Experimental porcine models of retinal ischemia

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2013

EXPERIMENTAL PORCINE MODELS OF RETINAL ISCHEMIA

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DOCTORAL DISSERTATION
by due permission of the Faculty of medicine, Lund University, Sweden.
To be defended at Segerfalksalen, Biomedical Centre, Sölvegatan 19, Lund, Sweden.
Date November 29, 2013 at 09.00

Faculty opponent
Professor Eyvind Rødahl, University of Bergen

Organization

LUND UNIVERSITY

Department of Ophthalmology

Clinical Sciences, Lund

Faculty of medicine at Lund University

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Document name

DOCTORAL DISSERTATION

Date of issue November 29, 2013

Sponsoring organization

Title and subtitle EXPERIMENTAL PORCINE MODELS OF RETINAL ISCHEMIA

Abstract

Retinal ischaemia resulting from e.g. diabetes, vein thrombosis or arterial occlusion, is one of the major causes of visual impairment and blindness. Although new methods of treatment are being developed, there is still a need for more effective pharmacological forms of treatment. The aim of this work was to develop an appropriate animal model of retinal ischaemia in which the intracellular signalling pathways involved in the development of retinal injury and neovascularization can be studied in the future.

The pig retina was used as it has a morphology and blood supply similar to those of humans. Two different approaches to inducing experimental retinal ischaemia were developed. In Study I, intraocular pressure was elevated, and in Study II, III and IV, an endovascular approach was used to access the retinal vasculature in order to achieve arterial occlusion. The eyes were analysed using indirect ophthalmoscopy, multifocal ERG with fundus imaging, fluorescence angiography and conventional angiography. The porcine model of pressure-induced retinal ischaemia resulted in multifocal ERG changes typical of retinal ischaemia although there may be a confounding problem of pressure-induced damage. Multifocal ERG may be a useful tool to evaluate retinal dysfunction after an ischaemic injury. The retinal circulation could be accessed by transfemoral endovascular catheterization, and the afferent arteries could be occluded using a balloon catheter for temporary occlusion, and a liquid embolic agent or coiling for permanent occlusion. The degree of ischaemia depended on the location of the occlusion. Occlusion of the proximal part of the ophthalmic artery caused little or no ischaemic effect, presumably due to collateral blood supply. Coiling of the distal parts of the ophthalmic artery caused more pronounced ischaemia. Angiographic evidence was found that blood supply to the pig retina may indeed be both ipsilateral and contralateral, due to an interconnecting artery between the eyes. Taken together, the results of this work represent a step in the development of experimental models of retinal ischaemia.

Key words

Classification system and/or index terms (if any)

Supplementary bibliographical information

Language English

ISSN and key title 1652-8220

ISBN 978-91-87651-23-6

Recipient's notes

Number of pages

Price

Security classification

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ISBN 978-91-87651-23-6
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2013

List of Publications

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

I. **Morén H**, Gesslein B, Andreasson S, Malmsjö M. "Multifocal electroretinogram for functional evaluation of retinal injury following ischemia-reperfusion in pigs", *Graefes Arch Clin Exp Ophthalmol*. 2010 May; 248(5):627-34.

II. **Morén H**, Undrén P, Gesslein B, Olivecrona GK, Andreasson S, Malmsjö M. "The porcine retinal vasculature accessed using an endovascular approach: a new experimental model for retinal ischemia", *Invest Ophthalmol Vis Sci*. 2009 Nov.; 50(11):5504-10.

III. **Morén H**, Gesslein B, Undrén P, Andreasson S, Malmsjö M. "Endovascular coiling of the ophthalmic artery in pigs to induce retinal ischemia", *Invest Ophthalmol Vis Sci*. 2011 Jul. 1;52(7):4880-5.

IV. **Morén H**, Gesslein B, Undrén P, Andreasson S, Malmsjö M. "Angiography and multifocal electroretinography show that blood supply to the pig retina may be both ipsilateral and contralateral", *Invest Ophthalmol Vis Sci*. 2013 Sep. 9; 54(9):6112-7.

Contents

Abbreviations 9

Introduction 11

- The anatomy of the eye and the retina 11
- Retinal ischaemia 14
- Current treatment of retinal ischaemia 16
  - Laser photocoagulation and cryotherapy 16
  - Pharmacological treatment 17
- Future pharmacological treatment modalities 20
- Animal models 21
  - Pig model of retinal ischaemia 22
- Experimental means of inducing retinal ischaemia 22
  - Elevation of IOP to induce retinal ischaemia 23
  - Retinal ischaemia induced by endovascular occlusion 23

Aims 25

Materials and methods 27

- Animal preparation and surgical procedure 27
- Induction of experimental retinal ischaemia by elevated IOP 29
- Fundus imaging and fluorescence angiography 29
- Induction of experimental retinal ischaemia using transfemoral endovascular catheterization 31
- Multifocal Electroretinography 34
- Statistical analysis 36

Results and discussion 37
Multifocal ERG for functional evaluation of retinal injury following ischaemia and reperfusion by elevating the IOP 37
Endovascular access to the porcine retinal vasculature 40
Occlusion of the retinal vasculature using a balloon catheter and a liquid embolic agent 41
Occlusion of the retinal vasculature using endovascular coiling 42
Cross midline collateral blood supply to the retina 45
Major conclusions and future outlook 49
Summary in Swedish - Populärvetenskaplig sammanfattning 51
Acknowledgements 55
References 57

Abbreviations

AMD	Age related macular degeneration
bFGF	Basic fibroblast growth factor
BRAO	Branch retinal artery occlusion
BRVO	Branch retinal vein occlusion
CNV	Choroidal neovascularization
CRAO	Central retinal artery occlusion
CRVO	Central retinal vein occlusion
ERG	Electroretinography
NVD	Neovascularization of the optic disc
NVE	Neovascularization elsewhere (in the retina)
NVI	Neovascularization of the iris
IOP	Intraocular pressure
RAO	Retinal artery occlusion
RPE	Retinal pigment epithelium
RVO	Retinal vein occlusion
VEGF	Vascular endothelial growth factor

Introduction

Retinal ischaemia is one of the major causes of visual impairment and blindness, and is most commonly caused by diabetes, vein thrombosis or arterial occlusion. As a result of ischaemia, neovascularization is induced by the release of growth factors. These newly formed blood vessels will be leaky and bleed, and will be unable to provide the blood flow required to ensure adequate oxygenation and nutrition of the retina. The ischemia may thus result in sight-threatening complications such as tractional retinal detachment, vitreous haemorrhage, neovascular glaucoma and macular oedema. It is therefore of the utmost importance to limit the extent of the ischaemic injury. Although new methods of treatment are being developed, there is still a need for more effective pharmacological forms of treatment. The aim of the thesis was to develop a large animal model of retinal ischaemia in which the intracellular signalling pathways involved in the development of retinal injury and neovascularization can be studied in the future. The field of research is described below, followed by the aims, the results and the conclusions of the thesis, in line with Paper I-IV and as agreed in my research group.

The anatomy of the eye and the retina

The cornea and lens of the eye focus light onto the retina (Figure 1). Light impinging on the photosensitive cells of the retina causes chemical changes that trigger nerve impulses to the brain. The retina in vertebrates has nine layers that originate from the inner wall of the primitive optic cup (Figure 1). A tenth layer, the pigment epithelium layer (RPE), lies just outside these layers, and is so closely associated with them in structure and function, that it can be considered part of the retina, although it has a different origin. The light-sensitive photoreceptors rods (night vision) and cones (sharpness, colour vision) are found in the outermost of the nine layers, and the light forming the image must therefore pass through the other eight layers before the neural signal can be generated. In the human retina sharp vision is generated in the macula, a central area with high cone density.

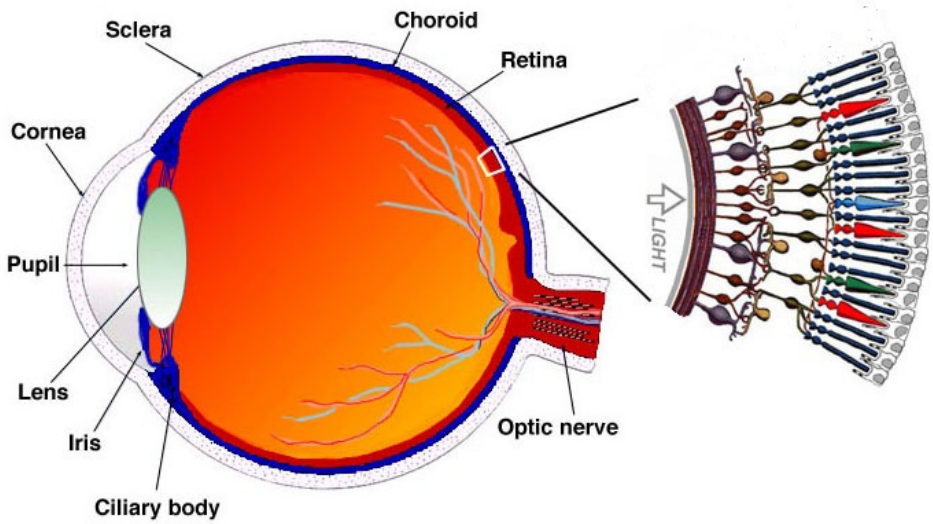

Figure 1.

Schematic cross sectional view of the human eye including a schematic drawing of the structure of the retinal cell layers. Adapted from Webvision, University of Utah.

The visual streak

The porcine eye has no macula as in the human eye. In a number of mammals, including the pig, cat, rabbit and horse, the equivalent of the macula is the visual streak. The visual streak is a horizontal region measuring approximately 2.5x15 mm situated slightly above the optic disc, parallel to the horizontal axis (Figure 2). The average ratio of rods/cones in the porcine retina is 8:1 (Gerke Jr *et al.*, 1995), compared to that of humans approx. 20:1 (Curcio *et al.*, 1990). The relatively high overall concentration of cones means that the pig retina is fairly similar to the human retina, although the human macula has a much higher peak concentration of cones than that found in the visual streak. Thus the visual acuity of the visual streak should be lower than that of the human macula. In the present work, multifocal electroretinography (mfERG), an electrophysiological method designed to measure localized cone function, was used to locate the visual streak in the porcine eye, and to investigate its response to ischaemia.

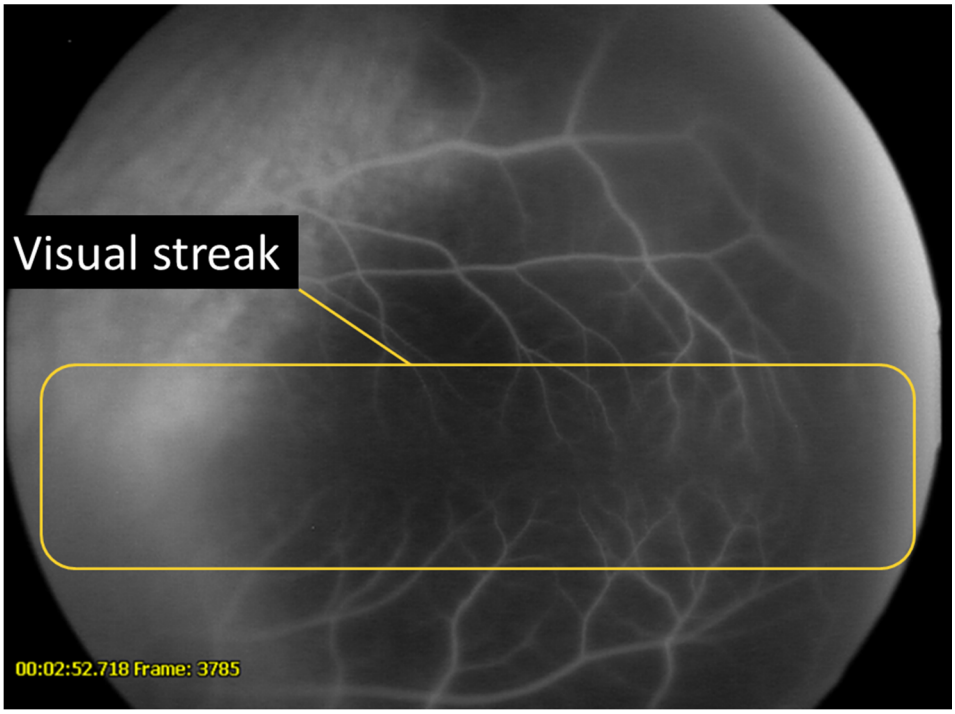

Figure 2.
Fluorescein angiography of the pig retina on which the visual streak area is marked

The retinal blood supply

In humans, the retinal artery branches from the ophthalmic artery and enters the optic nerve near the globe, close to the retinal vein. The retina has a dual blood supply. The photoreceptors, including their cell bodies in the outer retinal layers are nourished indirectly by the choroidal circulation. The inner retinal layers are nourished by branches of the central retinal artery. On leaving the optic disc the retinal arteries spread over the retina in a pattern unique to each individual. The main retinal vessels form two capillary plexuses: the superficial capillary network and the deep capillary network. The central 0.4mm of the macula, has a lower retinal thickness and is devoid of retinal vessels in order to permit optimal optical conditions - the foveal avascular zone. In the pig, the centre of the visual streak is devoid of major retinal vessels, in analogy with the foveal avascular zone in the human macula. Pigs have no central retinal artery at the nerve head. The porcine ophthalmic artery gives rise to the main ciliary artery, from which the retinal arteries derives. They may arise, as a single branch, that quickly divides, or as several branches. At the entry to the globe by the nerve head there are usually four retinal artery branches (Bloodworth et al., 1965). Apart from the course of

the retinal arteries the vasculature of the porcine retina closely resembles that of the human eye.

Retinal ischaemia

Retinal ischaemia ensues when the retinal circulation is insufficient to meet the metabolic demands of the retina. This is most commonly caused by local circulatory failure resulting from vascular disease. The major diagnostic entities are diabetes mellitus, retinal vein thrombosis and retinal artery occlusion. Ischaemia initiates a signalling cascade, which generates new blood vessels (neovascularization) in an attempt to restore the blood flow (Figure 3). The process of neovascularization is part of a physiologic process, angiogenesis, which controls the formation of new blood vessels from pre-existing vessels. Angiogenesis is an important process in wound healing, but is also involved in pathological processes such as tumour growth and neovascularization in the eye. The normal regulation of angiogenesis is controlled by a balance between factors that induce the formation of blood vessels (pro-angiogenic) and those that halt or inhibit the process (anti-angiogenic). In neovascular disease the balance is shifted and there is an excess of pro-angiogenic factors such as vascular endothelial growth factor (VEGF). In the pathological neovascularization of the eye, these newly formed blood vessels do not share the impermeability characteristics of normal retinal blood vessels. The proliferative vessels are prone to leakage and bleeding and will be unable to supply the necessary nutrients and oxygen. This may eventually lead to sight-threatening conditions such as vitreous haemorrhage, tractional retinal detachment, neovascular glaucoma and macular oedema (Dorrell et al., 2007; Osborne et al., 2004; Sapieha et al., 2010). A description of the most important diseases and conditions causing retinal ischaemia is given below.

Diabetic retinopathy

Diabetic retinopathy is one of the most debilitating disorders of the microvasculature of the retina, and one of the leading causes of vision loss worldwide. Hyperglycaemia causes microvascular complications in the retinal vessels resulting in diabetic retinopathy, but the exact biochemical pathways are not known. One of the major hallmarks of diabetic retinopathy is increased vascular permeability, which leads to haemorrhage and fluid accumulation in the macula, i.e. diabetic macular oedema. The ischemic retina may induce neovascularizations (proliferative retinopathy), causing vitreal hemorrhages and fibrovascular proliferations, which eventually may lead to tractional retinal detachments (Shah, 2008; Zhang et al., 2011). A review has recently be published by Kumar et al. (Kumar et al., 2012).

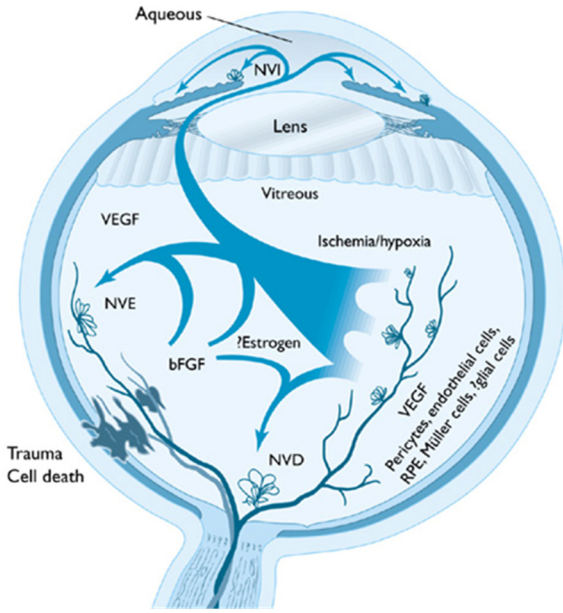

Figure 3.
Schematic illustration of the eye showing some of the growth factors involved in retinal ischaemia and neovascularization. Adapted from: Aiello L. Atlas of Clinical Endocrinology: Diabetes.

Retinal vein occlusion

Retinal vein occlusion (RVO) is the second most common retinal vascular disorder leading to visual impairment after diabetic retinopathy. The pathogenesis of RVO is multifactorial, with the same classic risk factors as those seen in other vascular diseases. A possible mechanism may be atherosclerosis, in which the adjacent retinal artery may compress the vein to the point of thrombus formation. RVO can be divided into two major categories, branch RVO (BRVO) and central RVO (CRVO), depending on the site of occlusion; BRVO being more common than CRVO. RVO usually causes retinal haemorrhage and often macular oedema. Depending on the degree of ischaemia, RVO may lead to neovascularization of the retina or the iris, the latter causing secondary glaucoma. In CRVO, the long-term incidence of neovascularization is about 15-20%, while in BRVO it is much less. For a review the reader is referred to Lattanzio et al. (Lattanzio et al., 2011).

Retinal artery occlusion

Retinal artery occlusion (RAO) is considerably less common than RVO. RAO is associated with atherosclerosis, embolic disease and often carotid occlusive disease. The entity is divided into central retinal artery occlusion (CRAO) and branch retinal artery

occlusion (BRAO), in analogy with CRVO and BRVO. RAO leads to acute ischaemia in the inner retina of the affected area. The risk of developing ocular neovascularization after CRAO is considerably lower than after CRVO.

Ocular ischaemic syndrome

Ocular ischaemic syndrome is a rare condition, caused by stenosis or occlusion of the common or internal carotid arteries, resulting in ocular hypoperfusion. The major cause of the changes in the carotid arteries is atherosclerosis. Ocular ischaemic syndrome may lead to neovascularization of the optic disc, the retina and iris. A review has recently been published by Terelak-Borys et al. (Terelak-Borys *et al.*, 2012).

Choroidal neovascularization in age-related macular degeneration

In contrast to the diagnoses of retinal ischaemia described above, in choroidal neovascularization (CNV) in age-related macular degeneration (AMD), or exudative AMD, the level of involvement and the role and origin of ischaemia in the pathogenesis is not known. However, CNV merits a description here as its treatment is similar to that of other kinds of neovascularization. CNV may occur in the context of AMD, after rupture of Bruch's membrane, a structure separating the choroid from the retina. The pathogenesis of CNV is multifactorial and not well understood. It is accompanied by upregulation of VEGF, but the exact cause of this upregulation is not clear. One component of the pathogenesis of CNV may be ischaemia of the overlying retinal pigment epithelial cells, due to either abnormalities in choroidal perfusion or the thickening of Bruch's membrane. VEGF upregulation is known to occur secondary to hypoxia, but may also be the result of oxidative stress, high glucose levels, protein kinase C activation, advanced glycation end products, activated oncogenes and a variety of cytokines.

Current treatment of retinal ischaemia

Laser photocoagulation and cryotherapy

One of the strategies adopted to minimize the risk of ischaemic injury is to take preventive measures by treating the patient's underlying disease. However, once ischaemic injury has been sustained by the retina, treatment is mainly focused on the secondary manifestations of ischaemia, i.e. neovascularization and macular oedema. These manifestations have previously mostly been treated by laser photocoagulation or cryotherapy. The main rationale behind these forms of treatment is destruction of the oxygen-consuming tissue in order to reduce hypoxia, thus balancing the oxygen

demand and delivery to the tissue. When treating ischemic areas of the retina this should theoretically also result in a lower number of ischemic cells able to contribute to the signalling cascade of ischemia. Clinical trials have demonstrated that laser photocoagulation can reduce the risk of visual loss by almost 50% in patients with proliferative diabetic retinopathy (ETDRS-Group, 1991). Convincing results have also been demonstrated in the treatment of retinal ischaemia secondary to circulatory failure such as central and branch venous thrombosis and artery embolization (Murdoch *et al.*, 1991).

The laser treatment method for neovascularizations involves treatment of regions of the peripheral retina with a pattern of laser effects applied to the ischemic retina, separated by the width of one burn (scatter treatment). The number of burns varies from 1000-3000 depending on the extent of treatment. Usually the entire midperipheral retina is treated (panretinal photocoagulation). The advantage of laser treatment is the good long-term effect, both in the prevention and treatment of neovascularization. The effect of treatment is permanent, although additional treatment may be needed. Side effects of this inherently destructive treatment include changes in peripheral visual field and decreased night vision. In rare cases, intense burns may cause CNV. The disease may progress leading to visual loss, despite timely and appropriate intervention.

Cryotherapy involves freezing of the peripheral retina with an external probe, resulting in destruction of the tissue, in a similar way to laser treatment. Advantages of cryotherapy include the possibility of treatment in cases of opacified media, and also the speed and efficiency of the treatment. However, cryotherapy is associated with several disadvantages: the effects are more confluent, leading to larger peripheral visual field defects, there is a risk of hypotension and the procedure is also more invasive. Cryotherapy has mainly been used to treat neovascular glaucoma in retinal occlusive disease, where a rapid effect is necessary and opacified media are common. For a recent review, the reader is referred to Kumar et al. (Kumar *et al.*, 2012).

Pharmacological treatment

Intense research has been devoted to identifying the ischaemia-stimulated signalling pathways in order to aid the development of pharmacological agents for the treatment of retinal ischaemia. The extent of retinal injury or neovascularization that follows an acute ischaemic insult may be reduced by pharmaceuticals that inhibit these cascades. A brief overview of the most important pharmaceuticals and the animal models that led to their development is given below.

Corticosteroids

Corticosteroids have traditionally been part of the treatment arsenal for diabetic macular oedema and diabetic retinopathy, due to their anti-inflammatory and anti-

angiogenic effects (Nauck *et al.*, 1997). However, their short duration has limited their use to an adjunct to panretinal photocoagulation. The primary method of administration of corticosteroids for the treatment of retinal disease is intravitreal injection. Intravitreal triamcinolone acetonide has been shown to improve diabetic macular oedema and AMD (Gopal *et al.*, 2007), although its use has not been uniformly spread. However, direct intravitreal injection of steroids is often associated with adverse effects, including cataracts and elevation of intraocular pressure (IOP). The treatment of diabetic macular oedema with triamcinolone acetonide is decreasing, due to the advent of anti-VEGF agents, which have considerably fewer side effects.

The lack of efficacy associated with long-term use and the adverse effects resulting from higher doses have resulted in the development of intravitreal steroid implants that release a small quantity of the drug over an extended period of time. Dexamethasone (Ozurdex®, Allergan) is an extended-release biodegradable dexamethasone intravitreal implant approved by the FDA in 2009 for the treatment of macular oedema secondary to RVO. Improvements in visual acuity and regression of macular oedema were reported in RVO patients participating in the GENEVA study (Haller *et al.*, 2010a). A phase II study on the performance of the implant for the treatment of diabetic macular oedema has shown good results (Haller *et al.*, 2010b), but the implant has not yet been approved for this condition.

Anti-VEGF treatment

VEGF has been shown to play a key role in ischaemia-induced neovascularization, and most advances in pharmaceutical treatment to date have been made in the use VEGF inhibitors (Zhang *et al.*, 2007). Anti-VEGF agents have revolutionized the treatment of angiogenesis in ocular disease since 2006, although all clinically used anti-VEGF agents share the disadvantage that they must be regularly injected intravitreally.

Bevacizumab

Bevacizumab (Avastin®, Genentech Inc.) is a complete full-length humanized antibody (149 kDa) that binds to all subtypes of VEGF. It was originally designed and used successfully in tumour therapy as a systemic drug (Ferrara et al., 2004). Due to the size of the molecule, penetration of the retina was believed to be low. Despite this, later studies demonstrated the effectiveness of intravitreal bevacizumab in the reduction of vascular permeability and fibrovascular proliferation in macular oedema secondary to central vein occlusion, retinal neovascularization secondary to proliferative diabetic retinopathy, and CNV secondary to AMD (Campa et al., 2011; Iturralde et al., 2006; Michels et al., 2005; Valiatti et al., 2011). Regression of iris and retinal neovascularization after intravitreal administration of bevacizumab has been reported previously (Avery et al., 2006; Mason et al., 2006). However, recurrence of neovascularization was noticed as early as two weeks after treatment, which is a major disadvantage compared to laser therapy. The BOLT study has shown that bevacizumab

is effective in the treatment of diabetic macular oedema (Rajendram et al., 2012). Thus, bevacizumab is now a clinical adjunct to laser treatment in patients with proliferative diabetic retinopathy, but does not constitute a substitute (Mirshahi et al., 2008; Schmidinger et al., 2011).

Ranibizumab

A further development of the bevacizumab molecule resulted in ranibizumab (Lucentis®, Genentech, Inc.). Ranibizumab is a recombinant humanized antibody fragment against VEGF-A, and was approved by the US Federal Drug Administration (FDA) for the treatment of exudative AMD in 2006 (Matsumiya *et al.*, 2011; Rosenfeld *et al.*, 2006); Ranibizumab has similar characteristics as bevacizumab but a smaller molecular weight (48kDa) and thus the penetration of the retina would theoretically be higher. The advent of anti-VEGF agents has revolutionized the treatment of exudative AMD, as no treatment was previously available. In 2010, ranibizumab was approved by the US FDA for the treatment of macular oedema resulting from RVO. The efficacy of ranibizumab in the treatment of macular oedema following BRVO and CRVO has been studied in two phase III clinical trials, BRAVO and CRUISE, respectively, showing good results (Brown *et al.*, 2010; Campochiaro *et al.*, 2010a). In 2012, ranibizumab was approved by the US FDA for the treatment of diabetic macular oedema. Two phase III clinical trials, RIDE and RISE, have shown ranibizumab to have good short-term effect in the treatment of diabetic macular oedema, although there are as yet no data on the long-term effects.

It was recently shown that bevacizumab and ranibizumab have comparable effects regarding the improvement of visual acuity and degree of oedema, although some safety concerns were raised (Martin *et al.*, 2012). Ranibizumab and bevacizumab are still widely used to treat the conditions described above, although newer anti-VEGF agents are becoming more common.

Aflibercept

The most recently approved anti-VEGF agent, aflibercept (VEGF Trap Eye - EYLEA®, Regeneron Pharmaceuticals, Inc. and Bayer HealthCare Pharmaceuticals) is being used increasingly. It is a recombinant fusion protein consisting of extracellular domains of the human VEGF receptors 1 and 2 fused to the tail region (Fc portion) of human immunoglobulin n1 that binds all forms of VEGF-A together with the related placental growth factor. It has been anticipated that the duration of the therapeutic effect will be longer due to its higher affinity for the VEGF molecule. It has been found in the VIEW 1 and VIEW 2 phase III clinical trials that an injection of aflibercept every two months provided the same effect as monthly injections of ranibizumab in the treatment of CNV (Heier *et al.*, 2012).

The future for VEGF inhibitors

Anti-VEGF agents are now being used successfully for the treatment of macular CNV secondary to AMD, macular oedema in diabetic retinopathy and central vein occlusion and secondary glaucoma in RVO. Promising results have been obtained in the treatment and prevention of neovascularization in diabetic retinopathy, although no anti-VEGF agent has yet been registered for this purpose. The major drawbacks of currently available anti-VEGF agents are the short duration of the therapeutic effect and the need for invasive drug delivery (intravitreal injections). Although rare, there is a risk of injection-related side effects such as retinal detachment, vitreous haemorrhage, and endophthalmitis. None of pharmacological agents has been shown to have the efficacy and duration of panretinal photocoagulation in preventing vision loss in the late stages of diabetic retinopathy. There is thus a need for pharmacological agents with a longer-lasting effect and non-invasive administration pathways, ideally a topically administered drug, with an effect that is at least comparable to those of current intravitreal drugs.

Future pharmacological treatment modalities

Multiple intracellular signalling cascades are activated by retinal ischaemia, and these pathways are currently being evaluated in experimental research in the search for effective pharmaceuticals. However some the most interesting candidates are found in area of angiogenesis. A number of new anti-angiogenetic agent prototypes are currently under development. Some of these are described below, with focus on the topically administrated candidates.

ATG-3 contains mecamylamine, an antagonist of the nicotinic acetylcholine receptor pathway, which mediates angiogenesis, and is administered as topical eye drops (Kiuchi *et al.*, 2008). It is the first kind of anti-angiogenic eye drops to be studied in humans. ATG-3 was evaluated in a phase II study for the treatment of diabetic macular oedema, showing promising results regarding tolerability and safety, but the study did not have sufficient power to evaluate effectiveness (Campochiaro *et al.*, 2010b).

Pazopanib is a tyrosine kinase inhibitor targeting VEGF-receptors 1 to 3, and also platelet-derived growth factor receptors (PDGFR), KIT and fibroblast growth factor receptor 1 (FGFR1). It has been shown to be effective in preclinical models of CNV (Takahashi *et al.*, 2009). The formulation is topical, and it has shown promising results in a phase II trial on CNV (Zhang *et al.*, 2012).

Several forms of treatment, shown to be theoretically feasible and efficacious in animal models, have, however, failed to demonstrate clinical significance in human testing (Comer *et al.*, 2004). There is, thus, evidence that pharmacological agents can be used

to treat neovascularization in the clinical setting. However, the duration of their efficiency is limited and frequent treatment may be required. They also have a limited effect on the more established vasculature observed in the later stages of disease. Therefore, more knowledge is needed to facilitate the development of effective pharmacological agents for treating retinal ischaemia.

Animal models

Extensive experimental research has been performed to examine the effects of pharmacological agents on the development of neovascularization. All the current and possible future treatment options mentioned above are designed to treat the indirect effects of retinal ischaemia. The objective should be to target the effects of retinal ischaemia further upstream in the signal transduction cascade. In order to do this an animal model that is similar to the human is needed. However, most of this research has been performed on small rodents and rabbits (Osborne *et al.*, 2004). These animals have a limited retinal vasculature, which is an important component of study in retinal ischaemia. The research on which the development of the anti-VEGF agents was largely carried out in a murine model and in the further development various animal models have been used: In the preclinical development of ranibizumab, the animal models used were mainly primates (Gaudreault et al., 2005; Husain et al., 2005; Kim et al., 2006). In the development of aflibercept, mice were mainly used (Saishin et al., 2003), and to a lesser extent, rabbits (Christoforidis et al., 2012). The preliminary animal studies on intravitreal dexamethasone implants were mostly performed on rabbits (Fialho et al., 2006; Ghosn et al., 2011; Morita et al., 1998). Later studies on the pharmacokinetics and pharmacodynamics of the registered implant were performed on monkeys (Chang-Lin et al., 2011a) and on rabbits (Chang-Lin et al., 2011b). Porcine models have been used rarely in previous experimental eye research, but have become more frequently used in recent years. Ranibizumab has mostly been tested *in vitro* on cell cultures from pigs (Miura et al., 2010). Bevacizumab has been studied *in vivo* in a porcine model (Iandiev et al., 2011; Lassota et al., 2010) and also in a rat model (Lu et al., 2009).

The studies of the present thesis were aimed to develop models of retinal ischaemia in pigs. The pig has an extensive retinal vasculature, comparable to that in humans. Clearly, the ability to extrapolate data from an animal model to the clinical situation requires an experimental model that closely resembles retinal ischaemia in humans. The clinical relevance of data obtained in the laboratory depends on the nature of extrapolation. If an experimental model of retinal ischaemia can replicate human pathology, and pharmacological treatment is seen to ameliorate the induced pathology, then it is a logical assumption that such treatment may be effective in the clinical setting, and thus merits further investigation. There are also limitations on the use of

pigs in experimental studies on ocular pathology. Pigs are large animals where the animal management and the experimental equipment, are expensive. It is also expensive and time-consuming to collect data from many animals for statistical comparisons. On the other hand due to the high similarity to human conditions, the development of an experimental porcine model for retinal ischaemia may prove to be of high validity and beneficial for future research.

Pig model of retinal ischaemia

The aim of the studies described in this thesis was to develop a pig model of retinal ischaemia. Pigs have similar retinal anatomy to humans. The porcine eye appears to have a typical primate-like architecture and is similar to the human eye regarding both size and retinal blood supply (Rootman, 1971). The pig has also proven to be a suitable animal for experimental analysis of the retina and retinal arteries (Buckley *et al.*, 1997). It has been suggested that the pig retina is suitable as a model of pathology based on its similarity with the human retina, as exemplified by the pattern of retinal ganglion cell death induced in the pig retina by episcleral vein cauterization, resembling that found in human glaucoma (Ruiz-Ederra *et al.*, 2005). The pig retina is large, and has a high cone density, including two types of cones, and has therefore been suggested to provide an excellent source for the biochemical analysis of cone proteins, and for *in vitro* approaches to understanding cone survival factors (Hendrickson *et al.*, 2002). A model of CNV in the pig has been tested with promising results (Lassota, 2008). The pig may, therefore, be a good animal model for testing various forms of ophthalmological treatment. If clear-cut retinal ischaemia can be created in the pig, without other confounding factors, this may provide an experimental model that closely resembles the pathology in humans.

Experimental means of inducing retinal ischaemia

Vascular ligation is a common method of inducing retinal ischaemia, which is achieved, in its simplest form, by placing a suture around the optic nerve bundle (Stefansson *et al.*, 1988). This occludes the blood flow, elevates the IOP (due to pressure on the globe), and constricts the optic nerves, which damages the axons (Osborne *et al.*, 2004). The posterior ciliary vessels can be ligated independently of the optic nerve in rats, although this is technically more demanding. This produces features similar to that seen after CRAO, but also causes uveal ischaemia. Partial ischaemia can be achieved by ligating more proximal arteries in the neck; the degree of ischaemia depending on the number of vessels ligated (Spertus *et al.*, 1984; Stevens *et al.*, 2002; Takamatsu *et al.*, 1984; Wakita *et al.*, 2002). This intervention may mimic carotid insufficiency, but

optic nerve ischaemia and cerebral infarction are also produced (Wakita *et al.*, 2002). Retinal vessels can be occluded by intravenous injection of rose bengal, a photosensitive dye, followed by intense retinal illumination, resulting in thrombus formation in the retinal vessels that can mimic BRAO (Daugeliene *et al.*, 2000; Mosinger *et al.*, 1991; Romano *et al.*, 1993), although this may result in secondary retinal injury due to phototoxicity. BRAO may also be produced by laser photocoagulation or transvitreal diathermia (Donati *et al.*, 2008; Noergaard *et al.*, 2008), but these interventions may have direct effects on the retina such as rupture of Bruch's membrane. Taken together, many of the experimental animal models commonly used for retinal ischaemia have limitations (Osborne *et al.*, 2004).

Elevation of IOP to induce retinal ischaemia

The induction of retinal ischaemia by elevating the IOP is used frequently in animal models of retinal ischeamia and has been described in a number of species including rats and rabbits. High IOP produces global ischaemia, with obstruction of both the retinal and uveal circulation, leading to whitening of the fundus and iris pallor. Ischaemia induced by elevating the intraocular pressure is known to produce ischaemia identical to that seen in central retinal artery occlusion (Buchi *et al.*, 1991; Osborne *et al.*, 2004; Smith *et al.*, 1952). In the first study described in this thesis (Paper I), retinal ischaemia was induced in pigs by elevated IOP. It was later realized that this method suffers from a drawback, namely that retinal injury may result from both ischaemia and pressure.

Retinal ischaemia induced by endovascular occlusion

In the present thesis it was hypothesized that one way of inducing clear-cut experimental model of retinal ischaemia may be occlusion of the retinal circulation by transfemoral endovascular catheterization. The cerebrovascular anatomy of domestic pigs differs from that in humans in that pigs do not have an internal carotid artery (Feng et al., 2005). The common carotid artery supplies the ascending pharyngeal artery, through a small side branch, which gives rise to the rete mirabilis at the cranial base, which is a network of fine arterioles resembling the gross morphology of a high-flow vascular malformation (Dondelinger *et al.*, 1998). The rete mirabilis then converges to form the intracranial carotid artery, which provides the major cerebral blood supply. The rete mirabilis cannot be accessed by an angiographic catheter (Dondelinger *et al.*, 1998; Reinert *et al.*, 2005), which has led to disappointment that the carotid circulation of the pig is not suitable for research in the field of cerebral infarction (Burbridge *et al.*, 2004). On the other hand, the pig has an extensive external carotid system that can be catheterized (Behrens-Baumann *et al.*, 1992; Scheurer *et al.*, 1992; Schroer *et al.*, 1992). In 1992, Scheurer et al. were successful in catheterizing the

external maxillary artery of the pig and injecting microparticles before the branching of the ophthalmic artery. However, since the injections were performed in the maxillary artery, which is a large artery supplying major parts of the head, ischaemia was presumably produced not only in the retina. Thus the ophtalmic artery in the pig is accessible for catheterization since it branches off the maxillary artery which derives from the external carotid circulation.

The aim of the studies presented in Papers II, III and IV was to explore the retinal circulation in the pig using a catheter-based transfemoral endovascular approach, and to investigate the possibility of inducing experimental retinal ischaemia using this technique. Occlusion techniques, including a balloon catheter, liquid embolic agent and coiling, were explored.

The incidence of ocular neovascularization after CRAO is considerably lower than in other vascular diseases such as ocular ischaemic syndrome (Jacobs et al., 1992). There is also little evidence that neovascularization in general is a result of CRAO per se, but may rather be correlated to the status of the blood vessels and general risk factors for vascular disease (Hayreh et al., 1982). After total CRAO without reperfusion, resulting in complete ischaemia of the inner retina, the rate of neovascularization should be lower, as most of the oxygen-demanding tissue has been permanently destroyed (Henkind et al., 1974). Therefore, an animal model with a high degree of ischaemia is not desirable if the objective is to create and study neovascularization. The objective should thus be to avoid total ischaemia, and instead to produce sufficient partial ischaemia to allow neovascularization. It was hypothesized that the endovascular occlusion in the pig model developed in this work would lead to a reduction in afferent blood flow to the retina, similar to that in ocular ischaemic syndrome, where the blood flow in the carotid arteries is impeded, but with a higher specificity of localization. If an experimental animal model can be developed that simulates human retinal ischaemia and neovascularization, this would allow studies of the effects of various kinds of pharmacological treatment, which may provide insight into which forms of treatment are likely to be effective in the clinic.

Aims

The general objective of the work presented in this thesis was to develop a porcine model of retinal ischaemia. Hopefully this may allow future studies of the signal transduction pathways involved in the development of retinal ischaemic injury and neovascularization.

The specific aims were:

- to confirm that multifocal ERG can be used to analyse the local functional response of the pig retina after inducing ischaemia,
- to evaluate pressure-induced retinal ischaemia as an experimental model of retinal ischaemia,
- to investigate whether the retinal vasculature can be accessed by a transfemoral endovascular approach aided by neuroradiological imaging,
- to explore the possibility of using this endovascular approach to create occlusions that would result in experimental retinal ischaemia,
- to evaluate occlusion of the vasculature by different techniques: a balloon catheter, a liquid embolic agent and coiling,
- to investigate whether the degree of retinal ischaemia can be determined by the location of occlusion, i.e. distal or proximal in the afferent vasculature of the eye,
- to confirm the hypothesis that there is collateral blood flow between the eyes, and that one ophthalmic artery can supply both the ipsi- and contralateral retina with blood via an interconnecting blood vessel, and
- to study the effects of such an interconnecting vessel on occlusion and the resulting retinal ischaemia, and to determine whether such findings can explain interindividual variations in the degree of ischaemia resulting from vascular occlusion.

Materials and methods

Animal preparation and surgical procedure

All studies were approved by the Ethics Committee at Lund University, Sweden. In total, 31 pigs were used in the four studies described in this thesis. The animals were under full anaesthesia during the entire duration of the experiments. Detailed descriptions of the anaesthesia can be found in the respective papers.

Both eyes in each pig were dilated with topical cyclopentolate hydrochloride (Cyclogyl 1%; Alcon Laboratories, Inc., Fort Worth, TX, USA) before the experiments were started. One eye in each pig was then subjected to elevated IOP (Paper I) or endovascular occlusion via transfemoral catheterization (Papers II, III and IV) in order to induce retinal ischaemia. The other eye served as an internal control. The eyes were examined using different methods.

- Indirect ophthalmoscopy and fundus examination for macroscopic evaluation of the retina. Blanching of the retinal arteries and a pale retina were considered indicative of ischaemia.
- Fundus imaging and fluorescence angiography were performed by our group previously to validate the technique of elevated IOP to produce retinal ischaemia.
- Multifocal ERG to evaluate the retinal function.
- The eyes that had undergone occlusion by liquid embolic agent were dissected to evaluate the position of the agent.
- Transfemoral endovascular catheterization and angiography.

Thesis at a glance

Study	Purpose	Methods	Number of pigs	Conclusions
I	To confirm the suitability of multifocal ERG for the analysis of the local functional response of the retina after ischaemia and reperfusion in the porcine eye	Retinal ischaemia induced by elevated IOP Analysis: -Fundus examination multifocal ERG -Fundus imaging and fluorescein angiography**	Total: 13 pigs* One ischaemic eye and one control eye in each pig. -Seven pigs with complete ischaemia -Six pigs with partial ischaemia	Multifocal ERG can be used to evaluate the retinal function following retinal ischaemia and reperfusion in pigs
II	To determine if the retinal circulation in the pig can be accessed using transfemoral endo-vascular catheterization and to explore the possibility of creating endovascular occlusions that result in experimental retinal ischaemia	Transfemoral endo-vascular catheterization and angiography Retinal ischaemia induced by endovascular arterial occlusion using: A balloon catheter Liquid embolic agent Analysis: -Fundus examination -Dissection of the eyes treated with liquid embolic agent -Multifocal ERG	Total: 6 pigs - two pigs with balloon catheter proximally - partial ischaemia - two pigs with liquid embolic agent proximally -partial ischaemia - two pigs with liquid embolic agent distally - complete ischaemia	Endovascular access to the retinal circulation may be a useful way of inducing retinal ischaemia. Temporary occlusion could be achieved with a balloon catheter and permanent occlusion with a liquid embolic agent. Distal occlusion of the ophthalmic artery produced more retinal ischaemia than proximal occlusion.
III	To determine whether endovascular coiling could be used to induce different degrees of experimental ischaemia, depending on the location of the occlusion	Transfemoral endo-vascular catheterization and angiography Retinal ischaemia induced by endovascular arterial occlusion using coils Analysis: -Fundus examination -Multifocal ERG	Total: 12 pigs*** One ischaemic eye and one control eye in each pig. -Six pigs with proximal occlusion -partial ischaemia -Six pigs with distal occlusion -partial ischaemia	Coils allow high precision in arterial occlusion. Confirmation that distal occlusion of the ophthalmic artery produced more retinal ischaemia than proximal occlusion.
IV	To explore the collateral vasculature of the porcine eye	Transfemoral endo-vascular catheterization and angiography Retinal ischaemia induced by endovascular arterial occlusion using coils Analysis: -Fundus examination -Multifocal ERG	Total: 6 pigs*** -two pigs with angiographic evidence of cross midline collateral vessels -four pigs with probable collateral vessels	Angiographic evidence of communication between the vascular systems of the eyes, i.e., cross midline collateral circulation.

*The retinas from these pigs were analysed with regard to intracellular signalling systems and these results are presented in separate publications. (Gesslein et al., 2010a; Gesslein et al., 2010b; Hakansson et al., 2010)

**Fundus imaging and fluorescence angiography were performed by our group previously to validate the technique of elevated IOP to produce retinal ischaemia (Gesslein *et al.*, 2010b).

***The pigs used in Studies III and IV were partly overlapping: the results from six of the pigs in Study III were analysed for the purpose of Study IV. I have had an active part in these experiments.

Induction of experimental retinal ischaemia by elevated IOP

Retinal ischaemia was induced by elevating the IOP (Paper I). The posterior chamber of each eye was cannulated with a 30-gauge needle. The IOP was increased to 80 mmHg in one eye. At this IOP level, the retinal blood flow was completely inhibited, as verified using a Tono-Pen®XL tonometer. Blanching of the retinal arteries was noted by ophthalmologic inspection. The other eye underwent the same surgical procedure without elevation of the IOP, and served as a control. The cannulation needle was removed 60 minutes later to allow reperfusion of the retinal vasculature, as one hour of occlusion has previously been found to cause persistent ischaemic damage to the retina in animal models (Osborne et al., 2004). Multifocal ERG was performed before the induction of ischaemia, and after 1 and 5 hours of reperfusion.

At the time when the experiments were performed there was no previous publication describing an IOP induced ischemia model in the pig with multifocal ERG evaluation but a similar set-up was developed simultaneously by another group (Kyhn et al., 2009).

Fundus imaging and fluorescence angiography

In a previous study by our group, including me, fundus imaging and fluorescein angiography were used to observe the structures of the retinal vasculature and the effects of elevated IOP (Gesslein *et al.*, 2010b). The IOP was gradually elevated from the normal level of 10-20 mmHg to 40, 60, 70 and 80 mmHg, and the fundus was imaged at each pressure. When the IOP was lowered from 80 mmHg to normal levels after ischaemia, fundus imaging showed that the retinal blood vessels were thinner at an IOP of 60 mmHg than at normal IOP, while at 80 mmHg there was no visible blood flow through the retinal blood vessels, indicating that a pressure of 80 mmHg is sufficient to prevent the perfusion of blood to the retina (Figure 4). It has been shown previously that this pressure is sufficient to induce ischaemia in large animal models (Osborne *et al.*, 2004).

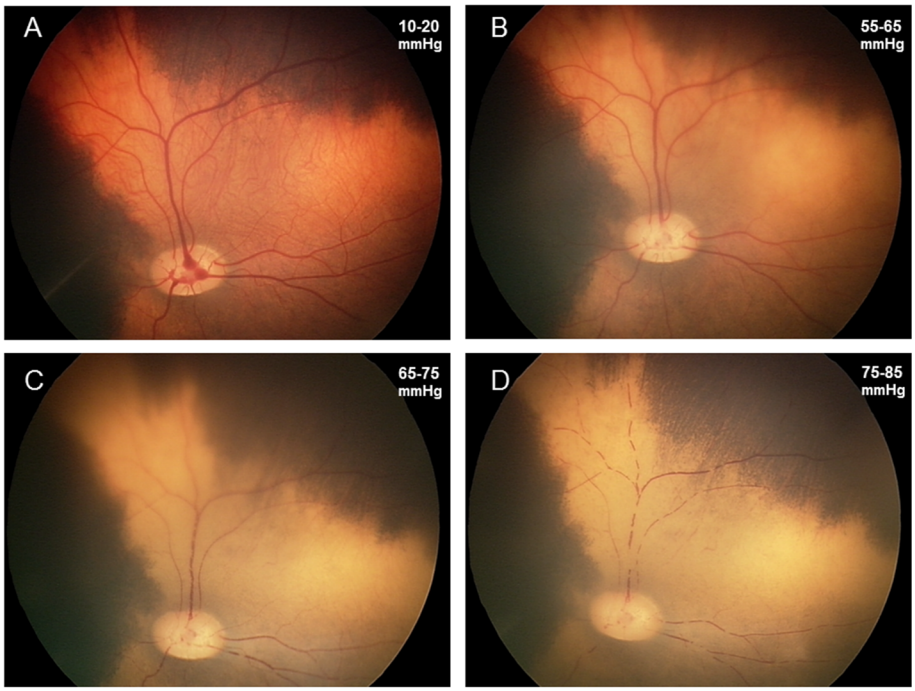

Figure 4.
Fundus images from a porcine eye subjected to gradually increasing IOP. At normal pressure (10-20 mmHg) the fundus was normal with filled blood vessels. The filling of the blood vessels decreased and the fundus became paler as the IOP was increased. At an IOP of 80 mmHg, blood flow was completely inhibited as evidenced by segmented blood in the vessels, verifying this method of elevated IOP to occlude the retinal vasculature. Figure adapted from (Gesslein et al., 2010b).

The reperfusion of the blood vessels was monitored with fluorescein angiography, which is a technique for visualizing the blood circulation in the retina using a dye. This involves the injection of the dye fluorescein into the systemic circulation, and then photographing the fluorescence emitted after illumination of the retina (Figure 5). Fluorescein was injected when the IOP was high (80 mmHg) and the fundus was imaged as the IOP was allowed to normalize. The retinal angiographic images and the colour fundus images were acquired using a RetCam®3 fundus camera (Clarity Medical Systems Inc., Pleasanton, CA, USA). During reperfusion the infusion-arterial time was considerably prolonged and time to full venous phase was approx. 7 minutes. The mobilization of microemboli in the retinal veins could be observed.

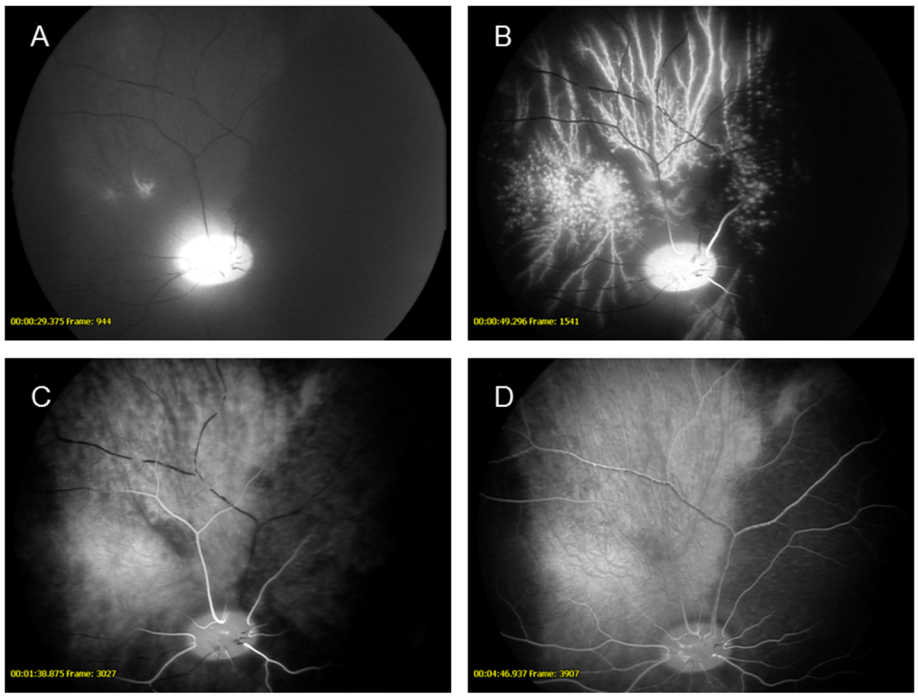

Figure 5.
Fundus images obtained during fluorescein angiography immediately after reperfusion of a retina exposed to high IOP (80 mmHg), showing reperfusion of the retina and choroid. The filling latencies of the angiograms were substantially prolonged. (A) The IOP is reduced to normal, but retinal blood flow is still completely inhibited; hyperfluorescence of the optic disc. (B) Filling of the large blood vessel of the choroidal circulation, but not the choriocapillaris. (C) Early arterial phase; distinct pulsation of retinal arteries, perfusion of the choriocapillaris; (D) arteriovenous phase; slow flow of microemboli in retinal veins. Similar results have been presented in a former study (Gesslein et al., 2010b).

Induction of experimental retinal ischaemia using transfemoral endovascular catheterization

The porcine model of pressure-induced retinal ischaemia resulted in multifocal ERG changes typical of retinal ischaemia. However, there was a possible confounding problem of pressure-induced damage. Therefore, retina ischemia was induced by arterial occlusion using transfemoral endovascular catheterization. An endovascular catheter is commonly used in neuroradiology to perform image-guided procedures to diagnose and treat diseases of the central nervous system. Similar endovascular

procedures, employing neuroradiological imaging, were used to investigate the retinal vasculature and to induce experimental retinal ischaemia (Papers II-IV). A vascular sheath was inserted into the right femoral artery, using an open or percutaneous approach. An angiographic catheter was then inserted via the external carotid artery into the maxillary artery using fluoroscopic guidance. Cerebral angiography of the external carotid system was performed, and sagittal and coronal road map images were obtained to guide the procedure. The ophthalmic artery was then catheterized. Endovascular arterial occlusion was induced in the ophthalmic artery or the main ciliary arteries using a balloon catheter, a liquid embolic agent or coiling (Figure 6). Angiography at different locations in the carotid system, i.e. the carotid artery, the maxillary artery and the ophthalmic artery, revealed the anatomical structures and the effects of arterial occlusion. For more detailed descriptions of the procedures, the reader is referred to Papers II-IV.

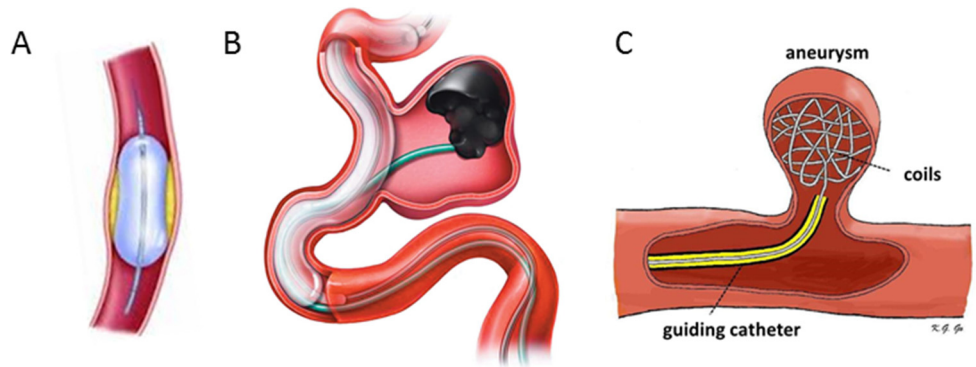

Figure 6
(A) Clinical use of balloon catheter for the treatment of vascular stenosis. Adapted from www.themedical-dictionary.thefreedictionary.com.(B) Liquid embolic agent used to fill an aneurysm in clinic use. Adapted from Covidien, MA, USA. (C) Coiling of an aneurysm. Adapted from K.G.Go, Department of Neurosurgery, University Hospital Groningen, the Netherlands

Balloon catheter

A balloon catheter is clinically used to dilate vessels exhibiting vascular stenosis. In Paper II, a balloon catheter was used to temporarily block the afferent blood flow to the retina in order to induce experimental retinal ischaemia. Occlusion of the main ciliary artery was achieved by inflating the balloon in the ophthalmic artery at the branching of the main ciliary artery.

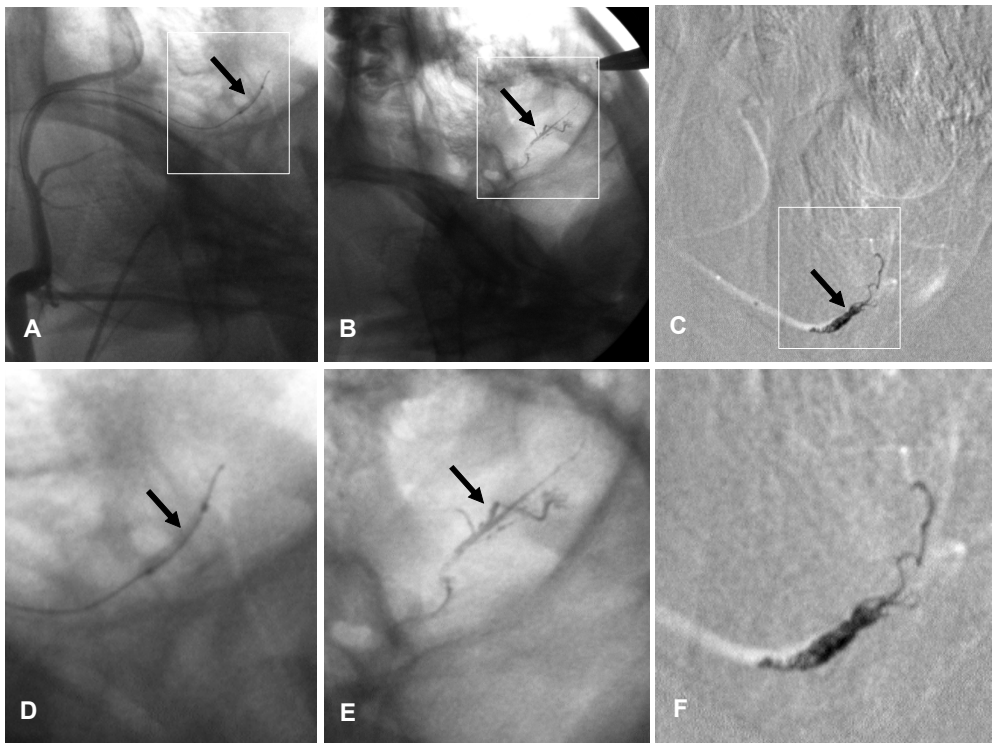

Figure 7.
Panel A shows an angiogram during balloon occlusion of the ophthalmic artery (arrow). Panel B shows an angiogram during liquid embolic agent occlusion of the main ciliary and retinal artery (arrow). Panel C shows a roadmap image of the positioning of the coils (arrows) in the distal part of the ophthalmic artery, over the branching of the main ciliary artery. Note that no contrast medium can be seen distally in the occluded arteries. The lower panels, D, E and F are enlargements of the insets in the corresponding upper panels.

Liquid embolic agent

A liquid embolic agent is a non-adhesive agent used to block arteriovenous malformations of the brain. It is injected slowly, and adapts to the shape of the injection site, but does not normally embolize or disappear from the site of injection. A liquid embolic agent (Onyx®,HD-500, ev3; Neurovascular) was used in the present work to create permanent occlusion of the arteries supplying the retina with blood, to induce experimental retinal ischaemia (Paper II). The embolic agent was injected at two different locations: proximally, at the branching of the main ciliary artery, and distally, into the ciliary artery and the branching retinal arteries. The eyes injected with liquid embolic agent were dissected and photographed under a microscope after termination of the experiments to determine the location of the occlusion.

Coiling

Coiling is clinically used to treat vascular aneurysms or other blood vessel malformations. In this context, it is used for experimental occlusions. A coil is a thin metallic thread contained in a catheter, which is deployed in the blood vessel at the desired site by an endovascular approach. Upon exiting the catheter, the coil takes on a 3-dimensional structure due to its intrinsic inclination to coil. These coils lower the blood flow to the point of clot formation, which produces complete occlusion. Between two and ten coils were needed to achieve occlusion. Coiling was performed at two different locations in the vascular tree: in the proximal part of the ophthalmic artery, before the branching of the main ciliary artery (which supplies the retina), and in the distal part of the ophthalmic artery, over the branching of the main ciliary artery, creating permanent occlusion of the arteries supplying the retina with blood in order to induce experimental retinal ischaemia (Papers III and IV).

After occlusion by the balloon catheter, liquid embolic agent or coiling, vascular occlusion was verified by performing local angiography proximal to the occlusion site. If a passage of contrast medium was seen, further occlusion was performed until no leakage was observed (Figure 7).

Multifocal Electroretinography

Electroretinography is a sensitive tool for the detection of ischaemic injury (Sabates et al., 1983). Research is often carried out to determine whether a certain drug can ameliorate ischaemic insult or injury. In the full field ERG all retinal cells are stimulated simultaneously, and the sum of all response is measured. The P1-wave (also denominated b-wave) amplitude, one component of the response and the implicit time, defined by the time-to-peak of the P1-wave, are sensitive to ischaemia, and are often used in laboratory-based research (Osborne et al., 2004). Full-field ERG was previously the golden standard for detecting retinal ischaemic injury. As full-field ERG measures the response from the whole retina, ischaemic changes in part of the retina cannot be examined in detail. Furthermore, different stimulation modes and light/dark adaptation states are required to discriminate between the different ERG responses (Marmor et al., 1998).

In 1992, Stutter & Tan introduced multifocal ERG (Sutter et al., 1992), which facilitates the evaluation of local retinal changes. Multifocal ERG is used to investigate the function of different areas of the retina, by measuring the electrical responses of various types of cells in the retina to light exposure. Small areas of the retina are stimulated separately, making it possible to extract signals from each area, thus providing local response measurements. Multifocal ERG has been used in various

animal studies, mostly in primates and rodents, but was recently also applied to pigs (Lalonde et al., 2006; Voss Kyhn et al., 2007).

Briefly, the pigs were first kept in normal room light for 1 hour before multifocal ERG stimulation. A Burian-Allen bipolar contact lens electrode with built-in infrared emitters was lubricated and applied to the eye, and a ground electrode needle was inserted into the skin behind the ear (Figure 8). The light stimulus consisted of a pattern containing 103 geometric hexagons. Each hexagon flickers in a predetermined sequence. The electrical voltage between the contact lens electrode and the ground electrical needle is measured, and the computer software extracts the signals from each area of the retina examined. The information is presented in a topographical map of the electrical activity, giving an objective measure of the local functional response. The fundus is visualized by an infrared camera to ensure that the stimulus pattern is consistently positioned with regard to the optic nerve head. After each recording, a fundus image derived from the infrared detection system is taken to document the position of the optic disc, for camera alignment (Figure 8).

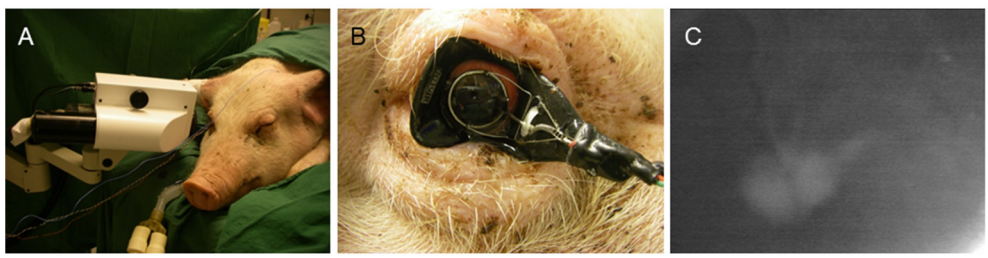

Figure 8
The experimental set-up showing the anaesthetised pig and the infrared illuminator (A), the Burian-Allen contact lens (B), and an infrared fundus image in which the optic nerve head is visible in the lower part of the image (C).

Different areas of the central part of the retina were analysed to determine local alterations. Area 1 corresponds to the visual streak, Area 2 corresponds to the area between the visual streak and the optic nerve head, and Area 3 corresponds to the area around the optic nerve head (Figure 9). The most important parameters are the amplitude and the implicit time (Figure 10). In retinal ischaemia, the amplitude is usually attenuated and/or the implicit time prolonged.

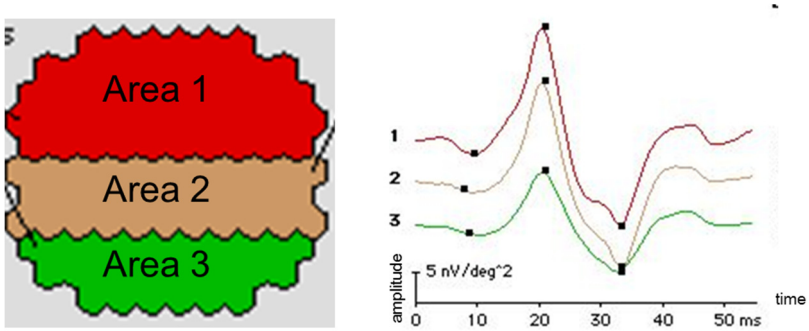

Figure 9.
The left panel shows an illustration of a topographical multifocal ERG map: Area 1, the visual streak, Area 2, the retina between the visual streak and the optic nerve head, and Area 3, the optic nerve head and the surrounding retina. The right panel shows a representative example of the multifocal ERG recordings in each of the anatomical areas.

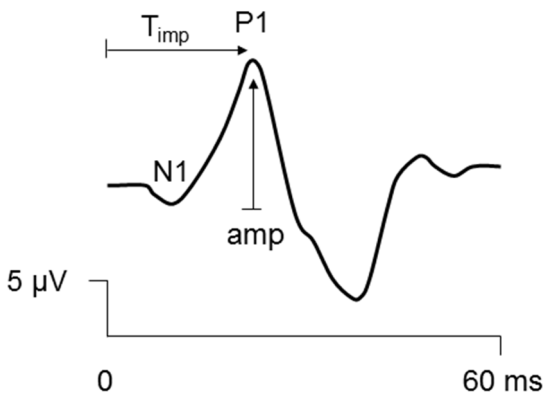

Figure 10.
A schematic illustration of a typical porcine multifocal ERG trace with the peaks N1 and P1. The traces are described by the peak time (implicit time, T_{imp}), and the amplitude (amp), as indicated by the arrows.

Statistical analysis

In Study I statistical analysis was performed using Student´s t-test. However, since normal distribution cannot be anticipated, due to the small number of observations it may have been better perform the statistical analysis using non-parametric test tests. In Study II-III the non-parametric Mann–Whitney test was used when comparing two groups.

Results and discussion

Multifocal ERG for functional evaluation of retinal injury following ischaemia and reperfusion by elevating the IOP

The aim of the first study (Paper I) was to establish whether multifocal ERG, together with simultaneous fundus monitoring, could be used to investigate the functional response of the porcine retina after ischaemia and reperfusion. Ischaemia was induced in one eye by elevating the IOP to 80 mmHg for one hour, while the other eye served as a control. The IOP was then normalized to allow reperfusion. Multifocal ERG was performed in both eyes, before inducing ischaemia, and after 1 and 5 hours of reperfusion. The multifocal ERG responses of three areas of the retina: the visual streak, the area between the visual streak and the optic nerve head, and the area around the optic nerve head were examined in relation to ischaemia and the duration of reperfusion.

Effects of ischaemia and reperfusion on multifocal ERG responses

The results of Study I show that the implicit time of the multifocal ERG recordings was prolonged after ischaemia induced by elevated IOP. This is in accordance with the results of previous studies on humans, using both full-field ERG and multifocal ERG, in which it was found that the implicit time was increased as a result of ischaemia resulting from central retinal vein occlusion (Hayreh, 1983; Kretschmann *et al.*, 1998; Larsson *et al.*, 2001). Furthermore, a reduction in the P1-wave amplitude was seen in the present study after retinal ischaemia.The results of Study I showed that in seven animals, the multifocal ERG signal in the eye subjected to ischaemia and reperfusion was practically zero (Figure 11C), suggesting complete ischaemia–reperfusion injury. In the other six animals, multifocal ERG signals were detected after ischaemia and reperfusion (Figure 11B), suggesting partial ischaemia–reperfusion injury. A reduction in P1-wave amplitude has long been known to be a poor prognostic sign for an ischaemic retina (Henkes, 1953; Karpe, 1945). The amplitude of the P1-wave has been considered the most sensitive indicator of ischaemic injury in the retina, and full-field ERG has therefore been used extensively to evaluate retinal ischaemia (Barnett *et al.*,

1995; Block *et al.*, 1998; Chao *et al.*, 2001; Grozdanic *et al.*, 2003; Rosenbaum *et al.*, 2001). In Study I, the relative changes in P1-wave amplitude and implicit time were similar in the area corresponding to the visual streak (Area 1) and the area between the visual streak and the optic nerve head (Area 2). This suggests that the effect of ischaemia on the central retina is uniform under the current conditions. Similar findings, of an overall effect of retinal ischaemia on the entire area examined with multifocal ERG, have been reported in patients with central retinal vein occlusion (Kretschmann *et al.*, 2000). In contrast, others have found that the retina exhibited regionalized sensitivity to ischaemia (Osborne *et al.*, 2004). In a study by Fortune et al. ischaemia following central retinal vein occlusion was shown to result in a more pronounced loss of macular activity, and local changes were found in regions with exudation in diabetic retinopathy (Fortune *et al.*, 1999).

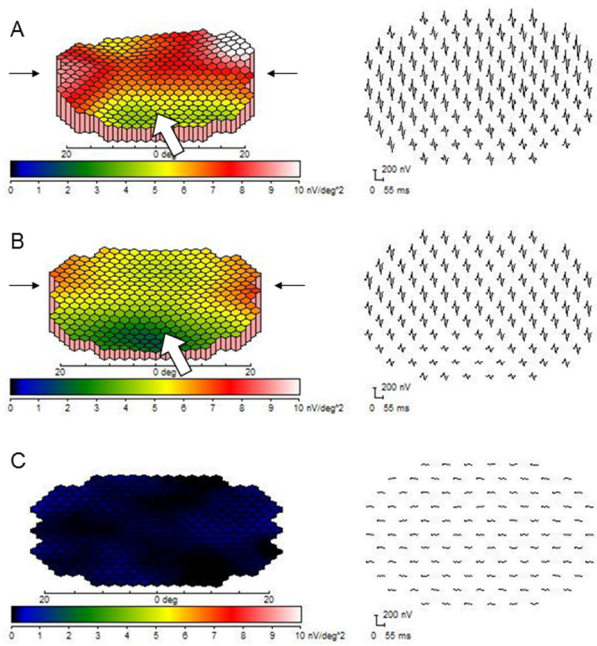

Figure 11.
Examples of multifocal ERG responses from the pig retina. The left panels are topographical maps, in which the optic nerve head (large arrows) and visual streak (small arrows) can be seen. The panels on the right show the individual recordings of each pulse. (A) shows the results of multifocal ERG before the induction of ischaemia. (B) and (C) show the results obtained from two different pigs after 5 hours of reperfusion following ischaemia. In one group of animals (B) ischaemia and reperfusion only resulted in partial loss of retinal function, while in the other group (C) the multifocal ERG recordings were completely flat.

Interindividual variation

The cause of the discrepancy in multifocal ERG response after ischemia perfusion between different animals cannot be deduced from the present study. The experimental settings used for all thirteen animals included in the study were very similar. The age, gender, weight and blood pressure of the pigs, and the IOP were approximately the same. Ischaemia was induced by elevating the IOP to 80 mmHg for 60 minutes. The ocular pressure was measured by a tonometer, and inhibition of retinal circulation was verified ophthalmologically by noting the blanching of the retinal arteries and a pale retina. Similar conditions have previously been shown to cause irreversible flattening of full-field ERG signals in a wide variety of animal models (Osborne et al., 2004). However, most ischaemia–reperfusion studies to date have been performed on smaller animals and rodents, and only few have examined the effects in higher primates (Osborne et al., 2004). The results of the present study on pigs suggest that there may be an interindividual variation in the retinal resistance to an ischaemic insult. Indeed, interindividual and inter-species variation have been reported, and there is a lack of consistency regarding ischaemic retinal tolerance times (Osborne et al., 2004). Furthermore, it has been reported that the primate retina can suffer up to 100 minutes of central retinal artery occlusion without permanent injury (Hayreh et al., 1980). It is therefore possible that interindividual variations in the resistance to an ischaemic insult may account for the differences in outcome in multifocal ERG findings, and thus retinal function, after ischaemia and reperfusion in the present experiments. It is possible that anatomical and structural differences, including variations in the vasculature could account for some of the differences in response to ischaemia. It has been observed in a previous study in rabbits, that elevated IOP did not produce a homogeneous pattern of retinal damage, even in the same animal (Marmor et al., 1993). It has been found that an IOP of 88 mm Hg was sufficient to reduce the oxygen tension at the optic nerve head to values not significantly different from zero in smaller pigs (25-33 kg), while 74 mmHg was not (la Cour et al., 2000). It is therefore possible that in the pigs used in the present study (70 kg), an IOP of 80 mmHg was not sufficient to completely inhibit blood flow in all the animals. Furthermore, the studies performed on smaller pigs also showed that the oxygen tension in the vicinity of the optic nerve head was maintained by autoregulatory mechanisms, even at relatively high IOPs (44 mmHg) (la Cour et al., 2000). Although the IOP used in the present study was considerably higher, it cannot be ruled out that autoregulation accounted for at least some of the variability seen in the results.

Influence of anaesthesia

It is well known that general anaesthesia may affect ERG responses (Andreasson et al., 1993; Whitacre et al., 1984). In Study I, thiopental was used for anaesthesia. Barbiturates and other kinds of drugs used for anaesthesia, including chloral hydrate, chlordiazepoxide, diazepam, ketamine and trichloroethylene, have been shown to

influence ERG recordings (Whitacre et al., 1984). The results of Study I show that the implicit time remained unchanged during the entire duration of the experiments in the control eyes, while the P1-wave amplitude seemed to decrease over time, although the latter did not reach statistical significance. As the results from each eye subjected to ischaemia were compared with the control eye in the same animal, the possible effects of anaesthesia on the multifocal ERG recordings should have been minimal, and the conclusions drawn from the results of this study should not have been affected.

Conclusions

Increasing the IOP to 80 mmHg for one hour resulted in ophthalmologically verified blanching of the arteries and a pale retina, indicating ischaemia. Multifocal ERG recordings at 1 and 5 hours of reperfusion after ischemia showed increased implicit times and decreased amplitudes, typical for retinal ischaemia. The results showed also that the amplitudes of the signals from the visual streak were significantly higher than those from the optic nerve head and the area in between. In conclusion the results suggest that multifocal ERG may be a sensitive tool to assess the extent of retinal injury after ischaemia, and to differentiate between local and overall functional changes in the retina.

Endovascular access to the porcine retinal vasculature

These studies were carried out to determine whether it is possible to obtain a clear-cut experimental model of retinal ischaemia by accessing the retinal circulation through transfemoral endovascular catheterization. An advantage of occlusion of the retinal circulation using an endovascular approach is that it only affects the blood supply, and does not have any unwanted side effects on the eye, such as those resulting from pressure in high-IOP ischaemia, or effects on the nerves as in optic nerve bundle ligation (Osborne *et al.*, 2004). In Study II it was shown for the first time in the pig, that the ophthalmic artery could be catheterized using a transfemoral endovascular approach. This was performed via the external carotid and maxillary artery. Injection of contrast medium into the ophthalmic artery produced a characteristic half-moon-shaped outline of the retina. The ophthalmic artery was demonstrated to give rise to the main ciliary artery, from which the retinal artery branched as a single or several arteries, as can be seen from the angiograms in Figure 12. Similar findings have been reported previously using light and electron microscopy (Bloodworth *et al.*, 1965). In addition to the ophthalmic artery, it was demonstrated that the main ciliary artery, and sometimes the retinal artery, could also be catheterized.

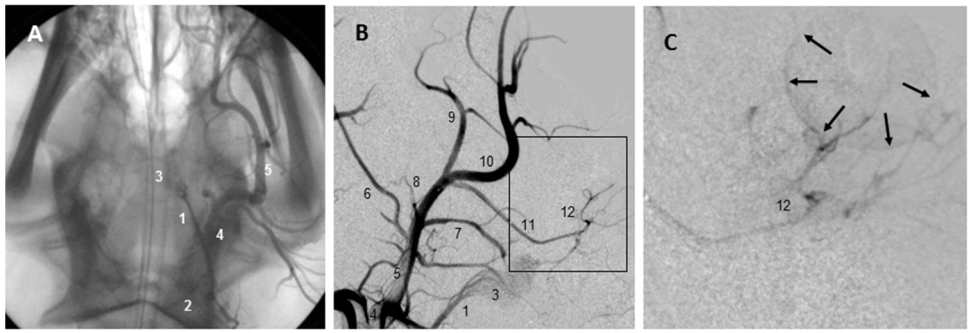

Figure 12.
Angiograms of the left common carotid artery in a pig. Panel A shows a basal view, panel B shows a lateral view, and panel C shows an enlargement of the inset in panel B. The ascending pharyngeal artery (1) originates as a small side branch from the common carotid artery (2) and feeds the rete mirabilis (3), which then converges to form the intracranial carotid artery. The external carotid artery (4) is a continuation of the common carotid artery. The maxillary artery (5) branches from the external carotid artery. The maxillary artery branches into the lingual (6), the auricular (7), the facial (8) and the buccinator artery (9). The maxillary artery gives rise to the infraorbital artery (10). The ophthalmic artery (11) branches off the infraorbital artery. After having accessed the ophthalmic artery, injection of contrast medium produced the characteristic half-moon-shaped outline of the retina, indicated by the arrows in panel C. The ophthalmic artery branches into the main ciliary artery (12), from which the retinal artery branches.

Occlusion of the retinal vasculature using a balloon catheter and a liquid embolic agent

In Study II, two different approaches were used to prevent the blood supply to the retina. Temporary occlusion was obtain by inserting a balloon catheter into the ophthalmic artery, proximally, before the over the branching of the main ciliary artery, and inflating it for one hour. The second approach included a liquid embolic agent, which was used to achieve permanent occlusion. It was injected into the ophthalmic artery, proximally, before the the branching of the main ciliary artery. Both approaches blocked blood flow to the whole vascular system, including the ciliary and retinal arteries that supply the retina, as seen by angiography (Figure 7). Neither of these procedures in the ophthalmic artery resulted in any ophthalmologically visible signs of retinal ischaemia, presumably due to collateral blood supply from the distal parts of the vasculature. However, the multifocal ERG recordings showed decreased amplitudes and increased implicit times, which reflects the retinal function in partial ischaemic injury (Hayreh, 1983; Henkes, 1953; Larsson et al., 2001; Sabates et al., 1983). Presumably, such a proximal occlusion allows collateral blood supply to the retina via anastomoses from e.g. the lingual artery, which may spare the retina from complete ischaemia and result in only partial ischaemia.

To rule out the contribution of blood flow from collateral arteries and to achieve total occlusion of retinal blood flow, the liquid embolic agent was also injected more distally, into the ciliary and retinal arteries. This prevented blood supply to the whole vascular system, including the ciliary and retinal artery branches that supply the retina. This resulted in blanching of the retinal arteries and a pale retina, when examined using indirect ophthalmoscopy. Furthermore, the signals from multifocal ERG were flat, suggesting complete ischaemia. The results of Study II showed that occlusion of the vasculature supplying the retina could be achieved using a liquid embolic agent. However, the liquid state of the agent makes it difficult to predict the exact location of occlusion. The agent may travel along the artery and produce a more distal occlusion than intended. It is also difficult to locate the agent precisely over a bifurcation. Further disadvantages include the risk of accidental embolization at other locations and the reproducibility is therefore limited.

Occlusion of the retinal vasculature using endovascular coiling

Study III was conducted to ascertain whether the vasculature of the porcine retina was accessible for endovascular coiling, and to investigate the possibility of creating occlusions at different sites in the vasculature in order to create stable controllable retinal ischaemia of different degrees of severity. Endovascular coiling was considered to be a suitable technique since it allows precise, repeatable location of the occlusion. This technique also decreases the risk of unintentional clotting in arteries outside the target location, leading to undesirable and adverse effects. The effects of occlusion on the retina were examined using angiography and multifocal ERG, 1 and 72 hours after the intervention, in order to determine whether the ischaemic injury was temporary or permanent. Coiling was performed to occlude the ophthalmic artery at two different locations in the vascular tree: in the proximal part of the ophthalmic artery, before the branching of the main ciliary artery (which supplies the retina), and in the distal part of the ophthalmic artery, over (to occlude) the branching of the main ciliary artery. The results show that the arteries supplying the retina could be occluded using endovascular coiling.

Coiling in the proximal part of the ophthalmic artery caused little or no ischaemia, presumably due to collateral blood supply. Angiography of the ophthalmic artery showed that blood flow through the artery was completely inhibited. However, the late angiogram frames revealed a faint retinal contour, suggesting that collateral arteries entering the vasculature distal to this site supplied the retina with blood (Figure 12). The multifocal ERG recordings showed a non-significant tendency towards prolonged implicit times and reduced amplitudes of the P1-wave, suggesting little or no ischaemia.

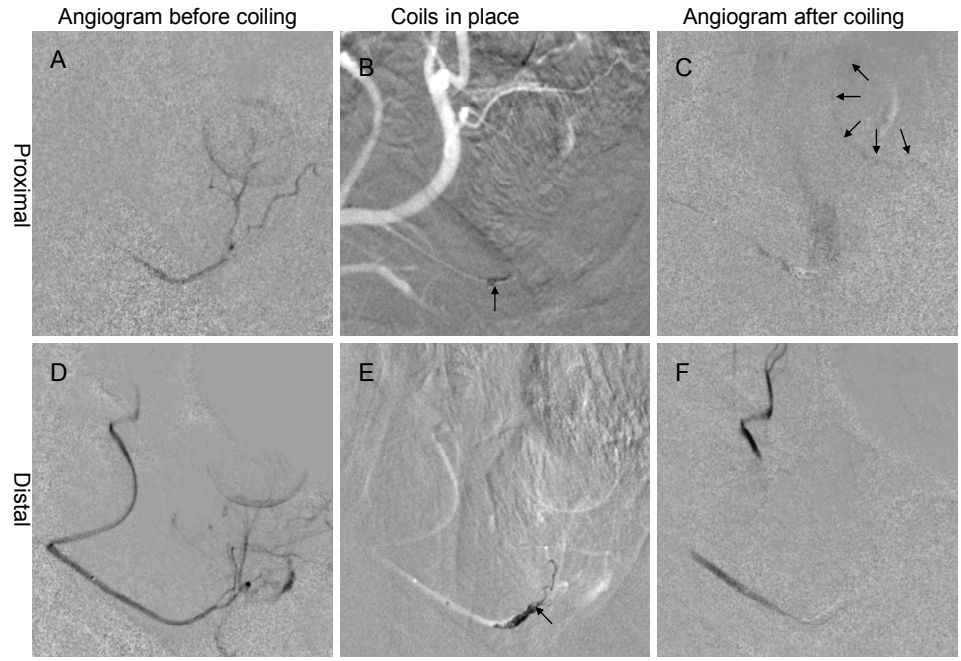

Figure 12.
Angiograms of the porcine ophthalmic artery (lateral projection) during coiling. Upper row: proximal occlusion, lower row: distal occlusion. The left panels show angiograms before coiling. The middle panels show the positioning of the coils (arrows): (B) in the proximal part of the ophthalmic artery, and (E) in the distal part of the ophthalmic artery, over the branching of the main ciliary artery. The right panels show angiograms after coiling of the ophthalmic artery. After proximal coiling (C), the retina shows weak contrast, indicating a collateral blood supply. After distal coiling (F) no noticeable contrast can be seen in the retina.

Coiling in the distal part of the ophthalmic artery, over the branching of the main ciliary artery, caused more severe retinal ischaemia. Angiography of the ophthalmic artery showed that blood flow through the artery was completely prevented and the retina could not be visualized, even in the late angiogram frames, suggesting ischaemia (Figure 12). The multifocal ERG recordings showed significant increases in implicit times and decreased amplitudes. The responses were similar 1 and 72 hours after coiling, indicating that permanent ischaemic injury had been induced.

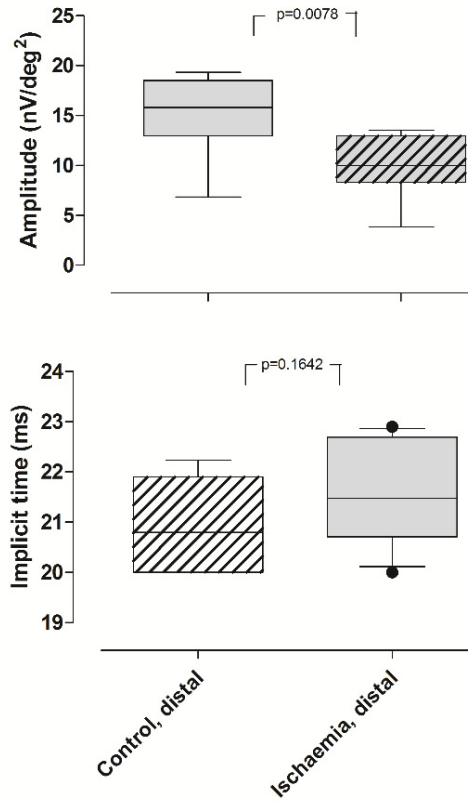

Figure 13.
The amplitude and implicit times of multifocal ERG signals obtained in the visual streak area from pigs' eyes in which a coil had been placed in the distal part of the ophthalmic artery, over the branching of the main ciliary artery, to induce ischaemia, compared with control eyes in the same animal. The mean value of the recordings in each pig was calculated and the results are shown as box plots with medians and percentiles. Statistical analysis was performed using Mann–Whitney test. It can be seen that the amplitude is decreased and the implicit time tends to be increased, indicating partial ischaemia.

To the best of our knowledge, this is the first study to demonstrate that endovascular coiling can be used to occlude the ophthalmic artery. Results show that coiling may be a suitable method of occluding the vasculature and that the degree of ischaemia can be varied by placing the coil at different locations in the vascular tree. The probable reason for this is that arteries anastomose to distal parts of the vasculature, allowing collateral blood supply to the retina, which may prevent complete retinal ischaemia, which was the result of a more distal occlusion by a liquid embolic agent in Paper II.

The technique of endovascular coiling in the pig thus provides the opportunity to vary the degree of ischaemic injury in order to create an optimal experimental model of retinal ischaemia. The technique may be used in the future in recovery experiments to evaluate the long-term effects of ischaemic injury. Performing recovery experiments in pigs, using transfemoral catheterization of the carotid circulation, is feasible and have been demonstrated in numerous previous studies (Dondelinger *et al.*, 1998; Feng *et al.*, 2005). The femoral artery can be located by palpation and then punctured transcutaneously. The advantage of this technique is that, after completing the

experiments, groin haemostasis can be achieved, and the animals will recover without any lasting effects (Burbridge *et al.*, 2004).

Cross midline collateral blood supply to the retina

One major advantage of occlusion of the retinal circulation using a transfemoral endovascular approach is that it only affects the retinal blood supply, and should not have any other, undesirable effects on the eye. Furthermore, the coiling technique allows precise and repeatable location of the desired occlusion. However, a substantial interindividual variation was observed in the degree of ischaemia in the hitherto studied animal models. It was hypothesized that a variation in the architecture of the vasculature could explain the variation in the results. Study IV was carried out to investigate the blood supply of the retina in detail.

The results of this study showed that the blood supply to the pig retina may originate from both the ipsilateral and contralateral ophthalmic arteries, and that a small blood vessel may connect the eyes. In one pig where the ophthalmic artery had been occluded by coils, the injection of contrast medium into the not coiled ophthalmic artery resulted in almost simultaneous filling of the ipsilateral and contralateral retinas with contrast medium. A small blood vessel (interconnecting artery) could be seen branching distally from the position of the tip of the injection catheter in the ophthalmic artery, supplying the retinal circulation of the contralateral eye (Figure 14).

Interestingly, angiography showed that the interconnecting vessel shunted blood to the contralateral retina, even in the presence of a coil in the ophthalmic artery of the contralateral eye. This excludes the possibility that retrograde flow may explain the results. The interconnecting artery presumably originates from a location distal to the tip of the catheter. An angiogram distally in the coiled ophthalmic artery showed bilaterally no sign of contrast passage to the retina. Multifocal ERG of the control eye showed no signs of ischemia, whereas the coiled eye showed only slight ischemia.

In another pig, before coiling in any eye, the injection of contrast medium into the ophthalmic artery of one eye resulted in almost simultaneous filling of both the ipsilateral and the contralateral retinas with contrast medium. In analogy with the first case above, a small interconnecting artery could be seen branching distally from the position of the tip of the injection catheter in the ophthalmic artery, supplying the retinal circulation of the contralateral eye. Consequent coiling of the ipsilateral ophthalmic artery was performed. Angiography of the distal ophthalmic artery after coiling of the same ipsilateral eye showed bilaterally no sign of contrast passage to the retinas. Angiography of the distal ophthalmic artery of the contralateral non coiled eye showed neither any signs of contrast passage. Multifocal ERG of the coiled eye showed significant signs of ischemia, whereas the control eye showed slight ischemia.

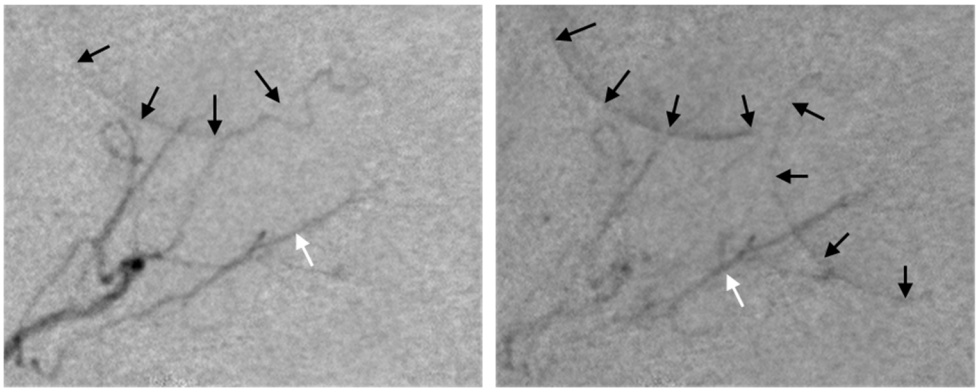

Figure 14.
Angiograms of the porcine ophthalmic artery (lateral projection): The left panel shows the contrast filling of the retinal circulation (black arrows) in the ipsilateral eye after contrast injection in the ipsilateral ophthalmic artery. The right panel shows the angiogram 0.5 seconds later in the same pig, which shows filling with contrast medium in both the psilateral and contralateral retina simultaneously (black arrows). The ophthalmic artery of the contralateral eye is occluded by coiling, thus the retina is presumably supplied by the small interconnecting vessel that can be seen (*white arrow*).

Evidence of communication between the vascular systems of the eyes has been reported in other laboratory animals. In 1961, Prince et al. found cross midline anastomoses of the internal ophthalmic artery in the rabbit (Prince *et al.*, 1960). Other possible cross midline vascular routes in laboratory animals have been described by Dondelinger et al. (Dondelinger *et al.*, 1998). They showed that, in the pig, the arteries originating from the external carotid artery, including the external ophthalmic artery, anastomose with the contralateral arteries across the midline. They also reported evidence of another possible cross midline route via the rete mirabile. The right and left rete mirabilia seem to be interconnected near the hypophysis, and the rete mirabile is connected to the external ophthalmic artery through the anastomic artery. The intracerebral circulation is also connected to the ocular circulation via the internal ophthalmic arteries, which are directly connected to the ciliary artery (Prince *et al.*, 1960). The anastomic pathways of the rete mirabile and the internal ophthalmic artery could be possible cross midline routes for the passage of contrast medium when performing angiography of the common carotid artery, although when performing angiography of the distal ophthalmic artery, the only possible pathway should be via the anastomoses of the external ophthalmic artery.

The results of the present study (Paper IV) may explain the considerable interindividual variations observed in the degree of ischaemia resulting from occlusion in the studies described in Papers II and III.

Cross midline collateral supply - implications for retinal ischaemia

Multifocal ERG recordings from two cases showed that occlusion of the ophthalmic artery by coiling may have a greater ischaemic effect in eyes that are mainly dependent on the ipsilateral ophthalmic artery for blood supply. Reciprocally, coiling may have less ischaemic effect in retinas that receive part of their blood supply from the ipsilateral ophthalmic artery and part from the contralateral ophthalmic artery, via an interconnecting vessel. These results suggest that there may be an interindividual variation in the architecture of the vascular system. However, this interpretation was made on the basis of two multifocal ERG experiments and more studies are needed before statistically tested conclusions can be drawn. Figure 5 in Paper IV is a, somewhat farfetched, hypothetical schematic illustration of how the experimental ischemia may be affected by anatomical variations in blood flow to the retina.

The findings in Paper IV may be important in the development of new animal models of experimental retinal ischaemia. Implications may include avoiding the use of the fellow eye as a control eye since the function may be influenced by the procedure on the contralateral eye. It may implicate that the blood supply of both eyes has to be impeded in order to create a reproducible level of ischemia. It should, however, be noted that extrapolation of these findings to the human eye is difficult.

Major conclusions and future outlook

Retinal ischaemia is one of the major causes of visual impairment and blindness, and is most commonly caused by diabetes, vein thrombosis or arterial occlusion (Osborne *et al.*, 2004). As a result of ischaemia, neovascularization is induced by the release of growth factors. These newly formed blood vessels will be leaky and bleed, and will be unable to provide the blood flow required to ensure adequate oxygenation and nutrition of the retina. The ischemia may thus result in sight-threatening complications such as tractional retinal detachment, vitreous haemorrhage, neovascular glaucoma and macular oedema. It is therefore of the utmost importance to limit the extent of the ischaemic injury. Although new methods of treatment are being developed, there is still a need for more effective pharmacological forms of treatment. The aim of this work was to develop an appropriate animal model of retinal ischaemia in which the intracellular signalling pathways involved in the development of retinal injury and neovascularization can be studied in the future. Two different approaches to occlude the retinal vasculature in the pig were investigated. In the first study (Paper I) elevation of the IOP was used to prevent perfusion of the retina. In the later studies a transfemoral endovascular approach was used to access the retinal vasculature in order to induce ischaemia.

Study I showed that retinal ischemia can be induced by elevating IOP and that the function effects can be evaluated by multifocal ERG. However, there was a possible confounding problem of pressure-induced damage.

The results of Study II showed that it was possible to access the retinal circulation by transfemoral endovascular catheterization. The afferent arteries could be occluded using a balloon catheter for temporary occlusion and a liquid embolic agent for permanent occlusion. The degree of ischaemia depended on the location of the occlusion. Occlusion of the proximal part of the ophthalmic artery caused little or no ischaemic effect, presumably due to collateral blood supply, and occlusion of the distal parts of the vasculature with the liquid embolic agent caused total ischemia. However, the liquid embolic agent used for creating permanent occlusion in this study was difficult to control. The liquid state of the agent made it difficult to predict the exact location of occlusion and hence the reproducibility was limited.

The purpose of Study III was to investigate whether endovascular coiling could be an alternative way to induce ischaemic damage to the retina, and whether it could be used to induce different degrees of ischaemic injury depending of the location of the coils. The coiling technique was shown to produce precise, repeatable location of the desired occlusion. Coiling in the proximal part of the ophthalmic artery caused little or no ischaemic effect according to multifocal ERG. It was hypothesized that this could be due to collateral blood supply. Coiling of the distal parts of the ophthalmic artery, over the branching of the main ciliary artery, caused more severe retinal ischaemia.

Study IV was performed to investigate the possibility of collateral blood supply, suggested by the findings in the previous studies. Angiographic evidence was found of collateral blood flow between the eyes. Thus it seems possible that one ophthalmic artery can supply both the ipsilateral and the contralateral retina. An occlusion of the afferent blood supply to one eye may not be sufficient to induce an appropriate and reproducible level of ischemia in that eye, as it may be supplied with blood from the vascular system of the other eye. These findings may have implications in the future development of an experimental animal model of retinal ischaemia.

The technique of transfemoral endovascular occlusion in the pig represents a step forward towards a large animal model of retinal ischemia. The technique may provide the opportunity to vary the degree of ischaemic injury in order to create an optimal experimental model of retinal ischaemia. However due to the presence of collaterals; cross midline collaterals and also collaterals presumably within the ipsilateral vasculature, the reproducibility of the model remains to be elucidated. Further studies of the blood supply of the porcine eye are needed before it can be used as a stable model for retinal ischemia

After having established an animal model for retinal ischaemia, future research should be carried out to identify which signal transduction pathways are activated or altered in the retina during retinal ischaemia. The development of a pharmacological modulator for the treatment of retinal ischaemia may prevent vision impairment and blindness in many individuals.

Summary in Swedish -

Populärvetenskaplig sammanfattning

Näthinnan (retina) ligger längst bak i ögat och fungerar som filmen i en kamera. Stavar och tappar registrerar ljus och signalen går via nerver till syncentra i hjärnan. Blodkärl löper genom näthinnan och förser cellerna med syre och näring samt transporterar bort slaggprodukter. Kärlsjukdomar, som t.ex. diabetes, blodproppar i näthinnan till följd av åderförkalkning och högt blodtryck kan skada näthinnan och resultera i synnedsättning eller blindhet. Vid diabetes blir blodkärlen sköra och kan börja läcka. Vid åderförkalkning bildas blodproppar som kan fastna i ögats blodkärl. Om blodkärlen i ögat läcker eller om blodflödet stoppas får inte näthinnan den mängd syre och näring som den behöver och man får syrebrist i näthinnan, s.k. retinal ischemi. Vid retinal ischemi sätts ett lokalt signalsystem igång för att nybilda blodkärl i näthinnan. De nya blodkärlen fungerar inte optimalt, utan växer onormalt och läcker vilket kan leda till synnedsättning. Retinal ischemi är en av de vanligaste orsakerna till blindhet i världen. Vanligtvis behandlar man näthinnan hos dessa patienter med laser. Laserbehandling förhindrar nybildningen av blodkärl, men är en grov metod som slår ut stora delar av näthinnan och påverkar synfältet och mörkerseendet. Nyligen har det kommit läkemedel som blockerar de signalvägar som stimulerar nybildningen av blodkärl i näthinnan, s.k. VEGF-hämmare. Vi tror att det finns stora möjligheter att finna nya och kompletterande läkemedel för att bromsa nybildning av blodkärl i näthinnan på ett mer effektivt sätt. Forskning om de signalvägar som är involverade i utvecklingen av skada efter retinal ischemi går snabbt framåt vad gäller stroke och hjärtinfarkt, som är ischemiska tillstånd i andra delar av kroppen. Vi vill applicera den kunskapen på ischemiska tillstånd i ögat.

Det övergripande målet med vårt forskningsprojekt är att upptäcka nya behandlingsmetoder för retinal ischemi. Det specifika syftet med denna avhandling är att skapa retinal ischemi på grisar där skadan på näthinnan så mycket som möjligt liknar den skada som uppkommer hos patienter. På dessa grisar med retinal ischemi kan man sedan kartlägga de cellulära signalsystem som är involverade i utvecklingen av skadan på näthinnan och kärlnybildningen till följd av retinal ischemi.

Vi har undersökt två olika metoder för att hindra blodflödet till näthinnan och på så sätt skapa retinal ischemi och en skada på näthinnan som liknar den hos människor. I Studie I använde vi en modell med förhöjt ögontryck för att stänga av blodtillförseln till ögat. I Studie II, III och IV använde vi en kateter som vi under röntgengenomlysning förde upp från ett kärl i ljumsken på grisen och hela vägen till ögats kärl och där stoppade till blodtillförseln till näthinnan.

Med ERG kan man mäta funktionen i näthinnan och den skada som uppstår till följd av ischemi. Man mäter den elektriska ström som uppstår när näthinnans celler aktiveras av ljus. När näthinnans celler skadas av ischemi så minskar denna ström. Hittills har man använt fullfälts-ERG för att mäta ischemisk skada i näthinnan. Med fullfälts ERG mäter man den totala strömmen som genereras i näthinnan vid aktivering av ljus. Vi har i våra studier använt multifokalt ERG, vilket är en vidareutveckling av fullfälts-ERG. Med multifokalt ERG mäter man strömmen i många punkter i näthinnan och kan på så sätt få fram en karta över hur näthinnan aktiveras av ljus. Man kan även se varifrån i näthinnan signalen kommer ifrån, och således kan man bestämma vilken del av näthinnan som skadats. Multifokalt ERG har använts i olika djurstudier, mestadels i primater (apor) och gnagare. I Studie I skapade vi retinal ischemi på grisar genom att höja ögontrycket med en vätskeinjektion i ögat. Vi använde mfERG för att mäta skadan som uppkom i näthinnan till följd av ischemi. Resultaten visar att högt tryck i ögat ger förändringar i multifokalt ERG som är typiska för retinal ischemi. Multifokalt ERG kan vara en användbar metod för att utvärdera och övervaka lokal skada i näthinnan till följd av ischemi.

Ett problem med att studera ischemi till följd av högt tryck i ögat är att man troligen inte bara får skador av blodbristen utan även av trycket. Syftet med Studie II var att skapa en mer renodlad djurmodell för retinal ischemi där skadan i näthinnan bara beror på blodbristen. Med hjälp av röntgengenomlysning letade vi oss upp in i kärlsystemet, från ljumsken och hela vägen till ögats blodkärl, med en kateter. En uppblåsbar ballongkateter användes för att stänga av (ockludera) det stora kärlet som leder till ögat (oftalmicaartären) tillfälligt. Ett lim (Onyx®) injicerades genom en injektionskateter för att stänga av de mindre kärlen som leder till ögat permanent. I gris heter dessa kärlen ”the main ciliary arteries”. Ocklusion i oftalmicaartären ledde till en mild ischemi i näthinnan, förmodligen på grund av att andra kärlsystem då kan förse ögat med blod genom att ansluta sig bortom ocklusionen. Ocklusion i ”the main ciliary arteries” orsakade fullständig ischemi. I Studie II var vi först i världen med att i gris nå hela vägen fram till ögats blodcirkulation med en kateter och att vi där kunde ockludera blodkärlen för att skapa retinal ischemi.

I Studie II gav ballongocklusion en för mild ischemi och limmet var svårt att reglera och det blev en stor variation i graden av ischemi. Syftet med Studie III var att vidareutveckla tekniken i Studie II med hjälp av s.k. ”coiling”. En coil är en liten metalltråd om man skjuter in i blodkärlet. Coilen har en förutbestämd tredimensionell

struktur och blir ett nystan när den kommer ut i blodkärlet. I nystanet koagulerar blodet och kärlet täpps till. Man använder coiling på kliniken för att bl a täppa till aneurysm (artärbråck) i hjärnan. Vi ville undersöka om coiling kan användas för att stänga av blodtillförseln till näthinnan och om man kan framkalla olika grader av ischemi genom att ockludera kärlet på olika avstånd från näthinnan. Hypotesen var att coiling kunde vara en lämplig teknik eftersom man kan bestämma platsen på vilken man sätter coilen med stor precision. Resultaten visade att graden av ischemi är relaterat till avståndet mellan ocklusionen och näthinna. Coiling i oftalmicaartären, långt från ögat, orsakade ingen eller liten ischemi, medan coiling i oftalmicaartären, nära ögat, orsakade en mer uttalad ischemi.

Trots den precision med vilken vi kunde placera coilen i kärlen så var variationen i graden av ischemi stor. Intressant nog så började vi i slutet av Studie III få klart för oss att det nog fanns ett blodflöde som gick mellan ögonen. Syftet med Studie IV var att undersöka detta närmare. På röntgen av blodkärlen kunde vi se ett kärl som gick från ena ögonartären till den andra. Om vi stängde av blodflödet till ena ögat så tror vi att funktionen i näthinnan på andra ögat påverkas. Denna upptäckt förklarar troligen den stora variationen i graden av retinal ischemi som vi får när vi ockluderar de tillförande kärlen och har betydelse för den fortsatta utvecklingen av denna djurmodell av retinal ischemi.

Vi hoppas att genom dessa studier ha tillfört kunskap om djurmodeller för retinal ischemi. På sikt hoppas vi få en stabil djurmodell för retinal ischemi och i denna hitta nya intressanta cellulära signalvägar som aktiveras av retinal ischemi och ta fram läkemedel mot dessa. Om man har en bra djurmodell så kan man undersöka läkemedlets effekt mot den skada och nybildning av blodkärl som uppkommer till följd av retinal ischemi. Först därefter kan man pröva dessa läkemedel på patienter med t.ex. diabetes och blodproppar i näthinnan. Förhoppningen är att hindra synnedsättning och blindhet till följd av retinal ischemi.

Acknowledgements

I wish to express my deep gratitude to the many people who have helped me throughout the course of this work:

My supervisor, Professor *Malin Malmsjö*, for her incredible energy in both research projects and clinical work, for always being so optimistic and having millions of new ideas, and for her patience, no matter what,

My assistant supervisor, Professor *Sten Andréasson*, for introducing me to the world of electrophysiology, in which he is an expert. Thanks for your kind advice and interest,

Bodil Gesslein, for being a trusted and excellent companion during long experiments, and for always being extremely well organized; without you, our experiments would not have been possible,

Per Undrén, an expert in interventional neuroradiology, thank you for guiding me to the right spot in the vascular labyrinth,

Gisela Håkansson, for her endurance during marathon sessions of experimental surgery,

Everyone at the *in vivo* lab: especially *Michael Kindler, Susanne Geres* and *Jeppe Stridh*, for their expert management of the pigs and help with the radiological imaging devices. Thank you for always being so helpful and cheerful,

Professor *Lars Edvinsson*, for his scrutiny of my work at my halftime control,

Sten Kjellström, for his support and encouragement at my halftime control,

Professor *Stefan Seregard*, for sharing his scientific and clinical skills,

Urban Eriksson, for giving me the time to pursue research and courses,

Manochehr Amani, for encouraging research and development in parallel with clinical work,

Eva Larsson, for solving the intricate puzzle of fitting research into the clinical schedule,

Gerd Holmström and *Hanna Åkerblom*, for sharing their enthusiasm in the field of ERG,

Charlotte Lindberg, for her kind help with the thesis,

All colleagues and staff at the Department of Vitreoretinal Diseases and the Department of Neuro-ophthalmology, St Erik's Eye Hospital, Stockholm and Department of Ophthalmology, Uppsala Akademiska Hospital for their inspiring motivation,

All former colleagues and staff at the Department of Ophthalmology at Västmanlands Hospital, Västerås, for sharing the early part of my career in clinical ophthalmology; special thanks for allowing me to attend research courses before starting my PhD,

All my dear friends for understanding my absence this autumn.

My parents, *Lena* and *Stellan*, for always being there, for their encouragement and support in everyday life,

My children, *Louise* and *Mathilda,* for giving me so much love, and for their interest in my research, especially when they pressed the secret "screen-up-side-down-button" on my laptop,

My beloved wife *Nadine*, for her love and support, and for her patience when I spent countless hours at the computer, or was "down south" in Lund.

Jan af Peterséns for teaching me the art of seamanship, both at sea and on dry land. I wish you could have been here.

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