BRAIN PROTECTION IN AORTIC ARCH SURGERY

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Abstract

Retrograde cerebral perfusion (RCP) techniques have been adopted in aortic arch surgery for clinical use. The clear benefits of RCP are that it reduces embolic injury and prolongs the permissible period of hypothermic circulatory arrest (HCA). At the same time, however, there is a great deal of evidence according to which RCP may be associated with an increased risk of fluid sequestration and cerebral edema. In the current study intermittent RCP was compared with continuous RCP and HCA alone to clarify if periodical RCP decreases fluid sequestration (I).

HCA is an effective method of cerebral protection, but is associated with long cardiopulmonary bypass times, and coagulation disturbances. We tested the hypothesis that deep hypothermic RCP could improve cerebral outcome during moderate HCA (II and III).

Glutamate excitotoxicity plays an important role in the development of ischemic brain injury. The purpose of the present study was to determine the efficacy of lamotrigine, a Na^+ channel blocker, to mitigate cerebral injury after HCA (IV). A chronic porcine model was used in the present series of studies. Hemodynamic, electrophysiologic, and metabolic monitoring were performed until four hours after the instigation of rewarming. S-100β was measured up to 20 hours. Daily behavioral assessment performed until death or elective sacrifice on the seventh postoperative day.

After continuous RCP the median fluid sequestration volume was 145 (0-250) ml compared with -50 (-100 - 0) ml after intermittent RCP (p = 0.04). In comparison of 15°C RCP to HCA alone during moderate 25°C hypothermia, 5/6 animals in the RCP group survived seven days compared with 2/6 in the HCA group (p=0.04).

The total histopathologic scores in the RCP(15°C) group were lower than those for the RCP(25°C) group during moderate 25°C hypothermia (p=0.04). EEG bursts were recovered better in the RCP(15°C) group at 3 hours after the start of rewarming compared to HCA group (p=0.05).

The rate of EEG burst recovery was higher in lamotrigine treated animals compared to placebo treated animals after 4 hours during the rewarming (p = 0.02). Among the animals that survived for 7 days, the median behavioral score was higher in the lamotrigine group (8) compared with controls (7) (p = 0.02). The results indicate that intermittent RCP decreases the rate of fluid sequestration after continuous RCP. The cold RCP at moderate systemic hypothermia seems to provide a better neurological outcome than that with moderate temperature RCP, a finding suggesting that enhanced cranial hypothermia is the major beneficial factor of RCP. The Na^+ channel blocker lamotrigine improves neurological outcome after a prolonged period of HCA. In conclusion, two refinements in the RCP concept are to administer it at low temperatures and if longer periods of perfusion are necessary, RCP should be applied intermittently.

Keywords: retrograde perfusion, hypothermic circulatory arrest, lamotrigine.

To Kaarin, Tuomas, Johannes and Elias

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Oulu, February, 2000   Vesa Anttila

Abbreviations

AMPA	$\propto$-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BAEP	Brainstem auditory evoked potential
CBF	Cerebral blood flow
$CMRO_2$	Cerebral metabolic rate of oxygen
CPB	Cardiopulmonary bypass
CPK-BB	Creatine kinase isoentzyme BB
C-RCP	Continuous retrograde cerebral perfusion
CVP	Central venous pressure
DNA	Deoxyribonucleic acid
EAA	Excitatory amino acid
EEG	Electroencephalography
HCA	Hypothermic circulatory arrest
IQR	Interquartile range
I-RCP	Intermittent retrograde cerebral perfusion
IVC	Inferior vena cava
KA	Kainate
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate reduced form
NMDA	*N*-Methyl-D-Aspartate
NO	Nitric oxide
NOS	Nitric oxide synthase
$PaCO_2$	Partial pressure of carbon dioxide
RNA	Ribonucleic Acid
RCP	Retrograde cerebral perfusion
SCP	Selective cerebral perfusion
SD	Standard error of mean
SEP	Somatosensory evoked potential
SVC	Superior vena cava

List of original publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:

I   Anttila V, Pokela M, Kiviluoma K, Rimpiläinen J, Vainionpää V, Hirvonen J & Juvonen T (2000) Intermittent retrograde cerebral perfusion during prolonged period of hypothermic circulatory arrest: A study in a chronic porcine model. Scandinavian Cardiovascular Journal 34: 116 – 123.

II   Anttila V, Kiviluoma K, Pokela M, Rimpiläinen J, Mäkiranta M, Jäntti V, Hirvonen J & Juvonen T (1999) Cold retrograde cerebral perfusion improves cerebral protection during moderate hypothermic circulatory arrest: A long-term study in a porcine model. The Journal of Thoracic and Cardiovascular Surgery 118: 938 - 945.

III   Anttila V, Pokela M, Kiviluoma K, Mäkiranta M, Hirvonen J & Juvonen T (2000) Is the maintained cranial hypothermia the only factor leading to improved outcome following retrograde cerebral perfusion? An experimental study using a chronic porcine model. The Journal of Thoracic and Cardiovascular Surgery 119: 1021 - 1029.

IV   Anttila V, Rimpiläinen J, Pokela M, Kiviluoma K, Mäkiranta M, Jäntti V, Vainionpää V, Hirvonen J & Juvonen T (2000) Lamotrigine improves cerebral outcome following hypothermic circulatory arrest: A study using a chronic porcine model. The Journal of Thoracic and Cardiovascular Surgery 120, in press.

Contents

1. Introduction

The brain has been under observation by cardiac surgeons since heart surgery was first practiced in the early 1950`s. The first clinical use of cardiopulmonary bypass (CPB) was reported in 1954 (Gibbon 1954), and a series of patients sustaining neurological and psychological dysfunction following cardiac surgery were documented in the same year (Fox *et al*. 1954). During aortic arch surgery, conventional CPB is not possible within the period in which the arch is excluded from the circulation. In this situation, cerebral circulation is cut off from the circulation going to the lower part of the body. DeBakey reported the first successful aortic arch aneurysm replacement in 1957 using normothermic CPB to supply the systemic circulation, and an isolated brachiocephalic and carotid artery perfusion to protect the brain (DeBakey *et al*. 1957). Subsequent techniques used multiple pumps for the selective perfusion of individual arch vessels at moderate hypothermia. HCA was introduced to protect the brain and other vital organ systems during interruptions in systemic perfusion (Niazi and Lewis 1957). The use of HCA during aortic arch replacement (Griepp *et al*. 1975, Gschnitzer 1973, Pierangeli *et al*. 1975) and the demonstration of an acceptable mortality rate (Griepp *et al*. 1991) using this technique led to its adoption and adaptation by others. Although interest in cerebral perfusion technique waned with the evolution of HCA, the complications arising from long and challenging aortic procedures have led to a renewed interest in perfusion of the cerebral circulation. Antegrade and retrograde perfusion techniques have been used during aortic arch surgery in an effort to prolong the "safe" period during which conventional cardiopulmonary bypass flow to the brain is interrupted.

Although retrograde cerebral perfusion (RCP) techniques have been adopted in clinical use, there are mixed opinions concerning its protective capacity and optimal modes. Proponents of RCP report that the method is effective in reducing embolic injury and in prolonging the permissible period of hypothermic circulatory arrest (HCA) (Coselli 1997, Safi *et al*. 1997, Ueda *et al*. 1994, Usui *et al*. 1997). At the same time, however, there is a great deal of evidence indicating that RCP may carry an increased risk of perfusion-induced cerebral injury, especially if high perfusion pressures are used (Juvonen *et al*. 1998a, Juvonen *et al*. 1998b, Okita *et al*. 1998). It would therefore appear that the details of the implementation of RCP are highly critical in order to provide benefit without inducing damage due to the cerebral edema which seems to be an almost inevitable consequence of its use. According to current information, the use of RCP for

cerebral protection during HCA in clinical setting is safe when flow rates and central venous pressures are maintained at relatively low levels (Nojima *et al.* 1994). This in turn indicates that the apparent superiority of RCP over HCA alone may be due to its improved cooling effect. This hypothesis is supported by the suggestion that RCP provides only a small percentage of the nutrient flow necessary to sustain cerebral metabolism, even in the presence of deep hypothermia (Boeckxstaens and Flameng 1995).

The increased understanding of the pathogenesis of ischemic neuronal damage has opened new avenues of research for improving protective methods through the addition of appropriate pharmacological agents. The failure of neurotransmitter transport is an essential step in the pathogenesis of ischemic cerebral injury. After release into the intercellular space, glutamate is taken up into glial cells and rapidly converted to glutamine after which it reenters the neuron ready to be used for the next message. Under conditions of hypoxia or ischemia, the conversion of glutamate to glutamine is interrupted. This leads to its accumulation in the intercellular space, where glutamate acts as a neurotoxic substance. Here it opens calcium channels, leading to an influx of calcium, which then initiates the catastrophic intracellular cascade leading to neuronal autodigestion and cell death (Lipton and Rosenberg 1994).

In the present experimental studies the metabolic and physiologic consequences of different interventions have been evaluated during operation using a chronic porcine model, and any possible cerebral injury has been assessed by electrophysiological recovery, behavioral evaluation and histopathological examination after elective sacrifice one week postoperatively. In the first study, continuous RCP, intermittent RCP and 75 minutes of HCA at systemic hypothermia of 20ºC were compared. In the second study, the efficacy of deep hypothermic RCP for improved cerebral outcome during moderate HCA was studied. The third study was conducted to compare two temperatures of RCP (15ºC and 25ºC) with HCA alone at a systemic temperature of 25ºC to clarify whether the possible benefit of RCP may only be due to an improved cooling effect. In the fourth study, the neuroprotective effects of lamotrigine during HCA were examined.

2. Review of the literature

2.1. The pathophysiology of ischemic brain injury

2.1.1. Cerebral metabolism

Although the mass of the brain is only 2 % of the total body mass, its blood flow accounts approximately 20% of the cardiac output and it consumes approximately 20% of the oxygen and glucose in the body. Because the cerebral stores of glucose are low, the brain is dependent on a steady supply of glucose and oxygen delivered by the arterial circulation. In the presence of an adequate amount of oxygen, a glucose molecule will give a high yield of adenosine triphosphate (ATP) molecules (38 mol of ATP for each mol of glucose) with final waste products of water and carbon dioxide. If glycolysis occurs under anaerobic conditions, the ATP yield will be low (2 mol of ATP per 1 mol of glucose) and the final product will be lactate which will subsequently make the cytosol acidic.

Different regions of the brain use energy at different rates, and some regions are therefore more vulnerable to ischemic injury than others. A prime example of this is the hippocampus, which performs memory processing or storage functions and appears to require a constant flow of energy to maintain its integrity. The earliest signs of histological ischemic injury are seen in the CA1 subfield in the hippocampus (Tabuchi *et al*. 1995), and the most subtle findings of cerebral injury in neuropsychometric testing involve memory functions (Buss *et al*. 1996, Volpe and Hirst 1983). In the pig model the caudate nucleus and the hippocampal CA4 region have been shown to be the most vulnerable to ischemia (Ye *et al*. 1996).

2.1.2. Cerebral blood flow

Cerebral blood flow (CBF) has been reported to be approximately 80 mL/100g/min in the cortex and 20 mL/100g/min for the white matter in conscious human (McHenry *et al.* 1978), with a 50 mL/100g/min rate on average for the whole brain (Lassen 1982). Under anesthesia, CBF will decrease by approximately 20% while a deep barbitural anesthesia will decrease flow by almost 50%. In the white matter, the CBF is substantially lower than that of the awake cortex, but is not markedly influenced by anesthesia (Hossmann 1988).

   The demand for a constant blood supply is met by autoregulation of the cerebral flow (Taylor 1998). The CBF will remain relatively constant when cerebral perfusion pressure is within a range of 50 to 170 mmHg (Berne *et al.* 1981, Harper 1966). The response rate of cerebral autoregulation is profoundly dependent on vascular tone (Aaslid *et al.* 1989). Common comorbid conditions such as long-lasting diabetes, hypertension, and most acute affections of the brain may be disturbed or alter the autoregulation of cerebral blood flow (Paulson *et al.* 1990).

2.1.3. Thresholds of ischemia

The brain will tolerate an acute reduction in blood flow below 20 ml/100g/min or even 10 ml/100g/min (*i.e.*,< 20% of normal values) in normothermia (Fig.1) (Astrup *et al.* 1977). Hypothermia lowers the ischemic threshold even further. Below that level, however, cerebral electrical activity decreases and ceases. This is followed by functional and cellular biochemical changes, with depletion of ATP stores, an accumulation of lactate and an impairment of glutamate transport, which will occur quite rapidly and eventually lead to the unrelenting biochemical cascade terminating in neuronal autodigestion and cell death (Astrup *et al.* 1981). Experiments in awake primates have shown that a gradual reduction in the blood flow of the middle cerebral artery at normothermia leads to a cessation of function and reversible paralysis at an approximately 50% reduction. At a cerebral blood flow of approximately 20 ml/100g/min, cell death is a function of time, and with a further reduction in flow neurons are lost exponentially earlier, with the time to cell death being approximately 5-8 minutes at zero flow (Jafar and Crowell 1987).

CBF (mL/100g/min)

```
50 ─                              NORMAL

40 ─
                                  OLIGEMIA
30 ─

20 ─
        Electrical Function Affected
        Electrical Function Ceases
        ATP ↓ Lactate ↑            ISCHEMIA
10 ─    Glutamate Transport ↓
        Biochemical Cascade
        Cell Death
```
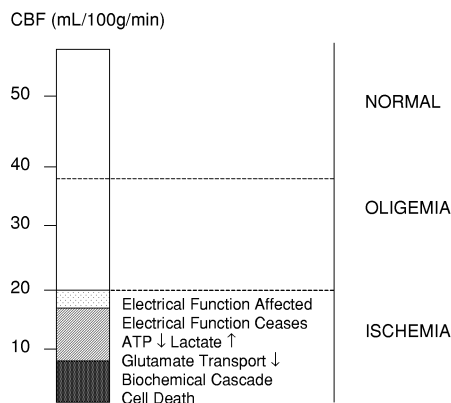
Fig. 1. Thresholds of cerebral ischemia (CBF = cerebral blood flow) (Modified from Astrup *et al.* 1977)

2.1.4. Loss of ion homeostasis

The working brain consumes about one-third of its energy in the maintenance of synaptic transmission, one-third in the transport of Na^+ and K^+, and one-third in preserving structural integrity including the biosynthesis of proteins, lipids, carbohydrates, and nucleic acids, the degradation of macromolecules, intracellular transport, and deoxyribonucleic acid repair. Normal cell function can be maintained even if plasma glucose concentrations decrease to half the normal rate (Lewis *et al.* 1974a, Lewis *et al.* 1974b). Energy failure leads rapidly to disorder in the membrane ion-transport mechanism. Under normal energy conditions, the extracellular concentration of Na^+ is 10-fold, Cl^- is 25-fold, and Ca^{2+} is 10.000-fold that of the intracellular concentrations, whereas K^+ will have a 30 times higher concentration intracellularly. The extracellular K^+ concentration increases 3 to 5-fold in the first few minutes following ischemia. Thereafter, extracellular Na^+ and Cl^- concentrations fall to half and Ca^{2+} to 10% of its normal rate while K^+ further increases to 20-fold. The increase of intracellular Na^+ and Ca^{2+} causes a passive shift of water into the cells leading to cellular swelling (cytotoxic edema). The extracellular space is reduced to half that of normal (normally 20% of brain water is in the extracellular space and 80% is located intracellularly).

EXCITATORY AMINO ACIDS

NMDA-RECEPTORS   AMPA RECEPTORS

DEPOLARIZATION   DEPOLARIZATION   HYDROLYSIS

Ca^{2+} ENTRY Na^+ ENTRY

PASSIVE
Cl^-/H_2O INFLUX

CELLULAR SWELLING

EAA
RELEASE Ca^{2+} ⬆

PROTEASE
ACTIVATION   LIPASE
ACTIVATION   FREE RADICALS ↑   ALTERED
TRANSCRIPTION   PROTEIN KINASE C
ACTIVATION
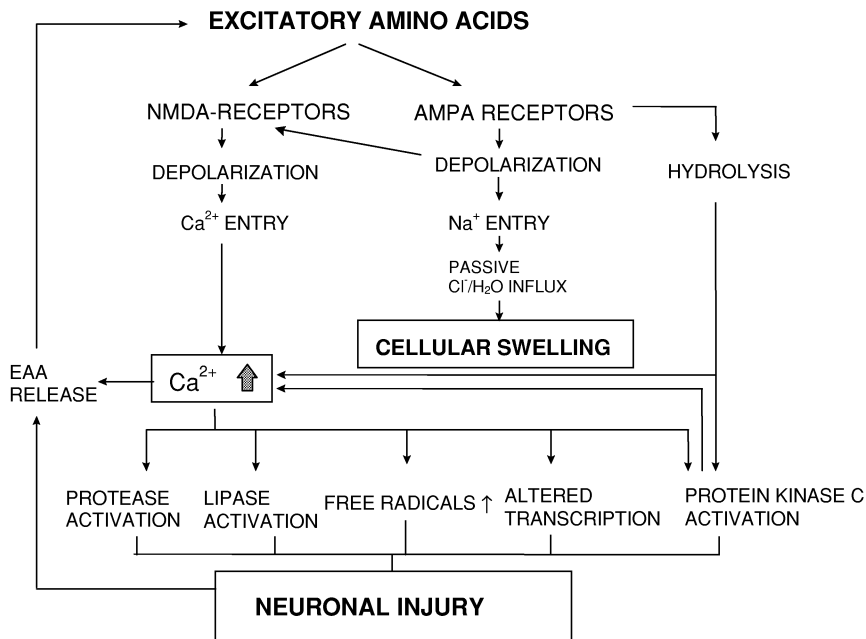
NEURONAL INJURY

Fig. 2. Pathophysiology of neuronal injury (Modified from McDonald and Johnston 1990).

2.1.5. The failure of neurotransmitter transport

The importance of the failure of neurotransmitter transport as a common pathway in the pathogenesis of ischemic cerebral injury, has been well demonstrated in the last few years. The ischemic cerebral injury follows a well-attested sequence of events. There is a loss of high-energy metabolites such as ATP and phosphocreatine, leading to increases in inorganic phosphate, lactate, and acidosis. The decrease of high-energy metabolites results in a failure to maintain ionic gradients and a normal hyperpolarized membrane potential. The cells of the presynaptic membrane depolarize and release a flood of neurotransmitters, *i. e.* glutamate. This depolarization is mediated by voltage-sensitive Na^+-channels, which carry electrical messages to the synapse. As a result of the depolarization, there is an influx of Ca^{2+} and a concomitant secretion of glutamate.

Extracellular glutamate levels increase in the penumbra from 7 µM to a peak of 180 µM during the first 20 to 30 minutes after the onset of ischemia and even more in the infarct core (Shimada *et al.* 1990, Takagi *et al.* 1993). Glutamate is a potent neurotoxin and exposure to 100 µM glutamate for 5 minute is sufficient to destroy large numbers of cultured cortical neurons (Choi 1987).

Two highly energy-demanding active transport mechanisms remove glutamate rapidly from the synapse. These mechanisms are disturbed during energy depletion after ischemia (Rhoades and Tanner 1995).

The excitatory amino acid (EAA) neurotransmitters bind to receptors at the membrane which themselves initiate a cascade of events. Glutamate is believed to be the predominant neurotransmitter involved in this excitotoxicity. The ionotropic glutamate receptors are divided into three groups based on which ligand preferentially activates the receptors; those that respond to N-methyl-D-aspartate (NMDA), $\propto$-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate (AMPA), and kainate (KA). Each of these receptor subtypes behaves differently in terms of both their pharmacology and biophysics (Small and Buchan 1996).

2.1.6. Cerebral edema

The development of brain infarction is often associated with cerebral edema, leading to volumetric enlargement of the tissue (Klatzo 1979). Edema aggravates the ischemic process by causing local compression of the microcirculation, a rise in intracranial pressure, and dislocation of parts of the brain (Hossmann 1988). Cerebral edema with subsequent tentorial herniation is the direct reason for mortality after supratentorial stroke in 31% of these patients (Bounds *et al.* 1981).

2.1.7. Lactic acidosis

Intracellular and extracellular pH is reduced during ischemia by a mismatch between glycolysis and oxidative phosphorylation. One molecule of glucose provides 2 ATP molecules and is converted to pyruvate. Under anaerobic conditions, pyruvate is converted to lactate without further ATP gain. During the conversion of one pyruvate molecule to lactate, 2 molecules of NADH lose one H^+ ion each, leading to extra- and intracellular acidosis. With ischemia the delivery of glucose is arrested, the amount of lactate accumulation depends on the pre-ischemic plasma glucose concentration. Lactic acidosis is worse when glucose levels are high (Ljunggren *et al.* 1974). In normoglycemic animals, intracellular pH in the brain falls to 6.2 − 6.4 (Behar *et al.* 1989) from the normal intracellular pH rate of 7.13 (Petroff *et al.* 1985) and it may decrease even further in hyperglycemic animals (Chopp *et al.* 1987). Notable acidosis enhances free radical formation triggered by the release of pro-oxidant iron from proteins such as transferrin (Siesjo *et al.* 1985).

2.1.8. Nitric oxide

Nitric oxide (NO) is a small second messenger molecule which plays a role in neuronal cell damage. After intracellular Ca^{2+} increases, Ca^{2+}/calmodulin-dependent nitric oxide synthase (NOS) is activated forming NO and citrulline from L-arginine and oxygen (Garthwaite *et al.* 1989). The reactions of NO with superoxide can lead to neurotoxicity through the formation of peroxynitrite. On the other hand, the reaction or transfer of NO^+ equivalents to thiols on the NMDA receptor can lead to neuroprotection by inhibiting Ca^{2+} influx (Lipton and Stamler 1994). NO is a potent dilator of cerebral blood vessels (Faraci and Brian 1994).

2.1.9. Ischemic core and penumbra

Experimental studies have demonstrated a gradual progression of a reversible degree of ischemia toward infarction (DeGirolami *et al.* 1984, Kaplan *et al.* 1991, Touzani *et al.* 1995, Weinstein *et al.* 1986). A central core with severely compromised CBF is surrounded by a circle or "penumbra" of moderate ischemic tissue with impaired electrical activity but preserved cellular metabolism and viability (Astrup *et al.* 1981, Astrup *et al.* 1977, Ginsberg and Pulsinelli 1994, Hakim 1987, Hossmann 1994, Siesjo 1992). The penumbra has been determined as follows: brain tissue in which the CBF has decreased to the point of causing electrophysiological silence and transient but recurrent losses of membrane ion gradients and energy metabolites (Ginsberg and Pulsinelli 1994). The penumbra has a variable outcome, and tissue recovery may be attained during reperfusion over the first 6 to 8 hours (Baron *et al.* 1995, Kaufmann *et al.* 1999).

2.1.10. Apoptosis

Necrosis and apoptosis are distinct mechanisms of cell death with different characteristics. Necrosis is caused by catastrophic toxic or traumatic events with passive cell swelling, injury to cytoplasmic organelles, and a rapid collapse of internal homeostasis. Necrosis leads to membrane lysis, the release of neuronal contents, and resulting inflammation. Apoptosis or "programmed cell death" is an active process requiring metabolic energy and protein and ribonucleic acid synthesis (Arends and Wyllie 1991). The apoptotic cell is characterized by cell shrinkage, nucleal collapse, a cleaving of chromatin into nucleosomal fragments while organelles retain their integrity and there is no associated inflammation (Arends *et al.* 1990).

2.1.11. Reperfusion injury

Reperfusion within 1 to 4 hours of ischemia is followed by hyperperfusion at even the core of the infarct, followed by moderate hypoperfusion or normal CBF (Kaplan *et al.* 1991, Muller *et al.* 1994). Regions of hyper- and hypoperfusion may even co-exist (Hossmann *et al.* 1985). Reperfusion delivers substrate to the tissue and removes the waste which has been accumulated during ischemia. Alterations or breakdown in blood-brain barrier permeability have been reported following global and focal ischemia. Increased brain water may increase intracranial pressure and lead to vascular compression and decreased tissue perfusion (Dobbin *et al.* 1989, Shigeno *et al.* 1985). Excessive delivery of oxygen to the ischemic tissue may produce a burst of free oxygen radicals (Traystman *et al.* 1991).

Interest has been directed toward the question of whether white blood cells, particularly polymorphonuclear leucocytes, act as mediators of secondary brain damage in models of cerebral ischemia. The temporal profile of polymorphonuclear leucocyte accumulation has been documented in ischemic models (Hallenbeck *et al.* 1986). White blood cells may obstruct microvessels, release vasoactive mediators, or migrate into the brain tissue where they may damage neurons by the release of cytotoxic enzymes or free radicals. Inflammatory processes influencing the pathological outcome after global ischemia have been reported (Dietrich *et al.* 1993).

2.1.12. Mechanisms of cerebral injury during aortic arch surgery

There are two basic mechanisms which lead to ischemic cerebral injury during aortic arch surgery. Global ischemia due to interrupted flow may lead to permanent or temporary neurological dysfunction. The temporary neurological complications is commonly believed to be self-limited and benign, but it may be associated with subtle permanent sequelae which become evident after more detailed neuropsychometric testing, especially of the memory function. Macro-embolic injury leads to strokes, which are the only neurological consequences of these operations reported by most investigators.

Studies on the possible or probable mechanism of injury following hypothermic circulatory arrest suffer from a lack of evidence that intraoperative and early postoperative changes correlate with the neurological outcome. Many studies purporting to analyze the pathophysiology of HCA continue to be published despite the fact that the connections between the observed phenomena and an adverse outcome are essentially speculative (Griepp and Griepp 1992). Findings which have been considered significant during HCA or immediately thereafter include acidotic intracellular pH, depletion of high energy phosphates (Aoki *et al.* 1993, Crittenden *et al.* 1991), decrease in CBF (Greeley *et al.* 1989a), decrease in the cerebral metabolic rate of oxygen (Greeley *et al.* 1989b) or loss of cerebral autoregulation, decrease in saturated hemoglobin or in cytochrome aa3 in near-infrared spectroscopy (Greeley *et al.* 1991a), low jugular venous oxygen saturation (Ausman *et al.* 1993, Croughwell *et al.* 1995), the increased levels of S-100β (Bhattacharya *et al.* 1999), the presence of enzymes such as CPK-BB (Ekroth *et al.*

1989) or of lactate in the cerebral venous blood or cerebrospinal fluid, and a delayed recovery of normal electroencephalographic patterns after surgery (Hsieh *et al.* 1990).

One of the consistent differences seen experimentally between low-flow cardiopulmonary bypass and hypothermic circulatory arrest is the presence of an interval of hyperemia immediately after HCA, raising the question of whether reperfusion injury may somehow be involved in the difference in outcome between the two groups (Mezrow *et al.* 1994, Mezrow *et al.* 1992). The presence of late changes in cerebrovascular resistance after hypothermia is also compatible with the possibility of reperfusion injury.

Cerebral metabolic demands tend to be met by increased oxygen and glucose extraction rather than increased CBF several hours after hypothermic circulatory arrest, suggesting that a "vulnerable period" for ischemia may exist after rewarming when the cerebral metabolism may be limited by CBF (Mezrow *et al.* 1992).

2.2. Methods of cerebral protection in aortic arch surgery

During routine cardiac surgery involving cardiopulmonary bypass (CPB), perfusion to the systemic and cerebral circulation is usually achieved through an arterial cannula in the ascending aorta or femoral artery. If the aortic arch requires repair or replacement due to an aneurysm or dissection, conventional CPB is not possible during the period in which the aortic arch is excluded from the circulation as the cerebral circulation is separated from that to the lower part of the body.

2.2.1. Hypothermic circulatory arrest

2.2.1.1. Safe duration of hypothermic circulatory arrest

Temperature is a major factor directly affecting cerebral metabolism (Greeley *et al.* 1991b) by reducing the cerebral metabolic rate of oxygen ($CMRO_2$) by approximately 7%/°C reduction in temperature (Govier *et al.* 1984, Kent and Peirce 1974). Despite the many years of discussion regarding hypothermia, the optimal temperature at which hypothermic circulatory arrest should be carried out continues to be controversial. Profound hypothermia (5°C to 7°C) gave better cerebral protection than deep hypothermia (18°C to 20°C) during prolonged hypothermic circulatory arrest in a canine model (Gillinov *et al.* 1993).

Cerebral oxygen consumption decreases progressively as the temperature is reduced, being 5% of the baseline at 8°C; 20% at 13°C; and 39% at 18°C (Mezrow *et al.* 1994). No clinical sequelae were seen following 90 minutes of HCA at 13°C, but at least transient neurological dysfunction were seen in all animals after 90 minutes of HCA at 18°C (Mezrow *et al.* 1995a), and both clinical and histological evidence of cerebral injury was demonstrated in pigs after 90 minutes of HCA at 20°C (Midulla *et al.* 1994).

CMRO₂
%

Duration of HCA
Min
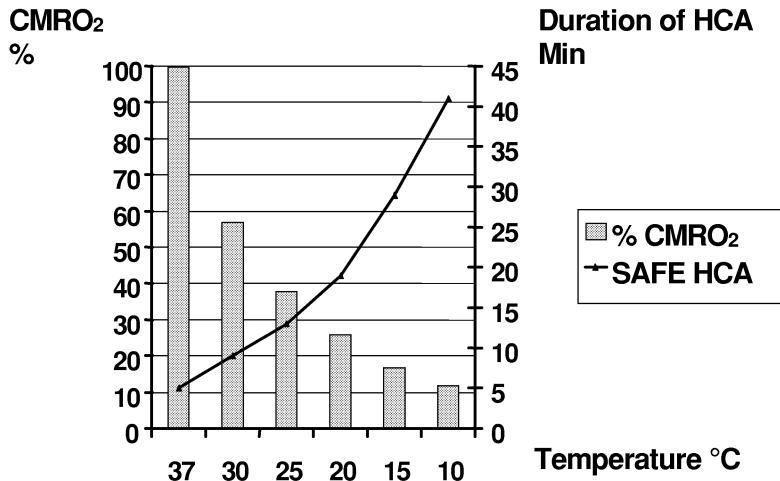
Fig. 3. The correlation between temperature and time during deep hypothermic arrest (Modified from McCullough *et al,* 1999).

The cerebral metabolic rate of O_2 in a study of 37 adult patients was measured with an ultrasonic carotid flow probe. The best available estimate for the period of safe arrest was then derived from the calculated Q_{10}. Twenty-five to 40 percent of the baseline metabolism is still present between 20 and 25 C°, while at 10 C° little over 10 percent of the metabolism is present and 40 minutes is safe (Fig. 3) (McCullough *et al.* 1999).

The main limitation imposed by circulatory arrest is the time factor. The "safe" duration of HCA has yet to be clearly defined, although times of 40 minutes have been associated with an increased incidence of stroke and 65 minutes with an increased mortality rate (Svensson *et al.* 1993). A prospective clinical study of HCA during repair of transposition of the great arteries revealed a high incidence of clinical neurological abnormalities at one year of age, and of both clinical and electrical seizure activity following an average of 53 minutes of HCA at 18°C (Newburger *et al.* 1993). Temporary neurological dysfunction correlated with a prolonged duration of HCA, and 60% of patients with a duration over 60 minutes had transient cerebral symptoms (Ergin *et al.* 1994a). In a randomized clinical trial in infants, surgery of transposition of the great arteries performed with HCA was associated with a higher risk of delayed motor development and neurologic abnormalities than surgery with low-flow bypass as the predominant support strategy (Bellinger *et al.* 1997).

The prolongation of extracorporeal circulation contributes to the consumption of clotting factors and interference with the coagulation pathway may result in coagulopathies and bleeding (Taylor *et al.* 1978). Hypothermia has been shown to impair the activity of the platelet activation enzymes and to reduce the enzymatic activity of clotting factors upon coagulation activation (Wilde 1997).

2.2.1.2. Acid-base balance and hypothermia

Cerebral vascular resistance depends on the partial pressure of carbon dioxide in arterial pressure (PaCO$_2$). Carbon dioxide diffuses across the blood brain barrier, reducing extracellular fluid pH and causing vasodilatation in cerebral vessels. Subsequent vasodilatation can disturb the normal autoregulatory response to changes in perfusion pressure (Strandgaard and Paulson 1984). Blood temperature can alter carbon dioxide solubility, causing a reciprocal change in PaCO$_2$ of approximately 4.5%/°C.

There are two protocols for acid-base control during cardiopulmonary bypass. In the pH-stat protocol, arterial pH is maintained during hypothermia, *i. e.* maintained at 7.40 irrespective of core temperature. As blood pH rises progressively with progressive reductions in temperature, pH-stat management requires the addition of CO$_2$ to the inflow gases of the CPB oxygenator to correct blood pH (Taylor 1997). This more acidic environment leads to improved oxygen delivery as a result of the rightward shift of the oxyhemoglobin dissociation curve, which might counteract the leftward shift induced by hypothermia. The lower pH promotes cerebral vasodilatation, and cerebral blood flow quickly exceeds that required for the maintenance of cerebral metabolic requirements, resulting in what has been termed "luxury perfusion" (Griepp and Griepp 1992). The advantage of pH-stat management is that it allows a more rapid cooling as a result of vasodilatation, but this may also predispose the patient to the steal phenomenon in the absence of autoregulation (Hiramatsu *et al.* 1995).

In the alpha-stat protocol, it is the "alpha" which is maintained, *i. e.* the ratio (termed alpha) of dissociated to nondissociated imidazole groups in the histidine buffer system. Because the imidazole group has a dissociation constant (pK) which parallels that of water with regard to a change in temperature, no adjustment of CO$_2$ content is required during the hypothermia or rewarming phases of CPB (Taylor 1998). In alpha-stat management, blood gases are regulated to remain pH neutral at normal body temperature, resulting in a relatively alkaline environment during hypothermia. Cerebral autoregulation is fairly well-preserved with this protocol and cerebral blood flow is reduced more or less in concert with diminishing cerebral oxygen requirements (Murkin *et al.* 1987). The advantage of alpha-stat management is a concurrent reduction in progressive tissue acidosis during and after a period of deep HCA (Watanabe *et al.* 1990).

In a retrospective clinical study, a higher incidence of choreoathetosis was observed following a change in anaesthetic strategy during cooling from the use of a pH-stat strategy to the use of an alpha-stat strategy. In another such study, the use of pH rather the alpha-stat strategy correlated with a better developmental outcome in a group of 16

pediatric patients who had undergone Senning repair of transposition of the great arteries (Jonas *et al.* 1993).

2.2.1.3. Cardiopulmonary bypass strategies

Pressures during CPB perfusion must be adjusted according to the predicted changes in cerebral autoregulation to avoid under- or over-perfusion. Impaired autoregulation leads to purely pressure-driven brain blood flow. This is usually associated with pH-stat management of the acid-base balance and is common in older patients and will expose the brain to relatively higher macro- or micro-embolic loads due to over perfusion. This in turn may in part explain the higher incidence of stroke in older patients (Taylor 1997).

Although physiologic blood flow rates are approximately 3.0 to 3.2 $L/m^2/min$, under normothermic conditions flow rates during CPB are often customarily set at 2.2 to 2.4 $L/m^2/min$ (Tarhan and Moffitt 1971). Studies have shown that the use of moderate hypothermia during CPB permit a reduction in flow rates safely to 1.6 $L/m^2/min$ at a core temperature of 28°C and flow rates of 1.2 $L/m^2/min$ are still adequate at 20°C (Fox *et al.* 1982). Experimental studies have demonstrated that even 0.5 $L/m^2/min$ flow rates are enough to perfusate the brain at 20°C systemic hypothermia (Fox *et al.* 1984).

2.2.1.4. Cooling and rewarming

Concern also exists regarding cooling as a consequence of clinical observations. In a study of children who had undergone repair of cyanotic congenital heart disease during early infancy with an average HCA duration of 64 minutes, shorter cooling periods (*i. e.* below 20 minutes) correlated significantly with lower developmental scores (Bellinger *et al.* 1991). This observation quickly resulted in a consensus that the interval of core cooling prior to an anticipated long duration of HCA must allow for thorough equilibration, and should be at least 20 minutes in neonates. Gaseous microemboli formation has been demonstrated during cooling and rewarming on CPB when the temperature gradient exceeds a critical threshold (Donald and Fellows 1961). Cooling gradients of 10°C or greater during CPB may be associated with gas emboli (Geissler *et al.* 1997). A study in a canine model showed that both extremely low-flow perfusion and excessive perfusion during cooling caused brain acidosis. Perfusion at a pressure of 20 mmHg at 20°C provides cerebral vasodilatation and aerobic metabolism (Watanabe *et al.* 1999).

2.2.1.5. Topical cooling

The use of topical cooling is also somewhat controversial, although several recent studies have demonstrated the value of packing the head in ice during the HCA interval. A study

on pigs clearly showed that there is an upward drift in epidural temperature if the head is not packed in ice during prolonged HCA, with a worse clinical outcome and greater evidence of histological damage (Midulla *et al.* 1994). A lower brain temperature and improved outcome was demonstrated in sheep after packing the head in ice for 2 hours of HCA, giving a temperature of 15°C (Crittenden *et al.* 1991). An improved recovery of metabolism was seen after packing the head in ice during HCA for 60 minutes at 18°C in a porcine model (Mault *et al.* 1993). Also in a study in man during HCA for repair of the aortic arch topical cooling of the head provided additional protection (Maas *et al.* 1997).

2.2.2. Selective cerebral perfusion

A technique that uses partial selective antegrade cerebral perfusion (SCP) with aortic arch aneurysms was described in the 1980`s (Frist *et al.* 1986). The SCP involves distal clamping of the aorta along with cannulation of the innominate, the left common carotid, and the left subclavian arteries. These arteries are perfused in an antegrade fashion in order to take advantage of the intrinsic cerebral autoregulation of blood flow that ensures a constant supply of oxygen over a broad range of metabolic demands and hydrostatic pressures (Ergin *et al.* 1994a). The current trend is to perfuse at least the innominate and left carotid arteries with a dedicated pump in a system with flows determined by target pressures measured at distal sites (Fig. 4). A system of low flow and profoundly hypothermic cerebral perfusion (10°C) has been used while keeping the rest of body at moderate hypothermia (25°C) in an attempt to reduce the time required for pump rewarming (Bachet *et al.* 1991).

Selective cerebral perfusion extends the "safe" period during which conventional systemic perfusion may be interrupted and reduces the risk of cerebral ischemia (Matsuda *et al.* 1989). Preservation of the cerebral autoregulatory system by SCP allows for longer periods of aortic arch repair. Any impairment of this autoregulation may lead to reperfusion injuries associated with regional over- and under-perfusion of the affected cerebral areas (Ergin *et al.* 1994b). Animal studies have shown improved cerebral protection using SCP compared with retrograde cerebral perfusion (RCP) (Mohri *et al.* 1993), (Crittenden *et al.* 1991). Better neurologic recovery, as well as improved electrophysiologic and histologic outcome were demonstrated using SCP when compared to RCP (Midulla *et al.* 1994).

Selective cerebral perfusion is technically more complicated than hypothermic circulatory arrest. Additional time and manipulation are required to isolate and cannulate the arch branches, and this may damage fragile arteries or dislodge atheromatous debris into the cerebral circulation. The use of SCP is limited in patients with severe carotid or brachiocephalic disease, traumatic aortic aneurysms, aortic rupture, infection or aortic dissection involving arch vessels, in patients with previous aortic, cardiac or mediastinal operations, or for those who require circulatory arrest before performing a sternotomy (Frist *et al.* 1986). Although a complicated method, SCP has been used in aortic arch operations with good neurological results (Veeragandham *et al.* 1998).

Dissatisfaction with the complications and time limits imposed by hypothermic circulatory arrest, as well as the complexity and limitations of selective cerebral perfusion has furthered interest in the more recent methods of cerebral protection.
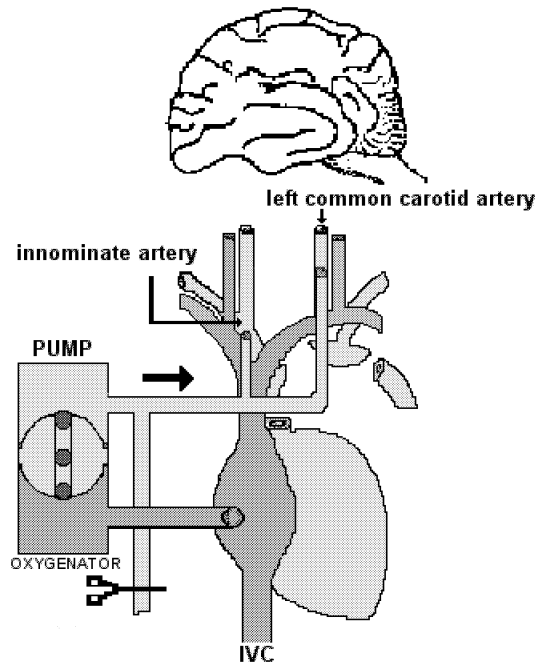
Figure 4. Selective antegrade perfusion

2.2.3. Retrograde cerebral perfusion

Retrograde cerebral perfusion was described in 1980 as a treatment for massive air embolism during CPB (Mills and Ochsner 1980). Thereafter intermittent use of this technique was used during the repair of a dissected thoracic aorta (Lemole *et al.* 1982). Intermittent and later continuous RCP was adopted as a method of cerebral protection during procedures involving the aortic arch (Ueda *et al.* 1990). Many reports in the Japanese literature followed (Murase *et al.* 1993, Yasuura *et al.* 1994), and the technique was soon tested by surgeons in all over the world.

RCP provides several theoretical advantages. The technique allows for smooth cerebral cooling, easy "de-airing" of the brachiocephalic vessels, the potential for limiting cerebral emboli, the ability to flush harmful neurological toxins and other byproducts of ischemic metabolism, and the possibility of direct cerebral metabolic substrate delivery.

The application of retrograde cerebral perfusion varies from one center to another and from one experiment to another. RCP techniques can be divided into four types (Nojima *et al.* 1994):

1. RCP via the superior vena cava (SVC) with the inferior vena cava (IVC) occluded and drainage from the aorta.
2. RCP via the SVC and systemic flow via the femoral artery with drainage from the IVC and aorta.
3. Retrograde perfusion via the SVC and IVC with drainage via the aorta (total body retrograde perfusion).
4. RCP by raising the central venous pressure (CVP) with the patient in the Trendelenburg position.

Furthermore, a simplified technique for achieving RCP via a small coronary sinus type catheter placed in the SVC has been described, especially when the need for circulatory arrest is recognized intraoperatively and a single venous cannula has already been placed for CPB (Cope *et al.* 1996).

The use of cold RCP (10°C) during moderate systemic hypothermia has been reported with good results in the surgical treatment of diseases of the proximal aorta (Imamaki *et al.* 1997, Lin *et al.* 1996, Moshkovitz *et al.* 1998). The advantages of this technique are shortened cooling and rewarming CPB times.

Although techniques within these general classifications may vary with respect to the perfusion pressure and flows used to generate RCP, the site at which this pressure is measured, the temperature of to cerebral perfusate, the temperature to which the patient is cooled and the site used to re-establish CPB after RCP, protection of cerebral function remains the common goal.

There is considerable variation in the site of perfusion, the mode of drainage and the perfusion pressures permitted in the larger clinical series (Bavaria *et al.* 1996, Coselli *et al.* 1995, Okamoto *et al.* 1993, Okita *et al.* 1996, Ueda *et al.* 1994). Most reports deal with perfusing through the SVC alone, with the exception of Takamoto`s group (Takamoto *et al.* 1992) who perfused via the femoral artery, and Okamoto`s report of total body retrograde perfusion (Okamoto *et al.* 1993).

There is sufficient experimental clinical and anatomic data, however, to suggest that the most effective retrograde perfusion of the brain is achieved when the entire venous system is pressurized. This emphasizes the important role which the valve-free azygos system plays as a connection between the central nervous system veins and the systemic venous plexus (Boeckxstaens and Flameng 1995, de Brux *et al.* 1995).
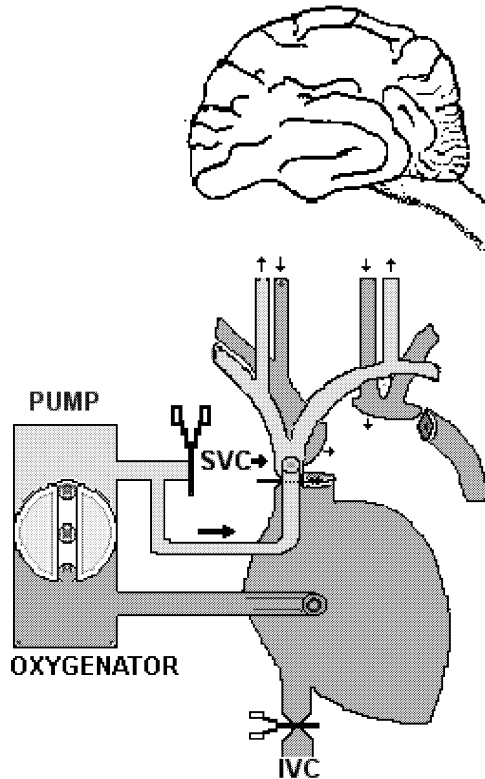
Figure 5. Retrograde cerebral perfusion

   Clamping the IVC during SVC perfusion improves retrograde flow to the brain. This maneuver was shown to increase the blood returning via the aortic arch from 22% in the IVC unclamped group to 83% in the clamped group (Nojima *et al.* 1994).
   An effective washout of particulate emboli from the brain was demonstrated when the IVC is occluded during RCP. The superiority of retrograde perfusion with the IVC occluded is reflected in a significantly higher aortic flow, a lower rate of oxygen extraction and a reduced microsphere retention in the brain as compared with RCP without IVC occlusion (Juvonen *et al.* 1998a).
   Although RCP may not be capable of maintaining aerobic metabolism, the addition of some oxygen may extend the safe duration of HCA and help prevent cerebral ischemic injury, particularly at lower temperatures (Midulla *et al.* 1994, Usui *et al.* 1994a). Animal studies of the ability of RCP to adequately perfuse cerebral tissue have produced controversial results (Midulla *et al.* 1994, Nojima *et al.* 1994, Usui *et al.* 1994a). It has been demonstrated in experimental studies that flow arriving in retrograde fashion will perfuse the cerebral venous system, but not the capillary system (Katz *et al.* 1999).

Whether RCP is of benefit owing to its cerebral perfusion capabilities (Safi *et al.* 1993, Ueda *et al.* 1990), the ability to prevent or reduce cerebral emboli (Ergin *et al.* 1994, Kouchoukos 1994, Nojima *et al.* 1994), the removal of cerebral waste products, or its ability to improve cerebral cooling (Usui *et al.* 1994a) remains unclear, but it can be used to provide an isolated cerebral flow that allows the brain temperature to be better maintained or cooled than with the systemic circulation (Lin *et al.* 1994). Manipulation of the arch vessels is avoided, thereby reducing the potential for embolization from debris and air.

RCP has been associated with cerebral edema, in which excessive venous pressure is generated (Mohri *et al.* 1993, Nojima *et al.* 1994, Usui *et al.* 1994a). Excessive RCP infusion pressure has also been associated with decreased cerebral blood flow caused by increased intracranial pressure (Usui *et al.* 1994a) and destruction of the blood-brain barrier (Mohri *et al.* 1993), and it has been suggested that a pressure of 20 mmHg (Nojima *et al.* 1994, Watanabe *et al.* 1996) or 25 mmHg (Usui *et al.* 1994b) provides the maximum retrograde cerebral flow with the least chance of producing increased intracranial pressure and cerebral edema.

Non-pulsatile and pulsatile RCP have been compared in an experimental study in which it was demonstrated that cerebral tissue water was significantly decreased in the pulsatile RCP group (Nojima *et al.* 1993). Although pulsatile RCP has not been used clinically, it may represent a useful fashion to reduce brain edema during prolonged periods of RCP.

In a recent analysis of risk factors associated with RCP, pump time, urgency of the surgery and age were shown to be the dominant risk factors for mortality and morbidity. In that study, the overall mortality was 25/249 (10%) patients, and 17% in emergency surgery. Cerebral stroke was described in 4% of patients (Ueda *et al.* 1999). In another retrospective analysis of 144 patients undergoing aortic arch surgery with RCP, it was shown that emergency surgery and the presence of untreated preoperative malperfusion were the only significant independent predictors for mortality (Deeb *et al.* 1999). In a 130 patient European material, overall mortality was 16.9% and the incidence of stroke was 6.9%. Age and HCA duration remained as risk factors for stroke and mortality, while RCP duration was not (Wong and Bonser 1999).

According to currently available information, the use of RCP for cerebral protection during HCA in the clinical setting is safe when flow rates and central venous (intracerebral) pressures are maintained at relatively low levels. Even if the only clinical benefits of RCP are its maintenance of cerebral hypothermia (Juvonen *et al.* 1998b) and the flushing of air and particulate emboli from the arterial circulation, thereby reducing the risk of embolism, its continued use and investigation would still be justified (Coselli 1997).

2.3. Pharmacological strategies to mitigate cerebral injury

2.3.1. Glutamate antagonists

Presynaptic Ca^{2+}-channel blockers are able to decrease the influx of Ca^{2+} and the subsequent release of neurotransmitters, primarily glutamate. SNX-111, a selective N-type Ca^{2+}-channel blocker, has been shown to be neuroprotective in both global and focal models of ischemia in rats and rabbits, but it has harmful sympatholytic activity to decrease blood pressure (Buchan *et al*. 1994, Perez-Pinzon *et al*. 1997).

Agents which block presynaptic voltage-sensitive Na^+-channels prevent membrane depolarization. Lamotrigine [3,5-diamino-6(2,3-dichlorophenyl)-1,2,4-triazine] is a Na^+-channel blocker used clinically as an anticonvulsant. It has few side effects and can also be administered orally. In view of the interaction of lamotrigine with Na^+-channels and its ability to penetrate the blood-brain barrier, interest has focused on its neuroprotective properties. In an experiment using a rabbit model, glutamate concentrations after lamotrigine treatment and in hypothermic animals were significantly lower than in the normothermic control group. Furthermore, glutamate levels in the lamotrigine group did not increase during the experiment (Bacher and Zornow 1997). Lamotrigine has been demonstrated to significantly attenuate excitatory neurotransmitter release in normothermic cerebral ischemia during CPB without improving other neurologic parameters (Conroy *et al*. 1999). Also BW619C89, riluzole, and lubeluzole has been referred to in the literature as glutamate release inhibitors (Leach *et al*. 1993). Clinical trials with lifarizine, a similar substance, although protective, were not continued due to cardiac side effects (Squire *et al*. 1995).

The mechanism of neuroprotection with NMDA antagonists in cell-culture models of excitotoxicity appears to be a blocking of the influx of Ca^{2+} and an arrestment of the Ca^{2+}-driven excitotoxicity (Small and Buchan 1996). The lipophilic non-competitive NMDA receptor antagonists currently under scrutiny are MK-801, dextromethorphan, dextrophan, CNS 1102 (Cerestat, Aptiganel), ketamine, memantine, remacemide desglycine, magnesium, zinc, and competitive NMDA receptor antagonists CPP, with its derivatives *d*-CPPene and CGS 19755 (Selfotel). Unfortunately, most NMDA antagonists exert too many harmful side effects to be of clinical use (Davis *et al*. 1997, Muir and Lees 1995).

A glycine receptor antagonist, ZD9379, 7-chlorokynuretic acid and its derivatives ACEA-1021, ACEA-1031, and ACEA-1416 have been demonstrated to reduce infarct volume in focal ischemia models (Takano 1997, Tatlisumak *et al*. 1998, Warner *et al*. 1995). The anticonvulsant felbamate has several potential sites of action which could promote its neuroprotective efficacy, but its clinical use has been associated with aplastic anemia, insomnia, headache, and altered taste (Sachdeo *et al*. 1992).

The competitive AMPA antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline (NBQX) or the non-competitive antagonist GYKI 52466 have been shown to be neuroprotective in both global and focal models of ischemia (Redmond *et al*. 1995). AMPA antagonists induce a dose-dependent respiratory

depression associated with a general depression of cerebral metabolism in animal models (Bullock *et al*. 1994).

2.3.2. Monosialogangliosides

Monosialogangliosides are naturally occurring sialic acid-containing glycosphingolipids and are to be found in abundance in neuronal plasma membranes. *In vitro* they have been shown to be efficacious in limiting EAA-induced neurotoxicity (Skaper *et al*. 1991). In an experimental study, a better behavioral outcome and less neuronal injury was seen in dogs pretreated with the monosialoganglioside GM1 (Redmond *et al*. 1993).

2.3.3. Ca^{2+}-channel blockers

Calcium-channel blockers have been demonstrated to possess promising characteristics as possible neuroprotective agents prior to HCA. They may be helpful in blocking the calcium influx into cells during the late stages of glutamate excitotoxicity as well as other mechanisms of ischemic injury, and also in promoting cerebral vasodilatation (Lipton and Rosenberg 1994, Shaw 1993). Nimodipine has been shown to have a beneficial effect on the neurological outcome after 3 hours of HCA at 10°C (Mazzoni M 1993). The size of the neocortical infarct was reduced, with increasing local cerebral blood flow in the calcium antagonist AT877 treated animals (Ohtaki and Tranmer 1994).

2.3.4. Free radical scavengers

The mechanisms of action for the agents in this group of compounds are to prevent the production of free radicals and the subsequent injury of membranes, to enhance enzymatic activity that would metabolize free radicals, and to trap or scavenge free radicals, thereby removing agents injurious to neurons, glial cells, and blood vessels (Small and Buchan 1996). Lipid peroxidation inhibitors stabilize membranes and may be more efficient as a vascular protectant than as a neuroprotectant (Hall *et al*. 1994). Positive results have been demonstrated with transgenic mice over-expressing superoxide dismutase (Kinouchi *et al*. 1991). The spin-trapping agent α-phenyl-tert-butyl-nitrone (PBN) has been shown to reduce infarct size and cerebral edema after focal ischemia in a rat model. The spin-trapping agents form longer living and more stable products through contact with free radicals (Cao and Phillis 1994). Tirilazad (U74006F) is in clinical trial for acute stroke and preliminary results are encouraging, with an absolute reduction in mortality of 14% (Haley 1998).

2.3.5. Calpain inhibitors

Calpain is a cytosolic neutral protease which normally exists in an inactive state. It is activated by increased intracellular calcium, which results in the irreversible proteolysis of the cytoskeletal membrane, and regulatory proteins (Bartus *et al.* 1998). Increasing evidence suggests that excessive activation of calpain is associated with the pathology of cerebral ischemia (Hong *et al.* 1994a). Intravenous injections of Cbz-Val-Phe-H, a calpain inhibitor have been shown to reduce infarct size, brain edema, and calcium-activated proteolysis in a focal ischemia model in rats (Hong *et al.* 1994b).

2.3.6. Caspase inhibitors

Caspases have been reported to be important for the execution of apoptosis in mammalian cells (Kuida *et al.* 1996). *In vitro* and *in vivo* studies demonstrated that caspase inhibition selectively reduces the apoptotic component of ischemic neuronal death. A study with cultured mouse cortical neurons demonstrated that caspase inhibition selectively reduces the apoptotic component of oxygen-glucose deprivation-induced neuronal death (Gottron *et al.* 1997). Peptide inhibitors of caspases have been shown to decrease infarct volume in focal ischemic animal models (Endres *et al.* 1998, Hara *et al.* 1997). Caspase inhibitors may have a place in future strategies for the prevention of delayed neuronal death in the hippocampus after global ischemia such as that seen during cardiac arrest.

2.3.7. Nitric oxide synthase inhibitors

Argine and oxygen are converted to the harmful NO and citrulline in the postsynaptic neuron by the action of nitric oxide synthase (NOS). It has been demonstrated in a canine model that 7-nitroindazole, a specific NOS inhibitor, reduced NO production and improved neurological outcome (Baumgartner *et al.* 1999). Neuronal apoptosis was decreased after 7-nitroindazole treatment (Tseng *et al.* 1997).

SNX-111
-sympatolytic

NBQX -nefrotoxicity
LY 293558 -respiratory depression

Ca²⁺ Channels

AMPA Receptor (Na⁺)

Phospholipases

Glutamate

NMDA Receptor (Ca²⁺)

Calpain ← *Calpain inhibitors*

Protein Kinase

Glycine site

Ca²⁺ → **Nitric Oxide Synthase** ← *7-nitroindazole*

MK801 -toxicity
Aptiganel -hallucinogenic
Selfotel -toxicity
Memantine

Na⁺ Channels

O2 + Arginine   **Citrulline + NO** → **Cell Death**

Lamotrigine   *Felbamate*

Kainate Receptor

Apoptosis   Necrosis

Caspase inhibitors

Presynaptic Membrane

Postsynaptic Membrane

Postsynaptic Neuron

Figure 6. Pharmacologic interventions to mitigate ischemic excitotoxic injury in the central nervous system.

2.4. Experimental models of retrograde cerebral circulation

Designing an experimental model meant for extrapolation to a clinical situation, dictates that the investigators must first clarify the anatomical differences between human and the animal species available for study. Also one of the most important prerequisites for the selection of the animal species in RCP set-up is that it must be big enough to place on cardiopulmonary bypass. Dogs, pigs and baboons have been used as laboratory animals in studying RCP (Boeckxstaens and Flameng 1995, Juvonen *et al*. 1998a, Usui *et al*. 1994). In this study the chronic porcine model used has been developed by professor Randall Griepp`s group at the Mount Sinai School of Medicine in New York (Midulla *et al*, 1994). There are certain differences in the details of the blood circulation of the brain between man and the pig.

2.4.1. The differences in the arterial circulation between man and the pig

In man, three side branches arise from the aortic arch: the brachiocephalic trunk, the left common carotid artery and the left subclavian artery. The internal carotid artery is one of the two branches of the bifurcation of the common carotid artery which on the right originates from the brachiocephalic trunk and on the left directly from the aortic arch. In the intracranial part, the internal carotid artery has three major branches: the anterior cerebral artery, the middle cerebral artery and the posterior communicating artery. The vertebral artery is one of the first tributaries of the subclavian artery. It follows an ascending course in the transverse foramina of the cervical spine and enters the skull on both sides of the foramen magnum. It has tributaries to the spinal cord, to the meningae, and to the cerebellum. Both vertebral arteries come together in the basilar artery, which supplies blood to the pons, the brain stem and cerebellum. Finally, the basilar artery ends up in an anastomosis with the posterior cerebral artery. All the arterial blood circulation to the brain, except for the cerebellum, pons and the brain stem, comes from tributaries of the Circle of Willis (Warwick and Williams 1973).

In the pig, the aortic arch has two major side branches to the upper part of the body: the brachiocephalic artery, which divides into the bicarotid trunk and the right subclavian artery, and the left subclavian artery (Fig. 7). The bicarotid trunk divides into the left and right common carotid artery, which lead to the head, giving side branches for the thyroid gland, the trachea and the esophagus. It divides at its end branches with the internal carotid artery for the brain and other side branches for the snout. As in man, the vertebral artery originates from the subclavian artery. The extracerebral system is more developed than in man and forms the largest part of the vasculature (Popesko 1977).

The arterial circulation in the cranium of the pig is similar to that of man. An arterial circle, formed by the internal carotid artery and the fusion of both vertebral arteries in a basilar artery, provides blood to the brain. The circle is completed by a very small communicating rostal artery, which is not as well developed as that in man.

2.4.2. The differences in the venous circulation between man and the pig

More important with regards the present experimental setting is the venous system. One of the most striking differences between man and animals is the absence of valves in the human cerebral venous circulation. However, a complete absence of valves has not been demonstrated. Studies examining human cadavers report that there are valves in the internal jugular vein when it enters the brachiocephalic trunk or approximately 2 cm above the junction in 88% (Dresser and McKinney 1987, Fisher *et al.* 1982), and that these valves can remain competent up to a pressure of 75 mmHg (Fisher *et al.* 1982, Sum-Ping 1994). Another important characteristic of the human system is the presence of a double outflow system, parallel to the arterial circulation, with an almost complete division between brain and extra-cranial venous return.

2.4.2.1. The venous system in man

The upper part of the sinus system consisting of the sinus sagittalis superior, sagittalis inferior, rectus occipitalis and transversus converges into the confluens sinuum, while the inferior part comes together in the sinus cavernosus. The blood from these sinuses exits the skull via the internal jugular vein. The much smaller external jugular vein drains the blood from the facial structures. An anterior jugular vein is often present, being very small and originates at the chin to drain the superficial structures of the face, and then forms an arcus venosus juguli with the vein of the other side. It also has branches to the external jugular vein. With the subclavian vein these three veins form the brachiocephalic trunk.

The venous drainage of the intracranial area is not completely parallel to the arterial system. Three different venous systems in the brain can be recognized: the superficial cerebral veins, the deep cerebral veins and the venous sinuses (Browder *et al.* 1972). The superficial cerebral veins drain the blood from the cerebral cortex and underlying white matter into the venous sinuses. Deep cerebral veins drain the blood in the centripetal direction from the deep white matter, the basal ganglia, and the diencephalon towards the lateral ventricles. Large subependymal veins empty into the internal cerebral veins and together form the great cerebral vein of Galen. This large vein empties into the dural sinuses. The dural sinuses and cerebral veins are rather unique in comparison to veins in other parts of the body. The walls of the cerebral veins are very thin, and they lack the three distinct layers usually associated with vascular structures. The dural sinuses have fibrous walls consisting of an endothelium surrounded by dura mater. There are no valves in the sinuses or cerebral veins (Browder *et al.* 1972).

The superior vena cava drains the blood from the upper part of body and the thoracic wall. It is formed from the left and right brachiocephalic vein, which in turn are formed from the subclavian and jugular veins. The jugular vein is derived from the internal, the external and anterior jugular veins and drains the blood of the head, whereas the subclavian vein drains the upper limb and a part of the thoracic wall. Before entering the right atrium, the superior vena cava receives flow from the azygos vein, which drains blood from the lateral and dorsal thoracic wall, and forms a connection with the inferior vena cava. A cadaver study shows that the valve-free azygos system is the major pathway to the central nervous system during retrograde cerebral perfusion (de Brux *et al.* 1995). The junction between the superior vena cava and the right atrium is sometimes protected by a valve.

There are various veno-venous anastomosis between the internal and external jugular system: the plexus venosus caroticus internus, the rete foraminis ovalis and the plexus pterigoideus, the plexus venosus canalis ovalis, and a direct anastomosis between both jugular veins through the retromandibular and posterior auricular vein. The jugular system is connected with the thoracic wall through an extended venous plexus around the spinal cord. Arteriovenous anastomosis are present at the level of the pia mater (Warwick and Williams 1973).

2.4.2.2. The venous system in the pig

Venous drainage is formed from the superficial and deep cerebral veins and cerebral venous sinuses. Venous sinuses are less developed than in man, and two major groups are identified with connections between them. They are drained into the internal jugular vein via two veins, the ventral cerebral vein and the dorsal cerebral vein (Fig. 8) (Getty 1975). In contrast to those of the dog and in part to man the internal jugular veins are valvulated in under 20% in the pig (Juvonen *et al*. 1998a). Although large subdivisions in venous classification are found, the venous drainage is less complex and developed than in man.

The extracerebral structures demand the main proportion of the vascularisation on the arterial as well as on the venous side and drainage is mostly performed by the maxillary vein. The extracerebral veins are composed of a complex network of plexuses and veins with many connections between the internal and external jugular and maxillary systems. External and internal jugular systems are united to the subclavian veins to form the superior vena cava which ends in the right atrium of the heart. The azygos and hemiazygos veins merges with the superior vena cava and form connections with the vena cava inferior and sinus coronarius of the heart (Fig. 7) (Stodkilde-Jorgensen *et al*. 1986). There are more connections between the internal and external cranial system, however, compared to that in man.

The most striking differences with the circulation of the human head are the poorer development of valves in the internal jugular veins and the sinus system of the brain, and the more highly developed arterial and venous circulation for the extracranial structures in comparison with the intracranial circulation.

Although the anatomy of the porcine venous system differs a little from the venous system in man, the porcine model is useful for studying retrograde cerebral perfusion (Midulla *et al*. 1994). The rate of competent jugular valves in pigs is less than 20%, which enable retrograde flow in most cases (Juvonen *et al*. 1998a). Limitations of the porcine model includes the greater circulation going to the extracranial structures and the smaller (approximately 80 g in 30 kg pig) brains compared to those in humans.
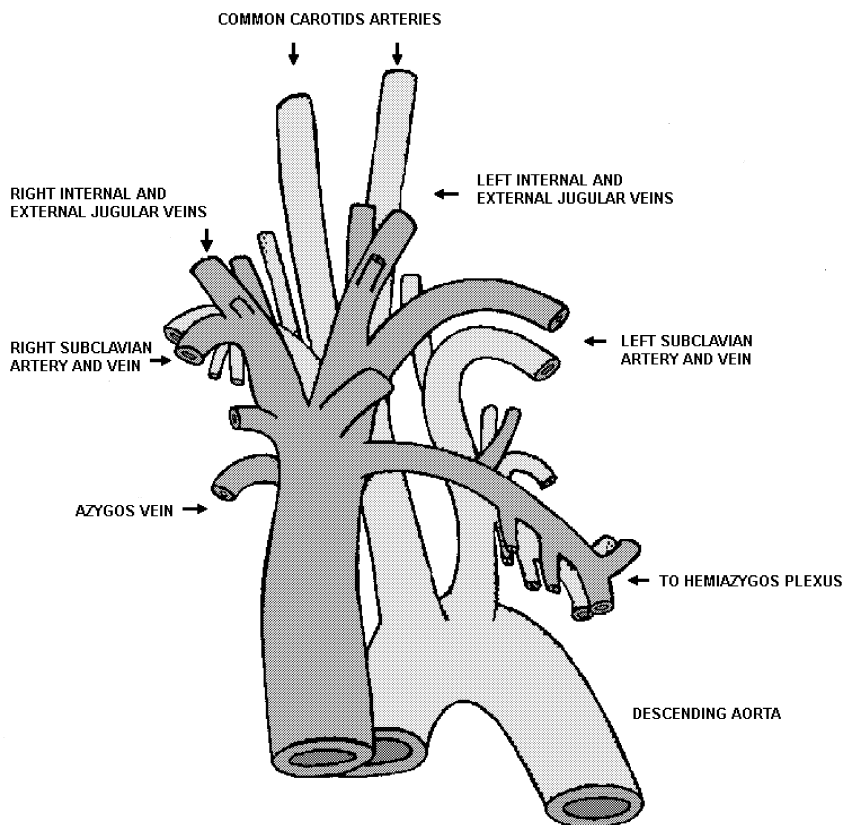
Figure 7. Main neck vessels of pig. The preparation has been turned 30 degrees to the left (Modified from Stodkilde-Jorgensen *et al.* 1986).

A.

Sagittal sinus

B.

Sagittal sinus

Cerebellum

Cortex

Ventral cerebral
veins

C.

D.

Ventral cerebral vein   Dorsal cerebral vein
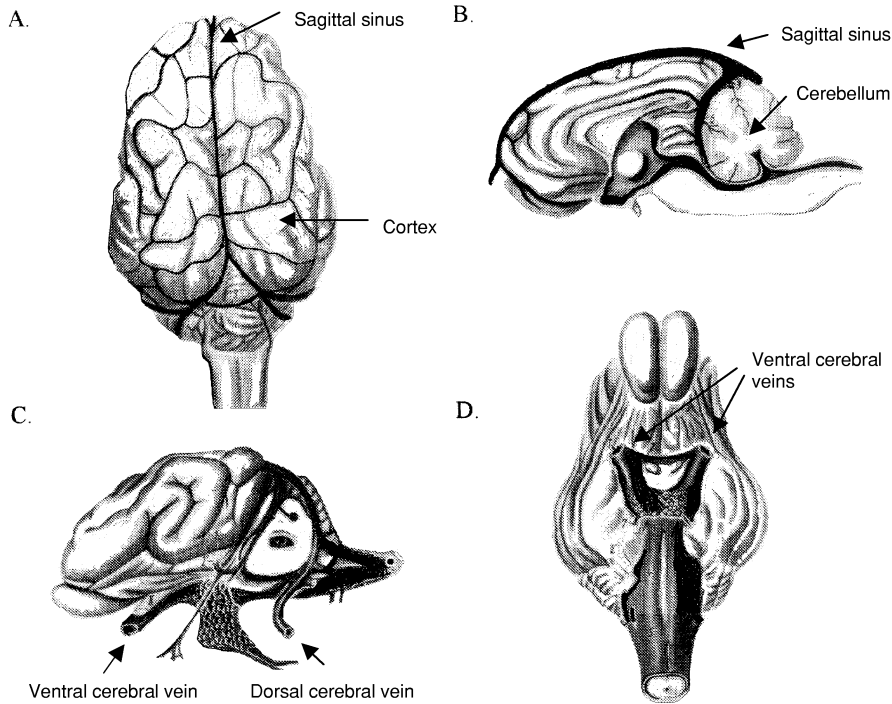
Figure 8. The venous sinuses of the brain of the pig (Modified from Boeckxstaens 1996). A. Dorsal view, B. Medial view, C. Lateral view, D. Ventral view.

2.4.3. Other animal models for the study of retrograde cerebral perfusion

2.4.3.1. The cerebral circulation of the dog

The cerebral circulation in dogs is very similar to that in man, but has its own characteristics. The circulation in the snout is proportionally the largest, which is similar to other animals when compared with primates due to a lesser development of the brain (Popesko 1977). The most important differences between the cerebral circulation in dogs and man are the absence of the double system with separate circulations for the brain and the extracerebral structures, the presence of multiple, well-developed valves in the venous drainage of the head and a poorer development of the venous sinuses (Miller *et al.* 1964). A canine model was used for studies of RCP and in these models the jugular venous

valves have been eliminated with the use of a valvulotome (Usui *et al*, 1992) or RCP flow was induced through the valveless maxillary veins (Nojima *et al*, 1993).

2.4.3.2. The cerebral circulation of the baboon

The cerebral arterial vascularisation of the baboon is very similar to that of man, the brain demanding the greatest proportion of blood going to the head and competent valves have not been documented (Symon and Russell 1971). Some experimental studies using a baboon have been made (Boeckxtaens and Flameng 1995). Naturally non-human primates would be ideal species to study retrograde cerebral perfusion, but ethical and economical points make this difficult.

2.5. Evaluation of ischemic cerebral damage

2.5.1. Histology of ischemic cerebral damage

The histological examination of tissues is one of the most accurate methods for investigating the discrete changes in organs and their reaction to externally applied stresses. It is very important to avoid any production of artifacts during fixation of the brain tissue for histological examination. It is also essential to avoid any time delay between death and fixation due to autolysis. The most common method of fixation is immersion in formaldehyde or formalin, but a perfusion fixation with a large volume of formaldehyde has also been used in experimental studies (van Reepts and Borgers 1990).

   A reduction and cessation of the cerebral blood flow induces cell necrosis in the brain which is seen as membrane disruption, protein disintegration, cytosol vesicularisation and nuclear chromatin clumping (Greenfield and Meyer 1979). These features are related to the site and duration of the ischemia and to the degree of flow reduction. Regardless of the localisation of the lesion within the brain or the time of the insult, cells (neurons and glial cells) will always show either edematous or coagulative changes. The vascular compartment will exhibit congestion, with endothelial damage, and dilatation of the perivascular space.

   Four different stages of cell necrosis can be detected; microvacularisation, ischemic cell change, severe cell change, and finally, cell loss. Microvacuolisation is represented by swollen mitochondria and dilatation of the endoplastic reticulum. Ischemic cell changes are described as the typical shrinkage or homogenization of the neuronal cytosol and nucleus, so the cell appears darker. Severe cell change corresponds with extremely swollen glial and to a lesser extent, neuronal cells. The clearly infarctive foci is characterized by disappearance of the cells with neoformation of the capillaries (Brown 1977).

2.5.2. Biochemical markers of cerebral damage

The measurement of biochemical markers of hypoxic and ischemic cerebral injury is of great value in acute clinical situations. They provide information on the injury of cerebral tissue and can sometimes be used to quantify the damage of the brain. In clinical settings, serial measurements of these markers are sometimes the only way available to evaluate the evolution of the damage.

2.5.2.1. Arterio-venous gradients and oxygen extraction

Analyzing blood gases can provide information on the metabolism of the organs through which the blood passes. In order to draw conclusions on the presence of brain ischemia samples from both the efferent and afferent blood vessels must be drawn simultaneously at different periods during the experiment or operation (Mezrow *et al*. 1995). The oxygen extraction ratio (OER) can be calculated from the formula: (O_2 content $_{inflow}$ - O_2 content $_{outflow}$) : O_2 content $_{inflow}$ (Cheung *et al*. 1999). Cerebral oxygen consumption can be evaluated during the experiment using the OER.

2.5.2.2. Lactate as a biochemical marker

Lactate is not specific for cerebral damage, but is closely correlated to glucose metabolism and appears in the cell during and after ischemic damage. The period of increased lactate concentration after an ischemic event is very short, concentration returning to the normal pre-ischemic levels within between 90 and 180 minutes (Frerichs *et al*. 1990). Hypothermia does not influence the lactate production despite the fact that the metabolism of glucose and glycogen falls to half of control values during cerebral hypothermia (Natale and D'Alecy 1989).

2.5.2.3. Glucose as a biochemical marker

Brain tissue is dependant on a continuous supply of oxygen and glucose, and glucose metabolism is normally aerobic. During hypothermia and cardiopulmonary bypass serum glucose levels have a tendency to increase (Ekroth *et al*. 1989, Kuntschen *et al*. 1986). Hyperglycemia during cerebral ischemia forces anaerobic glycolysis and leads to increased lactate production and aggravated cerebral damage (Anderson *et al*. 1992). By monitoring the arteriovenous glucose gradient cerebral ischemia can be estimated,

together with the measurement of lactate levels in the venous blood returning from the brain.

2.5.2.4. S-100β

The S-100 protein is a small (21 kDa) dimetric cytosolic calcium binding protein that exists in various forms depending on its chain (α or β) structure. The ββ-form occurs predominantly in astroglial and Schwann cells, and the αβ-form in astroglial cells, the concentrations in other cells being negligible, and the protein is involved in promoting axonal growth, glial proliferation, neuronal differentiation and calcium homeostasis (Johnsson 1996). Increased levels of S-100 protein have been measured following cardiac operations, stroke, and several other neurologic disorders (Buttner *et al.* 1997, Westaby *et al.* 1996). This marker has been enthusiastically adopted for clinical use by many cardiac surgeons with the expectation that repeated measurements could indicate brain injury postoperatively (Westaby *et al.* 1996).

2.5.3. Electroencephalography monitoring

The rationale behind quantitative electroencephalography (EEG) monitoring is its ability to non-invasively provide information on any process threatening damage to the brain. In experimental studies it can pinpoint the moment when cerebral damage occurs, and in a clinical setting it may show the time during an operation or after it when additional measures of brain protection should be instigated (Edmonds *et al.* 1992, Mezrow *et al.* 1995b, Mezrow *et al.* 1994). Other ways of electrophysiologically non-invasively indicating imminent damage, or the extent of damage which has already occurred are somatosensory-evoked potentials (SEPs), and auditory brainstem-evoked potentials (BAEPs) (Juvonen *et al.* 1998a, Rodriguez *et al.* 1995). One of the most accurate methods to quantify EEG is to measure burst durations, *i. e.* the effect on the brain can quantified even in hypothermia when EEG is very low amplitude (Lipping *et al.* 1995). The burst-suppression ratio can be calculated from continuously recorded EEG, *i. e.* the summation of high amplitude EEG burst lengths divided by the length of the recording interval in each time points (intervals). EEG suppression can be caused by general anesthesia, ischemic brain damage and hypothermia. Suppression is also seen postictally after epileptic seizure (Jantti *et al.* 1994). The first three fashions, at least, are additive both in whole brain or isolated cortex for reasons that are poorly understood. Probably all of these four fashions share similar mechanisms, the importance of which is in preventing the energy consuming and harmful activity such as seizure.

3. Aims of the present studies

The aims of the present research were:
1. to explore cerebral protection during RCP with interrupted flow, continuous RCP and HCA alone (I).
2. to test the efficacy of deep hypothermic RCP for improved cerebral outcomes during moderate HCA (II).
3. to clarify the possible benefit of RCP due to its improved cooling effect (III).
4. to test the neuroprotective efficacy of lamotrigine during HCA (IV).

4. Materials and methods

4.1. The chronic porcine model

The chronic porcine model has been developed by Professor Randall Griepp`s group at the Mount Sinai School of Medicine in New York during the 1990`s (Midulla *et al*. 1994, Juvonen *et al*. 1998a).

4.2. Preoperative management

All animals received humane care in accordance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication No. 85-23 revised 1985). The study was approved by the Research Animal Care and Use Committee of the University of Oulu.

4.3. Drug administration (Study IV)

An isethionate (2-hydroxyethanesulfonate) salt of lamotrigine [3,5-diamino-6 (2,3-dichlorophenyl)-1,2,4-triazine] (Glaxo-Wellcome, Great Britain) was diluted in saline to obtain a solution containing lamotrigine at 50 mg/ml and this was packed in 10 ml ampoules in the Pharmaceutical Laboratory of our institution. Saline placebo ampoules were prepared similarly. A dose of 20 mg/kg was measured and diluted to 50 ml in saline. This volume was given intravenously over a period of 20 minutes, starting two hours before HCA. Randomization was carried out by the chemist in the Pharmaceutical Laboratory of our institute.

4.4. Anesthesia and hemodynamic monitoring

Anesthesia was induced with ketamine hydrochloride (10 mg/kg intramuscularly) (Ketalar®, Parke-Davis, Sweden) and midazolam (1 mg/kg intramuscularly) (Dormicum®, Roche, Switzerland), and muscular paralysis was maintained with pancuronium bromide (0.1 mg/kg intravenously) (Pavulon®, Organon Teknika, Holland). Following endotracheal intubation, the animals were maintained on positive pressure ventilation with 100% oxygen; anesthesia was maintained with isoflurane (1.1 – 1.2 %) (Forene®, Abbot, Italy). An intravenous infusion of cefuroxime (1,5 g) (Lifurox®, Eli Lilly and Company, USA) was administered preoperatively. An arterial catheter was positioned in the left femoral artery and a Swan-Ganz catheter (CritiCath®, 7-F; Ohmeda GmbH & Co, Germany) was placed through the femoral vein to allow blood sampling and pressure monitoring in the pulmonary artery and for the recording of cardiac output. Temperature probes were placed in the esophagus and rectum to measure temperatures and a 10-Ch catheter (Braun Melsungen AG, Germany) into the urinary bladder to monitor urine output.

4.5. Electroencephalography monitoring (Studies II, III and IV)

Cortical electrical activity was registered from four stainless steel screw electrodes (5 mm in diameter) implanted in the skull over the parietal and frontal areas of the cortex using a digital EEG recorder (Nervus®, Iceland) and an amplifier (Magnus® EEG 32/8, Iceland). The sampling frequency was 1024 Hz and the bandwidth 0.03 – 256 Hz. All EEG recordings are referenced to a frontal screw electrode, which - together with a ground screw electrode - was implanted over the frontal sinuses. Continuous EEG activity was recorded for 10 minutes in anesthesia before the cooling period (baseline) and after HCA until 4 and half hours after the start of rewarming. During general anesthesia the EEG showed a burst-suppression pattern. Thus, the recovery of the EEG was measured by the EEG burst ratio. The burst ratio was calculated as the summation of burst lengths divided by the length of the recording.

4.6. Cardiopulmonary bypass

The heart and great vessels were exposed via a right thoracotomy in the fourth intercostal space, the right mammarian artery and azygos vein were ligated, and the homozygous vein was snared. The superior vena cava (SVC) was then mobilized and a membrane oxygenator (Midiflow® D 705, Dideco, Italy) was primed with 1 liter Ringer acetate and heparin (5000 IU) (Heparin LEO®, Lövens, Denmark). After heparinization (300 IU/kg), the ascending aorta was cannulated with a 18-French arterial cannula (Argyle®, Sherwood Medical, Belgium), and the right atrial appendage with a single 24-French atrial cannula

(DLP Inc, MI, USA). Non-pulsatile CPB was initiated at a flow rate of 100 ml/kg per minute and flow was subsequently adjusted to maintain a perfusion pressure of 50 mmHg. A 12-French intracardial sump cannula (DLP Inc, MI, USA) was positioned in the left ventricle for decompression of the left heart during CPB. A heat exchanger was used for core cooling. The pH was maintained using alpha-stat principles at 7.40 ± 0.05 with an arterial CO_2 tension of 3.5 to 4.0 kPa, uncorrected for temperature.

A cooling period of 45 minutes (study II), 50 minutes (study III) or 60 minutes (studies I and IV) was carried out to attain both rectal and esophageal temperatures at 20°C (study I) or rectal and epidural temperatures at 25°C (study II and III) or 20°C (study IV). Cardiac arrest was induced by injecting potassium chloride (1 mEq/kg) to the aortic cannula, and topical cardiac cooling was then begun and maintained throughout the aortic cross-clamp period. The ascending aorta was cross-clamped just proximal to the aortic cannula.

4.7. Experimental protocol

After cooling down to 20°C or 25°C depending on the protocol and cross clamping the aorta, the animals underwent an interval of HCA or RCP as dictated by the randomization protocol. The preparations for RCP involved inserting a 18-French cannula (Stockert Instrumente GmbH, Germany) into the SVC, advancing it as cranially as possible, tightening the rubber cord around the SVC and cannula and connecting it to the arterial line with a Y connector. The inferior vena cava (IVC) was not occluded. Retrograde flow was slowly increased and regulated to attain a SVC pressure of 20 mmHg. In the RCP groups, perfusate returning from the upper body to the ascending aorta was drained to the collecting chamber and returned to the pump once its volume had been measured. The amount of sequestered fluid was also measured (Studies I, II and III) (Fig. 10).
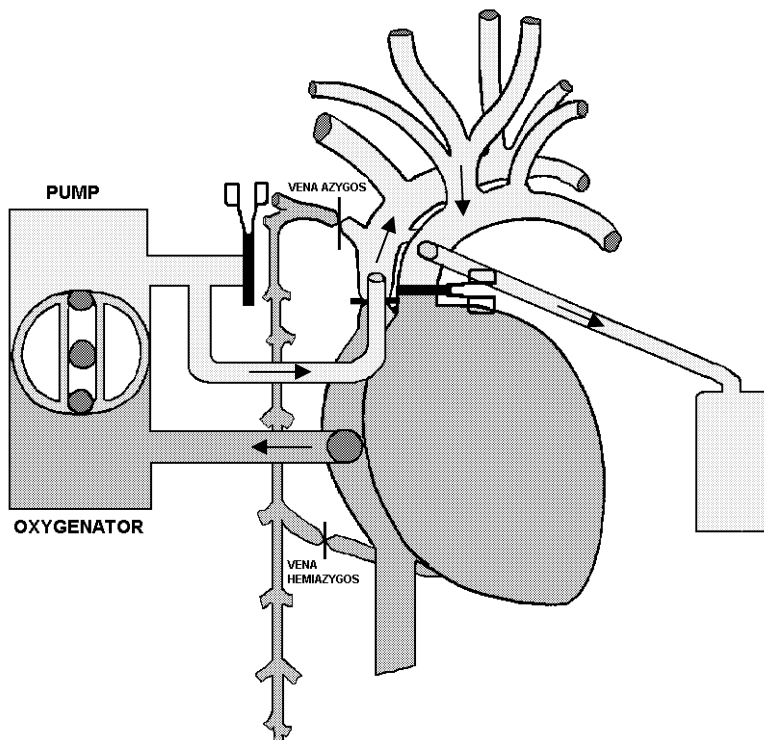
Figure 10. Experimental protocol of the RCP.

After intervention, rewarming was initiated, the SVC and the left ventricular cannulas were removed, and the snared hemiazygos vein was released. Weaning from CPB occurred approximately 60 minutes after the start of rewarming at a rectal temperature of 36.0 °C with the administration of furosemide (40 mg) (Furesis®, Orion, Finland), mannitol (15.0 g) (Mannitol® 150 mg/ml, Pharmacia & Upjohn, Belgium), methylprednisolone (80 mg) (Solu-Medrol®, Pharmacia & Upjohn, Belgium) and lidocaine (40 – 150 mg (Xylocain® 20 mg/ml, Astra, Sweden), depending on cardiac arrhythmias). Cardiac support was provided by dopamine (Abbodop®, Abbot, Italy) starting at a dose of 6.0 – 8.0 ml/min intravenous infusion; mean arterial pressure was kept approximately at 60 mmHg. Animals were kept under isoflurane (1.1 – 1.2%) anesthesia until the following morning, extubated, and moved into a recovery room. During anesthesia mean arterial pressure was kept over 60 mmHg with dopamine infusion and furosemide at a dose of 20 mg was administered twice and cumulative urine output was measured.

During the experiments hemodynamic and metabolic measurements were recorded at five different time points as follows: 1. At baseline, after the Swan-Ganz catheter was positioned, 2. At the end of cooling, at 25°C, immediately prior to institution of the

intervention, 3. During rewarming, at 30°C, 4. Two hours after the start of rewarming, 5. Four hours after the start of rewarming.

4.8. Postoperative evaluation

Postoperatively, all animals were evaluated daily utilizing a species-specific quantitative behavioral score (Juvonen *et al.* 1998a). The assessment quantified *mental status* (0 = comatose, 1 = stuporous, 2 = depressed, 3 = normal); appetite (0 = refuses liquids, 1 = refuses solids, 2 = decreased, 3 = normal), and *motor function* (0 = unable to stand, 1 = unable to walk, 2 = unsteady gait, 3 = normal). Numerical summing of these functions provides a final score: the maximum (score of 9) reflects apparently normal neurological function, while lower values indicating substantial neurologic damage. A score of 8 means that the animals were able to stand unassisted, and were likely to recover fully.

Each surviving animal was electively sacrificed by intravenous administration of thiopental (Pentothal Natrium®, Abbot, USA) overdose at day 7 after surgery. The entire brain was immediately harvested, weighed and stored for subsequent histological analysis.

4.9. Histopathological analysis

During autopsy the brain was excised immediately and the hemispheres were cut apart. One half was immersed in 10% neutral formalin and fixed there for one week en bloc. Thereafter 3-mm thick coronal samples were sliced from the frontal lobe, thalamus (including the adjacent cortex) and hippocampus (including the adjacent brain stem, and temporal cortex), and sagittal samples from the posterior brain stem (medulla oblongata and pons) and cerebellum. The pieces were fixed in fresh formalin for another week. After fixation, the samples were processed as follows: rinsing in water for 20 minutes, 2 hours immersion in 70% ethanol, 4 hours immersion in 94% ethanol and 9 hours in an absolute ethanol. Thereafter the pieces were kept one hour in absolute ethanol-xylene mixture, 4 hours in xylene and 6 hours for embedding in warm paraffin. The samples were sectioned at 6 μm and stained with haematoxylin eosin. The sections of the brain samples of each animal were screened by a single experienced senior pathologist, blinded both to the experimental design and to the identity and fate of the individual animals. Each section was carefully investigated for the presence or absence of any infarctive or other damage.

Visual estimation of the injuries in the sampled regions were made as follows: 0 = no morphological damage identified; 1 = edema and/or occasional dark neurons; 2 = numerous dark neurons (often also shrunk) and eosinophilic or dark/shrunk cerebellar Purkinje`s cells or haemorrhages, and 3 = clearly infarctive foci with neoformation of capillaries and the presence of macrophages and glia reaction.

To allow semiquantitative comparisons between the animals, a total histological score was calculated by adding the regional scores. A score of more than 4 means that the animal had a distinct brain injury.

4.10. Serum S-100β

Concentrations of serum S-100β were determined in mixed venous blood samples by using a luminescence immunoassay (Sangtec-100[®], LIA-mat) kit (Sangtec Medical AB, Bromma, Sweden). Serum S-100β protein levels were measured preoperatively, and 2, 4, 7 and 20 hours after the start of rewarming.

4.11. Other measurements

Systemic arterial and venous blood samples were obtained to determine pH, oxygen tension, carbon dioxide tension, oxygen saturation, oxygen content, hematocrit, hemoglobin and glucose (Ciba-Corning 288 Blood Gas System, Ciba-Corning Diagnostic Corp., MA, USA). Lactate was analyzed using a YSI 1500 (Yellow Springs Instrument Co, OH, USA). Hemodynamics, epidural, esophageal and rectal temperatures and respiratory gases were monitored by the Datex AS/3 anesthesia monitor (Datex Inc., Finland) throughout the study.

4.12. Statistical analysis

Summary statistics for continuous or ordinal variables are expressed as the median with the interquartile range (IQR, 25th and 75th percentile) or mean ± standard error of mean (SD). In figures values are shown as medians with IQR. The analysis were performed by analysis of variances for repeated measurements. Comparison between relevant time-points and the baseline (reference category) was performed by the paired samples t-test or Wilcoxon matched pairs signed rank test. Differences between groups were determined by the t-test or the Mann-Whitney U-test. Multiple comparison problem was controlled by the Bonferroni-method. Kendall`s (τ) correlation coefficient was used to determine correlation between histopathologic score and epidural temperature at the end of the HCA. Significance levels are reported for comparisons with $p < 0.05$. Analyses were performed using a standard, commercially available statistical program (SPSS 8.0, SPSS Inc, Ill., USA).

5. Results

5.1. Intermittent retrograde cerebral perfusion does not cause fluid sequestration during prolonged hypothermic circulatory arrest (I)

All animals were stable during surgical procedures, and 15 out of the 18 animals survived for 7 days after surgery and were electively sacrificed. At day 7, there were no significant differences in behavioral scores among the groups.

The major part of the blood perfused into the SVC during RCP was shunted through the low resistance venous bed, recirculating back to the right atrium. In the C-RCP group the median fluid sequestration volume was 145 (0 – 250) ml compared with –50 (-100 - 0) ml in the I-RCP group ($p = 0.04$).

The median histopathologic score tended to be lower in the HCA group (6.1) compared with the C-RCP (8.9) and the I-RCP groups (7.9), although these differences were not found to be statistically significant ($p = 0.2$ and $p = 0.2$).

5.2. Cold retrograde cerebral perfusion improves cerebral protection during moderate hypothermic circulatory arrest (II)

All animals were stable during the surgical procedures and survived to at least the first postoperative day. Seven out of the 12 animals survived 7 days after surgery and were electively sacrificed. In the RCP group 5/6 animals survived seven days compared with 2/6 in the HCA group ($p = 0.04$).

Complete behavioral recovery was seen in 4 out of 6 animals after RCP, compared with only 1 out of 6 in the HCA group. At day 7 there was a significant difference in the behavioral scores between the groups ($p = 0.04$).

Venous lactatemia increased significantly during cooling and especially after intervention in both groups and remained significantly greater in the HCA group. Oxygen extraction was significantly higher in the HCA group at the beginning of rewarming

(medians 4.85 vs. 3.30, $p = 0.001$). A similar difference, although not significant, was seen over the period of rewarming. Oxygen consumption did not differ significantly between the groups.

EEG bursts recovered quicker in the RCP group from time points 120 to 150 minutes after rewarming, but after this, a regression was seen at the time point 180 minutes after rewarming. No differences in burst ratios were found between the groups

The total histopathologic scores in the RCP group (3.5) tended to be lower than in the HCA group (5.0) although this difference was not found to be statistically significant.

5.3. A cranial hypothermia is an essential factor leading to improved outcome following retrograde cerebral perfusion (III)

Ten out of the 18 animals survived until 7 days after surgery and were electively killed. In the RCP(15°C) group, 4/6 animals survived for 7 days compared with 3/6 in both of the other two groups ($p > 0.2$).

Complete behavioral recovery was seen in 3/6 animals after RCP(15°C), compared with none in the other two groups. Of the animals who survived for 7 days, the median behavioral score was lower (5.0) in animals following RCP(15°) compared to RCP(25°) (7.0) or HCA (6.5) ($p = 0.02$ and $p = 0.03$).

EEG bursts were recovered significantly better in the RCP(15°C) group at 3 hours after the start of rewarming compared to HCA group ($p=0.05$), but a regression was seen at 3½ hours from the start of rewarming. After this bursts recovered similarly in both RCP groups, but slightly slower in the HCA group.

The most striking histopathological findings were tiny hemorrhages or extravasations, which were mostly located in the deeper axonal layer (white matter). Small infarctions were seen in the frontal cortex in three pigs following RCP(25°C) and one in the thalamus of an animal following HCA. The median of the histopathological score of the cerebellum was lower in the RCP(15°C) group (0.5) compared to the RCP(25°C) group (1.5) ($p = 0.01$).

The median of the total histopathologic score in the RCP(15°C) group (5.0) were significantly lower than in the group RCP(25°C) (7.0) ($p=0.04$). The median score in the RCP(15°C) group tended to be lower than in the HCA group (6.5) ($p=0.1$). The epidural temperature recorded at the end of the intervention was found to be positively correlated with the histopathologic score ($\tau = 0.27, p = 0.07$).

5.4. Lamotrigine improves cerebral outcome after hypothermic circulatory arrest (IV)

All animals were stable during the surgical procedures and survived to at least the first postoperative day. In the lamotrigine group 6 of the 8 animals survived seven days compared with 5 of the 8 animals in the placebo group ($p > 0.2$).

Complete behavioral recovery was seen in 5/8 after lamotrigine administration, compared with 1/8 in the placebo group ($p = 0.02$). Of the animals that survived for 7 days, the median behavioral score was higher in the lamotrigine group (8) compared with the controls (7) ($p = 0.02$).

The rate of EEG burst recovery was higher in the lamotrigine group, the median being 40% of the baseline compared with 17% in the placebo group at 4 hours ($p = 0.02$) and 80% compared with 20% at 4½ hours after the start of rewarming ($p = 0.01$).

The median of the total histopathological score in the lamotrigine group was 5.5 and in the placebo group 7.5 ($p = 0.06$). The median histopathological score in the hippocampus was lower in the lamotrigine group (0) than in the placebo group (0.5) although this difference was not statistically significant ($p = 0.09$).

An average 3-fold increase in S-100β levels in both groups compared with baseline was seen 2 hours after the start of rewarming ($p = 0.01$). The median S-100β concentration remained at a lower level in the lamotrigine group, and was statistically significant 20 hours after the start of rewarming (0.1 µg/L vs. 0.03 µg/L, $p = 0.01$).

6. Discussion

6.1. General discussion

RCP was adopted in aortic arch surgery before hard experimental data demonstrating its efficacy and safety was available. One reason for this may have been the fact that it is very difficult to study RCP in the laboratory, as there are various differences in the anatomy and physiology of the cerebral venous circulation among the species used in laboratory investigations (Griepp *et al.* 1997). The major question concerns the feasibility of generating an effective RCP over competent jugular valves (Watanabe *et al.* 1996). A study performed on monkeys indicated that less than 1% of blood returned to the aortic arch during RCP and more than 90% was circulating and shunting to the IVC (Boeckxstaens and Flameng 1995). This was supported by cadaver studies indicating that the internal jugular vein is valvulated by competent valves in the majority of human beings and that the valve-free azygos vein system is the major veno-venous collateral through which RCP flow reaches the central nervous system (de Brux *et al.* 1995, Dresser and McKinney 1987). Thus, it is possible that in humans an open IVC system would prevent or at least impair RCP flow to the brain. Competent jugular vein valves exist in under 20% in the pig and this model is therefore frequently used in RCP studies (Juvonen *et al.* 1998a).

The implementation of RCP was demonstrated to be a two-edge sword. With this chronic porcine model, effective RCP seems to require relatively high perfusion pressures in the laboratory, which are only obtainable through pressurization of the entire venous system. Using this method effective retrograde flow can be generated and significant removal of emboli can be achieved (Juvonen *et al.* 1998a). Clamping the IVC increases the risk of perfusion-induced cerebral injury, however, which is most likely a consequence of the development of cerebral edema (Juvonen *et al.* 1998a, Juvonen *et al.* 1998b). Even in the presence of deep hypothermia, retrograde flow is far too small to meet the metabolic demand of the brain (Griepp *et al.* 1997). It is possible, however, that the flow supplied by RCP may allow for the removal of some metabolites and toxins. It provides continued cooling during circulatory arrest, thereby delaying the development of severe acidosis in the ischemic brain.

Previous studies in the field of basic neuroscience have shed more light on the pathogenesis of ischemic brain injury, and the failure of neurotransmitter transport and

glutamate accumulation have been demonstrated to be toxic to neurons. Ischemic injury begins with depolarization of the presynaptic membrane. This depolarization is mediated by the metabolic failure caused by ischemia and by the voltage-sensitive Na^+-channels which carry electrical messages to the synapse. As a result of depolarization, there is an influx of Ca^{2+} via voltage-sensitive channels and a concomitant secretion of neurotransmitter (glutamate). In the normal environment, after release into the intercellular space, glutamate is normally rapidly removed from the synapse by an active transport mechanism to glial cells and neurons, ready to be utilized for the next message. Owing to a depletion of cellular energy during hypoxia and ischemia the high affinity of glutamate is compromised. This interrupts glutamate uptake and leads to its accumulation in the intercellular space, where it acts as a potent neurotoxic substance. It opens calcium channels, leading to an influx of calcium, which initiates the catastrophic intracellular activation of protease, lipase and kinase C, along with altered transcription and the release of free oxygen radicals. This eventually leads to neuronal autodigestion and cell death (Lipton and Rosenberg 1994). This knowledge has opened up new avenues of research to improve the current methods of protecting the brain during ischemia. If this biochemical cascade could be blocked by a suitable antagonist, the neurons might survive a period of depletion of oxygen and metabolites. The glutamate receptor blockers, and Ca^{2+}- and Na^+-channel antagonists are the antagonists under the most intense study. Despite promising results in experimental studies with various specific interventional agents, most of these have turned out to be neurotoxic in a clinical setting (Small and Buchan 1996).

Lamotrigine is used clinically as an antiepileptic drug. The proposed mechanism of this Na^+-channel blocker is the prevention of Na^+-dependent depolarization and subsequent neurotransmitter release (Small and Buchan 1996). Lamotrigine is rapidly absorbed after oral administration and the bioavailability of the oral formulation is about 98%.

6.2. Intermittent retrograde cerebral perfusion does not cause fluid sequestration during prolonged hypothermic circulatory arrest (I)

This study was undertaken to compare conventional continuous RCP, RCP with interrupted flow and HCA alone during a prolonged period of hypothermic circulatory arrest. The main purpose was to determine whether intermittently implemented RCP could decrease the rate of fluid sequestration and subsequent cerebral edema.

Although RCP has been demonstrated to improve cerebral protection and prolong the permissible period of HCA, it exposes the brain to perfusion-related injury, which is most likely a consequence of brain edema. The mechanism leading to brain edema after RCP is not clear. There are many possible explanations for this, such as brain ischemia, intracranial hypertension, inflammatory reaction, and reperfusion injury. It has been shown that cerebral ischemia itself causes edema (Klatzo 1979) and therefore ischemic cerebral injury after HCA may be further aggravated by edema. Edema impairs ischemia by causing local compression of the microcirculation and raising intracranial pressure

(Hossmann 1988). The high RCP driving pressure enhances the rate of precipitated cerebral edema and distinct edema was demonstrated to develop at a perfusion pressure level of 30 mmHg (Nojima *et al.* 1994). If the RCP pressure is kept below 25 mmHg, the risk of cerebral edema is reduced (Juvonen *et al.* 1998a). Therefore, the RCP pressure selected for use in the present study was 20 mmHg.

In previous studies, remarkable fluid sequestration has been associated with prolonged, 90 minutes continuous RCP, especially when the IVC has been occluded and the entire vascular bed is pressurized (Juvonen *et al.* 1998b). In the present study the IVC was not snared and the RCP pressure used was 20 mmHg line pressure, while that used in previous study was 20 mmHg as measured in the sagittal sinus (Juvonen *et al.* 1998b). However, the brain edema may also be related to the duration of prolonged continuous RCP and intermittently implemented RCP would therefore be better in this respect.

The selected fashion of the intermittent RCP, starting with 15 minutes arrest followed by 15 minutes RCP and repeated these with 15 minutes arrest in the end, can be simulated in a clinical situation with suturing anastomosis.

In the C-RCP group, 2 out of 6 animals died within the first 24 hours and the mean brain weight of these animals was significantly higher than in the animals which survived until elective sacrifice on day 7. This finding suggests that cerebral edema was more severe in the animals with early mortality. We must acknowledge, however, that the data from the two groups is not completely comparable as the control animals had a 7 day survival and therefore had time to get over any possible fluid retention. It must be born in mind that cerebral edema is almost constantly encountered following CPB (Taylor 1998).

This study demonstrates that the rate of fluid sequestration during RCP can be decreased if retrograde perfusion is given intermittently. In summary, this data suggest that if RCP is implemented intermittently the rate of cerebral edema can be decreased without compromising the potential benefits of this strategy.

6.3. Cold retrograde cerebral perfusion improves cerebral protection during moderate hypothermic circulatory arrest (II)

Good results have been reported using cold RCP during moderate hypothermia in the surgical treatment of the aortic arch (Imamaki *et al.* 1997, Lin *et al.* 1996). This method has been demonstrated to be safe for up 30 minutes of HCA (Moshkovitz *et al.* 1998). In this experimental study the efficacy of deep hypothermic (15°C) RCP for improved cerebral outcome during moderate (25°C) HCA was tested.

The major finding of this study was that cold RCP with a non-occluded IVC provides a distinctly better outcome compared to HCA with the head packed in ice at 25°C for 45 minutes. Behavioral assessment and survival were better in the RCP group, with an 83% survival rate in the RCP group compared with 33% in the HCA group. In terms of complete behavioral recovery, 67% recovered fully after RCP, whereas 17% recovered fully in the HCA group. This finding is attenuated by the fact that no statistically significant difference in histopathologic scoring between the groups could be demonstrated. One explanation for this might be that the high rate of early deaths in the

HCA group was related to the fact that clear morphological ischemic lesions did not have sufficient time to develop in these brains. The most striking finding in the HCA group was the severe cerebral edema in the animals which died on the first postoperative day. However, as discussed previously, cerebral edema exists almost constantly after an ischemic cerebral event and may be transient when the animal manages to survive over the first postoperative day.

While both groups had decreased pH levels after intervention, and two hours after the start of rewarming, the HCA group was significantly more acidotic. Venous lactate levels increased significantly during cooling and after intervention in both groups and these levels remained significantly higher in the HCA group. It has been demonstrated in an experimental study that cerebral serum lactate did not increase during RCP (Usui et al. 1994). Therefore, increased lactate levels in this study may indicate total body hypoxia during prolonged HCA. Lower lactate concentrations after rewarming in the RCP group may be due to better total body cooling and protection during cold RCP. In this study 95% of the cold RCP flow shunted through the venous bed back to the IVC and 5% drained from aortic cannula. The finding that lactate levels decreased to almost pre-ischemic levels during rewarming in this and in our subsequent studies is in agreement with the fact that an increased lactate concentration after ischemic event returns to the pre-ischemic levels in under 180 minutes (Frerichs et al. 1990). The lactate concentrations behaved similarly and were at the same level in all of our RCP studies.

The oxygen extraction rate was found to be higher in the HCA group during rewarming. We assume that this is a result of the at least minimal tissue oxygenation provided by RCP. On the other hand the cold RCP will also reduce tissue oxygen metabolism and the decreased oxygen extraction might be a result of a more effective cooling in this group. Previous studies have shown that RCP does not provide nutritive flow to the brain and that its most important benefit is its cooling effect (Usui et al. 1997) with a subsequent decrease in the metabolic rate.

There is a substantial amount of data suggesting that RCP related cerebral injury occurs during the reperfusion phase (Juvonen et al. 1998b). In that study, an almost complete recovery of brainstem evoked responses were seen shortly after the beginning of rewarming in animals who had undergone RCP, but this activity diminished over the following few hours. In terms of EEG recovery, a similar trend was seen in the present study. EEG activity recovered much faster following RCP compared with HCA, this difference being highest at two and a half hours after the start of rewarming. After that time point, however, a striking regression was seen in RCP group, a finding which emphasizes the previously set hypothesis that the RCP exposes the brain to reperfusion injury (Griepp et al. 1997). This phenomenon is most likely related to a high rate of fluid sequestration with a subsequent development of brain edema during and following RCP. This has also been documented by other investigators (Usui et al. 1994b, Yoshimura et al. 1995).

The results of this study suggest that cold continuous RCP during moderate HCA provides good cerebral protection and may negate the necessity to cool the entire body by CPB to deep hypothermia. The advantages of this technique are the shortened cooling and rewarming CPB times (Moshkovitz et al. 1998). Enhanced CPB time was in turn associated with an increased risk of stroke and increased mortality in a large clinical series of patients undergoing aortic arch surgery using RCP (Safi et al. 1997). This is of

particular importance in patients with long-lasting diabetes or hypertension who have an impaired autoregulation of cerebral blood flow, predisposing their brain to an embolic load during CPB. In addition, platelet dysfunction occurs during CPB and prolonged CPB time therefore increases the risk of bleeding complications (Woodman and Harker 1990). Deep hypothermia decreases the activity of the enzymes involved in platelet activation pathways and reduces the enzymatic activity of clotting factors upon coagulation activation. This ultimately leads to a retardation of fibrin/platelet clot generation. These phenomena are compounded by the presence of heparin, which may significantly contribute to a bleeding tendency (Wilde 1997).

6.4. Cranial hypothermia is an essential factor leading to improved outcome following retrograde cerebral perfusion (III)

As already discussed, the use of RCP is safe when flow rates and central venous pressures are maintained at relatively low levels. Our previous study suggested that the benefit of RCP in increasing the permissible period of HCA is most likely due to an improved cooling effect, even with the head packed in ice. In this continuation study we compared two temperatures of RCP (15°C and 25°C) with HCA at a systemic hypothermia of 25°C to determine whether the possible benefit of RCP may be due to an improved cooling effect.

The major finding of the present study was a distinctly better neurophysiological, neurological, and histopathological outcome in animals undergoing RCP(15°C) compared with RCP(25°C) and there tended to be a difference compared to HCA(25°C). The animals in the RCP(15°C) group had lower epidural temperatures during the intervention compared with both RCP(25°C) and HCA(25°C), which may have contributed to their improved outcome. The better temperature control must be the essential factor leading to improved outcome, as the animals undergoing RCP(25°C) did not have better outcome compared with HCA animals. Head packing in ice was not used in this study. These findings suggest that nutrient RCP flow was not generated in animals undergoing RCP(25°C) in the present experiments.

Total histopathological and cerebellum scores in the RCP(15°C) group were lower compared with the RCP(25°C) group. Brain infarctions were seen in three animals following RCP(25°C) and in one following HCA, but in none following RCP(15°C). It can be speculated, that there was no time for the development of the most severe infarctions in animals who died on the first day after surgery. The rates of early deaths did not differ between the groups and it is therefore difficult to believe that the central observation of this study might have been interpreted differently if histopathology were recorded on day seven in each animal.

The systemic temperature of 25°C at which the intervention was performed was selected as one of the demands addressed by RCP in the clinical setting is to shorten CPB time and thereby to avoid subsequent problems such as bleeding complications. At this temperature, the cerebral metabolism rate of oxygen has been shown to remain in both pig and humans at approximately 40% of baseline and the predicted safe interval of

circulatory arrest is 15 minutes (Ehrlich *et al.* 1998, McCullough *et al.* 1999). The poor outcome data in HCA animals seen in the present study indicates that the temperature and the length of intervention were appropriate.

The present EEG data supports the previous findings suggesting that RCP related cerebral injury will most likely occur during the reperfusion phase (Juvonen *et al.* 1998a, Juvonen *et al.* 1998b). We were able to repeat the EEG recovery pattern seen in our previous study (II). EEG activity normalized faster following cold RCP compared with both of the other two groups, the difference being highest three hours after the start of rewarming. After that time point, however, a striking regression was observed in animals who had undergone cold RCP, a finding emphasizing the previously set hypothesis that RCP exposes the brain to reperfusion injury (Griepp *et al.* 1997). We believe that this phenomenon is most likely related to the development of brain edema following RCP as also documented by other investigators (Nojima *et al.* 1994, Usui *et al.* 1994b, Yoshimura *et al.* 1995).

In conclusion, this study showed that the cold RCP at moderate systemic hypothermia seems to provide better neurologic outcome compared with moderate-temperature RCP, a finding suggesting that the enhanced cranial hypothermia is the major beneficial factor of RCP when careful attention is paid to its implementation. But despite reasonable experimental results, this method demands further development before it can be recommended for wide-spread clinical application.

6.5. Lamotrigine improves cerebral outcome after hypothermic circulatory arrest (IV)

Hypothermic circulatory arrest is a frequently used method of cerebral protection during operations on the aortic arch. Its main limitation is the time constraint. Selective antegrade and retrograde cerebral perfusions are other strategies used for brain protection, but unfortunately not all expectations addressed to these methods have been fulfilled, leading to renewed interest in search for other procedures to increase the permissible period of HCA.

Glutamate is believed to be the most important neurotransmitter involved in this excitotoxity. This knowledge has opened up new avenues of research to improve current methods to protect the brain during ischemia. If glutamate dependent biochemical cascade could be blocked by suitable antagonist, the neurons may survive a period of depletion of oxygen and metabolites. The most studied antagonist are the glutamate receptor blockers and Ca^{2+}- and Na^+-channel antagonists (Aoki *et al.* 1994, Baumgartner *et al.* 1999, Perez-Pinzon *et al.* 1997). Despite promising results in experimental studies with various specific interventional agents, most of these have turned out to be neurotoxic in a clinical setting, and trials have been stopped due to side effects, such as the sympatholytic effects of SNX-111, the nefrotoxicity of NBQX, and the hallucinogenic properties of MK-801 (Small and Buchan 1996). The reason for testing the Na^+-channel blocker lamotrigine in the present study was that it is a safely used clinically as an antiepileptic drug. Its proposed mechanism is the prevention of Na^+-

dependent depolarization and subsequent neurotransmitter release. Lamotrigine is demonstrated to decrease glutamate concentrations after ischemic events in experimental settings (Bacher and Zornow 1997, Shuaib *et al.* 1995).

Lamotrigine is rapidly absorbed and the bioavailability of the oral formula is about 98%. Peak serum levels are reached within three hours. The concentration of lamotrigine is the same in brain and plasma, its mean half-life being 23 to 37 hours (Ramsay *et al.* 1991). The present dose of lamotrigine (20 mg/kg) was higher than that recommended in antiepileptic medication, but this dose should be tolerated also by humans. The selected administration of lamotrigine was via an intravenous infusion, because it is more constant method to dose and time medicine in this kind of experiment. The timing for the drug administration was two hours before HCA.

The survival rate in the current study did not differ between the groups, being 75% in the lamotrigine group compared with 63% in the placebo group. In terms of behavioral assessment, however, a better outcome was seen in the lamotrigine group. Among the animals that survived for 7 days, the behavioral score was better in the lamotrigine group. The median score of 8 in the lamotrigine-treated animals indicates a mild disturbance or full recovery. Control animals showed more severe initial neurological impairment and recovery to a score of 8 was only seen in one animal of this group.

EEG bursts had recovered better in the lamotrigine group at four hours from the start of rewarming compared with the placebo group. Ischemic brain damage, surgical disconnection of cortical blocks and several anesthetics are known to produce burst suppression in EEG. The effects of anesthetics and brain damage are additive (Wennberg *et al.* 1997). In the present work, the additive effect of ischemic damage plus isoflurane-produced burst suppression proved to be a sensitive measure of recovery. The bursts are readily detectable and their duration can even be measured when EEG recordings are very low in amplitude as a result of hypothermia, close to the noise level of the recording system, when other quantification of EEG recordings is questionable. The fact that the burst suppression pattern became more severe with ischemic damage, and that EEG recovery was in line with other measures of brain damage suggests that it correctly reflects the neuroprotective effect of lamotrigine. It may also be capable of accurately pinpointing the time when lamotrigine-protected brains are recovering better than those in the placebo group. Previous studies using EEG in this series (II and III), have also suggested that a change in brain electrical activity approximately three hours after HCA correctly distinguishes between pigs who will suffer severe damage and those who will fare better. Whether this type of monitoring can be used in a clinical setting to identify patients in whom special measures should be carried out for brain protection remains to be seen.

Lamotrigine has an effect on glutamate receptors, and this neurotransmitter is involved in the generation of EEG. Furthermore, bursts and EEG seizure activity have similarities, and postictal suppression and anesthetic-induced suppression are related phenomena. As lamotrigine suppresses epileptic seizure activity, we hypothesize that it also suppresses bursts. Our control recording after drug administration, but before HCA, supported this view, although the difference was not statistically significant. We should expect the same after HCA during rewarming, but contrary results were obtained, *i. e.* less suppression in the lamotrigine group. Thus, if the effect on suppression had been as expected, and suppression was observed in the control recordings, the result should have been opposite

to those observed. It is therefore fair to assume that the EEG-result is due to the better cerebral protection than the effect of lamotrigine on the EEG generation mechanism alone. Nevertheless, the interactions of lamotrigine and isoflurane anesthetic is an interesting problem and should be further studied.

Serum S-100β levels increased significantly in response to ischemic brain insult shortly after the start of rewarming in both groups. The finding that S-100β concentrations decreased and were sustained at a lower level on day one after surgery in lamotrigine-treated animals is in line with other outcome data and supports the findings of better brain protection in these animals compared with controls.

Eleven (6 in the lamotrigine group and 5 in the placebo group) of the 16 animals survived for 7 days after surgery and were electively sacrificed. Three animals (1 lamotrigine and 2 placebo) died due to severe brain damage and were unable to resume spontaneous respiration after weaning from respirator. During the autopsy significant brain edema, especially in the cerebellum and brain stem was detected. One animal died to severe lung congestion and one due to acute myocardial infarction. This mortality rate was higher compared with the HCA group in our previous study (I) in which the same temperature and duration of HCA was used. This difference may be due to a more advanced brain temperature control by epidural temperature probe *i. e.* in the first study brains were cooler than we expected. However, there were no differences between these two studies in the histopathological scores and behavioral recovery.

In the present study, the total histopathological score tended to be lower in the lamotrigine group than in the placebo group. This difference was particularly visible in the hippocampus, where the structures important for memory are located. This anatomic region is known to be the most vulnerable with regards ischemic insult (Zola-Morgan *et al.* 1992). In a study on rats after cardiac arrest-induced global cerebral ischemia, lamotrigine has been demonstrated to reduce hippocampal CA1 cell injury to half that compared with controls (Crumrine *et al.* 1997).

In conclusion, the results suggest that lamotrigine improves cerebral protection during and following a prolonged period of HCA. The major finding was that pigs receiving lamotrigine prior to a 75-minute period of HCA at 20ºC had a better outcome than placebo-treated controls. The differences were seen in terms of neurophysiological, behavioral, S-100β, and histopathological data. The results confirm those of previous studies suggesting that lamotrigine has neuroprotective properties (Shuaib *et al.* 1995) and this study is the first to report a possible benefit of lamotrigine during HCA.

The good results in this experimental study raises the question of the clinical use of lamotrigine. It is clinically a reasonably well-tolerated antiepileptic drug, and its main adverse effect is skin eruption, which occurs in about 5% of treated patients, but in rare instances can be severe (Sachs *et al.* 1997). There are several published case reports of lamotrigine-induced severe skin reactions, such as toxic epidermal necrolysis and Stevens-Johnson syndrome (Chaffin and Davis 1997). These skin reactions appear to be related to the rate at which lamotrigine is introduced, which is why the recommended initial dose should be low (Wadelius *et al.* 1996). No intravenous form of lamotrigine is available. Consequently, the clinical use of lamotrigine in aortic arch surgery demands further studies.

7. Conclusions

Recalling the purpose of the present investigation in this chronic porcine model, the results can be summed up as follows:

1. The data suggest that the rate of cerebral edema can be decreased if RCP is implemented intermittently, without compromising the potential benefits of this strategy.
2. Cold RCP during moderate HCA seems to improve neurological outcome compared with moderate HCA alone.
3. Cold RCP at moderate systemic hypothermia seems to provide a better neurological outcome compared with moderate temperature RCP, a finding which suggests that enhanced cranial hypothermia is the major beneficial factor of RCP.
4. The Na^+-channel antagonist lamotrigine improves neurological outcome after a prolonged period of HCA.

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