Neuropeptides and diabetic retinopathy

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Diabetic retinopathy, a common complication of diabetes, develops in 75% of patients with type 1 and 50% of patients with type 2 diabetes, progressing to legal blindness in about 5%. In the recent years, considerable efforts have been put into finding treatments for this condition. It has been discovered that peptidergic mechanisms (neuropeptides and their analogues, activating a diverse array of signal transduction pathways through their multiple receptors) are potentially important for consideration in drug development strategies. A considerable amount of knowledge has been accumulated over the last three decades on human retinal neuropeptides and those elements in the pathomechanisms of diabetic retinopathy which might be related to peptidergic signal transduction. Here, human retinal neuropeptides and their receptors are reviewed, along with the theories relevant to the pathogenesis of diabetic retinopathy both in humans and in experimental models. By collating this information, the curative potential of certain neuropeptides and their analogues/antagonists can also be discussed, along with the existing clinical treatments of diabetic retinopathy. The most promising peptidergic pathways for which treatment strategies may be developed at present are stimulation of the somatostatin-related pathway and the pituitary adenyl cyclase-activating polypeptide-related pathway or inhibition of angiotensinergic mechanisms. These approaches may result in the inhibition of vascular endothelial growth factor production and neuronal apoptosis; therefore, both the optical quality of the image and the processing capability of the neural circuit in the retina may be saved.

Introduction

The visual world is the most important environmental information source for humans. None of the other sensory signals reaches the brain in such variety and none is processed by as many cortical areas as the visual cues [1, 2]. The first steps of visual processing, however, do not happen in the brain; they are performed by a thin sheath of neural tissue at the back of the eye, called the retina. After phototransduction by photoreceptors, light information is translated into neural signals and shaped by the retinal circuitry. There is no other source of processed visual signals to the brain than those that arise from the retina in the form of spike trains of retinal ganglion cells [3]; therefore, any damage to the retinal tissue immediately results in loss of vision and, in the worst case, causes total blindness.

Sight-threatening neurodegenerative diseases of the retina fall into two broad categories; one group is caused

by genetic deficits, e.g. retinitis pigmentosa and microphthalmia [4], whereas other retinodegenerative disorders, such as glaucoma, ischaemia, macular degeneration and diabetic retinopathy (DR), are thought to be consequences of pathological metabolic processes [5]. Metabolic insults can result from exposure to extremely strong light, changes in hormone/metabolite levels or in blood/ aqueous humour pressure. These processes lead to elevated extracellular glutamate levels and can provoke excitotoxic insults [6]. The balance between the neurotoxic and neuroprotective factors is crucial in determining the survival of retinal neurons [7].

Disorders of both genetic and metabolic origins have been reported to incur progressive loss of retinal neurons and, consequently, visual impairment. Based on my PubMed search, an impressive number of papers (more than 20 000) have been published in the last 10 years on the diagnosis, pathomechanism and treatment of the

above-mentioned conditions. However, very few of these papers have dealt with the possible role of neuropeptides in the pathomechanisms of human retinal degenerations; at the same time, a number of articles have provided evidence for structural and functional protection mediated by neuropeptides during conditions of metabolic stress in experimental models [8–15].

Aim

The aim of this review is to summarize knowledge accumulated over the last three decades on human retinal neuropeptides and those elements in the pathomechanisms of DR which might be related to peptidergic signal transduction. First, I compile a list of those neuropeptides and their receptors whose presence has been unequivocally proved in the human retina, then review the theories relevant to the pathogenesis of DR both in humans and in experimental models and, finally, collate this information. Furthermore, the curative potential of certain neuropeptides and their analogues/antagonists is also discussed, along with the existing clinical treatments of DR.

Neuropeptides in the human retina

The retina is a structure in the eye, in close connection with the choroid sheath and the fibrous layer. It consists of neural tissue, with the exception of its pigmented epithelium (RPE). Retinal nerve cells are organized into three cellular layers. Photoreceptor outer segments are embedded into the processes of the RPE; the cell bodies of these cells are found in the outer nuclear layer. Their role is phototransduction, which is the translation of light information into neural signals. The somata of the second-order neurons (bipolar and horizontal cells), the so-called amacrine cells, and the main glial element (Müller cells) constitute the inner nuclear layer. The innermost cellular layer contains the ganglion cells and the displaced amacrine cells and is termed the ganglion cell layer. Photoreceptors form synapses with bipolar and horizontal cell processes in the outer plexiform layer. This is the first place where shaping of the visual signals takes place; the antagonistic centre-surround properties of inner retinal neurons mostly originate in this layer. The inner plexiform layer consists of the axons of bipolar cells and the dendrites of the amacrine and ganglion cells. This layer is more than 10 times thicker than the outer plexiform layer and contains many more synapses. The synapses here compute such properties of the visual word as edges, motion, contrast, luminance and colours [3]. The so-called retinal straight-through pathway carrying the processed visual information to the brain can be described as a chain of neurons and their synapses (photoreceptor $\Rightarrow$ bipolar cell $\Rightarrow$ ganglion cell). These synapses use glutamate as their neuro-

transmitter [16], while the horizontal and amacrine cells use glycine and γ -aminobutyric acid (GABA) as their main transmitters [17, 18]. Neuropeptides are often found in colocalization with GABA and glycine in amacrine cells; wide-field amacrine cells tend to contain GABA rather than glycine [19].

Identification of neuropeptide-producing elements of the human retina

The presence of neuropeptides in the human retina has mostly been studied with immunocytochemical, radio-immunoassay and chromatographic methods. Early on, it was established that among retinal nerve cells only the amacrine and ganglion cells produce neuropeptides. However, it is noteworthy that only amacrine cells release their transmitter/neuropeptide content within the retinal tissue (to be precise, in the inner plexiform layer), because ganglion cells send their axons to the brain and they do not have recurrent collaterals. Furthermore, there is no evidence for dendritic transmitter release of ganglion cells. In contrast, neuropeptides may derive from non-neural cells of the retina, such as the RPE and the Müller glia, and some peptides can also be produced by extraretinal sources (Table 1). Nearly 20 neuropeptides have been firmly identified in the human retina to date; some of them are produced by non-neural elements (Müller cells or the RPE).

Some well-defined physiological actions of neuropeptides in the mammalian retina

There have been many efforts to define the physiological role of neuropeptides in the mammalian retina, with little success. Knowledge concerning the function of neuropeptides in the human retina is even more scarce. In this section, I provide a brief overview of the possible physiological role of those neuropeptides of the mammalian species which are localized in similar cell types to those in the human retina.

Angiotensin (AT) II In the last couple of years, it has become evident that some neuropeptides may be produced by non-neuronal cells of the human retina (e.g. angiotensin [20–22] and cortistatin [23]). Among ATs, angiotensin II and AT 1–7 are produced mostly by Müller glial cells and, in smaller quantities, also by the RPE/choroid complex [20]. Both major receptor types (AT1R and AT2R) are present in the retina, with AT1R being dominant. Local AT signalling may therefore regulate neurovascular function during normal and pathological conditions.

Cortistatin (Cst) Another prime example of a neuropeptide that is produced mostly by a non-neural cell of the retina is cortistatin. Its role may lie in activation of glial cells in stress conditions [23]. Its receptors are identical to those activated by somatostatin (Sst). The influence of Cst on neural information processing is unknown at present; however, it is presumed that Cst is a part of a signalling

Table 1

Neuropeptides in the human retina

Neuropeptide (abbreviation)	Neuronal	Müller cell	Pigment epithelium	Extrinsic, having receptors	Receptors
Angiotensin II (AT) [20–22]	U	+	+	++	AT1R and AT2R
Bradykinin (BK) [50, 51]		?	+	+	B1R
Cortistatin (Cst) [23]	U	—	++	—	SST 1, 2 and 4 receptors
Enkephalins (Enk) [27]	A	—	—	—	sigma
Erythropoietin (EPO) [52, 53]	?	+		++	EPO-R
Neurokinin A and B (NKA and NKB) [54]	A, G	—	—	—	NK-1R and NK-3R
Neuropeptide Y (NPY) [25–27]	A, G	—	—	—	Y1, Y2, Y4 and Y5
Neurotensin/LANT6 (NT) [55]	A, G	—	—	—	Not known
Orexin A and B (OXA and OXB) [56]	A, G	—	+	+	OX-R1
Pituitary adenylyl cyclase-activating peptide (PACAP) [32]	A, G	—	—	—	PAC-1R; VPAC1 and 2
Secretoneurin (SN) [57]	A, G	—	—	—	Not known
Somatostatin (Sst) [26, 36, 37, 39]	A, dA	—	—	—	SST1, 2 and 4 receptors
Substance P (SP) [27, 43, 47]	A, G	—	—	—	NK1R and NK3R
Thyrotrophin-releasing hormone (TRH) [58]	A	—	—	—	TRH-R1 and -R2
Urocortin (UCN) I, II and III [24, 59]	?	—	+	—	CRF-1R
Vasoactive intestinal polypeptide (VIP) [26, 46, 47]	A, dA	—	—	—	VPAC1 & 2

Abbreviations are as follows: A, amacrine cell; dA, displaced amacrine cell; G, ganglion cell; U, unidentified cell type; +, present; ++, present in high quantity; and ?, not certified.

system residing in the RPE of the retina which shows some parallel properties with the hypothalamo–pituitary–adrenal axis [24].

Neuropeptide Y (NPY) Neuropeptide Y is present in wide-field amacrine and large ganglion cells of the human retina [25–27], although it has to be noted that in some species it was found to be present in non-neural elements too (Müller glia, endothelial cells and microglia [28]). Through a purinergic paracrine mechanism, glutamate activates NPY, which in turn inhibits osmotic glial cell swelling, thus crucially influencing the volume homeostasis of the retina [29]. The most important known physiological actions of NPY on retinal neurons is that through Y1, Y4 and Y5 receptors it inhibits the increase of intracellular Ca^{2+} concentration in response to depolarizing stimuli [30] and it inhibits adenylyl cyclase activity through Y2 receptors [31].

Pituitary adenylyl cyclase-activating polypeptide (PACAP) Light and electron microscopic observations revealed PACAP immunopositivity in amacrine, horizontal and ganglion cells. At the ultrastructural level, PACAP-like immunoreactivity is visible near the plasma membrane, in the rough endoplasmic reticulum and cytoplasmic matrix [32]. Stimulation of cAMP production in the retina and various ophthalmic tissues has been shown in the rat, mouse, rabbit, pig and calf, where both forms of PACAP produced robust stimulation of adenylyl cyclase. PACAP38 was more potent than PACAP27, and both peptides were more effective than vasoactive intestinal polypeptide (VIP) [33]. Other studies revealed similar potency of PACAP27 and PACAP38 in cAMP formation and found that PACAP also increases levels of inositol monophosphate [34]. Pituitary

adenylyl cyclase-activating polypeptide is co-stored with glutamate in a subset of retinal ganglion cells which project to the suprachiasmatic nucleus and control the circadian cycle [35].

Somatostatin Somatostatin is mainly localized to a population of amacrine (in some species displaced amacrine) cells and, in a few species, it is also found in ganglion cells [26, 36, 37]. There is a general agreement that the distribution of Sst reflects its pleiotropic role in the retina. Physiological actions of Sst in the retina are multiple; primarily ganglion cell receptive field types are affected. Somatostatin causes general excitation, occurring with a threshold concentration of about 100 nM; it also increases the signal-to-noise ratio (ratio of light-evoked to spontaneous spiking, which results from a decrease in spontaneous activity and a concomitant increase in light-evoked spiking) and leads to a shift in centre-surround balance towards a more dominant centre [38]. In the outer retina, mostly SST2 receptors are found [39]. Phototransduction and light adaptation processes are influenced by Sst directly or through dopaminergic and NOergic pathways [40, 41], mainly activating SST2A receptors. Inner retinal cells, including some ganglion cell types, possess SST4 receptors. This receptor type seems to suppress calcium influx and, consequently, reduces spiking activity in ganglion cells [42]; thus, it may promote ganglion cell survival following metabolic insults.

Substance P (SP) There are detailed data on the circuitry of SP-positive amacrine cells in the primate retina, but little is known about their physiology. The SP-immunoreactive amacrine cell dendrites mostly target bipolar cell axon

terminals and ganglion cell dendrites [43]. In nonprimate species, SP acts as an excitatory neurotransmitter, raising the spontaneous activity level of ganglion cell responses [44]. It has also been described that SP is a physiological regulator of retinal development through its NK1 receptor-mediated actions [45].

Vasoactive intestinal polypeptide In the primate retina, a mosaic of VIP-positive cells was seen in both the inner nuclear layer and the ganglion cell layer [46, 47]. In retinal slices, VIP potentiated the GABA_A receptor-elicited currents in dissociated retinal ganglion cells [48]. It has also been shown that brain-derived neurotrophic factor (BDNF) regulates VIP expression in the retina, because mice lacking brain-derived neurotrophic factor expressed only 5% of the VIP in their retinæ compared with wild-type animals [49].

Other neuropeptides identified in the human retina Only neurochemical identification but not physiological roles in the human retina have been reported for the following neuropeptides: bradykinin [50, 51], enkephalin [27], erythropoietin (EPO) [52, 53], neurokinin A and B [54], neurotensin [55], orexin A and B [56], secretoneurin [57] thyrotrophin-releasing hormone [58] and urocortin [24, 59].

Diabetic retinopathy

Diabetic retinopathy, a common complication of diabetes, develops in 75% of patients with type 1 and 50% of patients with type 2 diabetes, progressing to legal blindness in about 5% [60, 61]. As the worldwide prevalence of diabetes continues to increase, diabetic retinopathy is a leading cause of loss of vision in developed countries [62]. The inability of the retina to adapt to metabolic stress leads to glucose-mediated microvascular disease along with chronic inflammation, which finally causes neurodegeneration and dysfunction in the retina. The retina is one of the most metabolically demanding tissues in the body and is therefore highly vulnerable. The interplay between the neuroretina and the vasculature is critical in developing neurological symptoms of disease. In DR, the rate of neuronal loss in the retina is slow, leading to a gradual, cumulative reduction, mostly of amacrine and ganglion cells [63]. Two forms of DR can be clearly distinguished; there is an early, nonproliferative form of DR, when neovascularization of the macula is not evident, while in proliferative DR the symptoms include macular neovascularization. Other vascular symptoms involve microangiopathy, with formation of microaneurysms, flame haemorrhages, leucocyte adherence to the vascular wall and formation of exudates in the extravascular space.

These observations have led to the theory that DR is primarily a vascular disorder whose degrading effects are due to the consequences of vascular failure, with ischaemia

being followed by increased production of reactive oxygen species, as in case of the retinal ischaemic diseases [64]. However, it has also been described that neuronal damage may precede any detectable microvascular changes [65], and the deterioration of the intrinsic time and also of oscillatory potentials in the electroretinogram start early, in some cases as early as 2 days after induction of diabetes in experimental animals [66–68]. These observations have led to formulation of the ‘neurodegeneration first’ hypothesis [69]. Patients suffering from DR experience gradual vision loss; after electroretinogram deterioration, the visually evoked potentials also start to decrease [70], which indicates cortical dysfunction. However, recent observations have led to the conclusion that inflammation may precede, or at least run parallel with, the vascular and neural events [63, 71, 72]. Inflammatory molecules in the retina can be produced not only by leucocytes, but also by glial cells; many of them are produced by the Müller glia [73].

Müller cells are primarily responsible for ion and volume regulation of the retina and also control extracellular glutamate levels through the excitatory amino acid transporters. In addition, they participate in protection against free radicals and hypoxic damage through glutathione synthesis. Usually, the first sign of Müller cell stress is upregulation of their glial fibrillary acidic protein (GFAP) content, which may be accompanied by hypertrophy and proliferation in certain damaging conditions. Diabetes itself is able to upregulate GFAP in Müller cells without the presence of other symptoms of diabetic retinopathy in humans [69], and this goes along well with what we see in experimental models, where 2 days after induction of diabetes GFAP upregulation is already apparent (K. Szabadfi, personal communication).

All the above observations may lead to the formation of a fourth hypothesis of initiation of diabetic retinopathy, the ‘glial cells first’ scenario. In this case, the Müller cells, sensing the elevated glucose level, would activate their volume- and ion-regulatory machinery, as well as releasing vaso-proliferative (vascular endothelial growth factor; VEGF [74–76]) and inflammatory substances (prostaglandins, tumour necrosis factor α and interleukins [72, 77, 78]), which would in turn initiate neurodegeneration, inflammation and vascular growth. Whatever the exact order of events may be, all the above evidence indicates that low oxygen supply and high blood glucose levels are important pro-DR parameters, and all signalling pathways converge to activate VEGF production.

Neuropeptides and diabetic retinopathy

During recent years, it has become clear that neuropeptides described formerly in the human retina fall into two categories regarding their contribution to the progress of

DR; some peptides promote the development of the symptoms, whereas others are able to slow down or eliminate them. First, I consider those that participate in the pathogenesis, followed by those that are potentially protective.

Pro-DR neuropeptides

Two peptides that were also found in the human retina are considered currently as important contributors to developing DR: AT [20–22] and erythropoietin [53]. However, both peptides are also found in the circulating blood. The role of erythropoietin is controversial because it has been listed among both pro- and anti-DR peptides by different authors; for historical reasons, I list it in the former group.

Angiotensin II Two major effects of AT in the development of DR have been described. First, AT contributes to neuronal dysfunction by promoting synapse degradation [79] through its type 1 receptor and, second, through VEGF activation it causes breakdown of the blood–retina barrier [80]. For the first mechanism, AT type 1 receptor blockade could prevent activation of extracellular signal-regulated kinase, which may potentially provide a therapeutic possibility. In the second mechanism, breakdown of the blood–retina barrier was accompanied by loss of tight junction proteins and upregulation of VEGF. Both AT type 1 and type 2 receptors were involved, and both effects could be prevented by blocking the angiotensin-converting enzyme.

Erythropoietin Erythropoietin is strongly upregulated in DR, both in the RPE and in the neuroretina [52, 53, 81, 82], implicating a role for EPO in exacerbating retinal angiogenesis and thrombosis. However, this upregulation is not followed by an increase in the quantity of EPO receptor [83]. Furthermore, overexpression of EPO is unrelated to mRNA expression of hypoxia-inducible factors; therefore, stimulating agents other than hypoxia are involved in this process [82]. Recently, it has been reported that a portion of the EPO molecule, the helix B-surface peptide, is non-erythrogenic and non-angiogenic, but exhibits tissue-protective properties [84]. These include attenuation of cytokine expression, upregulation of GFAP in Müller cells and inhibition of apoptosis. The authors concluded that treatment with helix B-surface peptide can significantly protect against neuroglial and vascular degenerative pathology after diabetes is fully established.

Anti-DR neuropeptides

Well-defined neuroprotective actions have been reported only for two neuropeptides/analogues: these are PACAP and somatostatin. There is only fragmented evidence for possible anti-DR effects of other neuropeptides such as Cst, NPY, SP and VIP.

Pituitary adenylyl cyclase-activating peptide Pituitary adenylyl cyclase-activating peptide belongs to the VIP/secretin/glucagon peptide superfamily and has a remark-

ably well-conserved structure throughout evolution. The PACAP receptors are G-protein coupled and can be divided as follows into two main groups: the PAC1 receptor, which binds PACAP with higher affinity than VIP; and the VPAC receptors, which bind PACAP and VIP with similar affinities [85]. Pituitary adenylyl cyclase-activating peptide induces phosphorylation of transcription factors by 5 min after treatment in neonatal rat retinas and is also important in maintaining antiapoptotic mechanisms in injured retinal tissue [6]. In induced diabetes, it rescues retinal photoreceptors, dopaminergic amacrine cells and ganglion cells from death by upregulating the PAC1 receptor [86]. Furthermore, PACAP restores B-cell lymphoma 2 and p53 levels to normal after 3 weeks of diabetes in experimental animals [87], suggesting a prominent antiapoptotic action. Taking all this evidence together, PACAP may have a substantial therapeutic potential in DR.

Somatostatin Somatostatin is a neuropeptide widely distributed in the nervous system. It comes in a 28 and a 14 amino acid-long version; the shorter one is cyclic and characteristic of the retina [88]. Besides its major effect in the pituitary (inhibition of growth hormone production), it strongly influences several processes, including memory formation, peripheral pain sensation and obesity [89, 90].

Five seven-transmembrane somatostatin receptor types have been cloned, of which SST1, 2 and 4 all upregulate phospholipase C and phospholipase A₂, while downregulating adenylyl cyclase [91]. In the retina, SST1, 2 and 4 receptors are present in the highest quantities; the SST1 receptor is an autoreceptor controlling somatostatin release, while the SST2 and 4 receptors are able to regulate voltage-gated ion channels of retinal neurons [39, 42, 92].

Somatostatin is a putative neuroprotective agent, with the ability to inhibit ischaemic degeneration and counteract cell death, as well as neovascularization, induced by various retinal disease conditions [88, 93]. It has been suggested that somatostatin receptor ligands may also counteract inflammation [90]; therefore, it is expected to have anti-DR actions. Indeed, Sst is downregulated in the diabetic retina and the vitreal fluid [94], and it has also been shown that Sst inhibits insulin-like growth factor-1-mediated VEGF production in human RPE cells [95]. These facts together indicate that a deficit of somatostatin will upregulate VEGF, leading to pathological angiogenesis. When considering how to counteract an undesirable increase VEGF, most studies have focused on the SST2 receptor and its ligand octreotide, which was applied with success in treatment of several experimentally induced retinal disorders involving angiogenic actions [8, 15, 96, 97]. This effect is supposedly mediated by protein kinase C β (PKC β), which is able to inactivate VEGF production, and this pathway seems especially important in diabetes [76].

Other neuropeptides A unique expression pattern of Cst was observed in the human retina, in that the bulk of the

Cst mRNA was found in the RPE. When Cst expression was found to be low in DR, the GFAP content in Müller cells rose and also the number of apoptotic cells rose substantially in the neural retina [23]. Given that Cst acts through the same set of receptors as Sst, it may share all the protective properties of Sst (see the previous subsection).

Given that NPY is present not only in neurons, but also in glial and endothelial cells of the retina, along with its different receptor subtypes [98], its possible effects include pathophysiological processes too. The Y1 receptor has been identified as a glial marker in proliferative vitreoretinopathy in the human retina [99], and several receptor types were found to be present in the RPE [98]. As the RPE is a source of a number of anti-DR agents [100], NPY-mediated release of these substances may play a part in the natural defence mechanisms.

Other neuropeptides, such as VIP and SP, show significant reductions in the retina in animal models of diabetes [101]. This finding may explain the lack of neovascularization in animal models of DR, because SP is known to be a potent vascular growth factor. Also, VIP and its receptors are downregulated after a transient increase in animals with induced diabetes, showing the importance of these signalling elements in normal retinal function [87].

However, it is currently not clear whether the protective effects of the above-discussed peptidergic mechanisms could be additive or not. Additivity of protection provided by an enriched environment (possibly mediated through

brain-derived neurotrophic factor-related pathways) and PACAP could not be proved [102].

Anti-DR drug treatments used currently in clinical practice

In clinical practice, the primary treatment for diabetic retinopathy is most often still surgery (photocoagulation, cryocoagulation, vitrectomy or even pituitary ablation); however, in the last 10–15 years it has often been supplemented by drug treatment [103]. In fact, there is a tendency nowadays that before or instead of surgical intervention ophthalmologists try to apply newly developed drugs for primary care, often with some success [104, 105]. A drug treatment strategy can be designed against the possible three major pathways leading to development of DR, namely disorders of the vascular, the immune and the neuroretinal components (including the glial and RPE damage). A fourth, relatively new approach is lipid-lowering medication. The major steps in developing these anti-DR strategies are collected in Table 2. Sporadic trials with drugs other than those targeting the above-mentioned processes are not discussed.

Antiangiogenesis treatments

In the retina, the primary cause of vascular proliferation is the upregulation of VEGF, which can be observed in many

Table 2

Drug treatments for diabetic retinopathy

Target	Agent	Drug name and application mode	Result
Vascular endothelial growth factor	Antivascular endothelial growth factor antibody fragments	Bevacizumab intravitreal injection, single [104, 116] or multiple (24 month intervals) [105, 114]	Nonpermanent improvement [116, 125]; or lasted for 6 months [105, 114]
		Ranibizumab [108, 118] and pegaptanib [108, 118]	After application alone, long-term (1 year) improvement
		Trap-Eye, single injection [115]	Well tolerated, bioactive
Inflammatory processes	Protein kinase C β blocker	Ruboxistaurin, oral [120]	Improved retinal circulation, high-dose tolerance
		Triamcinolone acetonide [123], intravitreal	Slight improvement of visual parameters*
		Triamcinolone acetonide in combination with bevacizumab [104], intravitreal	Additive effects were not seen
Neuropeptide receptors	Antitumour necrosis factor α antibody fragments	Adalimumab and infliximab [126], intravitreal	Visual acuity improvement at 3 months†
		Cyclo-oxygenase blocker	Lessened fluorescein leakage from retinal vessels
		Celecoxib, oral [127]	
Lipid-lowering medications	Angiotensin receptor blocker	Candesartan, oral [128]	No retinal outcome
		Angiotensin-converting enzyme inhibitor	No retinal outcome
		Somatostatin analogue	Retarded progress of diabetic retinopathy
Lipid-lowering medications	Somatostatin analogue	SOM230 [133]	No retinal outcome
		Atromid/clofibrate [134]	Improved retinal circulation
		Atorvastatin [135], oral	Improved vascular resistance
Lipid-lowering medications		Fenofibrate [136], oral	Increased time to first laser surgery

Side-effects of different drug treatments are as follows: *intraocular pressure increase; and †uveitis.

disease conditions, including DR [64]. To prevent VEGF-mediated angiogenesis, either the VEGF level should be reduced or the pathway leading to VEGF synthesis should be blocked. Both approaches are currently in use during clinical interventions.

Direct anti-VEGF therapies Preclinical and phase 1A clinical trials were initiated with pegylated anti-VEGF aptamers [106] against age-related macular degeneration. After establishing safety measures [107], this form of intervention slowly penetrated into the everyday clinical practice. From the original efforts, three lines of drug development led to three products that are now often used in ophthalmological practice: bevacizumab (Avastin), ranibizumab (Lucentis) and pegaptanib (Macugen) [108, 109]. All three are gaining popularity as an adjunct to photocoagulation in proliferative DR and diabetic macular oedema [109–112], or in combination with anti-inflammatory drugs, particularly triamcinolone acetonide [113, 114]. Recently, they have also been tried as primary care treatments [115–117]. More than 50 000 patients have been treated with pegaptanib in 1 year in the USA alone [118]. In the longest follow-ups, improvement has been seen for 1 year after treatment [108]. This approach therefore seems promising, because it is much less invasive than surgery; however, therapeutic schedules have to be worked out, and the active ingredients of the medications also need further research.

Blocking VEGF induction: drugs that inhibit PKC β activity It has been shown in animal models of DR that an upstream element of the VEGF induction pathway is the increased activity of PKC β [76]. If the activity of this enzyme can be blocked, one expects the VEGF induction to subside. Indeed, oral administration of a PKC β blocker, ruboxistaurin, reduced the diabetes-induced retinal haemodynamic abnormalities [119]. Later studies, however, added that without preliminary laser treatment (focal or grid photocoagulation) the drug treatment is much less effective [120]. Indeed, the therapeutic potential of PKC β inhibitors in treating DR is not yet fully explored, although this approach seems to be promising. Most recently, small-molecule atypical PKC blockers have also been tested *in vitro* and in rodent retinæ *in vivo*. The 2-amino-4-phehyl-tiophene derivatives are very promising for further drug development [121]. Clinical trials have not yet been initiated.

Anti-inflammatory agents

Anti-inflammatory agents in the treatment of DR fall into the following three categories: corticosteroid derivatives; tumour necrosis factor α inhibitors; and cyclo-oxygenase blockers.

Triamcinolone acetonide Triamcinolone acetonide, a corticosteroid derivative, has been in use for treating diabetic retinopathy for almost a decade [122], combined with

surgery or anti-VEGF treatment [104]. A warning has been issued that this drug may increase intraocular pressure [123]. Higher doses of the drug (up to 8 mg per eye) proved to be more beneficial and prolonged the positive effects in cases of diabetic macular oedema [124]. The current aim in improving the therapeutic potential of this drug is to design steroid delivery devices that release a small quantity over a long period of time, to avoid multiple intravitreal injections and unwanted side-effects [125].

Tumour necrosis factor α inhibitors Two antitumour necrosis factor α fragments (adalimumab and infliximab) were designed to counteract tumour necrosis factor α -mediated inflammatory processes [126]. Although the treatment was beneficial from the point of visual acuity, the treated eyes developed serious uveitis. This observation warrants further studies before antitumour necrosis factor α therapies could gain wider ground.

Cyclo-oxygenase inhibitors As a supplementary treatment to microdiode pulse laser treatment, celecoxib, a cyclo-oxygenase inhibitor, was administered orally. In the short-term follow-up, the authors did not find substantial visual benefits of the treatment. At the same time, a major observation of the study [127] was that fluorescein leakage from the retinal vessels was substantially reduced. Further studies are needed to ascertain the usefulness of this drug in treating DR.

Agents targeting neuropeptide receptors

From the description of the retinal neuropeptide system above, it is clear that there are four major candidates (AT, EPO, PACAP and Sst) to date that should be examined in detail in this review. Clinical trial data are available for two (AT and Sst) of these peptides, with the proviso that only Sst analogues were used to target DR; effects of angiotensinergic drugs were tried in diabetic nephropathies.

Angiotensinergic drugs There are two possible pathways; one is to block the AT receptor(s) and the other is to block the AT-converting enzyme. Both have been tried for diabetic nephropathy (e.g. [128, 129], respectively) but not in DR, possibly because effects on the circulation and kidney function are much greater than those exerted in DR.

Somatostatin analogues The first Sst analogues used in diabetic conditions were lanreotide (Somatuline) [130] to treat renal hyperfiltration and octreotide [131] to counteract vascular endothelial dysfunction. This latter drug soon was proved to be effective in delaying or to some extent reversing symptoms of DR [132]. Octreotide, acting through SST₂ receptors may possibly control inflammatory processes [90], endothelial [15, 131] and also, to some extent, neural symptoms [40, 41, 88, 96]. A more potent Sst analogue, SOM230, has been developed but is not yet available on the market for clinical trials in DR [133].

Lipid-lowering medications

The first ever approved clinical trial for drug treatment of DR has been with atorvastatin (2-(p-chlorophenoxy)-2-methylpropionate) [134], and this drug is still in use, although not in the area of retinal disorders. Recently, this line of clinical trials continued with more modern drugs, such as atorvastatin [135] and fenofibrate [136]. Two large sets of clinical data (FIELD and ACCORD-Eye studies), collected and compared, recently concluded that this medication can be recommended in the early stages of DR [137].

Conclusions

The symptoms of DR are very similar to conditions that are termed 'retinal ischaemic diseases'; they share all but one characteristic (i.e. high blood glucose levels). The most promising peptidergic pathways along which treatment strategies for DR may be further developed are the stimulation of Sst- and PACAP-related pathways or the inhibition of angiotensinergic mechanisms. Both approaches may result in the inhibition of VEGF and neuronal apoptosis. A fragment of EPO, helix B-surface peptide, may also be useful for treatment if diabetes is fully developed. By inhibiting VEGF production, the optical quality of the image falling on the retina can be preserved and the processing capability of the neural circuit in the retina may be saved, because neovascularization of the macula and formation of macular oedema can be prevented, while retinal neurons can be saved from apoptosis; thus, visual acuity can be maintained. Further studies should focus on the possible use of RPE-derived factors in fighting DR.

Competing Interests

The author has completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf and declares: no support from any organization for the submitted work or support of any organizations that might have an interest in the submitted work in the previous 3 years; and no other relationships or activities that could appear to have influenced the submitted work.

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