Progress in Retinal and Eye Research 34 (2013) 19–48

Contents lists available at SciVerse ScienceDirect

Progress in Retinal and Eye Research

journal homepage: www.elsevier.com/locate/prer

Molecular basis of the inner blood-retinal barrier and its breakdown in diabetic macular edema and other pathological conditions

Ingeborg Klaassen [a,1], Cornelis J.F. Van Noorden [b,1], Reinier O. Schlingemann [a,c,*,1]

[a] Ocular Angiogenesis Group, Department of Ophthalmology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
[b] Ocular Angiogenesis Group, Department of Cell Biology and Histology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
[c] Netherlands Institute for Neurosciences, Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Available online 13 February 2013

Keywords:
Blood-retinal barrier
Capillary permeability
Retina
Diabetic retinopathy
Diabetic macular edema
Tight junctions
Transcytosis
Caveolae
Endothelium
Pericytes
Vascular endothelial growth factor A/ antagonists & inhibitors

ABSTRACT

Breakdown of the inner endothelial blood-retinal barrier (BRB), as occurs in diabetic retinopathy, age-related macular degeneration, retinal vein occlusions, uveitis and other chronic retinal diseases, results in vasogenic edema and neural tissue damage, causing loss of vision. The central mechanism of altered BRB function is a change in the permeability characteristics of retinal endothelial cells caused by elevated levels of growth factors, cytokines, advanced glycation end products, inflammation, hyperglycemia and loss of pericytes. Subsequently, paracellular but also transcellular transport across the retinal vascular wall increases via opening of endothelial intercellular junctions and qualitative and quantitative changes in endothelial caveolar transcellular transport, respectively. Functional changes in pericytes and astrocytes, as well as structural changes in the composition of the endothelial glycocalyx and the basal lamina around BRB endothelium further facilitate BRB leakage. As Starling's rules apply, active transcellular transport of plasma proteins by the BRB endothelial cells causing increased interstitial osmotic pressure is probably the main factor in the formation of macular edema. The understanding of the complex cellular and molecular processes involved in BRB leakage has grown rapidly in recent years. Although appropriate animal models for human conditions like diabetic macular edema are lacking, these insights have provided tools for rational design of drugs aimed at restoring the BRB as well as for design of effective transport of drugs across the BRB, to treat the chronic retinal diseases such as diabetic macular edema that affect the quality-of-life of millions of patients.

Contents

* Corresponding author. Medical Retina Unit and Ocular Angiogenesis Group, Department of Ophthalmology, Academic Medical Center, Room A2-122, PO Box 22660, 1100 DD Amsterdam, The Netherlands. Tel.: +31205663682; fax: +31205669048.
E-mail address: r.schlingemann@amc.uva.nl (R.O. Schlingemann).

[1] Percentage of work contributed by each author in the production of the manuscript is as follows:Dr Ingeborg Klaassen designed the content of the review and was the first author of all versions of the manuscript including the final version. Prof. dr. Cornelis Van Noorden adviced in the design of the content of the review from a cell biological point of view and corrected and redesigned all versions of the manuscript including the final version. Prof. Dr. Reinier Schlingemann adviced in the design of the content of the review from a clinical/pathological point of view and corrected and redesigned all versions of the manuscript including the final version.

1350-9462/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.preteyeres.2013.02.001

Abbreviations

AGEs	advanced glycation end products
AMD	age-related macular degeneration
Ang	angiopoietin
AQP	aquaporin
BBB	blood-brain barrier
BL	basal lamina
BRB	blood-retinal barrier
BRECs	bovine retinal endothelial cells
CNS	central nervous system
CTGF	connective tissue growth factor
DME	diabetic macular edema
DR	diabetic retinopathy
ESAM	endothelial cell-specific adhesion molecule
GLUT1	glucose transporter 1
HGF	hepatocyte growth factor
HIF-1	hypoxia inducible factor 1
IL	interleukin
JAMs	junctional adhesion molecules
KSS	kallikrein-kinin system
MCP-1	monocyte chemotactic protein 1
MMP	matrix metalloprotease
NO	nitric oxide
NPDR	non-proliferative DR
PCDR	pre-clinical DR
PDGF	platelet-derived growth factor
PDR	proliferative DR
PEG	polyethylene glycol
PKC-β	protein kinase C beta
PLVAP	plasmalemma vesicle associated protein
PVR	proliferative vitreoretinopathy
ROS	reactive oxygen species
siRNA	small interfering RNA
STZ	streptozotocin
TEER	transendothelial electrical resistance
TGF-β	transforming growth factor beta
TIMP	tissue inhibitor of metalloproteases
TNF-α	tumor necrosis factor alpha
VEGF	vascular endothelial growth factor
ZO	zona occludens

1. Introduction

Retinal vascular leakage from loss of function of the blood–retinal barrier (BRB) and subsequent macular edema are the main causes of visual loss and blindness in major eye diseases such as diabetic retinopathy (DR), age-related macular degeneration (AMD), retinal vein occlusion and uveitis (Fig. 1). Despite recent advances, there is still a fundamental lack of understanding of the cellular mechanisms underlying both the function of the BRB in physiological conditions as well as its dysfunction in pathological conditions. However, it has become clear that the previously prevailing concept that BRB loss is the result of unspecified endothelial cell damage' has become obsolete. It should be replaced by the notion that dynamic adaptations of endothelial cells and other cell types involved in the BRB underlie vascular leakage in retinal disease.

A complex dual vascular system provides oxygen and nutrients to the metabolically highly active neural retina, a tissue that has a higher oxygen consumption per unit weight of tissue than any other human tissue (Arden et al., 2005). The choriocapillaris provides blood supply to the photoreceptors in the outer retina, while capillaries sprouting from the central retinal artery provide oxygen and nutrients to the inner retina. These two distinct vascular beds not only differ in embryonic origin, but also in their properties and functions in the adult eye. The endothelium of choroidal capillaries is highly fenestrated and permeable. The capillaries in the inner retina have a continuous endothelium with a barrier function and are organized in two parallel layers, whereas the outer retina is completely avascular.

Retinal neural tissue is protected from potentially harmful molecules in the circulation by the inner BRB that regulates the entry of molecules into the inner retina. To complete this protective

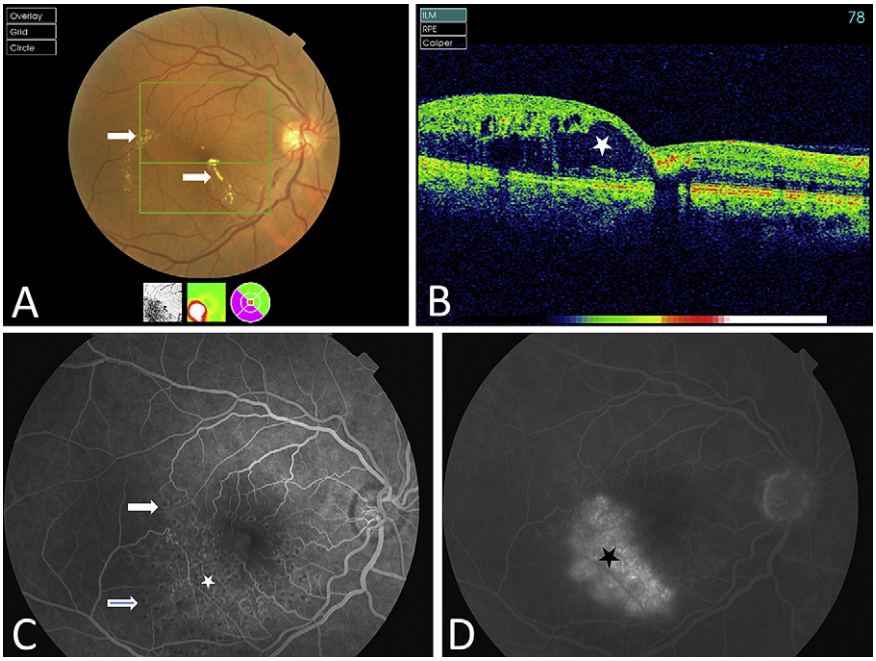

Fig. 1. Clinical observations in conditions with BRB loss. The figure shows a color fundus photograph (A), optical coherence tomography (OCT) (B) and early and late frames of fluorescein angiography (C and D) of a patient with macular edema due to branch retinal vein occlusion. Note the formation of hard exudates (A, arrows) and cystoid macular edema recognized by cystoid spaces on OCT (B, star) and cystoid pooling of extravasated fluorescein (D, star) due to leakage from tortuous and enlarged retinal capillaries in the affected retinal area (C, star). The leakage occurs despite previous laser photocoagulation (C, arrows) and this patient is likely to benefit from intravitreal injections with an anti-VEGF agent.

environment of the ocular interior, other blood-ocular barriers are formed by retinal pigment epithelium (outer BRB), epithelium of ciliary processes (blood-aqueous barrier), the capillaries of the optic nerve, with the exception of the pre-laminar part (Hofman et al., 2001b), and by endothelial cells of capillaries of the iris and ciliary muscle (Cunha-Vaz, 1997; Hofman et al., 2001a; Raviola, 1977). In this review, the focus is on the inner BRB, as other components of the blood-ocular barriers have been adequately discussed elsewhere (Freddo, 2012; Rizzolo et al., 2011; Simó et al., 2010).

The intercellular spaces between retinal endothelial cells that form the BRB are sealed by elaborate tight junctions, and the cells themselves lack fenestrations and have few pinocytotic vesicles (Bradbury, 1985; Raviola, 1977) (Fig. 2). These features of the BRB, which are comparable to those of the blood-brain barrier (BBB) endothelium, result in high transendothelial electrical resistance (TEER) and restricted paracellular permeability. The resistance of the BRB is not exactly known, but is likely similar to that of the BBB, with a TEER of 1500–2000 $\Omega \cdot cm^2$ (Butt et al., 1990). In comparison, human placental endothelial cells have a TEER of 22–52 $\Omega \cdot cm^2$ (Jinga et al., 2000), which permits rapid paracellular exchange of nutrients and waste products between mother and fetus, whereas urinary bladder epithelium has a resistance of 6000–30,000 $\Omega \cdot cm^2$, necessary to protect underlying tissues against the toxic urine and to preserve urine hyperosmolarity (Negrete et al., 1996). The neural retina is highly vulnerable and any vascular change leading to reduced barrier properties can be detrimental for visual function. In recent years, increasing insight has been gained in the molecular mechanisms of pathological BRB breakdown. In the present review, structural, cellular, molecular and mechanistic aspects of BRB functions and their loss in eye diseases are discussed.

Most cellular and molecular knowledge on the mechanisms of BRB breakdown comes from rodent and in vitro models. Although much progress has been achieved by the development of these models, it must be stressed that the extrapolation of this knowledge to the mechanisms involved in BRB dysfunction in human eye disease must be approached with caution. In fact, a huge knowledge gap exists between the descriptive data from the human clinical manifestations of ocular pathologies such as diabetic macular edema (DME), uveitis and venous occlusions and the knowledge gained from animal models and in vitro systems that endeavor to mimic these pathologies. On the clinical side, it is often difficult to make a clear-cut definition of the disease and from the research side it is difficult to mimic the pathology that is observed in the clinic. The success of drugs in the treatment of DME, such as anti-vascular endothelial growth factor (VEGF) therapies or corticosteroids has lead to hypotheses that point to the involvement of VEGF or inflammation, but the exact mechanisms have only partly been resolved. For example, no animal model thus far is able to mimic what really happens in the human retina in the context of DR or DME. The same applies for in vitro models of the BRB. Although these models provide a very useful tool for high-throughput testing and functional analysis of individual proteins, cells in culture rapidly lose their BRB properties, such as their high number of tight junctions and low number and specific cellular distribution of caveolae. Furthermore, it is virtually impossible to mimic the effects of chronic hyperglycemia in an in vitro system. Thus, in these models as well, the translation of insights obtained to understanding pathophysiology of human disease remains troublesome. Bearing this in mind, it is clear that the interpretation of results from in vivo and in vitro studies must be done with great care. Nevertheless, it is to be expected that new information from existing and novel models and confirmatory and complementary studies in human tissues and in patients will eventually close the current knowledge gap and will lead to the detailed understanding of the mechanisms of BRB breakdown in ocular pathologies.

2. Ocular pathology and BRB breakdown

2.1. Clinical pathology

In human ocular disorders with macular edema such as DR, AMD, retinal vein occlusion and uveitis, increased retinal vascular

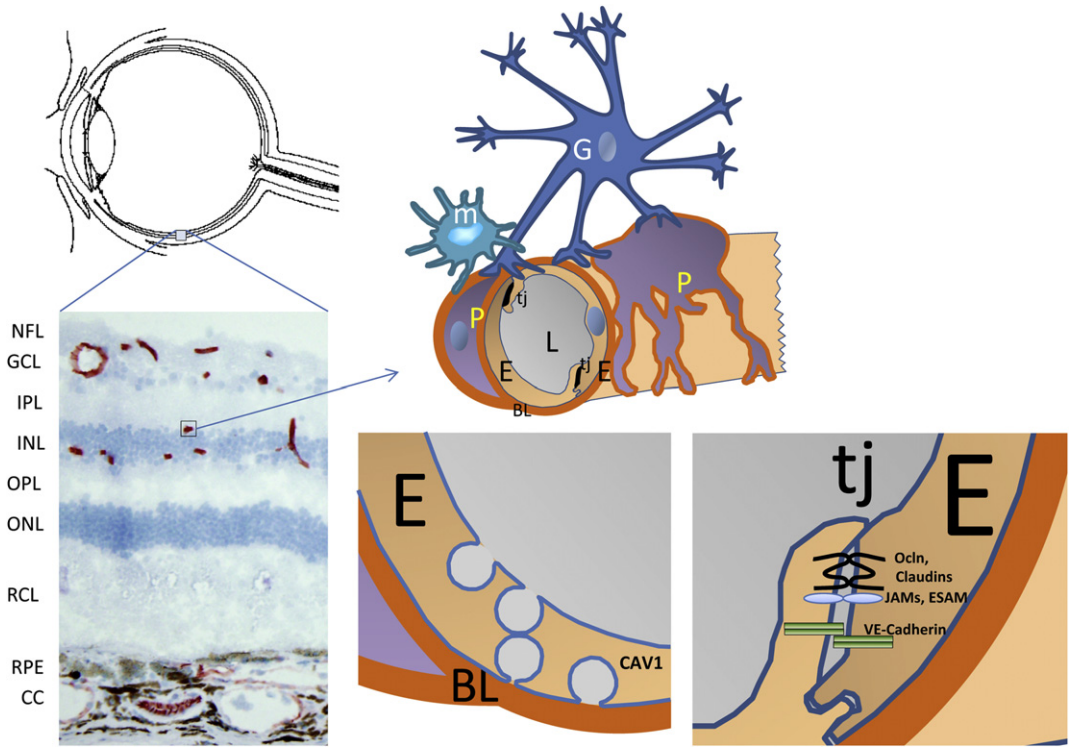

Fig. 2. Schematic diagram of the blood–retinal barrier (BRB). Upper panel: the BRB is composed of retinal capillary endothelial cells (inner BRB) and retinal pigment epithelial cells (RPE, outer BRB). Retinal capillary endothelial cells are surrounded by pericyte and glial cell foot processes. The left panel shows a cryosection of human retina stained for claudin-5 (red). Lower panels: two transport routes control the passage of molecules, the paracellular and the transcellular route. The paracellular route is sealed by tight junctions (tj) including occludin (Ocln), claudins, junctional adhesion molecules (JAMs) and endothelial cell-specific adhesion molecule (ESAM) and by adherens junctions, including VE-Cadherin. The transcellular route is restricted by selective transport by caveolar vesicles, of which caveolin-1 (CAV1) is the main constituent. BL, basal lamina; CC, choriocapillaries; E, endothelial cell; G, macroglial cell (astrocyte or Müller cell); GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; L, lumen; m, microglia; ONL, outer nuclear layer; OPL, outer plexiform layer; P, pericyte; RCL, rods and cones layer; RPE, retinal pigment epithelium.

permeability is involved. As in other vascular beds, the rules of Starling determine water homeostasis and edema formation in the retina. Net water transport over the endothelium is determined by the sum of hydrostatic pressure and osmotic pressure of the luminal and extraluminal compartments. Increased osmotic pressure of the interstitial compartment due to leakage of plasma solutes caused by BRB loss and/or an increased capillary hydrostatic pressure lead to edema. As smaller solutes can diffuse more easily over the vascular wall in both directions, in particular via the paracellular route, increasing concentrations of large proteins in the interstitium contribute mostly to increasing tissue osmotic pressure and subsequent edema formation.

DME is the major cause of loss of vision in patients with DR and, in particular in non-proliferative DR (NPDR) in type 2 diabetes mellitus. DME is characterized by vascular leakage, tissue edema and the deposition of hard exudates in the central retina. Before clinical signs of DR develop, long-term hyperglycemia leads to loss of the retinal vascular autoregulatory function and both arterioles and venules become dilated and elongated (Cheung et al., 2007; Gardiner et al., 2007; Quigley, 2007; Rogers et al., 2010). In DR, retinal arteriolar dilatation can increase capillary hydrostatic pressure contributing to tissue edema by Starling's rules, and can contribute to capillary wall dilatation (microaneurysm) and rupture (hemorrhage), which are all classical signs of DR (Cheung et al., 2007; Quigley, 2007). Loss of capillaries may increase the production of VEGF and other permeability factors, and induce breakdown of the BRB via several mechanisms, leading to extravasation of plasma proteins into the interstitium.

Although most DR patients show overlapping patterns (Browning et al., 2008), grossly two types of leakage can be distinguished clinically in eyes with DME: focal and diffuse (Aroca et al., 2004). Focal leakage from dilated capillaries and microaneurysms that are associated with areas of capillary occlusion causes local swelling of the retina. This is likely caused by loss of the BRB in the retinal capillaries near areas of ischemia, and is usually responsive to focal laser photocoagulation (Cunha-Vaz, 1998). Diffuse DME is characterized by swelling of the central retina, often with cystoid changes around the fovea and often without clinically visible capillary non-perfusion. In these cases, diffuse leakage in large areas of the retinal capillary bed is caused by massive breakdown of the BRB, possibly caused by loss of capillaries of the deep retinal plexus resulting in ischemia and release of inflammatory mediators. Before the introduction of anti-VEGF agents, laser photocoagulation, applied in a grid-like pattern around the fovea was the preferred treatment, but had in general little effect. Patients with diffuse leakage in the fovea have a relatively poor prognosis (Aroca et al., 2004).

In the last decade, other treatment strategies have been introduced in addition to laser. Corticosteroids such as triamcinolone administered by intravitreal injections have been used widely off-label in patients with DME, and often have a strikingly beneficial anatomical effect on retinal swelling and vascular leakage as detected by fluorescein angiography (Silva et al., 2009). The mechanisms involved are still not clearly understood, and steroids may act indirectly by inhibition of inflammatory cells or cytokines (Brooks et al., 2004; McAllister et al., 2009), through restoration of aquaporins in Müller cells and/or endothelial cells (see Section 5.2.2) or via a direct effect on tight junction molecules or other functions of endothelial cells. However, corticosteroids have severe side effects and their functional benefit is limited. A number of

recent clinical trials failed to show an advantage of steroids over standard laser therapy after longer follow-up (Beck et al., 2009).

More recently, anti-VEGF agents such as ranibizumab and bevacizumab were demonstrated to be beneficial in the treatment of DME (Mitchell et al., 2011; Witkin and Brown, 2011), in particular in patients with visual loss associated with thickening of the central fovea. These studies have shown that in a substantial number of DME patients, VEGF is a crucial pathogenic factor in BRB breakdown. The long-term side effects of anti-VEGF therapy are still unknown because the drugs are relatively new. Side effects may occur due to the interference of these agents with the neuroprotective and vasculoprotective roles of VEGF or with its role in wound healing.

In addition to the gross leakage occurring in DME, subtle changes in the BRB also occur in the early phase of pre-clinical DR (PCDR). PCDR precedes visible signs of clinical vision-threatening DR in the 8–15 years needed to develop DR, and is characterized by loss of pericytes, thickening of the basal lamina (BL) and vasoregression (Hammes et al., 2011; Lorenzi and Gerhardinger, 2001). The increased retinal vascular permeability in PCDR is characterized by the presence of extravascular albumin, reduced intercellular tight junctional complexes and increased numbers of intracellular vesicles in endothelial cells. Diffusely increased retinal vascular permeability has been suggested to be a crucial step in the progression to NPDR (Vinores et al., 1990). Therefore, a better understanding of the pathogenesis of these two types of BRB loss in the early and advanced clinical stages of DR may allow development of more effective therapeutic strategies for DME and possibly open avenues to prevent progression from PCDR to NPDR.

In several other major eye diseases, BRB loss is also crucial in causing blindness. Retinal vein occlusions (RVOs) lead to macular edema, and anti-VEGF agents have recently been shown to be effective in preventing visual loss in this condition (Al-Latayfeh et al., 2012; Kimoto and Kubota, 2012). RVOs are classified according to where the obstruction is located. Obstruction of the retinal vein at the optic nerve is referred to as central retinal vein occlusion (CRVO), subdivided into non-ischemic and ischemic type, and obstruction at a branch of the retinal vein is referred to as branch retinal vein occlusion (BRVO). Vision loss in RVOs is due to macular edema, macular ischemia, and retinal or iris neovascularization. Macular edema in RVOs is caused by BRB loss associated with retinal ischemia due to vascular stasis and capillary non-perfusion, but increased capillary pressure due to the venous outflow obstruction is likely to have an additional role in edema formation.

Retinopathy of prematurity (ROP) is a disease that may develop in immature eyes of premature babies. It can be mild with no visual defects, or it may become aggressive with new blood vessel formation (neovascularization) and progress to retinal detachment and blindness. As smaller and younger babies are surviving, the incidence of ROP has increased. Widespread BRB breakdown in this condition is probably caused by high levels of VEGF that eventually cause neovascularization, similarly to (pre-)proliferative DR (PDR). In addition, massive vascular leakage from neovascular blood vessels can be identified as focal points of fluorescein leakage by fluorescent angiography in proliferative DR and ROP.

Also in exudative AMD, loss of the inner and outer BRB, manifested by cystoid edema and retinal swelling, is a common finding in areas overlying subretinal neovascularization and wound healing tissue under the retina (Silva et al., 2009; Schlingemann, 2004). The beneficial effects of VEGF inhibitors on visual acuity in this condition, in particular visual gain, partly result from reduced retinal vascular leakage (El-Mollayess et al., 2011).

In uveitis, inflammation may also cause breakdown of the blood–aqueous barriers and BRB. Many infectious and noninfectious causes can incite episodes of uveitis. Topical corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs) remain the most effective class of drugs to treat uveitis. However, cystoid macular edema (CME) is the most common cause of visual loss in uveitis.

Macular edema may also occur after intraocular surgery or as a side effect of certain anti-glaucoma eye drops. Surgical trauma to the iris/ciliary body or lens epithelial cells induces the synthesis of prostaglandins, which in turn result in accumulation of other inflammatory mediators that may diffuse into the vitreous and reach the retina (Miyake and Ibaraki, 2002). At this point, the blood-aqueous barrier and the BRB are disrupted in parallel. Patients with diabetes, hypertension, old age or uveitis have an increased risk for the development of CME. The synthesis of prostaglandins can be effectively suppressed with NSAIDs (see also Section 6.1 and Table 3).

Taken together, vascular leakage by loss of the BRB is a major pathogenic mechanism causing loss of vision (Fig. 1). The eye diseases involved constitute a huge burden and seriously reduce quality-of-life for millions of patients, creating considerable health care expenditure and other societal costs. Better understanding of the BRB and its dysfunction in these conditions is a crucial step towards the development of better and individualized treatment strategies, but since the cause and pathophysiology of each condition vary, representative experimental models are typically lacking.

2.2. Experimental models used in BRB research

The major experimental models used in BRB research are in vitro systems and experimental conditions in animals. In vitro systems usually use bovine retinal endothelial cells (BRECs) cultured on filters, with or without other cells such as astrocytes and pericytes, which allow measurements of TEER values and permeability studies. Ideally, such a system would consists of cells with identical differentiation as retinal endothelial cells in vivo, both in tight junction composition and integrity, and in their specialized transcellular transport mechanisms. This should be complemented by a high TEER value and permeability characteristics similar to the BRB in vivo. Unfortunately, this ideal model has been found hard to generate, and many of the systems in the literature are difficult to reproduce in other laboratories for unknown reasons. We have recently described two models of the BRB, one simple system of BRECS cultured as a monolayer on transwell filters, and a more complex model where BRECS are co-cultured with rat astrocytes on the backside of the filter, and bovine retinal pericytes on the bottom of the culture well (Wisniewska-Kruk et al., 2012).

As already discussed in the Introduction, most experimental animal models employed to study mechanisms of BRB pathology in patients only partly mimic human disease. Streptozotocin (STZ)-induced diabetes in rats or mice is a widely used model to study signs of early DR, such as diffuse vascular leakage and

Table 1
Molecules identified in retinal endothelial junctions.

Category	Name	Gene name
Tight junctions	Occludin	OCLN
	Claudin 1	CLDN1
	Claudin 2	CLDN2
	Claudin 5	CLDN5
	Claudin 12	CLDN12
	F11 receptor (Jam1)	F11R
	Junctional adhesion molecule 2	JAM2
	Junctional adhesion molecule 3	JAM3
	Endothelial cell-specific adhesion molecule	ESAM
	Poliovirus receptor-related 1 (Nectin)	PVRL1
	Tight junction protein 1 (Zona occludens 1)	TJP1
	Tight junction protein 2 (Zona occludens 2)	TJP2
Adherens junctions	VE-Cadherin	CDH5
	β-Catenin	CTNNBIP1
	N-Cadherin	CDH2
Gap junctions	Gap junction protein, alpha 1, 43 kDa	GJA1

Table 2
Major categories of transcellular transport and its cargo in the BRB.

Category	Type		Cargo	Luminal	Abluminal
Carrier-mediated					
	Glucose transporter 1	GLUT-1	Glucose	+	+
	Monocarboxylate transporter 1	MCT1	Lactate	+	+
	Essential and non-essential amino acid transporters	AA, ASC, A, LNAA, EAAT, N	Amino acids	−/+	+
Ion transport					
	Sodium pump		Na^+ influx, K^+ efflux	−	+
	Sodium-hydrogen exchanger		Exchange Na^+ and H^+	+	−
	$Na+/K+/2Cl^-$ cotransporter		Exchange Na^+, K^+ and 2 Cl^-	+	−
	Chloride-bicarbonate exchanger		Exchange Cl^- and HCO_3^-	+	+
Active efflux transport					
	ABCA transporters	ABCA	Cholesterol, phospholipids and retinoids	+	+
	Multidrug resistance protein 1	MDR1/P-gp/ABCB1	Variety of drugs	+	−
	ABCC transporters	MRP4/ABCC4	Organic anions, including conjugates of lipophilic compounds with glutathione, glucuronate, or sulfate, cytotoxic and antiviral drugs	+	+
	Breast cancer resistance protein	BCRP/ABCG2	Xenobiotics, anticancer drugs	+	−
	Organic anion transporting polypeptide 2	OATP2/SLCO1A4/SLC21A	Organic anions	+	−
	Organic anion transporter	OAT/SLC22A	Organic anions	−	+
	Solute carrier transporters	SLC7, SLC16, SLC19, SLC29	Amino acids, chemotherapeutic agents, antibiotics	+	+
Receptor-mediated transport					
	Peptide transport system 1	PTS-1	Opioid peptides	−	+
	Peptide transport system 2	PTS-2	Arginine-vasopressin	−	+
	Peptide transport system 4	PTS-4	Arginine-vasopressin	+	−
Caveolae-mediated transport					
	Insulin receptor	IR	Insulin	+	−
	Transferrin receptor	TFR	Transferrin	+	+
	Albondin and/or absorptive transcytosis		Albumin	+	+
	Absorptive transcytosis		Other plasma proteins, SV40 virus	+	+

vasoregression, but signs of NPDR and PDR, such as DME and neovascularization, do not develop in this model. Nevertheless, results obtained with diffuse BRB loss and its inhibition by therapeutic agents in this model have often been used as the sole preclinical data underlying clinical studies in humans with DME. Other rodent diabetes models, whether transgenic, naturally occurring or induced, also fail to develop these advanced stages of DR, regardless of the age of onset or the duration of hyperglycemia. Intraocular injection of VEGF in rats or monkeys mimics signs of DR or DME, as BRB leakage, leukocyte adhesion, microaneurysms and capillary non-perfusion have been found in this model. However, these models have the disadvantage that the actions of VEGF have an acute and transient character and the normo-glycemic background reduces the value of these models in the study of DR. A third experimental animal model of BRB disruption is oxygen induced retinopathy in neonatal mice. However, BRB loss in this model occurs mainly in the context of retinal angiogenesis. Recently, a novel animal model was developed, the Akimba mouse model, which combines elevated VEGF expression and hyperglycaemia (Rakoczy et al., 2010). In the Akimba mouse many clinical signs of (pre)PDR are manifest, including microaneurysms, leaky capillaries, venous beading, tortuous vessels, capillary dropout, attenuation of vessels, edema and intraretinal neovascularization. Because of these features, this model may be very useful for further studies on mechanisms of pathological BRB loss.

3. Anatomical and molecular aspects of the normal BRB

3.1. Endothelial cells

The specific structural properties of the retinal vasculature provide the basis of the BRB function. Retinal capillaries that form the BRB consist of a single layer of tightly adherent endothelial cells, a basal lamina (BL) and surrounding pericytes, astrocytes and microglia (Fig. 3). This complex is called the neurovascular unit. Selectively regulated transport of molecules is possible across this barrier. Two pathways serve this purpose, the paracellular pathway which is regulated by dynamic opening and closing of inter-endothelial junctions, and the transcellular pathway which involves specialized transport vesicles (caveolae) and receptor-mediated transport.

3.1.1. The paracellular pathway: inter-endothelial cell junctions

The paracellular route restricts passage of solutes larger than 3 nm in radius and is the preferred pathway for water and small water-soluble compounds (Pappenheimer et al., 1951). Inter-endothelial cell junctions are complexes consisting of tight junctions, adherens junctions and gap junctions (Fig. 4). In addition to their role in cell-to-cell adhesion, these structures regulate contact inhibition of endothelial cell growth, cell survival, maintenance of cell polarity and paracellular permeability. In both tight junctions and adherens junctions, adhesion is mediated by homophylic interactions between adhesion proteins that form complexes at sites of cell-to-cell contacts organized in zipper-like structures along the entire cell-to-cell contact border (Cavey et al., 2008; Gumbiner, 2000; Tsukita et al., 2001). Endothelial cells express cell type-specific transmembrane proteins that are involved in adhesion, such as vascular endothelial cadherin (VE-cadherin) at adherens junctions (Dejana and Giampietro, 2012; Vestweber et al., 2009) and claudins at tight junctions (Furuse et al., 1998; Lal-Nag and Morin, 2009; Nitta et al., 2003; Paolinelli et al., 2011). Endothelial junctions are dynamic structures. Their molecular composition does not only vary during maturation and stabilization of endothelial junctions (Ayalon et al., 1994), but adhesion molecules cycle continuously between the plasma membrane and intracellular compartments, as well as along the plasma membrane (Shen et al., 2008). The molecular organization of endothelial junctions has been described in great detail in other reviews mainly focusing on the BBB (for reviews see Bazzoni and Dejana, 2004; Chiba et al., 2008; Dejana, 2004;

Table 3
Novel therapeutics in current clinical trials for the treatment of DME.

Drug	Other name	Function	Description	Administration	Phase	Results
ALG-1001		Anti-angiogenic	Integrin inhibitor	Intravitreal	Phase I, II	Ongoing
Minocycline		Antibiotic	Broad-spectrum tetracycline antibiotic	Oral	Phase I, II	Ongoing
FOV2304	Safotibant	Anti-inflammatory	Bradykinin receptor antagonist	Topical	Phase II	Ongoing
Betamethasone microsphere	DE-102	Anti-inflammatory	Steroid microsphere product for sustained release	Intravitreal	Phase II, III	Ongoing
Dextromethorphan	DXM or DM	Anti-inflammatory	Anti-inflammatory	Oral	Phase I, II	Ongoing
MS-R001 (rapamycin)	Sirolimus	Anti-inflammatory	Immunosuppressant drug, binds to mTOR	Subconjunctivally versus intravitreously	Phase I	Well tolerated
NOVA63035 “corticosteroid”		Anti-inflammatory	Anti-inflammatory	Intravitreal	Phase I	Ongoing
SAR 1118		Anti-inflammatory	Inhibits T-cell inflammation by blocking the binding of two key cellular surface proteins (LFA-1 and ICAM-1)	Topical	Phase I	Status unknown
Choline fenofibrate	SLV348/ABT-335, TriLipix™	Anti-lipid	Reduces triglycerides and increases HDL-C	Unknown	Phase II	Ongoing
Darapladib	SB435495	Anti-lipid	Lipoprotein-associated phospholipase A2 (lp-PLA2) inhibitor	Oral	Phase II	Ongoing
1,2 Dithiolane 3 valeric acid	Alpha lipoic acid (ALA)	Antioxidant	Antioxidant	Oral	Phase III	No effect
Infliximab	Remicade	Anti-TNFα	Monoclonal antibody against TNF-α	Intravitreal	Phase I, II	Adverse effects
Bevasiranib		Anti-VEGF	Cand5 selectively silences the mRNA encoding for VEGF	Intravitreal	Phase II	Completed
Pegaptanib	Macugen	Anti-VEGF	Anti-VEGF	Intravitreal	Phase III	Ongoing
KH902		Anti-VEGF	Recombinant human VEGF receptor-Fc fusion protein	Intravitreal	Phase I, II	Ongoing
MP0112		Anti-VEGF	Potentially long acting VEGF inhibitor	Intravitreal	Phase I	Terminated (company decision)
Mecamylamine	Inversine	nACh receptor inhibitor	Non-specific nicotinic acetylcholine (nACh) receptor inhibitor	Topical	Phase II	Heterogeneous effects
Bromfenac	Xibrom	NSAID	Non-steroidal anti-inflammatory drug (NSAID)	Topical	Phase IV	Effect
Diclofenac	Arthrotec, Voltaren, Voltarol, Diclon, Cataflam, Naclof	NSAID	NSAID	Intravitreal	Phase I	Status unknown
Nepafenac	Nevanac	NSAID	NSAID	Topical	Phase II	Ongoing
Tromethamine ketorolac	Toradol, Acular	NSAID	NSAID	Topical	Phase II	Ongoing
Ruboxistaurin	RBX	PKC-β inhibitor	PKC-β inhibitor	Oral	Phase III	Effect
Aliskiren	Tekturna, Rasilez	Renin inhibitor	Direct renin inhibitor	Oral	Phase II	Terminated (Inadequate enrollment)
PF-04523655	RTP801I-14	siRNA DDIT4	Short interfering RNA (siRNA) inhibitor of RTP801; targets DNA damage-inducible transcript 4	Intravitreal	Phase II	Not started

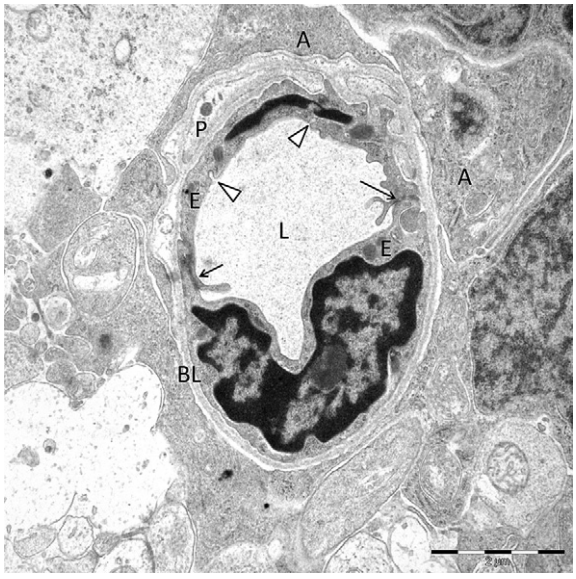

Fig. 3. Ultrastructural cross section of a retinal capillary in the inner nuclear layer. Electron microscopical image showing a retinal capillary with endothelial cells (E), surrounded by a basal lamina (BL), pericyte foot processes (P) and astrocyte foot processes (A). Tight junctional complexes appear as a dense black lining at the borders of the endothelial cells (arrows). Caveolar invaginations are indicated by arrow heads. L, lumen. Bar = 2 μm.

Lampugnani, 2012; Paolinelli et al., 2011; Paris et al., 2008; Wallez and Huber, 2008). Many of the molecules involved have also been identified in *retinal* endothelial cells (Harhaj and Antonetti, 2004; Klaassen et al., 2009; Luo et al., 2011; Runkle and Antonetti, 2011). These observations are summarized in Table 1.

3.1.1.1. Tight junctions. Tight junctions are multifunctional complexes, acting as (1) a gate involved in regulation of paracellular transport of ions, solutes and water and diapedesis of cells, (2) a fence that separates basolateral from apical cell membrane domains, and (3) a cell signaling coordination centre that affects differentiation, proliferation and polarity of cells.

Epithelial cells also have tight junctions, but the architecture and composition of tight junctions of endothelial cells are quite different in various ways (for review, see Dejana, 2004). Tight junctions of epithelial cells are a separate entity at the boundary between apical and basolateral compartments of the plasma membrane concentrated towards the apical side of the cells. In endothelial cells, tight junctions are frequently entangled with adherens junctions and gap junctions, and the tighter regulation of permeability is required, the more complex this system is structured. Endothelial cells of the retina (and brain) have the highest number of tight junction strands, with the highest complexity and the smallest intercellular gaps as compared to endothelial cells from other tissue beds.

The first protein that was found to be exclusively associated with tight junctions was ZO-1 (Stevenson et al., 1986), named after the "zonula occludens", the term that was originally used for tight junctions after their discovery (Farquhar and Palade, 1963). Two closely related proteins, ZO-2 and ZO-3, were identified later (Gumbiner et al., 1991; Haskins et al., 1998; Jesaitis and Goodenough, 1994). ZOs are adaptor proteins, which link tight junctional molecules intracellularly via cingulin to the actin cytoskeleton (Bauer et al., 2004; Wolburg and Lippoldt, 2002; Wolburg et al., 2009). The first protein associated with paracellular barrier function was occludin (Furuse et al., 1993). Occludin is expressed in both epithelial and endothelial cells (Saitou et al., 1997) and at much higher levels in barrier endothelium than in non-barrier endothelium (Hirase et al., 1997; Kevil et al., 1998). The findings that cells lacking occludin are still able to form a tight barrier (Balda et al., 1996) and that occludin knockout mice are viable and appear to form tight junctions (Saitou et al., 1998) indicate that the function of occludin in tight junctions can be taken over by other proteins.

Important new insights into the nature of these other proteins came in 1998 with the identification of claudin 1 and 2 (Furuse et al., 1998). Claudins, which are small transmembrane molecules of 18–27 kDa, form a family of 24 members in mouse and human (Lal-Nag and Morin, 2009; Tsukita et al., 2008; Van Itallie and Anderson, 2006). Claudins have no sequence homology with occludin, but possess four transmembrane domains like occludin and are able to copolymerize heterophilically or homofilically with other claudins and occludin (Furuse et al., 1998; Tsukita and Furuse, 1999). Some of the claudins have highly-restricted expression patterns. It was suggested that their distinct distribution patterns in different cell types reflect the diversity in barrier functions in the various epithelial and endothelial tissues.

Claudin-1 is ubiquitously expressed (Furuse et al., 1998), whereas claudin-5 is restricted to endothelial cells. In claudin-5-deficient mice, morphologically normal blood vessels are formed, but the vessels are more permeable for molecules that are 800 Da or less in size (Nitta et al., 2003), suggesting that claudin-5 is involved in the barrier function for smaller molecules. In brain, 4 claudins were identified: claudin-1, -3, -5 and -12 (Nitta et al., 2003; Wolburg et al., 2003). In the entire retina, mRNA of claudin-1, -2, -3, -4, -5, -12, -22, and −23 is found, but only claudin-1, -2, and -5 are located in the tight junctions of retinal vessels (Klaassen et al., 2009; Luo et al., 2011, Table 1). Other endothelial tight junctional proteins include junctional adhesion molecules (JAMs), a family of immunoglobulin-

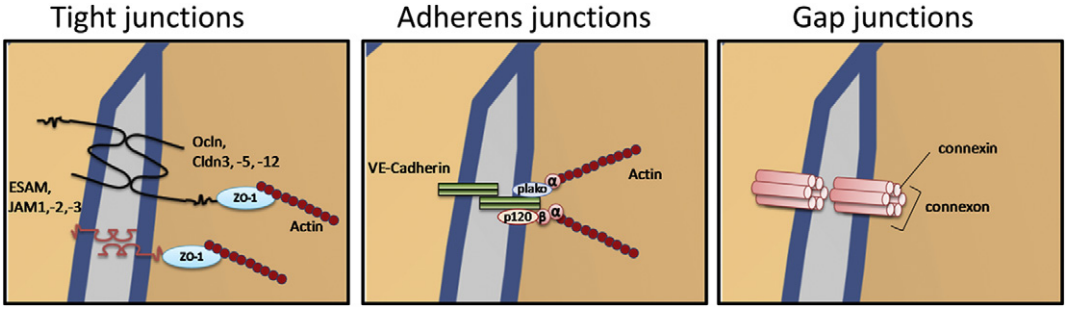

Fig. 4. Schematic overview of the three types of endothelial junctions of the BRB. Tight junction molecules such as occludin (Ocln) and claudins (Cldn) have four transmembrane domains and are able to copolymerize heterophilically or homophilically with each other. Tight junction molecules such as endothelial cell-specific adhesion molecule (ESAM) and junction adhesion molecules (JAMs) are immunoglobulin-like molecules that only bind homophilically. The adaptor protein zonula occludens (ZO) couples the tight junctions to the actin cytoskeleton. Adherens junctions are composed of VE-cadherin and a number of partnering compounds, including α- and β-catenin (α,β) plakoglobulin (plako) and p120. α-Catenin connects the adherens junctions to the cytoskeleton. Gap junctions consist of hemi-channels (connexons) that are formed by six identical or different connexins.

like molecules (reviewed in Ebnet et al., 2004), including JAM-A, JAM-B, JAM-C, JAM4, JAM-like (JAML), the endothelial cell-specific adhesion molecule (ESAM), and coxackie virus and adenovirus receptor (CAR). Only little is known of the specific functions of these molecules in maintaining the BRB.

3.1.1.2. Adherens junctions. Adherens junctions are another type of junction between BRB endothelial cells, which are formed in early stages of intercellular contact, before the formation of tight junctions, both in developing embryonic tissues (Collins and Fleming, 1995) and in cultured cells (Gumbiner, 1988). As a result, experimental disturbance of adherens junction organization as occurs in mice lacking genes of adherens junction components, causes major defects in early stages of development (reviewed in Nyqvist et al., 2008). VE-cadherin is the major compound of endothelial adherens junctions, and is bound to multiple intracellular partners, including β-catenin, p120, plakoglobin, density-enhanced phosphatase 1 (DEP-1) and vascular endothelial protein tyrosine phosphatase (VE-PTP) (Fig. 4; Dejana and Giampietro, 2012; Vestweber et al., 2009). The presence of adherens junctions stimulates the formation of tight junctions, since VE-cadherin at adherens junctions induces claudin-5 expression (Taddei et al., 2008). Therefore, changes in VE-cadherin adhesive properties will impact at multiple levels on the endothelial barrier function.

3.1.1.3. Gap junctions. The third type of intercellular junction involved in the BRB is the gap junction. It consists of a hemichannel (or connexon) on each of two neighboring cells that make contact (Fig. 4). The hemi-channels are formed by six identical or different connexins, creating homomeric or heteromeric connexons, respectively. Gap junctions mediate electrical and chemical communication between cells and allow small molecules (<1000 Da) to pass freely. In the brain and retina, gap junctions are found primarily in astrocytes, but also between adjacent endothelial cells and between endothelial cells and pericytes. Gap junctions are possibly involved in the barrier function of endothelial cells as gap-junction proteins facilitate the assembly of adherens and tight junctions. Overexpression of connexins in endothelial cells induces expression of occludin and claudins (Kojima et al., 1999, 2002; Morita et al., 2004), which is prevented by gap-junction blockers (Kojima et al., 2002). In addition, gap-junction blocking agents inhibit the barrier function of tight junctions in cells, as determined by measurement of TEER and paracellular flux of mannitol and inulin, without altering the expression and localization of tight-junction components (Nagasawa et al., 2006). Increased intracellular calcium ($[Ca^{2+}]_i$) is associated with increased barrier permeability (reviewed in Abbott, 2000). Recently, connexin channels were found to play a role in the regulation of $[Ca^{2+}]_i$ dynamics (De Bock et al., 2011). To fully understand the actual contribution of gap junctions in the regulation of BRB permeability more research is needed.

3.1.1.4. Relation of the junctions with the cytoskeleton and nucleus. Many tight junction or adherens junction components, such as ZO-1, cingulin, α- and β-catenin, α-actinin, and vinculin, interact directly or indirectly with actin. Binding of tight junctional proteins to actin microfilaments and signaling proteins allows the transfer of signals into the cell (Bazzoni et al., 1999; Braga, 2002; Matter and Balda, 2003; Wheelock and Johnson, 2003). Moreover, some intracellular junctional proteins, when released from junctions, translocate to the nucleus and modulate transcription (Bienz and Clevers, 2000; Matter and Balda, 2003). Besides signaling and stabilization of junctions through anchorage to the actin cytoskeleton, this association is also considered to be required for the dynamic opening and closing of the junctions. Small guanosine-5′-triphosphate hydrolase enzymes such as Rac, Rho and Rap1 play a critical role in this context (reviewed by Spindler et al., 2010).

In summary, the BRB endothelium forms a sophisticated junctional complex that regulates paracellular permeability and protects the neuronal environment against harmful substances from the blood. Dynamic opening and closing of these junctions appears to be important in regulating paracellular transport, in particular of smaller molecules and solutes. As stated above, tight junctions are intermingled with adherens junctions on endothelial cells and are formed after the formation of adherens junctions. Much less is known about the role of the gap-junctional complexes of endothelial cells. Thus far, the best characterized junctional proteins are occludin and claudins that form a barrier and regulate permeability, and ZO-1, an adaptor protein connected with the cytoskeleton which may have a role in contraction of cells and thereby regulate dynamic opening of tight junctions. Many more junctional proteins have been identified, but their exact roles still need to be elucidated.

3.1.2. The transcellular pathway: endothelial transcytosis

In general, transport across endothelial cells of the BRB is selective and regulated by membrane transporters and vesicular mechanisms. However, for a wide range of lipid-soluble molecules passive transport across the BRB is possible (Hosoya et al., 2010; Toda et al., 2011), and is proportional to their lipophilicity. Available lipophilicity trend lines enable the prediction of BRB permeability of drugs (Hosoya et al., 2010). All other types of transcellular transport across the barrier are energy-dependent and can be classified into five main categories: carrier-mediated transport, ion transport, active efflux transport, receptor-mediated transport, and caveolae-mediated transport (reviewed in Zlokovic, 2008). Examples of transporters in each category are shown in Fig. 5 and listed in Table 2.

Transcytosis, a concept first introduced by Nicolae Simionescu (1979), is the transport of molecules across endothelial cells via specialized plasmalemmal vesicles, now termed caveolae. After many years of research, the characteristics and functions of caveolae have begun to emerge (reviewed in Frank et al., 2009;

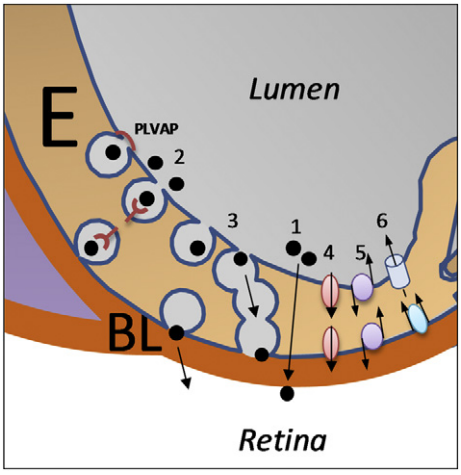

Fig. 5. Schematic overview of transcellular transport mechanisms of the BRB. Lipid-soluble molecules cross the BRB by passive transport (1), proportional to their lipophilicity. All other types of transcellular transport are energy-dependent and involve transport through caveolae with (2) or without (3) binding to cargo-specific receptors, carrier-mediated transport mechanisms (4), or other specific transporters such as ion and amino acid transporters (5) or involve active efflux pumps such as multidrug resistance pumps, e.g. P-glycoprotein (Pgp/MDR1) (6). Plasmalemma vesicle associated protein (PLVAP) forms a "cap" or stomotal diaphragm on top of caveolar flasks and is suggested to increase transcellular vascular permeability. BL, basal lamina; E, endothelial cell.

Komarova and Malik, 2010; Predescu et al., 2007; Simionescu et al., 2009; Tuma and Hubbard, 2003). First, molecules are taken up by invagination of the plasma membrane on either side of the cell to form a caveola, which is sealed off by membrane budding and fission. Then, the vesicle moves to the other side of the cell and after docking and fusion to the opposite cell membrane, the vesicular content is discharged into the extracellular space. Caveolar transport is necessary for regulation of the homeostasis of the retinal microenvironment. Transcytosis is the preferred pathway of transport of plasma macromolecules, such as albumin, LDL, metalloproteases and insulin, whereas water and ions are preferentially transported via the paracellular pathway.

Palade and Bruns were the first to propose that caveolae shuttle cargo across capillary endothelium from the vascular lumen to the abluminal side (Bruns and Palade, 1968; Palade and Bruns, 1968). Although it has been clearly shown that plasma macromolecules can travel across endothelial cells from one side of the cells to the other, and caveolae posses all the molecules necessary for budding and fusion (Schnitzer et al., 1995), such actual shuttling of caveolae is still a matter of controversy (reviewed in Predescu et al., 2004).

At first, caveolae were considered to be immobile structures and able to fuse with each other. Structures of fused pinocytotic vesicles had been observed using electron microscopy (Bundgaard et al., 1979; Gil and Magno, 1980). Later, it was demonstrated that caveolae can form chains of 2–3 linked vesicles that span the short distance across capillary endothelium, which is called a transendothelial channel (Simionescu, 1983). It has been reported that even a single plasmalemmal vesicle can open concomitantly at both sides of an endothelial cell (Simionescu et al., 1975). This is not a completely eccentric idea, since it has been observed that the cytoplasmic thickness between luminal and abluminal plasma membranes of endothelial cells outside the perinuclear region, is approximately the size of a caveola (50–80 nm) (Fig. 6).

Whether shuttling of caveolae occurs or not, compelling evidence has shown that electron dense tracers (Wagner and Chen, 1991), albumin (Milici et al., 1987), insulin (King and Johnson, 1985) and transferrin (Wagner et al., 1983) cross capillary endothelium by the transcellular route and not via paracellular transport. The technical difficulties in detecting and quantifying transcytosis have greatly slowed down progress in the understanding of the role and mechanisms of transcellular transport. The techniques used, often electron microscopy of serial sections, were very laborious and controversy still exists about the interpretation of the results obtained. In 2007, it was demonstrated for the first time by a live dynamic imaging technique, that caveolae in vivo can transport selective antibodies transcellularly across the barrier in seconds, with a speed that exceeds that of transcytosis or endocytosis of clathrin-coated vesicles (Oh et al., 2007). However, also these results provoked debate (Red-Horse and Ferrara, 2007; Anderson, 2008).

Albumin is definitely the best-studied protein in endothelial transcytosis. The exact mechanism of albumin transcytosis is not completely understood, but extensive biochemical and functional studies have proven the existence of albumin-binding proteins on the endothelial cell surface (reviewed by Stewart, 2000). It is currently assumed that binding of albumin to the 60 kDa glycoprotein (gp60) (Schnitzer, 1992) induces clustering of gp60 and binding to caveolin-1 in endothelium of the BRB (Minshall et al., 2000). The fact that normal BRB endothelium, like in the BBB, contains only few albumin-binding proteins (Stewart, 2000), emphasizes the selective regulation of passage of plasma macromolecules through barrier endothelium. There is more evidence that transcytosis is tightly regulated in barrier endothelia. The number of caveolae in endothelial cells is generally high as compared to other cell types, but brain and retinal barrier endothelium is

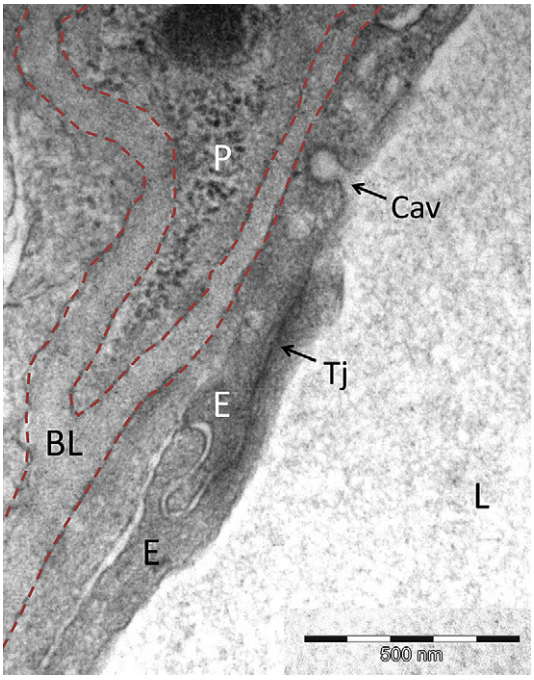

Fig. 6. Ultrastructural cell morphology of barrier endothelium in a retinal capillary of the inner nuclear layer. High power electron microscopical image showing a large tight junctional structure (Tj) between adjacent endothelial cells (E) and an open caveolar flask-like structure (Cav) that almost touches the abluminal membrane of the endothelial cell. The border of the basal lamina (BL), which is shared between endothelial cells and a pericyte (P), is indicated with a red dashed line. L, lumen. Bar = 500 nm.

characterized by a relatively low number of caveolae (Sagaties et al., 1987), with a preferential location at the abluminal cell surface (Hofman et al., 2000, 2001b; Peters et al., 1991). In contrast, non-barrier endothelium typically has more caveolae at the luminal surface. These features are considered to be typical of the BRB function. It was suggested that the preferential abluminal position of caveolae in barrier endothelium is consistent with a preferential direction of transcytosis from tissue to blood (Hofman et al., 2000, 2001a). For the BRB, this is supported by results of vitreous fluorometry experiments showing active transport of fluorescein from the extravascular to the vascular compartment in the normal eye (Sander et al., 2001).

Caveolae, which are a special type of lipid raft, are 50- to 100-nm cell-surface plasma membrane invaginations first described almost 60 years ago (Palade, 1953; Yamada, 1955). After the identification of caveolin-1 as the principal constituent and marker protein of caveolae (Glenney and Soppet, 1992; Kurzchalia et al., 1992; Rothberg et al., 1992), these vesicles are now associated with many functions besides transcellular transport, including endocytosis (Anderson, 1993), regulation of cholesterol levels (Rothberg et al., 1990), sensing of flow (Milovanova et al., 2008) and signal transduction (Lisanti et al., 1994; Parton and Richards, 2003). There is also evidence that two distinct pools of caveolae exist that have different functions, endocytotic caveolae and transcytotic caveolae (Simionescu, 1988). At present, still little is know of the molecular mechanisms that direct these caveolae to their specific destinations. Endothelial cells, including those of capillaries of brain and retina (Klaassen et al., 2009; Virgintino et al., 2002), express high levels of caveolin-1 (Lisanti et al., 1994).

The membranes of caveolae have a specific lipid composition with high levels of glycosphingolipids, sphingomyelin and cholesterol, and contain many lipoproteins, receptors and signal-transducing molecules. Caveolin-1 is the main constituent of caveolar membranes in most cell types, and binds proteins through the

caveolin-scaffolding domain, which recognizes a common motif within these signaling molecules (reviewed in Gratton et al., 2004). The caveolar membranes contain receptors for transferrin, insulin, albumin, RAGE, LDL, HDL, interleukin-1, and vesicle-associated membrane protein-2 (VAMP-2; Wolburg, 2006). Signaling complexes that bind to caveolin-1 include heterotrimeric G proteins, members of the MAPK pathway, Src kinases, Raf, PKC, endothelin, platelet-derived growth factor (PDGF) receptors, endothelial nitric oxide (NO) synthase (eNOS), VEGFR2 (Labrecque et al., 2003) and TGF-β receptors (Santibanez et al., 2008).

Another protein that was discovered as an important constituent of caveolae is cavin (Vinten et al., 2005). This protein is located at the cytosolic side of mature caveolae at the plasma membrane, but not at the Golgi complex, and is required for the stabilization of caveoale at the plasma membrane (Hill et al., 2008; Liu and Pilch, 2008).

In general, transcytosis of caveolae is guided by a cascade of intracellular signaling involving various molecules. Budding and fission from the plasma membrane is dependent on dynamin, which is recruited to the neck of caveolae after phosphorylation by Src kinase (Oh et al., 1998; Shajahan et al., 2004). Intersectin regulates the GTPase activity of dynamin, necessary for "pinching off" the caveolae from the plasmalemma (Predescu et al., 2003). Trafficking across the cell is regulated, according to the "SNARE hypothesis", through regulation by v-SNAREs on vesicles and t-SNAREs on the target membranes. In endothelial caveolae, essential SNARE proteins are VAMP-2, N-ethylmaleimide-sensitive factor (NSF), syntaxin and soluble NSF-attachment protein (SNAP25; Klaassen et al., 2009; Mehta and Malik, 2006) (Fig. 7). Ultrastructural studies showed that thin protein structures form a "cap" or stomatal diaphragm on top of caveolar flasks in non-barrier endothelia (Schlingemann et al., 1985; Stan et al., 1999). A major component of these diaphragms is plasmalemma vesicle associated protein (PLVAP), also known as the PAL-E antigen or PV-1. Although PLVAP has been suggested to play a role in barrier breakdown (as is discussed in Section 5.2), its exact function in transcellular transport remains unknown.

Taken together, in the normal BRB, the transcellular pathway is the preferred route for active transport of macromolecules, facilitated by caveolae and receptor-mediated transport mechanisms. In the BRB, transcytosis is specifically different from non-barrier endothelium. Numbers of caveolae, particularly at the luminal membrane, and expression of their main constituent caveolin-1, albumin receptors and other molecules, are relatively low in barrier endothelium as compared to other endothelial types.

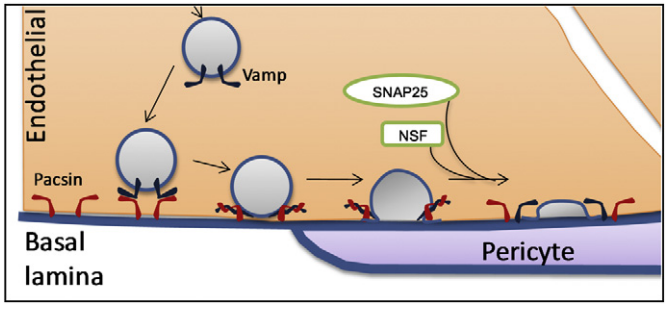

Fig. 7. Schematic overview of SNARE-mediated caveolar vesicle fusion to the abluminal membrane. SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins catalyze membrane fusion of vesicles with target membranes. Vesicles carry v-SNAREs, such as vesicle associated membrane proteins (Vamps) that pair with t-SNARE complexes, such as PKC and casein kinase substrate in neurons (Pacsin) at the target membrane to drive fusion. SNAP25 (synaptosome-associated protein of 25 kDa) and NSF (N-ethylmaleimide-sensitive factor) act together to disassemble SNARE complexes before and after fusion.

Furthermore, as macromolecules are the major determinants of differences in osmotic pressure between the intra- and extravascular compartment, tissue water homeostasis and edema formation in the retina are likely to be mainly regulated by direction and quantity of transcytosis. However, research on the relevance of the transcellular pathway in human BRB pathology is still at the very beginning.

3.2. The neurovascular unit: other cell types involved in regulation of the BRB

3.2.1. Pericytes

Pericytes play an important role in the BRB. They are extensively branched mural cells that partially cover capillary walls and are completely surrounded by a BL (Figs. 2 and 3). The role of pericytes in development and (patho)physiology was recently reviewed in detail (Armulik et al., 2011). Until recently, pericytes were considered to be of mesenchymal origin, but, lineage-tracing studies showed that pericytes in the central nervous system (CNS) have a different developmental origin than pericytes in other parts of the body and this may well be related with their specific functions in barrier induction and/or maintenance (Daneman et al., 2010; Korn et al., 2002). In vitro co-culture studies indicate that pericytes enhance barrier properties (Al-Ahmad et al., 2009; Deli et al., 2005; Dohgu et al., 2005; Cecchelli et al., 2007; Hori et al., 2004; Nakagawa et al., 2007, 2009; Wisniewska-Kruk et al., 2012). Ultrastructurally, the pericyte-to-endothelial ratio is relatively high in the retina (1:1) when compared to brain (1:3) or other microvascular beds (1:10) (Stewart and Tuor, 1994), suggesting important functions of pericytes in the BRB.

Pericytes are tightly wrapped around retinal capillaries, support them physically, and actively communicate with adjacent endothelial cells, astrocytes, microglia and neurons in a functional unit, called the neurovascular unit (Fig. 3). Endothelial cells and pericytes within a capillary are separated by a BL, but at some points they make direct contact through holes in the BL. These contacts are peg-socket contacts, containing cell-to-cell junction proteins including N-cadherin (Gerhardt et al., 2000), connexin 43 (Bobbie et al., 2010), and adhesion plaques, composed predominantly of fibronectin (FN; Díaz-Flores et al., 2009). Interactions between pericytes and endothelial cells are important for the maturation, remodeling and maintenance of the vascular system through autocrine and paracrine regulation by growth factors and modulation of the BL (Fisher, 2009). During early development and in angiogenesis, adhesion between endothelial cells and pericytes is mainly regulated by the release of chemotactic factors by endothelial cells, to induce pericyte recruitment and differentiation. On the other hand, pericytes secrete growth factors that regulate endothelial cell functions (Antonelli-Orlidge et al., 1989) or attract glial cells (Armulik et al., 2010).

Multiple ligand-receptor complexes are known to be involved in the intercellular signaling between the two cell types: PDGF subunit B (PDGF-B)/PDGFRβ, angiopoietin 1 (Ang-1)/tyrosine kinase receptor (Tie-2), stromal cell-derived factor-1 (SDF-1α)/C-X-C chemokine receptor type 4 (CXCR4), heparin binding epidermal growth factor (HB-EGF)/ErbB, Sonic hedgehog (Shh)/Patched (Ptc) receptor and TGF-β/TGF-βR (reviewed by Armulik et al., 2011; Gaengel et al., 2009).

Signaling by PDGF-B, secreted as a homodimer by endothelial cells, and PDGFRβ on the membrane of pericytes, is one of the best-studied interactions in which PDGF-B serves as an attractant for comigrating pericytes (reviewed by Andrae et al., 2008). Tip cells of angiogenic sprouts express higher PDGF-B levels than stalk cells, and pericytes are immediately attracted to angiogenic sprouts during development of the retinal vasculature (Gerhardt and

Betsholtz, 2003). Tight junction formation takes place immediately after recruitment of pericytes to endothelial cells (Kim et al., 2009).

Ang-1/Tie-2 interactions occur in the opposite direction, with Ang-1 expressed by pericytes and the tyrosine kinase receptor Tie-2 by endothelial cells. Ang-1 induces autophosphorylation of endothelial Tie-2 and promotes remodeling, maturation, barrier differentiation, and stabilization of blood vessels by recruiting pericytes and synthesis of extracellular matrix (Davis et al., 1996; Holash et al., 1999; Sato et al., 1995; Suri et al., 1996). On the other hand, Ang-2 is expressed by endothelial cells and is an antagonist of the Tie-2 receptor by inhibiting its autophosphorylation (Koh et al., 2002; Maisonpierre et al., 1997; Takagi et al., 2003). Ang-2 induces endothelial destabilization and promotes angiogenesis in the presence of VEGF (Asahara et al., 1998; Holash et al., 1999) (discussed in Section 5.4). In conditions of angiogenesis, destabilization by Ang-2 in the absence of VEGF leads to fragile vessels and vessel regression.

Thus, pericytes are important constituents of the BRB regulating homeostasis through communication with other cell types of the neurovascular unit.

3.2.2. Glial cells

In addition to pericytes, glial cells are also involved in regulating and maintaining the BRB. There are two major macroglial cell types in the retina, Müller cells and astrocytes (Fletcher et al., 2008). The somata of Müller cells are located in the inner nuclear layer and have processes that envelop all neurons and synapses extending from the inner to the outer limiting membranes. Astrocyte cell bodies and processes are almost entirely restricted to the nerve fiber layer of the retina (Schnitzer, 1988). Both types of glial cells have processes that wrap around retinal blood vessels (Fig. 8) forming a glia limitans, and they regulate a wide variety of endothelial cell functions. Glial cells and endothelial cells in the retina are separated only by a BL, in contrast to non-barrier capillaries that are surrounded by a peri-capillary space (Tao-Cheng et al., 1987).

The principal function of glial cells is the uptake of neurotransmitters from nerve terminals. However, glial cells also secrete neuroactive agents, including neurotransmitters, eicosanoids, steroids, neuropeptides, and growth factors such as VEGF (Bringmann et al., 2006; Gaucher et al., 2007). Glial cells are considered to play a critical role in the formation and maintenance of the BRB, in the uptake of nutrients and the disposal of waste products under normal conditions (Distler and Dreher, 1996; Reichenbach et al., 2007; Tout et al., 1993). Both physical contact and intercellular signaling are important in regulating the BRB (reviewed by Zlokovic, 2008). In cell cultures, endfeet-mediated contact with endothelial cells is required to increase barrier properties in vitro, such as formation of tight junctions (Gardner et al., 1997), increased mRNA expression of membrane transporters (Hayashi et al., 1997), increased TEER values and decreased paracellular transport (Gaillard et al., 2001; Nakagawa et al., 2009; Wisniewska-Kruk et al., 2012).

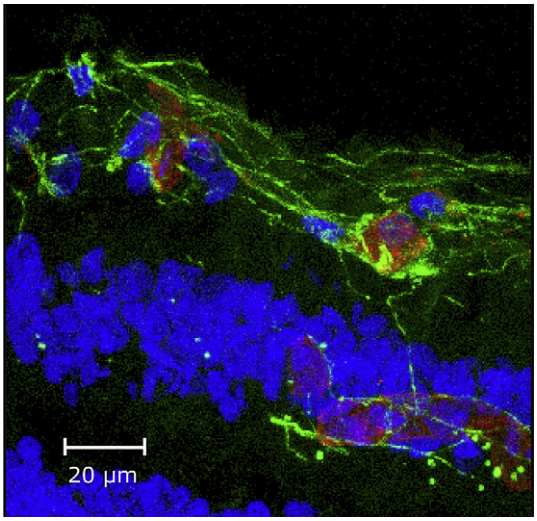

Fig. 8. Glial cells are wrapped around retinal blood vessels. Immunofluorescent staining of human retina with GFAP (green), a marker for glial cells, laminin (red), which stains the basal lamina around blood vessels, and DAPI (blue) nuclear staining. Blood vessels in the retina are wrapped by astrocytes. Bar = 20 µm.

In summary, macroglial cells (astrocytes and Müller cells) support the BRB, not only in a structural way but also by active communication between neural cells and vascular cells.

4. Mediators of BRB dysfunction and increased BRB permeability

Hyperglycemia, hypoxia, oxidative stress and/or inflammation are the main underlying processes in the human ocular diseases where dysfunction of the BRB is a major cause of loss of vision. In the later sections, their effects on BRB function and their use as an intervention target are discussed.

4.1. Diabetes and hyperglycemia-induced factors causing BRB alterations

Pre-clinical diabetic retinopathy (PCDR) is characterized by diffuse mild BRB loss that is not associated with edema formation, that is different from the localized profuse leakage in DME in the later clinical stage of NPDR. Hyperglycemia in diabetes is the major long-term determinant of vascular changes in DR. These initially lead to vasoregression and later to macular edema or neovascularization. Underlying molecular and cellular events are poorly understood, and various, often conflicting, theories exist. For example, several pathways have been shown to be involved in intracellular glucose toxicity, including non-enzymatic glycation, activation of PKC and activation of the polyol pathway (Brownlee, 2001, 2005; King et al., 1994; Lorenzi, 1992). Increased production of reactive oxygen species (ROS) in mitochondria in combination with impaired anti-oxidative defense systems has been proposed to be a common mechanism in these metabolic disturbances in endothelial cells caused by high glucose levels (Brownlee, 2001, 2005). Another effect of hyperglycemia is that plasma proteins, the BL and intracellular molecules undergo non-enzymatic glycation leading to formation of stable advanced glycation end products (AGEs) (Glenn and Stitt, 2009). AGEs can alter the function of intracellular and transmembrane proteins such as integrins and integrin receptors, disturbing crucial interactions with BL proteins. Glycated hemoglobin in red blood cells causes reduced oxygen delivery that may be compensated by an increased local blood flow (Ditzel, 1976).

AGE inhibitors, such as pyrodoxamine and aminoguanidine, were shown to successfully inhibit BRB dysfunction in diabetic animals (Hammes et al., 1991; Stitt et al., 2002; Hughes et al., 2007) but failed to delay progression of DR in patients (Bolton et al., 2004). Exposure of retinas to methylglyoxal (MGO), a precursor of AGEs, leads to BRB hyperpermeability (Giebel et al., 2005; Kim et al., 2012), as observed by increased albumin permeability and degradation of occludin. It has been speculated that occludin degradation is mediated by matrix metalloproteases (MMPs). Expression of MMPs is strongly induced by MGO (Kim et al., 2012) and was observed in animals with only 2 weeks of diabetes. MMPs can degradate VE-cadherin in vitro, and in experimental diabetes. Bastimastat (a potent MMP inhibitor)

prevented retinal vascular permeability and VE-cadherin breakdown (Navaratna et al., 2007), but this drug has not been tested in patients with DR.

In addition, several growth factors including VEGF-A, TGF-β, hepatocyte growth factor (HGF), PDGF, Ang-2 and the pro-fibrotic connective tissue growth factor (CTGF or CCN2) are induced by the diabetic milieu and are crucial for further development of (early) DR (Cui et al., 2007; Hinton et al., 2002; Hughes et al., 2007; Patel et al., 2005; Van Geest et al., 2010). VEGF-A is the best-studied inducer of ocular angiogenesis and vascular permeability (Schlingemann and van Hinsbergh, 1997; Siemerink et al., 2010; Witmer et al., 2003). It is involved in BRB loss and in the pathogenesis of many ocular diseases such as AMD and DME. In human DR, treatment with anti-VEGF agents significantly reduces the abnormal growth of microvessels and macular edema in DME (Aiello et al., 1995; Mitchell et al., 2011). In addition, anti-VEGF agents are generally used as adjuvant therapy to reduce bleeding during vitrectomy in eyes with PDR (Van Geest et al., 2012). The pro-angiogenic and permeability-inducing effects of VEGF on endothelial cells are mainly mediated by VEGFR2, whereas VEGFR1 has been identified as both a positive and negative regulator of VEGFR2 signaling depending on the tissue context (Bruns et al., 2009; Olsson et al., 2006). Both paracellular and transcellular pathways are affected in VEGF-induced vascular permeability, but only recently the exact molecular mechanisms have begun to be elucidated.

The role of *TGF-β* in BRB breakdown is complex and not fully understood, probably due to the multifunctional character and context-dependent actions of this growth factor. In the eye, TGF-β is overexpressed in the vitreous of patients with PDR and proliferative vitreoretinopathy (PVR) (Connor et al., 1989; Kita et al., 2007; Van Geest et al., 2010) and is also identified in proliferative membranes in these diseases (Bochaton-Piallat et al., 2000). Systemic inhibition of TGF-β causes numerous abnormalities in the retinal microcirculation, with impaired perfusion of the superficial vascular plexus and vascular leakage (Walshe et al., 2009), indicating that TGF-β has protective effects on vessel walls. However, TGF-β is also able to induce permeability in vitro by mechanisms that involve MMP9 (Behzadian et al., 2001) or Smad2 mediated RhoA activation (Lu et al., 2006).

Protein kinase C-beta (PKC-β) is a protein involved in intracellular signaling pathways that are increased by hyperglycemia (Aiello, 2002) and also acts as a downstream mediator of VEGF (Xia et al., 1996). In STZ-induced diabetic rats, the PKC-β inhibitor ruboxistaurin mesylate ameliorated diabetes-induced abnormalities, including retinal vascular permeability (Aiello et al., 1997; Ishii et al., 1996), but this compound failed to prevent DR progression significantly in humans (PKC-DRS Study Group, 2005; PKC-DMES Study Group, 2007). In vitro studies demonstrated that activation of PKC-β by VEGF is required for phosphorylation and reorganization of the tight junction complex, but inhibition of PKC-β could only partly prevent VEGF-induced vascular permeability (Harhaj et al., 2006), indicating the involvement of additional signaling pathways. Recently, a new atypical type of PKC-β inhibitor showed promising effects by preventing VEGF-induced retinal endothelial permeability in rats (Titchenell et al., 2012).

The kallikrein-kinin system (the KSS), a proteolytic complex stimulating the release of kinins such as bradykinin and kallidin, has also been recognized as a potential mediator of BRB loss in DME (Pruneau et al., 2010). This was based on indirect evidence, as components of the KKS are increased in the vitreous of patients with PDR (Gao et al., 2007), and by studies in experimental models of STZ-induced PCDR, where bradykinin, kallidin and their metabolites were found to increase vascular permeability via opening of tight junctions and increased transcellular permeability, possibly via a VEGF-independent manner (Gao et al., 2007; Pruneau et al., 2010). Clinical trials are underway to investigate whether this system is also involved in BRB loss causing DME.

Finally, hyperglycemia-induced endothelial cell dysfunction may also involve upregulation of expression of adhesion proteins on endothelial cells, and promote attachment of monocytes and leukocytes (leukostasis) that may cause vascular cell death (Joussen et al., 2001, 2002; Rahman et al., 2007). Furthermore, circulating endothelial progenitor cells, which are important for the repair of impaired vessels, are reduced in number in diabetic subjects as well as in healthy subjects exposed to high glucose levels (Chen et al., 2007; Churdchomjan et al., 2010).

In conclusion, several factors, including AGEs, VEGF, HGF, TGF-β, PKC-β and the KSS system, increased by the hyperglycemic milieu, may play a role in initiating the breakdown of the BRB in DR and DME, but their relative roles are poorly understood. The in vivo experimental evidence is often limited to the documentation of a role in diffuse BRB loss that is characteristic for PCDR and is observed in the STZ model of early diabetes. Direct extrapolation of such observations to mechanisms of BRB loss in human DME does not seem appropriate. In addition, although the cellular working mechanisms of most of these compounds are largely unraveled, inhibition in patients is either difficult to achieve or is associated with severe side effects. Anti-VEGF treatment is the most successful treatment at present, as it is now widely used in the treatment of DME whereas thus far the other factors have not been targeted therapeutically with success to any comparable extent.

4.2. Hypoxia, ischemia and oxidative stress

Diseases of the retina associated with impaired capillary or venous circulation lead to increased microvascular permeability and retinal neovascularization (D'Amore, 1994; Ferrara, 1995). Ischemia in retinal tissue in these conditions seems to be a prerequisite for production of vasogenic edema and an angiogenic response, which is probably triggered by hypoxia. Oxygen measurements in the retina of experimental animals and patients indeed have shown that the pO_2 is very low in ischemic retinal conditions (Arden et al., 2005; D'Amore, 1994; Hogeboom van Buggenum et al., 1996). Surprisingly, panretinal photocoagulation, which is an effective treatment modality for proliferative retinopathies, can restore retinal pO_2 levels, most likely through a decrease of outer retinal oxygen consumption and improved oxygen diffusion from the choroid (Hogeboom van Buggenum et al., 1996). Physiological retinal hypoxia varies throughout the day, as it is mainly due to dark-adapted rod cells that require more oxygen than any other cell type (Arden et al., 2005). The effects of hypoxic ischemic conditions and oxidative stress on the BRB have been accurately reviewed (Kaur et al., 2008), and therefore are not discussed here. However, we like to indicate that hypoxia-inducible factor-1 (HIF-1) may well play a role here.

HIF-1 is a crucial player in the regulation of cellular oxidative metabolism and mediates many adaptive endogenous mechanisms during hypoxia by transcriptional activation of specific target genes that function to restore oxygen supply and/or improve survival of cells in a hypoxic environment (Semenza, 2003; Ziello et al., 2007). These target genes include VEGF, glucose transporter 1 (GLUT1), erythropoietin-1 (EPO-1), carbonic anhydrase IX (CAIX), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Forsythe et al., 1996; Gleadle and Ratcliffe, 1997; Iyer et al., 1998; Jiang et al., 1996). Furthermore, HIF-1 activates the production of inducible nitric oxide synthase (iNOS) in glial cells (Kaur et al., 2006).

HIF-1 is a likely mediator of barrier disruption, probably through the actions of VEGF, and indeed inhibition of HIF-1α resulted in reduced vascular permeability (Choi et al., 2007; Yeh et al., 2007).

However, a recent study showed that despite prevention of barrier disruption, inhibition of HIF-1α did not prevent edema formation and neuronal damage (Yan et al., 2011).

It is generally assumed that HIF-1α is only expressed in hypoxia associated with pathological conditions, but we recently observed that HIF-1α is also active in the retina under normal physiological conditions (Hughes et al., 2010). Since many of the downstream effector genes of HIF-1α are neuroprotective, this suggests that HIF-1α may also regulate neuronal cell survival in the normal retina. These findings indicate that caution should be taken when considering HIF-1α as a therapeutic target for ocular diseases such as DR and AMD. The pleiotropic function of HIF-1α on vascular barrier integrity, and its consequences for therapeutic intervention, were recently described in detail elsewhere (Ogunshola and Al-Ahmad, 2012).

In conclusion, the threshold to develop pathological levels of hypoxia in the retina is probably relatively low as compared to other tissues, due to its relative hypoxic environment. HIF-1α, which is induced by hypoxia, is the main regulator of oxygen homeostasis and plays not only a role in stimulating angiogenesis in order to induce reoxygenation, but also in the survival of neuronal cells.

4.3. Inflammation and inflammatory mediators

In both the early and later stages of experimental DR in rodents, signs of inflammatory responses are observed: increased expression of inflammatory mediators, macrophage infiltration, increased leukocyte adhesion, complement activation, and acute phase response protein expression (Joussen et al., 2004; Zhang et al., 2002). These findings have been extrapolated to the clinical forms of human DR, which has been considered to be an "inflammatory disease" (Adamis and Berman, 2008) or has been described as a response to "para-inflammation" (Xu et al., 2009).

However, for human DR, much less evidence is available about the exact role of inflammation, particularly in PCDR. Most relevant results were obtained in the STZ-diabetes models in rodents, showing upregulation of adhesion molecules leading to leukostasis. However, other models, including the db/db mouse and spontaneous diabetes type 2 rhesus monkeys did not show increased leukostasis (Kim et al., 2005; Tadayoni et al., 2003) and in human PCDR and early NPDR, upregulation of leukocyte adhesion molecules was absent (Hughes et al., 2004). Clinical studies show that treatment with triamcinolone and other corticosteroids is effective in reducing DME (Paccola et al., 2008; Shimura et al., 2008) and this was interpreted as major evidence that inflammation plays an important role in DME. In the context of aquaporin-mediated macular edema (see Section 5.2.2), steroids prevented osmotic swelling of Müller cells (Reichenbach et al., 2007). However, a direct effect of steroids on BRB endothelium has not been ruled out as the main mechanism of reduced macular edema, given the well-known direct barrier-enhancing effect of steroids on cell culture models of the BBB or BRB (Deli et al., 2005; Nakagawa et al., 2009; Wisniewska-Kruk et al., 2012).

Several inflammatory factors have been investigated in models of early DR, mostly the STZ-induced diabetes model. Although inhibition of inflammatory factors is successful in these models to prevent diffuse BRB leakage and progression of PCDR, the effects in patients with DME were disappointing (Gardner et al., 2006; Suhler et al., 2005, 2009; Wu et al., 2011). *Histamine*, a strong inflammatory factor and neurotransmitter that causes blood vessels to leak, is increased in the retina of rats with experimental diabetes (Gardner et al., 1995). Studies in diabetic rats have shown that early diffuse vascular leakage in PCDR can be reduced with histamine receptor blockers, such as astemizole and loratidine (Hollis et al., 1992). A preliminary study showed that antihistamines also reduce BRB leakage in patients with NPDR (Gardner et al., 1995). However, in the Astemizole Retinopathy Trial, no beneficial effects were noted in patients with DME (Gardner et al., 2006), providing further evidence that results from the STZ-induced diabetes models cannot be extrapolated directly to human DME.

The procoagulant serine protease *thrombin* can amplify inflammation induced by other stimuli, either indirectly through generation of downstream mediators such as activated protein C, or directly via signaling through protease-activated receptors (PARs) that can bind thrombin, of which PAR-1 is best characterized with respect to endothelial permeability. Argatroban, a direct thrombin inhibitor, was shown to prevent increased vascular permeability after laser photocoagulation in rats (Musashi et al., 2005). However, argatroban has serious side effects and therefore cannot be used as therapeutic agent in DR.

In several studies investigating vitreous of human patients with NPDR or PDR, levels of *monocyte chemotactic protein (MCP)-1*, a chemokine with monocyte chemotactic activity, as well as inflammatory cytokines such as interleukin (IL)-6, IL-8, IL-1β, tumor necrosis factor-alpha (TNF-α) and interferon-induced protein-10 were reported to be elevated. In previous reports, IL-1β was shown to induce a number of alterations in the BRB which includes leukocyte recruitment, increased permeability, and changes in endothelial cell morphology (Brosnan et al., 1989; Martiney et al., 1990, 1992; Claudio et al., 1994). However, its mechanism of action is still unresolved. These observations suggest activation of inflammatory cells and activity of these cytokines in the human eye with DR (Abu el Asrar et al., 1992; Demircan et al., 2006; Elner et al., 1998; Funatsu et al., 2005; Murugeswari et al., 2008; Oh et al., 2010; Wakabayashi et al., 2011), and a possible role in DME (Funatsu et al., 2003; Funk et al., 2010).

TNF-α is an important mediator of (para-)inflammation in tissues under stress. It is also elevated in the vitreous of human eyes with established clinical DR (Demircan et al., 2006; Limb et al., 1996) and is implicated in inflammatory changes in early PCDR as well. TNF-α is an important mediator of leukostasis induced by VEGF, IL-1β, and platelet-activating factor in the rodent retinal vasculature (Vinores et al., 2007), and it also mediates apoptosis of retinal neurons and vascular endothelial cells in rodent PCDR (Joussen et al., 2009). Expression of TNF-α is elevated in diabetic animals (Joussen et al., 2002; Zhang et al., 2006) and its inhibition prevents the pathologic events of PCDR including BRB breakdown (Joussen et al., 2002). TNF-α has therefore been suggested to be a potential target in the treatment of DME. In humans with DME, systemic infusions with the TNF-α inhibitor infliximab led to visual improvement and decrease in central macular thickness in four of six eyes with refractory DME to macular laser photocoagulation (Sfikakis et al., 2005). Although no complications were observed in this small study, systemic TNF-α inhibition may have severe side effects (Suhler et al., 2005, 2009) indicating that local treatment may be a better approach. However, intravitreal injections with infliximab or adalimumab (another TNF-α inhibitor) did not show any beneficial effects in patients with refractory DME in a study including 39 eyes (Wu et al., 2011).

As indicated above, the long-term clinical efficacy of steroids, as single treatment or in combination with other treatment modalities, such as laser photocoagulation and anti-VEGF treatment, has been disappointing.

Taken together, it can be concluded that there is still insufficient evidence for the specific causal involvement of leukocytes and/or inflammatory cytokines in the development and progression of human PCDR. Inflammatory mechanisms most likely do play an aspecific role in para-inflammation associated with ischemia, as a response to protein leakage and hard exudates in DME, and in the

wound-healing response in PDR. However, the exact mechanisms and specific roles of the various cytokines and inflammatory factors still need to be resolved, as well as the therapeutic potential of these inflammatory aspects of DR.

5. Mechanisms of barrier breakdown

In BRB loss and leakage in pathological conditions, the specialized properties of the neurovascular unit regulating barrier integrity are altered. The endothelial cells of the normal BRB possess well-developed intracellular tight junctions, have few caveolar vesicles, located at their abluminal side, and express selective transporters, and all of these properties may be changed in BRB loss, but a true understanding of these mechanisms in human disease is still lacking. The tight junctions and increased paracellular transport have been studied in detail, but evidence is accumulating that caveolar transport is also a major mechanism of pathological vascular barrier breakdown. Besides endothelial cells, pericytes and macroglia contribute significantly to BRB maintenance as part of the neurovascular unit. Therefore, loss or dysfunction of these perivascular cells under pathological conditions may be another important cause of BRB breakdown.

5.1. Increased paracellular permeability

The paracellular pathway of transport is formed and regulated by tight junctions, adherens junctions and gap junctions. In general, junctional proteins are disrupted in pathological conditions in the eye, leading to defects in endothelial permeability. Disruption of tight junctions has been widely studied, in vitro and in animal models, including STZ-induced diabetes. However, only recently the exact molecular mechanisms have become clear.

5.1.1. Dynamic modulation of tight junctions

Disruption of tight junctions has been identified as a possible mechanism of BRB breakdown in a number of ocular pathologies, including DR, ROP, RVO, AMD, and inflammation. Many growth factors, cytokines and other mediators are involved in these pathologies (see also Section 4). Consequently, paracellular leakage has been proposed to induce macular edema leading to vision loss.

Reduced protein levels of occludin but not claudin-5 were found after 2 and 12 weeks in PCDR in STZ-induced diabetic rats (Antonetti et al., 1998; Barber and Antonetti, 2003; Brankin et al., 2005). Transcription of these genes was also reduced in this model, but not at all times, suggesting a temporal effect (Klaassen et al., 2009). Whereas claudin-5 mRNA levels were significantly reduced after 6 weeks, occludin was significantly reduced after 12 weeks of diabetes. Rats intra-ocularly injected with VEGF, as a model of early DR, showed reduced levels of occludin, but again claudin-5 protein levels were not decreased (Antonetti et al., 1999). However, we observed a significant downregulation of claudin-5 mRNA levels at 1 and 6 h after VEGF injection and had returned to basal levels after 24 h (unpublished results), indicating a transient effect. In cultured bovine retinal endothelial cells (BRECs) stimulated with VEGF, we also observed a transient downregulation of claudin-5, as well as of occludin and expression of other tight junction genes (Klaassen et al., 2009).

These findings support the concept that endothelial junctions are dynamic structures undergoing continuous remodeling and that they may not be irreversibly affected under diabetic conditions and in the presence of elevated levels of VEGF.

Among the various tight junction molecules, downregulation of occludin has been reported most consistently in the context of BRB breakdown, especially in relation with VEGF. VEGF signaling activates downstream proteins including PKC and Src family kinases (Scheppke et al., 2008) that induce phosphorylation, ubiquitination and internalization of occludin (see Section 5.1.2).

Glucocorticoids have a direct effect on tight junction protein expression, and this may explain their clinical potency to reduce macular edema. In vitro, hydrocortisone and dexamethasone enhance barrier integrity of retinal endothelial cells by upregulating claudin-5 and occludin protein expression (Antonetti et al., 2002; Felinski et al., 2008). Similar effects were observed in in vitro models of the blood-nerve and BBB barriers (Forster et al., 2008; Kashiwamura et al., 2011).

In summary, downregulation of tight junction mRNA and protein levels is a major mechanism in BRB breakdown. However, given that most findings have been obtained from models of early DR or from in vitro models with short duration exposure to barrier disrupting factors, it is not known whether in chronic BRB loss such as occurs in DME, tight junction disruption has an important role.

5.1.2. Phosphorylation of tight junctions

Molecular analysis suggests that the regulation of endothelial paracellular permeability involves phosphorylation of junctional molecules, which is followed by their internalization and degradation (Antonetti et al., 1999; Esser et al., 1998; González-Mariscal et al., 2008; Murakami et al., 2012). Several kinases and phosphatases were found to modulate phosphorylation of tight junctional proteins and vascular permeability in vivo and in vitro (reviewed by Dörfel and Huber, 2012). Increased endothelial barrier permeability is associated with increased phosphorylation of occludin and decreased amounts of occludin in the tight junctions indicating again that phosphorylation of occludin induces its removal from tight junction complexes (Antonetti et al., 1999). VEGF (Antonetti et al., 1999; Harhaj et al., 2006), MCP-1 (Stamatovic et al., 2006), cytokines (Hirase et al., 2001), oxidized phospholipids (DeMaio et al., 2006), PKC-β (Murakami et al., 2012), and shear stress (DeMaio et al., 2001) all induce phosphorylation of occludin and subsequent breakdown of the BRB. PKC-β activation by VEGF is necessary and sufficient for occludin phosphorylation in vitro (Harhaj et al., 2006). Five VEGF-inducible phosphorylation sites were identified on occludin (Sundstrom et al., 2009), of which the serine 409 phosphorylation site appeared to be responsible for VEGF-induced permeability (Murakami et al., 2009; Raleigh et al., 2011). STZ-induced diabetes in rats causes occludin phosphorylation in the retina as well (Harhaj et al., 2006). The integrity of the junctions can also be modified indirectly by interference in actin dynamics (e.g. by small GTPases such as Rho and Rac, or by cytochalasin D) (Bazzoni and Dejana, 2004; Harhaj and Antonetti, 2004; Spindler et al., 2010).

Taken together, the results described here suggest that the functions of the tight junction molecules are regulated by various protein kinases and phosphatases, and that phosphorylation induces removal of the proteins from tight junctional complexes leading to increased paracellular permeability.

5.1.3. Disruption of adherens junctions

Permeability-enhancing agents also alter adherens junctions. VE-cadherin and β-catenin protein expression in endothelial cells in vitro is decreased after exposure to VEGF (Kevil et al., 1998; Wright et al., 2002) and AGEs (Otero et al., 2001). Moreover, phosphorylation of the VE-cadherin/β-catenin complex is associated with increased endothelial permeability (Esser et al., 1998). When β-catenin is removed from this complex, it can translocate to the nucleus and regulate the activity of transcription factors (Lampugnani, 2012). It seems that proteolytic degradation of VE-cadherin rather than reduced expression is associated with increased permeability (Navaratna et al., 2007). VEGF-induced phosphorylation of VE-cadherin through VEGFR2 signaling

promotes the removal of VE-cadherin from adherence junctions and results in internalization, leading to impaired barrier function (Gavard and Gutkind, 2006).

Several growth factors counteract the effects of VEGF on VE-cadherin. Recently, it was shown that pigment epithelium-derived factor (PEDF) inhibits VEGF-induced permeability, through γ-secretase, both in cultured microvascular endothelial cell monolayers and in vivo in the mouse retinal vasculature (Cai et al., 2011a). PEDF acted by (a) prevention of dissociation of adherens and tight junction proteins and (b) by regulation of both the association of VEGF receptors with adherens junction proteins and the subsequent phosphorylation of the adherens junction proteins, VE-cadherin and β-catenin. The same group reported that placental growth factor (PlGF)-1 is able to prevent VEGF-induced permeability as well, by stabilizing VE-cadherin, but only 6 h after VEGF-exposure (Cai et al., 2011b).

In line with these observations, evidence is accumulating that VE-cadherin is a major player in the control of vascular permeability (Dejana et al., 2008; Vestweber, 2008). Clustering of VE-cadherin with growth factor receptors such as VEGFR2 (Cavallaro and Christofori, 2004) and TGF-β receptor (Rudini et al., 2008) is involved in keeping endothelial cells in a quiescent state. Moreover, endothelial cells are contact inhibited in their growth and lose the capacity to respond to growth factors when they reach confluence. For example, VEGF signaling was significantly reduced by confluence (Lampugnani et al., 2006). Normally, VEGF signaling is induced by internalization of VEGFR2 by its phosphorylation. In contrast to VE-cadherin internalization, which leads to its degradation, the internalization of VEGFR2 leads to activation of signaling. However, internalization of VEGFR2 was prevented by binding to VE-cadherin in confluent cells (Lampugnani et al., 2006). This suggests that breakdown of the endothelial barrier first needs an initial challenge that decreases adherens junction integrity and causes dissociation of VE-cadherin from VEGFR2, making the VEGF receptor available for signaling. However, in vitro results can only partly mimic the in vivo situation and do not take the differences between different types of vessels along the vascular three into account. Fortunately, recent work of Orsenigo et al. (2012) contributed to our understanding of VE-cadherin regulation in vivo. These researchers found that VE-cadherin is constitutively tyrosine phosphorylated in veins but not in arteries and that this is most likely mediated by shear stress induced by venous blood flow. The results gave new insight, since they indicate that tyrosine phosphorylation alone is not sufficient to cause barrier breakdown. The authors suggest that VE-cadherin phosphorylation may act as a priming mechanism to sensitize veins to permeability-inducing agents, which is in line with our interpretation of the study of Lampugnani described above.

Taken together, VE-cadherin and β-catenin are not only part of adherens junctions, but also play an important role in intracellular signaling and in the regulation of vascular permeability in the BRB. This introduces another level of complexity in the regulation of permeability of the BRB and needs to be studied in more detail.

5.2. Increased transcellular permeability

5.2.1. Changes in caveolar transport

There is growing evidence that BRB loss can occur in pathological conditions due to increased transcellular permeability, with or without changes in paracellular transport.

Evidence for VEGF-induced caveolar transcytosis in the BRB was first provided by Feng et al. (1999a, b). In cultured BRECs, VEGF increased permeability by stimulating caveolae-mediated transcytotic transport in an NO-dependent process (Feng et al., 1999b).

In an in vivo model in non-human primates, we observed increased numbers of vesicles at the luminal surface of BRB endothelial cells in VEGF-induced vascular permeability in the retina (Hofman et al., 2000). In this model, paracellular transport via opening of tight junctions of endothelial cells of the leaky retinal vessels was not observed. Our results indicated that increased permeability of the BRB as induced by VEGF is predominantly associated with increased vesicular transport. Particularly interesting is the finding that BRB loss is associated in this model with a shift of the distribution of caveolae from a predominantly abluminal localization to a predominantly luminal localization (Fig. 9; Hofman et al., 2000), which mimics the normal distribution of vesicles in non-barrier continuous endothelium. This phenomenon was also observed in retinal capillaries in rats treated with AGE-modified albumin. In these rats, increased numbers of caveolae at the luminal plasma membrane of retinal vascular continuous endothelium and intact tight junctions were found (Stitt et al., 2000), suggesting that AGEs can also induce permeability by increased transcytosis, possibly without affecting the paracellular pathway. It is tempting to speculate that the reversed distribution of caveolae in BRB loss is associated with an active reversal of transcytosis from a predominantly outward direction to an inward

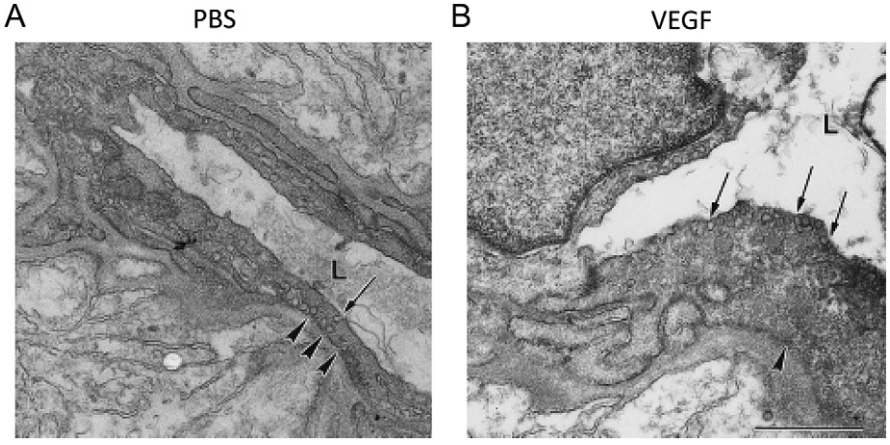

Fig. 9. VEGF induces a shift in pinocytotic vesicle localization. Electron micrographs of a retinal capillary in monkey eyes after 12 PBS injections (A), and after 12 VEGF (B) injections. Endothelium in the PBS-treated eye shows more pinocytotic vesicles at the abluminal side (arrowheads) than at the luminal side of the endothelial cell (arrows, A). In contrast, more pinocytotic vesicles are located at the luminal side (arrows) than at the abluminal side of cells (arrowheads) in the VEGF-treated eye (B). Lumen (L). Bar = 1 μm. Reproduced from Hofman et al. (2001a).

direction carrying proteins preferentially towards the retinal tissue interstitium.

In addition, Lightman and Greenwood (1992) showed that damage to the retinal endothelial cell tight junctions did not occur at any stage of experimental autoimmune uveoretinitis, but that vesicular transport was the mechanism of protein extravasation. In STZ-induced diabetes in rats, increased endocytosis of horseradish peroxidase (HRP) was shown in retinal endothelial cells (Gardiner et al., 1995), whereas no HRP was detected within the junctional complexes, intercellular clefts or the vascular BL.

Outside the eye, it was shown that in the vasculature of the blood–air barrier in lung alveolae of diabetic rats, increased expression of caveolin-1 and increased permeability were associated with elevated albumin transcytosis, whereas paracellular transport remained unchanged (Pascariu et al., 2004).

In summary, a large number of studies indicate that increased vascular permeability induced by VEGF or AGEs in the retina is associated with increased caveolar transport, with a shift in caveolar distribution to a predominantly luminal membrane localization, possibly without affecting the paracellular pathway.

Caveolin-1-knockout mice have been studied in an attempt to elucidate the role of caveolae in transcytosis in more detail, but this has shown that caveolar transport is more complex than previously assumed. Caveolin-1-deficient mice are viable and fertile but indeed lack caveolae and show various types of vascular dysfunction, including impaired NO and calcium signaling (Drab et al., 2001; Medina et al., 2006; Park et al., 2002; Razani et al., 2001; Schubert et al., 2007). Caveolin-1-null mice are also systemically hyperpermeable for plasma albumin (DeWever et al., 2007; Rosengren et al., 2006; Schubert et al., 2002). This was an unexpected finding, since caveolae were presumed to be necessary for transcellular transport of plasma proteins. The barrier function of the lung microvasculature was heavily compromised in caveolin-1-null mice, and the endothelial cells showed loss of caveolae-mediated transcytosis, which was compensated by an increased paracellular transport of macromolecules such as albumin (Schubert et al., 2002). Alternatively, alterations in capillary pressure or the glycocalyx were suggested as a cause for the increased paracellular leak (Rosengren et al., 2006). On the other hand, others failed to find any changes in adherence junctions in hyperpermeable abdominal wall capillaries or skin capillaries in caveolin-1-null mice (Rosengren et al., 2006). These discrepancies suggest that the vascular beds of different tissues respond differently to the lack of the caveolin-1 gene and protein.

In experimental studies, it is difficult to separate the contribution of transcellular transport from paracellular transport, for several reasons. First, caveolae are not only involved in transcytosis but also in processes such as endocytosis, regulation of cholesterol levels, sensing of flow and signal transduction (Simionescu, 1988). Secondly, tight junctions are localized at cholesterol-enriched regions associated with caveolin-1 (Nusrat et al., 2000). This means that caveolin-1 not only plays a role in transcellular transport, but that it is also associated with tight junctions. Suppression of caveolin-1 expression by siRNA in an in vitro model of the BBB not only affected the transcellular pathway but also affected the paracellular pathway. In this study, occludin, VE-cadherin and β-catenin were present in lower amounts and were dissociated from the cytoskeleton, whereas paracellular permeability was increased (Song et al., 2007). Another recent study has also provided evidence that transcellular and paracellular vascular leakage are co-regulated via dynamin and Rac (Armstrong et al., 2012).

Taken together, caveolin-1 undoubtedly plays a role in vascular permeability. However, its role may be different depending on type of endothelium or tissue. Furthermore, overexpression of caveolin-1 does not affect the paracellular pathway, whereas inhibition of caveolin-1 does, possibly by providing an alternative route of transport. This suggests that caveolin-1 is important for both transport via the transcellular pathway and for regulation of expression of tight junctional proteins and thus paracellular transport.

More evidence for a role of transcellular transport in the BRB comes from studies on another protein associated with caveolae, plasmalemma vesicle associated protein 1 (PLVAP), also known as PV-1 (Schlingemann et al., 1985; Stan et al., 1999), which was identified as the ligand of the human endothelium-specific monoclonal antibody PAL-E (Niemela et al., 2005; Schlingemann et al., 1985) and the mouse-specific monoclonal MECA-32 (Hallmann et al., 1995). Although its function is unknown, expression of PLVAP is completely lacking in barrier endothelia in brain, the eye and testis (Hnasko et al., 2002; Schlingemann et al., 1988, 1997). In pathological conditions, such as brain tumors, DME, and experimental VEGF-induced retinopathy, its expression co-localizes with barrier loss at the individual capillary level (Carson-Walter et al., 2005; Hofman et al., 2001a; Ruiter et al., 1993; Schlingemann et al., 1988, 1991, 1999; Shue et al., 2008; Strickland et al., 2005). PLVAP is induced by VEGF in vivo (Hofman et al., 2001a) and in vitro (Klaassen et al., 2009; Strickland et al., 2005). This suggests that PLVAP is a marker and possibly functional determinant of BRB disruption (Witmer et al., 2002). This is further supported by our studies in which PAL-E staining was positively related in DR with vascular leakage in individual capillaries, as recognized by the presence of fibrinogen, albumin and IgG around these capillaries as endogenous markers of vascular leakage (Schlingemann et al., 1999). Taken together, the observations on PV-1 support a role of transcellular vascular permeability via caveolae in BRB dysfunction.

On a completely different note, a role for the transcellular pathway may also be predominantly involved in BRB pathology associated with leukocyte diapedesis. In the BBB, mononuclear cells do not only migrate via the paracellular pathway, as has always been assumed, but also penetrate directly through the cytoplasm of endothelial cells (Engelhardt and Wolburg, 2004; Muller, 2011; Wolburg et al., 2005). Extravasation through intercellular junctions is a rapid and regulated process, during which the leukocyte squeezes through the cleft (diapedesis), followed by rapid junctional restoration. Transcellular diapedesis enables the leukocytes to cross the BRB without disrupting adhesion and tight junction integrity. Leukocytes enter the endothelial cell with the luminal membrane closing over it before it creates an opening in the abluminal membrane, so a fluid-filled channel is never created and the permeability barrier remains intact (Wolburg et al., 2005; Carman and Springer, 2008).

These data show that during inflammation, leukocytes are able to transverse the endothelial wall via both transcellular and paracellular routes (Anthony et al., 1997; Bolton et al., 1998). However, in experimental rat models of autoimmune uveoretinitis and in IL-1β-mediated acute inflammation, lymphocytes, granulocytes and monocytes cross the BRB exclusively via a transcellular pathway (Bamforth et al., 1997; Greenwood et al., 1994). These findings suggest that the transcellular route is used preferably by leukocytes to cross the BRB.

Finally, the formation of extracellular edema in the retina is associated with vascular leakage due to BRB breakdown and the specific anatomy of the retina. The predominant fluid accumulation in the retinal layers around the fovea has been attributed to the specific retinal anatomy, where the central macula is (relatively) avascular. Retinal pigment epithelium has a limited capacity to remove water from the retina to the choroid, and movement of proteins out of the retina into the vitreous is restricted by the external limiting membrane. Starling's rules, which govern the movement of fluids across capillary walls, apply to this type of

edema (Cunha-Vaz and Travassos, 1984). According to these rules, an increase in tissue osmotic pressure and/or an increase in luminal hydrostatic pressure can lead to edema. Theoretically, large plasma proteins play the most important role in formation of tissue edema. When large plasma proteins cross the BRB by leakage, tissue osmotic pressure increases. In contrast to smaller solutes, large plasma proteins do not easily diffuse across the vascular wall via the paracellular route, but are actively transported by caveolar vesicles, implying that, theoretically, altered (direction of) transport via the transcellular route plays the major role in the formation of edema.

In conclusion, in this section we have provided evidence that BRB damage in ocular disease does not only involve paracellular transport by changes in tight junctions but is also caused by altered transcellular transport. In certain conditions the transcellular route appears even exclusively affected. In experimental models of BRB loss, the numbers of caveolar vesicles are increased and caveolae are shifted from the abluminal to the luminal side of endothelial cells, reflecting the characteristics of permeable non-barrier endothelium. In DME, extravasation of large plasma proteins via the transcellular route is likely to be the main cause of edema formation.

5.2.2. Modulation of aquaporins

As discussed above, pathological edema of the retina is generally considered to be caused by leakage of plasma solutes through a dysfunctional BRB into the retinal interstitium. Recently, evidence for an alternative theory was presented, suggesting that through interactions of retinal endothelial cells and Müller glia cells, changes in regulation of water homeostasis occur in the retina, involving aquaporin 4 (AQP4). This would cause intracellular edema and swelling of Müller cells. AQP4 knockdown in retinas of diabetic rats leads to exacerbation of retinopathy, including enhanced retinal vascular permeability, increased retinal thickness, and elevated levels of pro-inflammatory factors, VEGF and glial fibrillary acidic protein (GFAP) (Cui et al., 2012).

The aquaporins are integral membrane proteins. Their main function is to transport water across cell membranes in response to osmotic gradients, and are therefore also called “osmotic sensors”. AQP1 is expressed in microvascular endothelia of all mammalian tissues except in brain and retina (Nielsen et al., 1993; Hasegawa et al., 1994; Verkman, 2006; Motulsky et al., 2010), as well as in proliferating microvessels in various tumors (Endo et al., 1999; Saadoun et al., 2002; Vacca et al., 2001; Verkman et al., 2008). This suggests that AQP1 is associated with non-barrier type of permeability. In non-barrier endothelium, at least 40% of water transport is regulated by aquaporins (Michel and Curry, 1999). The predominant AQP in the mammalian retina is AQP4, which is expressed by the Müller glia cells (Goodyear et al., 2008; Hamann et al., 1998) with the highest density at their perivascular and perisynaptic membrane domains (Bosco et al., 2005; Da and Verkman, 2004; Nagelhus et al., 2004).

Two studies reported that diabetes causes an alteration in the type of AQPs that surround the superficial vessels of the rat retina. These vessels are surrounded by AQP4 in the normal retina and by AQP1 in diabetic retina (Fukuda et al., 2010; Iandiev et al., 2007).

Dysregulation of water movement across the glio–vascular interface (the space between the glial and endothelial cells that is used for their communication) may cause edema. Under normal physiological conditions, Müller cells absorb fluid from the retinal tissue and secrete it into the blood by a co-transport of water, facilitated by AQP4 (Goodyear et al., 2009; Nagelhus et al., 1999) and osmolytes, especially potassium ions facilitated by Kir4.1 channels (Kucheryavykh et al., 2007). This ion channel is compromised very early in experimental diabetes in rats (Pannicke et al., 2006) and in a model of branch retinal vein occlusion in rats (Rehak et al., 2009).

In conclusion, under pathological conditions, such as retinal ischemia, ocular inflammation, retinal detachment, and diabetes, fluid transport through Müller cells is disturbed and has been suggested to lead to intracellular edema by massive swelling of the Müller cells (Reichenbach et al., 2007). The corticosteroid triamcinolone acetonide was not only found to inhibit vascular leakage but also to prevent osmotic swelling of Müller cells (Pannicke et al., 2006). Whether this is a result of restored water management by glial cells or by restored BRB integrity by mechanisms in endothelial cells, or both, is still unclear. More research is necessary to clarify the exact function of aquaporins in BRB disruption and DME.

5.3. Endothelial cell damage or death

In contrast to the above described adaptative changes in an otherwise intact endothelial layer, it has often been suggested that in DR, retinal endothelial cell loss or structural damage, i.e. as occurs in microaneurysms, may also be a direct cause of BRB dysfunction (Cunha-Vaz, 1983; Gardner et al., 2002; Joussen et al., 2007). Death of endothelial cells was observed in diabetic humans and rats, before any other retinal histopathology was evident (Mizutani et al., 1996), and finally leads to formation of ghost capillaries. Oxidative stress, leukostasis, endothelial progenitor dysfunction and senescence are underlying mechanisms of retinal endothelial cell death. AGEs accumulate as a consequence of diabetes and in turn generate ROS. Oxidative stress plays a major role in DR and sustained production of ROS is believed to lead to apoptosis of retinal endothelial cells (Brownlee, 2005; Hammes et al., 2003). Finally, it has been postulated that, due to the hyperglycemic and hypoxic environment, endothelial cells have a higher proliferation rate than under normal conditions and may end up in a state of replicative senescence, an irreversible cell cycle block (Mizutani et al., 1996; Von Zglinicki, 2000). Cells trapped in replicative senescence function differently, and some senescent cell types are more likely to undergo apoptosis when exposed continuously to oxidative stress (Demerath et al., 2004; Edo and Andres, 2005; Stefanec, 2004).

However, the exact relation between microvascular cell death and early BRB loss in PCDR and vascular leakage in DME has not been established.

5.4. Pericyte loss and dysfunction

In recent years, interesting new insights have emerged with respect to the contribution of pericytes to the regulation and integrity of the BRB. Density and relative surface coverage of pericytes on capillaries are positively correlated with endothelial barrier properties (Stewart and Tuor, 1994). Furthermore, several in vitro co-culture models have shown that addition of pericytes increases the TEER of endothelial cell monolayers (Al-Ahmad et al., 2009; Cecchelli et al., 2007; Deli et al., 2005; Dohgu et al., 2005; Hori et al., 2004; Nakagawa et al., 2007, 2009; Wisniewska-Kruk et al., 2012). The TEER reflects the physiological endothelial membrane electrical potential, which is determined by both paracellular and transcellular “pores”, but is mostly regarded as a measure for the tightness of endothelial junctions. Therefore, the positive effects of pericytes on TEER have mainly been attributed to modulation of junctional integrity by pericytes. However, we recently found a much greater reduction in permeability of large tracers (FD40) than of small tracers (Cy3) in a co-culture of retinal endothelial cells and pericytes, indicating a lesser involvement of the paracellular “pores” (Wisniewska-Kruk et al., 2012). Other recent in vivo studies in BBB development and in adult mice strengthen

the idea of regulation of transcellular permeability by pericytes (Armulik et al., 2010; Bell et al., 2010; Daneman et al., 2010). All these studies showed that brain vessel permeability was directly correlated to the density of pericytes. Increased permeability due to low density of pericytes was not caused by reduced tight junctional integrity, but by upregulated endothelial transcytosis, as increased numbers of cytoplasmic vesicles and endocytosis of biotin was demonstrated (Daneman et al., 2010). During aging, the pericyte-deficient mice reduce cerebral blood flow. This causes diminished perfusion of the brain capillaries and BBB breakdown accompanied with deposition of plasma proteins (Bell et al., 2010). The finding that pericyte loss has an effect on blood flow is interesting, since it suggests that the contractile properties of pericytes are responsible for regulation of capillary blood flow. Contraction of pericytes may thus ultimately lead to BBB breakdown. Besides these physical properties of pericytes, also soluble factors have been suggested to play a role in their ability to maintain the BBB. Microarray analysis of pericyte-deficient blood vessels identified upregulated expression of several permeability-related factors, including VEGF-A, Ang-2 and PLVAP, whereas Ang-1 was downregulated (Armulik et al., 2010; Daneman et al., 2010).

The role of pericytes in the retina during development of the BRB or during retinal pathology is less clear than in brain. In DR, pericyte loss is an early feature. In an endothelium-specific knockout of PDGF-B, reduced pericyte coverage of the endothelium coincided with a wide range of retinal microvascular abnormalities that mimic DR, such as diameter variability of microvessels, microaneurysms, increased vascular regression and proliferative retinopathy (Enge et al., 2002). These findings support the theory that loss of pericytes contributes to the progression of DR. How diabetes causes pericyte loss is still poorly understood. Ang-2-deficient mice fail to develop hyperglycemia-induced pericyte loss and/or migration, whereas overexpression of Ang-2 induces pericyte migration and vascular pathology as is found in DR (Pfister et al., 2010). Production of Ang-2 is upregulated by hypoxia, VEGF and hyperglycemia (Mandriota and Pepper, 1998; Oh et al., 1999; Rangasamy et al., 2011). In the vitreous of patients with DR, both Ang-1 and Ang-2 levels are increased, but concentrations of Ang-2 were twice as high as that of Ang-1 (Patel et al., 2005). Therefore, pericyte loss may not only be the result of apoptosis, but also of migration of pericytes (Pfister et al., 2008), induced by Ang-2.

These observations suggest that increased Ang-2 levels in DR prevent adhesion of pericytes on endothelial cells by occupying the Tie-2 receptor. This causes inhibition of binding of Ang-1, leading to decreased production of PDGF-B (Fig. 10). Supporting evidence comes from a study in Ang-1-knockout mice (Suri et al., 1996). Endothelial cells of developing blood vessels in these mice show a poor association between endothelial cells and surrounding perivascular cells and matrix, suggesting that Ang-1 indeed is necessary for adhesion of pericytes to endothelial cells via its effect on endothelial cells.

Ang-2 and Ang-1 may also have direct effects on endothelial junctions in the BRB (Rangasamy et al., 2011). Intravitreal injection of Ang-2 in rats increased retinal vascular permeability, and exposure of cultured human retinal endothelial cells to Ang-2 increased permeability, decreased VE-cadherin expression and the formation of intercellular gaps. Ang-2-induced changes were associated with increased phosphorylation of VE-cadherin (Rangasamy et al., 2011), whereas Ang-1 prevented VE-cadherin phosphorylation (Gavard et al., 2008; Lee et al., 2011).

In summary, Ang-1 and Ang-2 appear to have opposite effects in the retina, on adhesion of pericytes to endothelial cells, phosphorylation of VE-cadherin and vascular permeability. The

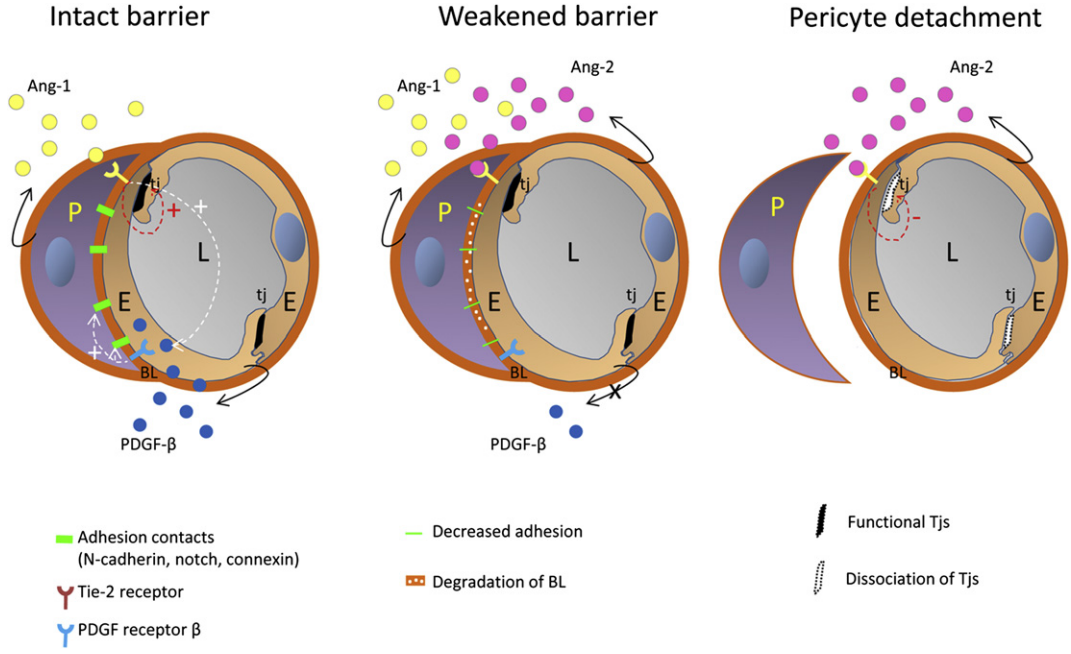

Fig. 10. Hypothetical model of the role of retinal pericyte detachment in BRB loss. Left panel: in an intact barrier, angiopoietin (Ang)-1 is produced by pericytes, which binds to the Tie-2 receptor. Activation of the Tie-2 receptor leads to strengthening of endothelial junctions (tight junctions and adherens junctions) and production of PDGF-β in endothelial cells. Binding of PDGF-β to its receptor results in strengthening of adhesion contacts between pericytes and endothelial cells. Middle panel: During retinal pathology endothelial cells start to produce Ang-2. Ang-1 and Ang-2 are now competing for binding to the Tie-2 receptor. When Ang-2 binds to Tie-2, no downstream activation of Tie-2 takes place. This results in reduced stabilization of junctional complexes and reduced PDGF-β production, consequently leading to decreased intercellular adhesion and degradation of the basal lamina between pericytes and endothelial cells. Right panel: in a situation where Ang-2 becomes dominant, Ang-2 completely blocks signaling through the Tie-2 receptor. As a result, endothelial junctions are no longer stabilized and VE-cadherin is phosphorylated, leading to internalization of VE-cadherin and increased vascular permeability. Production of PDGF-β is now completely stopped, resulting in downregulation of adhesion contacts, which may lead to pericyte detachment. BL, basal lamina; E, endothelial cell; L, lumen; P, pericyte; tj, tight junction complex.

mechanisms are complex and dependent on the context, such as local VEGF levels, but these mechanisms appear to have an important role in PCDR and the associated diffuse BRB loss.

TGF-β may also play a role in the interactions between pericytes and endothelial cells regulating BRB integrity. In vitro studies have demonstrated that contact between endothelial cells and pericytes leads to activation of latent TGF-β (Garcia et al., 2004; Murphy-Ullrich and Poczatek, 2000) which induces antiproliferative and antimigratory responses. These responses are even further increased in the presence of VE-cadherin (Rudini et al., 2008). Thus, the actions of TGF-β seem to induce tight association of pericytes to endothelial cells, thereby increasing BRB integrity.

Pericytes are surrounded by the capillary BL and this BL may have an important role in BRB function. In diabetes, components of the BL undergo glycation and AGE formation due to high glucose levels (Beltramo et al., 2002). The glycation of proteins as well as the structural and compositional changes of the BL are likely to act synergistically on the permeability of the BL. High glucose-induced accumulation of extracellular matrix and altered composition underlie structural and functional changes that allow increased permeability (Chronopoulos et al., 2011; Oshitari et al., 2006; Roy et al., 2003). Several matrix components of the BL are altered during diabetes. These alterations may lead to decreased binding of pericytes to endothelial cells and increased vascular permeability. BL of retinal capillaries in diabetic rats contain increased amounts of collagen IVα1, laminin-β1, laminin-γ and fibronectin, as early as 8 weeks after induction of diabetes, indicating increased expression of matrix components (Nishikawa et al., 2000). Pericytes produce increased amounts of pro-fibrotic proteins, such as CTGF and TIMP-1 under diabetic conditions and due to high levels of VEGF, causing BL thickening (Hughes et al., 2007; Kuiper et al., 2004, 2006, 2007, 2008a, b; Van Geest et al., 2010; Witmer et al., 2004). In STZ-induced diabetic CTGF$^{+/-}$ mice, BL thickening was prevented as compared to wild type mice (Kuiper et al., 2008b), suggesting an important role for CTGF in this process. Increased levels of CTGF and glycation of retinal matrix components reduce adhesion of pericytes that in turn destabilizes capillaries. Combined downregulation of the expression of fibronectin, laminin and collagen type IV using antisense oligos injected into the eyes of rats with STZ-induced diabetes reduced vascular leakage (Oshitari et al., 2006). Cultured retinal pericytes exposed to AGEs showed increased expression of Cyr61 and CTGF, two members of the CCN family of genes, which was accompanied with pericyte detachment and subsequent cell death (Liu and Pilch, 2008). Taken together, these studies suggest that alterations of the BL result in increased BRB permeability in diabetes and other conditions, which may be related to detachment and migration of pericytes from the BL.

It can be concluded that pericytes have an important role in stabilizing retinal capillaries and in keeping their endothelial cells in a quiescent and differentiated state. In pathological conditions associated with pericyte loss or dysfunction, such as PCDR, loss of pericytes may be an important factor in BRB loss.

5.5. Loss of glial cells

Abnormalities in glial cell functions are also associated with retinal pathology, including neuronal dysfunction and death, retinal swelling and breakdown of the BRB (Bringmann et al., 2006; Dyer and Cepko, 2000). Under pathological conditions, glial cells are activated and overexpress angiogenic cytokines such as VEGF, basic fibroblast growth factor, TNF-α and MMPs (Behzadian et al., 2001; Bringmann et al., 2006). Recently, a direct relation between glial disruption, increased levels of VEGF and breakdown of the BRB was reported (Shen et al., 2010).

It has also been suggested that overexpression of angiogenic cytokines by glial cells may directly induce breakdown of the BRB via proteolytic degradation of tight junctional proteins such as occludins and claudins (Barber et al., 2000; Eichler et al., 2000; Giebel et al., 2005; Kaur et al., 2006; Shen et al., 2010; Tretiach et al., 2005).

As described in Section 5.2.2, glial cells, and especially Müller cells, may also contribute to edema formation due to their role as controllers of water homeostasis in the retina. Under normal physiological conditions, Müller cells transport fluid from retinal tissue into the blood by a co-transport of water, facilitated by AQP4 (Goodyear et al., 2009; Nagelhus et al., 1999). Under pathological conditions, such as retinal ischemia, ocular inflammation, retinal detachment, and diabetes, fluid transport through Müller cells is disturbed, and this was suggested to contribute to retinal degeneration and intracellular edema formation (Reichenbach et al., 2007). In fact, these authors even postulate that cystoid macular edema is caused by massive swelling of Müller cells, in contrary to the common idea that retinal edema is associated with increased extracellular fluid.

In summary, activation of glial cells in pathological conditions results in overexpression of angiogenic cytokines that disrupt the BRB, and, although controversial, in altered regulation of water homeostasis that leads to edema.

5.6. Loss of the endothelial glycocalyx

Loss or dysfunction of the endothelial glycocalyx may also contribute to increased vascular permeability and inflammation (Elkin et al., 2001). The glycocalyx is a network of membrane-bound proteoglycans and glycoproteins at the luminal side of endothelium, which constitutes the first permeability barrier for plasma proteins and adhering leukocytes (Vink and Duling, 1996, 2000). The glycosaminoglycan hyaluronan in the glycocalyx has been shown to be a principal determinant of vascular permeability because selective removal of hyaluronan by hyaluronidase from the vessel wall causes a profound increase in macromolecular transport (Henry and Duling, 1999). Second, elevated hyaluronidase activity increases vascular permeability in mouse models (Ikegami-Kawai et al., 2004; Wang and Hascall, 2004).

Under hyperglycemic conditions, the glycocalyx is significantly reduced in thickness. This coincides with endothelial dysfunction and a general systemic increased vascular permeability in humans (Nieuwdorp et al., 2006). Recently, we found that type 2 diabetes is associated with glycocalyx perturbations and increased vascular permeability in ocular tissues, which can be partially restored by administration of sulodexide, a compound that restores the glycocalyx and attenuates hyperglycemia-associated endothelial permeability (Broekhuizen et al., 2010).

These findings suggest that an intact glycocalyx is necessary for the normal function of the BRB, and that dysfunction of the glycocalyx by itself may be sufficient for BRB loss. However, the exact role and relative contribution of the glycocalyx to BRB function in normal and pathological conditions such as PCDR and DME remains largely unknown.

6. Future perspectives

6.1. Therapeutic modulation of the BRB

The present treatment modalities for macular edema are laser therapy in DR and branch retinal vein occlusion, and intravitreal injections with corticosteroids and anti-VEGF agents (reviewed in Wenick and Bressler, 2012; Witkin and Brown, 2011) in several

conditions. Corticosteroids and anti-VEGF agents have shown efficacy in reducing macular edema, suggesting involvement of VEGF-A and/or inflammatory mechanisms in the underlying pathophysiology. However, as indicated above, the exact working mechanisms of corticosteroids remain elusive, and a direct effect on endothelial cells of the BRB, independent of inflammation, may play a crucial role. In addition to these local treatments, DME can also be modulated by systemic factors such as reduced blood pressure and vascular overload. The success of steroids and anti-VEGF has led to the development of a large number of new compounds that are aimed at the treatment of DME (Table 3) (for example, Callanan and Williams, 2008; Sivaprasad et al., 2012; partially reviewed in Wenick and Bressler, 2012).

Most of these new drugs are now in phase I or II clinical studies and are targeted, by various approaches, to inflammatory mechanisms. Unfortunately, an adequate model of human DME, which would be characterized by capillary loss, retinal ischemia and retinal edema, with or without microaneurysms, is lacking. For this reason, the pre-clinical data on which these clinical studies are based is often limited to results obtained in experimental models of rodent PCDR (1–6 months Streptozotocin-induced diabetes). However, such models only allow studies of the effects of potential drugs on leukocyte adhesion and other signs of para-inflammation, and diffuse BRB breakdown. Therefore, they may be inadequate for predicting the desired inhibition of established clinical leakage and edema resulting from massive BRB breakdown as occurs in human DME.

6.2. Enhanced drug delivery through the BRB

On a completely different note, strategies to improve delivery of systemic drugs across the BRB are being developed, now that the understanding of the function of the BRB is increasing (reviewed in Hosoya et al., 2011). At present, drug delivery to the retina is possible, but the techniques used have their disadvantages (reviewed by Chen and Liu, 2011). Topical administration using eye drops is hindered by a low diffusion (<5%) through the cornea into the vitreous (Geroski and Edelhauser, 2000). Drug delivery by intraocular injections is easy and widely used. However, in addition to being a burden for the patient, multiple injections carry an increasing risk of endophthalmitis (Myles et al., 2005).

Systemic drug delivery to the retina is limited by the strict barrier properties of both the inner (retinal capillaries) and outer BRB (retinal pigment epithelium). Strategies currently employed to enhance drug delivery over the BBB may be applicable in the eye as well. Basically, two methods have been described in the literature to enhance drug delivery across the BBB: employing endogenous transport systems and reversible disruption of BBB.

6.2.1. Modulation of endogenous BRB transporters

The strategy to use endogenous transport systems for the delivery of drugs over the BBB has been shown to be successful in experimental models. For example, GLUT1 has been used for transport of glycosylated neuropeptides such as L-serinyl-β-D-glucosed analogues of Met^5 -enkephalin over the BBB (Polt et al., 1994). GLUT1 is also expressed at both the luminal and abluminal membranes of the inner BRB and on the brush border and basolateral membranes of retinal pigment epithelial cells (Takata et al., 1992). So, this mode of facilitated transport using GLUT1 may also be effective in the BRB, but this has as yet not been reported. L-dopa, a drug used in clinical treatment of Parkinson's disease, is efficiently transported across both BRB and BBB by the influx transporter LAT1, also known as SLC7A5 (solute carrier family 7 (amino acid transporter light chain, L system), member 5) (Hosoya et al., 2010). Other influx transporters, such as ENT2, RFC1/folate receptor α/PCFT and MCT/SMCT, also play a role in transport of physiological substrates and drugs across the BRB (Nagase et al., 2006). Novel approaches in this field apply nanotechnology by the use of nanoparticles, liposomes, and antibodies for delivery across the BBB (Alam et al., 2010). Liposomes are non-toxic, biocompatible and biodegradable lipid body carriers consisting of lipids such as phospholipids and sphingolipids. The advantage of liposomes is that they can carry hydrophilic, lipophilic as well as amphoteric drug molecules either entrapped inside the liposomes or bound to the micellar surface (Samad et al., 2007; Vyas and Sihorkar, 2001).

Synthetic polymeric materials including polyethylene glycol (PEG) have been used to produce sterically-stablilized liposomes with prolonged half-life that are not opsonized by serum opsins (Alam et al., 2010). Gene transfer of DNA constructs encapsulated in these PEG-stabilized liposomes that are conjugated to monoclonal antibodies is currently in development and is called the "Troyan Horse Liposome" technology (Boado and Pardridge, 2011). Binding of the antioxidant reduced glutathione to PEG-coated liposomes was found to even further increase efficiency of drug delivery over the BBB.

The development of drug delivery across the BBB is fully in progress, whereas few data have been reported on drug delivery across the BRB so far. Whether enhanced drug delivery over the human BRB has clinical value remains to be determined, in particular in the light of alternative possibilities of drug delivery in the eye employing slow release devices or sophisticated eye drops.

6.2.2. Opening of the BRB for delivery of therapeutic agents to the retina

Opening of the BBB or BRB for delivery of therapeutic agents to the brain or retina may be performed by inducing osmotic imbalance, the use of ultrasound, or via vasoactive compounds (e.g. bradykinin or P-glycoprotein inhibitors). These strategies have the disadvantage that disruption of BBB causes non-specific leakage of plasma proteins into the brain or retina that may damage neurons.

An alternative strategy for the delivery of systemic drugs to the retina is to genetically-modulate tight junction proteins (Campbell et al., 2010). A systemic hydrodynamic (high-volume) delivery of siRNA molecules via the mouse tail-vein was applied to reversibly inhibit claudin-5 mRNA expression and enhance permeability of the BRB (Campbell et al., 2008). Increased permeability was observed for small molecules up to 742 Da, but not for molecules of 4400 Da, using tracer molecule perfusion and MRI analysis. Efficacy of this approach was shown using the small neuropeptide thyrotropin-releasing hormone (360 Da), which could be delivered successfully to the brain of mice at 48 h post-injection of siRNA targeting claudin-5.

7. Concluding remarks and directions for future research

The current literature on the molecular basis of the inner BRB and its breakdown in DME and other pathological conditions shows that in addition to paracellular transport, evidence for an important additional or even predominant role of transcellular transport across the endothelial cells is substantial.

Macular edema, as the main clinical result of altered BRB permeability, is still a major cause of blindness as a final common pathway of a large variety of ocular diseases. Our understanding of the BRB has increased tremendously in the last decades, providing hope for development of more effective treatments of these diseases. Although the pathophysiology and inciting factors of macular edema may vary between these conditions, the data presented here show that both paracellular and transcellular routes of increased permeability are involved in altered BRB function. In the literature,

opening of tight junctions and increased paracellular transport are usually regarded as the cause of retinal edema. However, results of studies investigating paracellular transport have not excluded that the transcellular pathway may be affected as well in the tested conditions. Co-regulation of paracellular and transcellular transport is a likely mechanism of BRB loss. Theoretically, according to Starling's rules, macromolecules are the main determinant of increased interstitial osmotic pressure and therefore of edema formation. As BRB loss for macromolecules is thought to depend mainly on increased transcellular transport by actively enhanced transcytosis, this further emphasizes the importance of this form of leakage for edema formation in the retina. More research is needed to investigate this possibility. In addition, other factors such as dysfunction or loss of the glycocalyx and water transport by glial cells may play additional but presently poorly understood roles.

What can we learn from the literature on BRB pathophysiology in the light of new drug design? The data show that there is more out there than just 'inflammation' as a target for the treatment of macular edema. Clearly, the cellular mechanisms involved in altered paracellular and transcellular transport described in this review provide several leads for the development of alternative strategies for drug development and deserve more basic and translational research, as do the specific cellular effects of steroids on BRB endothelium.

Detailed understanding of these mechanisms will allow targeted drug design specifically aimed at restoration of the BRB integrity. This provides a rationale for increasing hope to treat ocular diseases more effectively in order to improve the quality-of-life of the millions of patients with chronic retinal diseases caused by BRB loss.

Contribution of each author

Dr Ingeborg Klaassen designed the content of the review and was the first author of all versions of the manuscript including the final version.

Prof. Dr. Cornelis Van Noorden adviced in the design of the content of the review from a cell biological point of view and corrected and redesigned all versions of the manuscript including the final version.

Prof. Dr. Reinier Schlingemann adviced in the design of the content of the review from a clinical/pathological point of view and corrected and redesigned all versions of the manuscript including the final version.

Acknowledgements

We thank Kees Hoeben for his help with electron microscopy work and Monique Arendse for help with preparing the manuscript. This study was supported by grants of the Diabetes Fonds Nederland (Grant 1999.050) and by grants of the Landelijke Stichting voor Blinden en Slechtzienden (LSBS); Stichting Blinden-Penning; Stichting Oogfonds Nederland; Vereniging Blindenbelangen Rotterdam; Stichting Blindenhulp; Prof. Hoppenbrouwers Fonds, Stichting Nederlands Oogheelkundig Onderzoek (SNOO). Fig. 7 is reproduced with permission of the Informa Healthcare Publishing Group in respect of earlier publication in Current Eye Research.

References

Abbott, N.J., 2000. Inflammatory mediators and modulation of blood–brain barrier permeability. Cell. Mol. Neurobiol. 20, 131–147.

Abu el Asrar, A.M., Maimone, D., Morse, P.H., Gregory, S., Reder, A.T., 1992. Cytokines in the vitreous of patients with proliferative diabetic retinopathy. Am. J. Ophthalmol. 114, 731–736.

Adamis, A.P., Berman, A.J., 2008. Immunological mechanisms in the pathogenesis of diabetic retinopathy. Semin. Immunopathol. 30, 65–84.

Aiello, L.P., Pierce, E.A., Foley, E.D., Takagi, H., Chen, H., Riddle, L., Ferrara, N., King, G.L., Smith, L.E., 1995. Suppression of retinal neovascularization in vivo by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins. Proc. Natl. Acad. Sci. U.S.A. 92, 10457–10461.

Aiello, L.P., Bursell, S.E., Clermont, A., Duh, E., Ishii, H., Takagi, C., Mori, F., Ciulla, T.A., Ways, K., Jirousek, M., Smith, L.E., King, G.L., 1997. Vascular endothelial growth factor-induced retinal permeability is mediated by protein kinase C in vivo and suppressed by an orally effective beta-isoform-selective inhibitor. Diabetes 46, 1473–1480.

Aiello, L.P., 2002. The potential role of PKC beta in diabetic retinopathy and macular edema. Surv. Ophthalmol. 47 (Suppl 2), S263–S269.

Al-Ahmad, A., Gassmann, M., Ogunshola, O.O., 2009. Maintaining blood–brain barrier integrity: pericytes perform better than astrocytes during prolonged oxygen deprivation. J. Cell. Physiol. 218, 612–622.

Alam, M.I., Beg, S., Samad, A., Baboota, S., Kohli, K., Ali, J., Ahuja, A., Akbar, M., 2010. Strategy for effective brain drug delivery. Eur. J. Pharm. Sci. 40, 385–403.

Al-Latayfeh, M., Silva, P.S., Sun, J.K., Aiello, L.P., 2012. Antiangiogenic therapy for ischemic retinopathies. Cold Spring Harb. Perspect. Med. 2, a006411.

Anderson, R.G., 1993. Caveolae: where incoming and outgoing messengers meet. Proc. Natl. Acad. Sci. U.S.A. 90, 10909–10913.

Anderson, R.G., 2008. Transendothelial movement and caveolae. Nat. Biotechnol. 26, 380–381.

Andrae, J., Gallini, R., Betsholtz, C., 2008. Role of platelet-derived growth factors in physiology and medicine. Genes Dev. 22, 1276–1312.

Anthony, D.C., Bolton, S.J., Fearn, S., Perry, V.H., 1997. Age-related effects of interleukin-1 beta on polymorphonuclear neutrophil-dependent increases in blood–brain barrier permeability in rats. Brain 120, 435–444.

Antonelli-Orlidge, A., Smith, S.R., D'Amore, P.A., 1989. Influence of pericytes on capillary endothelial cell growth. Am. Rev. Respir. Dis. 140, 1129–1131.

Antonetti, D.A., Barber, A.J., Khin, S., Lieth, E., Tarbell, J.M., Gardner, T.W., 1998. Vascular permeability in experimental diabetes is associated with reduced endothelial occludin content: vascular endothelial growth factor decreases occludin in retinal endothelial cells. Penn State Retina Research Group. Diabetes 47, 1953–1959.

Antonetti, D.A., Barber, A.J., Hollinger, L.A., Wolpert, E.B., Gardner, T.W., 1999. Vascular endothelial growth factor induces rapid phosphorylation of tight junction proteins occludin and zonula occluden 1. A potential mechanism for vascular permeability in diabetic retinopathy and tumors. J. Biol. Chem. 274, 23463–23467.

Antonetti, D.A., Wolpert, E.B., DeMaio, L., Harhaj, N.S., Scaduto Jr., R.C., 2002. Hydrocortisone decreases retinal endothelial cell water and solute flux coincident with increased content and decreased phosphorylation of occludin. J. Neurochem. 80, 667–677.

Arden, G.B., Sidman, R.L., Arap, W., Schlingemann, R.O., 2005. Spare the rod and spoil the eye. Br. J. Ophthalmol. 89, 764–769.

Armstrong, S.M., Khajoee, V., Wang, C., Wang, T., Tigdi, J., Yin, J., Kuebler, W.M., Gillrie, M., Davis, S.P., Ho, M., Lee, W.L., 2012. Co-regulation of transcellular and paracellular leak across microvascular endothelium by dynamin and Rac. Am. J. Pathol. 180, 1308–1323.

Armulik, A., Genove, G., Mae, M., Nisancioglu, M.H., Wallgard, E., Niaudet, C., He, L., Norlin, J., Lindblom, P., Strittmatter, K., Johansson, B.R., Betsholtz, C., 2010. Pericytes regulate the blood–brain barrier. Nature 468, 557–561.

Armulik, A., Genove, G., Betsholtz, C., 2011. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. Dev. Cell. 21, 193–215.

Aroca, P.R., Salvat, M., Fernandez, J., Mendez, I., 2004. Risk factors for diffuse and focal macular edema. J. Diabetes Complications 18, 211–215.

Asahara, T., Chen, D., Takahashi, T., Fujikawa, K., Kearney, M., Magner, M., Yancopoulos, G.D., Isner, J.M., 1998. Tie2 receptor ligands, angiopoietin-1 and angiopoietin-2, modulate VEGF-induced postnatal neovascularization. Circ. Res. 83, 233–240.

Ayalon, O., Sabanai, H., Lampugnani, M.G., Dejana, E., Geiger, B., 1994. Spatial and temporal relationships between cadherins and PECAM-1 in cell-cell junctions of human endothelial cells. J. Cell Biol. 126, 247–258.

Balda, M.S., Whitney, J.A., Flores, C., Gonzalez, S., Cereijido, M., Matter, K., 1996. Functional dissociation of paracellular permeability and transepithelial electrical resistance and disruption of the apical-basolateral intramembrane diffusion barrier by expression of a mutant tight junction membrane protein. J. Cell Biol. 134, 1031–1049.

Bamforth, S.D., Lightman, S.L., Greenwood, J., 1997. Ultrastructural analysis of interleukin-1 beta-induced leukocyte recruitment to the rat retina. Invest. Ophthalmol. Vis. Sci. 38, 25–35.

Barber, A.J., Antonetti, D.A., Gardner, T.W., 2000. Altered expression of retinal occludin and glial fibrillary acidic protein in experimental diabetes. The Penn State Retina Research Group. Invest. Ophthalmol. Vis. Sci. 41, 3561–3568.

Barber, A.J., Antonetti, D.A., 2003. Mapping the blood vessels with paracellular permeability in the retinas of diabetic rats. Invest. Ophthalmol. Vis. Sci. 44, 5410–5416.

Bauer, H.-C., Traweger, A., Bauer, H., 2004. Proteins of the tight junction in the blood–brain barrier. In: Sharma, H.S., Westman, J. (Eds.), Blood–Spinal Cord and Brain Barriers in Health and Disease. Elsevier, San Diego, pp. 1–10.

Bazzoni, G., Dejana, E., Lampugnani, M.G., 1999. Endothelial adhesion molecules in the development of the vascular tree: the garden of forking paths. Curr. Opin. Cell Biol. 11, 573–581.

Bazzoni, G., Dejana, E., 2004. Endothelial cell-to-cell junctions: molecular organization and role in vascular homeostasis. Physiol. Rev. 84, 869–901.
Beck, R.W., Edwards, A.R., Aiello, L.P., Bressler, N.M., Ferris, F., Glassman, A.R., Hartnett, E., Ip, M.S., Kim, J.E., Kollman, C., 2009. Three-year follow-up of a randomized trial comparing focal/grid photocoagulation and intravitreal triamcinolone for diabetic macular edema. Arch. Ophthalmol. 127, 245–251.
Behzadian, M.A., Wang, X.L., Windsor, L.J., Ghaly, N., Caldwell, R.B., 2001. TGF-beta increases retinal endothelial cell permeability by increasing MMP-9: possible role of glial cells in endothelial barrier function. Invest. Ophthalmol. Vis. Sci. 42, 853–859.
Bell, R.D., Winkler, E.A., Sagare, A.P., Singh, I., LaRue, B., Deane, R., Zlokovic, B.V., 2010. Pericytes control key neurovascular functions and neuronal phenotype in the adult brain and during brain aging. Neuron 68, 409–427.
Beltramo, E., Pomero, F., Allione, A., D'Alu, F., Ponte, E., Porta, M., 2002. Pericyte adhesion is impaired on extracellular matrix produced by endothelial cells in high hexose concentrations. Diabetologia 45, 416–419.
Bienz, M., Clevers, H., 2000. Linking colorectal cancer to Wnt signaling. Cell 103, 311–320.
Boado, R.J., Pardridge, W.M., 2011. The Trojan Horse liposome technology for nonviral gene transfer across the blood–brain barrier. J. Drug Deliv. 2011, 296151.
Bobbie, M.W., Roy, S., Trudeau, K., Munger, S.J., Simon, A.M., Roy, S., 2010. Reduced connexin 43 expression and its effect on the development of vascular lesions in retinas of diabetic mice. Invest. Ophthalmol. Vis. Sci. 51, 3758–3763.
Bochaton-Piallat, M.L., Kapetanios, A.D., Donati, G., Redard, M., Gabbiani, G., Pournaras, C.J., 2000. TGF-beta1, TGF-beta receptor II and ED-A fibronectin expression in myofibroblast of vitreoretinopathy. Invest. Ophthalmol. Vis. Sci. 41, 2336–2342.
Bolton, S.J., Anthony, D.C., Perry, V.H., 1998. Loss of the tight junction proteins occludin and zonula occludens-1 from cerebral vascular endothelium during neutrophil-induced blood–brain barrier breakdown in vivo. Neuroscience 86, 1245–1257.
Bolton, W.K., Cattran, D.C., Williams, M.E., Adler, S.G., Appel, G.B., Cartwright, K., Foiles, P.G., Freedman, B.I., Raskin, P., Ratner, R.E., Spinowitz, B.S., Whittier, F.C., Wuerth, J.P., 2004. Randomized trial of an inhibitor of formation of advanced glycation end products in diabetic nephropathy. Am. J. Nephrol. 24, 32–40.
Bosco, A., Cusato, K., Nicchia, G.P., Frigeri, A., Spray, D.C., 2005. A developmental switch in the expression of aquaporin-4 and Kir4.1 from horizontal to Muller cells in mouse retina. Invest. Ophthalmol. Vis. Sci. 46, 3869–3875.
Bradbury, M.W., 1985. The blood–brain barrier. Transport across the cerebral endothelium. Circ. Res. 57, 213–222.
Braga, V.M., 2002. Cell-cell adhesion and signalling. Curr. Opin. Cell Biol. 14, 546–556.
Brankin, B., Campbell, M., Canning, P., Gardiner, T.A., Stitt, A.W., 2005. Endostatin modulates VEGF-mediated barrier dysfunction in the retinal microvascular endothelium. Exp. Eye Res. 81, 22–31.
Bringmann, A., Pannicke, T., Grosche, J., Francke, M., Wiedemann, P., Skatchkov, S.N., Osborne, N.N., Reichenbach, A., 2006. Muller cells in the healthy and diseased retina. Prog. Retin. Eye Res. 25, 397–424.
Broekhuizen, L.N., Lemkes, B.A., Mooij, H.L., Meuwese, M.C., Verberne, H., Holleman, F., Schlingemann, R.O., Nieuwdorp, M., Stroes, E.S., Vink, H., 2010. Effect of sulodexide on endothelial glycocalyx and vascular permeability in patients with type 2 diabetes mellitus. Diabetologia 53, 2646–2655.
Brooks Jr., H.L., Caballero Jr., S., Newell, C.K., Steinmetz, R.L., Watson, D., Segal, M.S., Harrison, J.K., Scott, E.W., Grant, M.B., 2004. Vitreous levels of vascular endothelial growth factor and stromal-derived factor 1 in patients with diabetic retinopathy and cystoid macular edema before and after intraocular injection of triamcinolone. Arch. Ophthalmol. 122, 1801–1807.
Brosnan, C.F., Litwak, M.S., Schroeder, C.E., Selmaj, K., Raine, C.S., Arezzo, J.C., 1989. Preliminary studies of cytokine-induced functional effects on the visual pathways in the rabbit. J. Neuroimmunol. 25, 227–239.
Browning, D.J., Altaweel, M.M., Bressler, N.M., Bressler, S.B., Scott, I.U., 2008. Diabetic macular edema: what is focal and what is diffuse? Am. J. Ophthalmol. 146, 649–655.
Brownlee, M., 2001. Biochemistry and molecular cell biology of diabetic complications. Nature 414, 813–820.
Brownlee, M., 2005. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 54, 1615–1625.
Bruns, A.F., Bao, L., Walker, J.H., Ponnambalam, S., 2009. VEGF-A-stimulated signalling in endothelial cells via a dual receptor tyrosine kinase system is dependent on co-ordinated trafficking and proteolysis. Biochem. Soc. Trans. 37, 1193–1197.
Bruns, R.R., Palade, G.E., 1968. Studies on blood capillaries. II. Transport of ferritin molecules across the wall of muscle capillaries. J. Cell Biol. 37, 277–299.
Bundgaard, M., Frokjaer-Jensen, J., Crone, C., 1979. Endothelial plasmalemmal vesicles as elements in a system of branching invaginations from the cell surface. Proc. Natl. Acad. Sci. U.S.A. 76, 6439–6442.
Butt, A.M., Jones, H.C., Abbott, N.J., 1990. Electrical resistance across the blood–brain barrier in anaesthetized rats: a developmental study. J. Physiol. 429, 47–62.
Cai, J., Wu, L., Qi, X., Li, C.S., Caballero, S., Shaw, L., Ruan, Q., Grant, M.B., Boulton, M.E., 2011a. PEDF regulates vascular permeability by a gamma-secretase-mediated pathway. PLoS One 6, e21164.
Cai, J., Wu, L., Qi, X., Shaw, L., Li, C.S., Caballero, S., Jiang, W.G., Vinores, S.A., Antonetti, D., Ahmed, A., Grant, M.B., Boulton, M.E., 2011b. Placenta growth factor-1 exerts time-dependent stabilization of adherens junctions following VEGF-induced vascular permeability. PLoS One 6, e18076.
Callanan, D., Williams, P., 2008. Topical nepafenac in the treatment of diabetic macular edema. Clin. Ophthalmol. 2, 689–692.
Campbell, M., Kiang, A.S., Kenna, P.F., Kerskens, C., Blau, C., O'Dwyer, L., Tivnan, A., Kelly, J.A., Brankin, B., Farrar, G.J., Humphries, P., 2008. RNAi-mediated reversible opening of the blood–brain barrier. J. Gene Med. 10, 930–947.
Campbell, M., Ozaki, E., Humphries, P., 2010. Systemic delivery of therapeutics to neuronal tissues: a barrier modulation approach. Expert Opin. Drug Deliv. 7, 859–869.
Carman, C.V., Springer, T.A., 2008. Trans-cellular migration: cell-cell contacts get intimate. Curr. Opin. Cell Biol. 20, 533–540.
Carson-Walter, E.B., Hampton, J., Shue, E., Geynisman, D.M., Pillai, P.K., Sathanoori, R., Madden, S.L., Hamilton, R.L., Walter, K.A., 2005. Plasmalemmal vesicle associated protein-1 is a novel marker implicated in brain tumor angiogenesis. Clin. Cancer Res. 11, 7643–7650.
Cavallaro, U., Christofori, G., 2004. Cell adhesion and signalling by cadherins and Ig-CAMs in cancer. Nat. Rev. Cancer 4, 118–132.
Cavey, M., Rauzi, M., Lenne, P.F., Lecuit, T., 2008. A two-tiered mechanism for stabilization and immobilization of E-cadherin. Nature 453, 751–756.
Cecchelli, R., Berezowski, V., Lundquist, S., Culot, M., Renftel, M., Dehouck, M.P., Fenart, L., 2007. Modelling of the blood–brain barrier in drug discovery and development. Nat. Rev. Drug Discov. 6, 650–661.
Chen, Y., Liu, L., 2011. Modern methods for delivery of drugs across the blood–brain barrier. Adv. Drug Deliv. Rev. 64, 640–665.
Chen, Y.H., Lin, S.J., Lin, F.Y., Wu, T.C., Tsao, C.R., Huang, P.H., Liu, P.L., Chen, Y.L., Chen, J.W., 2007. High glucose impairs early and late endothelial progenitor cells by modifying nitric oxide-related but not oxidative stress-mediated mechanisms. Diabetes 56, 1559–1568.
Cheung, N., Tikellis, G., Wang, J.J., 2007. Diabetic retinopathy. Ophthalmology 114, 2098–2099.
Chiba, H., Osanai, M., Murata, M., Kojima, T., Sawada, N., 2008. Transmembrane proteins of tight junctions. Biochim. Biophys. Acta 1778, 588–600.
Choi, Y.K., Kim, J.H., Kim, W.J., Lee, H.Y., Park, J.A., Lee, S.W., Yoon, D.K., Kim, H.H., Chung, H., Yu, Y.S., Kim, K.W., 2007. AKAP12 regulates human blood–retinal barrier formation by downregulation of hypoxia-inducible factor-1alpha. J. Neurosci. 27, 4472–4481.
Chronopoulos, A., Trudeau, K., Roy, S., Huang, H., Vinores, S.A., Roy, S., 2011. High glucose-induced altered basement membrane composition and structure increases trans-endothelial permeability: implications for diabetic retinopathy. Curr. Eye Res. 36, 747–753.
Churdchomjan, W., Kheolamai, P., Manochantr, S., Tapanadechopone, P., Tantrawatpan, C., Pratya, Y., Issaragrisil, S., 2010. Comparison of endothelial progenitor cell function in type 2 diabetes with good and poor glycemic control. BMC Endocr. Disord. 10, 5.
Claudio, L., Martiney, J.A., Brosnan, C.F., 1994. Ultrastructural studies of the blood–retina barrier after exposure to interleukin-1 beta or tumor necrosis factor-alpha. Lab. Invest. 70, 850–861.
Collins, J., Fleming, T., 1995. Specific mRNA detection in single lineage-marked blastomeres from preimplantation embryos. Trends Genet. 11, 5–7.
Connor Jr., T.B., Roberts, A.B., Sporn, M.B., Danielpour, D., Dart, L.L., Michels, R.G., de, B.S., Enger, C., Kato, H., Lansing, M., 1989. Correlation of fibrosis and transforming growth factor-beta type 2 levels in the eye. J. Clin. Invest. 83, 1661–1666.
Cui, B., Sun, J.H., Xiang, F.F., Liu, L., Li, W.J., 2012. Aquaporin 4 knockdown exacerbates streptozotocin-induced diabetic retinopathy through aggravating inflammatory response. Exp. Eye Res. 98, 37–43.
Cui, J.Z., Chiu, A., Maberley, D., Ma, P., Samad, A., Matsubara, J.A., 2007. Stage specificity of novel growth factor expression during development of proliferative vitreoretinopathy. Eye (Lond) 21, 200–208.
Cunha-Vaz, J.G., 1983. Studies on the pathophysiology of diabetic retinopathy. The blood–retinal barrier in diabetes. Diabetes 32 (Suppl 2), 20–27.
Cunha-Vaz, J.G., Travassos, A., 1984. Breakdown of the blood–retinal barriers and cystoid macular edema. Surv. Ophthalmol. 28, 485–492.
Cunha-Vaz, J.G., 1997. The blood–ocular barriers: past, present, and future. Doc. Ophthalmol. 93, 149–157.
Cunha-Vaz, J., 1998. Diabetic macular edema. Eur. J. Ophthalmol. 8, 127–130.
D'Amore, P.A., 1994. Mechanisms of retinal and choroidal neovascularization. Invest. Ophthalmol. Vis. Sci. 35, 3974–3979.
Da, T., Verkman, A.S., 2004. Aquaporin-4 gene disruption in mice protects against impaired retinal function and cell death after ischemia. Invest. Ophthalmol. Vis. Sci. 45, 4477–4483.
Daneman, R., Zhou, L., Kebede, A.A., Barres, B.A., 2010. Pericytes are required for blood–brain barrier integrity during embryogenesis. Nature 468, 562–566.
Davis, S., Aldrich, T.H., Jones, P.F., Acheson, A., Compton, D.L., Jain, V., Ryan, T.E., Bruno, J., Radziejewski, C., Maisonpierre, P.C., Yancopoulos, G.D., 1996. Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. Cell 87, 1161–1169.
De Bock, M., Culot, M., Wang, N., Bol, M., Decrock, E., De, V.E., da, C.A., Dauwe, I., Vinken, M., Simon, A.M., Rogiers, V., De, L.G., Evans, W.H., Bultynck, G., Dupont, G., Cecchelli, R., Leybaert, L., 2011. Connexin channels provide a target to manipulate brain endothelial calcium dynamics and blood–brain barrier permeability. J. Cereb. Blood Flow Metab. 31, 1942–1957.
Dejana, E., 2004. Endothelial cell-cell junctions: happy together. Nat. Rev. Mol. Cell Biol. 5, 261–270.
Dejana, E., Orsenigo, F., Lampugnani, M.G., 2008. The role of adherens junctions and VE-cadherin in the control of vascular permeability. J. Cell Sci. 121, 2115–2122.

Dejana, E., Giampietro, C., 2012. Vascular endothelial-cadherin and vascular stability. Curr. Opin. Hematol. 19, 218–223.
Deli, M.A., Abraham, C.S., Kataoka, Y., Niwa, M., 2005. Permeability studies on in vitro blood–brain barrier models: physiology, pathology, and pharmacology. Cell. Mol. Neurobiol. 25, 59–127.
DeMaio, L., Chang, Y.S., Gardner, T.W., Tarbell, J.M., Antonetti, D.A., 2001. Shear stress regulates occludin content and phosphorylation. Am. J. Physiol. Heart Circ. Physiol. 281, H105–H113.
DeMaio, L., Rouhanizadeh, M., Reddy, S., Sevanian, A., Hwang, J., Hsiai, T.K., 2006. Oxidized phospholipids mediate occludin expression and phosphorylation in vascular endothelial cells. Am. J. Physiol. Heart Circ. Physiol. 290, H674–H683.
Demerath, E.W., Cameron, N., Gillman, M.W., Towne, B., Siervogel, R.M., 2004. Telomeres and telomerase in the fetal origins of cardiovascular disease: a review. Hum. Biol. 76, 127–146.
Demircan, N., Safran, B.G., Soylu, M., Ozcan, A.A., Sizmaz, S., 2006. Determination of vitreous interleukin-1 (IL-1) and tumour necrosis factor (TNF) levels in proliferative diabetic retinopathy. Eye (Lond) 20, 1366–1369.
Dewever, J., Frerart, F., Bouzin, C., Baudelet, C., Ansiaux, R., Sonveaux, P., Gallez, B., Dessy, C., Feron, O., 2007. Caveolin-1 is critical for the maturation of tumor blood vessels through the regulation of both endothelial tube formation and mural cell recruitment. Am. J. Pathol. 171, 1619–1628.
Diaz-Flores, L., Gutierrez, R., Madrid, J.F., Varela, H., Valladares, F., Acosta, E., Martin-Vasallo, P., Diaz-Flores Jr., L., 2009. Pericytes. Morphofunction, interactions and pathology in a quiescent and activated mesenchymal cell niche. Histol. Histopathol. 24, 909–969.
Distler, C., Dreher, Z., 1996. Glia cells of the monkey retina–II. Muller cells. Vis. Res. 36, 2381–2394.
Ditzel, J., 1976. Oxygen transport impairment in diabetes. Diabetes 25, 832–838.
Dohgu, S., Takata, F., Yamauchi, A., Nakagawa, S., Egawa, T., Naito, M., Tsuruo, T., Sawada, Y., Niwa, M., Kataoka, Y., 2005. Brain pericytes contribute to the induction and up-regulation of blood–brain barrier functions through transforming growth factor-beta production. Brain Res. 1038, 208–215.
Dorfel, M.J., Huber, O., 2012. Modulation of tight junction structure and function by kinases and phosphatases targeting occludin. J. Biomed. Biotechnol. 2012, 807356.
Drab, M., Verkade, P., Elger, M., Kasper, M., Lohn, M., Lauterbach, B., Menne, J., Lindschau, C., Mende, F., Luft, F.C., Schedl, A., Haller, H., Kurzchalia, T.V., 2001. Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. Science 293, 2449–2452.
Dyer, M.A., Cepko, C.L., 2000. Control of Muller glial cell proliferation and activation following retinal injury. Nat. Neurosci. 3, 873–880.
Ebnet, K., Suzuki, A., Ohno, S., Vestweber, D., 2004. Junctional adhesion molecules (JAMs): more molecules with dual functions? J. Cell Sci. 117, 19–29.
Edo, M.D., Andres, V., 2005. Aging, telomeres, and atherosclerosis. Cardiovasc. Res. 66, 213–221.
Eichler, W., Kuhrt, H., Hoffmann, S., Wiedemann, P., Reichenbach, A., 2000. VEGF release by retinal glia depends on both oxygen and glucose supply. Neuroreport 11, 3533–3537.
El-Mollayess, G.M., Noureddine, B.N., Bashshur, Z.F., 2011. Bevacizumab and neovascular age related macular degeneration: pathogenesis and treatment. Semin. Ophthalmol. 26, 69–76.
Elkin, M., Ilan, N., Ishai-Michaeli, R., Friedmann, Y., Papo, O., Pecker, I., Vlodavsky, I., 2001. Heparanase as mediator of angiogenesis: mode of action. FASEB J. 15, 1661–1663.
Elner, S.G., Strieter, R., Bian, Z.M., Kunkel, S., Mokhtarzaden, L., Johnson, M., Lukacs, N., Elner, V.M., 1998. Interferon-induced protein 10 and interleukin 8. C-X-C chemokines present in proliferative diabetic retinopathy. Arch. Ophthalmol. 116, 1597–1601.
Endo, M., Jain, R.K., Witwer, B., Brown, D., 1999. Water channel (aquaporin 1) expression and distribution in mammary carcinomas and glioblastomas. Microvasc. Res. 58, 89–98.
Enge, M., Bjarnegard, M., Gerhardt, H., Gustafsson, E., Kalen, M., Asker, N., Hammes, H.P., Shani, M., Fassler, R., Betsholtz, C., 2002. Endothelium-specific platelet-derived growth factor-B ablation mimics diabetic retinopathy. EMBO J. 21, 4307–4316.
Engelhardt, B., Wolburg, H., 2004. Mini-review: transendothelial migration of leukocytes: through the front door or around the side of the house? Eur. J. Immunol. 34, 2955–2963.
Esser, S., Lampugnani, M.G., Corada, M., Dejana, E., Risau, W., 1998. Vascular endothelial growth factor induces VE-cadherin tyrosine phosphorylation in endothelial cells. J. Cell Sci. 111, 1853–1865.
Farquhar, M.G., Palade, G.E., 1963. Junctional complexes in various epithelia. J. Cell Biol. 17, 375–412.
Felinski, E.A., Cox, A.E., Phillips, B.E., Antonetti, D.A., 2008. Glucocorticoids induce transactivation of tight junction genes occludin and claudin-5 in retinal endothelial cells via a novel cis-element. Exp. Eye Res. 86, 867–878.
Feng, Y., Venema, V.J., Venema, R.C., Tsai, N., Behzadian, M.A., Caldwell, R.B., 1999a. VEGF-induced permeability increase is mediated by caveolae. Invest. Ophthalmol. Vis. Sci. 40, 157–167.
Feng, Y., Venema, V.J., Venema, R.C., Tsai, N., Caldwell, R.B., 1999b. VEGF induces nuclear translocation of Flk-1/KDR, endothelial nitric oxide synthase, and caveolin-1 in vascular endothelial cells. Biochem. Biophys. Res. Commun. 256, 192–197.
Ferrara, N., 1995. Vascular endothelial growth factor. The trigger for neovascularization in the eye. Lab. Invest. 72, 615–618.
Fisher, M., 2009. Pericyte signaling in the neurovascular unit. Stroke 40, S13–S15.
Fletcher, E.L., Downie, L.E., Ly, A., Ward, M.M., Batcha, A.H., Puthussery, T., Yee, P., Hatzopoulos, K.M., 2008. A review of the role of glial cells in understanding retinal disease. Clin. Exp. Optom. 91, 67–77.
Forster, C., Burek, M., Romero, I.A., Weksler, B., Couraud, P.O., Drenckhahn, D., 2008. Differential effects of hydrocortisone and TNFalpha on tight junction proteins in an in vitro model of the human blood–brain barrier. J. Physiol. 586, 1937–1949.
Forsythe, J.A., Jiang, B.H., Iyer, N.V., Agani, F., Leung, S.W., Koos, R.D., Semenza, G.L., 1996. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol. Cell. Biol. 16, 4604–4613.
Frank, P.G., Pavlides, S., Lisanti, M.P., 2009. Caveolae and transcytosis in endothelial cells: role in atherosclerosis. Cell. Tissue Res. 335, 41–47.
Freddo, T.F., 2013. A contemporary concept of the blood–aqueous barrier. Prog. Retin. Eye Res. 32, 181–195.
Fukuda, M., Nakanishi, Y., Fuse, M., Yokoi, N., Hamada, Y., Fukagawa, M., Negi, A., Nakamura, M., 2010. Altered expression of aquaporins 1 and 4 coincides with neurodegenerative events in retinas of spontaneously diabetic Torii rats. Exp. Eye Res. 90, 17–25.
Funatsu, H., Yamashita, H., Ikeda, T., Mimura, T., Eguchi, S., Hori, S., 2003. Vitreous levels of interleukin-6 and vascular endothelial growth factor are related to diabetic macular edema. Ophthalmology 110, 1690–1696.
Funatsu, H., Yamashita, H., Noma, H., Mimura, T., Nakamura, S., Sakata, K., Hori, S., 2005. Aqueous humor levels of cytokines are related to vitreous levels and progression of diabetic retinopathy in diabetic patients. Graefes Arch. Clin. Exp. Ophthalmol. 243, 3–8.
Funk, M., Schmidinger, G., Maar, N., Bolz, M., Benesch, T., Zlabinger, G.J., Schmidt-Erfurth, U.M., 2010. Angiogenic and inflammatory markers in the intraocular fluid of eyes with diabetic macular edema and influence of therapy with bevacizumab. Retina 30, 1412–1419.
Furuse, M., Hirase, T., Itoh, M., Nagafuchi, A., Yonemura, S., Tsukita, S., Tsukita, S., 1993. Occludin: a novel integral membrane protein localizing at tight junctions. J. Cell Biol. 123, 1777–1788.
Furuse, M., Sasaki, H., Fujimoto, K., Tsukita, S., 1998. A single gene product, claudin-1 or -2, reconstitutes tight junction strands and recruits occludin in fibroblasts. J. Cell Biol. 143, 391–401.
Gaengel, K., Genove, G., Armulik, A., Betsholtz, C., 2009. Endothelial-mural cell signaling in vascular development and angiogenesis. Arterioscler. Thromb. Vasc. Biol. 29, 630–638.
Gaillard, P.J., Voorwinden, L.H., Nielsen, J.L., Ivanov, A., Atsumi, R., Engman, H., Ringbom, C., de Boer, A.G., Breimer, D.D., 2001. Establishment and functional characterization of an in vitro model of the blood–brain barrier, comprising a co-culture of brain capillary endothelial cells and astrocytes. Eur. J. Pharm. Sci. 12, 215–222.
Gao, B.B., Clermont, A., Rook, S., Fonda, S.J., Srinivasan, V.J., Wojtkowski, M., Fujimoto, J.G., Avery, R.L., Arrigg, P.G., Bursell, S.E., Aiello, L.P., Feener, E.P., 2007. Extracellular carbonic anhydrase mediates hemorrhagic retinal and cerebral vascular permeability through prekallikrein activation. Nat. Med. 13, 181–188.
Garcia, C.M., Darland, D.C., Massingham, L.J., D'Amore, P.A., 2004. Endothelial cell-astrocyte interactions and TGF beta are required for induction of blood–neural barrier properties. Brain Res. Dev. Brain Res. 152, 25–38.
Gardiner, T.A., Stitt, A.W., Archer, D.B., 1995. Retinal vascular endothelial cell endocytosis increases in early diabetes. Lab. Invest. 72, 439–444.
Gardiner, T.A., Archer, D.B., Curtis, T.M., Stitt, A.W., 2007. Arteriolar involvement in the microvascular lesions of diabetic retinopathy: implications for pathogenesis. Microcirculation 14, 25–38.
Gardner, T.W., Eller, A.W., Friberg, T.R., D'Antonio, J.A., Hollis, T.M., 1995. Antihistamines reduce blood–retinal barrier permeability in type I (insulin-dependent) diabetic patients with nonproliferative retinopathy. A pilot study. Retina 15, 134–140.
Gardner, T.W., Lieth, E., Khin, S.A., Barber, A.J., Bonsall, D.J., Lesher, T., Rice, K., Brennan Jr., W.A., 1997. Astrocytes increase barrier properties and ZO-1 expression in retinal vascular endothelial cells. Invest. Ophthalmol. Vis. Sci. 38, 2423–2427.
Gardner, T.W., Antonetti, D.A., Barber, A.J., LaNoue, K.F., Levison, S.W., 2002. Diabetic retinopathy: more than meets the eye. Surv. Ophthalmol. 47 (Suppl 2), S253–S262.
Gardner, T.W., Sander, B., Larsen, M.L., Kunselman, A., Tenhave, T., Lund-Andersen, H., Reimers, J., Hubbard, L., Blankenship, G.W., Quillen, D.A., Brod, R.D., Wilmarth, M.H., Post-Hansen, H., Parving, H.H., Davis, M.D., 2006. An extension of the Early Treatment Diabetic Retinopathy Study (ETDRS) system for grading of diabetic macular edema in the Astemizole Retinopathy Trial. Curr. Eye Res. 31, 535–547.
Gaucher, D., Chiappore, J.A., Paques, M., Simonutti, M., Boitard, C., Sahel, J.A., Massin, P., Picaud, S., 2007. Microglial changes occur without neural cell death in diabetic retinopathy. Vis. Res. 47, 612–623.
Gavard, J., Gutkind, J.S., 2006. VEGF controls endothelial-cell permeability by promoting the beta-arrestin-dependent endocytosis of VE-cadherin. Nat. Cell Biol. 8, 1223–1234.
Gavard, J., Patel, V., Gutkind, J.S., 2008. Angiopoietin-1 prevents VEGF-induced endothelial permeability by sequestering Src through mDia. Dev. Cell 14, 25–36.
Gerhardt, H., Wolburg, H., Redies, C., 2000. N-cadherin mediates pericytic-endothelial interaction during brain angiogenesis in the chicken. Dev. Dyn. 218, 472–479.
Gerhardt, H., Betsholtz, C., 2003. Endothelial-pericyte interactions in angiogenesis. Cell. Tissue Res. 314, 15–23.

Geroski, D.H., Edelhauser, H.F., 2000. Drug delivery for posterior segment eye disease. Invest. Ophthalmol. Vis. Sci. 41, 961–964.
Giebel, S.J., Menicucci, G., McGuire, P.G., Das, A., 2005. Matrix metalloproteinases in early diabetic retinopathy and their role in alteration of the blood–retinal barrier. Lab. Invest. 85, 597–607.
Gil, J., Magno, M., 1980. Fusion of pinocytotic vesicles in liquid filled lungs: a mechanism of cellular damage. Exp. Lung Res. 1, 43–56.
Gleadle, J.M., Ratcliffe, P.J., 1997. Induction of hypoxia-inducible factor-1, erythropoietin, vascular endothelial growth factor, and glucose transporter-1 by hypoxia: evidence against a regulatory role for Src kinase. Blood 89, 503–509.
Glenn, J.V., Stitt, A.W., 2009. The role of advanced glycation end products in retinal ageing and disease. Biochim. Biophys. Acta 1790, 1109–1116.
Glenney Jr., J.R., Soppet, D., 1992. Sequence and expression of caveolin, a protein component of caveolae plasma membrane domains phosphorylated on tyrosine in Rous sarcoma virus-transformed fibroblasts. Proc. Natl. Acad. Sci. U.S.A. 89, 10517–10521.
Gonzalez-Mariscal, L., Tapia, R., Chamorro, D., 2008. Crosstalk of tight junction components with signaling pathways. Biochim. Biophys. Acta 1778, 729–756.
Goodyear, M.J., Junghans, B.M., Giummarra, L., Murphy, M.J., Crewther, D.P., Crewther, S.G., 2008. A role for aquaporin-4 during induction of form deprivation myopia in chick. Mol. Vis. 14, 298–307.
Goodyear, M.J., Crewther, S.G., Junghans, B.M., 2009. A role for aquaporin-4 in fluid regulation in the inner retina. Vis. Neurosci. 26, 159–165.
Gratton, J.P., Bernatchez, P., Sessa, W.C., 2004. Caveolae and caveolins in the cardiovascular system. Circ. Res. 94, 1408–1417.
Greenwood, J., Howes, R., Lightman, S., 1994. The blood–retinal barrier in experimental autoimmune uveoretinitis. Leukocyte interactions and functional damage. Lab. Invest. 70, 39–52.
Gumbiner, B., 1988. Cadherins: a family of $Ca2^{+}$-dependent adhesion molecules. Trends Biochem. Sci. 13, 75–76.
Gumbiner, B., Lowenkopf, T., Apatira, D., 1991. Identification of a 160-kDa polypeptide that binds to the tight junction protein ZO-1. Proc. Natl. Acad. Sci. U.S.A. 88, 3460–3464.
Gumbiner, B.M., 2000. Regulation of cadherin adhesive activity. J. Cell. Biol. 148, 399–404.
Hallmann, R., Mayer, D.N., Berg, E.L., Broermann, R., Butcher, E.C., 1995. Novel mouse endothelial cell surface marker is suppressed during differentiation of the blood brain barrier. Dev. Dyn. 202, 325–332.
Hamann, S., Zeuthen, T., La, C.M., Nagelhus, E.A., Ottersen, O.P., Agre, P., Nielsen, S., 1998. Aquaporins in complex tissues: distribution of aquaporins 1-5 in human and rat eye. Am. J. Physiol. 274, C1332–C1345.
Hammes, H.P., Martin, S., Federlin, K., Geisen, K., Brownlee, M., 1991. Aminoguanidine treatment inhibits the development of experimental diabetic retinopathy. Proc. Natl. Acad. Sci. U.S.A. 88, 11555–11558.
Hammes, H.P., Du, X., Edelstein, D., Taguchi, T., Matsumura, T., Ju, Q., Lin, J., Bierhaus, A., Nawroth, P., Hannak, D., Neumaier, M., Bergfeld, R., Giardino, I., Brownlee, M., 2003. Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. Nat. Med. 9, 294–299.
Hammes, H.P., Feng, Y., Pfister, F., Brownlee, M., 2011. Diabetic retinopathy: targeting vasoregression. Diabetes 60, 9–16.
Harhaj, N.S., Antonetti, D.A., 2004. Regulation of tight junctions and loss of barrier function in pathophysiology. Int. J. Biochem. Cell Biol. 36, 1206–1237.
Harhaj, N.S., Felinski, E.A., Wolpert, E.B., Sundstrom, J.M., Gardner, T.W., Antonetti, D.A., 2006. VEGF activation of protein kinase C stimulates occludin phosphorylation and contributes to endothelial permeability. Invest. Ophthalmol. Vis. Sci. 47, 5106–5115.
Hasegawa, H., Lian, S.C., Finkbeiner, W.E., Verkman, A.S., 1994. Extrarenal tissue distribution of CHIP28 water channels by in situ hybridization and antibody staining. Am. J. Physiol. 266, C893–C903.
Haskins, J., Gu, L., Wittchen, E.S., Hibbard, J., Stevenson, B.R., 1998. ZO-3, a novel member of the MAGUK protein family found at the tight junction, interacts with ZO-1 and occludin. J. Cell Biol. 141, 199–208.
Hayashi, Y., Nomura, M., Yamagishi, S., Harada, S., Yamashita, J., Yamamoto, H., 1997. Induction of various blood–brain barrier properties in non-neural endothelial cells by close apposition to co-cultured astrocytes. Glia 19, 13–26.
Henry, C.B., Duling, B.R., 1999. Permeation of the luminal capillary glycocalyx is determined by hyaluronan. Am. J. Physiol. 277, H508–H514.
Hill, M.M., Bastiani, M., Luetterforst, R., Kirkham, M., Kirkham, A., Nixon, S.J., Walser, P., Abankwa, D., Oorschot, V.M., Martin, S., Hancock, J.F., Parton, R.G., 2008. PTRF-Cavin, a conserved cytoplasmic protein required for caveola formation and function. Cell 132, 113–124.
Hinton, D.R., He, S., Jin, M.L., Barron, E., Ryan, S.J., 2002. Novel growth factors involved in the pathogenesis of proliferative vitreoretinopathy. Eye (Lond) 16, 422–428.
Hirase, T., Staddon, J.M., Saitou, M., Ando-Akatsuka, Y., Itoh, M., Furuse, M., Fujimoto, K., Tsukita, S., Rubin, L.L., 1997. Occludin as a possible determinant of tight junction permeability in endothelial cells. J. Cell Sci. 110, 1603–1613.
Hirase, T., Kawashima, S., Wong, E.Y., Ueyama, T., Rikitake, Y., Tsukita, S., Yokoyama, M., Staddon, J.M., 2001. Regulation of tight junction permeability and occludin phosphorylation by Rhoa-p160ROCK-dependent and -independent mechanisms. J. Biol. Chem. 276, 10423–10431.
Hnasko, R., McFarland, M., Ben-Jonathan, N., 2002. Distribution and characterization of plasmalemma vesicle protein-1 in rat endocrine glands. J. Endocrinol. 175, 649–661.
Hofman, P., Blaauwgeers, H.G., Tolentino, M.J., Adamis, A.P., Nunes Cardozo, B.J., Vrensen, G.F., Schlingemann, R.O., 2000. VEGF-A induced hyperpermeability of blood–retinal barrier endothelium in vivo is predominantly associated with pinocytotic vesicular transport and not with formation of fenestrations. Vascular endothelial growth factor-A. Curr. Eye Res. 21, 637–645.
Hofman, P., Blaauwgeers, H.G., Vrensen, G.F., Schlingemann, R.O., 2001a. Role of VEGF-A in endothelial phenotypic shift in human diabetic retinopathy and VEGF-A-induced retinopathy in monkeys. Ophthalmic Res. 33, 156–162.
Hofman, P., Hoyng, P., vanderWerf, F., Vrensen, G.F., Schlingemann, R.O., 2001b. Lack of blood–brain barrier properties in microvessels of the prelaminar optic nerve head. Invest. Ophthalmol. Vis. Sci. 42, 895–901.
Hogeboom van Buggenum, I.M., van der Heijde, G.L., Tangelder, G.J., Reichert-Thoen, J.W., 1996. Ocular oxygen measurement. Br. J. Ophthalmol. 80, 567–573.
Holash, J., Maisonpierre, P.C., Compton, D., Boland, P., Alexander, C.R., Zagzag, D., Yancopoulos, G.D., Wiegand, S.J., 1999. Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science 284, 1994–1998.
Hollis, T.M., Sill, H.W., Butler, C., Campos, M.J., Gardner, T.W., 1992. Astemizole reduces blood–retinal barrier leakage in experimental diabetes. J. Diabetes Complications 6, 230–235.
Hori, S., Ohtsuki, S., Hosoya, K., Nakashima, E., Terasaki, T., 2004. A pericyte-derived angiopoietin-1 multimeric complex induces occludin gene expression in brain capillary endothelial cells through Tie-2 activation in vitro. J. Neurochem. 89, 503–513.
Hosoya, K., Yamamoto, A., Akanuma, S., Tachikawa, M., 2010. Lipophilicity and transporter influence on blood–retinal barrier permeability: a comparison with blood–brain barrier permeability. Pharm. Res. 27, 2715–2724.
Hosoya, K., Tomi, M., Tachikawa, M., 2011. Strategies for therapy of retinal diseases using systemic drug delivery: relevance of transporters at the blood–retinal barrier. Expert Opin. Drug Deliv. 8, 1571–1587.
Hughes, J.M., Brink, A., Witmer, A.N., Hanraads-de, R.M., Klaassen, I., Schlingemann, R.O., 2004. Vascular leucocyte adhesion molecules unaltered in the human retina in diabetes. Br. J. Ophthalmol. 88, 566–572.
Hughes, J.M., Kuiper, E.J., Klaassen, I., Canning, P., Stitt, A.W., Van Bezu, J., Schalkwijk, C.G., Van Noorden, C.J., Schlingemann, R.O., 2007. Advanced glycation end products cause increased CCN family and extracellular matrix gene expression in the diabetic rodent retina. Diabetologia 50, 1089–1098.
Hughes, J.M., Groot, A.J., van der Groep, P., Sersansie, R., Vooijs, M., van Diest, P.J., Van Noorden, C.J., Schlingemann, R.O., Klaassen, I., 2010. Active HIF-1 in the normal human retina. J. Histochem. Cytochem. 58, 247–254.
Iandiev, I., Pannicke, T., Reichenbach, A., Wiedemann, P., Bringmann, A., 2007. Diabetes alters the localization of glial aquaporins in rat retina. Neurosci. Lett. 421, 132–136.
Ikegami-Kawai, M., Okuda, R., Nemoto, T., Inada, N., Takahashi, T., 2004. Enhanced activity of serum and urinary hyaluronidases in streptozotocin-induced diabetic Wistar and GK rats. Glycobiology 14, 65–72.
Ishii, H., Jirousek, M.R., Koya, D., Takagi, C., Xia, P., Clermont, A., Bursell, S.E., Kern, T.S., Ballas, L.M., Heath, W.F., Stramm, L.E., Feener, E.P., King, G.L., 1996. Amelioration of vascular dysfunctions in diabetic rats by an oral PKC beta inhibitor. Science 272, 728–731.
Iyer, N.V., Kotch, L.E., Agani, F., Leung, S.W., Laughner, E., Wenger, R.H., Gassmann, M., Gearhart, J.D., Lawler, A.M., Yu, A.Y., Semenza, G.L., 1998. Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. Genes Dev. 12, 149–162.
Jesaitis, L.A., Goodenough, D.A., 1994. Molecular characterization and tissue distribution of ZO-2, a tight junction protein homologous to ZO-1 and the Drosophila discs-large tumor suppressor protein. J. Cell Biol. 124, 949–961.
Jiang, B.H., Rue, E., Wang, G.L., Roe, R., Semenza, G.L., 1996. Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor 1. J. Biol. Chem. 271, 17771–17778.
Jinga, V.V., Gafencu, A., Antohe, F., Constantinescu, E., Heltianu, C., Raicu, M., Manolescu, I., Hunziker, W., Simionescu, M., 2000. Establishment of a pure vascular endothelial cell line from human placenta. Placenta 21, 325–336.
Joussen, A.M., Murata, T., Tsujikawa, A., Kirchhof, B., Bursell, S.E., Adamis, A.P., 2001. Leukocyte-mediated endothelial cell injury and death in the diabetic retina. Am. J. Pathol. 158, 147–152.
Joussen, A.M., Poulaki, V., Qin, W., Kirchhof, B., Mitsiades, N., Wiegand, S.J., Rudge, J., Yancopoulos, G.D., Adamis, A.P., 2002. Retinal vascular endothelial growth factor induces intercellular adhesion molecule-1 and endothelial nitric oxide synthase expression and initiates early diabetic retinal leukocyte adhesion in vivo. Am. J. Pathol. 160, 501–509.
Joussen, A.M., Poulaki, V., Le, M.L., Koizumi, K., Esser, C., Janicki, H., Schraermeyer, U., Kociok, N., Fauser, S., Kirchhof, B., Kern, T.S., Adamis, A.P., 2004. A central role for inflammation in the pathogenesis of diabetic retinopathy. FASEB J. 18, 1450–1452.
Joussen, A.M., Smyth, N., Niessen, C., 2007. Pathophysiology of diabetic macular edema. Dev. Ophthalmol. 39, 1–12.
Joussen, A.M., Doehmen, S., Le, M.L., Koizumi, K., Radetzky, S., Krohne, T.U., Poulaki, V., Semkova, I., Kociok, N., 2009. TNF-alpha mediated apoptosis plays an important role in the development of early diabetic retinopathy and long-term histopathological alterations. Mol. Vis. 15, 1418–1428.
Kashiwamura, Y., Sano, Y., Abe, M., Shimizu, F., Haruki, H., Maeda, T., Kawai, M., Kanda, T., 2011. Hydrocortisone enhances the function of the blood–nerve barrier through the up-regulation of claudin-5. Neurochem. Res. 36, 849–855.

Kaur, C., Sivakumar, V., Foulds, W.S., 2006. Early response of neurons and glial cells to hypoxia in the retina. Invest. Ophthalmol. Vis. Sci. 47, 1126–1141.
Kaur, C., Foulds, W.S., Ling, E.A., 2008. Blood–retinal barrier in hypoxic ischaemic conditions: basic concepts, clinical features and management. Prog. Retin. Eye Res. 27, 622–647.
Kevil, C.G., Okayama, N., Trocha, S.D., Kalogeris, T.J., Coe, L.L., Specian, R.D., Davis, C.P., Alexander, J.S., 1998. Expression of zonula occludens and adherens junctional proteins in human venous and arterial endothelial cells: role of occludin in endothelial solute barriers. Microcirculation 5, 197–210.
Kim, J., Kim, C.S., Lee, Y.M., Jo, K., Shin, S.D., Kim, J.S., 2012. Methylglyoxal induces hyperpermeability of the blood–retinal barrier via the loss of tight junction proteins and the activation of matrix metalloproteinases. Graefes Arch. Clin. Exp. Ophthalmol. 250, 691–697.
Kim, J.H., Kim, J.H., Yu, Y.S., Kim, D.H., Kim, K.W., 2009. Recruitment of pericytes and astrocytes is closely related to the formation of tight junction in developing retinal vessels. J. Neurosci. Res. 87, 653–659.
Kim, S.Y., Johnson, M.A., McLeod, D.S., Alexander, T., Hansen, B.C., Lutty, G.A., 2005. Neutrophils are associated with capillary closure in spontaneously diabetic monkey retinas. Diabetes 54, 1534–1542.
Kimoto, K., Kubota, T., 2012. Anti-vegf agents for ocular angiogenesis and vascular permeability. J. Ophthalmol. 2012, 852183.
King, G.L., Johnson, S.M., 1985. Receptor-mediated transport of insulin across endothelial cells. Science 227, 1583–1586.
King, G.L., Shiba, T., Oliver, J., Inoguchi, T., Bursell, S.E., 1994. Cellular and molecular abnormalities in the vascular endothelium of diabetes mellitus. Annu. Rev. Med. 45, 179–188.
Kita, T., Hata, Y., Kano, K., Miura, M., Nakao, S., Noda, Y., Shimokawa, H., Ishibashi, T., 2007. Transforming growth factor-beta2 and connective tissue growth factor in proliferative vitreoretinal diseases: possible involvement of hyalocytes and therapeutic potential of Rho kinase inhibitor. Diabetes 56, 231–238.
Klaassen, I., Hughes, J.M., Vogels, I.M., Schalkwijk, C.G., Van Noorden, C.J., Schlingemann, R.O., 2009. Altered expression of genes related to blood–retina barrier disruption in streptozotocin-induced diabetes. Exp. Eye Res. 89, 4–15.
Koh, G.Y., Kim, I., Kwak, H.J., Yun, M.J., Leem, J.C., 2002. Biomedical significance of endothelial cell specific growth factor, angiopoietin. Exp. Mol. Med. 34, 1–11.
Kojima, T., Sawada, N., Chiba, H., Kokai, Y., Yamamoto, M., Urban, M., Lee, G.H., Hertzberg, E.L., Mochizuki, Y., Spray, D.C., 1999. Induction of tight junctions in human connexin 32 (hCx32)-transfected mouse hepatocytes: connexin 32 interacts with occludin. Biochem. Biophys. Res. Commun. 266, 222–229.
Kojima, T., Spray, D.C., Kokai, Y., Chiba, H., Mochizuki, Y., Sawada, N., 2002. Cx32 formation and/or Cx32-mediated intercellular communication induces expression and function of tight junctions in hepatocytic cell line. Exp. Cell Res. 276, 40–51.
Komarova, Y., Malik, A.B., 2010. Regulation of endothelial permeability via paracellular and transcellular transport pathways. Annu. Rev. Physiol. 72, 463–493.
Korn, J., Christ, B., Kurz, H., 2002. Neuroectodermal origin of brain pericytes and vascular smooth muscle cells. J. Comp. Neurol. 442, 78–88.
Kucheryavykh, Y.V., Kucheryavykh, L.Y., Nichols, C.G., Maldonado, H.M., Baksi, K., Reichenbach, A., Skatchkov, S.N., Eaton, M.J., 2007. Downregulation of Kir4.1 inward rectifying potassium channel subunits by RNAi impairs potassium transfer and glutamate uptake by cultured cortical astrocytes. Glia 55, 274–281.
Kuiper, E.J., Witmer, A.N., Klaassen, I., Oliver, N., Goldschmeding, R., Schlingemann, R.O., 2004. Differential expression of connective tissue growth factor in microglia and pericytes in the human diabetic retina. Br. J. Ophthalmol. 88, 1082–1087.
Kuiper, E.J., de Smet, M.D., van Meurs, J.C., Tan, H.S., Tanck, M.W., Oliver, N., van Nieuwenhoven, F.A., Goldschmeding, R., Schlingemann, R.O., 2006. Association of connective tissue growth factor with fibrosis in vitreoretinal disorders in the human eye. Arch. Ophthalmol. 124, 1457–1462.
Kuiper, E.J., Hughes, J.M., Van Geest, R.J., Vogels, I.M., Goldschmeding, R., Van Noorden, C.J., Schlingemann, R.O., Klaassen, I., 2007. Effect of VEGF-A on expression of profibrotic growth factor and extracellular matrix genes in the retina. Invest. Ophthalmol. Vis. Sci. 48, 4267–4276.
Kuiper, E.J., van Nieuwenhoven, F.A., de Smet, M.D., van Meurs, J.C., Tanck, M.W., Oliver, N., Klaassen, I., Van Noorden, C.J., Goldschmeding, R., Schlingemann, R.O., 2008a. The angio-fibrotic switch of VEGF and CTGF in proliferative diabetic retinopathy. PLoS One 3, e2675.
Kuiper, E.J., van, Z.R., Roestenberg, P., Lyons, K.M., Goldschmeding, R., Klaassen, I., Van Noorden, C.J., Schlingemann, R.O., 2008b. Connective tissue growth factor is necessary for retinal capillary basal lamina thickening in diabetic mice. J. Histochem. Cytochem. 56, 785–792.
Kurzchalia, T.V., Dupree, P., Parton, R.G., Kellner, R., Virta, H., Lehnert, M., Simons, K., 1992. VIP21, a 21-kD membrane protein is an integral component of trans-Golgi-network-derived transport vesicles. J. Cell Biol. 118, 1003–1014.
Labrecque, L., Royal, I., Surprenant, D.S., Patterson, C., Gingras, D., Beliveau, R., 2003. Regulation of vascular endothelial growth factor receptor-2 activity by caveolin-1 and plasma membrane cholesterol. Mol. Biol. Cell 14, 334–347.
Lal-Nag, M., Morin, P.J., 2009. The claudins. Genome Biol. 10, 235.
Lampugnani, M.G., Orsenigo, F., Gagliani, M.C., Tacchetti, C., Dejana, E., 2006. Vascular endothelial cadherin controls VEGFR-2 internalization and signaling from intracellular compartments. J. Cell Biol. 174, 593–604.
Lampugnani, M.G., 2012. Endothelial Cell-to-cell Junctions: Adhesion and Signaling in Physiology and Pathology. Cold Spring Harb. Perspect. Med. 2.
Lee, S.W., Won, J.Y., Lee, H.Y., Lee, H.J., Youn, S.W., Lee, J.Y., Cho, C.H., Cho, H.J., Oh, S., Chae, I.H., Kim, H.S., 2011. Angiopoietin-1 protects heart against ischemia/reperfusion injury through VE-cadherin dephosphorylation and myocardiac integrin-beta1/ERK/caspase-9 phosphorylation cascade. Mol. Med. 17, 1095–1106.
Lightman, S., Greenwood, J., 1992. Effect of lymphocytic infiltration on the blood–retinal barrier in experimental autoimmune uveoretinitis. Clin. Exp. Immunol. 88, 473–477.
Limb, G.A., Chignell, A.H., Green, W., LeRoy, F., Dumonde, D.C., 1996. Distribution of TNF alpha and its reactive vascular adhesion molecules in fibrovascular membranes of proliferative diabetic retinopathy. Br. J. Ophthalmol. 80, 168–173.
Lisanti, M.P., Scherer, P.E., Vidugiriene, J., Tang, Z., Hermanowski-Vosatka, A., Tu, Y.H., Cook, R.F., Sargiacomo, M., 1994. Characterization of caveolin-rich membrane domains isolated from an endothelial-rich source: implications for human disease. J. Cell Biol. 126, 111–126.
Liu, L., Pilch, P.F., 2008. A critical role of cavin (polymerase I and transcript release factor) in caveolae formation and organization. J. Biol. Chem. 283, 4314–4322.
Lorenzi, M., 1992. Glucose toxicity in the vascular complications of diabetes: the cellular perspective. Diabetes Metab. Rev. 8, 85–103.
Lorenzi, M., Gerhardinger, C., 2001. Early cellular and molecular changes induced by diabetes in the retina. Diabetologia 44, 791–804.
Lu, Q., Harrington, E.O., Jackson, H., Morin, N., Shannon, C., Rounds, S., 2006. Transforming growth factor-beta1-induced endothelial barrier dysfunction involves Smad2-dependent p38 activation and subsequent RhoA activation. J. Appl. Physiol. 101, 375–384.
Luo, Y., Xiao, W., Zhu, X., Mao, Y., Liu, X., Chen, X., Huang, J., Tang, S., Rizzolo, L.J., 2011. Differential expression of claudins in retinas during normal development and the angiogenesis of oxygen-induced retinopathy. Invest. Ophthalmol. Vis. Sci. 52, 7556–7564.
Maisonpierre, P.C., Suri, C., Jones, P.F., Bartunkova, S., Wiegand, S.J., Radziejewski, C., Compton, D., McClain, J., Aldrich, T.H., Papadopoulos, N., Daly, T.J., Davis, S., Sato, T.N., Yancopoulos, G.D., 1997. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science 277, 55–60.
Mandriota, S.J., Pepper, M.S., 1998. Regulation of angiopoietin-2 mRNA levels in bovine microvascular endothelial cells by cytokines and hypoxia. Circ. Res. 83, 852–859.
Martiney, J.A., Litwak, M., Berman, J.W., Arezzo, J.C., Brosnan, C.F., 1990. Pathophysiologic effect of interleukin-1b in the rabbit retina. Am. J. Pathol. 137, 1411–1423.
Martiney, J.A., Berman, J.W., Brosnan, C.F., 1992. Chronic inflammatory effects of interleukin-1 on the blood–retina barrier. J. Neuroimmunol. 41, 167–176.
Matter, K., Balda, M.S., 2003. Holey barrier: claudins and the regulation of brain endothelial permeability. J. Cell Biol. 161, 459–460.
McAllister, I.L., Vijayasekaran, S., Chen, S.D., Yu, D.Y., 2009. Effect of triamcinolone acetonide on vascular endothelial growth factor and occludin levels in branch retinal vein occlusion. Am. J. Ophthalmol. 147, 838–846.
Medina, F.A., de Almeida, C.J., Dew, E., Li, J., Bonuccelli, G., Williams, T.M., Cohen, A.W., Pestell, R.G., Frank, P.G., Tanowitz, H.B., Lisanti, M.P., 2006. Caveolin-1-deficient mice show defects in innate immunity and inflammatory immune response during *Salmonella enterica* serovar Typhimurium infection. Infect. Immun. 74, 6665–6674.
Mehta, D., Malik, A.B., 2006. Signaling mechanisms regulating endothelial permeability. Physiol. Rev. 86, 279–367.
Michel, C.C., Curry, F.E., 1999. Microvascular permeability. Physiol. Rev. 79, 703–761.
Milici, A.J., Watrous, N.E., Stukenbrok, H., Palade, G.E., 1987. Transcytosis of albumin in capillary endothelium. J. Cell Biol. 105, 2603–2612.
Milovanova, T., Chatterjee, S., Hawkins, B.J., Hong, N., Sorokina, E.M., Debolt, K., Moore, J.S., Madesh, M., Fisher, A.B., 2008. Caveolae are an essential component of the pathway for endothelial cell signaling associated with abrupt reduction of shear stress. Biochim. Biophys. Acta 1783, 1866–1875.
Minshall, R.D., Tiruppathi, C., Vogel, S.M., Niles, W.D., Gilchrist, A., Hamm, H.E., Malik, A.B., 2000. Endothelial cell-surface gp60 activates vesicle formation and trafficking via G(i)-coupled Src kinase signaling pathway. J. Cell Biol. 150, 1057–1070.
Mitchell, P., Bandello, F., Schmidt-Erfurth, U., Lang, G.E., Massin, P., Schlingemann, R.O., Sutter, F., Simader, C., Burian, G., Gerstner, O., Weichselberger, A., 2011. The RESTORE study: ranibizumab monotherapy or combined with laser versus laser monotherapy for diabetic macular edema. Ophthalmololgy 118, 615–625.
Miyake, K., Ibaraki, N., 2002. Prostaglandins and cystoid macular edema. Surv. Ophthalmol. 47 (Suppl 1), S203–S218.
Mizutani, M., Kern, T.S., Lorenzi, M., 1996. Accelerated death of retinal microvascular cells in human and experimental diabetic retinopathy. J. Clin. Invest. 97, 2883–2890.
Morita, K., Tsukita, S., Miyachi, Y., 2004. Tight junction-associated proteins (occludin, ZO-1, claudin-1, claudin-4) in squamous cell carcinoma and Bowen's disease. Br. J. Dermatol. 151, 328–334.
Motulsky, E., Koch, P., Janssens, S., Lienart, M., Vanbellinghen, A.M., Bolaky, N., Chan, C.C., Caspers, L., Martin-Martinez, M.D., Xu, H., Delporte, C., Willermain, F., 2010. Aquaporin expression in blood–retinal barrier cells during experimental autoimmune uveitis. Mol. Vis. 16, 602–610.
Muller, W.A., 2011. Mechanisms of leukocyte transendothelial migration. Annu. Rev. Pathol. 6, 323–344.
Murakami, T., Felinski, E.A., Antonetti, D.A., 2009. Occludin phosphorylation and ubiquitination regulate tight junction trafficking and vascular endothelial growth factor-induced permeability. J. Biol. Chem. 284, 21036–21046.

Murakami, T., Frey, T., Lin, C., Antonetti, D.A., 2012. Protein kinase cbeta phosphorylates occludin regulating tight junction trafficking in vascular endothelial growth factor-induced permeability in vivo. Diabetes 61, 1573–1583.
Murphy-Ullrich, J.E., Poczatek, M., 2000. Activation of latent TGF-beta by thrombospondin-1: mechanisms and physiology. Cytokine Growth Factor Rev. 11, 59–69.
Murugeswari, P., Shukla, D., Rajendran, A., Kim, R., Namperumalsamy, P., Muthukkaruppan, V., 2008. Proinflammatory cytokines and angiogenic and anti-angiogenic factors in vitreous of patients with proliferative diabetic retinopathy and eales' disease. Retina 28, 817–824.
Musashi, K., Kiryu, J., Miyamoto, K., Miyahara, S., Katsuta, H., Tamura, H., Hirose, F., Yoshimura, N., 2005. Thrombin inhibitor reduces leukocyte-endothelial cell interactions and vascular leakage after scatter laser photocoagulation. Invest. Ophthalmol. Vis. Sci. 46, 2561–2566.
Myles, M.E., Neumann, D.M., Hill, J.M., 2005. Recent progress in ocular drug delivery for posterior segment disease: emphasis on transscleral iontophoresis. Adv. Drug Deliv. Rev. 57, 2063–2079.
Nagasawa, K., Chiba, H., Fujita, H., Kojima, T., Saito, T., Endo, T., Sawada, N., 2006. Possible involvement of gap junctions in the barrier function of tight junctions of brain and lung endothelial cells. J. Cell. Physiol. 208, 123–132.
Nagase, K., Tomi, M., Tachikawa, M., Hosoya, K., 2006. Functional and molecular characterization of adenosine transport at the rat inner blood–retinal barrier. Biochim. Biophys. Acta 1758, 13–19.
Nagelhus, E.A., Horio, Y., Inanobe, A., Fujita, A., Haug, F.M., Nielsen, S., Kurachi, Y., Ottersen, O.P., 1999. Immunogold evidence suggests that coupling of K+ siphoning and water transport in rat retinal Muller cells is mediated by a coenrichment of Kir4.1 and AQP4 in specific membrane domains. Glia 26, 47–54.
Nagelhus, E.A., Mathiisen, T.M., Ottersen, O.P., 2004. Aquaporin-4 in the central nervous system: cellular and subcellular distribution and coexpression with KIR4.1. Neuroscience 129, 905–913.
Nakagawa, S., Deli, M.A., Nakao, S., Honda, M., Hayashi, K., Nakaoke, R., Kataoka, Y., Niwa, M., 2007. Pericytes from brain microvessels strengthen the barrier integrity in primary cultures of rat brain endothelial cells. Cell. Mol. Neurobiol. 27, 687–694.
Nakagawa, S., Deli, M.A., Kawaguchi, H., Shimizudani, T., Shimono, T., Kittel, A., Tanaka, K., Niwa, M., 2009. A new blood–brain barrier model using primary rat brain endothelial cells, pericytes and astrocytes. Neurochem. Int. 54, 253–263.
Navaratna, D., McGuire, P.G., Menicucci, G., Das, A., 2007. Proteolytic degradation of VE-cadherin alters the blood–retinal barrier in diabetes. Diabetes 56, 2380–2387.
Negrete, H.O., Lavelle, J.P., Berg, J., Lewis, S.A., Zeidel, M.L., 1996. Permeability properties of the intact mammalian bladder epithelium. Am. J. Physiol. 271, F886–F894.
Nielsen, S., Smith, B.L., Christensen, E.I., Agre, P., 1993. Distribution of the aquaporin CHIP in secretory and resorptive epithelia and capillary endothelia. Proc. Natl. Acad. Sci. U.S.A 90, 7275–7279.
Niemela, H., Elima, K., Henttinen, T., Irjala, H., Salmi, M., Jalkanen, S., 2005. Molecular identification of PAL-E, a widely used endothelial-cell marker. Blood 106, 3405–3409.
Nieuwdorp, M., van Haeften, T.W., Gouverneur, M.C., Mooij, H.L., van Lieshout, M.H., Levi, M., Meijers, J.C., Holleman, F., Hoekstra, J.B., Vink, H., Kastelein, J.J., Stroes, E.S., 2006. Loss of endothelial glycocalyx during acute hyperglycemia coincides with endothelial dysfunction and coagulation activation in vivo. Diabetes 55, 480–486.
Nishikawa, T., Giardino, I., Edelstein, D., Brownlee, M., 2000. Changes in diabetic retinal matrix protein mRNA levels in a common transgenic mouse strain. Curr. Eye Res. 21, 581–587.
Nitta, T., Hata, M., Gotoh, S., Seo, Y., Sasaki, H., Hashimoto, N., Furuse, M., Tsukita, S., 2003. Size-selective loosening of the blood–brain barrier in claudin-5-deficient mice. J. Cell Biol. 161, 653–660.
Nusrat, A., Parkos, C.A., Verkade, P., Foley, C.S., Liang, T.W., Innis-Whitehouse, W., Eastburn, K.K., Madara, J.L., 2000. Tight junctions are membrane microdomains. J. Cell Sci. 113, 1771–1781.
Nyqvist, D., Giampietro, C., Dejana, E., 2008. Deciphering the functional role of endothelial junctions by using in vivo models. EMBO Rep. 9, 742–747.
Ogunshola, O.O., Al-Ahmad, A., 2012. HIF-1 at the blood–brain barrier: a mediator of permeability? High Alt. Med. Biol. 13, 153–161.
Oh, H., Takagi, H., Suzuma, K., Otani, A., Matsumura, M., Honda, Y., 1999. Hypoxia and vascular endothelial growth factor selectively up-regulate angiopoietin-2 in bovine microvascular endothelial cells. J. Biol. Chem. 274, 15732–15739.
Oh, P., McIntosh, D.P., Schnitzer, J.E., 1998. Dynamin at the neck of caveolae mediates their budding to form transport vesicles by GTP-driven fission from the plasma membrane of endothelium. J. Cell Biol. 141, 101–114.
Oh, P., Borgstrom, P., Witkiewicz, H., Li, Y., Borgstrom, B.J., Chrastina, A., Iwata, K., Zinn, K.R., Baldwin, R., Testa, J.E., Schnitzer, J.E., 2007. Live dynamic imaging of caveolae pumping targeted antibody rapidly and specifically across endothelium in the lung. Nat. Biotechnol. 25, 327–337.
Oh, I.K., Kim, S.W., Oh, J., Lee, T.S., Huh, K., 2010. Inflammatory and angiogenic factors in the aqueous humor and the relationship to diabetic retinopathy. Curr. Eye Res. 35, 1116–1127.
Olsson, A.K., Dimberg, A., Kreuger, J., Claesson-Welsh, L., 2006. VEGF receptor signalling - in control of vascular function. Nat. Rev. Mol. Cell Biol. 7, 359–371.
Orsenigo, F., Giampietro, C., Ferrari, A., Corada, M., Galaup, A., Sigismund, S., Ristagno, G., Maddaluno, L., Young, K.G., Franco, D., Kurtcuoglu, V., Poulikakos, D., Baluk, P., McDonald, D., Grazia, L.M., Dejana, E., 2012. Phosphorylation of VE-cadherin is modulated by haemodynamic forces and contributes to the regulation of vascular permeability in vivo. Nat. Commun. 3, 1208.
Oshitari, T., Polewski, P., Chadda, M., Li, A.F., Sato, T., Roy, S., 2006. Effect of combined antisense oligonucleotides against high-glucose- and diabetes-induced overexpression of extracellular matrix components and increased vascular permeability. Diabetes 55, 86–92.
Otero, K., Martinez, F., Beltran, A., Gonzalez, D., Herrera, B., Quintero, G., Delgado, R., Rojas, A., 2001. Albumin-derived advanced glycation end-products trigger the disruption of the vascular endothelial cadherin complex in cultured human and murine endothelial cells. Biochem. J. 359, 567–574.
Paccola, L., Costa, R.A., Folgosa, M.S., Barbosa, J.C., Scott, I.U., Jorge, R., 2008. Intravitreal triamcinolone versus bevacizumab for treatment of refractory diabetic macular oedema (IBEME study). Br. J. Ophthalmol. 92, 76–80.
Palade, G.E., 1953. Fine structure of blood capillaries. J. Appl. Physiol. 24, 1424.
Palade, G.E., Bruns, R.R., 1968. Structural modulations of plasmalemmal vesicles. J. Cell Biol. 37, 633–649.
Pannicke, T., Iandiev, I., Wurm, A., Uckermann, O., Vom, H.F., Reichenbach, A., Wiedemann, P., Hammes, H.P., Bringmann, A., 2006. Diabetes alters osmotic swelling characteristics and membrane conductance of glial cells in rat retina. Diabetes 55, 633–639.
Paolinelli, R., Corada, M., Orsenigo, F., Dejana, E., 2011. The molecular basis of the blood brain barrier differentiation and maintenance. Is it still a mystery? Pharmacol. Res. 63, 165–171.
Pappenheimer, J.R., Renkin, E.M., Borrero, L.M., 1951. Filtration, diffusion and molecular sieving through peripheral capillary membranes; a contribution to the pore theory of capillary permeability. Am. J. Physiol. 167, 13–46.
Paris, L., Tonutti, L., Vannini, C., Bazzoni, G., 2008. Structural organization of the tight junctions. Biochim. Biophys. Acta 1778, 646–659.
Park, D.S., Woodman, S.E., Schubert, W., Cohen, A.W., Frank, P.G., Chandra, M., Shirani, J., Razani, B., Tang, B., Jelicks, L.A., Factor, S.M., Weiss, L.M., Tanowitz, H.B., Lisanti, M.P., 2002. Caveolin-1/3 double-knockout mice are viable, but lack both muscle and non-muscle caveolae, and develop a severe cardiomyopathic phenotype. Am. J. Pathol. 160, 2207–2217.
Parton, R.G., Richards, A.A., 2003. Lipid rafts and caveolae as portals for endocytosis: new insights and common mechanisms. Traffic 4, 724–738.
Pascariu, M., Bendayan, M., Ghitescu, L., 2004. Correlated endothelial caveolin overexpression and increased transcytosis in experimental diabetes. J. Histochem. Cytochem. 52, 65–76.
Patel, J.I., Hykin, P.G., Gregor, Z.J., Boulton, M., Cree, I.A., 2005. Angiopoietin concentrations in diabetic retinopathy. Br. J. Ophthalmol. 89, 480–483.
Peters, A., Palay, S., Webster, H., 1991. The Fine Structure of the Nervous System: Neurons and Their Supporting Cells. Oxford University Press, New York.
Pfister, F., Feng, Y., Vom, H.F., Hoffmann, S., Molema, G., Hillebrands, J.L., Shani, M., Deutsch, U., Hammes, H.P., 2008. Pericyte migration: a novel mechanism of pericyte loss in experimental diabetic retinopathy. Diabetes 57, 2495–2502.
Pfister, F., Wang, Y., Schreiter, K., Vom, H.F., Altvater, K., Hoffmann, S., Deutsch, U., Hammes, H.P., Feng, Y., 2010. Retinal overexpression of angiopoietin-2 mimics diabetic retinopathy and enhances vascular damages in hyperglycemia. Acta Diabetol. 47, 59–64.
PKC-DMES Study Group, 2007. Effect of ruboxistaurin in patients with diabetic macular edema: thirty-month results of the randomized PKC-DMES clinical trial. Arch. Ophthalmol. 125, 318–324.
PKC-DRS Study Group, 2005. The effect of ruboxistaurin on visual loss in patients with moderately severe to very severe nonproliferative diabetic retinopathy: initial results of the Protein Kinase C beta Inhibitor Diabetic Retinopathy Study (PKC-DRS) multicenter randomized clinical trial. Diabetes 54, 2188–2197.
Polt, R., Porreca, F., Szabo, L.Z., Bilsky, E.J., Davis, P., Abbruscato, T.J., Davis, T.P., Harvath, R., Yamamura, H.I., Hruby, V.J., 1994. Glycopeptide enkephalin analogues produce analgesia in mice: evidence for penetration of the blood–brain barrier. Proc. Natl. Acad. Sci. U.S.A 91, 7114–7118.
Predescu, D., Vogel, S.M., Malik, A.B., 2004. Functional and morphological studies of protein transcytosis in continuous endothelia. Am. J. Physiol. Lung Cell. Mol. Physiol. 287, L895–L901.
Predescu, S.A., Predescu, D.N., Timblin, B.K., Stan, R.V., Malik, A.B., 2003. Intersectin regulates fission and internalization of caveolae in endothelial cells. Mol. Biol Cell. 14, 4997–5010.
Predescu, S.A., Predescu, D.N., Malik, A.B., 2007. Molecular determinants of endothelial transcytosis and their role in endothelial permeability. Am. J. Physiol. Lung Cell. Mol. Physiol. 293, L823–L842.
Pruneau, D., Belichard, P., Sahel, J.A., Combal, J.P., 2010. Targeting the kallikrein-kinin system as a new therapeutic approach to diabetic retinopathy. Curr. Opin. Invest. Drugs 11, 507–514.
Quigley, M.G., 2007. Prognosis and retinal vessel features. Ophthalmology 114, 1796–1797.
Rahman, S., Rahman, T., Ismail, A.A., Rashid, A.R., 2007. Diabetes-associated macrovasculopathy: pathophysiology and pathogenesis. Diabetes Obes. Metab. 9, 767–780.
Rakoczy, E.P., Ali Rahman, I.S., Binz, N., Li, C.R., Vagaja, N.N., de Pinho, M., Lai, C.M., 2010. Characterization of a mouse model of hyperglycemia and retinal neovascularization. Am. J. Pathol. 177, 2659–2670.
Raleigh, D.R., Boe, D.M., Yu, D., Weber, C.R., Marchiando, A.M., Bradford, E.M., Wang, Y., Wu, L., Schneeberger, E.E., Shen, L., Turner, J.R., 2011. Occludin S408 phosphorylation regulates tight junction protein interactions and barrier function. J. Cell Biol. 193, 565–582.

Rangasamy, S., Srinivasan, R., Maestas, J., McGuire, P.G., Das, A., 2011. A potential role for angiopoietin 2 in the regulation of the blood–retinal barrier in diabetic retinopathy. Invest. Ophthalmol. Vis. Sci. 52, 3784–3791.
Raviola, G., 1977. The structural basis of the blood–ocular barriers. Exp. Eye Res. 25, 27–63.
Razani, B., Engelman, J.A., Wang, X.B., Schubert, W., Zhang, X.L., Marks, C.B., Macaluso, F., Russell, R.G., Li, M., Pestell, R.G., Di Vizio, D., Hou Jr., H., Kneitz, B., Lagaud, G., Christ, G.J., Edelmann, W., Lisanti, M.P., 2001. Caveolin-1 null mice are viable but show evidence of hyperproliferative and vascular abnormalities. J. Biol. Chem. 276, 38121–38138.
Red-Horse, K., Ferrara, N., 2007. Vascular targeting via caveolae. Nat. Biotechnol. 25, 431–432.
Rehak, M., Hollborn, M., Iandiev, I., Pannicke, T., Karl, A., Wurm, A., Kohen, L., Reichenbach, A., Wiedemann, P., Bringmann, A., 2009. Retinal gene expression and Muller cell responses after branch retinal vein occlusion in the rat. Invest. Ophthalmol. Vis. Sci. 50, 2359–2367.
Reichenbach, A., Wurm, A., Pannicke, T., Iandiev, I., Wiedemann, P., Bringmann, A., 2007. Muller cells as players in retinal degeneration and edema. Graefes Arch. Clin. Exp. Ophthalmol. 245, 627–636.
Rizzolo, L.J., Peng, S., Luo, Y., Xiao, W., 2011. Integration of tight junctions and claudins with the barrier functions of the retinal pigment epithelium. Prog. Retin. Eye Res. 30, 296–323.
Rogers, S., McIntosh, R.L., Cheung, N., Lim, L., Wang, J.J., Mitchell, P., Kowalski, J.W., Nguyen, H., Wong, T.Y., 2010. The prevalence of retinal vein occlusion: pooled data from population studies from the United States, Europe, Asia, and Australia. Ophthalmology 117, 313–319.
Rosengren, B.I., Rippe, A., Rippe, C., Venturoli, D., Sward, K., Rippe, B., 2006. Transvascular protein transport in mice lacking endothelial caveolae. Am. J. Physiol. Heart Circ. Physiol. 291, H1371–H1377.
Rothberg, K.G., Ying, Y.S., Kamen, B.A., Anderson, R.G., 1990. Cholesterol controls the clustering of the glycophospholipid-anchored membrane receptor for 5-methyltetrahydrofolate. J. Cell. Biol. 111, 2931–2938.
Rothberg, K.G., Heuser, J.E., Donzell, W.C., Ying, Y.S., Glenney, J.R., Anderson, R.G., 1992. Caveolin, a protein component of caveolae membrane coats. Cell 68, 673–682.
Roy, S., Sato, T., Paryani, G., Kao, R., 2003. Downregulation of fibronectin overexpression reduces basement membrane thickening and vascular lesions in retinas of galactose-fed rats. Diabetes 52, 1229–1234.
Rudini, N., Felici, A., Giampietro, C., Lampugnani, M., Corada, M., Swirsding, K., Garre, M., Liebner, S., Letarte, M., ten, D.P., Dejana, E., 2008. VE-cadherin is a critical endothelial regulator of TGF-beta signalling. EMBO J. 27, 993–1004.
Ruiter, D.J., Schlingemann, R.O., Westphal, J.R., Denijn, M., Rietveld, F.J., de Waal, R.M., 1993. Angiogenesis in wound healing and tumor metastasis. Behring Inst. Mitt. 92, 258–272.
Runkle, E.A., Antonetti, D.A., 2011. The blood–retinal barrier: structure and functional significance. Methods Mol. Biol. 686, 133–148.
Saadoun, S., Papadopoulos, M.C., Davies, D.C., Bell, B.A., Krishna, S., 2002. Increased aquaporin 1 water channel expression in human brain tumours. Br. J. Cancer 87, 621–623.
Sagaties, M.J., Raviola, G., Schaeffer, S., Miller, C., 1987. The structural basis of the inner blood–retina barrier in the eye of *Macaca mulatta*. Invest. Ophthalmol. Vis. Sci. 28, 2000–2014.
Saitou, M., Ando-Akatsuka, Y., Itoh, M., Furuse, M., Inazawa, J., Fujimoto, K., Tsukita, S., 1997. Mammalian occludin in epithelial cells: its expression and subcellular distribution. Eur. J. Cell Biol. 73, 222–231.
Saitou, M., Fujimoto, K., Doi, Y., Itoh, M., Fujimoto, T., Furuse, M., Takano, H., Noda, T., Tsukita, S., 1998. Occludin-deficient embryonic stem cells can differentiate into polarized epithelial cells bearing tight junctions. J. Cell Biol. 141, 397–408.
Samad, A., Sultana, Y., Aqil, M., 2007. Liposomal drug delivery systems: an update review. Curr. Drug Deliv. 4, 297–305.
Sander, B., Larsen, M., Moldow, B., Lund-Andersen, H., 2001. Diabetic macular edema: passive and active transport of fluorescein through the blood–retina barrier. Invest. Ophthalmol. Vis. Sci. 42, 433–438.
Santibanez, J.F., Blanco, F.J., Garrido-Martin, E.M., Sanz-Rodriguez, F., del Pozo, M.A., Bernabeu, C., 2008. Caveolin-1 interacts and cooperates with the transforming growth factor-beta type I receptor ALK1 in endothelial caveolae. Cardiovasc. Res. 77, 791–799.
Sato, T.N., Tozawa, Y., Deutsch, U., Wolburg-Buchholz, K., Fujiwara, Y., Gendron-Maguire, M., Gridley, T., Wolburg, H., Risau, W., Qin, Y., 1995. Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation. Nature 376, 70–74.
Scheppke, L., Aguilar, E., Gariano, R.F., Jacobson, R., Hood, J., Doukas, J., Cao, J., Noronha, G., Yee, S., Weis, S., Martin, M.B., Soll, R., Cheresh, D.A., Friedlander, M., 2008. Retinal vascular permeability suppression by topical application of a novel VEGFR2/Src kinase inhibitor in mice and rabbits. J. Clin. Invest. 118, 2337–2346.
Schlingemann, R.O., Dingjan, G.M., Emeis, J.J., Blok, J., Warnaar, S.O., Ruiter, D.J., 1985. Monoclonal antibody PAL-E specific for endothelium. Lab. Invest. 52, 71–76.
Schlingemann, R.O., Bots, G.T.A.M., Van Duinen, S.G., Ruiter, D.J., 1988. Differential expression of endotheliumspecific antigen PAL-E in vasculature of brain tumors and preexistent brain capillaries. In: Strand, F. (Ed.), Blood–Brain Transfer. Ann. N.Y. Acad. Sci. 465, 111–114.
Schlingemann, R.O., Hofman, P., Anderson, L., Troost, D., van der, G.R., 1997. Vascular expression of endothelial antigen PAL-E indicates absence of blood-ocular barriers in the normal eye. Ophthalmic Res. 29, 130–138.
Schlingemann, R.O., Rietveld, F.J., Kwaspen, F., van de Kerkhof, P.C., de Waal, R.M., Ruiter, D.J., 1991. Differential expression of markers for endothelial cells, pericytes, and basal lamina in the microvasculature of tumors and granulation tissue. Am. J. Pathol. 138, 1335–1347.
Schlingemann, R.O., van Hinsbergh, V.W., 1997. Role of vascular permeability factor/vascular endothelial growth factor in eye disease. Br. J. Ophthalmol. 81, 501–512.
Schlingemann, R.O., Hofman, P., Vrensen, G.F., Blaauwgeers, H.G., 1999. Increased expression of endothelial antigen PAL-E in human diabetic retinopathy correlates with microvascular leakage. Diabetologia 42, 596–602.
Schlingemann, R.O., 2004. Role of growth factors and the wound healing response in age-related macular degeneration. Graefes Arch. Clin. Exp. Ophthalmol. 242, 91–101.
Schnitzer, J., 1988. Astrocytes in mammalian retina. Prog. Ret. Eye Res. 7, 209–212.
Schnitzer, J.E., 1992. gp60 is an albumin-binding glycoprotein expressed by continuous endothelium involved in albumin transcytosis. Am. J. Physiol. 262, H246–H254.
Schnitzer, J.E., Liu, J., Oh, P., 1995. Endothelial caveolae have the molecular transport machinery for vesicle budding, docking, and fusion including VAMP, NSF, SNAP, annexins, and GTPases. J. Biol. Chem. 270, 14399–14404.
Schubert, W., Frank, P.G., Woodman, S.E., Hyogo, H., Cohen, D.E., Chow, C.W., Lisanti, M.P., 2002. Microvascular hyperpermeability in caveolin-1 (-/-) knockout mice. Treatment with a specific nitric-oxide synthase inhibitor, L-name, restores normal microvascular permeability in Cav-1 null mice. J. Biol. Chem. 277, 40091–40098.
Schubert, W., Sotgia, F., Cohen, A.W., Capozza, F., Bonuccelli, G., Bruno, C., Minetti, C., Bonilla, E., Dimauro, S., Lisanti, M.P., 2007. Caveolin-1(-/-)- and caveolin-2(-/-)-deficient mice both display numerous skeletal muscle abnormalities, with tubular aggregate formation. Am. J. Pathol. 170, 316–333.
Semenza, G.L., 2003. Targeting HIF-1 for cancer therapy. Nat. Rev. Cancer 3, 721–732.
Sfikakis, P.P., Markomichelakis, N., Theodossiadis, G.P., Grigoropoulos, V., Katsilambros, N., Theodossiadis, P.G., 2005. Regression of sight-threatening macular edema in type 2 diabetes following treatment with the anti-tumor necrosis factor monoclonal antibody infliximab. Diabetes Care 28, 445–447.
Shajahan, A.N., Timblin, B.K., Sandoval, R., Tiruppathi, C., Malik, A.B., Minshall, R.D., 2004. Role of Src-induced dynamin-2 phosphorylation in caveolae-mediated endocytosis in endothelial cells. J. Biol. Chem. 279, 20392–20400.
Shen, L., Weber, C.R., Turner, J.R., 2008. The tight junction protein complex undergoes rapid and continuous molecular remodeling at steady state. J. Cell Biol. 181, 683–695.
Shen, W., Li, S., Chung, S.H., Gillies, M.C., 2010. Retinal vascular changes after glial disruption in rats. J. Neurosci. Res. 88, 1485–1499.
Shimura, M., Nakazawa, T., Yasuda, K., Shiono, T., Iida, T., Sakamoto, T., Nishida, K., 2008. Comparative therapy evaluation of intravitreal bevacizumab and triamcinolone acetonide on persistent diffuse diabetic macular edema. Am. J. Ophthalmol. 145, 854–861.
Shue, E.H., Carson-Walter, E.B., Liu, Y., Winans, B.N., Ali, Z.S., Chen, J., Walter, K.A., 2008. Plasmalemmal vesicle associated protein-1 (PV-1) is a marker of blood–brain barrier disruption in rodent models. BMC Neurosci. 9, 29.
Siemerink, M.J., Augustin, A.J., Schlingemann, R.O., 2010. Mechanisms of ocular angiogenesis and its molecular mediators. Dev. Ophthalmol. 46, 4–20.
Silva, P.S., Sun, J.K., Aiello, L.P., 2009. Role of steroids in the management of diabetic macular edema and proliferative diabetic retinopathy. Semin. Ophthalmol. 24, 93–99.
Simionescu, N., 1979. The microvascular endothelium. Segmental differentiation transcytosis: selective distribution of anionic sites. In: Weissman, G., Samuelson, B., Paoletti, R. (Eds.), Advances in Inflammation Research. Raven, New York, pp. 61–70.
Simionescu, M., 1988. Receptor-mediated transcytosis of plasma molecules by vascular endothelium. In: Simionescu, N., Simionescu, M. (Eds.), Endothelial Cell Biology in Health and Disease. Plenum, New York, pp. 69–104.
Simionescu, M., Popov, D., Sima, A., 2009. Endothelial transcytosis in health and disease. Cell Tissue Res. 335, 27–40.
Simionescu, N., Siminoescu, M., Palade, G.E., 1975. Permeability of muscle capillaries to small heme-peptides. Evidence for the existence of patent transendothelial channels. J. Cell Biol. 64, 586–607.
Simionescu, N., 1983. Cellular aspects of transcapillary exchange. Physiol. Rev. 63, 1536–1579.
Sivaprasad, S., Bunce, C., Crosby-Nwaobi, R., 2012. Non-steroidal anti-inflammatory agents for treating cystoid macular oedema following cataract surgery. Cochrane Database Syst. Rev. 2, CD004239.
Simó, R., Villarroel, M., Corraliza, L., Hernández, C., Garcia-Ramírez, M., 2010. The retinal pigment epithelium: something more than a constituent of the blood-retinal barrier–implications for the pathogenesis of diabetic retinopathy. J. Biomed. Biotechnol. 2010, 190724.
Song, L., Ge, S., Pachter, J.S., 2007. Caveolin-1 regulates expression of junction-associated proteins in brain microvascular endothelial cells. Blood 109, 1515–1523.
Spindler, V., Schlegel, N., Waschke, J., 2010. Role of GTPases in control of microvascular permeability. Cardiovasc. Res. 87, 243–253.
Stamatovic, S.M., Dimitrijevic, O.B., Keep, R.F., Andjelkovic, A.V., 2006. Protein kinase Calpha-RhoA cross-talk in CCL2-induced alterations in brain endothelial permeability. J. Biol. Chem. 281, 8379–8388.
Stan, R.V., Ghitescu, L., Jacobson, B.S., Palade, G.E., 1999. Isolation, cloning, and localization of rat PV-1, a novel endothelial caveolar protein. J. Cell Biol. 145, 1189–1198.

Stefanec, T., 2004. How the endothelium and its bone marrow-derived progenitors influence development of disease. Med. Hypotheses 62, 247–251.
Stevenson, B.R., Siliciano, J.D., Mooseker, M.S., Goodenough, D.A., 1986. Identification of ZO-1: a high molecular weight polypeptide associated with the tight junction (zonula occludens) in a variety of epithelia. J. Cell Biol. 103, 755–766.
Stewart, P.A., Tuor, U.I., 1994. Blood–eye barriers in the rat: correlation of ultrastructure with function. J. Comp. Neurol. 340, 566–576.
Stewart, P.A., 2000. Endothelial vesicles in the blood–brain barrier: are they related to permeability? Cell. Mol. Neurobiol. 20, 149–163.
Stitt, A.W., Bhaduri, T., McMullen, C.B., Gardiner, T.A., Archer, D.B., 2000. Advanced glycation end products induce blood–retinal barrier dysfunction in normoglycemic rats. Mol. Cell. Biol. Res. Commun. 3, 380–388.
Stitt, A., Gardiner, T.A., Alderson, N.L., Canning, P., Frizzell, N., Duffy, N., Boyle, C., Januszewski, A.S., Chachich, M., Baynes, J.W., Thorpe, S.R., 2002. The AGE inhibitor pyridoxamine inhibits development of retinopathy in experimental diabetes. Diabetes 51, 2826–2832.
Strickland, L.A., Jubb, A.M., Hongo, J.A., Zhong, F., Burwick, J., Fu, L., Frantz, G.D., Koeppen, H., 2005. Plasmalemmal vesicle-associated protein (PLVAP) is expressed by tumour endothelium and is upregulated by vascular endothelial growth factor-A (VEGF). J. Pathol. 206, 466–475.
Suhler, E.B., Smith, J.R., Wertheim, M.S., Lauer, A.K., Kurz, D.E., Pickard, T.D., Rosenbaum, J.T., 2005. A prospective trial of infliximab therapy for refractory uveitis: preliminary safety and efficacy outcomes. Arch. Ophthalmol. 123, 903–912.
Suhler, E.B., Smith, J.R., Giles, T.R., Lauer, A.K., Wertheim, M.S., Kurz, D.E., Kurz, P.A., Lim, L., Mackensen, F., Pickard, T.D., Rosenbaum, J.T., 2009. Infliximab therapy for refractory uveitis: 2-year results of a prospective trial. Arch. Ophthalmol. 127, 819–822.
Sundstrom, J.M., Tash, B.R., Murakami, T., Flanagan, J.M., Bewley, M.C., Stanley, B.A., Gonsar, K.B., Antonetti, D.A., 2009. Identification and analysis of occludin phosphosites: a combined mass spectrometry and bioinformatics approach. J. Proteome Res. 8, 808–817.
Suri, C., Jones, P.F., Patan, S., Bartunkova, S., Maisonpierre, P.C., Davis, S., Sato, T.N., Yancopoulos, G.D., 1996. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. Cell 87, 1171–1180.
Tadayoni, R., Paques, M., Gaudric, A., Vicaut, E., 2003. Erythrocyte and leukocyte dynamics in the retinal capillaries of diabetic mice. Exp. Eye Res. 77, 497–504.
Taddei, A., Giampietro, C., Conti, A., Orsenigo, F., Breviario, F., Pirazzoli, V., Potente, M., Daly, C., Dimmeler, S., Dejana, E., 2008. Endothelial adherens junctions control tight junctions by VE-cadherin-mediated upregulation of claudin-5. Nat. Cell Biol. 10, 923–934.
Takagi, H., Koyama, S., Seike, H., Oh, H., Otani, A., Matsumura, M., Honda, Y., 2003. Potential role of the angiopoietin/tie2 system in ischemia-induced retinal neovascularization. Invest. Ophthalmol. Vis. Sci. 44, 393–402.
Takata, K., Kasahara, T., Kasahara, M., Ezaki, O., Hirano, H., 1992. Ultracytochemical localization of the erythrocyte/HepG2-type glucose transporter (GLUT1) in cells of the blood–retinal barrier in the rat. Invest. Ophthalmol. Vis. Sci. 33, 377–383.
Tao-Cheng, J.H., Nagy, Z., Brightman, M.W., 1987. Tight junctions of brain endothelium in vitro are enhanced by astroglia. J. Neurosci. 7, 3293–3299.
Titchenell, P.M., Lin, C.M., Keil, J.M., Sundstrom, J.M., Smith, C.D., Antonetti, D.A., 2012. Novel atypical PKC inhibitors prevent vascular endothelial growth factor-induced blood–retinal barrier dysfunction. Biochem. J. 446, 455–467.
Toda, R., Kawazu, K., Oyabu, M., Miyazaki, T., Kiuchi, Y., 2011. Comparison of drug permeabilities across the blood–retinal barrier, blood–aqueous humor barrier, and blood–brain barrier. J. Pharm. Sci. 100, 3904–3911.
Tout, S., Chan-Ling, T., Hollander, H., Stone, J., 1993. The role of Muller cells in the formation of the blood–retinal barrier. Neuroscience 55, 291–301.
Tretiach, M., Madigan, M.C., Wen, L., Gillies, M.C., 2005. Effect of Muller cell coculture on in vitro permeability of bovine retinal vascular endothelium in normoxic and hypoxic conditions. Neurosci. Lett. 378, 160–165.
Tsukita, S., Furuse, M., 1999. Occludin and claudins in tight-junction strands: leading or supporting players? Trends Cell Biol. 9, 268–273.
Tsukita, S., Furuse, M., Itoh, M., 2001. Multifunctional strands in tight junctions. Nat. Rev. Mol. Cell Biol. 2, 285–293.
Tsukita, S., Yamazaki, Y., Katsuno, T., Tamura, A., Tsukita, S., 2008. Tight junction-based epithelial microenvironment and cell proliferation. Oncogene 27, 6930–6938.
Tuma, P.L., Hubbard, A.L., 2003. Transcytosis: crossing cellular barriers. Physiol. Rev. 83, 871–932.
Vacca, A., Frigeri, A., Ribatti, D., Nicchia, G.P., Nico, B., Ria, R., Svelto, M., Dammacco, F., 2001. Microvessel overexpression of aquaporin 1 parallels bone marrow angiogenesis in patients with active multiple myeloma. Br. J. Haematol. 113, 415–421.
Van Geest, R.J., Klaassen, I., Vogels, I.M., Van Noorden, C.J., Schlingemann, R.O., 2010. Differential TGF-{beta} signaling in retinal vascular cells: a role in diabetic retinopathy? Invest. Ophthalmol. Vis. Sci. 51, 1857–1865.
Van Geest, R.J., Lesnik-Oberstein, S.Y., Tan, H.S., Mura, M., Goldschmeding, R., Van Noorden, C.J., Klaassen, I., Schlingemann, R.O., 2012. A shift in the balance of vascular endothelial growth factor and connective tissue growth factor by bevacizumab causes the angiofibrotic switch in proliferative diabetic retinopathy. Br. J. Ophthalmol. 96, 587–590.
Van Itallie, C.M., Anderson, J.M., 2006. Claudins and epithelial paracellular transport. Annu. Rev. Physiol. 68, 403–429.
Verkman, A.S., 2006. Roles of aquaporins in kidney revealed by transgenic mice. Semin. Nephrol. 26, 200–208.
Verkman, A.S., Ruiz-Ederra, J., Levin, M.H., 2008. Functions of aquaporins in the eye. Prog. Retin. Eye Res. 27, 420–433.
Vestweber, D., 2008. VE-cadherin: the major endothelial adhesion molecule controlling cellular junctions and blood vessel formation. Arterioscler. Thromb. Vasc. Biol. 28, 223–232.
Vestweber, D., Winderlich, M., Cagna, G., Nottebaum, A.F., 2009. Cell adhesion dynamics at endothelial junctions: VE-cadherin as a major player. Trends Cell Biol. 19, 8–15.
Vink, H., Duling, B.R., 1996. Identification of distinct luminal domains for macromolecules, erythrocytes, and leukocytes within mammalian capillaries. Circ. Res. 79, 581–589.
Vink, H., Duling, B.R., 2000. Capillary endothelial surface layer selectively reduces plasma solute distribution volume. Am. J. Physiol. Heart Circ. Physiol. 278, H285–H289.
Vinores, S.A., McGehee, R., Lee, A., Gadegbeku, C., Campochiaro, P.A., 1990. Ultrastructural localization of blood–retinal barrier breakdown in diabetic and galactosemic rats. J. Histochem. Cytochem. 38, 1341–1352.
Vinores, S.A., Xiao, W.H., Shen, J., Campochiaro, P.A., 2007. TNF-alpha is critical for ischemia-induced leukostasis, but not retinal neovascularization nor VEGF-induced leakage. J. Neuroimmunol. 182, 73–79.
Vinten, J., Johnsen, A.H., Roepstorff, P., Harpoth, J., Tranum-Jensen, J., 2005. Identification of a major protein on the cytosolic face of caveolae. Biochim. Biophys. Acta 1717, 34–40.
Virgintino, D., Robertson, D., Errede, M., Benagiano, V., Tauer, U., Roncali, L., Bertossi, M., 2002. Expression of caveolin-1 in human brain microvessels. Neuroscience 115, 145–152.
Von Zglinicki, T., 2000. Role of oxidative stress in telomere length regulation and replicative senescence. Ann. N.Y. Acad. Sci. 908, 99–110.
Vyas, S.P., Sihorkar, V., 2001. In: Vyas, S.P., Dixit, V. (Eds.), Advances in Liposomal Therapeutics. CBS Publishers, New Delhi, p. 230.
Wagner, R.C., Robinson, C.S., Cross, P.J., Devenny, J.J., 1983. Endocytosis and exocytosis of transferrin by isolated capillary endothelium. Microvasc. Res. 25, 387–396.
Wagner, R.C., Chen, S.C., 1991. Transcapillary transport of solute by the endothelial vesicular system: evidence from thin serial section analysis. Microvasc. Res. 42, 139–150.
Wakabayashi, Y., Usui, Y., Okunuki, Y., Kezuka, T., Takeuchi, M., Iwasaki, T., Ohno, A., Goto, H., 2011. Increases of vitreous monocyte chemotactic protein 1 and interleukin 8 levels in patients with concurrent hypertension and diabetic retinopathy. Retina 31, 1951–1957.
Wallez, Y., Huber, P., 2008. Endothelial adherens and tight junctions in vascular homeostasis, inflammation and angiogenesis. Biochim. Biophys. Acta 1778, 794–809.
Walshe, T.E., Saint-Geniez, M., Maharaj, A.S., Sekiyama, E., Maldonado, A.E., D'Amore, P.A., 2009. TGF-beta is required for vascular barrier function, endothelial survival and homeostasis of the adult microvasculature. PLoS One 4, e5149.
Wang, A., Hascall, V.C., 2004. Hyaluronan structures synthesized by rat mesangial cells in response to hyperglycemia induce monocyte adhesion. J. Biol. Chem. 279, 10279–10285.
Wenick, A.S., Bressler, N.M., 2012. Diabetic macular edema: current and emerging therapies. Middle East. Afr. J. Ophthalmol. 19, 4–12.
Wheelock, M.J., Johnson, K.R., 2003. Cadherins as modulators of cellular phenotype. Annu. Rev. Cell Dev. Biol. 19, 207–235.
Wisniewska-Kruk, J., Hoeben, K.A., Vogels, I.M., Gaillard, P.J., Van Noorden, C.J., Schlingemann, R.O., Klaassen, I., 2012. A novel co-culture model of the blood–retinal barrier based on primary retinal endothelial cells, pericytes and astrocytes. Exp. Eye Res. 96, 181–190.
Witkin, A.J., Brown, G.C., 2011. Update on nonsurgical therapy for diabetic macular edema. Curr. Opin. Ophthalmol. 22, 185–189.
Witmer, A.N., Blaauwgeers, H.G., Weich, H.A., Alitalo, K., Vrensen, G.F., Schlingemann, R.O., 2002. Altered expression patterns of VEGF receptors in human diabetic retina and in experimental VEGF-induced retinopathy in monkey. Invest. Ophthalmol. Vis. Sci. 43, 849–857.
Witmer, A.N., Vrensen, G.F., Van Noorden, C.J., Schlingemann, R.O., 2003. Vascular endothelial growth factors and angiogenesis in eye disease. Prog. Retin. Eye Res. 22, 1–29.
Witmer, A.N., van Blijswijk, B.C., Van Noorden, C.J., Vrensen, G.F., Schlingemann, R.O., 2004. In vivo angiogenic phenotype of endothelial cells and pericytes induced by vascular endothelial growth factor-A. J. Histochem. Cytochem. 52, 39–52.
Wolburg, H., Lippoldt, A., 2002. Tight junctions of the blood–brain barrier: development, composition and regulation. Vascul. Pharmacol. 38, 323–337.
Wolburg, H., Wolburg-Buchholz, K., Kraus, J., Rascher-Eggstein, G., Liebner, S., Hamm, S., Duffner, F., Grote, E.H., Risau, W., Engelhardt, B., 2003. Localization of claudin-3 in tight junctions of the blood–brain barrier is selectively lost during experimental autoimmune encephalomyelitis and human glioblastoma multiforme. Acta Neuropathol. 105, 586–592.
Wolburg, H., Wolburg-Buchholz, K., Engelhardt, B., 2005. Diapedesis of mononuclear cells across cerebral venules during experimental autoimmune encephalomyelitis leaves tight junctions intact. Acta Neuropathol. 109, 181–190.
Wolburg, H., 2006. The endothelial frontier. In: Dermietzel, R., Spray, D.C., Nedergaard, M. (Eds.), Blood–Brain Interface: From Ontogeny to Artificial Barriers. Wiley-VCH, Weinheim, Germany, pp. 77–109.
Wolburg, H., Noell, S., Mack, A., Wolburg-Buchholz, K., Fallier-Becker, P., 2009. Brain endothelial cells and the glio-vascular complex. Cell Tissue Res. 335, 75–96.
Wright, T.J., Leach, L., Shaw, P.E., Jones, P., 2002. Dynamics of vascular endothelial-cadherin and beta-catenin localization by vascular endothelial growth factor-induced angiogenesis in human umbilical vein cells. Exp. Cell Res. 280, 159–168.

Wu, L., Hernandez-Bogantes, E., Roca, J.A., Arevalo, J.F., Barraza, K., Lasave, A.F., 2011. Intravitreal tumor necrosis factor inhibitors in the treatment of refractory diabetic macular edema: a pilot study from the Pan-American Collaborative Retina Study Group. Retina 31, 298–303.

Xia, P., Aiello, L.P., Ishii, H., Jiang, Z.Y., Park, D.J., Robinson, G.S., Takagi, H., Newsome, W.P., Jirousek, M.R., King, G.L., 1996. Characterization of vascular endothelial growth factor's effect on the activation of protein kinase C, its isoforms, and endothelial cell growth. J. Clin. Invest. 98, 2018–2026.

Xu, H., Chen, M., Forrester, J.V., 2009. Para-inflammation in the aging retina. Prog. Retin. Eye Res. 28, 348–368.

Yamada, E., 1955. The fine structure of the gall bladder epithelium of the mouse. J. Biophys. Biochem. Cytol. 1, 445–458.

Yan, J., Zhou, B., Taheri, S., Shi, H., 2011. Differential effects of HIF-1 inhibition by YC-1 on the overall outcome and blood–brain barrier damage in a rat model of ischemic stroke. PLoS One 6, e27798.

Yeh, W.L., Lu, D.Y., Lin, C.J., Liou, H.C., Fu, W.M., 2007. Inhibition of hypoxia-induced increase of blood–brain barrier permeability by YC-1 through the antagonism of HIF-1alpha accumulation and VEGF expression. Mol. Pharmacol. 72, 440–449.

Zhang, J., Gerhardinger, C., Lorenzi, M., 2002. Early complement activation and decreased levels of glycosylphosphatidylinositol-anchored complement inhibitors in human and experimental diabetic retinopathy. Diabetes 51, 3499–3504.

Zhang, S.X., Wang, J.J., Gao, G., Shao, C., Mott, R., Ma, J.X., 2006. Pigment epithelium-derived factor (PEDF) is an endogenous antiinflammatory factor. FASEB J. 20, 323–325.

Ziello, J.E., Jovin, I.S., Huang, Y., 2007. Hypoxia-Inducible Factor (HIF)-1 regulatory pathway and its potential for therapeutic intervention in malignancy and ischemia. Yale J. Biol. Med. 80, 51–60.

Zlokovic, B.V., 2008. The blood–brain barrier in health and chronic neurodegenerative disorders. Neuron 57, 178–201.