Acta Ophthalmologica

Review Article

The pathophysiology of retinopathy of prematurity: an update of previous and recent knowledge

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ABSTRACT.

Retinopathy of prematurity (ROP) is a disease that can cause blindness in very low birthweight infants. The incidence of ROP is closely correlated with the weight and the gestational age at birth. Despite current therapies, ROP continues to be a highly debilitating disease. Our advancing knowledge of the pathogenesis of ROP has encouraged investigations into new antivasculogenic therapies. The purpose of this article is to review the findings on the pathophysiological mechanisms that contribute to the transition between the first and second phases of ROP and to investigate new potential therapies. Oxygen has been well characterized for the key role that it plays in retinal neoangiogenesis. Low or high levels of pO_2 regulate the normal or abnormal production of hypoxia-inducible factor 1 and vascular endothelial growth factors (VEGF), which are the predominant regulators of retinal angiogenesis. Although low oxygen saturation appears to reduce the risk of severe ROP when carefully controlled within the first few weeks of life, the optimal level of saturation still remains uncertain. IGF-1 and Epo are fundamentally required during both phases of ROP, as alterations in their protein levels can modulate disease progression. Therefore, rhIGF-1 and rhEpo were tested for their abilities to prevent the loss of vasculature during the first phase of ROP, whereas anti-VEGF drugs were tested during the second phase. At present, previous hypotheses concerning ROP should be amended with new pathogenetic theories. Studies on the role of genetic components, nitric oxide, adenosine, apelin and β-adrenergic receptor have revealed new possibilities for the treatment of ROP. The genetic hypothesis that single-nucleotide polymorphisms within the β-ARs play an active role in the pathogenesis of ROP suggests the concept of disease prevention using β-blockers. In conclusion, all factors that can mediate the progression from the avascular to the proliferative phase might have significant implications for the further understanding and treatment of ROP.

Key words: erythropoietin – hypoxia-inducible factor 1 – insulin-like growth factor-1 – neovascularization – pathophysiology – placental growth factor – retinopathy of prematurity – vascular endothelial growth factor – β-adrenergic receptors

Acta Ophthalmol.

doi: 10.1111/aos.12049

Introduction

Over the past two decades, the survival of premature infants has markedly increased in industrialized countries (Vermont Oxford Network Database, https://nightingale.vtoxford.org), whereas the neonatal mortality rate worldwide – approximately 4 million deaths each year – has remained virtually unchanged (Lawn et al. 2005, 2009; Black et al. 2010; Carlo et al. 2010; Målqvist 2011). Ninety-nine per cent of these neonatal deaths affect underdeveloped and developing countries (Lawn et al. 2005).

Unfortunately, survival is affected by premature death (1%) and several forms of neurological impairment, such as cerebral palsy (8%), cognitive delay (10%), deafness (3%) and blindness (1%) (Vermont Oxford Network Database, https://nightingale.vtoxford.org; Fanaroff et al. 2007; Wilson-Costello et al. 2007; Wood et al. 2000). In extremely low birthweight (ELBW) neonates, morbidity is elevated, and retinopathy of prematurity (ROP) can develop in the survivors. Severe bronchopulmonary dysplasia (BPD), brain injury and ROP, together or separately, represent strong prognostic indicators in ELBW infants (Koo et al. 2010).

In 2010, the Vermont Oxford Network Database (VON) estimated that

the incidence of any form of ROP in all very low birthweight (VLBW) infants was 33.2% (Vermont Oxford Network Database, https://nightingale.vtoxford.org). The incidence of this disease is closely correlated with the weight and the gestational age at birth; ROP is more severe and more frequent in extremely premature infants and those with very low birthweights. Indeed, the incidence of any ROP increases to 84.7% and 85%, and the incidence of severe ROP (stage >2) is 40.7% and 39.2%, in infants weighing <501 g and in those with a gestational age <24 w, respectively (Koo et al. 2010).

A recent Norwegian study using the Braille system in people with visual loss revealed that ROP continues to be one of the leading causes of blindness in children (Augestad et al. 2012), although several studies have demonstrated the importance and contribution of unpreventable or untreatable disorders (including cerebral visual impairment, hereditary retinal dystrophies and optic nerve atrophy and hypoplasia) (Rahi & Cable 2003; Boonstra et al. 2012).

Although the incidence rates and age distribution of the risk population for ROP are well established, to date, the pathogenetic mechanisms underlying ROP remain poorly understood.

This review summarizes the previous and new pathogenic hypotheses on the development of ROP, involving factors such as hypoxia-inducible factor 1 (HIF-1), vascular endothelial growth factors (VEGFs), insulin-like growth factor-1 (IGF-1), erythropoietin (Epo), placental growth factor (PlGF), nitric oxide (NO), adenosine, apelin, as well as the most recent and innovative theory on the role of the sympathetic nervous system and polymorphisms of the beta-adrenoreceptors (β-ARs). The theoretical mechanisms responsible for retinal neovasculogenesis are illustrated in Fig. 1. In addition, we describe the new potentially preventive and therapeutic modalities that arise from these hypotheses.

Normal Retinal Vascular Development

In the human retina, five cell types form the 'vascular complex': vascular endothelial cells, astrocytes, microglia, pericytes and a complex of three cell types (neuronal substance P (SP)-

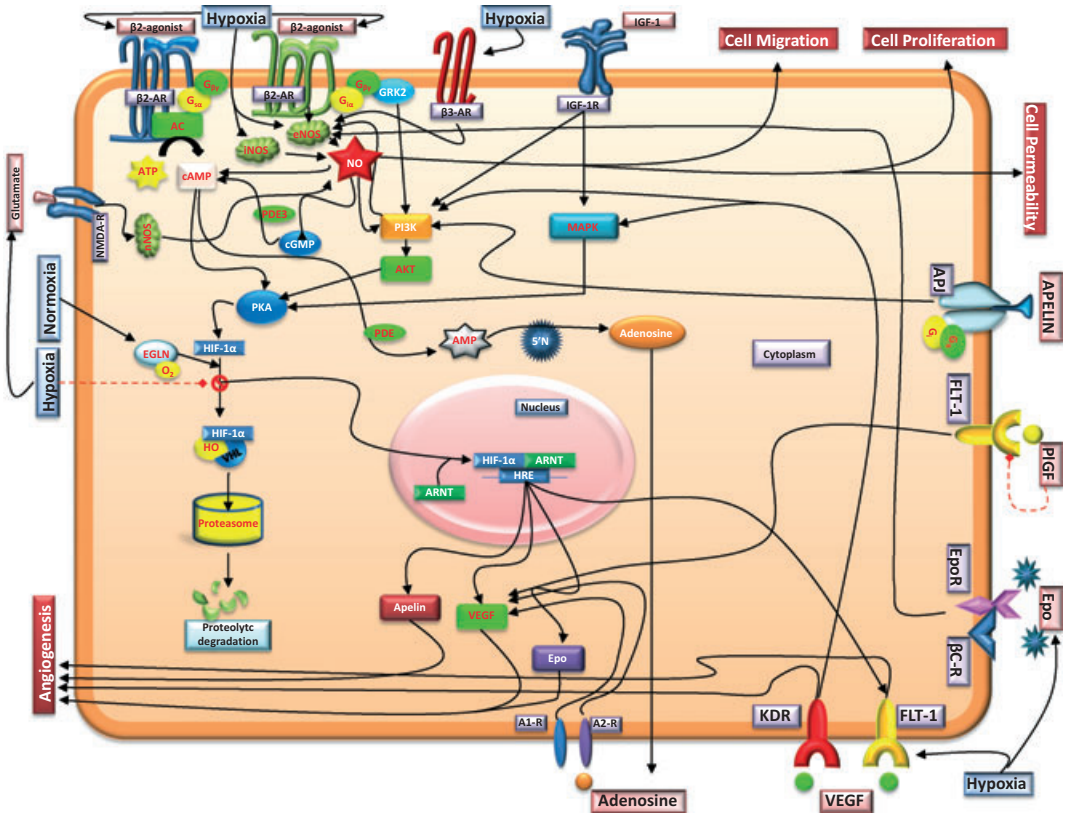

Fig. 1. The mechanisms induced by hypoxia to produce proangiogenic factors. 5′N: 5′ nucleotidase; A1-R: Adenosine A_1 receptor; A2-R: adenosine A_2 receptor; AC: adenylate cyclise; Akt: non-specific serine/threonine protein kinase; AMP: adenosine monophosphate; APJ: apelin receptor; ARNT: aryl hydrocarbon receptor nuclear translocator; ATP: adenosine-5′-triphosphate; cAMP: cyclic adenosine monophosphate; cGMP: cyclic guanosine monophosphate; EGLN: prolyl hydroxylase; eNOS: endothelial nitric oxide synthase; Epo: erythropoietin; EpoR: erythropoietin receptor; FLT-1: fms-related tyrosine kinase 1 receptor; $G_{i\alpha}$: α subunit of the inhibitory G protein; G_q: G protein q; GRK2: G protein–coupled receptor kinase 2; $G_{s\alpha}$: α subunit of the stimulatory G protein; $G_{\beta\gamma}$: β and γ subunit of the G protein; HIF-1: hypoxia-inducible factor 1; HRE: hypoxia response element; IGF-1: insulin-like growth factor-1; IGF-1R: insulin-like growth factor-1 receptor; iNOS: inducible nitric oxide synthase; KDR: kinase insert domain–containing receptor; MAPK: mitogen-activated protein kinase; NMDA-R: *N*-methyl-D-aspartate receptor; nNOS: neuronal nitric oxide synthase; NO: nitric oxide; O_2: oxygen; PDE: phosphodiesterase; PDE3: phosphodiesterase 3; PI3K: phosphatidylinositol 3-kinases; PKA: protein kinase A; PlGF: placental growth factor; VEGF: vascular endothelial growth factor; VHL: von Hippel–Lindau protein; β2-AR: β2-adrenergic receptor; β3-AR: β3-adrenergic receptor; βC-R: common β receptor.

containing, nitric oxide synthase (NOS)-containing and dopaminergic amacrine-like cells) (Provis 2001).

Endothelial and microglial cells proliferate and invade into the retina during fetal development. The glial fibrillary acidic protein (GFAP)-immunoreactive astrocytes are involved in the process of vasculogenesis. In fact, they precede the front of vascularization by a few hundred microns, and their proliferation appears to be associated with the release of proliferative factors by the endothelial cells (Provis 2001). These astrocytes are highly sensitive to hypoxia, and under conditions of low oxygen saturation, they release VEGF, which in turn stimulates the migration, differentiation and proliferation of endothelial cells (Provis 2001).

Vascular development comprises two phases (Hughes et al. 2000): vasculogenesis and angiogenesis. The former phase is characterized by the denovo formation of blood vessels from endothelial precursor cells within the central retina, whereas the latter phase is characterized by the development of new blood vessels that bud from existing blood vessels. During the vasculogenic period, four vascular arcades are formed. The tissue hypoxia that stimulates the production of VEGF does not appear to be necessary for this stage of retinal vascular development.

Angiogenesis is responsible for increasing the vascular density and peripheral vascularization of the superficial retina and for forming the outer plexus and radial peripapillary capillaries. The mechanisms underlying the generation of the retinal vasculature have been found to be similar to those of the central nervous system (Hughes et al. 2000). Similar to what is observed in brain tissues, angiogenic sprouting represents the predominant mechanism of retinal vascularization, although there are additional modes of vascular growth, such as intussusception (Flower et al. 1985; Gariano 2003; Gariano & Gardner 2005). The angiogenic system is controlled by a balance between the activation and the inhibition of regulatory factors (Carmeliet & Jain 2000; Talks & Harris 2000; Chader 2001). During fetal development, relative hypoxia promotes retinal development (Chen & Smith 2007). Indeed, hypoxia stimulates the production of HIF-1, a nuclear transcription factor that regulates VEGF. HIF-1 is rapidly degraded during normoxia, but in moderately hypoxic conditions, such as those present during fetal life, its half-life is prolonged, promoting its nuclear accumulation. After HIF-1 stimulation, VEGF is secreted predominantly by retinal astrocytes and Muller cells, thereby inducing angiogenesis.

Pathological Retinal Development

The relationship between oxygen supply and ROP has been well known since the 1950s (Campbell 1951; Patz et al. 1952; Ashton et al. 1953; Kinsey 1956; Patz & Eastham 1957). Furthermore, several retrospective and prospective studies on the restrictive use of oxygen have revealed a decrease in the incidence and severity of ROP (Tin et al. 2001; Chow et al. 2003; Anderson et al. 2004; VanderVeen et al. 2006; Wright et al. 2006; Sears et al. 2009; Tokuhiro et al. 2009; SUPPORT Study Group of the Eunice Kennedy Shriver NICHD Neonatal Research Network 2010; Wiwatwongwana et al. 2010), although some studies have failed to establish such a correlation (Deulofeut et al. 2006; Wallace et al. 2007; Tlucek et al. 2010). Low oxygen saturation might appear to reduce the risk of severe ROP when carefully controlled within the first weeks of life, but the optimal level of saturation remains uncertain. A meta-analysis conducted by Chen et al. (2010) revealed that the use of low oxygen saturation (70–96%) during the first 8 weeks resulted in a 52% reduction in the ROP risk compared to the use of high concentrations (94–99%). The beneficial effects of low oxygen concentrations can be explained by the pathogenesis of ROP (Smith 2004). Moreover, hyperoxia also induces the formation of reactive oxygen species (ROS), such as peroxynitrite. Hyperoxia-induced peroxynitrite might trigger apoptosis of retinal endothelial cells (Gu et al. 2003).

Historically, ROP is defined as a two-stage disease, although the development of neovascularization might be gradual and not well defined. The first stage begins with the interruption of normal retinal development at the time of preterm birth, accompanied by a sudden reduction in insulin-like growth factor-1 (IGF-1) and VEGF (Smith 2004). Smith observed the importance of IGF-1 during the development of the two phases. VEGF and IGF-1 contribute to the development of the disease. Indeed, IGF-1 was found to be significantly lower in infants who developed ROP than those who did not, suggesting that the administration of IGF-1 within the first stage of ROP might inhibit the progression to the second stage (Smith 2004).

The second phase of ROP begins at 32–34 weeks postmenstrual age (PMA) and is characterized by increased hypoxia of the avascularized retina. The vascular obliteration that occurs secondary to hypoxic stimulation both up-regulates VEGF and erythropoietin and stimulates neovascularization (Chan-Ling et al. 1995). Furthermore, IGF-1 regulates the neovascularization within this stage by acting as an amplifying factor for VEGF (Smith 2004). The progression from the avascular to the proliferative phase is attributed to the gap between the insufficient capillary circulation and the increased metabolic demands of the developing retina. Neovascularization can regress if adequate oxygenation is administered. The neovascularization and secondary cicatricial fibrosis are responsible for retinal detachment and the associated blindness.

However, this interpretation of the progression from the first to the second phase of ROP is quite imprecise; all possible explanations of this transition could have significant implications for understanding and treating ROP.

The Role of Hypoxia-Inducible Factor 1α (HIF-1α)

HIF-1 is a heterodimer consisting of α- and β-subunits that, in humans, are encoded by the *Hif-1α* gene. Whereas the α-subunit is regulated by hypoxia, the β-subunit, known as the aryl hydrocarbon receptor nuclear translocator (ARNT), is constitutively expressed (Wood et al. 1996). HIF-1

mediates the transcription of VEGF. During normoxic conditions, HIF-1 is hydroxylated by prolyl hydroxylases (EGLNs). This post-translational modification facilitates its binding to the von Hippel–Lindau protein (VHL), which results in the ubiquitination and rapid degradation of HIF-1. During hypoxia, hydroxylation becomes less efficient, resulting in HIF-1 accumulation and binding to a hypoxia response element (HRE) within the VEGF promoter. HIF-1α protein degradation is extremely fast, making HIF-1α one of the most well-known short-lived proteins. At low oxygen concentrations, the EGLNs become less effective, and HIF-1α is no longer hydroxylated. This impairs the ability of VHL to bind to HIF-1, thereby inhibiting the process of ubiquitination and degradation. HIF-1α accumulates in the nucleus, ultimately increasing VEGF expression (Fig. 1). HIF-1α expression is induced during normal retinal development, is down-regulated by hyperoxia and is up-regulated upon a return to normoxic conditions – a pattern that is temporally and spatially correlated with VEGF expression. During fetal development, the low oxygen concentrations inhibit the activity of EGLNs, concomitantly increasing local HIF-1α and VEGF levels, which in turn improve vascularization (Ozaki et al. 1999). Premature exposure to relative hyperoxia at birth from 30–35 mmHg (in the uterus) to 50–80 mmHg (in the extrauterine environment), which is further increased in many cases by supplemental oxygen therapy, suppresses HIF-1α levels, thus reducing VEGF expression and inducing the obliteration of retinal capillaries.

The reduction in HIF-1 is essential for the initiation and progression of the first phase of ROP, whereas its increase is essential for the second phase. The decrease of HIF-1 activity and/or expression by mutations, polymorphisms or drugs reduces the oxygen-induced neovascularization. Kim et al. (2008) showed that treatment with deguelin blocked the neovascularization induced by oxygen by reducing HIF-1 expression. Similarly, Brafman et al. (2004) demonstrated that oxygen-induced neovascularization was reduced in *RTP801* knockout mice. Therefore, the drug-induced down-regulation of HIF-1 might represent a new therapeutic target for ROP (Xia et al. 2012).

Table 1. Action of different factors on the development of retinopathy of prematurity (ROP).

Factor	Role in ROP development	Phase of ROP development
HIF-1 (↓)	+	1°
HIF-1 (↑)	+	2°
VEGF (↓)	+	1°
VEGF (↑)	+	2°
IGF-1 (↓)	+	1°
IGF-1 (↑)	+	2°
NO (↓)	+	1°
NO (↑)	+	2°
PlGF (↑)	+	1°
Epo (↓)	+	1°
Epo (↑)	?	2°
Adenosine (↓)	+	1°
Adenosine (↑)	+	2°
Apelin	?	?
β2-AR (↑)	+	2°
β3-AR (↑)	?	2°

↓ = decrease; ↑ = increase; + = stimulation; ? = not known; Epo = erythropoietin; HIF-1 = hypoxia-inducible factor 1; IGF-1 = insulin-like growth factor-1; NO = nitric oxide; PlGF = placental growth factor; VEGF = vascular endothelial growth factor; β2-AR = β2-adrenergic receptor; β3-AR = β3-adrenergic receptor.

In conclusion, both the negative and positive modulations of HIF-1 play key roles in ROP development (Table 1).

The Roles of Vascular Endothelial Growth Factors (VEGFs)

Vascular growth factors are a family of proteins composed of placenta growth factor (PlGF), VEGF-A, VEGF-B, VEGF-C, VEGF-D and the viral VEGF homologue VEGF-E (Olofsson et al. 1998; Eriksson & Alitalo 1999; Ferrara 1999a; Persico et al. 1999). VEGF is known to play a key role in angiogenesis (Ferrara & Davis-Smyth 1997; Ferrara 1999b).

Various combinations of the eight exons that constitute the VEGF gene (*Vegf*) allow for the generation of three predominant isoforms of human VEGF (VEGF121, VEGF165 and VEGF189). Each isoform binds to two receptors, the fms-related tyrosine kinase 1 (FLT-1, also known as VEGFR-l) and the kinase insert domain–containing receptors (KDR, also known as FLK-1 or VEGFR-2). Heparin sulphate proteoglycans (HSPGs), neuropilin 1 (NRP-l) and NRP-2 are specific receptors for VEGF165.

FLT-1 and KDR are present in all embryonic tissues, although their expression levels vary in relation to gestational age (Peters et al. 1993). Hypoxia regulates the expression of the *Flt-1* gene by inducing the binding of HIF-1α to its promoter (Gerber et al. 1997; Bellik et al. 2005). Whereas the up-regulation of *Flt-1* by hypoxia promotes remodelling and vascular tone (Bellik et al. 2005), in contrast, the *Kdr* gene does not appear to be regulated by HIF-1α (Gerber et al. 1997). *Kdr* and *Flt-1* undergo changes in expression in vascular endothelial cells during development: they are both highly expressed during early gestation and are significantly reduced by the end of gestation (Shalaby et al. 1995).

Kdr is essential for vasculogenesis and haematopoiesis; the loss of its function during embryogenesis leads to premature fetal death (Shalaby et al. 1995). In endothelial cells, the interaction between VEGF and KDR results in a phosphorylation process that is more efficient than that induced by the interaction between VEGF and FLT-1. Therefore, KDR is the main receptor that induces the proliferation, migration, differentiation and maturation of endothelial cells, as well as vascular permeability (Terman et al. 1992; Quinn et al. 1993; Waltenberger et al. 1994; Bernatchez et al. 1999).

Models of retinal vasculogenesis suggest that the maturation of photoreceptors and neurons leads to the development of a 'physiological hypoxia' in the retina, which in turn induces the expression of VEGF by astrocytes and the proliferation of the vascular endothelium (Chan-Ling et al. 1995). Under the hypoxic conditions during development, the astrocytes are a source of VEGF, which stimulates retinal growth towards the periphery (Provis 2001). This angiogenesis consequently leads to a reduction of the negative-feedback effects of hypoxia and VEGF. Similarly, the expression of VEGF by Muller cells is thought to regulate the development of the deep vascular plexus (Stone & Maslim 1997).

Vascular endothelial growth factors plays a key role in ROP development

(Fig. 1). Indeed, VEGF expression is down-regulated by hyperoxia during the first phase of ROP, resulting in the stunting and obliteration of the vasculature, whereas it is up-regulated by hypoxia in the second phase (Table 1). Pierce et al. (1996) showed that supplemental oxygen significantly reduces the level of VEGF mRNA, whereas in contrast, vessel obliteration is reduced by pretreatment with exogenous VEGF. Similarly, treatment with hyperoxia reduces the VEGF in the ischaemic retina during the second phase of ROP. The retinal neovascularization observed during the second phase of ROP is reduced by the application of VEGF inhibitors. The use of intravitreal antisense oligodeoxynucleotides was demonstrated to reduce neovascularization by approximately 31% (Robinson et al. 1996). Jiang et al. (2009) observed that VEGF- and HIF-1α-targeted small interfering RNAs (siRNAs) were also able to inhibit their effects on neovascularization. The use of intravitreal bevacizumab, a humanized monoclonal antibody that inhibits VEGF and that is commonly used for age-related macular degeneration (AMD), reduces the development of severe ROP and simultaneously allows blood vessels to grow towards the periphery (Mintz-Hittner & Kuffel 2008; Dorta & Kychenthal 2010; Mintz-Hittner et al. 2011; Wu et al. 2011; Spandau et al. 2012). More recently, the ranibizumab was reported to be a preferred therapeutic agent due to its relatively shorter half-life (Hoerster et al. 2012). Indeed, whereas bevacizumab can decrease serum levels of VEGF for several weeks (Matsuyama et al. 2010; Lee et al. 2011; Sato et al. 2012), ranibizumab reduces it only for a few weeks, with a detectable increase by the fourth week after intravitreal treatment (Hoerster et al. 2012).

The Role of Insulin-Like Growth Factor-1 (IGF-1)

IGF-1 is a maternally derived factor that is provided via the placenta and amniotic fluid (Langford et al. 1998). During the fetal development, IGF-1 is a potent growth factor that also acts as a mediator of several growth hormone signalling pathways.

Hellstrom et al. (2001) demonstrated that IGF-1 plays a key role in the normal development of the retina and in the pathogenesis of ROP. The deficiency of IGF-1 during the early stages of postnatal life induces abnormal retinal vascularization and the development of ROP. IGF-1 null mice exhibit significantly delayed retinal growth, despite normal levels of VEGF (Hellstrom et al. 2001). However, patients with a genetic defect in IGF-1 production exhibit reduced retinal vascularization (Hellstrom et al. 2002) that is not restored upon the administration of VEGF alone. IGF-1, which is repressed at birth, rapidly increases in premature infants who do not develop ROP. Thus, it has been assumed that if IGF-1 levels remain low then the vasculogenesis will stop; the resulting avascular retina becomes hypoxic, and VEGF accumulates in the vitreous. High VEGF levels and normal IGF-1 levels stimulate the neovasculogenesis that is characteristic of ROP (Hellstrom et al. 2001).

The normal VEGF and IGF-1 concentrations activate Akt signalling, thus promoting the survival of endothelial cells. Hellstrom et al. showed that VEGF and IGF-1 can activate Akt and that its activation is fivefold higher if both cytokines are present compared to either alone. However, if IGF-1 is low, as observed during the first phase of ROP, then VEGF cannot activate Akt. This leads to an increase in apoptosis despite normal levels of VEGF (Hellstrom et al. 2001). In contrast, high IGF-1 levels, as observed during the second phase of ROP, block endothelial cell apoptosis and promote neoangiogenesis, presumably by activating Akt signalling (Table 1).

IGF-1 also regulates neovascularization through the p44/42 MAPK pathway, which allows for an increase in VEGF activity and the resultant stimulation of retinal endothelial cell growth (Smith et al. 1999). Moreover, Fukuda et al. (2002) showed that IGF-1 stimulates HIF-1 expression through the PI3K/Akt and MAPK pathways (Fig. 1).

The phases of ROP and the roles that VEGF and IGF-1 play at different stages are suggestive of the time and approaches for intervention. For instance, the inhibition of VEGF and IGF-1 might prevent normal vascular growth during the first phase, whereas it might support the regression of the disease during the second phase (Smith 2004). Similarly, recombinant human IGF-1 (rhIGF-1) might reduce neovascularization when administered during the first phase of ROP, whereas it would increase neovascularization if administered during the second phase (Smith 2004; Vanhaesebrouck et al. 2009).

The Role of Placental Growth Factor (PlGF)

Vascular endothelial growth factors and placental growth factor (PlGF) are members of a large family of peptides that share many biochemical and molecular characteristics (Olofsson et al. 1996; Nicosia 1998). In contrast to VEGF, PlGF has not been thoroughly studied in the context of angiogenesis. One study, by Carmeliet & Jain (2000), showed that PlGF affects the angiogenic response by binding to FLT-1.

Placental growth factor is a homodimeric glycoprotein that plays crucial roles in promoting monocyte chemotaxis, collateral vessel development, adult pathophysiological neovascularization and endothelial cell proliferation and migration (Rakic et al. 2003). It also acts as an important cofactor during retinal neovascularization by increasing the activity and expression of VEGF (Luttun et al. 2002; Nagy et al. 2003). VEGF and PlGF create a heterodimer that binds to and activates FLT-1, leading to angiogenesis (Michels et al. 2006; Cao 2009).

The action of PlGF is still unclear. Some studies argue that it dimerizes with VEGF to limit the binding of VEGF to KDR (Cao et al. 1996; Cunningham et al. 1999), thereby reducing the migration and proliferation induced by VEGF. However, a study by Carmeliet and Park showed that the binding of PlGF to FLT-1 increases the amount of circulating VEGF available to activate KDR (Park et al. 1994; Carmeliet et al. 2001). Adini et al. (2002) showed that VEGF-deficient mice die during the first 2 weeks of life, whereas PlGF-deficient mice develop normally. These data indicate that PlGF is not essential for normal development. More-

over, Michels et al. (2006) revealed that PlGF deficiency inhibits the pathological angiogenesis and vascular leakage observed in ischaemia, cancer and wound healing. PlGF induces tumour angiogenesis by recruiting circulating haematopoietic progenitor cells and macrophages to the site of the growing tumours (Schlingemann 2004). PlGF can be found together with VEGF in retinal endothelial cells and pericytes (Yonekura et al. 1999). Simpson et al. (1999) observed that all members of the VEGF family are up-regulated during hypoxic conditions, except for PlGF, which decreases during hypoxia and increases during hyperoxia (Fig. 1; Table 1). PlGF is up-regulated during angiogenesis (Khaliq et al. 1998; Nomura et al. 1998; Yamashita et al. 1999; Bottomley et al. 2000; Carmeliet et al. 2001), although its temporal expression depends on the affected tissue and the nature of the pathology. Specifically, the up-regulation of PlGF in the retina is delayed during ROP, suggesting that it can contribute to the neoangiogenesis induced by VEGF (Khaliq et al. 1998; Carmeliet et al. 2001). Adini et al. (2002) demonstrated an antiapoptotic action of PlGF. Moreover, a study conducted in an oxygen-induced retinopathy (OIR) model by Shih et al. (2003) confirmed the antiapoptotic action of PlGF during hyperoxia. Zhao et al. (2003) showed that VEGF and hyperglycaemia can induce PlGF expression through the MAPK signalling pathway and, partially, through protein kinase C (PKC). Akrami et al. (2011) showed that PlGF acts as a proangiogenic factor for retinal endothelial cells. The authors speculated that the suppression of the PlGF gene might reduce pathological angiogenesis, suggesting a new therapeutic strategy for ocular neovascular diseases. The blockade of PlGF function through the use of antagonists to FLT-1 (Bae et al. 2005) or by monoclonal antibodies against FLT-1 (Luttun et al. 2002) suppresses the neovascularization of tumoural tissues and of ischaemic retinas.

The Role of Erythropoietin (Epo)

Erythropoietin (Epo) is a glycoprotein that is produced in the adult kidney and the fetal liver (Jacobson et al. 1957; Zanjani et al. 1977). Epo binds to a homodimeric Epo receptor (EpoR), which stimulates erythropoiesis, and to a heterodimeric receptor, which contains EpoR and the common β receptor (βC-R) and which performs all of the other functions of Epo (Sautina et al. 2010).

Epo might function in non-erythroid cells. EpoR is present in endothelial cells, smooth muscle cells, B cells, rodent placenta cells, embryonic stem cells, megakaryocytes and neuronal cells (Ishibashi et al. 1987; Sawyer et al. 1989; Anagnostou et al. 1990, 1994; Kimata et al. 1991; Schmitt et al. 1991; Heberlein et al. 1992; Carlini et al. 1993, 1995; Masuda et al. 1993; Gogusev et al. 1994; Digcaylioglu et al. 1995). Some neuronal cells express EpoR on their surface, and Epo binding to EpoR increases intracellular Ca^{2+} and monoamine concentrations (Masuda et al. 1993). Konishi et al. (1993) demonstrated that choline acetyltransferase expression increases after Epo stimulation in cultured embryonic neurons, promoting the *in vivo* survival of cholinergic neurons in adult rats. Thus, Epo has been hypothesized to act as a neurotrophic factor.

The presence of Epo and EpoR in the retina supports the hypothesis that these molecules might play different roles (Koury & Bondurant 1990). Several authors have demonstrated the protective effect of Epo against light-induced retinal degeneration, ischaemia–reperfusion injury, neuronal cell death and even against human stroke (Konishi et al. 1993; Sakanaka et al. 1998; Siren et al. 2001; Ehrenreich et al. 2002; Grimm et al. 2002; Junk et al. 2002).

Yamaji et al. (1996) suggested that Epo is a mitogenic factor for the endothelial cells of brain capillaries, showing that Epo performs an endocrine function in kidney and testis cells and a paracrine function in brain astrocytes during hypoxic conditions.

Anagnostou et al. (1990) demonstrated that recombinant human Epo (rhEpo) elicits chemotactic and proliferative effects in human umbilical vein endothelial cells. Carlini et al. observed that Epo increases endothelin-1 expression in bovine pulmonary artery endothelial cells and stimulates angiogenesis in rat thoracic aorta (Carlini et al. 1993, 1995).

Hypoxia stimulates Epo secretion, which in turn increases erythrocyte numbers and angiogenesis. Epo and VEGF are both stimulated by HIF-1α during hypoxia. Studies in cystic fibrosis patients showed that increased VEGF is associated with increased Epo during hypoxia (Watts & McColley 2011). Gess et al. (1996) showed that the expression of Epo mRNA increased 20-fold, whereas the VEGF 188, 164 and 120 mRNAs increased two-, three- and sixfold, respectively, in rat cultured hepatocytes exposed to extreme hypoxia. Epo and VEGF exhibit similar characteristics, as both are induced by hypoxia (Krantz 1991). Similarly, Epo increases during ischaemia, and its inhibition reduces retinal oxygen-induced angiogenesis (Watanabe et al. 2005).

Watanabe et al. observed high vitreal Epo in patients with diabetic retinopathy compared to healthy patients. The study showed that vitreal Epo was higher than vitreal VEGF and than serum Epo levels, demonstrating that increased Epo is unrelated to VEGF and that retinal tissues are able to produce Epo. Therefore, Epo inhibition during the growth phase inhibited neovascularization in a mouse model of proliferative retinopathy (Watanabe et al. 2005).

Chen et al. (2008) studied the contribution of Epo to every phase of oxygen-induced retinopathy and found that Epo deficiency contributes to the development of the disease during the first phase of retinopathy. The administration of Epo prevents the loss of vasculature and ischaemia at this stage. These results suggest that treating the patients at an early stage of retinopathy might prevent the damage following retinal neovascularization. In contrast, treatment during the late stage of neovascularization might exacerbate the disease by promoting endothelial cell proliferation (Chen et al. 2008) (Table 1).

The mechanisms by which Epo induces angiogenesis are not fully known. There is evidence that it increases NO production through direct stimulation of endothelial nitric oxide synthase (eNOS) and by stimulating the proliferation, differentiation, mobilization and the adhesion of endothelial progenitor cells (Heeschen

et al. 2003; Beleslin-Cokic et al. 2004; Westenbrink et al. 2007). NO induction depends on βC-R and KDR stimulation and the interaction between βC-R and KDR receptors, and βC-R blockade can decrease NO production (Sautina et al. 2010; Su et al. 2011) (Fig. 1).

Over the past 20 years, Epo treatment has become an important part of premature baby care. Its use has increased to reduce the number of blood transfusions required by these small patients. Studies on the use of Epo during the neonatal period have increased in recent years (Shannon et al. 1991; Gumy-Pause et al. 2005). Some studies did not find an increase of retinopathy associated with Epo use, whereas others observed an increase in ROP incidence with the administration of rhEpo (Donato et al. 2000; Romagnoli et al. 2000; Maier et al. 2002; Turker et al. 2005; Brown et al. 2006; Shah et al. 2010). A meta-analysis conducted by Ohlsson and Aher found an increase in severe ROP (stage 3) in patients treated with rhEpo compared to control-treated patients and an increase in all stages when the treatments were started within the first 8 days of life (Aher & Ohlsson 2006; Ohlsson & Aher 2006). Brown et al. (2006) observed that the use of higher cumulative doses of rhEpo was associated with an increased risk of retinopathy progression. Suk et al. (2008) assessed the effects of the dosage and duration of administration of Epo on the development of ROP and reported that babies who received more than 20 doses of rhEpo and who started this treatment after 20 days of life exhibited an increased risk of ROP compared with those who received less than 20 doses and started this drug before the 20th day of life. A recent retrospective analysis of 718 very low birthweight babies showed that early use of Epo did not lead to an increased risk of severe ROP (only stage 1). The authors found that severe ROP was associated with lower birthweight and gestational age and required more aggressive therapy. They speculated that the increase in stage 3 ROP observed in other studies might be due to the concomitant actions of Epo and high doses of iron (Figueras-Aloy et al. 2010). Excessive levels of iron are toxic to tissues due to the Fenton reaction; production of hydroxyl radicals can cause oxidative damage to lipids, proteins and DNA (Sullivan 1988; Hesse et al. 1997; Loh et al. 2009).

New Hypothesis

At present, new pathogenetic hypotheses are associated with previous knowledge. The studies on the role of genetic components, nitric oxide, adenosine, apelin and β-adrenergic receptor (β-ARs) have opened new gates to the treatment of ROP.

The Genetic Components of ROP

Although ROP exhibits the same incidence rates in Caucasian and Black populations, the progression to severe stages is more frequent in Caucasian than in Black infants and in males than in females (Saunders et al. 1997; Good et al. 2005, 2012; Csak et al. 2006). The major susceptibility factor for ROP progression in Caucasian is the increased frequency of polymorphisms of β-adrenoreceptors (β-ARs) in Black than in Caucasian infants (Saunders et al. 1997; Good et al. 2005, 2012). Indeed, the ROP was once considered as a result of increased retinal pigmentation (Saunders et al. 1997) appears to be attributed to a polymorphism of G protein–coupled receptor kinase 5 (GRK5), which desensitizes β-ARs (Liggett et al. 2008, Good et al. 2012). The GRK5 polymorphism blocks β-ARs in Black patients, causing resistance to noradrenergic stimuli and conferring protection against heart failure, myocardial ischaemia and also against ROP (Good et al. 2012). The protective polymorphism suggests that pharmacological blockage of β-ARs might decrease ROP progression by reducing the neovascularization induced by β-stimuli (Good et al. 2012).

Therefore, the gene mutations and single-nucleotide polymorphisms (SNP) of ROP-related factors might play significant roles in the development of this multifactorial disease. Several genetic mutations are associated with the development of ROP. Studies by Shastry et al. (1997) and by Hiraoka et al. (2001) found that the mutation of the *Norrie disease protein gene* (*NDP*) increased the risk of severe ROP. In contrast, Haider et al. (2000, 2001) failed to demonstrate an association with the mutations of *NDP* (R121W and L108P, A105T and Val60Glu) with the progression of ROP in Kuwaiti populations (Haider et al. 2000, 2001). Furthermore, a more frequent correlation between ROP and a C597A polymorphism was reported (Haider et al. 2002).

More recently, mutations in the three genes (NDP, FZD4 and LRP5) involved in the function of the wingless/integrated (Wnt) receptor signalling pathway, which have been associated with familial exudative vitreoretinopathy, were also correlated with an increased risk of severe ROP (Hiraoka et al. 2010; Shastry 2010).

ROP is also associated with SNPs of the eNOS gene (*T-786C* and *G894T*) (Yanamandra et al. 2010), complement factor H (CFH) genes and of the *EPAS1* gene (Mohamed et al. 2009). Genetic factors, along with prematurity and early exposure to oxygen, appear to play key roles in the development of ROP. Future investigations of SNPs may provide valuable insight into the pathogenetic mechanisms underlying the development of ROP.

The Role of Nitric Oxide (NO)

Nitric oxide (NO) is a key signalling molecule that mediates several basic physiological processes, including neurotransmission, vasodilatation and host cell defence (Christopherson & Bredt 1997; MacMicking et al. 1997; Nathan 1997). NO is synthesized from L-arginine by NOS. There are two isoforms of NOS, constitutive (cNOS) and inducible (iNOS) (Stuehr 1999). The neuronal NOS (nNOS or NOS1) and endothelial NOS (eNOS or NOS3) are constitutive enzymes that synthesize NO following an increase in Ca^{2+} or after exposure to Ca^{2+} -independent stimuli, such as shear stress (Fleming et al. 1998). iNOS, or NOS2, is Ca^{2+} -independent and constitutively expressed only in select tissues, such as the lung epithelium (Dweik et al. 1998). It is usually synthesized following the exposure to proinflammatory stimuli (Kroncke et al. 1995; Hierholzer et al. 1998).

NOS isoforms have been identified in the eyes of both animals and humans (Goureau et al. 1993, 1994; Yamamoto et al. 1993; Kobayashi et al. 2000; Neufeld et al. 2000). NOS isoforms are also present in the vascular endothelium and in pericytes (Chakravarthy et al. 1995; Meyer et al. 1999). Martin et al. (2000) described how pericytes control endothelial cell growth by stimulating or inhibiting cNOS or iNOS, respectively. nNOS is expressed at low levels in the lamina cribrosa and in the astrocytes of the optic nerve head (Neufeld et al. 1997; Shareef et al. 1999). eNOS is present in the endothelium of the optic nerve head and choroidal blood vessels but is scarcely detectable in nonvascular choroidal and scleral endothelial cells (Neufeld et al. 1997; Poukens et al. 1998; Meyer et al. 1999; Shareef et al. 1999).

NO is a principal determinant of choroidal and retinal blood flow (Seligsohn & Bill 1993; Ostwald et al. 1997; Granstam et al. 1998; Granstam & Granstam 1999; Schmetterer & Polak 2001). Additionally, NO is involved in the pathogenesis of retinal and brain injury after a hypoxic ischaemic insult (Dawson et al. 1991; Iadecola 1992; Samdani et al. 1997; Adachi et al. 1998). Furthermore, hypoxic injury increases retinal mRNA and protein expression of eNOS, leading to increased NO production and consequently vasodilatation and angiogenesis. Hashiguchi et al. (2004) demonstrated that eNOS expression elicits a neuroprotective function during ischaemic conditions. Kaur et al. (2006) postulated the existence of a balance between harmful and protective factors after hypoxia. An imbalance between these factors might promote the adverse effects that result in hypoxic retinal damage and subsequent neovascularization.

Recently, He et al. (2007) showed that iNOS modulates the activity of HIF-1 via PI3K/Akt signalling and VEGF expression in the OIR mouse model (Fig. 1). The authors observed that a selective iNOS inhibitor decreased the neoangiogenesis in ROP animals treated with aminoguanidine hemisulphate. This led them to hypothesize a therapeutic role of iNOS inhibitors for the treatment of ROP (He et al. 2007).

Yuan and Ziche showed that NO stimulates the proliferation and migration of endothelial cells (Yuan et al. 1993; Ziche et al. 1993, 1997). The NO pathway might therefore be the core component that is affecting VEGF in this context. Cellular proliferation occurs after VEGF stimulation through the accumulation of NO and cGMP (Morbidelli et al. 1996). The inhibition of NO synthesis inhibits cell proliferation and the stimulation of protein kinase mitogenic activity. Similarly, the increase in cell permeability induced by VEGF appears to depend on the action of NO (Wu et al. 1996).

Although it is constitutively expressed, eNOS expression can also be regulated by different stimuli (Inoue et al. 1995; Liao et al. 1995; Uematsu et al. 1995). Hood et al. (1998) showed that there is a dose-dependent increase in NO production following VEGF stimuli. This increase has a biphasic pattern, with peaks at 1 and 24 hr. Simultaneously, NO activates the transcription of the VEGF gene, thereby playing a crucial role in facilitating VEGF-induced angiogenesis (Jośko & Mazurek 2004).

Wu et al. (1996) observed that VEGF induces vascular hyper-permeability in a NO-dependent manner. Lakshminarayanan et al. (2000) suggested that the increased permeability of the endothelial cells induced by NO is attributed to the modulation of tight junction proteins in response to VEGF.

Data regarding the roles of eNOS and NO in proliferative retinopathies are apparently contradictory. On the one hand, NO appears to inhibit angiogenesis and to protect against proliferative retinopathies. In fact, the genetic polymorphisms and reduced gene transcription of eNOS, which are associated with lower serum levels of NO metabolites (Li et al. 2004), are significantly correlated with the development of severe ROP in preterm infants (Rusai et al. 2008; Yanamandra et al. 2010). The reduced NO production might aggravate the retinal vascular obliteration observed during the first phase of ROP, when the capillaries of the developing retina of premature infants experience high concentrations of oxygen, and thereby promoting the neoangiogenesis observed during the second phase of ROP (Rusai et al. 2008) (Table 1).

On the other hand, NO appears to increase neoangiogenic activity. Endothelial NO synthase plays a predominant role in VEGF-induced angiogenesis, as well as in vascular permeability, and the lack of eNOS contributes to the reduced angiogenic response to VEGF observed in eNOS-deficient mice (Fukumura et al. 2001).

The developing retina of eNOS-deprived mice, induced either by gene disruption or by pharmacological inhibition (with the NOS inhibitor *N*-nitro-L-arginine), is significantly protected from hyperoxia-induced damage and subsequent retinopathy (Brooks et al. 2001; Ando et al. 2002) by a mechanism that appears to be independent of VEGF expression (Brooks et al. 2001).

In the premature retina, impaired circulation, which leads to ischaemia, predisposes the retina to abnormal preretinal neovascularization (Hardy et al. 2000). The increased production of NO and prostacyclin (PGI2) improves ocular blood flow, resulting in oxygen delivery to an immature retina that is devoid of antioxidant defences; tissue oxygenation induces ROS production, which significantly contributes to the pathogenesis of ROP (Toda & Nakanishi-Toda 2007).

NO production was proposed to represent a compensatory mechanism to induce vasodilation effects and to reduce the vascular obliteration observed in the early stages of OIR. However, as OIR progresses to a later stage, the excessive production of NO might become deleterious, promoting neovascularization. However, it is difficult to explain why a strong activation of NOS and consequent NO production occur during the proliferative phase of ROP.

The Role of Adenosine

Adenosine is an endogenous purine nucleotide that has fundamental biological functions, such as the transfer of energy (conversion of ATP to ADP), signal transduction (cAMP) and inhibitory neurotransmission (Collis & Hourani 1993; Johnson et al. 1999; Wurm et al. 2008; Ibrahim et al. 2011). The adenosine level increases with increasing tissue activ-

ity, stress or hypoxia. In fact, the activity of adenosine is higher where oxygen demand is highest, such as in the retina. Adenosine is derived from hydrolysis of cytoplasmic *S*-adenosylhomocysteine in the myocardium, whereas it is produced from the hydrolysis of AMP by the action of the 5′ nucleotidase (5′N) in the retinal Muller cells. Adenosine binds four different receptors (A1, A2A, A2B and A3), which are all coupled to G proteins (Blazynski 1987; Freissmuth et al. 1991; Stefanovic et al. 1993; Abbracchio et al. 1995; Palmer et al. 1995; Liang 1996; Zhao et al. 1997, 2000; Abebe & Mustafa 1998; Marala & Mustafa 1998; Li & Wong 2000; Montesinos et al. 2002; Lutty & McLeod 2003).

Adenosine can act as a vasoconstrictor or vasodilator, depending on the tissue involved and the receptors that are present (Tabrizchi & Bedi 2001). Vasodilation is induced by the direct stimulation of NO production (Olanrewaju & Mustafa 2000). In addition, adenosine appears to elicit anti-inflammatory activities, as it is present in high amounts in inflamed tissues (Cronstein et al. 1983, 1985, 1992; Ralevic & Burnstock 1998; Awad et al. 2006; Liou et al. 2008).

Studies in chicken chorioallantoic membranes have also demonstrated that hypoxia stimulates angiogenesis through adenosine production and uptake (Dusseau & Hutchins 1988; Grant et al. 1999; Taomoto et al. 2000; Grant et al. 2001; Lutty et al. 2000). Adenosine is a mitogenic, chemotactic and proliferative factor (Teuscher & Weidlich 1985; Meininger et al. 1988; Lutty et al. 1998). Furthermore, adenosine directly stimulates VEGF production during hypoxia (Fischer et al. 1995; Takagi et al. 1996a,b; Grant et al. 2001).

Adenosine and the A2A receptor present on angioblasts and endothelial cells are elevated during retinal development. The hyperoxic phase of ROP is characterized by a reduction in the 5′N activity and adenosine levels. The subsequent hypoxic vasoproliferative phase is characterized by angiogenesis, high activity of 5′N and high levels of adenosine (Fig. 1; Table 1).

Inhibition of adenosine or, preferably, of A2 receptors might represent a therapeutic target to block the retinal angiogenesis. The use of systemic A2 receptor antagonists might elicit adverse effects not only on the heart or on the central nervous system but also on the retina because they block normal vasculogenesis, resulting in persistent avascular retina. Therefore, the therapeutic blockade of A2 receptors should be local and short term (Lutty & McLeod 2003).

The Role of Apelin

Apelin is a peptide transcribed by a gene on chromosome Xq25–26.1 in humans (Tatemoto et al. 1998). When it binds to the cognate apelin receptor (APJ), it inhibits cAMP-induced forskolin production, suggesting that the receptor is coupled to the inhibitory G protein (Gi). Activation of the APJ receptor also mediates Ras-independent activation of extracellular regulated kinases (ERKs) by PKC (Hosoya et al. 2000; Reaux et al. 2001; Masri et al. 2002; Zhou et al. 2003). The APJ/apelin pathway is active in several cells, organs and peripheral tissues (lung, heart, liver, kidney, adipose tissue, intestine, brain, endothelium, mammary gland and plasma) and regulates cardiovascular activity, cell growth, apoptosis, metabolism, fluid homeostasis and immune response. This pathway plays an angiogenic role. It is likely that apelin regulates vasculogenesis and angiogenesis (Devic et al. 1999; Saint-Geniez et al. 2002; Kasai et al. 2004; Cox et al. 2006; Sorli et al. 2007).

Kasai et al. (2004) showed that the APJ/apelin pathway is highly active in RF/6A retinal endothelial cells. The administration of apelin promotes migration, proliferation and capillary formation. *APJ* genes are highly expressed during embryonic vasculogenesis and postnatal retinal vessel development (Devic et al. 1999; Saint-Geniez et al. 2002). Similarly, this pathway is active in many tumoural tissues, which are areas of intense neovascularization (Sorli et al. 2007). Cox et al. (2006) showed that apelin is required during normal development of blood vessels in frog embryos. This activity is VEGF-independent because the suppression of VEGF with a KDR inhibitor does not inhibit the cell proliferation induced by apelin.

The molecular mechanisms that regulate the release of apelin remain unknown, although recent data suggest that HIF-1 promotes the expression of apelin in adipocytes and cardiomyocytes (Kalin et al. 2007; Eyries et al. 2008).

Kasai et al. (2008) showed delayed development and a reduced angiogenic response to VEGF in apelin knockout mice, supporting the co-operative activities between VEGF and apelin. Apelin induces retinal neovascularization during the hypoxic phase in OIR mice. However, apelin stimulates angiogenesis through APJ directly and not through growth factors (Kasai et al. 2010). The detailed mechanism by which this occurs is still unknown, but it appears that apelin/APJ binding activates eNOS through PI3K/Akt signalling, which results in increased NO production (Fig. 1) (Katugampola et al. 2001; Tatemoto et al. 2001; Jia et al. 2007; Kojima & Quertermous 2008). Therefore, apelin induces the proliferation of endothelial cells independently of VEGF (Kasai et al. 2010). Thus, it appears that hypoxia, and specifically HIF-1α, stimulates the expression of HRE-containing genes to activate apelin production (Fig. 1) (Eyries et al. 2008).

The vitreal apelin concentration also increases in patients with diabetic retinopathy, whereas it is not significantly associated with increased VEGF or plasma apelin concentrations. Therefore, apelin is hypothesized to be produced locally and elicits autocrine functions in endothelial cells (Tao et al. 2010).

Taken together, these data strongly suggest that the apelin/APJ pathway plays a vital role in normal and pathological retinal angiogenesis (Table 1).

The Role of β-Adrenergic Receptors (β-ARs)

Adrenaline and noradrenaline (NA) are catecholamines that are actively involved in several age-related diseases. The adrenergic receptors are metabotropic G protein–coupled receptors. Several cells express these receptors on their surfaces, and their binding to agonists induces a sympathetic response. There are two classes of adrenergic receptors (α-AR and β-AR) and five major subtypes (α1-AR, α2-AR, β1-AR, β2-AR and β3-AR) (Hieble et al. 1995). Ageing is associ-

ated with sympathetic denervation (Burnstock 1990). In the retina, the level of dopamine β-hydroxylase, the enzyme that converts dopamine to NA, decreases during ageing, and the expression of β1-AR consequently increases as a consequence of age-related denervation (Smith et al. 2007). Sympathetic innervation plays a fundamental role in regulating ocular vascular architecture. Steinle et al. (2002) showed that a superior cervical ganglionectomy could increase choroidal thickness and vascularity. Moreover, the blockade of β-AR recapitulated the results obtained with denervation. Therefore, the activation of β-AR is essential for the prevention of an abnormal vasculature (Steinle & Smith 2002).

Studies have demonstrated that angiogenesis is controlled by the adrenergic system through its regulation of proangiogenic factors. β-ARs are widely expressed in vascular endothelial cells (Guimaraes & Moura 2001), and β2-ARs can regulate neoangiogenesis in response to chronic ischaemia. In fact, in the endothelium of the rat femoral artery, hind limb ischaemia induces β2-AR overexpression in endothelial cells, promoting VEGF production and function, including cell proliferation and revascularization. This observation suggests a novel and physiologically relevant role of such receptors in neoangiogenesis in response to ischaemia (Iaccarino et al. 2005). In several systems, hypoxia causes catecholaminergic overstimulation, which in turn alters signalling pathways that are associated with β-ARs (Lindgren & Altimiras 2009).

Propranolol, a well-tolerated, non-selective β-AR blocker, reduces the growth of infantile capillary haemangiomas, the most common tumour that affects infants (Leaute-Labreze et al. 2008). Although there is no generally accepted mechanism for this function, it has been hypothesized that propranolol might act by reducing VEGF levels (Sans et al. 2009; Storch & Hoeger 2010). The possibility of a relationship between the adrenergic system and angiogenesis is supported by the data from solid tumours and tumoural cell lines, in which NA promotes tumour progression by up-regulating VEGF (Guo et al. 2009; Yang et al. 2009). In addition, in human umbilical vein endothelial cells, NA stimulates VEGF production (Seya et al. 2006). Finally, NA has been found to stimulate angiogenesis by up-regulating VEGF in neonatal rat cardiac myocytes (Weil et al. 2003).

A role of β-ARs in the vascular remodelling of the rat choroid has been demonstrated (Steinle & Smith 2002), and β-AR expression in the retina has been established (Kubrusly et al. 2007; Smith et al. 2007; Walker & Steinle 2007). β-AR messengers and proteins are expressed in the retina, including β1- and β2-ARs in rats (Smith et al. 2007) and β1-ARs in birds (Kubrusly et al. 2007). β1- and β2-ARs are expressed in rat Muller cells, in which they influence cytokine production in response to hyperglycaemia (Walker & Steinle 2007). In the human retinal pigment epithelium, β-ARs appear to regulate the production of the antiangiogenic protein pigment epithelium-derived factor (Lashbrook & Steinle 2005). β1- and β3-ARs, but not β2-ARs, are expressed in human retinal and choroidal endothelial cells. In these cells, a role of β3-ARs in angiogenic processes has been demonstrated (Fig. 1) (Steinle et al. 2003, 2005). In addition, pharmacological evidence shows that β2- and β3-ARs are expressed in the retinal blood vessels of the rat (Mori et al. 2010).

Considering that β-AR stimulation up-regulates VEGF and that the second phase of ROP is promoted by increased VEGF production, the overexpression of VEGF during ROP has been hypothesized to be induced by β-AR stimulation, and furthermore, that β-blockers might represent useful agents in the treatment of ROP (Filippi et al. 2010) (Fig. 2). This hypothesis is supported by the observation that infantile haemangiomas are associated with ROP development, suggesting a possible pathogenic relationship between the two diseases (Praveen et al. 2009).

However, data regarding the roles of β-ARs in proliferative retinopathies appear to be contradictory. In human choroidal endothelial cells, the β-AR agonist isoproterenol leads to increased levels of growth factors implicated in ocular diseases (Steinle et al. 2008), but on the other hand, isoproterenol inhibits diabetic-like changes in the rat retina, suggesting that loss of β-AR signalling might be a key factor in early diabetic retinopathy (DR) (Jiang et al. 2010). Dal Monte et al. (2012) observed, in an OIR model using C57BL/6J mice, that isoproterenol reduced the levels of VEGF and the formation of neovascular tufts as well as promoted the down-regulation of β2-ARs. Such antiangiogenic activity was due to the reduced sensitivity of β-receptors following an increase in β-arrestin-1, β-arrestin-2 and G protein–coupled receptor kinase 2 (GRK2) (Dal Monte et al. 2012).

Propranolol does not affect the VEGF retinal levels in rats with DR (Zheng et al. 2007). In contrast, a decrease in VEGF has been observed in the retinas of neonatal OIR rats, in which a reduction in intraocular pressure induced by the β-AR antagonist timolol diminishes the severity of OIR (Ricci et al. 1991, 1995, 2000). This discrepancy might be explained by either the different drug administration routes or different dosages or the possibility that the control of angiogenesis by β-ARs can be regulated by distinct mechanisms in OIR and DR.

In addition, there are conflicting and opposing data on experimental models of OIR. In C57BL/6 OIR mice, treatment with propranolol partially restores the hypoxia-induced increase in IGF-1 mRNA and VEGF mRNA (Ristori et al. 2011). Furthermore, VEGF protein is dose-dependently reduced without affecting FLT-1, KDR or IGF-1R mRNA levels (Chen & Smith 2007; Ristori et al. 2011). Propranolol reduces VEGF overproduction in hypoxic retinas but does not affect the VEGF level in normoxic retinas, suggesting different patterns of regulation of VEGF transcription during normoxic and hypoxic conditions. This possibility is supported by the additional finding that β-AR blockade does not influence the VEGF level in the brain, lung or heart, where VEGF expression is not regulated by hypoxia, indicating that these organs most likely do not experience hypoxia in the OIR model. These data suggest that only VEGF production during hypoxia-ischaemia, which is most likely induced by HIF-1α, might be affected by the inhibition of β-ARs (Ristori et al. 2011). On the other hand, Chen et al. (2012) recently observed that in an OIR model performed in 129S6 mice,

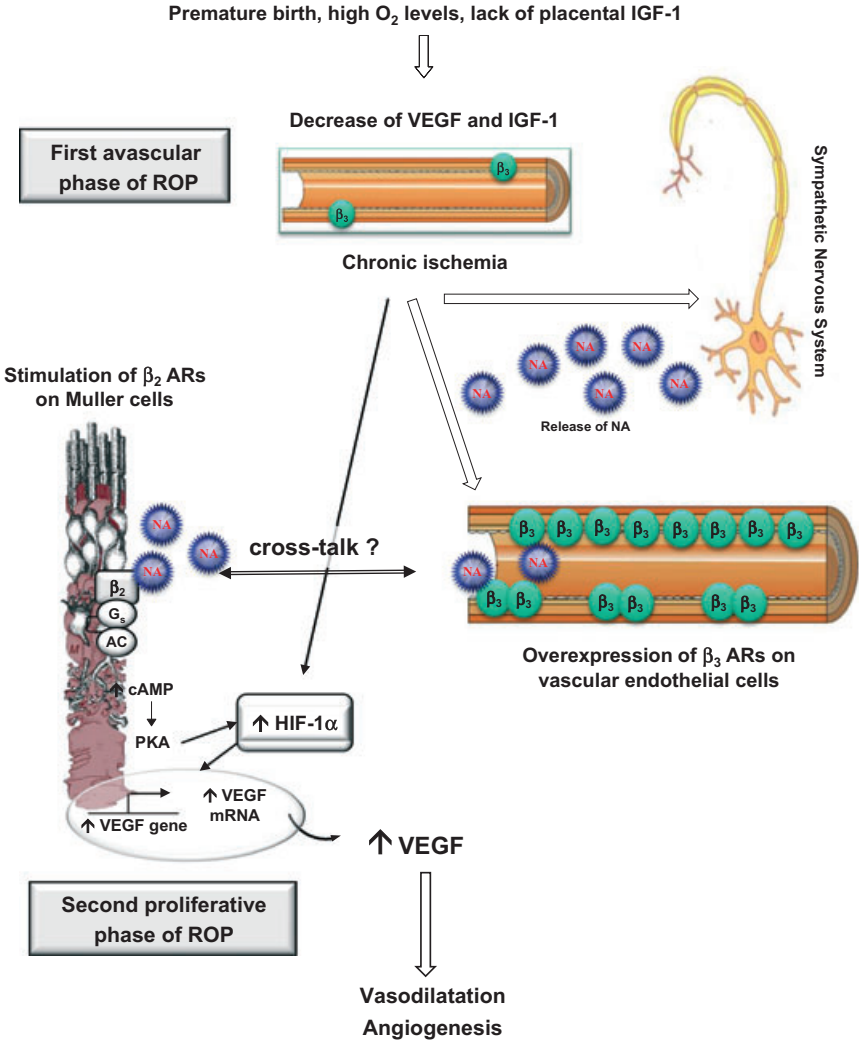

Fig. 2. Beta-adrenoreceptor stimulation and ROP. *β*-AR stimulation induces VEGF up-regulation and overexpression. O_2: oxygen; HIF-1: hypoxia-inducible factor 1; VEGF: vascular endothelial growth factor; IGF-1: insulin-like growth factor-1; *β*2-AR: *β*2-adrenergic receptor; *β*3-AR: *β*3-adrenergic receptor; mRNA: messenger ribonucleic acid; NA: noradrenaline; cAMP: cyclic adenosine monophosphate; PKA: protein kinase A; AC: adenylate cyclise; Gs: stimulatory G protein; ROP: retinopathy of Prematurity.

the administration of propranolol did not reduce VEGF levels or the extent of neovascularization. These apparent contradictions might be explained by the use of different strains of mice. In fact, the severity of the OIR varies according to the strain used, and occasionally, in the case of the same strain and vendor, on the origin of the strains (Europe, Asia, America) (Stahl et al. 2010). In addition, the 129 strain is significantly more susceptible to retinal neovascularization induced by hypoxia, exhibiting increased intraretinal and intravitreal angiogenesis and amplified VEGF mRNA expression compared to the C57BL strain (Chan et al. 2005). Ultimately, the difference in gene expression of the different strains, 129 and C57BL, changes the role of the beta-receptors in hippocampal synaptic plasticity (Schimanski et al. 2007). Moreover, it is possible that these differences are attributed to a SNP harboured between the two strains, consistent with what Good et al. (2012) have reported to exist between Caucasian and Black infants. This would explain the predisposition of 129 mice to develop more aggressive neovascularization than that of C57BL.

That propranolol (Ristori et al. 2011) and ICI 118,55, a selective *β*2-AR blocker (Martini et al. 2011), significantly inhibited hypoxia-induced VEGF production provides the first demonstration that *β*-ARs are coupled to the modulation of VEGF in the OIR model. Similarly, carvedilol, another non-selective *β*-AR blocker, reduces the expression of both HIF-1α and VEGF in a rat model of cardiac hypertrophy (Shyu et al. 2005). In conclusion, these findings imply an antiangiogenic and antipermeability effect of *β*-AR blockade that is most likely mediated through the down-regulation of VEGF and IGF-1 expression. The effect of propranolol on VEGF expression is likely mediated by HIF-1α. These results suggest a theoretical activity of catecholamines on the progression from the first to the second stage of ROP.

Whereas the role of *β*2-ARs in the pathogenesis of ROP might not be probable, the involvement of *β*3-ARs appears even less likely. An analysis of *β*-AR expression in the retina revealed that hypoxia up-regulates the *β*3-ARs, which appeared to colocalize with engorged retinal tufts in the inner capillary network that reside within the ganglion cell layer (Filippi et al. 2010). This observation is suggestive of the possibility that *β*3-ARs might mediate the onset of angiogenesis and the antiangiogenic effects of propranolol on the retina of the OIR mice. *β*3-ARs play a key role in regulating the proliferation and migration of human retinal endothelial cells (Steinle et al. 2003). Additionally, in human choroidal endothelial cells, *β*3-ARs play a role in cellular invasion and elongation (Steinle et al. 2005). Intriguingly, in this animal model, chronic ischaemia is hypothesized to induce the overexpression of *β*3-ARs in vascular endothelial cells, which in turn could stimulate the activation (crosstalk) of *β*2-ARs (Filippi et al. 2010). It is therefore likely that the transition between the first and the second phases of ROP is correlated with the up-regulation of these *β*-ARs (Fig. 2).

Further studies are needed to understand the role of the *β*3-ARs in the retina. In fact, the antagonists used to evaluate their roles might act as partial agonists (Vrydag & Michel 2007) or might not suppress their constitutive activity (Perrone & Scilimati 2010).

A pilot randomized trial is actively being conducted to verify whether propranolol a well-tolerated, non-selective *β*-AR blocker might reduce

the progression of ROP when administered to preterm newborns exhibiting a precocious phase of ROP (Filippi et al. 2010). Any favourable results of this research might open new avenues of research into the treatment or the prevention of this and other proliferative retinopathies (Table 1).

Conclusions

Retinopathy of prematurity is a multifactorial disease that potentially results in blindness. The increased survival of extremely premature infants is associated with increased risk for the development of ROP, prompting research for early screening methods and treatments that can reduce such complications. The limitations of ablative laser therapy and our growing knowledge of the pathogenesis of ROP have encouraged investigations into new antivasculogenic therapies.

The recent development of HIF-1 inhibitors has triggered great interest in their potential therapeutic application. The inhibition of HIF-1 should inhibit the production of proangiogenic factors that cause neoangiogenesis in numerous pathologies, including ROP (Xia et al. 2012). In premature babies, rhIGF-1 and rhEpo were tested for their abilities to prevent the loss of vasculature during the first phase of ROP (Smith 2004; Chen et al. 2008; Vanhaesebrouck et al. 2009), whereas anti-VEGF drugs have been administered during the second phase of ROP. However, few studies have evaluated their efficacy and safety (Darlow et al. 2011; Hård & Hellström 2011). Although the effectiveness of anti-VEGF treatment was recently demonstrated (Mintz-Hittner et al. 2011; Spandau et al. 2012), there remain concerns and limitations about its systemic safety. The anti-VEGF drug persists in the blood long after the initial intravitreal injection (Matsuyama et al. 2010; Lee et al. 2011; Sato et al. 2012). Little is known about its possible anti-VEGF effects in the brain and the lungs. Based on these uncertainties, it is necessary to study new therapeutic strategies.

The notion of a gene or drug therapy that inhibits PlGF is extremely attractive but far from clinically applicable in the near future (Luttun et al. 2002; Bae et al. 2005; Akrami et al. 2011). Similarly, more detailed studies of adenosine receptors and their antagonists will provide a solid basis for targeted local therapy (Lutty & McLeod 2003).

The genetic hypothesis that SNPs of β-ARs actively contribute to the pathogenesis of ROP supports the notion that the prevention of this disease might be possible using β-blockers (Good et al. 2012).

Under these new pathogenic hypotheses, the sympathetic nervous system plays a central role by releasing NA during hypoxic conditions, triggering the β2 and β3-ARs, which in turn should increase NO release. Nitric oxide likely plays a predominant role in the progression from the first to the second stage of ROP. In fact, its increase might release the proangiogenic factors that stimulate retinal neoangiogenesis (Fig. 1). Therefore, the local administration of propranolol or of selective β2-AR blockers, which would reduce the possible side-effects associated with their systemic administration, might represent ideal treatment modalities for the prevention of ROP from the earliest stage of the disease. Local propranolol, which elicits anti-VEGF activity by blocking the excess circulating VEGF without affecting the normal vasculature of other organs and systems, might be the drug of choice to understand and to prevent the pathogenesis of ROP.

References

Abbracchio MP, Brambilla R, Ceruti S et al. (1995): G protein-dependent activation of phospholipase C by adenosine A3 receptors in rat brain. Mol Pharmacol **48**: 1038–1045.

Abebe W & Mustafa SJ (1998): A1 adenosine receptor mediated Ins(1,4,5)P3 generation in allergic rabbit airway smooth muscle. Am J Physiol **275**: L990–L997.

Adachi K, Fujita Y, Morizane C et al. (1998): Inhibition of NMDA receptors and nitric oxide synthase reduces ischemic injury of the retina. Eur J Pharmacol **30**: 53–57.

Adini A, Kornaga T, Firoozbakht F et al. (2002): Placental growth factor is a survival factor for tumor endothelial cells and macrophages. Cancer Res **62**: 2749–2752.

Aher SM & Ohlsson A (2006): Early versus late erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Database Syst Rev **3**: CD004865.

Akrami H, Soheili ZS, Sadeghizadeh M et al. (2011): PlGF gene knockdown in human retinal pigment epithelial cells. Graefes Arch Clin Exp Ophthalmol **249**: 537–546.

Anagnostou A, Lee ES, Kessimian N et al. (1990): Erythropoietin has a mitogenic and positive chemotactic effect on endothelial cells. Proc Natl Acad Sci USA **87**: 5978–5982.

Anagnostou A, Liu Z, Steiner M et al. (1994): Erythropoietin receptor mRNA expression in human endothelial cells. Proc Natl Acad Sci USA **91**: 3974–3978.

Anderson CG, Benitz WE & Madan A (2004): Retinopathy of prematurity and pulse oximetry: a national survey of recent practices. J Perinatol **24**: 164–168.

Ando A, Yang A, Mori K et al. (2002): Nitric oxide is proangiogenic in the retina and choroid. J Cell Physiol **191**: 116–124.

Ashton N, Ward B & Serpell G (1953): Role of oxygen in the genesis of retrolental fibroplasia; a preliminary report. Br J Ophthalmol **37**: 513–520.

Augestad LB, Klingenberg O & Fosse P (2012): Braille use among Norwegian children from 1967 to 2007: trends in the underlying causes. Acta Ophthalmol **90**: 428–434.

Awad AS, Huang L, Ye H et al. (2006): Adenosine A2A receptor activation attenuates inflammation and injury in diabetic nephropathy. Am J Physiol Renal Physiol **290**: F828–F837.

Bae DG, Kim TD, Li G et al. (2005): Anti-Flt1 peptide, a vascular endothelial growth factor receptor 1-specific hexapeptide, inhibits tumor growth and metastasis. Clin Cancer Res **11**: 2651–2661.

Beleslin-Cokic BB, Cokic VP, Yu X et al. (2004): Erythropoietin and hypoxia stimulate erythropoietin receptor and nitric oxide production by endothelial cells. Blood **104**: 2073–2080.

Bellik L, Vinci MC, Filippi S et al. (2005): Intracellular pathways triggered by the selective FLT-1-agonist placental growth factor in vascular smooth muscle cells exposed to hypoxia. Br J Pharmacol **46**: 568–575.

Bernatchez PN, Soker S & Sirois MG (1999): Vascular endothelial growth factor effect on endothelial cell proliferation, migration, and platelet-activating factor synthesis is Flk-1-dependent. J Biol Chem **274**: 31047–31054.

Black RE, Cousens S, Johnson HL et al. (2010): Global, regional, and national causes of child mortality in 2008: a systematic analysis. Lancet **375**: 1969–1987.

Blazynski C (1987): Adenosine A1 receptor-mediated inhibition of adenylate cyclase in rabbit retina. J Neurosci **7**: 2522–2528.

Boonstra N, Limburg H, Tijmes N et al. (2012): Changes in causes of low vision between 1988 and 2009 in a Dutch population of children. Acta Ophthalmol **90**: 277–286.

Bottomley MJ, Webb NJ, Watson CJ et al. (2000): Placenta growth factor (PlGF): induces vascular endothelial growth factor

(VEGF): secretion from mononuclear cells and is co-expressed with VEGF in synovial fluid. Clin Exp Immunol **119**: 182–188.

Brafman A, Mett I, Shafir M et al. (2004): Inhibition of oxygen-induced retinopathy in RTP801-deficient mice. Invest Ophthalmol Vis Sci **45**: 3796–3805.

Brooks SE, Gu X, Samuel S et al. (2001): Reduced severity of oxygen-induced retinopathy in eNOS-deficient mice. Invest Ophthalmol Vis Sci **42**: 222–228.

Brown MS, Baron AE, France EK et al. (2006): Association between higher cumulative doses of recombinant erythropoietin and risk for retinopathy of prematurity. J AAPOS **10**: 143–149.

Burnstock G (1990): Changes in expression of autonomic nerves in aging and disease. J Auton Nerv Syst **30**: S25–S34.

Campbell K (1951): Intensive oxygen therapy as a possible cause of retrolental fibroplasia; A clinical approach. Med J Aust **2**: 48–50.

Cao Y (2009): Positive and negative modulation of angiogenesis by VEGFR1 ligands. Sci Signal **2**: 1–11.

Cao Y, Chen H, Zhou L et al. (1996): Heterodimers of placenta growth factor/vascular endothelial growth factor: endothelial activity, tumor cell expression, and high affinity binding to Flk-1/KDR. J Biol Chem **271**: 3154–3162.

Carlini RG, Dusso AS, Obialo CI et al. (1993): Recombinant human erythropoietin (rHuEPO): increases endothelin-1 release by endothelial cells. Kidney Int **43**: 1010–1014.

Carlini RG, Reyes AA & Rothstein M (1995): Recombinant human erythropoietin stimulates angiogenesis in vitro. Kidney Int **47**: 740–745.

Carlo WA, Goudar SS, Jehan I et al. (2010): High mortality rates for very low birth weight infants in developing countries despite training. Pediatrics **126**: e1072–e1080.

Carmeliet P & Jain RK (2000): Angiogenesis in cancer and other diseases. Nature **407**: 249–257.

Carmeliet P, Moons L, Luttun A et al. (2001): Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. Nat Med **7**: 575–583.

Chader GJ (2001): PEDF: raising both hopes and questions in controlling angiogenesis. Proc Natl Acad Sci USA **98**: 2122–2124.

Chakravarthy U, Stitt AW, McNally J et al. (1995): Nitric oxide synthase activity and expression in retinal capillary endothelial cells and pericytes. Curr Eye Res **14**: 285–294.

Chan CK, Pham LN, Zhou J et al. (2005): Differential expression of pro- and antiangiogenic factors in mouse strain-dependent hypoxia-induced retinal neovascularization. Lab Invest **85**: 721–733.

Chan-Ling T, Gock B & Stone J (1995): The effect of oxygen on vasoformative cell division: evidence that ''physiological hypoxia'' is the stimulus for normal retinal vasculogenesis. Invest Ophthalmol Vis Sci **36**: 1201–1214.

Chen J & Smith LE (2007): Retinopathy of prematurity. Angiogenesis **10**: 133–140.

Chen J, Connor KM, Aderman CM et al. (2008): Erythropoietin deficiency decreases vascular stability in mice. J Clin Invest **118**: 526–533.

Chen ML, Guo L, Smith LE et al. (2010): High or low oxygen saturation and severe retinopathy of prematurity: a metaanalysis. Pediatrics **125**: e1483–e1492.

Chen J, Joyal JS, Hatton CJ et al. (2012): Propranolol inhibition of β-adrenergic receptor does not suppress pathologic neovascularization in oxygen-induced retinopathy. Invest Ophthalmol Vis Sci **53**: 2968–2977.

Chow LC, Wright KW, Sola A et al. (2003): Can changes in clinical practice decrease the incidence of severe retinopathy of prematurity in very low birth weight infants? Pediatrics **111**: 339–345.

Christopherson KS & Bredt DS (1997): Nitric oxide in excitable tissues: physiological roles and disease. J Clin Invest **100**: 2424–2429.

Collis MG & Hourani SM (1993): Adenosine receptor subtypes. Trends Pharmacol Sci **14**: 360–366.

Cox CM, D'Agostino SL, Miller MK et al. (2006): Apelin, the ligand for the endothelial G-protein-coupled receptor, APJ, is a potent angiogenic factor required for normal vascular development of the frog embryo. Dev Biol **296**: 177–189.

Cronstein BN, Kramer SB, Weissmann G et al. (1983): Adenosine: a physiological modulator of superoxide anion generation by human neutrophils. J Exp Med **158**: 1160–1177.

Cronstein BN, Rosenstein ED, Kramer SB et al. (1985): Adenosine; a physiologic modulator of superoxide anion generation by human neutrophils. Adenosine acts via an A2 receptor on human neutrophils. J Immunol **135**: 1366–1371.

Cronstein BN, Levin RI, Philips MR et al. (1992): Neutrophil adherence to endothelium is enhanced via adenosine A1 receptors and inhibited via adenosine A2 receptors. J Immunol **148**: 2201–2206.

Csak K, Szabo V, Szabo A et al. (2006): Pathogenesis and genetic basis for retinopathy of prematurity. Front Biosci **11**: 908–920.

Cunningham SA, Tran TM, Arrate MP et al. (1999): Characterization of vascular endothelial cell growth factor interactions with the kinase insert domain-containing receptor tyrosine kinase: a real time kinetic study. J Biol Chem **274**: 18421–18427.

Dal Monte M, Martini D, Latina V et al. (2012): Beta-adrenoreceptor (β-AR) agonism influences retinal responses to hypoxia in a mouse model of retinopathy of prematurity. Invest Ophthalmol Vis Sci **53**: 2181–2192.

Darlow BA, Ells AL, Gilbert CE et al. (2011): Are we there yet? Bevacizumab therapy for retinopathy of prematurity Arch Dis Child Fetal Neonatal Ed. [Epub ahead of print].

Dawson T, Bredt D, Fotuki M et al. (1991): Nitric oxide synthase and neuronal NADPH diaphorase are identical in brain and peripheral tissues. Proc Natl Acad Sci USA **88**: 7797–7801.

Deulofeut R, Critz A, Adams-Chapman I et al. (2006): Avoiding hyperoxia in infants $\leq$ 1250 g is associated with improved short- and longterm outcomes. J Perinatol **26**: 700–705.

Devic E, Rizzoti K, Bodin S et al. (1999): Amino acid sequence and embryonic expression of msr/apj. Mech Dev **84**: 199–203.

Digcaylioglu M, Bichet S, Marti HH et al. (1995): Localization of specific erythropoietin binding sites in defined areas of the mouse brain. Proc Natl Acad Sci USA **92**: 3717–3720.

Donato H, Vain N, Rendo P et al. (2000): Effect of early versus late administration of human recombinant erythropoietin on transfusion requirements in premature infants: results of a randomized, placebo-controlled, multicenter trial. Pediatrics **105**: 1066–1072.

Dorta P & Kychenthal A (2010): Treatment of type 1 retinopathy of prematurity with intravitreal bevacizumab (Avastin). Retina **30**: S24–S31.

Dusseau JW & Hutchins PM (1988): Hypoxia-induced angiogenesis in chick chorioallantoic membranes: a role for adenosine. Respir Physiol **71**: 33–44.

Dweik RA, Laskowski D, Abu-Soud HM et al. (1998): Nitric oxide synthesis in the lung. Regulation by oxygen through a kinetic mechanism. J Clin Invest **101**: 660–666.

Ehrenreich H, Hasselblatt M, Dembowski C et al. (2002): Erythropoietin therapy for acute stroke is both safe and beneficial. Mol Med **8**: 495–505.

Eriksson U & Alitalo K (1999): Structure, expression and receptor binding properties of novel vascular endothelial growth factors. Curr Top Microbiol Immunol **237**: 41–57.

Eyries M, Siegfried G, Ciumas M et al. (2008): Hypoxia-induced apelin expression regulates endothelial cell proliferation and regenerative angiogenesis. Circ Res **103**: 432–440.

Fanaroff AA, Stoll BJ, Wright LL et al. (2007): Trends in neonatal morbidity and mortality for very low birth weight infants. Am J Obstet Gynecol **196**: 147.

Ferrara N (1999a): Molecular and biological properties of vascular endothelial growth factor. J Mol Med **77**: 527–543.

Ferrara N (1999b): Vascular endothelial growth factor: molecular and biological aspects. Curr Top Microbiol Immunol **237**: 1–30.

Ferrara N & Davis-Smyth T (1997): The biology of vascular endothelial growth factor. Endocr Rev **18**: 4–25.

Figueras-Aloy J, Alvarez-Dominguez E, Morales-Ballus M et al. (2010): Early administration of erythropoietin in the extreme premature, a risk factor for retinopathy of prematurity? An Pediatr (Barc) **73**: 327–333.

Filippi L, Cavallaro G, Fiorini P et al. (2010): Study protocol: safety and efficacy of propranolol in newborns with Retinopathy of Prematurity (PROP-ROP): ISRCTN18523491. BMC Pediatr **10**: 83.

Fischer S, Sharma HS, Kaliczek GF et al. (1995): Expression of vascular permeability factor/vascular endothelial growth factor in pig cerebral microvascular endothelial cells and its upregulation by adenosine. Mol Brain Res **28**: 141–148.

Fleming I, Bauersachs J, Fisslthaler B et al. (1998): Ca^{2+} -independent activation of the endothelial nitric oxide synthase in response to tyrosine phosphatase inhibitors and fluid shear stress. Circ Res **82**: 686–695.

Flower RW, McLeod DS, Lutty GA et al. (1985): Postnatal retinal vascular development of the puppy. Invest Ophthalmol Vis Sci **26**: 957–968.

Freissmuth M, Schutz W & Linder ME (1991): Interactions of the bovine brain A-adenosine receptor with recombinant G protein a-subunits. Selectivity for rGiOa-3. J Biol Chem **266**: 17778–17783.

Fukuda R, Hirota K, Fan F et al. (2002): IGF-1 induces HIF-1-mediated VEGF expression that is dependent on MAP kinase and PI-3-kinase signaling in colon cancer cells. J Biol Chem **277**: 38205–38211.

Fukumura D, Gohongi T, Kadambi A et al. (2001): Predominant role of endothelial nitric oxide synthase in vascular endothelial growth factor-induced angiogenesis and vascular permeability. Proc Natl Acad Sci USA **98**: 2604–2609.

Gariano RF (2003): Cellular mechanisms in retinal vascular development. Prog Retinal Eye Res **22**: 295–306.

Gariano RF & Gardner TW (2005): Retinal angiogenesis in development and disease. Nature **438**: 960–966.

Gerber HP, Condorelli F, Park J et al. (1997): Differential transcriptional regulation of the two vascular endothelial growth factor receptor genes. Flt-1, but not Flk-1/KDR, is up-regulated by hypoxia. J Biol Chem **272**: 23659–23667.

Gess B, Sandner P & Kurtz A (1996): Differential effects of kinase inhibitors on erythropoietin and vascular endothelial growth factor gene expression in rat hepatocytes. Pflugers Arch Eur J Physiol **432**: 426–432.

Gogusev J, Zhu DL, Herembert T et al. (1994): Effect of erythropoietin on DNA synthesis. proto-oncogene expression and phospholipase activity in rat vascular smooth muscle cells. Biochem Biophys Res Commun **199**: 977–983.

Good WV, Hardy RJ, Dobson V et al. (2005): The incidence and course of retinopathy of prematurity: findings from the early treatment for retinopathy of prematurity study. Pediatrics **116**: 15–23.

Good WV, Hardy RJ, Wallace DK et al. (2012): β-Blocking and racial variation in the severity of retinopathy of prematurity. Arch Ophthalmol **130**: 117–118.

Goureau O, Lepoivre M, Bequet F et al. (1993): Differential regulation of inducible nitric oxide synthase by fibroblast growth factors and transforming growth factor in bovine retinal pigmented epithelial cells: inverse correlation with cellular proliferation. Proc Natl Acad Sci USA **90**: 4276–4280.

Goureau O, Hicks D & Courtouis Y (1994): Human retinal pigmented epithelial cells produce nitric oxide in response to cytokines. Biochem Biophys Res Commun **198**: 120–126.

Granstam E & Granstam SO (1999): Regulation of uveal and retinal blood flow in STZ-diabetic and non-diabetic rats; involvement of nitric oxide. Curr Eye Res **19**: 330–337.

Granstam E, Granstam SO, Fellstrom B et al. (1998): Endothelium-dependent vasodilation in the uvea of hypertensive and normotensive rats. Curr Eye Res **17**: 189–196.

Grant MB, Tarnuzzer RW, Caballero S et al. (1999): Adenosine receptor activation induces vascular endothelial growth factor in human retinal endothelial cells. Circ Res **85**: 699–706.

Grant MB, Davis MI, Caballero S et al. (2001): Proliferation, migration, and ERK activation in human retinal endothelial cells through A (2B) adenosine receptor stimulation. Invest Ophthalmol Vis Sci **42**: 2068–2073.

Grimm C, Wenzel A, Groszer M et al. (2002): HIF-1-induced erythropoietin in the hypoxic retina protects against light-induced retinal degeneration. Nat Med **8**: 718–724.

Gu XG, El-Remessy AB, Brooks SE et al. (2003): Hyperoxia induces retinal vascular endothelial cell apoptosis through formation of peroxynitrite. Am J Physiol Cell Physiol **285**: C546–C554.

Guimaraes S & Moura D (2001): Vascular adrenoceptors: an update. Pharmacol Rev **53**: 319–356.

Gumy-Pause F, Ozsahin H, Mermillod B et al. (2005): Stepping up versus standard doses of erythropoietin in preterm infants: a randomized controlled trial. Pediatr Hematol Oncol **22**: 667–678.

Guo K, Ma Q, Wang L et al. (2009): Norepinephrine-induced invasion by pancreatic cancer cells is inhibited by propranolol. Oncol Rep **22**: 825–830.

Haider MZ, Devarajan LV, Al-Essa M et al. (2000): Missense mutations in Norrie disease gene are not associated with advanced stages of retinopathy of prematurity in Kuwaiti Arabs. Biol Neonate **77**: 88–91.

Haider MZ, Devarajan LV, Al-Essa M et al. (2001): Retinopathy of prematurity: mutations in the Norrie disease gene and the risk of progression to advanced stages. Pediatr Int **43**: 120–123.

Haider MZ, Devarajan LV, Al-Essa M et al. (2002): A C597 $\rightarrow$ A polymorphism in the Norrie disease gene is associated with advanced retinopathy of prematurity in premature Kuwaiti infants. J Biomed Sci **9**: 365–370.

Hård AL & Hellström A (2011): On safety, pharmacokinetics and dosage of bevacizumab in ROP treatment - a review. Acta Paediatr **100**: 1523–1527.

Hardy P, Dumont I, Bhattacharya M et al. (2000): Oxidants, nitric oxide and prostanoids in the developing ocular vasculature: a basis for ischemic retinopathy. Cardiovasc Res **47**: 489–509.

Hashiguchi A, Yano S, Morioka M et al. (2004): Up-regulation of endothelial nitric oxide synthase via phosphatidylinositol 3-kinase pathway contributes to ischemic tolerance in the CA1 subfield of gerbil hippocampus. J Cereb Blood Flow Metab **24**: 271–279.

He T, Ai M, Zhao XH et al. (2007): Inducible nitric oxide synthase mediates hypoxia-induced hypoxia-inducible factor-1 alpha activation and vascular endothelial growth factor expression in oxygen-induced retinopathy. Pathobiology **74**: 336–343.

Heberlein C, Fischer KD, Stoffel M et al. (1992): The gene for erythropoietin receptor is expressed in multipotential hematopoietic and embryonal stem cells: evidence for differentiation stage-specific regulation. Mol Cell Biol **12**: 1815–1826.

Heeschen C, Aicher A, Lehmann R et al. (2003): Erythropoietin is a potent physiologic stimulus for endothelial progenitor cell mobilization. Blood **102**: 1340–1346.

Hellstrom A, Perruzzi C, Ju M et al. (2001): Low IGF-I suppresses VEGF-survival signaling in retinal endothelial cells: direct correlation with clinical retinopathy of prematurity. Proc Natl Acad Sci USA **98**: 5804–5808.

Hellstrom A, Carlsson B, Niklasson A et al. (2002): IGF-I is critical for normal vascularization of the human retina. J Clin Endocrinol Metab **87**: 3413–3416.

Hesse L, Eberl W, Schlaud M et al. (1997): Blood transfusion. Iron load and retinopathy of prematurity. Eur J Pediatr **156**: 465–470.

Hieble JP, Bondinell WE & Ruffolo RR (1995): Alpha- and beta-adrenoceptors: from the gene to the clinic. 1. Molecular biology and adrenoceptor subclassification. J Med Chem **38**: 3415–3444.

Hierholzer C, Harbrecht B, Menezes J et al. (1998): Essential role of induced nitric oxide in the initiation of the inflammatory response after hemorrhagic shock. J Exp Med **187**: 917–928.

Hiraoka M, Berinstein D, Trese M et al. (2001): Insertion and deletion mutations in the dinucleotide repeat region of the Norrie disease gene in patient with advanced retinopathy of prematurity. J Hum Genet **46**: 178–181.

Hiraoka M, Takahashi H, Orimo H et al. (2010): Genetic screening of Wnt signaling factors in advanced retinopathy of prematurity. Mol Vis **16**: 2572–2577.

Hoerster R, Muether P, Dahlke C et al. (2012): Serum concentrations of vascular endothelial growth factor in an infant treated with ranibizumab for retinopathy of prematurity. Acta Ophthalmol **91**: e74–75.

Hood JD, Meininger CJ, Ziche M et al. (1998): VEGF upregulates ecNOS message, protein, and NO production in human endothelial cells. Am J Physiol **274**: H1054–H1058.

Hosoya M, Kawamata Y, Fukusumi S et al. (2000): Molecular and functional characteristics of APJ. Tissue distribution of mRNA and interaction with the endogenous ligand apelin. J Biol Chem **275**: 21061–21067.

Hughes S, Yang H & Chan-Ling T (2000): Vascularisation of the human fetal retina: roles of vasculogenesis and angiogenesis. Invest Ophthalmol Vis Sci **41**: 1217–1228.

Iaccarino G, Ciccarelli M, Sorriento D et al. (2005): Ischemic neoangiogenesis enhanced by beta2-adrenergic receptor overexpression: a novel role for the endothelial adrenergic system. Circ Res **97**: 1182–1189.

Iadecola C (1992): Does nitric oxide mediate the cerebrovasodilation elicited in hypercapnia? Proc Natl Acad Sci USA **89**: 3913–3916.

Ibrahim AS, El-shishtawy MM, Zhang W et al. (2011): A2A Adenosine Receptor (A2AAR) as a Therapeutic Target in Diabetic Retinopathy. Am J Pathol **178**: 2136–2145.

Inoue N, Venema RC, Sayegh HS et al. (1995): Molecular regulation of the bovine endothelial cell nitric oxide synthase by transforming growth factor-beta1. Arterioscler Thromb Vasc Biol **15**: 1255–1261.

Ishibashi T, Koziol JA & Burstein SA (1987): Human recombinant erythropoietin promotes differentiation of murine megakaryocytes in vitro. J Clin Invest **79**: 286–289.

Jacobson LO, Goldwasser E, Fried W et al. (1957): Role of the kidney in erythropoiesis. Nature **179**: 633–634.

Jia YX, Lu ZF, Zhang J et al. (2007): Apelin activates L-arginine/nitric oxide synthase/nitric oxide pathway in rat aortas. Peptides **28**: 2023–2029.

Jiang J, Xia XB, Xu HZ et al. (2009): Inhibition of retinal neovascularization by gene transfer of small interfering RNA targeting HIF-1alpha and VEGF. J Cell Physiol **218**: 66–74.

Jiang Y, Walker RJ, Kern TS et al. (2010): Application of isoproterenol inhibits diabetic-like changes in the rat retina. Exp Eye Res **91**: 171–179.

Johnson SM, Patel S, Bruckner FE et al. (1999): 5′-nucleotidase as a marker of both general and local inflammation in rheumatoid arthritis patients. Rheumatology **38**: 391–396.

Jośko J & Mazurek M (2004): Transcription factors having impact on vascular endothelial growth factor (VEGF) gene expression in angiogenesis. Med Sci Monit **10**: RA89–RA98.

Junk AK, Mammis A, Savitz SI et al. (2002): Erythropoietin administration protects retinal neurons from acute ischemia-reperfusion injury. Proc Natl Acad Sci USA **99**: 10659–10664.

Kalin RE, Kretz MP, Meyer AM et al. (2007): Paracrine and autocrine mechanisms of apelin signaling govern embryonic and tumor angiogenesis. Dev Biol **305**: 599–614.

Kasai A, Shintani N, Oda M et al. (2004): Apelin is a novel angiogenic factor in retinal endothelial cells. Biochem Biophys Res Commun **325**: 395–400.

Kasai A, Shintani N, Kato H et al. (2008): Retardation of retinal vascular development in apelin-deficient mice. Arterioscler Thromb Vasc Biol **28**: 1717–1722.

Kasai A, Ishimaru Y, Kinjo T et al. (2010): Apelin is a crucial factor for hypoxia-induced retinal angiogenesis. Arterioscler Thromb Vasc Biol **30**: 2182–2187.

Katugampola SD, Maguire JJ, Matthewson SR et al. (2001): [(125)I]-(Pyr(1))Apelin-13 is a novel radioligand for localizing the APJ orphan receptor in human and rat tissues with evidence for a vasoconstrictor role in man. Br J Pharmacol **132**: 1255–1260.

Kaur C, Sivakumar V & Foulds WS (2006): Early response of neurons and glial cells to hypoxia in the retina. Invest Ophthalmol Vis Sci **47**: 1126–1141.

Khaliq A, Foreman D, Ahmed A et al. (1998): Increased expression of placenta growth factor in proliferative diabetic retinopathy. Lab Invest **78**: 109–116.

Kim JH, Kim JH, Yu YS et al. (2008): Deguelin inhibits retinal neovascularization by down-regulation of HIF-1alpha in oxygen-induced retinopathy. J Cell Mol Med **12**: 2407–2415.

Kimata H, Yoshida A, Ishioka C et al. (1991): Human recombinant erythropoietin directly stimulates B cell immunoglobulin production and proliferation in serum-free medium. Clin Exp Immunol **85**: 151–156.

Kinsey VE (1956): Retrolental fibroplasia; Cooperative study of retrolental fibroplasia and the use of oxygen. AMA Arch Ophthalmol **56**: 481–543.

Kobayashi M, Kuriowa T, Shimokawa R et al. (2000): Nitric oxide synthase expression in ischemic rat retinas. Jpn J Ophthalmol **44**: 235–244.

Kojima Y & Quertermous T (2008): Apelin-APJ signaling in retinal angiogenesis. Arterioscler Thromb Vasc Biol **28**: 1687–1688.

Konishi Y, Chui DH, Hirose H et al. (1993): Trophic effect of erythropoietin and other hematopoietic factors on central cholinergic neurons in vitro and in vivo. Brain Res **609**: 29–35.

Koo KY, Kim JE, Lee SM et al. (2010): Effect of severe neonatal morbidities on long term outcome in extremely low birth-weight infants. Korean J Pediatr **53**: 694–700.

Koury MJ & Bondurant MC (1990): Erythropoietin retards DNA breakdown and prevents programmed death in erythroid progenitor cells. Science **248**: 378–381.

Krantz SB (1991): Erythropoietin. Blood **77**: 419–434.

Kroncke KD, Fehsel K & Kolb-Bachofen V (1995): Inducible nitric oxide synthase and its product nitric oxide, a small molecule with complex biological activities. Biol Chem Hoppe-Seyler **376**: 327–343.

Kubrusly RC, Ventura AL, de MeloReisRA et al. (2007): Norepinephrine acts as D1-dopaminergic agonist in the embryonic avian retina: late expression of beta1-adrenergic receptor shifts norepinephrine specificity in the adult tissue. Neurochem Int **50**: 211–218.

Lakshminarayanan S, Antonetti DA, Gardner TW et al. (2000): Effect of VEGF on retinal microvascular endothelial hydraulic conductivity: the role of NO. Invest Ophthalmol Vis Sci **41**: 4256–4261.

Langford K, Nicolaides K & Miell JP (1998): Maternal and fetal insulin-like growth factors and their binding proteins in the second and third trimesters of human pregnancy. Hum Reprod **13**: 1389–1393.

Lashbrook BL & Steinle JJ (2005): Beta-adrenergic receptor regulation of pigment epithelial-derived factor expression in rat retina Auton. Neuroscience **121**: 33–39.

Lawn JE, Cousens S, Zupan J et al. (2005): 4 million neonatal deaths: when? Where? Why? Lancet **365**: 891–900.

Lawn JE, Kerber K, Enweronu-Laryea C et al. (2009): Newborn survival in low resource settings - are we delivering? BJOG **116**: 49–59.

Leaute-Labreze C, Dumas delaRoqueE, Hubiche T et al. (2008): Propranolol for severe hemangiomas of infancy. N Engl J Med **358**: 2649–2651.

Lee SJ, Kim SY, Yoo B et al. (2011): Plasma Level Of Vascular Endothelial Growth Factor In Retinopathy Of Prematurity After Intravitreal Injection Of Bevacizumab. Invest Ophthalmol Vis Sci **52**: 3165, E-abstract 3165.

Li SN & Wong PT (2000): The adenosine receptor agonist, APNEA, increases Ca++ influx into rat cortical synaptosomes through N-type channels associated with A2a receptors. Neurochem Res **25**: 457–459.

Li R, Lyn D, Lapu-Bula R et al. (2004): Relation of endothelial nitric oxide synthase gene to plasma nitric oxide level, endothelial function, and blood pressure in African Americans. Am J Hypertens **17**: 560–567.

Liang BT (1996): Direct preconditioning of cardiac ventricular myocytes via adenosine A1 and KATP channel. Am J Physiol **271**: H1769–H1777.

Liao JK, Zulueta JJ, Yu FS et al. (1995): Regulation of bovine endothelial constitu-

tive nitric oxide synthase by oxygen. J Clin Invest **96**: 2661–2666.

Liggett SB, Cresci S, Kelly RJ et al. (2008): A GRK5 polymorphism that inhibits beta-adrenergic receptor signaling is protective in heart failure. Nat. Med **14**: 510–517.

Lindgren I & Altimiras J (2009): Chronic prenatal hypoxia sensitizes beta-adrenoceptors in the embryonic heart but causes postnatal desensitization. Am J Physiol Regul Integr Comp Physiol **297**: R258–R264.

Liou GI, Auchampach JA, Hillard CJ et al. (2008): Mediation of cannabidiol anti-inflammation in the retina by equilibrative nucleoside transporter and A2A adenosine receptor. Invest Ophthalmol Vis Sci **49**: 5526–5531.

Loh A, Hadziahmetovic M & Dunaief JL (2009): Iron homeostasis and eye disease. Biochim Biophys Acta **1790**: 637–649.

Luttun A, Tjwa M & Carmeliet P (2002): Placental growth factor (PlGF) and its receptor Flt-1 (VEGFR-1): novel therapeutic targets for angiogenic disorders. Ann N Y Acad Sci **979**: 80–93.

Lutty GA & McLeod DS (2003): Retinal vascular development and oxygen-induced retinopathy: a role for adenosine. Progr Retin Eye Res **22**: 95–111.

Lutty GA, Mathews MK, Merges C et al. (1998): Adenosine stimulates canine retinal microvascular endothelial cell migration and tube formation. Curr Eye Res **17**: 594–607.

Lutty GA, Merges C & McLeod DS (2000): 5'nucleotidase and adenosine during retinal vasculogenesis and oxygen induced retinopathy. Invest Ophthalmol Vis Sci **41**: 218–229.

MacMicking J, Xie QW & Nathan C (1997): Nitric oxide and macrophage function. Annu Rev Immunol **15**: 323–350.

Maier RF, Obladen M, Muller-Hansen I et al. (2002): Early treatment with erythropoietin beta ameliorates anemia and reduces transfusion requirements in infants with birth weights below 1000 g. J Pediatr **141**: 8–15.

Målqvist M (2011): Neonatal mortality: an invisible and marginalised trauma. Glob Health Action **4**: 5724.

Marala RB & Mustafa SJ (1998): Immunological characterization of adenosine A2a receptors in human and porcine cardiovascular tissue. J Pharmacol Exp Ther **286**: 1051–1057.

Martin AR, Bailie JR, Robson T et al. (2000): Retinal pericytes control expression of nitric oxide synthase and endothelin-1 in microvascular endothelial cells. Microvasc Res **59**: 131–139.

Martini D, Dal MonteM, Ristori C et al. (2011): Antiangiogenic effects of beta2-adrenergic receptor blockade in a mouse model of oxygen-induced retinopathy. J Neurochem **119**: 1317–1329.

Masri B, Lahlou H, Mazarguil H et al. (2002): Apelin (65–77) activates extracellular signal-regulated kinases via a PTX-sensitive G protein. Biochem Biophys Res Commun **290**: 539–545.

Masuda S, Nagao M, Takahata K et al. (1993): Functional erythropoietin receptor of the cells with neural characteristics. J Biol Chem **268**: 11208–11216.

Matsuyama K, Ogata N, Matsuoka M et al. (2010): Plasma levels of vascular endothelial growth factor and pigment epithelium-derived factor before and after intravitreal injection of bevacizumab. Br J Ophthalmol **94**: 1215–1218.

Meininger CJ, Schelling ME & Granger HJ (1988): Adenosine and hypoxia stimulate proliferation and migration of endothelial cells. Am J Physiol **255**: H554–H562.

Meyer P, Champion C, Schlotzer-Schrehardt U et al. (1999): Localization of nitric oxide synthase isoforms in porcine ocular tissues. Curr Eye Res **18**: 375–380.

Michels S, Schmidt-Erfurth U & Rosenfeld PJ (2006): Promising new treatments for neovascular age-related macular degeneration. Expert Opin Investig Drugs **15**: 779–793.

Mintz-Hittner HA & Kuffel RR Jr (2008): Intravitreal injection of bevacizumab (Avastin) for treatment of stage 3 retinopathy of prematurity in zone I or posterior zone II. Retina **28**: 1374.

Mintz-Hittner HA, Kennedy KA, Chuang AZ et al. (2011): Efficacy of intravitreal bevacizumab for stage 3+ retinopathy of prematurity. N Engl J Med **364**: 603–615.

Mohamed S, Schaa K, Cooper ME et al. (2009): Genetic contributions to the development of retinopathy of prematurity. Pediatr Res **65**: 193–197.

Montesinos MC, Desai A, Chen JF et al. (2002): Adenosine Promotes Wound Healing and Mediates Angiogenesis in Response to Tissue Injury Via Occupancy of A2A Receptors. Am J Pathol **160**: 2009–2018.

Morbidelli L, Chang CH, Douglas JG et al. (1996): Nitric oxide mediates mitogenic effect of VEGF on coronary venular endothelium. Am J Physiol **270**: H411–H415.

Mori A, Miwa T, Sakamoto K et al. (2010): Pharmacological evidence for the presence of functional beta(3)-adrenoceptors in rat retinal blood vessels. Naunyn Schmiedebergs Arch Pharmacol **382**: 119–126.

Nagy JA, Dvorak AM & Dvorak HF (2003): VEGF-A164/165 and PlGF. Roles in angiogenesis and arteriogenesis. Trends Cardiovasc Med **13**: 169–175.

Nathan C (1997): Inducible nitric oxide synthase: what difference does it make? J Clin Invest **100**: 2417–2423.

Neufeld AH, Hernandez R & Gonzalez M (1997): Nitric oxide synthase in the human glaucomatous optic nerve head. Arch Ophthalmol **115**: 497–503.

Neufeld AH, Shareef S & Pena J (2000): Cellular localization of neuronal nitric oxide synthase (NOS-1) in the human and rat retina. J Comp Neurol **4**: 269–275.

Nicosia RF (1998): What is the role of vascular endothelial growth factor related molecules in tumor angiogenesis? Am J Pathol **153**: 11–16.

Nomura M, Yamagishi S, Harada S et al. (1998): Placenta growth factor (PlGF) mRNA expression in brain tumors. J Neurooncol **40**: 123–130.

Ohlsson A & Aher SM (2006): Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Database Syst Rev **3**: CD004863.

Olanrewaju HA & Mustafa SJ (2000): Adenosine A(2A) and A(2B) receptors mediated nitric oxide production in coronary artery endothelial cells. Gen Pharmacol **35**: 171–177.

Olofsson B, Pajusola K, von EulerG et al. (1996): Genomic organization of the mouse and human genes for vascular endothelial growth factor B (VEGF-B) and characterization of a second splice isoform. J Biol Chem **271**: 19310–19317.

Olofsson B, Korpelainen E, Pepper MS et al. (1998): Vascular endothelial growth factor B (VEGF-B) binds to VEGF receptor-1 and regulates plasminogen activator activity in endothelial cells. Proc Natl Acad Sci USA **95**: 11709–11714.

Ostwald P, Park SS, Toledano AY et al. (1997): Adenosine receptor blockade and nitric oxide synthase inhibition in the retina: impact upon post-ischemic hyperemia and the electroretinogram. Vision Res **37**: 3453–3461.

Ozaki H, Yu AY, Della N et al. (1999): Hypoxia-inducible factor 1α is increased in ischemic retina: Temporal and spatial correlation with VEGF expression. Invest Ophthal Vis Sci **40**: 182–189.

Palmer TM, Gettys TW & Stiles GL (1995): Differential interaction with and regulation of multiple G-proteins by the rat A3 adenosine receptor. J Biol Chem **270**: 16895–16902.

Park JE, Chen HH, Winer J et al. (1994): Placenta growth factor: potentiation of vascular endothelial growth factor bioactivity, in vitro and in vivo, and high affinity binding to Flt-1 but not to Flt-1/KDR. J Biol Chem **269**: 25646–25654.

Patz A & Eastham AB (1957): Oxygen studies in retrolental fibroplasia. VI. The effect of concentration and duration of exposure to oxygen on the immature mouse eye. Am J Ophthalmol **44**: 110–115.

Patz A, Hoeck LE & De LaCruzE (1952): Studies on the effect of high oxygen administration in retrolental fibroplasia. I. Nursery observations. Am J Ophthalmol **35**: 1248–1253.

Perrone MG & Scilimati A (2010): β(3)-Adrenoceptor agonists and (antagonists as) inverse agonists history, perspective, constitutive activity, and stereospecific binding. Methods Enzymol **484**: 197–230.

Persico MG, Vincenti V & DiPalma T (1999): Structure, expression and receptor-binding properties of placenta growth factor (PlGF). Curr Top Microbiol Immunol **237**: 31–40.

Peters KG, De VriesC & Williams LT (1993): Vascular endothelial growth factor receptor expression during embryogenesis and tissue repair suggests a role in endothelial differentiation and blood vessel growth. Proc Natl Acad Sci USA **90**: 8915–8919.

Pierce EA, Foley ED & Smith LE (1996): Regulation of vascular endothelial growth factor by oxygen in a model of retinopathy of prematurity. Arch Ophthalmol **114**: 1219–1228.

Poukens V, Glasgow BJ & Demer JL (1998): Nonvascular contractile cells in sclera and choroid of humans and monkeys. Invest Ophthalmol Vision Sci **39**: 1765–1774.

Praveen V, Vidavalur R, Rosenkrantz TS et al. (2009): Infantile hemangiomas and retinopathy of prematurity: possible association. Pediatrics **123**: e484–e489.

Provis JM (2001): Development of the primate retinal vasculature. Prog Retin Eye Res **20**: 799–821.

Quinn TP, Peters KG, De VriesC et al. (1993): Fetal liver kinase 1 is a receptor for vascular endothelial growth factor and is selectively expressed in vascular endothelium. Proc Natl Acad Sci USA **90**: 7533–7537.

Rahi JS & Cable N (2003): Severe visual impairment and blindness in children in the UK. Lancet **362**: 1359–1365.

Rakic JM, Lambert V, Devy L et al. (2003): Placental growth factor, a member of the VEGF family, contributes to the development of choroidal neovascularization. Invest Ophthalmol Vis Sci **44**: 3186–3193.

Ralevic V & Burnstock G (1998): Receptors for purines and pyrimidines. Pharmacol Rev **50**: 413–492.

Reaux A, De MotaN, Skultetyova I et al. (2001): Physiological role of a novel neuropeptide, apelin, and its receptor in the rat brain. J Neurochem **77**: 1085–1096.

Ricci B, Calogero G, Caprilli A et al. (1991): Reduced severity of oxygen-induced retinopathy in the newborn rat after topical administration of timolol maleate. A preliminary study. Doc Ophthalmol **77**: 47–56.

Ricci B, Minicucci G, Manfredi A et al. (1995): Oxygen-induced retinopathy in the newborn rat: effects of hyperbarism and topical administration of timolol maleate. Graefes Arch Clin Exp Ophthalmol **233**: 226–230.

Ricci B, Ricci F & Maggiano N (2000): Oxygen-induced retinopathy in the newborn rat: morphological and immunohistological findings in animals treated with topical timolol maleate. Ophthalmologica **214**: 136–139.

Ristori C, Filippi L, Dal MonteM et al. (2011): Role of the adrenergic system in a mouse model of oxygen-induced retinopathy: antiangiogenic effects of beta-adrenoreceptor blockade. Invest Ophthalmol Vis Sci **52**: 155–170.

Robinson GS, Pierce EA, Rook SL et al. (1996): Oligodeoxynucleotides inhibit retinal neovascularization in a murine model of proliferative retinopathy. Proc Natl Acad Sci USA **93**: 4851–4856.

Romagnoli C, Zecca E, Gallini F et al. (2000): Do recombinant human erythropoietin and iron supplementation increase the risk of retinopathy of prematurity? Eur J Pediatr **159**: 627–628.

Rusai K, Vannay A, Szebeni B et al. (2008): Endothelial nitric oxide synthase gene T-786C and 27-bp repeat gene polymorphisms in retinopathy of prematurity. Mol Vis **14**: 286–290.

Saint-Geniez M, Masri B, Malecaze F et al. (2002): Expression of the murine msr/apj receptor and its ligand apelin is upregulated during formation of the retinal vessels. Mech Dev **110**: 183–186.

Sakanaka M, Wen TC, Matsuda S et al. (1998): In vivo evidence that erythropoietin protects neurons from ischemic damage. Proc Natl Acad Sci USA **95**: 4635–4640.

Samdani AF, Dawson TM & Dawson VL (1997): Nitric oxide synthase in models of focal ischemia. Stroke **28**: 1283–1288.

Sans V, Dumas delaRoqueE, Berge J et al. (2009): Propranolol for Severe Infantile Hemangiomas: Follow-Up Report. Pediatrics **124**: e423–e431.

Sato T, Wada K, Arahori H et al. (2012): Serum concentrations of bevacizumab (avastin) and vascular endothelial growth factor in infants with retinopathy of prematurity. Am J Ophthalmol **153**: 327–333.

Saunders RA, Donahue ML, Christmann LM et al. (1997): Racial variation in retinopathy of prematurity. Cryotherapy for Retinopathy of Prematurity Cooperative Group. Arch Ophthalmol **115**: 604–608.

Sautina L, Sautin Y, Beem E et al. (2010): Induction of nitric oxide by erythropoietin is mediated by the {beta} common receptor and requires interaction with VEGF receptor 2. Blood **115**: 896–905.

Sawyer ST, Krantz SB & Sawada KI (1989): Receptors for erythropoietin in mouse and human erythroid cells and placenta. Blood **74**: 103–109.

Schimanski LA, Ali DW, Baker GB et al. (2007): Impaired hippocampal LTP in inbred mouse strains can be rescued by beta-adrenergic receptor activation. Eur J Neurosci **25**: 1589–1598.

Schlingemann RO (2004): Role of growth factors and the wound healing response in age-related macular degeneration. Graefes Arch Clin Exp Ophthalmol **242**: 91–101.

Schmetterer L & Polak K (2001): Role of nitric oxide in the control of ocular blood flow. Prog Retin Eye Res **20**: 823–847.

Schmitt RM, Bruyns E & Snodgrass HR (1991): Hematopoietic development of embryonic stem cells in vitro: cytokine and receptor gene expression. Genes Dev **5**: 728–740.

Sears JE, Peitz J, Sonnie C et al. (2009): Change in oxygen supplementation can decrease the incidence of retinopathy of prematurity. Ophthalmology **116**: 513–518.

Seligsohn EE & Bill A (1993): Effects of NG-nitro-l-arginine methyl ester on the cardiovascular response to thyrotropin-releasing hormone. Br J Pharmacol **109**: 1219–1225.

Seya Y, Fukuda T, Isobe K et al. (2006): Effect of norepinephrine on RhoA, MAP kinase, proliferation and VEGF expression in human umbilical vein endothelial cells Eur. J Pharmacol **553**: 54–60.

Shah N, Jadav P, Jean-Baptiste D et al. (2010): The effect of recombinant human erythropoietin on the development of retinopathy of prematurity. Am J Perinatol **27**: 67–71.

Shalaby F, Rossant J, Yamaguchi TP et al. (1995): Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature **376**: 62–66.

Shannon KM, Mentzer WC, Abels RI et al. (1991): Recombinant human erythropoietin in the anemia of prematurity: results of a placebo-controlled pilot study. J Pediatr **118**: 949–955.

Shareef S, Sawada A & Neufeld AH (1999): Isoforms of nitric oxide synthase in the optic nerves of rat eyes with chronic moderately elevated intraocular pressure. Invest Ophthalmol Vis Sci **40**: 2884–2891.

Shastry BS (2010): Genetic susceptibility to advanced retinopathy of prematurity (ROP). J Biomed Sci **17**: 69.

Shastry BS, Pedergrast SD, Hartzer MK et al. (1997): Identification of missense mutations in the Norrie disease gene associated with advanced retinopathy of prematurity. Arch Ophthal **115**: 651–655.

Shih SC, Ju M, Liu N et al. (2003): Selective stimulation of VEGFR-1 prevents oxygen-induced retinal vascular degeneration in retinopathy of prematurity. J Clin Invest **112**: 50–57.

Shyu KG, Liou JY, Wang BW et al. (2005): Carvedilol prevents cardiac hypertrophy and overexpression of hypoxia-inducible factor-1alpha and vascular endothelial growth factor in pressure-overloaded rat heart. J Biomed Sci **12**: 409–420.

Simpson DA, Murphy GM, Bhaduri T et al. (1999): Expression of VEGF gene family during retinal vaso-obliteration and hypoxia. Biochem Biophys Res Commun **262**: 333–340.

Siren AL, Fratelli M, Brines M et al. (2001): Erythropoietin prevents neuronal apoptosis after cerebral ischemia and metabolic stress. Proc Natl Acad Sci USA **98**: 4044–4049.

Smith LE (2004): Pathogenesis of retinopathy of prematurity. Growth Horm IGF Res **14**: S140–S144.

Smith LE, Shen W, Perruzzi C et al. (1999): Regulation of vascular endothelial growth factor-dependent retinal neovascularization by insulin-like growth factor-1 receptor. Nat Med **5**: 1390–1395.

Smith CP, Sharma S & Steinle JJ (2007): Age-related changes in sympathetic neurotransmission in rat retina and choroid. Exp Eye Res **84**: 75–81.

Sorli SC, Le GonidecS, Knibiehler B et al. (2007): Apelin is a potent activator of tumour neoangiogenesis. Oncogene **26**: 7692–7699.

Spandau U, Tomic Z, Ewald U et al. (2012): Time to consider a new treatment protocol for aggressive posterior retinopathy of prematurity? Acta Ophthalmol **91**: 170–175.

Stahl A, Connor KM, Sapieha P et al. (2010): The mouse retina as an angiogenesis model. Invest Ophthalmol Vis Sci **51**: 2813–2826.

Stefanovic V, Vlahovic P, Savic V et al. (1993): Adenosine stimulates 50-nucleotidase activity in rat mesangial cells via A2 receptor. FEBS Lett **331**: 96–100.

Steinle JJ & Smith PG (2002): Role of adrenergic receptors in vascular remodelling of the rat choroid. Br J Pharmacol **136**: 730–734.

Steinle JJ, Pierce JD, Clancy RL et al. (2002): Increased ocular blood vessel numbers and sizes following chronic sympathectomy in rat. Exp Eye Res **74**: 761–768.

Steinle JJ, Booz GW, Meininger CJ et al. (2003): Beta 3-adrenergic receptors regulate retinal endothelial cell migration and proliferation. J Biol Chem **278**: 20681–20686.

Steinle JJ, Zamora DO, Rosenbaum JT et al. (2005): Beta 3-adrenergic receptors mediate choroidal endothelial cell invasion, proliferation, and cell elongation. Exp Eye Res **80**: 83–91.

Steinle JJ, Cappocia FC Jr & Jiang Y (2008): Beta-adrenergic receptor regulation of growth factor protein levels in human choroidal endothelial cells. Growth Factors **26**: 325–330.

Stone J & Maslim J (1997): Mechanisms of Retinal Angiogenesis. Prog Retinal Eye Res **16**: 157–181.

Storch CH & Hoeger PH (2010): Propranolol for infantile haemangiomas: insights into the molecular mechanisms of action Br. J Dermatol **163**: 269–274.

Stuehr DJ (1999): Mammalian nitric oxide synthases. Biochim Biophys Acta **1411**: 217–230.

Su KH, Shyue SK, Kou YR et al. (2011): β common receptor integrates the erythropoietin signaling in activation of endothelial nitric oxide synthase. J Cell Physiol **226**: 3330–3339.

Suk KK, Dunbar JA, Liu A et al. (2008): Human recombinant erythropoietin and the incidence of retinopathy of prematurity: a multiple regression model. J AAPOS **12**: 233–238.

Sullivan JL (1988): Iron, plasma antioxidants, and the ''oxygen radical disease of prematurity''. Am J Dis Child **142**: 1341–1344.

SUPPORT Study Group of the Eunice Kennedy Shriver NICHD Neonatal Research Network (2010): Target ranges of oxygen saturation in extremely preterm infants. N Engl J Med **362**: 1959–1969.

Tabrizchi R & Bedi S (2001): Pharmacology of adenosine receptors in the vasculature. Pharmacol Ther **91**: 133–147.

Takagi H, King GL, Ferrara N et al. (1996a): Hypoxia regulates vascular endothelial growth factor receptor KDR/Flk gene expression through adenosine A2 receptors in retinal capillary endothelial cells. Invest Ophthalmol Vis Sci **37**: 1311–1321.

Takagi H, King GL, Robinson GS et al. (1996b): Adenosine mediates hypoxic induction of vascular endothelial growth factor in retinal pericytes and endothelial cells. Invest Ophthalmol Vis Sci **37**: 2165–2176.

Talks KL & Harris AL (2000): Current status of antiangiogenic factors. Br J Haematol **109**: 477–489.

Tao Y, Lu Q, Jiang YR et al. (2010): Apelin in plasma and vitreous and in fibrovascular retinal membranes of patients with proliferative diabetic retinopathy. Invest Ophthalmol Vis Sci **51**: 4237–4242.

Taomoto M, McLeod DS, Merges C et al. (2000): Localization of adenosine A2a receptor in retinal development and oxygen-induced retinopathy. Invest Ophthalmol Vis Sci **41**: 230–243.

Tatemoto K, Hosoya M, Habata Y et al. (1998): Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor, Biochem. Biophys Res Commun **251**: 471–476.

Tatemoto K, Takayama K, Zou MX et al. (2001): The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism. Regul Pept **99**: 87–92.

Terman BI, Dougher-Vermazen M, Carrion ME et al. (1992): Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor. Biochem Biophys Res Commun **187**: 1579–1586.

Teuscher E & Weidlich V (1985): Adenosine nucleotides, adenosine and adenine as angiogenesis factors. Biomed Biochim Acta **44**: 493–495.

Tin W, Milligan DW, Pennefather P et al. (2001): Pulse oximetry, severe retinopathy, and outcome at one year in babies of less than 28 weeks gestation. Arch Dis Child Fetal Neonatol Ed **84**: F106–F110.

Tlucek PS, Corff KE, Bright BC et al. (2010): Effect of decreasing target oxygen saturation on retinopathy of prematurity. J AAPOS **14**: 406–411.

Toda N & Nakanishi-Toda M (2007): Nitric oxide: ocular blood flow, glaucoma, and diabetic retinopathy. Prog Retin Eye Res **26**: 205–238.

Tokuhiro Y, Yoshida T, Nakabayashi Y et al. (2009): Reduced oxygen protocol decreases the incidence of threshold retinopathy of prematurity in infants\33 weeks gestation. Pediatr Int **51**: 804–806.

Turker G, Sarper N, Gokalp AS et al. (2005): The effect of early recombinant erythropoietin and enteral iron supplementation on blood transfusion in preterm infants. Am J Perinatol **22**: 449–455.

Uematsu M, Ohara Y, Navas JP et al. (1995): Regulation of endothelial cell nitric oxide synthase mRNA expression by shear stress. Am J Physiol **269**: C1371–C1378.

VanderVeen DK, Mansfield TA & Eichenwald EC (2006): Lower oxygen saturation alarm limits decrease the severity of retinopathy of prematurity. J AAPOS **10**: 445–448.

Vanhaesebrouck S, Daniëls H, Moons L et al. (2009): Oxygen-induced retinopathy in mice: amplification by neonatal IGF-I deficit and attenuation by IGF-I administration. Pediatr Res **65**: 307–310.

Vermont Oxford Network Database, Nightingale Internet Web site. Available at: https://nightingale.vtoxford.org Accessed September 19, 2011.

Vrydag W & Michel MC (2007): Tools to study beta3-adrenoceptors. Naunyn Schmiedebergs Arch Pharmacol **374**: 385–398.

Walker RJ & Steinle JJ (2007): Role of beta-adrenergic receptors in inflammatory marker expression in Muller cells. Invest Ophthalmol Vis Sci **48**: 5276–5281.

Wallace DK, Veness-Meehan KA & Miller WC (2007): Incidence of severe retinopathy of prematurity before and after a modest reduction in target oxygen saturation levels. J AAPOS **11**: 170–174.

Waltenberger J, Claesson-Welsh L, Siegbahn A et al. (1994): Different signal transduction properties of KDR and Fltl, two receptors for vascular endothelial growth factor. J Biol Chem **269**: 26988–26995.

Watanabe D, Suzuma K, Matsui S et al. (2005): Erythropoietin as a retinal angiogenic factor in proliferative diabetic retinopathy. N Engl J Med **353**: 782–792.

Watts KD & McColley SA (2011): Elevated vascular endothelial growth factor is correlated with elevated erythropoietin in stable, young cystic fibrosis patients. Pediatr Pulmonol **46**: 683–687.

Weil J, Benndorf R, Fredersdorf S et al. (2003): Norepinephrine upregulates vascular endothelial growth factor in rat cardiac myocytes by a paracrine mechanism. Angiogenesis **6**: 303–309.

Westenbrink BD, Lipsic E, van derMeerP et al. (2007): Erythropoietin improves cardiac function through endothelial progenitor cell and vascular endothelial growth factor mediated neovascularization. Eur Heart J **28**: 2018–2027.

Wilson-Costello D, Friedman H, Minich N et al. (2007): Improved neurodevelopmental outcomes for extremely low birth weight infants in 2000–2002. Pediatrics **119**: 37–45.

Wiwatwongwana A, Kersey JP & Gardiner JA (2010): The effect of changing oxygen saturation protocols on the incidence of laser treatment for retinopathy of prematurity. Can J Ophthalmol **45**: 585–589.

Wood SM, Gleadle JM, Pugh CW et al. (1996): The role of the aryl hydrocarbon receptor nuclear translocator (ARNT) in hypoxic induction of gene expression. Studies in ARNT-deficient cells. J Biol Chem **271**: 15117–15123.

Wood NS, Marlow N, Costeloe K et al. (2000): Neurologic and developmental disability after extremely preterm birth. N Engl J Med **343**: 378–384.

Wright KW, Sami D, Thompson L et al. (2006): A physiologic reduced oxygen pro-

tocol decreases the incidence of threshold retinopathy of prematurity. Trans Am Ophthalmol Soc **104**: 78–84.

Wu HM, Huang Q, Yuan Y et al. (1996): VEGF induces NO-dependent hyperpermeability in coronary venules. Am J Physiol **271**: H2735–H2739.

Wu WC, Yeh PT, Chen SN et al. (2011): Effects and complications of bevacizumab use in patients with retinopathy of prematurity: a multicenter study in Taiwan. Ophthalmology **118**: 176–183.

Wurm A, Iandiev I, Hollborn M et al. (2008): Purinergic receptor activation inhibits osmotic glial cell swelling in the diabetic rat retina. Exp Eye Res **87**: 385–393.

Xia Y, Choi HK & Lee K (2012): Recent advances in hypoxia-inducible factor (HIF)-1 inhibitors. Eur J Med Chem **49**: 24–40.

Yamaji R, Okada T, Moriya M et al. (1996): Brain capillary endothelial cells express two forms of erythropoietin receptor mRNA. Eur J Biochem **239**: 494–500.

Yamamoto R, Bredt DS, Dawson TM et al. (1993): Enhanced expression of nitric oxide synthase by rat retina following pterygopalatine parasympathetic denervation. Brain Res **631**: 83–88.

Yamashita H, Eguchi S, Watanabe K et al. (1999): Expression of placenta growth factor (PIGF) in ischaemic retinal diseases. Eye **13**: 372–374.

Yanamandra K, Napper D, Pramanik A et al. (2010): Endothelial nitric oxide synthase genotypes in the etiology of retinopathy of prematurity in premature infants. Ophthalmic Genet **31**: 173–177.

Yang EV, Kim SJ, Donovan EL et al. (2009): Norepinephrine upregulates VEGF, IL-8, and IL-6 expression in human melanoma tumor cell lines: implications for stress-related enhancement of tumor progression. Brain Behav Immun **23**: 267–275.

Yonekura H, Sakurai S, Liu X et al. (1999): Placenta growth factor and vascular endothelial growth factor B and C expression in microvascular endothelial cells and pericytes. Implication in autocrine and paracrine regulation of angiogenesis. J Biol Chem **274**: 35172–35178.

Yuan Y, Granger HJ, Zawieja DC et al. (1993): Histamine increases venular permeability via a phospholipase C-NO synthase-guanylate cyclase cascade. Am J Physiol **264**: H1734–H1739.

Zanjani ED, Poster J, Burlington H et al. (1977): Liver as the primary site of erythropoietin formation in the fetus. J Lab Clin Med **89**: 640–644.

Zhao Z, Francis CE & Ravid K (1997): An A3-subtype receptor is highly expressed in rat smooth muscle cells: Its role in attenuating adenosine-induced increase in camp. Microvasc Res **54**: 243–252.

Zhao Z, Makaritsis K, Francis CE et al. (2000): A role for the A3 adenosine receptor in determining tissue levels of cAMP and blood pressure: Studies in knockout mice. Biochem Biophys Acta **1500**: 280–290.

Zhao B, Cai J & Boulton M (2003): Expression of placenta growth factor is regulated by both VEGF and hyperglycaemia via VEGFR-2. Microvasc Res **68**: 239–246.

Zheng Z, Chen H, Xu X et al. (2007): Effects of angiotensin-converting enzyme inhibitors and beta-adrenergic blockers on retinal vascular endothelial growth factor expression in rat diabetic retinopathy. Exp Eye Res **84**: 745–752.

Zhou N, Fan X, Mukhtar M et al. (2003): Cell–cell fusion and internalization of the CNS-based, HIV-1 co-receptor, APJ. Virology **307**: 22–36.

Ziche M, Morbidelli L, Masini E et al. (1993): Nitric oxide promotes DNA synthesis and cyclic GMP formation in endothelial cells from postcapillary venules. Biochem Biophys Res Commun **92**: 1198–1203.

Ziche M, Parenti A, Ledda F et al. (1997): Nitric oxide promotes proliferation and plasminogen activator production by coronary venular endothelium through endogenous bFGF. Circ Res **80**: 845–852.

Received on December 17th, 2011.
Accepted on October 27th, 2012.

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