 80 – 100 mmHg during CPB and found that combined cardiac and neurological outcome was improved in the high pressure group (Gold et al., 1995). Others have found an association between cognitive decline and the combination of high age and arterial hypotension during CPB (Newman et al., 1995).

The published data are, however, conflicting. A large retrospective study of 2862 patients after coronary bypass surgery concluded that there was an association between low pressure during CPB and decreased incidence of stroke and coma (van Wermeskerken et al., 2000).

Children undergoing cardiac surgery were examined with bispectral index (BIS) and near infrared spectroscopy (NIRS) to detect possible cerebral ischemia. The authors reported that children below the age of four years were likely to have cerebral ischemia caused by hypotension (Hayashida et al., 2004). Quite recently a drop in MAP from preoperative baseline was shown to put the patients at risk for early cognitive dysfunction after coronary artery bypass surgery (Gottesman et al., 2007).

Finally, the current large interest in peroperative neurological monitoring prompted a prospective randomized study of 200 patients admitted to coronary bypass surgery. The interventional group was treated according to a protocol directed by brain oxygen saturation levels as measured with NIRS. One of the steps to be taken in case of cerebral desaturation was to raise MAP to values above 50 mmHg. The authors concluded that the interventional

group had significantly lower incidence of peroperative cerebral desaturation and major postoperative organ dysfunction (Murkin et al., 2007).

The animals studied in paper IV and V were subjected to a sustained hypotension for 150 minutes. The duration probably is an important factor determining the severity of the cerebral injury. Major pathological changes in intracerebral biochemistry was present in paper IV and V after 60 min of normothermic CPB.

A MAP of 40 – 45 mmHg is frequently seen in clinical practice. If this level of cerebral perfusion pressure is inadequate, then even shorter episodes could prove to be harmful and cause subtle neurological dysfunction.

Conclusions

The studies included in the thesis have led us to the following main conclusions:

- Elevation or lowering of the mean arterial pressure during combined normothermic and hypothermic CPB by use of alpha-adrenergic agents does not seem to affect peroperative net fluid balance or fluid extravasation rate.

- Increasing CPB perfusion flow rate may lead to a more positive net fluid balance and higher fluid extravasation rate during combined normothermic and hypothermic CPB.

- Mean arterial pressure of 40 – 45 mmHg during combined normothermic and hypothermic CPB with crystalloid prime may lead to cerebral ischemia and cellular injury.

Future aspects

The presented data on fluid shifts and cerebral biochemical and structural changes need to be confirmed and further elaborated before any firm conclusions can be drawn regarding clinical practice.

The future plans for this research group depends on which experimental endpoints that will be available. We will need relevant, sensitive and reliable physiological endpoints.

Measurement of tissue oxygen tension is increasingly available and highly relevant in this line of research. The microdialysis technique opens up for isolation of multiple biochemical markers of metabolic and inflammatory disturbances during CPB. Some examples are excitotoxic neurotransmitters, ATP degradation products, markers of reactive oxygen species formation and nitric oxide (Hillered, 2005).

One of the questions raised in this thesis is whether MAP of 40 – 45 mmHg during CPB is more harmful when Hct simultaneously is reduced to 18 – 20 %. Another issue is whether stabilizing ICP by infusion of hyperosmolar-colloidal solution could render the patient more tolerant to a low MAP during CPB. These questions are all available for assessment in experimental studies.

The implementation of experimental data in clinical routine has to be done in the context of clinical studies. Numerous observational and randomized controlled studies have addressed neurological dysfunction after CPB. Study populations are, however, often heterogeneous and it may be difficult to prove the advantage of a standardized treatment regime. Future clinical studies should therefore also evaluate treatment algorithms that provide individualized patient management. Such algorithms would have to be directed by the clinical condition and by continuous monitoring of the patient.

Technology for monitoring regional oxygenation is rapidly developing and may in the future gain a major impact on the management of our patients in ICU and the operating theatre. The method of monitoring cerebral oxygenation by near infrared spectroscopy (NIRS) is a promising new tool in this setting (Hoffman, 2006).

By providing the clinicians with early signs of warning whenever cerebral oxygen supply is inadequate, we may, in the future, be able to attenuate cerebral ischemic injury and improve neurological outcome.

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Errata

1. In paper II, the section "Materials and methods", the priming volume of the CPB circuit is said to be 1000 ml. Actually it was 1115ml.

2. In paper II, the section "Materials and methods", the time used for surgical preparation was falsely stated to be 40 – 45 min while it was in the range of 20 – 30 min.

3. In paper IV, the section "Material and methods", the paragraph: "Study groups and interventions" the target MAP of the LP-group was reported to be 40 – 50 mmHg while it was 40 – 45 mmHg. In the "Result" section, the dose of nitroprusside was reported as follows: "In the LP-group nitroprusside at an average infusion rate of 6.95 (2.0) µg/kg/min was required to keep MAP in the range of 40 – 50 mmHg". It should be: "…to keep MAP in the range of 40 – 45 mmHg."