Abstract
Pathological angiogenesis is a key feature of many diseases including retinopathies such as ROP (retinopathy of prematurity) and DR (diabetic retinopathy). There is considerable evidence that increased production of ROS (reactive oxygen species) in the retina participates in retinal angiogenesis, although the mechanisms by which this occurs are not fully understood. ROS is produced by a number of pathways, including the mitochondrial electron transport chain, cytochrome P450, xanthine oxidase and uncoupled nitric oxide synthase. The family of NADPH oxidase (Nox) enzymes are likely to be important given that their primary function is to produce ROS. Seven isoforms of Nox have been identified named Nox1–5, Duox (dual oxidase) 1 and Duox2. Nox1, Nox2 and Nox4 have been most extensively studied and are implicated in the development of conditions such as hypertension, cardiovascular disease and diabetic nephropathy. In recent years, evidence has accumulated to suggest that Nox1, Nox2 and Nox4 participate in pathological angiogenesis; however, there is no clear consensus about which Nox isofrom is primarily responsible. In terms of retinopathy, there is growing evidence that Nox contribute to vascular injury. The RAAS (renin–angiotensin–aldosterone system), and particularly AngII (angiotensin II), is a key stimulator of Nox. It is known that a local RAAS exists in the retina and that blockade of AngII and aldosterone attenuate pathological angiogenesis in the retina. Whether the RAAS influences the production of ROS derived from Nox in retinopathy is yet to be fully determined. These topics will be reviewed with a particular emphasis on ROP and DR.

Key words: aldosterone, diabetic retinopathy, NADPH oxidase, oxidative stress, renin-angiotensin-aldosterone system (RAAS), retinopathy of prematurity

INTRODUCTION
Pathological angiogenesis is a hallmark feature of ROP (retinopathy of prematurity) and DR (diabetic retinopathy) and is a major contributor to vision loss due to breakdown of the blood–retinal barrier, which can result in haemorrhage, oedema and retinal detachment. A number of factors are implicated in the development of pathological retinal angiogenesis, including the up-regulation of angiogenic factors such as VEGF (vascular endothelial growth factor) [1–3], angiopoietin [4–7], erythropoietin [8–11] and SDF-1 (stromal-derived factor-1) [12,13]. Other agents are also involved and include the RAAS (renin–angiotensin–aldosterone system) [14–18], invading and resident inflammatory cells [19–27], IGF (insulin-like growth factor) [28,29], omega-3 fatty acids [30], and activation of PKC (protein kinase C) [31,32]. In DR, increased flux through the polyol pathway [33,34] and production of AGEs (advanced glycation end-products) participate [18,35–37].

A common underlying mechanism in many of these pathways is the increased production of ROS (reactive oxygen species), which, through the stabilization of the transcription factor HIF (hypoxia-inducible factor)-1, leads to the increased expression of angiogenic and inflammatory mediators (reviewed in [38,39]). The increased production of ROS and subsequent pathological angiogenesis is documented in retinopathy [40–47], and antioxidant treatments, such as vitamin C and vitamin E, reduce vascular injury in experimental models of ROP [48,49] and DR [50,51]. Although these interventions have not necessarily been as
successful in individuals with ROP [52,53] or DR [54,55], there has been interest in the utility of antioxidant therapy for the treatment of a variety of diseases. This is based on expanding knowledge about NADPH oxidase (Nox) as a major source of ROS and the role of this family of enzymes in angiogenesis. A growing body of evidence indicates that specific isoforms of Nox promote endothelial cell migration and tubulogenesis in vitro [56–61] and angiogenesis in vivo [61–66]. Attention has also been given to the role of the RAAS in Nox-mediated cell damage [67–72], which may be relevant to pathological angiogenesis in ROP and DR, given the established role of the RAAS in both these conditions (reviewed [14]).

**PATHOGENESIS OF ROP**

**Incidence of ROP in children**

ROP was first described in the 1940s and 1950s as the survival rate of pre-term infants increased in industrialized countries [73]. The unmonitored use of supplemental oxygen was considered the main causal factor in the development of ROP [74]. In the 1950s, the restriction of high oxygen levels to pre-term infants resulted in a decline in the incidence of ROP. However, as subsequent advances in the care of pre-term babies have occurred, smaller and younger infants are surviving in both industrialized and low-to-middle income countries, and the incidence of ROP has increased [75,76]. It is estimated that the overall incidence rate of ROP is approximately 68% among infants born less than 1251 g in weight and 98% among infants born with a birth weight less than 750 g [75]. The main consequence for some infants with ROP is damage to the retinal microvasculature, which may lead to pathological angiogenesis, vascular leakage and retinal detachment, resulting in ROP being a major cause of vision loss and blindness in infants. ROP is also associated with complications, including retinal fold, dragging of the macula, glaucoma, cataract and strabismus [77,78]. ROP is a life-long disease and represents a significant health burden with severe consequences for education, employment, earning potential and socio-economic development. Alarminglly, the World Health Organization has identified ROP as the leading cause of vision impairment in children of the developing world [79,80].

**Pathological angiogenesis in children with ROP**

In humans, the retinal vasculature normally develops in utero and is guided by contributions from astrocytes, microglia and contractile cells (pericytes/smooth muscle cells), and VEGF [81]. Much of what is known about the retinal vasculature in development and ROP comes from animal studies [3,82,83]. In the developing retina, the hypoxic tissue environment is viewed to be the main driver for VEGF-mediated physiological angiogenesis [82]. When the retinal vasculature is established, there is an increase in retinal oxygen levels, which results in a down-regulation of VEGF expression. ROP is a condition that can occur in some pre-term infants due to a changing oxygen environment in the retina, which is initiated by exposure to hyperoxia to assist respiration. Studies in animal models of ROP have demonstrated that hyperoxia causes the down-regulation of retinal VEGF and cessation of normal vascular growth, resulting in areas of vaso-obliteration [3,83]. The subsequent exposure to room air causes the non-vascularized retina to become increasingly metabolically active and hypoxic. This stimulates retinal VEGF production and the growth of abnormally formed blood vessels into the vitreous cavity, which may haemorrhage [2,84]. VEGF is likely to be critical for pathological angiogenesis in children with ROP, as increased levels of VEGF mRNA in retina have been detected in advanced ROP [85], and the level of retinal VEGF gene expression increases with the extent of vascularity in ROP [13]. Vision loss in children with ROP is principally due to traction arising from the formation of a fibrovascular scar, which has the potential to detach the retina from the wall of the eye. ROP is comprised of five progressive stages based on the extent of pathological retinal angiogenesis and retinal detachment [86]. According to the ICROP (International Classification of ROP), these stages are: Stage 1, demarcation line; Stage 2, ridge; Stage 3, extraretinal fibrovascular proliferation; Stage 4, partial retinal detachment; Stage 5, total retinal detachment [87].

**ROP in animal models**

Our knowledge about the pathogenesis of ROP has been greatly assisted by animal models of the condition, with numerous studies performed in mice and rats [8,28,30,88,89]. There are a few differences between ROP in children and ROP in animals. In animals, normal vascularization of the retina occurs post-natally rather than pre-natally as in humans, resulting in ROP being induced in animals in the few weeks after birth. In animals, ROP develops over two phases rather than five successive stages as in children with ROP. Phase I comprises exposure to hyperoxia and down-regulation of VEGF. The degree of hyperoxia is significantly higher in humans and is associated with vaso-obliteration in the central retina in mice and the peripheral retina in rats and humans [86]. Phase II of ROP involves exposure of animals to room air, and a hypoxic-induced pathological angiogenesis occurs which is similar in nature to that in children with ROP: however, a fibrous lesion does not develop. Despite these differences between ROP in humans and animals, ROP models in animals have provided a wealth of information about the contribution of VEGF and a variety of factors to pathological retinal angiogenesis, including the participation of oxidative stress.

Studies in animal models of ROP have revealed that, in normal physiological angiogenesis of the retina, astrocytes are a major source of VEGF and provide a template for developmental vascularization of the retina [90], although recent evidence suggests that retinal neurons also participate [91]. This also occurs in phase II of ROP, where, following astrocyte apoptosis, ganglion cells and macroglial Müller cells are the major sites of increased VEGF production [90,92–94]. Hence, in this regard, vasculopathy in ROP comprises a complex interplay between vascular, neuronal and glial cell components. Other angiogenic factors also contribute to pathological angiogenesis in ROP [8–13]. Furthermore, inflammation is involved in the vascular injury of ROP based on findings that inflammatory cytokines and mediators are up-regulated in the retina and that leucocyte adhesion occurs [22,27,95]. Migrating microglia and activation of resident...
microglia may also contribute to this pro-inflammatory proliferation and angiogenesis [23,96,97].

Treatments for ROP in children

Current treatment strategies for ROP aim to either prevent the development of retinal angiogenesis or ablate it when present. The standard approaches are (i) tight control of oxygen saturation to help prevent abnormal retinal neovascularization, and (ii) timely laser photocoagulation to cause regression of abnormal vessels. Obviously, oxygen restriction needs to be carefully monitored to prevent negative effects on other developing systems [98] and, unfortunately, laser surgery does not completely reduce the incidence of vision impairment. As aberrant levels of VEGF are considered to be critical to the vaso-obliteration and pathological angiogenesis that occurs in ROP [1,86], therapies that inhibit VEGF (e.g. avastin) have gained attention as a possible treatment and have been reported to have benefits where laser treatment has failed [99]. However, as VEGF is also a retinal neuroprotective factor [100–102] and stimulates normal vascularization, there is some concern that anti-VEGF therapies may interfere with these processes. Furthermore, given the enhanced permeability of the retinal circulation in ROP, it is possible that anti-VEGF therapies may reach other organs and have negative effects [101]. Until these issues are resolved, it is unclear whether anti-VEGF strategies are suitable as a first-line treatment for ROP and therefore alternative approaches are required.

PATHOGENESIS OF DR

Incidence and vascular abnormalities in individuals with DR

DR is the major cause of vision loss and blindness in people of working age and develops over approximately 10 to 25 years. During the first two decades of disease, nearly all individuals with Type 1 diabetes and approximately 60% of individuals with Type 2 diabetes will have some degree of retinopathy. DR is largely a disease of the retinal microvasculature [103], although damage to neurons and glia also occurs [104]. DR is a progressive disease that is classified into either non-proliferative DR or proliferative DR based on the extent of microvascular damage. Non-proliferative DR is comprised of three successive stages of mild, moderate and severe DR, with the latter also known as pre-proliferative DR. The clinically visible vascular lesions that develop in non-proliferative DR include cotton wool spots, venous beading and loops, blood vessel closure, tissue ischaemia and the formation of intra-retinal microvascular abnormalities. Proliferative DR features the most serious microvascular damage of pathological angiogenesis [103]. Vision loss in DR principally occurs from breakdown of the blood–retinal barrier, resulting in macular oedema, tractional retinal detachment and inner retinal and vitreous haemorrhage. Similar to ROP, the pathological angiogenesis and vascular leakage that develops in DR is to a large extent driven by VEGF, which is sourced from retinal ganglion cells, glia and retinal vascular pericytes [105–107]. Inflammatory mediators are also implicated in the development of microvascular damage in DR (reviewed in [108]).

A recent study has highlighted the global prevalence of DR as being 34.5% for DR (approximately 93 million people), 6.96% for proliferative DR (approximately 17 million people), 6.81% for diabetic macular oedema (approximately 21 million people) and 10.2% for vision-threatening DR (approximately 28 million people) [109]. The main risk factors for the development of DR are the duration of diabetes, poor glycaemic control and hypertension [110–112].

DR in animal models

As for ROP, animal models have provided important insights into the mechanisms involved in the pathogenesis of DR. There are, however, some limitations of studying these models which have been described in detail by others [113]. Perhaps most relevant to the topic of the present review is that mouse and rat models do not progress to the proliferative angiogenic stage of DR even after years of diabetes [113]. Nevertheless, they do develop important structural and functional changes that are similar to these which occurs in individuals with DR, including vascular leakage, inflammation, pericyte loss, acellular capillaries, neuronal and glial cell damage, and a decline in retinal function as measured by the electroretinogram [113].

To evaluate diabetic pathological angiogenesis in the retina, researchers often make correlates with animal models of ROP. Pathological retinal angiogenesis in ROP and DR does have common underlying mechanisms, such as the hypoxic tissue environment that induces the up-regulation of oxidative stress and inflammatory angiogenic factors (Figure 1). However, some metabolic influences may be only relevant to DR, such as the role of the AGEs, which largely form in a hyperglycaemic environment or as a consequence of aging and environmental stimuli [18,35–37,114]. ROP and DR also differ in the nature of the pathological angiogenesis that develops, with ROP featuring disturbances to a developing vasculature, whereas, in DR, the retinal vasculature is established.

Treatments for individuals with DR

Despite intensive research, treatments for DR are limited. As for ROP, the main treatment for DR is laser photocoagulation, which is applied at the most advanced stage of the disease, proliferative DR, in order to remove abnormal blood vessels [115]. Although photocoagulation can retard the progression of vision loss, it is associated with complications such as reduced central vision, alterations in colour vision, and, in some cases, the stimulation of pathological angiogenesis in the posterior part of the eye [115–117]. Unfortunately, proliferative DR often advances despite intensive photocoagulation. New treatments are now sought that prevent the progression of DR and target both retinal vasculopathy and neuroglial injury. Approaches such as anti-VEGF therapies are being trailed for the treatment of macula oedema and proliferative DR, and may provide some benefits in these situations [118,119]. However, they do not target the early stages of DR and hence do not prevent disease progression, and, as described for ROP, may be associated with potential
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Figure 1  Proposed events that lead to ROS derived from Noxes being involved in pathological angiogenesis and inflammation in ROP and DR

Tissue hypoxia/hyperglycaemia is viewed to increase the stabilization of HIF-1α, with ROS participating in this process [157]. The source of ROS is most likely to be mitochondria [38,39,157], although it is unclear whether Nox could also be involved (green arrow). Activation of HIF-1α leads to the increased production of angiogenic and inflammatory genes such as VEGF, erythropoietin and SDF-1 (stromal-cell-derived factor-1) resulting in pathological angiogenesis and vascular leakage. VEGF may amplify the entire system with interactions between VEGFR2 and Nox, leading to further production of ROS and stabilization and activation of HIF-1α (green arrow) [65,171]. Other amplifiers include the ROS induction of NF-κB, which in turn leads to the production of TNFα and subsequent generation of inflammatory mediators such as IL-6, MCP-1 and ICAM-1. Cross-talk between NF-κB and HIF-1α can also occur [173,174]. Other feedback mechanisms are possible, but are not shown here. AngII and aldosterone may participate in the development of ROP and DR by the induction of Nox and subsequent generation of ROS. The signalling mechanisms by which this occurs in the retina are not defined.

Ocular and systemic risks, including intraocular inflammation and stroke [120,121].

OXIDATIVE STRESS

Although a number of factors contribute to the development of ROP and DR, it is likely that oxidative stress is an important underlying mechanism. ROS are integral as signalling molecules and are produced continuously in cells to support normal cellular functions such as proliferation and migration [122]; however, the excess production of ROS can result in cell injury. ROS are highly reactive molecules due to the presence of unpaired valence shell electrons and include O₂⁻ (superoxide anion) and H₂O₂. O₂⁻ is produced during oxygen metabolism and can directly damage DNA through oxidation, inactivate antioxidants, such as glutathione peroxidase, and activate the pro-inflammatory agent NF-κB (nuclear factor κB) [123]. O₂⁻ can be rapidly reduced to H₂O₂, which, due to its lipid-soluble properties, can diffuse through membranes to exert widespread actions [124]. H₂O₂ modifies cellular proteins, lipids and RNA and DNA, and can be reduced by transition metals to form the highly reactive OH⁻ (hydroxide anion), which oxidizes DNA nucleotides and lipids (reviewed in [125]). O₂⁻ may also react with NO to rapidly form OHOO⁻ (peroxynitrite), which influences the properties of a variety of proteins including iNOS (inducible NOS (NO synthase)) and eNOS (endothelial NOS) [126]. In the vasculature, ROS are involved in a number of pathological processes, including vascular hypertrophy and remodelling, hypertension and inflammation. ROS influence these events via signalling pathways including MAPKs (mitogen-activated protein kinases), tyrosine kinases, Rho kinase, transcription factors [NF-κB, AP-1 (activator protein-1) and HIF-1], pro-inflammatory genes, protein tyrosine phosphatases and increases in intracellular free calcium [127–130].

The main sources of intracellular ROS are the mitochondrial electron transport chain, cytchrome P450, xanthine oxidase, uncoupled NOS and Nox [131–134]. ROS levels in tissues may also be elevated by the compromised activity of antioxidant systems, such as reduced glutathione, haem oxygenase, thioredoxin peroxidase, vitamin E, SOD (superoxide dismutase) and catalase [131]. The retina is particularly susceptible to oxidative stress because of its high consumption of oxygen and glucose oxidation,
high levels of polyunsaturated fatty acids and exposure to light [135]. Indeed the oxygen consumption of the human retina has been estimated to be 50% higher than kidney, 300% higher than cerebral cortex and 600% higher than cardiac muscle [136].

Oxidative stress and ROP in children
The premature infant with ROP is prone to ROS-mediated damage due to an inadequate ability to scavenge ROS with reduced levels of antioxidants such as vitamins C and E, Cu,Zn-SOD (copper/zinc SOD), metallothionein, haem oxygenase and catalase [19,48,137,138]. Furthermore, the hyperoxic and subsequent hypoxic environment of the retina in ROP is viewed to lead to abnormal levels of tissue ROS. The effects of antioxidant treatments have been evaluated in children with ROP, although with varying success. For example, recombinant human Cu,Zn-SOD reduced the risk of developing ROP in infants participating in the ELGAN (Extremely Low Gestational Age Newborns) trial [139]. A meta-analysis of six randomized trials administering vitamin E to pre-term infants concluded that there was a 50% reduction in severe ROP. However, the dose of vitamin E needed to protect infants from severe ROP was associated with a significant risk of infection, which precluded the widespread use of this therapy [52,53].

Oxidative stress and ROP in animal models
A causal role for elevated oxidative stress in ROP has clearly been established in both phase I and phase II of animal models of the condition. In the hyperoxic phase I of ROP, elevated levels of O$_2^-$, NO and OHOO$^-$ have been reported [40,41]. These molecules are thought to play a key role in the apoptosis of capillary endothelial cells [140–142] by reducing the pro-survival signals of VEGF, although the mechanisms by which this occurs are not fully understood [1,142]. A role for ROS in this pathology has been demonstrated in studies where the intravitreal administration of liposome-encapsulated SOD [143] and systemically administered Trolox (a water-soluble analogue of vitamin E) [49] reduced capillary degeneration. In the hypoxic phase II of ROP, an elevation in ROS occurs and contributes to pathological angiogenesis in the retina [42]. Antioxidant therapy provides protection against this ROS-mediated retinal vascular damage [141,144]. For instance, inhibition of tyrosine nitration with the green tea extract epicatechin, or blockade of OHOO$^-$ formation with N-acetylcysteine, decreased both vaso-oblitervation and pathological angiogenesis in ROP [144]. Of interest is recent work by Okuno et al. [43], which suggests that, although the ROS that accumulates in new blood vessels in ROP most likely contributes to pathological angiogenesis, excessive amounts of ROS may have suppressive effects. These authors identified ATM (ataxia telangiectasia mutated) kinase, a master regulator of the DNA damage response that also has functions in oxidative defence, to play a role in this suppressive effect on retinal angiogenesis.

Sources of increased oxidative stress in patients with DR and animal models of DR
In DR, increased retinal ROS can be generated by a number of mechanisms, including the hyperglycaemic induction of glucose auto-oxidation, the polyol pathway and activation of PKC isoforms (reviewed in [50,135,145]). Furthermore, AGEs, which are formed by the non-enzymatic reaction of glucose and other glycating compounds with proteins, are known to play a causal role in DR [18,37,114,146], which is linked to ROS. As in ROP, ROS levels such as O$_2^-$ and H$_2$O$_2$ are elevated in animal models of DR [44–47]. Inhibition of AGEs and the administration of antioxidant supplements, such as vitamin C and vitamin E, are effective in reducing retinal microvascular damage in experimental DR [50,51,147]. However, these micronutrient strategies have not had widespread benefits in patients with DR [54,55]. Overall, in both ROP and DR, it is difficult to determine which ROS-generating pathway makes the major contribution to retinal vascular and neuro-glial damage, and it is likely that many of the pathways interact to exacerbate cell injury.

NADPH Oxidase (NOX) and ROS
Nox enzymes are likely to play an important role in the development of ROP and DR given that they are haem-containing multi-subunit transmembrane proteins whose primary function is the production of O$_2^-$ and H$_2$O$_2$. Nox catalyses the production of O$_2^-$ by the one-electron reduction of oxygen using NADH or NAPDH as the electron donor. Seven isoforms of Nox have been identified and each contains a core catalytic unit of Nox and Duox and up to five regulatory subunits. Details about the general characteristics of the Nox isoforms can be found in the following reviews [132,148,149]. Briefly, the prototypical oxidase is phagocytic Nox, which is comprised of p91$^{phox}$ (Nox2) and p22$^{phox}$ and three cytosolic regulators (p47$^{phox}$, p67$^{phox}$ and p40$^{phox}$) [132,148,149]. Nox2 is membrane bound in sites such as plasma membranes and the endoplasmic reticulum to p22$^{phox}$ in a heterodimer named cytochrome b-558. The C-terminus of p22$^{phox}$ interacts with p47$^{phox}$, a process that is necessary for Nox2 activation. p47$^{phox}$ is required for the recruitment of another cytosolic protein, p67$^{phox}$, which binds the small GTPase Rac. Nox2 is widely expressed with particularly high expression in inflammatory cells, such as macrophages, and is also present in endothelial cells.

Nox1 shares 56% structural similarity with Nox2 and is expressed in a variety of cell types, including vascular smooth muscle cells, lung epithelium and colon. Nox3 has a similar structure to Nox1 and Nox2 and is highly expressed in the inner ear and fetal tissue. Nox4 is most highly expressed in kidney and originally termed Renox because of this location. Nox4 shares 39% sequence similarity with Nox2; however, unlike Nox1, Nox2, Nox3 and Nox5, its activity results in the constitutive release of H$_2$O$_2$ rather than O$_2^-$ [150]. Similar to Nox1, Nox4 requires cytosolic subunits for its regulation. Nox5 displays little structural similarity to Nox1 and Nox4 and does not require p22$^{phox}$ for activation nor any of the other known regulatory subunits. Nox5 is expressed in human tissues, such as lymphoid organs, testis, spleen and endothelial cells [149], and differs from other Noxes by being able to generate O$_2^-$ in response to an increase in intracellular calcium [149]. Duox1 and Duox2 are largely localized to the thyroid gland and lung epithelial cells of adults where...
they are thought to be involved in hormone biosynthesis and host defence through the production of H$_2$O$_2$. The pathological roles of each particular Nox isoform are not completely understood; however, there is evidence for roles in angiogenesis, inflammation, cell growth and fibrosis [132,151], all of which are relevant to the development of ROP and DR.

**ROS AND HYPOXIA**

**ROS, Nox, HIF-1 and angiogenesis**

HIF-1 is a transcription factor comprised of $\alpha$- and $\beta$-subunits that respond to changes in the cellular oxygen environment. Under normoxic conditions, the $\alpha$-subunits of HIF are hydroxylated at conserved protein residues by HIF PHDs (prolyl hydroxylases), which allows them to be rapidly degraded. Under hypoxic conditions, such as in ROP and DR, PHD is inhibited since it requires oxygen for its function. This event results in the stabilization of HIF-$\alpha$,$\beta$, which leads to the up-regulation of hypoxia-sensitive genes such as VEGF [1–3], angiopoietin [4], erythropoietin [8, 9] and SDF-1 [12,13], all of which have angiogenic properties in the retina [152–155] (Figure 1). The importance of HIF-1 in oxygen-induced retinal angiogenesis has been demonstrated in studies in which a deficiency in PHD proteins protected retinal microvessels from damage in phases I and II of experimental ROP [153–155]. Of note is evidence that classical HIF targets such as VEGF can also be up-regulated through HIF-independent mechanisms [156].

ROS may participate in the stabilization and activation of HIF-$\alpha$, which leads to the up-regulation of hypoxia-sensitive genes. Although the mechanisms by which this occur have not been fully defined, there is evidence that ROS can directly ligate to the active ferrous iron centre of PHDs and may promote phosphorylation-dependent stabilization of HIF-$\alpha$ [157]. The source of ROS, which contributes to HIF-$\alpha$ stabilization, is most likely mitochondria [158–160], with a role for ROS derived from Nox yet to be defined (Figure 1). However, hypoxic up-regulation of Nox1 and subsequent ROS production has been reported to participate in the activation of HIF-1 [161]. The transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) may be involved in this process with Nox1 activation of Nrf2 resulting in the induction of the cytoprotective enzyme thioredoxin-1 and subsequent enhanced HIF-$\alpha$ signalling [162]. A similar relationship between Nrf2 and Nox4 has been reported in the hearts of mice that overexpress Nox4 [163]. Nox4 has been shown to be important for HIF-$\alpha$ transcriptional activity in von Hippel–Lindau renal cell carcinoma, suggesting a potential interaction between HIF-$\alpha$/HIF2$\alpha$ and Nox4 [164,165]. Furthermore, HIF-$\alpha$ is able to enhance the Nox4 promoter at a hypoxic regulator element to stimulate Nox4 promoter activity [166]. Interestingly, Nox can also be activated by hyperoxia, with evidence for involvement of calcium signalling and Rac1 activation [167], Src-dependent tyrosine phosphorylation of p47$^{phox}$ [168] and MAPK pathways [169].

Of interest and probable relevance to ROP and DR is the amplification of HIF-$\alpha$ and downstream events. A recent study in metastatic melanoma tumour cells suggests that there are two phases of HIF-$\alpha$ stabilization in response to hypoxia. In the first phase, mitochondrial-derived ROS leads to HIF-$\alpha$ stabilization and subsequent increased VEGF production [170]. In the second phase, the increased VEGF stimulates the generation of ROS-derived Nox resulting in further stabilization of HIF-$\alpha$ and an amplified signal. Relevant to this idea is that VEGF interacting with VEGFR (VEGF receptor) 2 leads to activation and translocation of the small GTPase Rac1, which stimulates Nox2 [65,171]. Whether these events occur in ROP and DR is yet to be determined (Figure 1).

**ROS, HIF-1 and inflammation**

An important mediator of inflammation is the NF-$\kappa$B family of transcription factors [172]. In resting cells, members of the NF-$\kappa$B family are sequestered into the cytosol due to their association with a family of inhibitors called I$\kappa$B (inhibitor of $\kappa$B). Activation of NF-$\kappa$B occurs via the kinase IKK (I$\kappa$B kinase), which subsequently becomes degraded and liberates NF-$\kappa$B for translocation to the nucleus. There is considerable evidence that ROS not only participates in the stabilization and activation of HIF-$\alpha$, but also in the activation of NF-$\kappa$B. Furthermore, there are complex links between the HIF-1 and NF-$\kappa$B pathways with the presence of an NF-$\kappa$B-binding site in the HIF-$\alpha$ promoter [173] and evidence that HIF-$\alpha$ can contribute to the activation of the NF-$\kappa$B pathway [174]. These relationships may result in the amplification of signals by both pathways. Further information about this topic can be found in the review by Gorlach and Bonello [173].

The activation of NF-$\kappa$B leads to the increased production of hundreds of genes [175], including inflammatory factors that are implicated in ROP and DR such as TNF-$\alpha$, tumour necrosis factor-$\alpha$, IL (interleukin)-6, IL-8, COX2 (cyclo-oxygenase-2), ICAM-1 (intercellular adhesion molecule-1) and MCP-1 (monocyte chemoattractant protein-1) [23,40,95,108,176–178]. These inflammatory factors may themselves induce angiogenic responses and/or enhance the expression of angiogenic factors such as VEGF [179–181]. Few studies have examined the relationship between ROS/Nox/HIF-$\alpha$/NF-$\kappa$B in the retina, although there is a report that ischaemic-induced retinal ganglion cell death was reduced in mice with a suppressed NF-$\kappa$B pathway, accompanied by reduced expression of TNF-$\alpha$ and subunits of Nox [182]. A further study from these authors indicated that activation of NF-$\kappa$B following ischaemia/reperfusion injury in the retina activates Nox in neurons [183].

**NOX ISOFORMS AND ANGIogenesis**

The contribution of the various Nox isoforms to both physiological and pathological angiogenesis has yet to be fully defined. To date most studies have been performed in vitro with results indicating that Nox1, Nox2 and Nox4 can all stimulate endothelial cell proliferation and tubulogenesis. Of the small number of in vivo studies most have utilized mice deficient in Nox1, Nox2 or Nox4 isoforms and have reported reductions in angiogenesis in models of hindlimb ischaemia and sponge implants. However, there is not...
a clear consensus about which Nox isofrom is more important in the angiogenic process. The following section summarizes these findings with additional detail found in Table 1. It is clear that further work is required to understand the role of specific Nox isoforms in pathological angiogenesis with a particular emphasis on experimental models that have applicability to the disease state in humans.

**Nox1**

*In vitro* studies have indicated that inhibition of Nox1 reduced endothelial cell migration and tubulogenesis [56,57,62]. In one *in vitro* study, Nox1 siRNA (small interfering RNA) decreased endothelial cell migration and tubulogenesis through the inhibition of PPAR (peroxisome-proliferation-activated receptor) α, a regulator of NF-κB [62]. The same authors compared the angiogenic responses of Nox1-, Nox2- and Nox4-deficient mice [62]. Only in Nox1-deficient mice was angiogenesis reduced in bFGF (basic fibroblast growth factor)-loaded matrigel implants [62]. Furthermore, administration of a dual Nox1 and Nox4 inhibitor (GKT136901) reduced angiogenesis and tumour growth in a PPARα-dependent manner [62]. These findings are consistent with a report by Arbiser et al. [63], who demonstrated that overexpression of Nox1 increased VEGF and VEGFR expression and MMP (matrix metalloproteinase) activity through increased ROS, which resulted in tumour angiogenesis.

**Nox2**

*In vitro* studies have indicated that Nox2 overexpression increased the proliferation of endothelial cells, whereas inhibition of Nox2 reduced endothelial cell proliferation and tubulogenesis [58,59,184]. *In vivo*, a deficiency in Nox2 reduced angiogenesis in the ischaemic hindlimb [64] and sponge implant model [65], and attenuated the homing and mobilization of EPCs (endothelial progenitor cells) [185].

**Nox4**

*In vitro*, Nox4 overexpression promoted endothelial cell proliferation, migration and tubulogenesis [59–61,186], whereas Nox4 siRNA reduced these events [58,59,184]. *In vivo*, Nox4 overexpression increased myocardial capillary density [184] and, in transgenic mice with endothelial-specific Nox4 overexpression, there was increased recovery from hindlimb ischaemia and increased aortic sprouting [61]. A deficiency in Nox4 is anti-angiogenic, with a recent study by Schroder et al. [66] having demonstrated that mouse lung endothelial cells from Nox4-knockout mice undergo less tubulogenesis *in vitro* when exposed to H2O2 compared with endothelial cells from wild-type mice [66].

**Nox isoforms and pathological angiogenesis in the retina**

The majority of studies evaluating Nox and retinal angiogenesis have utilized the experimental ROP model and used either apocynin or DPI (diphenyleneiodonium) as Nox inhibitors to evaluate retinal pathology. These studies have provided interesting and useful information about the anti-angiogenic and antioxidant effects of these treatments in retina [187]. However, both agents are now considered to not be specific Nox inhibitors but rather general ROS inhibitors. Selemidis et al. [188] provide an excellent in depth review about the characteristics of these inhibitors and others.

Currently, the roles of specific Nox isoforms in the retinal angiogenesis of ROP and DR have not been fully determined, although there is evidence from studies of Nox-knockout mice that Nox isoforms have pro-inflammatory effects in the retina. Inflammation contributes to the development of microvascular disease in both ROP and DR as leucocytes migrate and adhere to the retinal vasculature where they may cause injury by releasing cytokines, inflammatory factors and ROS [108,189]. The inflammatory state of the retina can be exacerbated by the activation of resident inflammatory cells, microglia, which can also release cytokines, inflammatory factors and ROS to injure the microvasculature, neurons and glia [40,190–192]. The following section summarizes the known effects of apocynin and Nox in ROP and DR. Given the established role of Nox2 in phagocytic defence and inflammation, this isoform has been most extensively studied in ROP and DR.

**ROP**

There is clear evidence that apocynin reduces vascular injury in animal models of ROP. For instance, apocynin reduced vasoobliteration and cleaved caspase 3 (expressed during apoptosis) in rats with ROP, although it had no effect on retinal angiogenesis and the elevated levels of VEGF [193]. A study from the same group reported that, when rats with ROP were exposed to supplemental oxygen (28 % oxygen) instead of room air (approximately 21 % oxygen) in phase II of ROP, Nox activation was exacerbated and contributed to retinal angiogenesis [194]. A subsequent study demonstrated that Nox activation in this model works through activation of the JAK/STAT (Janus kinase/signal transducer and activator of transcription) signalling pathway and particularly STAT3 [195]. Nox2 has been implicated in the increased levels of O2− in retina observed in ROP. In mice, increased retinal O2− was reduced when retinal sections were incubated with either apocynin or gp91ds-tat (a peptide which inhibits Nox assembly by binding to p47−/− and blocking its interaction with Nox2) [187,196]. In the same paper, the authors reported that, in cultured retinal endothelial cells exposed to hypoxia, both apocynin and gp91ds-tat reduced O2− levels [187], but this did not occur in rat Müller cells exposed to hypoxia [187]. Tawfik et al. [197] demonstrated an interesting association between Nox2 and PPARγ in experimental models of ROP and DR. Down-regulation of PPARγ has been implicated in retinal vascular injury via inflammatory mechanisms involving the up-regulation of NF-κB [198]. Tawfik et al. [197] also reported that, in Nox2-knockout mice, PPARγ levels were restored with a concomitant reduction in NF-κB.

**DR**

Evidence for a role for Nox2 in DR comes from *in vitro* studies showing that glucose-induced apoptosis of cultured bovine retinal pericytes is associated with increased Nox2 expression, which is reduced with apocynin [199]. Al-Shabrawey et al. [200] reported in a study of short-term streptozotocin diabetes
Table 1  Summary of evidence supporting a pro-angiogenic effect of particular Nox isoforms
HGF, hepatocyte growth factor; HUVEC, human umbilical vein endothelial cells; KNRK, K-Ras transformed normal rat kidney cells; shRNA, short hairpin RNA.

<table>
<thead>
<tr>
<th>Cell type/tissue</th>
<th>Treatment</th>
<th>Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro: Nox1 studies</strong></td>
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<tr>
<td>Sinusoid endothelial cells</td>
<td>VEGF</td>
<td>Fivefold increase in Nox1 expression</td>
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<tr>
<td>Non-tubulogenic endothelial cell line</td>
<td>Nox1 overexpression</td>
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<td>[56]</td>
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<tr>
<td>KNRK cells</td>
<td>Nox1 siRNA</td>
<td>Reduced synthesis of VEGF protein and mRNA</td>
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<tr>
<td>KNRK-derived tumours</td>
<td>Nox1 siRNA transfected</td>
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</tr>
<tr>
<td>Lung endothelial cells, HUVECs and endothelioma cells</td>
<td>VEGF and bFGF</td>
<td>Increased Nox1 mRNA</td>
<td>[62]</td>
</tr>
<tr>
<td>Lung endothelial cells, HUVECs and endothelioma cells exposed to VEGF or bFGF</td>
<td>GKT136901 (dual Nox1 and Nox4 inhibitor) or Nox siRNA</td>
<td>Reduced ROS</td>
<td>[62]</td>
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<tr>
<td>Lung endothelial cells</td>
<td>Nox1 siRNA</td>
<td>Reduced endothelial cell migration and tubulogenesis through the inhibition of PPARα</td>
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<td><strong>In vitro: Nox2 studies</strong></td>
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<td>EaHy926 cells and human microvascular endothelial cells</td>
<td>Nox2 overexpression</td>
<td>Increased proliferation</td>
<td>[59]</td>
</tr>
<tr>
<td>EaHy926 cells and human microvascular endothelial cells</td>
<td>Nox2 siRNA</td>
<td>Reduced proliferation</td>
<td>[59]</td>
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<tr>
<td>Human endothelial cells</td>
<td>Nox2 siRNA</td>
<td>Reduced cell proliferation, and increased apoptosis and cytoskeletal disorganization</td>
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<tr>
<td>HUVECs overexpressing phosphodiesterase 2</td>
<td>Nox2 siRNA</td>
<td>Reduced proliferation and tubulogenesis</td>
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<tr>
<td>HUVECs exposed to urotensin II</td>
<td>Nox2 shRNA</td>
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<td><strong>In vitro: Nox4 studies</strong></td>
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<tr>
<td>EaHy926 cells and human microvascular endothelial cells</td>
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<tr>
<td>Human microvascular cells</td>
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<td>Endothelial cells</td>
<td>Conditioned medium from Nox4-overexpressing cardiomyocytes</td>
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<tr>
<td>Human and bovine endothelial cells</td>
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<td>EaHy926 cells and human microvascular endothelial cells</td>
<td>Nox4 siRNA</td>
<td>Reduced proliferation</td>
<td>[59]</td>
</tr>
<tr>
<td>Human microvascular cells</td>
<td>Nox4 siRNA</td>
<td>Reduced tubulogenesis</td>
<td>[60]</td>
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<tr>
<td>Cultured ovarian cancer cells</td>
<td>Nox4 siRNA</td>
<td>Reduced angiogenesis, VEGF and HIF-1</td>
<td>[277]</td>
</tr>
<tr>
<td>Lung artery endothelial cells</td>
<td>Nox4 siRNA</td>
<td>Reduced cell migration and tubulogenesis</td>
<td>[278]</td>
</tr>
<tr>
<td>Human endothelial cells</td>
<td>Nox4 siRNA</td>
<td>Reduced proliferation and ERK1/2 phosphorylation</td>
<td>[58]</td>
</tr>
<tr>
<td>HUVECs</td>
<td>Nox4 siRNA</td>
<td>Reduced proliferation involving prostacyclin 1/2 and cAMP signalling</td>
<td>[279]</td>
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<tr>
<td>Lung endothelial cells</td>
<td>From Nox4 −/− mice</td>
<td>Reduced tubulogenesis</td>
<td>[66]</td>
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<tr>
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<tr>
<td>Human endothelial cells</td>
<td>Nox5 proteins</td>
<td>Increased proliferation and capillary-like structures</td>
<td>[214]</td>
</tr>
<tr>
<td>Human endothelial cells</td>
<td>Nox5 siRNA</td>
<td>Reduced proliferation and capillary-like structures</td>
<td>[214]</td>
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<tr>
<td><strong>In vivo: Nox1, 2 and 4 studies</strong></td>
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<tr>
<td>NIH 3T3 cells injected into athymic mice to generate tumours</td>
<td>Nox1 expressing</td>
<td>Increased vascularity of tumours, and increased VEGF, VEGFR1 and VEGFR2 mRNA and MMP activity</td>
<td>[63]</td>
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<tr>
<td>Tumour cells injected</td>
<td>Nox1 −/− mice</td>
<td>Reduced angiogenesis and tumour growth via PPARα</td>
<td>[62]</td>
</tr>
<tr>
<td>Matrigel loaded with bFGF</td>
<td>Nox1, Nox2 and Nox4 −/− mice</td>
<td>Angiogenesis only reduced in Nox1 −/− mice</td>
<td>[62]</td>
</tr>
</tbody>
</table>
that the increased retinal leukostasis, vascular leakage, ICAM-1 and ROS levels were all reduced in Nox2-knockout mice and mice treated with apocynin [200]. Furthermore, in LPS (lipopolysaccharide)-induced retinal inflammation, leucocyte adherence and ICAM-1 expression were reduced in Nox2-knockout mice [200]. The beneficial effects of HMG-CoA reductase (3-hydroxy-3-methylglutarlyl-CoA reductase) inhibition (statins) on experimental DR have been linked to Nox2. Simvastatin reduced the increased retinal levels of Nox2, VEGF and ICAM-1 and preserved the integrity of the blood–retinal barrier [201]. In the same study, Nox2 protein levels were increased in retinal blood vessels and were associated with increased oxidative stress in the retina [201]. An interesting study by Jarajapu et al. [202] indicated that Nox2 expression, Nox activity and O₂⁻ levels are higher in EPCs from diabetic individuals than non-diabetic individuals [202]. Nox inhibition with apocynin or gp91ds-tat in EPCs from diabetic individuals restored their capacity to migrate to the ischaemic retina and hence influence blood vessel repair [202].

Oxidative stress is closely linked to the formation of AGEs [203]. Whether there is interplay between ROS derived from Nox and AGE-related damage in DR is not completely understood. There is evidence that AGEs induce the expression of p47phox in cultured retinal endothelial cells [204]. A number of studies have evaluated the vasculoprotective effects of PEDF (pigment epithelium-derived factor) and linked these effects to a reduction in oxidative stress in the retina [205]. For example, in diabetic rats, AGEs induced the breakdown of the blood–retinal barrier, increased retinal VEGF expression and reduced the expression of PEDF [206]. In these animals, co-administration of AGEs and PEDF reduced vascular pathology with a concomitant reduction in p22phox, Nox2 and oxidative stress [206]. These protective effects of PEDF have been reported to occur via the PI3K (phosphoinositide 3-kinase) signalling pathway [207]. Finally, puerarin, an isoflavone glycoside with anti-AGE properties, inhibited AGE–BSA-induced apoptosis of bovine retinal pericytes in culture and in vivo. In cultured retinal pericytes, puerarin reduced the AG-E–BSA-induced up-regulation of Nox activity and ROS and reduced the phosphorylation of p47phox and Rac1 [208].

Few studies have evaluated Nox4 in either ROP or DR. In db/db mice, a model of Type 2 diabetes, the expression of Nox4 and VEGF was significantly increased in the retina and was reduced with the hypolipidaemic agent lovastatin and the Nox inhibitor DPI [209]. Intravitreal delivery of Nox4 siRNA decreased retinal Nox4 and VEGF expression, Nox activity and retinal vascular permeability [209]. Studies in bovine retinal endothelial cells exposed to high glucose elicited similar results and showed that Nox4 inhibition reduced the phosphorylation of STAT3, a transcription factor that activates VEGF expression. These results suggest that Nox4 contributes to the vascular dysfunction of early DR [209].

### Cellular location of Nox isoforms in the retina

The cellular location of the various Nox isoforms in the retina is yet to be fully elucidated. In other vascular beds, Nox1 [210–212], Nox2 [210,211,213], Nox4 [210–213] and Nox5 [214] have been identified in endothelial cells. There is a report of Nox1, Nox2 and Nox4 expression in primary retinal capillary endothelial cells [209] and p22phox, p47phox, p67phox. Nox1 subunits, but not Nox2, in pericytes from rat adipose tissue [215]. In general, expression levels in endothelial cells are much higher for Nox4 than Nox1 or Nox2 (at least 100-fold in retinal endothelial cells) [209], suggesting that Nox4 may be the primary ‘vascular Nox‘. However, whether this expression profile described in vitro for retinal endothelial cells accurately reflects the expression profile in ROP and DR is yet to be confirmed.
relevance is a report of immunolabelling for Nox2 in angiogenic blood vessels in mice with ROP [187]. In some angiogenic situations, including ROS derived from Nox. In tumours, Noxes are expressed not only in endothelial cells, but also tumour cells (reviewed in [148]). In terms of retinopathy, a recent study has indicated that cultured retinal ganglion cells express Nox1, Nox2 and Nox4, as well as regulatory subunits of Nox [216]. Of interest is that Nox 1 mRNA levels were higher than other Noxes, and that Nox 1 was the predominant Nox isoform in ganglion cells in retina following transient ischaemia [216]. Given that retinal ganglion cells are a source of VEGF in ROP and DR [90,94,217,218], the location of Noxes in ganglion cells may be important for retinal angiogenesis. Supporting this idea is evidence from a model of retinal ischaemic reperfusion injury, which features damage to ganglion cells and activation of ERK (extracellular-signal-regulated kinase) and NF-κB signalling mechanisms, that these events were attenuated in Nox2-knockout mice [219]. Retinal microglia are also a probable source of ROS [40,192], although whether ROS is generated from Nox in these cells in ROP and DR is not yet understood.

Nox, the RAAS and retinopathy

A wide range of factors are activators of Nox, including shear stress [220–222] and inflammatory mediators such as TGF-α (transforming growth factor-α), IFN (interferon)-γ, IL-1, IL-17 and the bacterial toxin LPS [213,223–227]. There is substantial evidence that AngII (angiotensin II) is an important stimulator of Nox [67,68]. AngII is a key effector of the RAAS and elicits wide-ranging effects, including regulation of blood pressure and promotion of fibrosis, inflammation, cell hypertrophy and angiogenesis (reviewed [14]). AngII also stimulates the production of aldosterone from the adrenal gland, which can participate in these events via the mineralocorticoid receptor [14]. AngII has been reported to influence the expression of Nox subunits, such as Nox 1 [69,228–232], Nox2 [70, 232–234], Nox4 [71,230,232] and Nox5 [72,235], and contribute to ROS-mediated damage in a number of pathologies, including hypertension and cardiovascular disease (reviewed in [134,151,236]). Aldosterone can stimulate Nox, with reports that aldosterone/salt infusion increased the expression of Nox isoforms and ROS production [237–240]. The mineralocorticoid receptor has been linked to the AngII-induced up-regulation of Nox isoforms and ROS production [241,242], and mineralocorticoid receptor blockade reduced Nox4 expression in kidneys from aldosterone/salt infused salt-sensitive Dahl rats [243].

It is well known that the RAAS is involved in the development of ROP and DR. This is based on findings that blockade of ACE (angiotensin-converting enzyme), the AT,R (angiotensin type 1 receptor) and renin attenuate pathological angiogenesis, inflammation and neuronal injury in animal models of ROP [15,244–246] and improve neurovascular pathology in experimental DR [17,18,246–248]. Clinical studies also indicate benefits of ACE and AT,R blockade in individuals with DR [249–253]. Although less studied, recent work has demonstrated improvements in ROP in animals treated with mineralocorticoid receptor antagonists and aldosterone synthase inhibition [16,254]. Of interest is that the mineralocorticoid receptor antagonist eplerenone has been reported to reduce choriovascularopathy in patients [255]. Whether the effects of AngII and aldosterone in the retina are predominately due to their local formation or contributions from the circulation is not entirely clear, and is clouded by the possible uptake of both agents in the retina when the blood–retinal barrier is compromised. Nevertheless, local actions of the RAAS in the retina are most likely, given substantial evidence of a retinal RAAS [14,16,254,256,257] and that components of the system are up-regulated in the retina in both ROP and DR (reviewed in [14]).

Although not extensively studied, it is likely that interactions exist between the RAAS and Nox within the retina. Intravitreal administration of AngII increased leukostasis and retinal levels of IL-6, O2− and Nox activity and these changes were decreased in IL-6-deficient mice [258]. Furthermore, AngII infusion resulted in elevated levels of VEGF and p22phox in retina [259], and apocynin reduced AngII-induced leukostasis in diabetic rats [260]. In spontaneously diabetic Torii rats, a Type 2 diabetic model, VEGF and p22phox were elevated in the retina and were reduced with the AT,R blocker candesartan [259]. In a rat model of retinal ischaemia, retinal p47phox and p67phox expression and ROS levels were increased and were subsequently reduced with AT,R blockade [261]. The effect of aldosterone on retinal Nox is unknown, although aldosterone infusion does modulate retinal Nox4 mRNA levels in ROP [254].

The signalling mechanisms by which AngII induces Nox activity are complex and have not been defined in the retina. Studies in other tissues may provide some clues with respect to retinal vascular and neuronal function. For instance, AngII stimulates vascular smooth muscle cell proliferation and migration via AT,R/Nox1-dependent IL-18 induction, and this process may involve the physical association of the AT,R and Nox 1 [262]. In endothelial cells, AngII through PKC activation induces the up-regulation of Nox1 [263]. Studies in brain neuronal cells indicate that Nox activation requires Rac1 [264] and PKC [265], p38 MAPK and ERK1/2 may also be involved [266], and changes in intracellular calcium [265,267] and the delayed rectifier potassium current [268] may contribute to neuronal activity. The review by Cai et al. [269] provides further details about this and other mechanisms by which AngII influences Nox.

The contribution of ROS derived from Nox in hypertension (reviewed in [236]) and its impact on retinopathy may need to be considered given that hypertension is a known risk factor for DR [111,115]. Briefly, AngII-induced hypertension stimulates Nox activity. For example, AngII-induced hypertension is reduced in mice deficient in Nox1 [270] and following blockade of the association between Nox2 and p47phox [196]. As pointed out by Montezano and Touyz [236], in hypertension, AngII alone is unlikely to induce oxidative stress and other factors, such as activation of the sympathetic and/or central nervous system and the adaptive immune system, may be involved [271–273]. Little is known about the role of Nox in hypertensive retinopathy. There are reports that, in spontaneously hypertensive rats, ROS in retinal ganglion cells and retinal Nox activity are increased compared with normotensive Wistar–Kyoto rats [274]. Furthermore, in retinal pericytes exposed to cyclic stretch, which is a mimic of
hypertension, ROS production is increased and there is increased association of PKCδ with p47phox [222].

CONCLUSIONS
The contribution of the ROS derived from Nox to pathological angiogenesis is an emerging and exciting area of research that is likely to be important in the development of retinal vascular pathologies such as ROP and DR. Further work is required to determine how the RAAS influences ROS derived from Nox and the roles of specific Nox isoforms in pathological angiogenesis in retinopathy and other pathologies (Figure 1). With respect to ROP and DR, the contribution of ROS derived from Nox in vascular cells and non-vascular cells (e.g. ganglion cells, Müller cells and microglia) is likely to be important given that cross-talk between these cell types is implicated in the development of pathological retinal angiogenesis [86,275].

FUNDING
Our own work was supported by the Juvenile Diabetes Research Foundation. J.L.W.-B. is a Senior Research Fellow of the National Health and Medical Research Council (NHMRC) of Australia.

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Received 1 October 2012/28 November 2012; accepted 30 November 2012

Published on the Internet 4 February 2013, doi: 10.1042/CS20120212