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The β3 adrenoceptor in proliferative retinopathies: "Cinderella" steps out of its family shadow

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ABSTRACT

In the retina, hypoxic condition leads to overgrowing leaky vessels resulting in altered metabolic supply that may cause impaired visual function. Hypoxia-inducible factor-1 (HIF-1) is a central regulator of the retinal response to hypoxia by activating the transcription of numerous target genes, including vascular endothelium growth factor, which acts as a major player in retinal angiogenesis. In the present review, oxygen urge by the retina and its oxygen sensing systems including HIF-1 are discussed in respect to the role of the beta-adrenergic receptors (β-ARs) and their pharmacologic manipulation in the vascular response to hypoxia. In the β-AR family, β1- and β2-AR have long been attracting attention because their pharmacology is intensely used for human health, while β3-AR, the third and last cloned receptor is no longer increasingly emerging as an attractive target for drug discovery. Here, β3-AR, a main character in several organs including the heart, the adipose tissue and the urinary bladder, but so far a supporting actor in the retina, has been thoroughly examined in respect to its function in retinal response to hypoxia. In particular, its oxygen dependence has been taken as a key indicator of β3-AR involvement in HIF-1-mediated responses to oxygen. Hence, the possibility of β3-AR transcription by HIF-1 has been discussed from early circumstantial evidence to the recent demonstration that β3-AR acts as a novel HIF-1 target gene by playing like a putative intermediary between oxygen levels and retinal vessel proliferation. Thus, targeting β3-AR may implement the therapeutic armamentarium against neovascular pathologies of the eye.

1. Introduction

Hyperoxia and hypoxia, *i.e.*, excess and insufficient oxygen availability with respect to needs, are thought to trigger opposite responses that are, however, strictly dependent on each other. In particular, in organs with high-rate metabolic activity such as the retina, the tissue response to high oxygenation is characterized by the reduction of the local blood flow as vessel growth stops along with a degeneration of existing blood vessels in order to mitigate local build-up of oxygen and its complications [1]. In preterm infants, for instance, exposure to hyperoxia can lead to ocular damage characterized by disorganized growth of retinal blood vessels which may result in retinal detachment (see Section 4). In particular, reduced blood flow following vessel regression may drastically decrease metabolic supply to the tissue, ultimately establishing a hypoxic condition, which in turn leads to adaptive responses including angiogenesis, which is responsible for most of the sight-threatening complications characterizing human proliferative retinopathies [2]. Among them, retinopathy of prematurity (ROP) and proliferative diabetic retinopathy (PDR) are characterized by an excessive growth of retinal vessels that, although originally established to compensate for a lack of oxygen and nutrients, may result in the formation of thin-caliber vessels that lack tight junctions and are very

Abbreviations: β-AR, beta-adrenergic receptor; BRB, blood-retinal barrier; DMOG, dimethyloxalylglycine; EPC, endothelial progenitor cell; ERG, electroretinogram; GCL, ganglion cell layer; GPCR, G protein-coupled receptor; GRK, G protein-coupled receptor kinases; HBS, HIF-1 binding site; HIF, hypoxia-inducible factor; HRE, hypoxia response element; IH, infantile hemangioma; INL, inner nuclear layer; MAP, mitogen-activated protein; nAMD, neovascular age-related macular degeneration; NMDA, N-methyl-D-aspartate; NO, nitric oxide; OIR, oxygen induced retinopathy; ONL, outer nuclear layer; PD, postnatal day; PDR, proliferative diabetic retinopathy; PHD, prolyl hydroxylase domain; PKA, protein kinase A; RGC, retinal ganglion cell; ROP, retinopathy of prematurity; RP, retinitis pigmentosa; RPE, retinal pigment epithelium; STAT3, signal transducer and activator of transcription 3; TM, transmembrane domain; VEGF, vascular endothelial growth factor; VHL, Von Hippel-Lindau.

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prone to leakage [3]. Abnormal vessel growth also characterizes subretinal vascular diseases including the wet or neovascular form of age-related macular degeneration (nAMD) in which growing choroidal vessels invade the avascular outer retina and subretinal space [4]. Proliferative retinopathies are the leading causes of blindness in the working age and their incidence is rapidly increasing mostly due to their delayed treatment because their symptoms can be easy to miss at first.

Retinal response to hypoxia is centrally regulated by hypoxia-inducible factor (HIF)-1, which activates the transcription of numerous target genes, including vascular endothelial growth factor (VEGF), a major in neovessel proliferation [5]. Growing evidence support the use of anti-VEGF agents for the treatment of hypoxia-associated retinopathies [6] although there are concerns regarding their adverse effects [7]. In this respect, due to the growing burden of proliferative retinopathies, optimizing our understanding about mechanisms underlying proliferative retinopathies may help to develop additional treatments to counteract sight-threatening neovascularization. In particular, the identification of novel oxygen-dependent pathways would deserve a particular attention if one considers that the fundamental discovery of genes targeted by HIF-1 including VEGF has drastically transformed the therapeutic protocol of proliferative retinopathies in a relatively short time span of less than 15 years.

The present review aims to highlight the role of the β-adrenoceptor (β-AR) system in proliferative retinopathies including the current state of the art on β3-AR, the third less popular and last cloned adrenoceptor, starting from the preliminary findings on its localization to retinal endothelial cells [8]. In the β-AR system, the role of β2-AR in retinal angiogenesis has been apparently clear since the very beginning while β3-AR has gained increasing attention over time for its peculiar oxygen-dependent regulation. The history of β3-AR can be compared to Cinderella's story……. Since β2-AR blockade was found to inhibit retinal angiogenesis, β3-AR was put aside remaining hidden in the BAR family–just like Cinderella, treated by her stepsisters as a maid. Nevertheless, because of its upregulation by hypoxia at the level of proliferating vessels [9] and its HIF-1 dependence [10], β3-AR has achieved some recognition for its involvement in angiogenesis-associated pathological conditions similarly to Cinderella, who rose into a new role after a period of neglect.

2. Oxygen urge by the retina

The retina is one of the highest oxygen-consuming tissues in the human body with a metabolic activity higher than that of the brain. In particular, more than 60 % of the oxygen delivered to the retina is consumed by the mitochondria located in the inner segment of photoreceptors to maintain the dark current and to provide for light transduction [11]. The retinal pigment epithelium (RPE) is an additional oxygen consumer because its involvement in regenerating the photopigments. Additionally, the plexiform layers of the retina require oxygen to maintain synaptic transmission and among retinal cells, retinal ganglion cells (RGCs) have a particularly active metabolism and are highly vulnerable to an insufficient oxygen supply. Since oxygen cannot be stored in the retina, the high oxygen demand is supported by a continuous oxygen supply through the choroidal and retinal circulations, which support the outer and the inner part of the retina, respectively [12].

The choroidal blood vessels mainly originate from the long posterior, short posterior and anterior ciliary arteries that, once entered the choroid, produce smaller branches finally dividing in choriocapillaris that accomplish several functions, including metabolic support to photoreceptors, thermal regulation of the posterior part of the eye and filtration of the waste produced by the outer retina. Fenestrated choriocapillaris are separated by the retinal surface through the RPE that forms the outer blood retinal barrier (BRB), which regulates the environment of the outer retina [13].

The retinal blood vessels originate from the central retina artery, a branch of the ophthalmic artery, that arborizes into the retinal parenchyma forming three capillary layers, the superficial, near the nerve fiber layer, the intermediate, at the border between the inner plexiform and the inner nuclear layers, and the deep, at the level of the outer plexiform layer [14].

The prominent function of retinal blood vessels is to nourish the inner retina through the capillary meshwork that perfuses the retinal parenchyma. The tight junctions located between endothelial cells participate to the formation of the inner BRB that prevents the free diffusion of substances between the circulating blood and the neural retina thus maintaining retinal homeostasis [15]. Protective mechanisms to compensate for reduced oxygen availability include new blood vessels formation that, instead of repairing the problem, may rather exacerbate it (see below). In this respect, neovascularization in response to hypoxic conditions, may lead to the disruption of the inner BRB associated with increased vascular permeability with consequent retinal cell damage and adverse effects upon vision [16].

In Fig. 1, a schematic representation of the retina and its vascularization is shown together with enlarged magnification of the inner and the outer BRB.

3. HIF-1/VEGF as an oxygen sensing system

Although the retina is well vascularized, the high oxygen demand of retinal cells exposes this tissue to the risk of developing a dangerous hypoxic state. Despite the fact that, within the body, several tissues have oxygen reserves that enables them to face acute hypoxia, the retina has a limited oxygen reserve that derives from increasing extraction of oxygen from oxygen-loaded red blood cells and that in humans, lasts for few seconds only [17]. A master regulator of the metabolic switch that adapts retinal cells to a hypoxic environment is represented by the family of HIFs. Their activity is increased under hypoxia leading to an extensive modification of transcriptome that, through coordinated action on gene expression, attempts to reinstate oxygen homeostasis. The HIF family is composed by three members, each of them constituted by an α labile subunit and by a β stable subunit. Among the members of the HIF family, HIF-1, discovered in 1995 [18], is the master of transcription factors and physiologically regulates cell adaptation to reduced oxygen tension. When oxygen is available, HIF-1α is hydroxylated on key proline residues (Pro^{402} and Pro^{564}, which are present in the C-terminus domain of the protein) by the prolyl hydroxylase activity of enzymes belonging to the α-ketoglutarate dioxygenase superfamily, named prolyl hydroxylase domain proteins (PHDs; [19]). After being hydroxylated, proline residues are bound by the Von Hippel-Lindau (VHL) protein, the substrate recognition subunit of a protein complex endowed with E3 ubiquitin ligase activity that, in turn, targets HIF-1α for proteasomal degradation. In the presence of a decreased amount of oxygen, however, PHDs do not hydroxylate HIF-1α that escapes proteasomal degradation, moves into the nucleus, dimerizes with HIF-1β and participates to the transactivation of an enormous number of target genes after its coupling with the co-activators p300 and cAMP response element-binding protein that act as histone acetyl transferases thus promoting gene transcription [20].

Within the promoter of its target genes, HIF-1 binds to cis-acting elements defined hypoxia response elements (HREs), with a core consensus sequence 5'-(A/G)CGTG-3' commonly defined as HIF-1 binding site (HBS). HREs are normally distributed in the genome and occur in promoter, enhancer and 3' untranslated regions of a gene sequence [21]. The number of HREs in the genome is incredibly high, although less than 1 % of the potentially functional HREs bind HIF-1 in hypoxic conditions [22].

The number of HIF-1 target genes is gradually increasing and many of them are regulated not only in hypoxia but also in normoxia indicating that they may serve to maintain cell basal activity [23]. Under hypoxia, genes targeted by HIF-1 are involved in metabolic reprogramming and stimulation of angiogenesis, both allowing the cell to

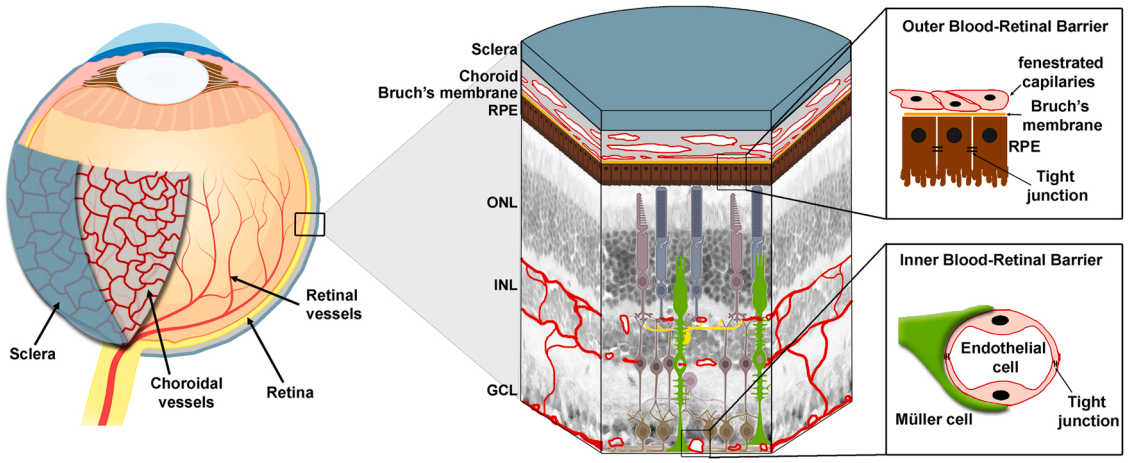

Fig. 1. Scheme of the eye and sectional view of the layered retina structure. The globe of the eye is composed by a wall enclosing a cavity filled with fluid with three coats: the sclera, the choroid and the retina including the choroidal vessels and the retinal vessels. In the tridimensional representation of a coronal section through the eye, the different coats are shown together with the choroidal vessels, which supply both the RPE and the photoreceptors in the ONL, and the retinal vessels that are organized in three layers tightly coordinated with cells in the inner retina. Müller cells endfeet at high magnification are shown to envelope capillary endothelial cells where they regulate the tightness of the inner BRB that is formed by tight junctions between retinal capillary endothelial cells. As shown by the high magnification, the outer BRB is formed by the tight junctions at the apical periphery of RPE cells, the Bruch's membrane, and the fenestrated choriocapillaris. RPE, retinal pigment epithelium; ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer.

adapt for surviving in conditions of reduced oxygen tension. For instance, HIF-1 transactivates genes that shift glucose metabolism from the Krebs' cycle towards glycolysis as well as those involved in oxygen delivery to the tissues, including among others VEGF and erythropoietin [21]. Diagrammatic representation in Fig. 2 summarizes the cascade of events leading from HIF-1α dimerization in response to low oxygen tension up to the formation of new vessels through VEGF release.

Considering the high demand of oxygen supply required by photoreceptors, it is likely that the retina is under a physiological hypoxia, with an oxygen gradient declining from the outer to the inner retina [24]. In particular, outer nuclear layer (ONL), inner nuclear layer (INL) and ganglion cell layer (GCL) reveal HIF-1α immunopositive profiles in human and rats, with at least 75 % of positive cells in the GCL, more than 50 % of positive cells in the INL and less than 50 % of positive cells in the ONL, in accordance with the oxygen gradient from the outer to the inner retina [25]. In addition, there is evidence that HIF-1α is abundantly expressed in the photoreceptor layer [26]. These findings suggest that the constitutive presence of HIF-1 may play a role in the healthy retina as for instance during development or to maintain cell homeostasis. On the other hand, HIF-1 is more abundantly expressed under pathological conditions associated with hypoxia with an increased expression of HIF-1α in macroglial cells, both Müller cells and astrocytes, and RGCs [27–29].

3.1. HIF-1 pharmacology

HIF-1 plays critical roles in proliferative retinopathies by activating the transcription of a large battery of genes encoding proteins that play key roles in several processes including angiogenesis and inflammation. The increased expression of HIF-1α in hypoxia-associated retinal pathologies supports the use of HIF-1α inhibitors as therapeutical strategies although their use must be considered with caution given the important role that HIF-1 plays in the retina. Although much progress has been made to validate the pharmacology of HIF-1α inhibitors against tumor angiogenesis [30], their application against proliferative retinopathies remains matter of debate as any of them exhibit significant side effects and toxicities due to their lack of specificity [31]. On the one hand, inhibiting HIF-1 may prevent the upregulation of HIF-1-dependent angiogenic factors without influencing their physiologic levels thus ensuring ocular homeostasis [32]. In this respect, HIF-1 inhibitors may act at different levels by suppressing *HIF-1α* gene transcription, preventing HIF-1α mRNA translation, promoting HIF-1 α degradation, inhibiting the dimerization of the α and β subunits or hampering the association of HIF-1 to DNA [33].

However, HIF-1α inhibitors would also block the activity of a plethora of target genes thus disrupting specific associations of HIF with cellular components that are essential for retinal function. Although pharmacological inhibitors of HIF are currently tested at the preclinical level, increasing the list of HIF-1 target genes activated in response to

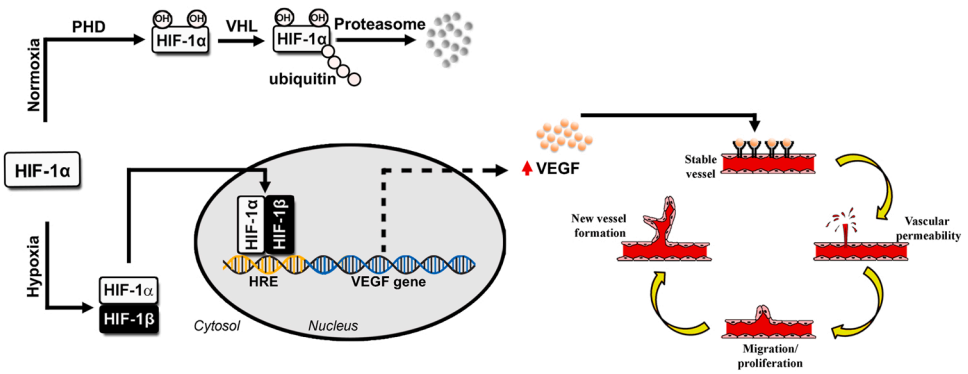

Fig. 2. Diagram depicting the regulation of HIF-1α levels. In normoxic conditions, PHD enzymes hydroxylate HIF-1α, which, after being targeted by the E3 ubiquitin ligase VHL, is degraded by the proteasome. In hypoxic conditions, HIF-1α escapes proteasome degradation and dimerizes with HIF-1β, thus generating the active form of HIF-1. Into the nucleus, HIF-1 binds to HRE sequences activating, in turn, the transcription of a plethora of target genes. Among them, the transcription of the *VEGF* gene leads to the accumulation of VEGF protein, a condition triggering the activation of the angiogenic cascade.

hypoxia remains the best way forward to develop novel target therapies. In this respect, the anti-VEGF drugs have been instrumental in treating retinal diseases previously thought to be untreatable as for instance age-related macular degeneration [34]. Therefore, targeting selected proteins whose expression is increased by HIF-1 during the hypoxic drive may be a better strategy to counteract hypoxia-associated retinal diseases. Thus, uncovering novel HIF-1-regulated genes both in physiological and pathological states may be critical for developing novel therapeutics for the treatment of hypoxia-related diseases in the retina. On the other hand, HIF-1 target genes beside to ensure that retinal cells survive in conditions of low oxygen tension by increasing oxygen delivery, may also drive potentially dangerous scenarios by promoting the formation of an inefficient neovessel network, which aggravates the pathological state of the hypoxic retina.

3.2. VEGF pharmacology

Of the hypoxia-inducible proangiogenic factors, VEGF, which is the paradigmatic proangiogenic cytokine acting in the retina, is produced by several retinal cell sources including RPE, endothelial and Müller cells, astrocytes and neurons [35]. In a physiological setting, endogenous VEGF, mainly released by astrocytes, stimulates blood vessel growth to meet the increasing demand of oxygen and nutrients of the developing retina while during the adult life, VEGF released by Müller cells is required to maintain retinal cell homeostasis acting mainly on neurons, glial cells and vessels [36]. Under hypoxia, VEGF accumulation mainly by Müller cells originates as a compensatory mechanism to overcome tissue suffering by the formation of adequate microvasculature in response to ischemic conditions although VEGF overexpression may lead to neovessel formation that is the leading cause of angiogenesis-related ocular diseases [37]. From classical anti-VEGF, corticosteroids, and photocoagulation the trail narrows to novel therapies including RNA-based therapies that are currently explored as alternative therapeutics for the management of proliferative retinopathies due to their role in the regulation of VEGF gene expression [38]. In particular, RNA interference therapy inhibits the activity of the VEGF gene at the post transcriptional level and reduces its protein expression with high specificity. In preclinical studies, bioreducible lipidoid nanoparticles conveying VEGF siRNA have been shown to effectively inhibit retinal vessel proliferation by reducing the expression of VEGF mRNA and protein [39].

Despite the essential role of anti-VEGF therapies, attention should be paid to the fact that VEGF also exerts some neuroprotective role to defend retinal cells against the hypoxic insult thus somehow limiting the well-recognized use of anti-VEGF therapies against proliferative retinopathies. Therefore, considerable interest remains in developing new treatments for angiogenesis-associated retinal pathologies without intervening on VEGF protective action.

4. Pathological vessel proliferation: the case of retinopathy of prematurity

The incidence of angiogenesis-related diseases in the retina is rapidly increasing with increasing rate of risk factors as for instance metabolic disorders that have increased to epidemic proportions in industrialized countries. A typical example of hypoxia-driven pathology is the retinopathy of prematurity (ROP), a disease that recapitulates those events characterizing vascular proliferation in the retina, which, unless counteracted at their early stages, bring to potential irreversible blindness caused by retinal detachment. ROP is characterized by altered neurovascular growth of the retina in preterm immature infants, even though the interest has been historically focused on the vascular aspect of the disease [40]. Suppression of proangiogenic factors due to premature exposure to a relatively hyperoxic environment and loss of the maternal-fetal interaction results in an arrest of retinal vascularization followed by vascular regression from the 16th week of gestation to the 32–34th week. Over this period, increasing retinal metabolism is not fulfilled by still poorly developed vascularization thus progressively developing hypoxic environment, which leads to an overproduction of growth factors, particularly VEGF, that induce the growth, differentiation, and migration of endothelial cells to ensure adequate perfusion. Thus, an avascular disease driven by a premature exposure to a hyperoxic environment progressively becomes characterized by tumultuous retina vascularization in response to low oxygen tension (typically classified from stage 1 to stage 5) [41]. Waiting to understand if and how it is possible to dissociate (and thus modulate pharmacologically) the close link between oxygen levels and vascularity, limiting the use of oxygen immediately after birth represents the most effective strategy to reduce the onset of ROP, even though controlled oxygen administration reduces but does not eliminate ROP [42].

The greatest efforts in clinical research are directed towards the identification of the most effective treatments able to reduce the risk of retinal detachment [43]. Over the last decades, ROP therapy has evolved from cryotherapy and laser photocoagulation to the use of anti-VEGF agents for the treatment of severe ROP [44] although VEGF relevance in retinal maturation and microvascular maintenance has arisen some concerns about developmental consequences of anti-VEGF therapy [43]. In this respect, new ocular and systemic therapies are under investigation with partially proven results issues and much preclinical work is ongoing to identify novel therapeutics with minimal adverse effects for the treatment of ROP.

4.1. Mimicking ROP: the oxygen induced retinopathy (OIR) model

The scientific interest in ROP is also attributable to the availability of animal models capable of effectively mimicking the development of this disease, which have proved useful in defining the mechanisms by which abnormal angiogenesis occurs in the retina. In particular, the mouse model of OIR unequivocally demonstrates the fundamental role played by the different oxygen levels inside the retina and their close coupling with the vascularization processes [45]. In the typical OIR model, mice pups with their nursing mothers are exposed to high oxygen tension (75 % oxygen) between postnatal day (PD)7 and PD12, before return to room air between PD12 and PD17. Between PD7 and PD12, hyperoxia reduces the activity of HIF-1 (due to the degradation of its labile α subunit) and drives down expression of its target gene VEGF. Downregulated levels of VEGF result in reduced endothelial cell survival in immature vessels leading to large capillary obliteration in the central retina with the formation of an avascular zone. After returning to normoxia, perceived as relative hypoxia, the retina becomes immediately hypoxic because oxygen tension rapidly decreases from the capillary-depleted center to the periphery thus triggering the stabilization of HIF-1α and the increase in VEGF production. In the peripheral retina, the remaining capillaries and veins begin to form vascular sprouts that fail to regenerate the capillary network while they are likely to form leaky neovascular tufts towards the vitreous. Impairment of vascular permeability leads to retinal blood barrier breakdown that contributes to retinal dysfunction as demonstrated by altered electroretinogram (ERG) [46].

In Fig. 3, ROP stages are summarized in respect to the different phases of the OIR model in which oxygen manipulation allows to reproduce major events that characterize ROP at the aim to understand the mechanisms underlying retinal vessel proliferation with the final scope to intervene on them.

The adoption of an experimental model based on the deliberate manipulation of oxygen exposure indisputably demonstrates the decisive role played by oxygen levels in the modulation of retinal vascularization and consequently in the pathogenesis of ROP. In the OIR model, rodents pups are used for this purpose because their retina at birth is incompletely vascularized, thus mimicking the same condition as in preterm newborns. However, the similarity of the two models is partial, since the physiological vascularization of the retina of rodents

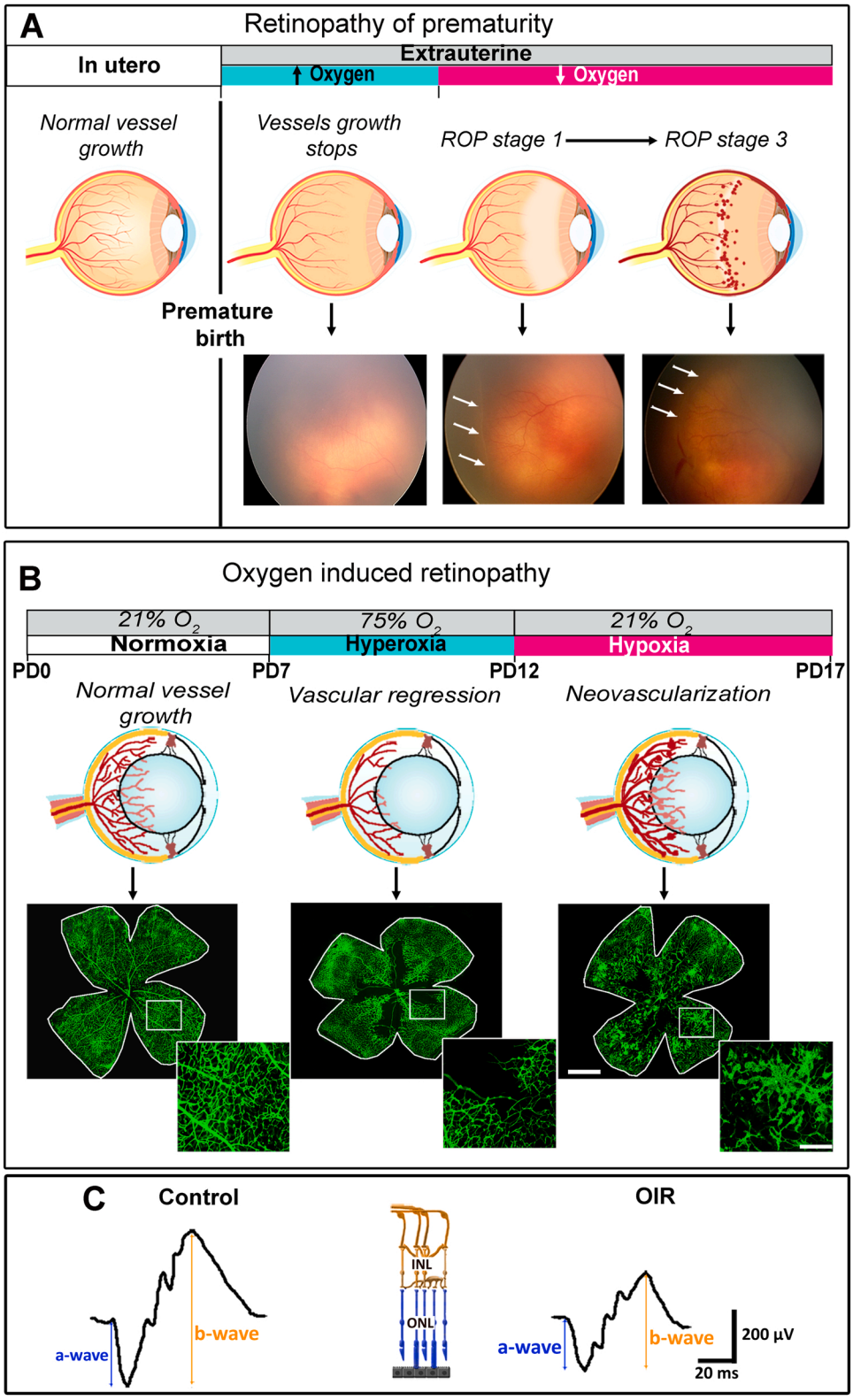

Fig. 3. ROP as mimicked by the mouse model of OIR. In panel **A**, ROP progression is schematically represented. In infants, after premature birth, retinal vessel growth stops thus reducing retinal oxygen tension that causes ROP stage1 characterized by a faint demarcation line at the junction between the vascularized and the avascular retina. Through stage 2 in which the demarcation line acquires the shape of a ridge, ROP reaches stage 3 characterized by abnormal growing vessels. RetCam fundus images showing the initial phase in which vessel growth stops after premature birth followed by ROP stages 1 and 3 in which arrows indicate the demarcation line that separates the avascular retina from progressively increasing retinal vessels. RetCam images have been generously provided by Dr. Anna Bendinelli of the Ophthalmic Surgery Division at the University of Pisa. In the OIR model represented in panel **B**, 7 days of environmental oxygen lead to normal vessel growth at PD7 while additional 5 days exposure to hyperoxia leads to large capillary obliteration in the retinal center with the formation of an avascular zone at PD12. The subsequent exposure to environmental oxygen sensed as hypoxia causes vessel proliferation in the midperipheral retina. Representative images of retinal whole mounts refer to PD7, PD12 and PD17 (scale bar:1 mm), with higher magnifications as insets (scale bar 100 µm). As shown in panel **C**, visual dysfunction in ROP is mimicked in OIR by drastically reduced amplitude of the a and b waves of the electroretinogram in respect to their control amplitude as indicated in the graphic representation together with the a and b wave generators in the retinal circuitry.

occurs in an extrauterine environment and therefore in normoxia [47], while the physiological vascularization of human fetuses occurs during intrauterine life, in a hypoxic environment [48]. Such difference explains why in order to experimentally arrest retinal vascularization in rodents it is necessary to expose rodent pups to oxygen concentrations significantly higher than those usually required for human preterm newborns in which premature exposure to atmospheric oxygen stops retinal vascularization. Despite this important distinction, the OIR model provides a clear demonstration of the role played by oxygen in modulating retinal vascularization.

Hypoxia-associated retinal neovascularization is not limited to ROP (as experimentally mimicked by the OIR model) but can be extended to much largely diffused retinal pathologies in which HIF-1 and its target genes have been shown to contribute to retinal neovascularization [49].

This is the case of human proliferative diabetic retinopathy (PDR), a serious condition that can cause blindness in diabetic patients. Though rodent models develop the most similar retinal lesions as those seen in early DR in humans, neovascularization, the hallmark of PDR which occurs at the later stages of the disease, cannot be mimicked by rodents due to their short lifespan [50]. In this respect, the OIR model is the most acknowledged model for proliferative retinopathies and it is widely used to explore new potential antiangiogenic therapies.

5. The beta-adrenoceptor system in the retina

Stress conditions, such as hypoxia, may cause cathecolaminergic overstimulation, which, in turn, activates adrenoceptors. In the retina of OIR mice, for instance, norepinephrine levels increase by about 90 % in hypoxic condition as compared to normoxia [51]. One possible source of norepinephrine are sympathetic nerve terminals directed to the choroidal circulation as removal of the superior cervical ganglion, which provides sympathetic input to the choroid, drastically reduces norepinephrine concentration in the retina [52]. This finding indicates that norepinephrine, originating from sympathetic nerve terminals in the choroid, reaches its receptors by paracrine diffusion. On the other hand, norepinephrine, since the first demonstration of its neurotransmitter role in the retina [53], has been localized to the inner retinal layers including the ganglion cell layer although its accumulation by dopaminergic amacrines has been also demonstrated [54]. Despite some role of noradrenergic neurotransmission in visual processing, the adrenergic signaling pathways mainly act as pivotal regulators of the sympathetic input to the retina with a predominant role as potent regulators of vascular function.

Among the receptors activated by norepinephrine, the β-AR family includes β1-, β2- and β3-ARs, acting through downstream pathways that are in part distinct and in part overlapped with β1-AR and β2-AR, which activate the stimulatory G protein leading to the activation of protein kinase A and its downstream signaling pathways [55]. Among β-ARs, β3-AR deserves a separate discussion either in terms of its molecular structure and its signaling complex or in terms of its function in response to hypoxia (see below).

5.1. The distinct players in the β-AR family

Since the first autoradiographic studies that had allowed to localize β-ARs to the mammalian retina [56], radioligand binding studies and additional Western blot analysis demonstrated β1- and β3-AR expression by human retinal endothelial cells [8], while β1-, β2- and β3-AR were immunohistochemically localized to human choroidal endothelial cells [57] with β1- and β2-AR also expressed by Müller cells isolated from the rodent retina [58]. Since then, the three β-ARs have been localized to distinct elements of the rodent retina with β3-AR located to the outer and the inner capillary plexus of the mouse retina [9] in which β2-AR-expressing Müller cells have been also identified with additional β2-AR-expressing cells presumably bipolars and amacrines [59]. The fact that β1-AR and β2-AR are indeed expressed by Müller cells, which are the main contributors to VEGF production [60], adds some functional implication about the role of these receptors in response to norepinephrine. In particular, hypoxia-induced norepinephrine increase has been shown to upregulate VEGF through β2-AR-induced accumulation of HIF-1α indicating a fundamental role of β-ARs in the molecular and physiological responses to hypoxia [61].

In the mouse retina, propranolol, a β1-/β2-AR non-selective blocker, effectively inhibits the hypoxia-induced increase in HIF-1α and VEGF expression and the consecutive neovascular response suggesting that β1-/β2-AR blockade is protective against retinal angiogenesis and blood-retinal barrier dysfunction [9]. In addition, propranolol has been shown to reduce mouse choroidal neovascularization [62] presumably through recruitment of monocyte-derived macrophages [63]. By the transitive property, β1-AR and β2-AR activation in response to hypoxia-induced norepinephrine accumulation would act as a proangiogenic switch by participating to the upregulation of HIF-1α and VEGF both of them playing a key role in the formation of pathogenic blood vessels. At variance with the protective efficacy of β-AR blockade in the hypoxic retina, norepinephrine loss in non-diabetic animals leads to retinal damages typical of DR [64] suggesting a key role of the noradrenergic pathway in the retina.

5.2. β1-/β2-AR blockade as a novel therapeutic approach in ROP

From preclinical studies demonstrating propranolol efficacy to clinical trials for ROP treatment the step was short as propranolol has already had great resonance as peak therapy against infantile hemangioma [65], a hypoxia-induced pathology, which is often associated with ROP in preterm infants. In clinical trials, oral propranolol has been demonstrated to slow down ROP progression [66–69] although some safety concerns have been evidenced [70]. In particular, a recent meta-analysis of randomized controlled trials verifying clinical efficacy and safety of propranolol in pre-term newborns with ROP reveals that oral propranolol is effective although its administration may have some potential safety issues [71]. On the other hand, repurposing propranolol administration as eye drops has been demonstrated to reduce ROP progression without compromising safety and tolerability [72,73]. In this respect, four study trials have been completed, three in Italy and one in Israel while one study trial at the University Hospital of Zurich (Switzerland) is still recruiting and it is estimated to complete in November 2024.

5.3. Propranolol efficacy

The mechanisms underlying the success of propranolol in the treatment of ROP remain elusive. The fact that in the OIR model propranolol-induced efficacy is mimicked by a β2-AR specific antagonist (ICI-118,551) while the β1-AR blocker atenolol has no effect on proangiogenic factors and pathogenic neovascularization indicates β2-AR as a major player in the angiogenic response to hypoxia [59] although its selective antagonism still remains at the preclinical level.

If the pathway from catecholamines to angiogenesis passing through β2-AR and HIF-1α can be affordable as β2-AR blockade reduces both HIF-1α levels and retinal vessel proliferation, then HIF-1 coupling to catecholamine release remains to be demonstrated. Although peripheral catecholamine release may also depend on orthosympathetic activation by central oxygen sensing, the possibility that oxygen sensing in the brain is associated with HIF-1-mediated orthosympathetic activation in response to hypoxia remains to be clarified. On the other hand, the finding that HIF-1 plays a critical role in the development of the sympathetic nervous system [74] suggests the possibility that HIF-1 may be involved in sympathetic function including its response to hypoxia.

How propranolol ameliorates hypoxia-induced retinal damage remains a less answered question. In OIR mice, HIF-1α and signal transducer and activator of transcription 3 (STAT3) are likely involved in the mechanisms that couple β-ARs to VEGF regulation as propranolol reduces VEGF upregulation by destabilizing HIF-1α and inhibiting STAT3 phosphorylation [9]. In this line, STAT3 involvement in β-AR signaling has been also demonstrated in models of cardiac hypertrophy in which STAT3 deletion reduces cardiac contractility [75]. The finding that propranolol counteracts retinal neovessel growth in response to hypoxia suggests the possibility that β-AR overstimulation may promote angiogenesis. In the case of sustained sympathetic drive, the pan-β-AR agonist isoproterenol points to benefit of downregulating β2-AR expression by preventing prolonged β2-AR activation through β-arrestin-2 recruitment and receptor internalization thus behaving like a β2-AR antagonist [76]. In the OIR model, for instance, isoproterenol ameliorates retinal angiogenesis by agonist-induced β2-AR desensensitization and downregulation thus supporting the notion that downregulated receptor signaling acts as a protective mechanism against excessive sympathetic

transmission [51].

That β-ARs are responsible of triggering retinal angiogenesis in response to hypoxia is further supported by the finding that in β1-/β2-AR knock out mice, hyperoxia-induced vaso-obliteration and the subsequent neovascular tuft overgrowth are almost completely prevented by β1-/β2-AR deletion suggesting that β1-/β2-AR overstimulation by increased catecholamine levels may potentially act as a proangiogenic switch in response to hypoxia [77]. This is in line with the anti-angiogenic efficacy of β1-/β2-AR blockade that functionally reflects on restored electroretinographic responses [46], as therapeutic interventions to prevent or recover severe visual impairment consequent to proliferative retinopathies are still limited.

Among the intracellular processes regulating endothelial cell homeostasis, a correct balance between autophagy and apoptosis is critically implicated in vascular function in response to stress conditions [78]. In this line, in the OIR model of neovessel proliferation, the efficacy of propranolol has been shown to be mediated by its coordinated action on apoptosis and autophagy [46]. Specifically, hypoxic retinas are characterized by an altered crosstalk between apoptosis and autophagy that results in decreased protective mechanisms essential for retinal cell survival in response to injury. Propranolol acts by regulating these processes in a coordinated manner thus reinstating apoptosis/autophagy balance, a critical step to determine the final response of the retina to low oxygen tension. Overall, these results demonstrate that β1-/β2-ARs are key regulators of apoptosis/autophagy and that propranolol efficiently protects the retina from hypoxic damage by preserving retinal cells from apoptotic death and increasing their protective mechanisms. In this respect, autophagy would represent an additional mechanism through which β-blockers act as potential therapies in pathologic conditions involving uncontrolled angiogenesis.

Whether propranolol directly acts on retinal cells expressing β1-/β2-ARs or exerts an indirect protective action by improving retinal vascularization remains to be established. In this respect, activated autophagy has been shown to mediate the efficacy of β-blockers in hemangioma-derived endothelial cells thus providing a mechanism through which propranolol restores angiogenesis in proliferative vascular diseases [79]. Activated autophagy has been also shown to mediate the efficacy of β3-AR activation in models of hypertrophic cardiomyopathies in which β3-AR overexpression exerts protective effects against hypertrophy promoting autophagic flux that counteracts harmful protein accumulation and reactive oxygen species production [80].

6. β3-AR characterization

Despite the major gap between β1-/β2-AR pharmacological characterization in the late sixties and the cloning and characterization of β3-AR in humans at the end of the eighties [81], studies on β3-AR role in diseased organs have been rapidly increasing with more recent investigations mainly focused on β3-AR function in ischemic conditions with a prevalent address to β3-AR role in cancer. As reviewed below, β3-AR profile is quite distinct from that of β1-AR and β2-AR and its peculiarities render it an attractive target for developing novel therapies against ischemic diseases. The low sensitivity of β3-AR to catecholamines characterizes its major role to bind noradrenaline secreted from sympathetic nerve endings when its concentration in the synaptic cleft is very high and/or when the high affinity β1-ARs are desensitized by prolonged sympathetic stimulation [82]. Additional characteristics differentiate β3-AR from β1-/β2-ARs. The human β3-AR is composed by a single peptide chain of 408 amino acid residues with a homology of 51 % and 46 % with β1-AR and β2-AR, respectively. β3-AR is made up by 7 transmembrane domains linked by 3 intracellular and 3 extracellular loops. Of the transmembrane domains, TM3, TM4, TM5, and TM6 together with the disulfide bond between extracellular loops 2 and 3 are essential for ligand binding and activity of the receptor. TM2 and TM7 are instead involved in the G-protein coupling [83]. In this respect, unlike β1- and β2-AR that activate more efficiently the stimulatory G protein, β3-AR can interchangeably couple to both stimulating and $G_{\alpha s}$ [84]. On the other hand, alternative coupling of β2-AR to inhibiting G proteins has been shown to play important roles in cardiac physiology [85,86].

Depending on the subunit of G proteins to which β3-AR is coupled, receptor activation can either stimulate or inhibit the adenylyl cyclase/cAMP pathway through $G_{\alpha s}$ or $G_{\alpha i}$ proteins, respectively. Coupling to inhibiting G proteins leads to nitric oxide (NO) production that results in cGMP accumulation by the soluble guanylyl cyclase [87]. The N-terminal region of β3-AR is glycosylated and exposed to the extracellular environment whereas the C-terminal is intracellular and, unlike β1- and β2-AR, lacks phosphorylation sites for protein kinase A (PKA) and G protein-coupled receptor (GPCR) kinases (GRKs) that are key regulators of GPCR signaling. This crucial difference renders β3-AR less incline to be desensitized through internalization/degradation, as it occurs for β1- and β2-AR [88]. However, desensitization of β3-AR may rather occur through transcriptional mechanisms or the down regulation of its downstream effectors [89].

6.1. β3-AR pharmacology: pros and cons

After an extensive review of β3-AR pharmacology by Ursino et al. [90], increasing preclinical investigations on β3-AR function made this receptor an interesting target for novel therapeutic approaches. However, β3-AR unique properties and its restricted expression in human tissues render difficult to extrapolate rodent findings to humans in which β3-AR is a less-understood subtype because of its late cloning and the relative disinterest as compared to β1-/β2-ARs [91].

The situation is additionally complicated by the fact that the gene encoding the human β3-AR is polymorphic thus requiring clinical trials with a large number of participants to compare therapeutic responses to β3-AR agonists across genotypes [92]. Unlike β3-AR gene polymorphism has been associated to overactive bladder [93], obesity [94] and insulin resistance [95], any indication on whether β3-AR polymorphism impacts angiogenic properties is far to be provided as further studies should be performed to demonstrate potential association of Trp64Arg mutation with receptor function. Another complication is that, unlike β1- and β2-AR, the expression of β3-AR in humans has often resulted barely above the detection limit in healthy tissues [96], while its expression significantly increases in pathological conditions associated to hypoxia as, for instance, in the human failing myocardium [97]. Finally, the limited specificity of β3-AR ligands and the fact that the binding pocket apparently differs between the human and rodent receptor have constrained the development of pharmacological applications targeting β3-AR [91]. On the other hand, clinically used drugs such as mirabegron and vibegron have a pretty good selectivity in humans and almost as good in rodents [98,99]. In addition, the β3-AR agonist CL 316,243 has a fairly good selectivity in preclinical studies using rodents [100]. In addition, new developments in β3-AR pharmacology should be expected thanks to the recent characterization of the β3-AR-G_s signaling complex by cryo-electron microscopy [101,102].

At the preclinical level, β3-AR pharmacology has been used to demonstrate the functional role of β3-AR in models of pathologies characterized by catecholaminergic overstimulation in which β3-AR antagonism may represent a useful tool to counteract the adverse cathecolaminergic environment. The additional finding that β3-AR activation may positively correlate with healthy conditions suggests that β3-AR likely serves as a buffer or “rescue” function from the effects of excess in catecholamines thus acting as a kind of auto-break for the sympathetic tone [103,104]. β3-AR has been primarily localized to the adipose tissue where it has traditionally been considered as a metabolic receptor involved in the lipolysis and thermogenesis [105,106]. After activation by norepinephrine released by sympathetic nerve endings, β3-AR, couples to $G_{\alpha s}$ protein and triggers the cAMP-PKA pathway thus promoting the activity of lipid droplet protein such as perilipin and hormone-sensitive lipase that initiate the lipolytic process. Interestingly,

the adrenergic activation of lipases can also occur *via* β3-AR coupling to $G_{\alpha i}$-protein and consequent initiation of the ERK1/2 MAP kinase cascade [107]. Strong preclinical evidence converges to the demonstration of β3-AR role in obesity and diabetes [108] with particular emphasis to the finding that β3-AR agonism increases the energy expenditure mainly in the brown adipose tissue, thus promoting the reduction of adiposity, the prevention of ectopic fat accumulation, and the decrement in insulin resistance [109]. While preclinical findings encouraged to explore the potential role of β3-AR agonizts as anti-obesity and anti-diabetic agents, clinical studies ultimately failed to achieve the desired effects. Data on the efficacy of β3-AR agonism are indeed derived from small pilot studies, usually not placebo-controlled [110,111] thus requiring to be confirmed in larger randomized controlled trials. The lack of potency and selectivity of β3-AR agonizts, which still need drug approval, their questionable role in brown adipose tissue, which is present in limited amount in adult humans and the additional fact that obesity is associated with diminished β3-AR expression and decreased β-AR responses might explain clinical failure of β3-AR agonizts in obesity [112]. On the other hand, the recent finding that counteracting epigenetic suppressors of β3-AR expression in adipocytes can recover β3-AR levels, thereby reinstating their responsiveness to β-adrenergic stimulation, is highly suggestive of the possibility that recovering molecular axis leading to enhanced β-adrenergic signaling may result in an overall increase in energy expenditure to prevent fat accumulation and obesity thus increasing therapeutic options for treating obesity and related metabolic diseases [113].

Additional localization studies in the urinary system had a more successful epilogue with β3-AR located to nephron segments sensitive to vasopressin where its activation allows the reabsorption of salts and water [114]. In addition, β3-AR antagonism improves the renal function in a mouse model of polycystic kidney disease in which β3-AR overexpression is in line with what found in human biopsies of polycystic kidneys [115]. Finally, β3-AR localization to the urinary bladder, the fact that its activation strikingly promotes the myorelaxation of the detrusor muscle and the increment in bladder capacity allowed to develop β3-AR agonism as the primary strategy in the treatment of overactive bladder [116]. At a very advanced stage is also the evidence of the role of β3-AR in the mammalian cardiac tissue in which the discovery of its transcript in the early 90's prompted international effort to decipher the β3-AR role in cardiovascular physiology and pathology [87] including an extended view of β3-AR-dependent cardiac modulation in non-mammalian vertebrates [117]. Particular interest originates from the finding that β3-AR is overexpressed in the endothelium of human coronary arteries of the ischemic heart in which its activation leads to increased generation of NO, a key mediator of heart protection through mechanisms involving endothelial cell proliferation and vasodilation [118]. In this respect, β3-AR has been assumed as a promising therapeutic target for the treatment or prevention of cardiovascular diseases [119] and its specific agonist, mirabegron, when chronically administered, appears to be rather effective in heart failure as demonstrated by the SPHERE-HF trial, which, unfortunately, missed the primary endpoint of β3-AR-mediated decrease in pulmonary vascular resistance [87,120]. β3-AR relatively low tendency to desensitization as it lacks the consensus motif for GRK-mediated phosphorylation renders β3-AR agonists as potential appealing drugs for a prolonged pharmacological stimulation. On the other hand, β3-AR pharmacology turned out to be less simple than expected. Many agonists and antagonists classically considered as being β3-AR-selective actually are not and much work is still in progress to unravel the knots. In the first generation of β3-AR agonists, BRL 37344, although displaying higher selectivity towards β3-AR as compared with β1- and β2-AR in transfected CHO cells [121], may activate also β2-AR as demonstrated by the finding that part of its relaxation effects on bovine iris and ciliary is inhibited by β2-AR antagonism [122]. Similarly, the myorelaxant role of the β3-AR agonist mirabegron on rat urinary bladder and porcine ureteral contractility has been recently questioned since either β2-AR agonism or α1-AR antagonism seem also to participate to its relaxation responses [123,124]. However, these effects were not identified in human tissues, and not on bladder specifically. As compared with mirabegron, vibegron displays high specificity to β3-AR and reduced specificity against β1- and β2-AR in transfected CHO and HEK cells [125]. Within β3-AR agonists, GW427353 or solabegron may evoke bladder relaxation and facilitate bladder storage in dogs [126] while SAR150640 regulates myometrial inflammation in humans suggesting its potential therapeutic relevance to the treatment of uterine inflammatory conditions [127]. In addition, CL-316,243, although selective for β3-AR and efficiently acting on thermogenesis and energy expenditure, cannot be translated to human medications as its selectivity is much higher at the mouse than at the human β3-AR [100].

Clinical trials aimed at evaluating the efficacy of β3-AR agonists on overactive bladder and ischemic heart are summarized in Table 1. As mirabegron is on the bench site yet to cure overactive bladder, ongoing clinical trials using vibegron have been included in the Table. In three additional clinical trials, mirabegron efficacy has been compared with that of anti-muscarinic receptor drugs including solifenacin succinate and tolterodine tartrate both used in the overactive bladder before the advent of mirabegron. In the ischemic heart, four clinical trials have been included. Of them BEAT-HF, BEAT-HF II and SPHERE-HF results have been published [120,128,129] while the results of Beta3_LVH have not yet published although a preliminary report has been presented to American Heart Association Scientific Session 2022 [130]. Despite growing interest on β3-AR agonists that are already on the market, more-selective agents with higher specificity may be hopefully designed based on the recently characterized cryo-electron microscopic structure of the β3-AR -G_s signaling complexes with the selective agonists mirabegron and solabegron in comparison with the nonselective agonist, isoproterenol [101,102].

The situation is more complicated for β3-AR antagonists that have revealed important function of β3-AR in cardiovascular diseases although exhibiting many degrees of freedom in terms of selectivity and specificity. Among β3-AR antagonists, SR59230A exerts beneficial effects on ventricular function in rat models of heart diseases in which alleviates pulmonary arterial hypertension [131]. On the other hand, SR59230A has been found to display partial agonist properties in β3-AR transfected cells although its selectivity has been questioned in light of its affinity also for β1, β2 and α1-ARs [100]. In a pig model, L-748,337 has been recently shown to attenuate dobutamine-induced cardiac inefficiency without ameliorating the vascular resistance as expected by its inhibitory effect on NO signaling [132]. In this respect, L-748,337 has been shown to provide a higher selectivity for β3-AR than SR59230A with apparently lower affinity for the rat than the human β3-AR [133,134]. Together, except for their application to counteract tumor growth, the success of β3-AR antagonists has been very limited. Generally, receptor antagonists have different structure-activity relationships than agonists for which their ligands can be used as a starting point. In this respect, the design of specific β3-AR antagonists is complicated by the fact that their layout is based on prototypical β-AR blockers such as propranolol, whose affinity for β3-AR is much lower than that for β1- and β2-ARs [135].

7. β3-AR in the retina

7.1. β3-AR localization

Soon after the identification of the β1-/β2-AR role in the retina, it became clear that some apparently β-AR-mediated responses did not fit either of these two subtypes [136]. In concomitance, the promising findings on β3-AR role in tissue ischemia laid the foundations for investigating β3-AR function in the diseased retina. Localization studies of β3-AR in the retina have been limited by several factors including its low expression in the rodent retina unless in response to ischemic conditions [9,137], the poor availability of data on β3-AR expression in the

Table 1
Ongoing clinical trials on overactive bladder and completed clinical trials on heart failure in which the efficacy of β3-AR agonists has been tested. Data are derived from clinicaltrials.gov database (updated February 9th, 2023).

NCT Number	Title	Conditions	Interventions	Phase	Study start	Expected end	Study results
NCT 03902080	Study to Evaluate the Efficacy, Safety and Tolerability of Vibegron in Men with Overactive Bladder (OAB) Symptoms on Pharmacological Therapy for Benign Prostatic Hyperplasia (BPH)	Overactive Bladder	Vibegron Placebo	Phase: 3	March 2019	June 2023	No Results Available
NCT 05067478	Composure: Study to Understand the Performance of Vibegron in Participants with Overactive Bladder (OAB)	Overactive Bladder	Vibegron		October 2021	December 2023	No Results Available
NCT 05491525	A Study of Vibegron in Pediatric Participants 2 Years to Less Than (<) 18 Years of Age With NDO and on CIC (KANGUROO)	Neurogenic Detrusor Overactivity	Vibegron	Phase 2 Phase 3	August 2022	September 2027	No Results Available
NCT 03632772	Comparison of Solifenacin and Mirabegron in Treatment of Overactive Bladder Symptoms in Men After TURP	Overactive Bladder	Solifenacin Mirabegron	Phase 2	August 2018	July 2019	No Results Available
NCT 04693897	Effect of beta3-adrenoceptor Agonist on Patients with Overactive Bladder and as a Urinary Biomarker	Overactive Bladder	Solifenacin Mirabegron		March 2021	December 2022	No Results Available
NCT 05362292	Treating Incontinence for Underlying Mental and Physical Health (TRIUMPH)	Overactive Bladder	Tolterodine Tartrate Mirabegron Placebo	Phase 4	October 2022	December 2026	No Results Available
NCT 01876433	Beta 3 Agonist Treatment in Heart Failure (BEAT-HF)	Chronic Heart Failure With Reduced Ejection Fraction (HFrEF)-NYHA class II–III	Mirabegron Placebo		September 2013	September 2015	[128]
NCT 02775539	Beta3 Agonist Treatment in Chronic Pulmonary Hypertension Secondary to Heart Failure (SPHERE-HF)	Pulmonary Hypertension Heart Failure Pulmonary Vascular Resistance Abnormality	Mirabegron Placebo	Phase 2	June 2016	June 2019	[120]
NCT 03926754	Beta 3 Agonist Treatment in Heart Failure-2 (BEAT-HF II)	Heart Failure With HFrEF-NYHA Class III-IV	Mirabegron	Phase 2 Phase 3	January 2017	January 2021	[129]
NCT 02599480	Assessment of Efficacy of Mirabegron, a New beta3-adrenergic Receptor in the Prevention of Heart Failure (Beta3_LVH)	Hypertrophy, Left Ventricular	Mirabegron	Phase 2	April 2016	September 2022	[130]

posterior segment of the human eye and the low target selectivity of antibodies raised against GPCR, including β3-AR. In this respect, the literature on antibody-based findings including immunohistochemical studies is highly contaminated by methodological flaws as many β3-AR antibodies fail to display relevant target selectivity when tested with stringent criteria [138]. As an additional limitation, some of these antibodies exhibit specificity between rodent and human β3-AR thus possibly leading to false-positive results. Since the early demonstration that β3-AR plays a role in the eye by evoking relaxation of bovine iris sphincter [139], investigations on the role of the β3-AR in the retina started much later when β3-AR had been just proven to mediate adrenaline-induced vasodilation of retinal arterioles in rat models [136]. At that time, the observation that in the retina, β3-AR responds to hypoxia with a relevant upregulation of its expression in the growing endothelium of engorged retinal tufts [9] was in line with what found in failed hearts in which β3-AR is upregulated in response to ischemic conditions [87]. In Fig. 4, β-AR localization to distinct retinal elements is shown with β1-/β2-AR located mostly to Müller cells while β3-AR to endothelial cells of the superficial vascular plexus. In hypoxic conditions, Müller glia displays reactivity in a process termed gliosis characterized by the upregulation of cytoskeletal proteins. Gliotic Müller cells produce VEGF that promotes neovessel proliferation with the formation of engorged retinal tufts to which dense β3-AR immunolabeling is localized.

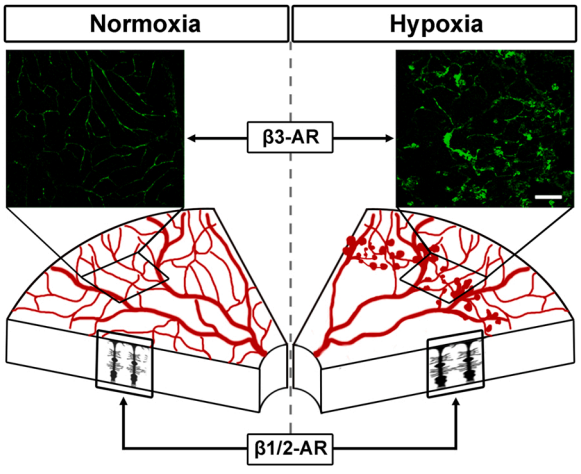

Fig. 4. β-AR localization to the retina. In respect to β1-/β2-AR localization to Müller cells, β3-AR is barely expressed by the superficial vascular plexus in environmental oxygen. As oxygen tension decreases, Müller cells become gliotic thus releasing VEGF that promotes the formation of engorged vascular tufts where dense β3-AR immunolabeling is localized. High magnifications of β3-AR immnolabeling correspond to the boxed areas of the superficial vascular plexus while Müller cells either normal or gliotic are schematically represented in the retinal thickness. Adapted from Ristori et al. [9] in which β3-AR immunolabeling was detected using the goat polyclonal antibody sc-1473. Scale bar: 100 μm.

7.2. *β3-AR function*

Since the landmark paper of Ristori et al. [9], much work has been performed at the aim to answer the question of whether β3-AR upregulation in the hypoxic retina may serve as a compensatory mechanism to counteract hypoxia-associated retinal damage or as a crucial event

triggering hypoxia-induced angiogenic processes. The finding that β1-/β2-AR blockade with propranolol efficiently counteracts hypoxia-induced retinal damage seems to exclude a proangiogenic role of β3-AR since propranolol affinity to β3-AR is about two log units lower than that of β1- or β2-AR [91] and speaks in favor of a major contribution of β2-AR as also demonstrated by the efficacy of its selective antagonism with ICI-118,551 [59]. On the other hand, the finding that in hypoxic retinal explants of mice β3-AR antagonism with SR59230A inhibits VEGF release presumable through the NO pathway speaks in favor of β3-AR role in angiogenesis [140]. This finding was contradicted by the fact that in the OIR model, SR59230A does not affect hypoxia-induced proangiogenic factors, including VEGF [59] thereby raising the issue of whether β3-AR upregulation may have a functional role in the hypoxic retina. The discrepancy between *in vivo* and *ex vivo* findings can be explained by assuming that, at variance with retinal explants in which quantifiable amounts of SR59230A are locally administered, in OIR mice, systemic administration of that antagonist may not provide the retina with a sufficient drug concentration to interfere with VEGF release. On the other hand, the fact that SR59230A affects PKA activity both in the retina of OIR mice and in hypoxic retinal explants speaks in favor of its comparable efficacy in the two models [59]. Further complications arise from the fact that SR59230A, beside acting as a partial agonist, may not suppress the receptor constitutive activity, which is typical of β3-AR [141]. Additional findings in favor of β3-AR role in angiogenic processes include the fact that β3-AR activation increases the expression of proangiogenic factors in *in vitro* preparations of human retinal and choroidal endothelial cells [8,57]. In addition, potential proangiogenic effects of β3-AR agonism have been recently demonstrated in human patients suffering from ocular angiogenesis in the posterior segment of the eye in which systemic mirabegron has been found to cause increased thickness and vascularity of the choroidal layer [142]. The additional finding that β3-AR agonism causes neovessel growth in response to hypoxia in the absence of β1- and β2-AR as in β1-/β2-AR knock out mice, suggests that β3-AR activity may replace β1-/β2-AR function [77]. In this line, one can assume that β1-/β2-AR may normally mask β3-AR activity suggesting a redundancy of the β-AR system that, through β3-AR, may provide the retina with a sort of compensation to the absence of β1-/β2-AR. The same applies to canine models of heart volume overload in which the cardiac β3-AR becomes functionally effective after β1-/β2-AR downregulation [143]. Recent evidence in the endothelium of the rat cremaster muscle artery demonstrates that β3-AR-mediated vasodilation is only evident after blockade of β1-/β2-AR suggesting that β1-/β2-AR activity may somehow inhibit β3-AR function [144].

Some possibility to identify the role of β3-AR in the retina has emerged from findings that have debated the anti-angiogenic efficacy of propranolol in OIR mice thus raising the issue of whether β1-/β2-AR blockade may be a therapeutic approach for treating ROP. Propranolol, in fact, proved ineffective in mice of the 129S6 strain that is indeed characterized by a massive response to hypoxia as compared to the C57BL/6J strain that is, instead, responsive to β1-/β2-AR blockade [137]. Interestingly, in mice of the 129S6 background, β-AR activity is higher than in mice of the C57BL/6 strain and this may explain the predisposition of 129S6 mice to develop a more aggressive neovascularization. Even more interestingly is that the 129S6 strain is characterized by a dramatic upregulation of the β3-AR messenger in response to hypoxia indicating some role of this receptor in the massive neovessel formation and suggesting the possibility that unresponsiveness to β1-/β2-AR blockade might be related to exceeding presence of β3-AR [145]. In this respect, the expression of β3-AR is lower in biopsies from IH patients responding to propranolol than in biopsies from non-responders, suggesting a possible association between β3-AR overexpression with nonresponse to propranolol [146].

The possibility that β3-AR may play a role in angiogenesis-related retinal diseases and may represent a drug target in ophthalmology has been previously questioned [147] although its role as an attractive therapeutic target for the treatment of ischemic retinal diseases has been recently reconsidered [148]. On the other hand, the restricted expression pattern of β3-AR in comparison to that of the other subtypes and the paucity of data about β3-AR expression and function in the human retina renders even more difficult the extrapolation of findings from rodent models to ophthalmological applications. Furthermore, β3-AR coupling to both $G_{\alpha s}$ and $G_{\alpha i}$ [84] therefore activating multiple signaling pathways, strongly limits the effectiveness of β3-AR ligands as the same compound may act as a biased agonist by activating a given pathway while antagonizing another signaling response [149]. Beside the limitations of pharmacological approaches targeting β3-AR that may prevent further investigation about β3-AR function in the retina, another open question is how β3-AR expressed by engorged retinal vessels of the OIR model may affect VEGF release in response to hypoxia. Although a major source of retinal VEGF is represented by Müller cells, particularly under hypoxic-ischemic conditions [150], there is some evidence that retinal endothelial cells express and release VEGF [151] indicating the possibility that β3-AR may directly influence VEGF release. Aside from VEGF sources, β3-AR, by modulating VEGF release, would not only participate to hypoxia-driven vessel proliferation but would also serve to regulate neuroprotective action of VEGF. In fact, in the mouse hypoxic retina, β3-AR antagonism increases retinal cell death presumably through decreased VEGF production while β3-AR agonism reduces apoptotic profiles through increased VEGF release that acts as a neurotrophic factor [55]. Independently on VEGF mediation, β3-AR agonism has been demonstrated to reduce N-methyl-D-aspartate-(NMDA)-induced damage in the rat retina suggesting protective efficacy of β3-AR activation on NMDA-associated excitotoxicity [152]. Direct neuroprotective effects on retinal neurons would imply their expression of β3-AR, a fact yet to be proven. In this respect, β3-AR localization to neuronal structures has recently highlighted its role as a potentially critical target in the adrenergic modulation of brain processes. Since the first indication of anxiolytic- and antidepressant-like activities of β3-AR activation that involve an increase of serotonergic and noradrenergic neurotransmission [153], additional findings have been provided about the role of β3-AR in the modulation of high brain function. In the rat hippocampus, for instance, the activation of functional β3-AR in CA1 neurons participates to increased neuronal excitability that is crucial in cognition and memory [154]. The recent evidence that in a mouse model of Alzheimer's disease, β3-AR agonism improves memory deficits in concomitance with ameliorated metabolic and thermoregulatory defects provides some arguments for repurposing β3-AR agonists in neurodegenerative diseases [100]. In addition, in the rat prefrontal cortex, β3-AR activation is capable of recovering stress-induced recognition memory defects [155] while in the mouse cerebellum, β3-AR expressed by Purkinje cell soma and dendrites participates to the adrenergic modulation of motor learning [156].

7.3. β3-AR as a HIF-1 target gene: indirect evidence

β3-AR upregulation in response to ischemic/hypoxic conditions likely suggests the possibility that β3-AR participates to oxygen sensing in the mouse retina. That β3-AR responds to low oxygen tension is also supported by early findings demonstrating its high expression in the initial hypoxic phase of the rat developing embryo until to decrease over time [157]. When oxygen increases after birth, β3-AR maintains a restricted expression [96] unless in association with hypoperfusion when β3-AR levels drastically increase in response to ischemic conditions. There are several examples of β3-AR upregulation in response to ischemic insult: in proliferating tumor cells, in ischemic heart and in the hypoxic retina where β3-AR is expressed by angiogenic vessels [158] all indicating that β3-AR participates to oxygen sensing. In the mouse retina, β3-AR expression decreases progressively over the first postnatal week following a developmental time course comparable to that of HIF-1α and VEGF, which are both regulated by oxygen levels. During the intrauterine life, retinal hypoxia, which precedes the appearance of

retinal vessels, induces VEGF production by astrocytes that enter the retina through the optic nerve head. VEGF attracts growing endothelial cells as they migrate radially from the optic disc. VEGF accumulation by astrocytes is further enhanced by HIF-1α production by retinal precursor cells in response to hypoxia [159] that interact with epithelial cells to induce their differentiation [160]. Detailed information about the developmental time course of the HIF-1/VEGF axis and β2-/β3-AR expression in relation to the maturation of retinal vessels are provided by the unpublished results in Fig. 5.

According to Stahl et al. [47], the superficial vessels increase in density from the perinatal phase to PD8/10 when vascular growth in the superficial plexus is almost completed as shown by the diagrammatic representation of retinal whole mounts at different postnatal stages. Retinal vessel maturation occurs in concomitance with a progressive increase of retinal oxygenation that causes a concurrent decrease of HIF-1α and VEGF expression as determined by Western blot analysis of HIF-1α/VEGF proteins and their quantitation. The fact that vessel appearance coincides with HIF-1/VEGF reduction in response to postnatal oxygen increase suggests the possibility that the development of retinal vascularization would require an intrauterine hypoxic phase in which VEGF production precedes vessel formation and an extrauterine phase in which oxygen increase would promote endothelial cell differentiation. In this respect, increasing oxygen levels have been shown to promote the differentiation of progenitor cells into mature endothelial cells [162]. As also shown in Fig. 5, in line with the postnatal decrease of HIF-1α/VEGF expression, but in contrast with the time course of retinal vessel maturation, β3-AR expression, which is extremely high in the hypoxic intrauterine phase, gradually declines after birth when the litters are exposed to environmental oxygen thus suggesting β3-AR participation to the HIF-1/VEGF axis. The absence of vasculature in concomitance with high levels of β3-AR argues against β3-AR localization to retinal vessels where, in any case, it is barely expressed unless in the presence of low oxygen tension. One possibility is that in the prevascular phase, β3-AR is expressed by circulating endothelial precursor cells (EPCs) that are known to migrate from blood circulation to neovascular sites where they differentiate into endothelial cells to promote the formation of new blood vessels. In this line, previous findings from infantile hemangiomas have demonstrated β3-AR localization to EPCs of the tumor microenvironment [146] where β3-AR may likely participate to EPC recruitment to form vessel-like structures as also demonstrated in human melanoma cells [163]. As an additional possibility, β3-AR might be localized to retinal astrocytes in line with previous findings demonstrating β3-AR expression by astrocytes in the mouse brain [164]. As also evidenced in Fig. 5, in contrast to β3-, β2-AR

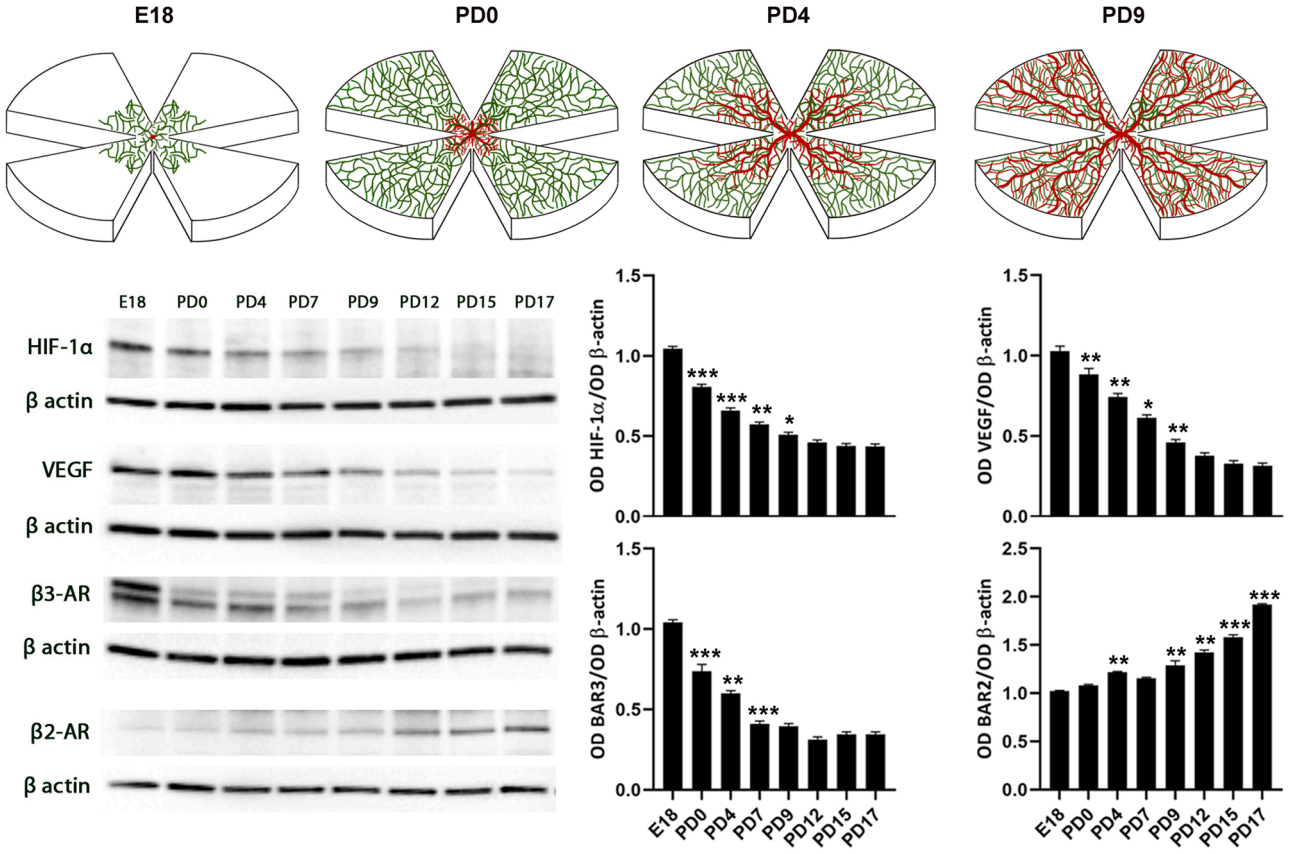

Fig. 5. Developmental expression of β2- and β3-AR in respect to progressively decreasing levels of HIF-1α and VEGF from embryonal (E) 18 to PD 17 in the mouse retina. Schematic representation of developing retinal vasculature. Vessel development is preceded by an invasion of migrating astrocytes that at E18 are found to emerge from the optic nerve head and spread in a centrifugal fashion across the inner surface of the retina, forming a cellular network that provides a template for the blood vessels in their wake (green). Sprouting astrocytes guide the maturation of retinal vessels (red) with a relatively uniform plexus in the central retina at birth (PD0) that progressively expands toward the periphery at PD4 to cover the entire inner surface at PD8. Protein levels, as represented by blots with their densitometric analysis, have been evaluated at distinct time windows characterized by increased vessel density in the superficial plexus. Increased density of retinal vessels is inversely correlated with decreasing expression of HIF-1α/VEGF/β3-AR while it is directly related to increasing levels of β2-AR. Protein levels of HIF-1α, VEGF, β3-AR, β2-AR and β were detected using the rabbit polyclonal antibody ab2185, the rabbit polyclonal antibody ab9570, the mouse monoclonal antibody sc-515763, the mouse monoclonal antibody sc-81577 and the mouse monoclonal antibody A2228, respectively (for Refs. see [10,161]). N = 4 retinas for each experimental condition, one-way ANOVA with Tukey's post-hoc test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to the previous value. Experiments were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, the EU Directive (2010/63/EU), and the Italian guidelines for animal care (DL 26/14; Permission number: 132/2019-PR).

expression progressively increases along a progressive decrease of HIF-1α and VEGF with a specular trend in respect to that of β3-AR suggesting that the two β-ARs play a different role depending on the different stages of postnatal retinal maturation. The fact that increased β2-AR expression occurs after the development of the superficial vascular plexus is completed and in concomitance with steady oxygen levels suggests β2-AR minor role in retinal vessel maturation. On the other hand, β2-AR increased expression from PD10 on may rather reflect the time course of maturation of Müller cells to which β2-AR is localized and where this receptor can be associated to VEGF production. Given the opposite time-dependent expression of β2- and β3-AR, it is legitimate to speculate that in the mature retina, β2-AR would mediate retinal vessel proliferation in response to ischemic conditions through VEGF production by Müller cells. In contrast, in the immature retina, hypoxia-dependent β3-AR accumulation would participate to trigger a cascade of events leading to the early phases of vessel development.

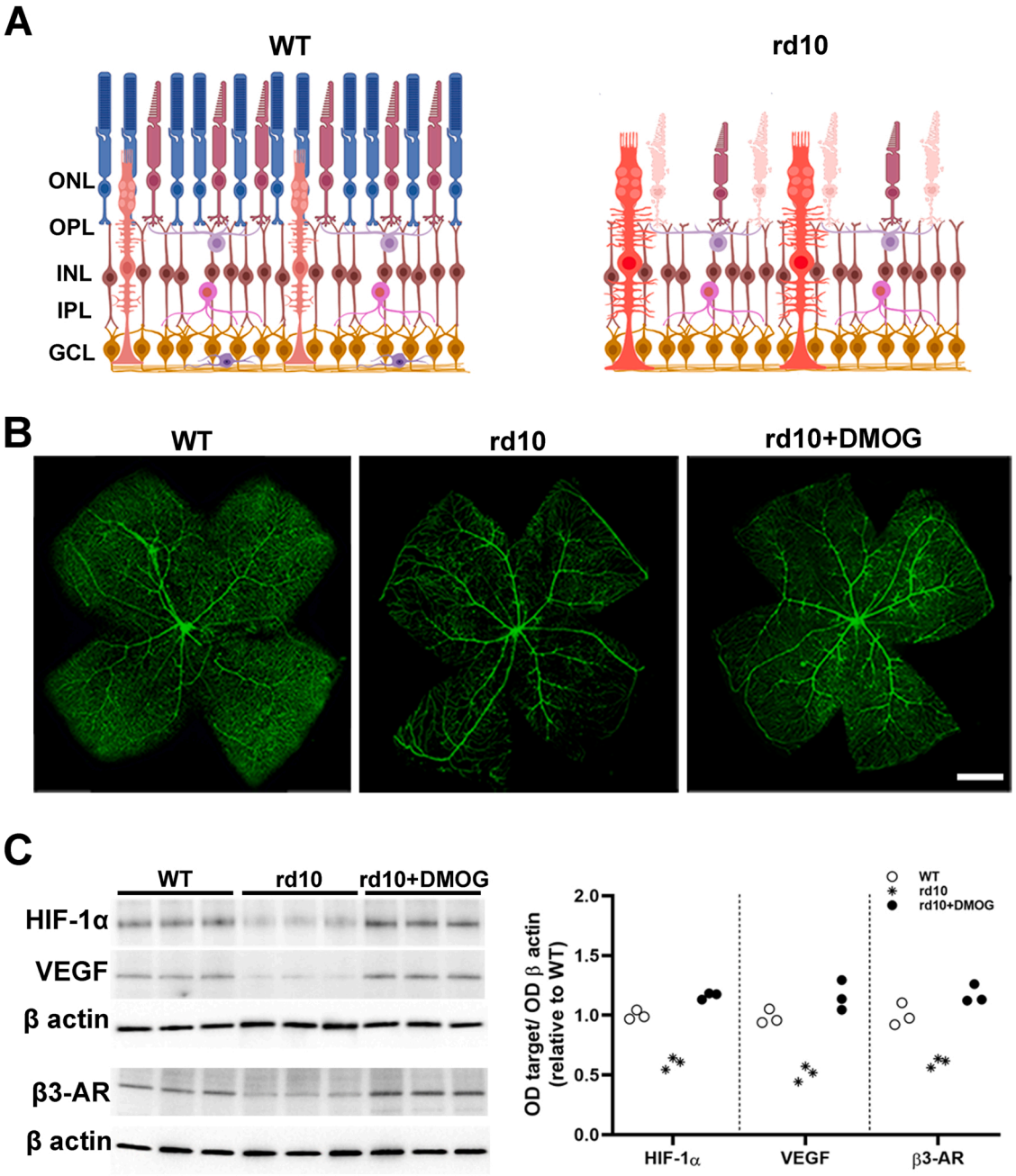

Fig. 6. β3-AR expression in the mouse rd10 retina. In panel **A**, schematic representation of photoreceptor loss that characterizes the retina of the rd10 model. In panel **B**, representative images of the superficial vascular plexus of retinal whole mounts stained with the endothelial cell marker IB4 from WT and rd10 mice either untreated or treated with DMOG. In panel **C**, representative blots with their densitometric analysis showing protein levels of HIF-1α, VEGF and β3-AR as evaluated by Western blot in retinal extracts. Protein levels of HIF-1α, VEGF, β3-AR and β actin were detected using the rabbit polyclonal antibody ab2185, the rabbit polyclonal antibody ab9570, the mouse monoclonal antibody sc-515763 and the mouse monoclonal antibody A2228, respectively (for Ref. see [10]). β-actin was used as the loading control. As compared to the WT retina, in the rd10 retina, hyperoxia leads to decreased vessel density that is recovered by DMOG administration reaching a consistence almost comparable to that in WT. In panel **C**, variations in vessel density are strictly related to corresponding variations in the level of HIF-1α that coincides with the course of both VEGF and β3-AR with decreased levels in rd10 and recovered levels after HIF-1α stabilization with DMOG. Scale bar: 1 mm. N = 3 retinas for each experimental condition. Experiments were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, the EU Directive (2010/63/EU), and the Italian guidelines for animal care (DL 26/14; Permission number: 132/2019-PR).

Additional results of Amato et al. [10] from the OIR model further support oxygen dependence of β3-AR expression with β3-AR upregulation by hypoxia specularly to its downregulation by hyperoxia thus mirroring HIF-1α and VEGF trend of expression. In addition, decoupling HIF-1α from oxygen tension with dimethyloxalylglycine (DMOG), a widely used HIF-1α stabilizer, prevents β3-AR and VEGF downregulation in response to hyperoxia thus inhibiting their upregulation in response to hypoxia and supporting the possibility of a close correlation between HIF-1α levels and β3-AR transcription. This is further supported by additional findings from the rd10 mouse model of retinitis pigmentosa (RP, unpublished data in Fig. 6). In this model, photoreceptor degeneration is induced by *Pde6b* mutation with massive rod loss that precedes cone death [165,166], as schematically represented in Fig. 6**A**. Photoreceptor loss causes less oxygen consumption by retinal cells with the establishment of a hyperoxic environment that reduces HIF-1α and VEGF levels leading, successively, to vessel attenuation [167] as demonstrated by the qualitative evaluation of isolectin B4 (IB4) staining of retinal vessels. Retinal vasculature is partially recovered after inhibiting HIF-1α degradation by DMOG (Fig. 6**B**) in concomitance with restored levels of HIF-1α, VEGF and β3-AR (Fig. 6**C**) suggesting the possibility that β3-AR participates to retinal vessel formation.

7.4. β3-AR as a HIF-1 target gene: direct demonstration

To address the possibility that HIF-1 regulates transcriptionally β3-AR expression, a previous study in the rodent β3-AR gene failed to identify a functional HRE with which HIF-1 could physically interact [168] suggesting that hypoxia-induced β3-AR upregulation may derive exclusively from the post-transcriptional regulation of β3-AR mRNA and/or from the stabilization of β3-AR protein. On the other hand, the determination of HRE may be limited by its highly variable flanking sequences in contrast to that of the HBS characterized instead by highly conserved nucleotide sequence. A recent *in silico* analysis in the β3-AR gene has allowed to identify 6 different potential HBSs. Of them, in the OIR model, HBS#1 has been demonstrated to physically interact with HIF-1 shortly after hypoxia onset when HIF-1α is maximally upregulated thus leading to drastic β3-AR expression [10] in line with the time course of VEGF transcription by HIF-1 [169,170]. Fig. 7 summarizes the hypoxia-associated cascade of events leading to the synthesis of β3-AR after its transcriptional regulation by HIF-1.

In addition to transcriptionally activate β3-AR under hypoxia, HIF-1 is also coupled post-transcriptionally to β2-AR upregulation under hyperoxia. As mimicked by the mouse rd10 model of RP, preventing HIF-1α drop in response to hyperoxia counteracts the upregulation of β2-AR without intervening on its transcription [161]. In particular, hyperoxia-associated low levels of HIF-1α result in β-arrestin 2 downregulation that uncouples β2-AR from G proteins thus resulting in β2-AR accumulation at the cell membrane that may serve as a compensatory mechanism to counteract reduced blood flow under hyperoxia. In addition to HIF-1-dependent regulation of angiogenesis-associated genes, HIF-1-independent mechanisms are also involved in hypoxia-induced gene expression. For instance, upon VEGF stimulation, the VEGF receptor 2, the predominant receptor in angiogenic signaling, translocates to the nucleus where activates its own promoter independently on HIF-1-related transcriptional mechanisms [171].

8. β3-AR as a putative intermediary between oxygen levels and vessel proliferation

Despite the peculiar features that would make of β3-AR a promising target for drug development, β3-AR still remains the black sheep of the β-AR family as compared to the success of β1- and β2-AR as well-established targets for cardiovascular and respiratory drugs. Of the one side, β3-AR low tendency to desensitization and the fact its

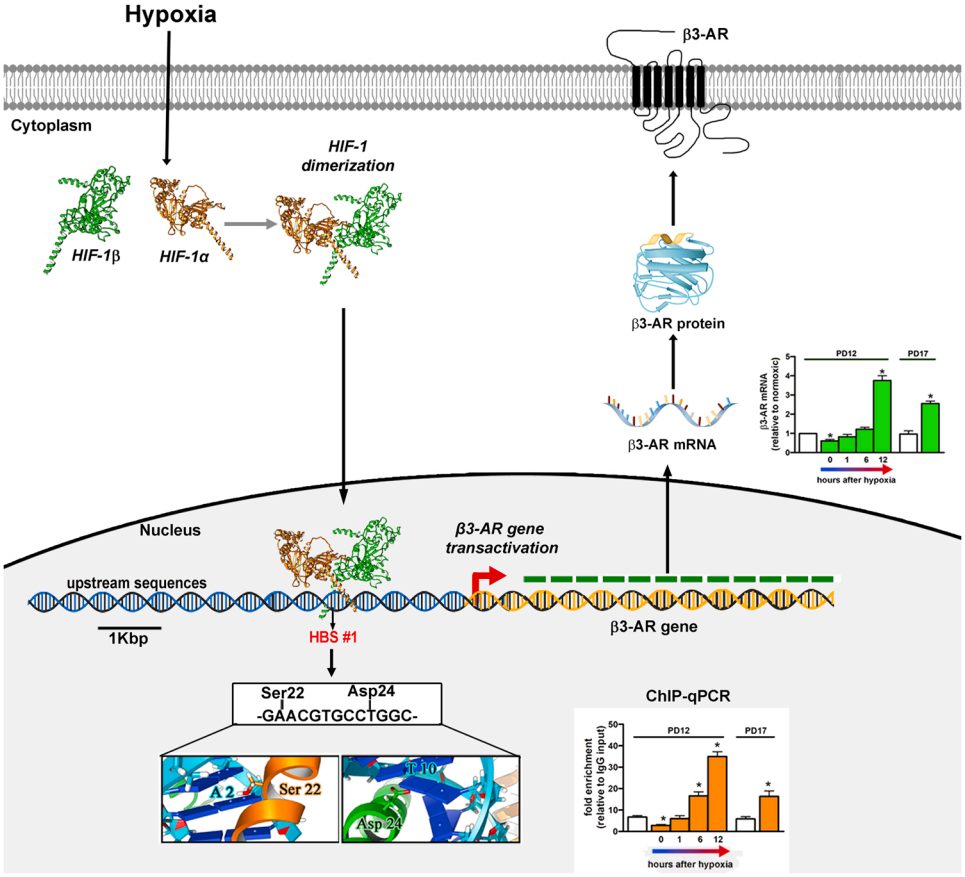

Fig. 7. Schematic representation of transcriptional regulation of β3-AR by HIF-1. In response to hypoxia, HIF-1α dimerizes with HIF-1β to enter the nucleus where physically interacts with HBS#1 a few hours after hypoxia onset as demonstrated by chromatin immunoprecipitation coupled to quantitative PCR. As simulated by molecular dynamics, Ser22 and Asp24 of the HIF-1 subunit interact with A2 and T10, respectively, in the DNA sequence. β3-AR gene transactivation produces a messenger RNA that is translated into β3-AR protein to then becoming embedded in the phospholipid bilayer of the cell membrane with its hydrophobic region spanning the bilayer while the hydrophilic region extending out on both the intracellular and extracellular sides of the membrane.
Adapted from Amato et al. [10].

activation requires sustained amounts of catecholamines have attracted much attention as peculiar features of potential appealing drugs for a prolonged pharmacological stimulation. On the other side, the lack of an efficient ligand battery renders still difficult the development of β3-AR-based pharmacology. In this respect, the clinical targeting of β3-AR has had its highs and lows except for the presence on the market of β3-AR agonists as most essential drugs against overactive bladder.

Since the demonstration that β3-AR regulates both thermogenesis in brown adipose tissue and lipolysis in white adipose tissue of rodents [105,106] much work has been done to demonstrate that β3-AR might serve as an attractive target for the treatment of diabetes and obesity. Since the demonstration that in rodent models, β3-AR exerts negative inotropic effect in cardiomyocytes, causes relaxation in blood vessels and promotes new vessel proliferation in heart injury, its targeting in cardiovascular diseases attracted much interest [87]. However, the development of β3-AR ligands as potential therapeutic tools has not been remarkably successful. An example for all goes back to early nineties when the initial excitement in developing potential drugs to increasing energy expenditure in obese patients *via* β3-AR agonizts has been limited by their modest and short-lived efficacy although significant interest for β3-AR ligands in obesity and metabolic diseases has been recently revived [111]. The same applies to the numerous preclinical studies that, since twenty years, have produced more and more evidence on the role of β3-AR on the contractility, remodeling and metabolism of the cardiac muscle although the development of β3-AR-based drugs against heart diseases still remains a "promising" issue.

Even more difficult appears the definition of β3-AR role in the retina although the strict correlation between β3-AR levels and the state of retinal vascularization (negligible levels of β3-AR with vessel regression under hyperoxia in concomitance with low levels of the HIF/VEGF axis; upregulated levels of β3-AR with neovessel proliferation under hypoxia in concomitance with high levels of the HIF/VEGF axis) is highly suggestive of β3-AR role in retinal angiogenesis. In this line, β3-AR activation is involved in the re-vascularization of failed heart through its efficacy on the hypoxic endothelium of coronary arteries [87]. This is in line with additional findings in a mouse model of limb ischemia in which β3-AR agonism increases angiogenesis through ameliorated NO/redox balance [172]. Finally, in the 129S6 mouse strain, a breed with a predisposition to accumulate higher levels of VEGF in response to hypoxia leading to a particularly aggressive retinal neovascularization, β3-AR mRNA is massively upregulated suggesting some role of this receptor in retinal vessel proliferation [137]. Although deserving a separate discussion, the finding that β3-AR expression affects the VEGF-associated angiogenic response in some tumors in which β3-AR blockade acts by inhibiting vessel proliferation in response to hypoxia, further supports an angiogenic role of β3-AR [173]. In addition, the identification of β3-AR as a component of the HIF-1 target gene family represents a key clue to infer its role into the complex HIF-1-driven responses aimed to increase oxygen availability under hypoxic conditions. In particular, upregulated β3-AR, which is expressed by growing vessels of the mouse hypoxic retina, through its binding to catecholamines that are accumulated in response to stress condition, would trigger a compensatory response by promoting VEGF production that causes the proliferation of new vessels and the vasodilation of the existing ones, thus playing a functional role during the early phase of oxygen deprivation in support of suffering cells. In this case, VEGF expression in response to hypoxia would be regulated through at least two interdependent mechanisms: one involving direct VEGF activation by HIF-1 and the other through HIF-1-mediated β3-AR gene expression indicating a key role of physiological redundancy in the adaptive responses to reduced oxygen tension. Similarly to β3-AR, for instance, the HIF-1-regulated adenosine receptor A2a is overexpressed in the endothelial cells of the mouse hypoxic retina where it is located to pathological neovascular tufts in which its binding to increased extracellular adenosine in response to stress condition participates to endothelial cell proliferation and migration through the stimulation of VEGF production [174,175].

9. Conclusions and future directions

Proliferative ischemic retinopathies including PDR and ROP are the leading causes of visual impairment in the working age and pediatric populations of industrialized countries although they are rapidly expanding to developing countries. These pathologies, despite generally treated with intraocular injections of anti-VEGF drugs, are still object of active investigations aimed at clarifying their molecular mechanism to provide the basis for identifying novel therapeutic targets for treatment. Major effort is therefore directed towards the identification of additional players in the complex mechanism ultimately leading to retinal vessel proliferation. The present review retraces the main steps of the research work investigating the role of the β-AR system in models of hypoxia-associated retinal angiogenesis with a particular emphasis to the role of β3-AR. β3-AR is not a novel target in pharmacotherapy as its agonists have already been approved for the treatment of overactive bladder and could be repurposed for the treatment of ischemic heart diseases. Although the SPHERE-HF study failed to test the efficacy of β3-AR agonism in patients with chronic pulmonary hypertension secondary to heart failure, treatment with mirabegron resulted in a significant improvement in ventricular ejection thus the need of repurposing this study. Future investigations will also require additional research to develop more potent β3-AR agonists including the assessment of their safety, tolerability and efficacy.

More challenging appears the possibility to direct the β3-AR pharmacotherapy towards the management of proliferative retinopathies in which using small molecules to antagonize β3-AR upregulation in response to hypoxia would likely inhibit new vessel proliferation. However, the development of novel β3-AR antagonists is currently limited by a number of factors including target selection, efficacy and delivery technologies. In this respect, monoclonal antibody (mAb) therapy against β3-AR upregulation would have major advantages over small molecule therapy in that mAbs are more selective because of their antigen specificity while having a longer duration of action because of their longer half-life in plasma. Unfortunately, it is extremely difficult to create antibodies against GPCRs using traditional approaches, especially for clinical applications. In this respect, gene therapy targeting β3-AR may provide an alternative approach as the most interesting feature of siRNAs and miRNAs is their ability to virtually modulate the expression of any gene and its mRNA transcripts. Although there are strong indications that miRNAs play important roles in the pathogenesis of ocular diseases their clinical application is still under evaluation. In contrast, siRNAs are successfully used in ocular pathologies mostly in respect to their non-viral intraocular delivery, thus minimizing systemic effects, and avoiding delivery vector toxicity. Despite the scarce information on β3-AR expression in the human retina may render premature the progress of translating β3-AR as a therapeutic target in ophthalmology, investigations on β3-AR role in retinal angiogenesis deserve further effort as their results may help to clarify the involvement the HIF-1/β3-AR/VEGF loop in complementing the master pathways through which cells and tissues adapt to reduced oxygen levels. As the discovery of HIF-1 target genes including VEGF has implemented the therapeutic armamentarium against uncontrolled angiogenesis, then β3-AR transactivation by HIF-1 renders its potential targeting as a promising tool for developing novel therapeutical approaches against neovascular pathologies.

To date, β3-AR has been better understood as an important component of the β-AR family. In respect to its more popular fellows, β3-AR displays some of the features that would allow for its prolonged pharmacological stimulation with low systemic off-target effects. In fact, its restricted tissue expression, the low tendency to desensitization and its low sensitivity to catecholamines point to β3-AR as an innovative therapeutic target that deserves further studies. As Cinderella got rid of the stepsisters, β3-AR acquired over time the role of an important regulator of various physiological functions thus highlighting its targeting as a potential therapy in the treatment of several human diseases.

CRediT authorship contribution statement

PB conceptualized the manuscript and supervised the research work; MC and RA analyzed the literature; PB, MC, RA, MDM and LF set up the original draft; PB, MC and RA wrote the manuscript and edited its final version.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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