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Research Article

Targeting of Signal Transduction Pathway Components to Mitigate Selected Ocular Disorders

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Abstract

Coordinated communication between and within cells forms the basis for life in health and disease of all tissues and organs. Such relaying of information is mediated by neurotransmitters, hormones, bacteria, viruses, steroids and a host of cyto- and chemokines via specific receptors, ion-channels, and transporters located in cellular membranes and on intracellular organelles including the nuclear membrane and within the nucleus itself. Activation of such transmembrane components generates intracellular second messengers such as cAMP, cGMP, inositol phosphates, diacyl glycerol, Ca^{2+}, and gaseous transmitters such as nitric oxide, carbon monoxide and hydrogen sulfide that modulate activity of cytoplasmic proteins, lipids and other substances via phosphorylation, dephosphorylation, glycation, and acetylation. Additional communication is achieved by modulation of genetic machinery, via transcription factors, various chaperones, and through secretome/exosome-mediated release of growth factors, microRNAs, and via direct transfer of many of the afore-mentioned chemicals between neighboring cells down nanotunnels. Dysfunctions within any component of this signal transduction machinery, whether in excess or deficiency or by mutation, results in some form of disease and thus represent targets for intervention by small molecule drugs, peptides, antibodies, genetic manipulation and/or via cell-replacement therapeutics. A brief outline of some of these elements will be discussed.

Keywords: Receptors; Signal Transduction; Ion-channels; GPCRs; Enzyme; Agonist; Antagonist; Inhibitor

Introduction

Based on the human genome project discoveries, the human body contains a huge array of proteins with diverse functions some of which relate to signal transduction at the cellular and molecular levels. Receptors are cellular proteins that mediate responses to signaling molecules that nature has designed to promote cellular communication amongst cells and tissues within each organ and

system that permits the physiological, biochemical and pathological expression of biological responses. The latter encompass such activities as neurotransmitter release from sympathetic and parasympathetic nerves and neuroaxonal generation of action potentials encoded by the signaling molecule(s), muscle contraction or relaxation, hormone release, sleep, and much broader functions as olfactory, auditory and visual communication.

Only 2-4% of proteins form the basis of safe and effective drugs approved by world health agencies for treatment of various diseases and disorders. However, the potential druggable cellular protein targets are represented as follows: 45% are membrane-bound receptors, 28% are enzymes, 11% are hormones and related factors, 5% are ion-channels, 2% are nuclear receptors, 2% are DNA-related proteins, and the remainder perform unknown functions (e.g. [1]). Four major types of signal transducing receptors exist in the human body (Table 1) [2-7]. Proteins embedded in the cell membranes that function as transporters of solutes, amino acids, nutrients and waste products are also known [2-7]. A brief account and properties of the four major receptor-types that participate in transduction of signaling via the four receptor types will now be provided. The latter include:

	Adenylyl Cyclase (cAMP System)	**PLC-mediated Phosphoinositide(PI) System (PI Turnover)**	**Arachidonic Acid System**	**Guanylyl Cyclase (cGMP System)**	**Tyrosine Kinase System**
First messenger (Neuro-transmitter)	Epinephrine (α-2; β-1; β-2); Acetylcholine (M_2)	Histamine (H-1); Acetylcholine (M1,M3) Bradykinin (B1; B2)	Histamine	Nitric oxide	-
First messenger ligand (e.g. Hormone)	Luteinizing hormone; Glucagon; Parathyroid hormone	Oxytocin; Thyrotrophin-releasing hormone	-	Atrial natriuretic Peptide; Nitric oxide	Vascular endothelial Growth Factor; Platelet Derived Growth factor
Signal transducer (e.g. G-protein)	GPCR/G_S (β-1; β-2); GPCR/G_I (α-2; M2)	GPCR/G_q	Unknown G-protein	-	Receptor Tyrosine Kinase (RTK)
Primary effector	Adenylyl cyclase	Phospholipase C	Phospholipase A	Guanylyl cyclase	RasGEF
Second messenger(s)	cAMP (cyclic adenosine monophosphate)	IP_3; Diacylglycerol; Ca^{2+} (intracellular)	Arachidonic acid	cGMP	Ras.GTP (small GTPase)
Secondary effector influenced by 2^{nd}-messenger	Protein Kinase A	Protein kinase C; Calmodulin	Cyclooxygenase; 5-lipoxygenase; 12-lipoxygenase	Protein Kinase G	Myosin-Actin Phospho-3 Kinase (c-Raf)
Examples of downstream events	Muscle relaxation; Inhibition of hormone release	Muscle contraction; Secretion of hormone or neurotransmitter	Production of Prostaglandins and Leukotrienes; Vasoconstriction or vasorelaxation	Ion-channel Activity modulation; Muscle Relaxation;	Modulation of gene expression; Cell proliferation/ migration

Table 1: Major signal transduction systems associated with various endogenous/exogenous ligands and GPCRs and their downstream actions - some examples.

- G-protein-coupled receptors (GPCRs; >800 present in human genome) that contain 7-transmembrane domains and which are linked to effector enzymes on the inner leaflet of the cellular membranes [3-7]. Activation of these GPCRs generates intracellular second messengers such as cAMP, cGMP, nitric oxide (NO; [8], hydrogen sulfide (H_2S) [9], lipid metabolites like inositol phosphates [10-14] and intracellular Ca^{2+} $[Ca^{2+}]_i$ [15] (e.g. prostaglandin [PG] receptors [16,17]; histamine-1 receptor, [18], the former involving adenylate/guanylate cyclase [10,19], and the latter involving hydrolysis of membrane lipids by phospholipase C (PLC) (e.g. during vasodilation and vasoconstriction; Figure 1). Activation of phospholipase A-1/2 or PLD by certain ligand-receptor stimulants generates lysophosphatidic acid (LPA) which has the ability to then interact with up to 6 sub-types of LPA receptors which in turn recruit different G-proteins to generate some of the afore-mentioned second messengers and influence additional down-stream signal transduction elements, including numerous cytoplasmic enzymes [5] to modulate cell-cell interactions, cell migration, cell proliferation, cytoskeletal changes, etc. (e.g. Figure 2 and 3) [3-5].

Figure 1: This figure illustrates examples of some major GPCRs and their intracellular signal transduction pathways in mediating vasodilation and vasoconstriction of blood vessels.

Figure 2: Food- and cell membranes-derived lipid mediators generated by the action of various membrane-bound and cytosolic phospholipase enzymes is depicted. Additional downstream signaling associated with various lysophosphatidic acid (LPA) receptor subtypes is also shown to illustrate how recruitment of different cytosolic proteins occurs to amplify the signal transduction processes.

Figure 3: This figure illustrates the complexity and intricacy of the signal transduction events that may occur upon activation of cell surface and intracellular/nuclear receptors by their cognate ligands. The signal amplification and divergence is also apparent from this diagram.

- Receptors that penetrate the plasma membrane of cells and which possess intrinsic enzymatic activity or are enzyme-linked (~ 500 protein kinases are identified) which upon stimulation cause phosphorylation of cytoplasmic proteins (e.g. receptor tyrosine kinases [RTKs]; Figure 3) [3,5]. Various growth factors and other proteinaceous factors mediate their cellular effects via RTKs [20,21] (Table 1).
- Receptors that function as ligand-gated ion-channels that facilitate movement of ions across the plasma membrane (glutamate-receptor-linked ion-channel; Ca^{2+}-activated K^+-channel) [7,22,23]. Agonist binding to these ionotropic receptors changes the conformation of the multimeric channel sub-units to permit ions such as Na^+, K^+, Cl^-, and Ca^{2+} to flow through the channel. Examples of ionotropic receptors nicotinic acetyl choline receptor [24], purinergic receptors [25], serotonin-3 receptor, γ-amino butyric acid receptor, glycine receptor, N-methyl-D-aspartate sub-type of glutamate receptor [23,25] (Table 1).
- Receptors that are located within the nucleus itself and which mediate and regulate genetic transcription by altering the production of mRNAs for various proteins. About 50 types of nuclear receptors (NRs; [4,26]) are known including steroid receptors and thyroid hormone receptors) (Table 1). Four major mechanistic classes of NRs exist, for example the Type-1 and Type-II classes (Figure 4). Type-I NR: a steroid, such as a gonadal hormone or a glucocorticoids, being highly lipophilic, readily crosses the cell membrane and binds to NR/heat shock protein (HSP) complex within the cell cytoplasm thereby causing the HSPs to dissociate away. The NR-steroid complex dimerizes and moves to the cell nucleus and attaches to hormone-response-elements on the DNA and modulates transcription to generate specific mRNAs (Figure 4). Type II NR ligands include thyroid hormone and retinoic acid. NRs for these ligands remain within the nucleus bound to DNA as heterodimers complexed with corepressor proteins. Binding of the hormone to the ligand binding domain of the NR causes the corepressor to leave and coactivator proteins to bind to the complex. Transcription of DNA to mRNA then occurs when enzymes like RNA polymerase act on the NR/DNA complex (Figure 4). Histone acetyltransferase activity is often intrinsic to many activator proteins which weakens the association of histones to DNA, and therefore promotes gene transcription to liberate mRNA for requisite proteins. Conversely, antagonist ligands binding to NRs change the receptor conformation that preferentially binds corepressor proteins. These proteins then recruit histone deacetylases, which enhances the linkage of histones to DNA, and therefore suppresses gene transcription. Type III NRs are essentially a sub-family of Type-I NRs, while Type-IV NRs differ from the above in that they can bind as monomers or dimers.

Figure 4: Nuclear receptor subtypes and their signaling pathways are displayed in this figure. As can be seen, homo- and heterologous dimerization of receptors and their ligand complexes and the involvement of coactivator and repressor proteins adds to the complexity and elegance of such cellular activity regulation within the cell cytosol and at the DNA/mRNA levels.

Thus, cellular communication is performed by a variety of signal transduction pathways that are ultimately responsible for many diverse biological response outcomes including cellular metabolism, cell division/differentiation/death, hormone release, vision, taste, hearing, touch, sleep, muscle or blood vessel contraction/relaxation and movement. However, in a more constrained context, there are at least five types of sensory receptors including pain receptors which are stimulated by tissue damage; thermoreceptor which are stimulated by changes in temperature; chemoreceptors which are stimulated by changes in the chemical concentration of substances; photoreceptors which are stimulated by light energy; and mechanoreceptors which are stimulated by changes in pressure or movement. In addition, the innate and adaptive immune system utilizes specialized receptors to respond to attack by bacteria, viruses and fungi. The ability of the immune system to recognize molecules that are broadly shared by pathogens is, partially due to the prevalence of immune receptors termed toll-like receptors (TLRs; Figure 5) that are expressed on the cell membranes of leukocytes including dendritic cells, macrophages, natural killer cells, cells of the adaptive immunity T-cells and B- cells, and non-immune cells (e.g. fibroblasts, and epithelial and endothelial cells). The binding of ligands, either as adjuvants used in vaccines or infecting agents, to the TLRs represents the key molecular events that ultimately result in innate immune responses and the development of antigen-specific acquired immunity. Upon activation, TLRs recruit adaptor proteins from the cytoplasmic milieu of the immune cell in order to propagate the antigen-induced signal transduction pathway. These recruited proteins activate other downstream proteins (e.g. protein kinases like IKKi, IRAK1, IRAK4, and TBK1; [27]) that further amplify the signal and modulate genes expression to affect inflammatory responses and other transcriptional events (e.g. cytokine production, proliferation) (Figure 5). In the case of a bacterial factor ligand, the pathogen might be phagocytosed and digested and its antigens presented to CD4+ T-cells for creating adaptive immunity. However, if the ligand is a viral factor, this may lead to the infected cell terminating its protein synthesis and it may die by apoptosis. Immune cells that have detected a virus may also release anti-viral factors such as interferons to signal this invasion. TLRs have also been shown to be an important link between innate and adaptive immunity through their presence in dendritic cells. Flagellin, a TLR5 ligand, induces cytokine secretion on interacting with TLR5 on human T- cells, for example.

Figure 5: This figure displays the role of ion-channel-coupled purinergic receptors and Toll-like receptors in mediating cellular toxicity, and creation of inflammatory mediators, activation of the transcription factors and responses to the inciting agents such as bacterial toxins. Both cytosolic and nuclear elements coordinate the final responses to the initial signals.

As can be ascertained from above, the complexity of the various signaling pathways are highly ligand-(antigen)-receptor-specific and permit finely tuned biological responses. The interplay amongst the different receptors via a range of signaling molecules, packaging and distribution of signaling substances (microRNAs; [28], exosomes and micro-vesicles [29], creation of novel inter-cellular connections (nanotunnels; [30]), generation and exploitation of intracellular organelles (inflammasome; proteosome; [25]), and prevalence of receptor sub-types (EP_1, EP_2, EP_3 and EP_4 PG receptor sub-types; [14-17]) ensures additional regulation of the signaling cascades within and between cells. New discoveries are constantly expanding our understanding of how and why homeostatic regulation of cellular activities depend on proper functioning of receptors and their associated ligands and

signaling components. Malfunctions at any level of such signal transduction mechanisms leads to disease states that require appropriate correction by therapeutic interventions. Accordingly, modern drug discovery and development [11-14] and introduction into clinical medicine of suitable drugs, devices and procedures form the basis of treating these diseases as is discussed in the rest of this book. A brief overview of how receptor-mediated signal transduction pathways cause some major ocular diseases, and how therapeutic intervention can abrogate the signs and symptoms of them, will now be described for selected eye disorders.

Allergic conjunctivitis and involvement of Phospholipase-C-Coupled receptors, janus kinase pathway, transcription and immunogenic factors

As the name implies, conjunctivitis is the inflammation of the conjunctiva, a highly vascularized tissue that is an extension of the cornea and which forms the inner lining of the eyelids [31]. The conjunctival epithelium contains many mast cells, dendritic cells and macrophages which respond rapidly to airborne pollen, dust, pathogens and other pollutants. The latter cells are responsible for the innate and adaptive immunity of the conjunctiva and are responsible for causing seasonal allergic conjunctivitis (SAC) and perennial allergic conjunctivitis (PAC; [31]). As an allergen (antigen) such as pollen binds to a B-lymphocyte and is cross-linked to the immunoglobulin-E (IgE) in a sensitized individual, the latter binds to the high-affinity IgE receptor on the mast cells in the conjunctiva and triggers the mast cell to immediately empty its content of preformed mediators such as histamine, platelet activating factor (PAF; Sharif, 2020), serotonin, bradykinin (BK; [14]), tryptase and cathepsin G, onto the surrounding tissues (Figure 6A). The immediate actions of histamine, and of BK and PAF (e.g. Figure 6B) are to trigger PLC-mediated release of IPs that release intracellular $[Ca^{2+}]_i$ from the endoplasmic reticulum (Figure 6) that causes vasodilation of conjunctival blood vessels (Figure 1) and enhances vascular permeability. This acute early-phase response also results in secretion of various cytokines (e.g. interleukin-1 [IL-1], IL-6, IL-8, and tumor neurosis factor-α [TNF-α]; [13]) from the ocular surface epithelial cells. These inflammatory factors act through a janus kinase (JAK)-mediated enhancement of nuclear transcription to generate and release more cytokines in an ever-increasing positive feed-forward loop, thereby amplifying the inflammatory cascade. Over the next few minutes to hours, the mast cells release newly generated PGs (mainly PGD_2), leukotrienes and cytokines as part of a delayed late-phase secondary response to the allergen. The cytokines in turn activate their cognate receptors, via JAK-mediated signaling cascade (Figure 3), to induce IgE synthesis/release by B-cells and cause inflammatory eosinophils to infiltrate the conjunctiva. This results in leukocyte adhesion, migration and activation, thereby amplifying and exacerbating the local inflammation. Allergic inflammation of the ocular surface is patently visible now and the patient feels the edematous eyelids becoming more itchy, red and increasingly painful/irritated. The patient seeks swift relief to overcome this malady and to feel somewhat “normal” again.

Figure 6A: Involvement of various mediators of allergic conjunctivitis and the feed-forward and feed-back mechanisms of numerous signaling molecules and pathways.

Figure 6B: PAF-induced mobilization of intracellular Ca^{2+} in human conjunctival epithelial cells is shown over time in a cell cluster. Note the time-dependence and differing magnitudes of responses in the 3 cells, and how the Ca^{2+}-waves are generated and propagated.

Years of research has led to the conclusion that histamine is the major culprit in causing the afore-mentioned signs and symptoms of SAC/PAC [13,31]. Accordingly, since the human conjunctival epithelial (HCE; Sharif., *et al*. 1996), human corneal epithelial (HCEPI; [13,32], and human corneal fibroblasts mainly express functionally active histamine H_1-type receptors, the latter receptor became the target for therapeutic intervention despite the fact that four histamine receptor sub-types exist (H_1-H_4; [13,31]). Based on the pharmacological properties of the H_1-receptors which are PLC-coupled [3,10] and their location and actions on HCF/HCE/HCEPI cells [10,18,32,33], and their defined signal transduction pathways, it was possible to address the signs and symptoms of allergic conjunctivitis using potent, selective and efficacious H_1-receptor antagonists specifically formulated for topical ocular use. These drugs directly bind to the H_1-receptor, change its conformation and inactivate its signal transducing ability. The most notable drugs utilized today to treat SAC/PAC constitute emedastine (Emadine®; Sharif, 2020), olopatadine (Patanol®/Pataday®/Pazeo®; [13,18,33], epinastine (Elastat®) and alcaftadine (Lastacaft®), all being nanomolar affinity and potency H_1-receptor antagonists that directly prevent histamine from binding to its receptors on the ocular surface receptors (Figure 6A). The long residence-time of olopatadine within the H_1-receptor binding pocket allows it to have a long duration of action affording 24-hrs of relief from ocular itching and conjunctival redness and inflammation. Fortuitously, drugs such as olopatadine, epinastine and alcaftadine also possess membrane-stabilizing activity which slows and eventually prevents mast cell degranulation within the conjunctiva and thereby restoring homeostasis [13,30]. The patients' eyes become quiescent, and the ocular itch and redness subside.

On the other hand, vernal kerotoconjunctivitis (VKC) has a different etiology to SAC/PAC and mostly affects children. VKC is considered both immunoglobulin (IgE)- and non-IgE-mediated with about half of the patients manifesting an IgE-allergic sensitization. VKC symptoms consist of severe itching, photophobia, mucous discharge, foreign body sensation, and blurring of vision. The increased expression of several chemotactic factors and co-stimulatory signals required for T-cell activation, confirms that VKC is mostly eosinophil-mediated. Pattern recognition receptors, that are pathogen-derived and that also exist on sensitive tissues of humans, such as TLRs, may be partly responsible for causing this ocular surface disease. As can be seen from Figure 5, TLR-activation results in transcriptional increase of inflammatory cytokine production using a variety of cytoplasmic mediators such as JAK-family of tyrosine/serine/threonine kinases (e.g. receptor-interacting-kinase [RIP]; phosphoinositide-3-kinase [PI3K]; protein kinase B [Akt]) and transcription factors

(e.g. nuclear factor-κB [NF-κB]; [34]) acting within the nucleus of ocular surface cells. Activation of the NF-κB pathway is initiated in response to pro-inflammatory stimuli (e.g. lipopolysaccharide, LPS) expressed on the surface of pathogens, or by the secretion of pro-inflammatory cytokines (e.g. TNFα; IL-1) (Figure 3 and 5). Immune cell stimulation leads to activation of the homologous kinase subunits IKK-α and IKK-β. This complex phosphorylates serine residues in the amino-terminal domain of inhibitor of NF-κB (IκBα) upon activation, thus resulting in ubiquitination and its degradation by the proteasome apparatus. IκBα degradation liberates p50-p65 dimer for translocation to the nucleus where it binds to κB sites and directs NF-κB-dependent transcription of appropriate genes (Figure 5). Ultimately, immunoregulatory factor activation promotes lymphocyte proliferation, differentiation, growth, and survival. However, in the context of VKC, mostly detrimental inflammatory components are increased to propagate this deleterious signal transduction cascade (Figure 5). At this time, dual-action drugs with both H_1-receptor-blocking mechanism and mast-cell stabilization (such as olopatadine at 0.2% and 0.7%) provide the best benefits since they are effective immediately and possess long-term disease modifying activity. Topical corticosteroids are typically quite effective since they dampen gene expression of inflammatory mediators (Figure 4) but obviously their serious side-effects prohibit long-term use. Durable immunomodulation with steroid-sparing drugs such as cyclosporine and tacrolimus is often needed. Topical ocular cyclosporin-A in concentrations of 0.05% to 2% reduces inflammatory cytokines and the signs and symptoms of VKC. Tacrolimus 0.1% topical ocular also improves signs and symptoms of VKC. Additionally, adult patients with VKC may respond more favorably to topical cyclosporin therapy. Omalizumab, an anti-IgE monoclonal antibody, has also shown some efficacy in patients with VKC recalcitrant to other treatment strategies but more research is needed to support its protracted and more widespread use.

Lymphocytic T-cell activity is reduced by cyclosporin, a natural product with immunomodulator activity. It inhibits calcineurin in the calcineurin–phosphatase pathway and prevents the mitochondrial permeability transition pore from opening. Cyclosporin binds to the cytosolic protein cyclophilin (immunophilin) of lymphocytes, especially of T-cells. This cyclosporin-cyclophilin complex inhibits calcineurin, which is normally responsible for activating the transcription of the IL-2. In T-cells, activation of the T-cell receptor normally increases $[Ca^{2+}]_i$, which acts via calmodulin to activate calcineurin. Calcineurin then dephosphorylates the transcription factor NF-AT (nuclear factor of activated T-cells), which moves to the T-cell nucleus and increases the transcription of genes to enhance production of IL-2 and related cytokines [34]. Cyclosporin reduces T-cell function by preventing the dephosphorylation of NF-AT. Tacrolimus is an inhibitor of the mechanistic target of rapamycin (mTOR; [35]) (Figure 7), a kinase of the PI3K-related kinase family of protein kinases. Tacrolimus works similarly to cyclosporin but has a greater efficacy than the latter. The signaling elements involved in the action of tacrolimus and other mTOR inhibitors is shown in figure 7. Another mTOR inhibitor, sirolimus, is currently being targeted as a therapeutic agent in the treatment of non-infectious posterior uveitis [35].

Figure 7: This figure illustrates the diversity of cellular and biological responses mediated by mTOR components of the signal transduction processes involving different types of enzymes within cells of the inflammatory/immune/cancer cells.

Ocular hypertension and glaucoma: role of multiple of receptor-signaling pathways

The optic neuropathy that robs the patients of their peripheral vision is known as glaucoma, a collection of similarly related ocular diseases where the retinal ganglion cells and their axons are damaged. Visual impairment due to primary open-angle glaucoma (POAG) is the 2nd leading cause of blindness worldwide (WHO; [36]). Ocular hypertension (OHT), due to accumulation of excessive aqueous humor in the anterior chamber of the eye [37], is often associated with POAG, although other risk factors such as age, African-American heritage, and low cerebrospinal fluid pressure are also involved [11-14]. The elevated intraocular pressure (IOP) causes mechanical damage to the back of the eye structures which are weakest at the area (lamina cribosa, LC; [38-40]) where unmyelinated optic nerve fibers leave the eye in the optic nerve to connect to the brain. Inflammatory (e.g. cytokines like IL-1, IL-6, TNFα; [41,42]), immunogenic (e.g. complement; activated microglia; [42,43]) and vasoconstrictor (e.g. endothelin) factors are released at the deformation of LC and when the retinal blood supply is interrupted/decreased due to vasoconstriction and/or due to tortuosity of the retinal blood vessels [44,45]. All these deleterious substances initiate a series of receptor-mediated signal transduction cascades that ultimately cause the death of RGCs and their axons and hence vision loss [43,46,47]. In normal tension glaucoma, IOP is not such a major factor but nevertheless the patient's eyes experience the majority of the latter events indicating that other triggers causing RGC/optic nerve damage are involved. It's been hypothesized that perhaps the normal tension glaucoma patients have an increased sensitivity their RGCs and optic nerve components to local toxins (e.g. endothelin; glutamate; NO; [47-49]), compromised ocular blood flow [44,47-50], lower than normal intracranial fluid pressure, increased susceptibility to oxidative stress, heightened autoimmunity, etc. [45,47-52].

However, since IOP is the single most validated modifiable risk factor for OHT/POAG [53], attention has been directed at pharmaceutically and physically (laser therapy; AQH microshunts;[11,12,14]) lowering the IOP by enhancing the outflow of the accumulated AQH from the anterior chamber of the eye. Since the inflow and outflow of AQH can be modified [37], development of drugs to inhibit the production of AQH (beta-blockers [54], carbonic anhydrase inhibitors [CAIs]; alpha-2-adrenergic agonists and to increase AQH outflow via the TM system (e.g. muscarinic agonists; rho kinase inhibitors; and NO-activators [8,14]), and via the uveoscleral outflow pathway (e.g. prostaglandin FP-receptor agonists [55,56]; and EP2-receptor agonists [57,58]) has been accomplished and many drugs have been introduced into medical management of OHT and glaucoma. Briefly, in the context of receptors and cell signaling, for example, the prostaglandin FP-receptor agonists (e.g. latanoprost [Xalatan® 0.005%]; travoprost [Travatan® 0.004%]; [55,56]; Tafluprost [Taflutan®]; [59]) and EP2-receptor agonists (e.g. omidenepag isopropyl [Eybelis®] 0.002%; [57,58]) administered once-daily in the evening lower IOP for up to 24-hrs. Specifically, the mechanism of action of FP-receptor agonists can be described as follows to illustrate the cell signaling mechanism: thus, upon topical ocular dosing of the isopropyl ester drugs like travoprost on the cornea, the lipophilic ester penetrates the latter and is hydrolyzed by esterases to release the free acid form (travoprost acid; TA) into the AQH. TA then binds to FP-receptors in both the ciliary muscle (CM) cells [15,16], and the TM cells [17] to generate IPs and diacylglycerol (DAG) via PLC-induced phosphoinositide lipid breakdown [12-17] into the cytoplasm of the cells which in turn mobilize $[Ca^{2+}]_i$ and activate protein kinases, respectively (Figure 1, 8). The net result of these activities is the transcriptional increase and release of matrix metalloproteinases (MMPs; [12,13,16] (and additional endogenous PGs that amplify the signal transduction pathways) (Figure 8) into the extracellular space which then digest the accumulated extracellular collagen and other matrix components to create and expand spaces between the CM muscle bundles and between TM cells to promote the egress of AQH, thereby lowering IOP [12-14]. Various FP-receptors of differing agonist potencies, intrinsic activities (full *vs* partial agonists) and clinical efficacies are used as first-line therapeutics to treat OHT/POAG (Figure 8) [13,14,56]. Whilst apparently simple in its design, exploitation of this receptor-mediated signal transduction mechanism does indeed elegantly address the disease process and provide a solution of reducing OHT that helps slow down the damage to the visual system and preserve vision for millions of patients suffering from these ocular disorders. In some ways, these drugs overcome an intrinsic deficiency of either FP receptors being recruited and/or a deficit of endogenous PGs being produced and secreted in OHT/POAG patient's eyes.

On the other hand, drugs that lower IOP by suppression of AQH production, such as brimonidine/ apraclonidine, do so by activating pre-synaptic alpha-2 receptors which trigger intracellular

Figure 8: The diagram in the left panel shows how the effects of FP-receptor-PLC activation in human ciliary muscles/ TM cells leads to generation of intracellular second messengers and with the eventual stimulation of AQH outflow from the anterior chamber of the eye to lower IOP. The panel on the right depicts the relative concentration-response curves for typical FP-receptor agonists in stimulating the production of intracellular inositol phosphates via PLC-induced PI turnover. Note how the relative potencies and the intrinsic activities of the different drugs varies, thereby illustrating full- and partial agonist nature of these drugs.

adenylate cyclase to generate cAMP and thereby inhibit release of norepinephrine. This prevents the ciliary epithelial cells producing AQH. Likewise, beta-adrenergic antagonists such as timolol and betaxolol and levobetaxolol [54] reduce the intraocular pressure (IOP) by blockade of sympathetic nerve endings in the ciliary epithelium causing a fall in AQH generation. Lastly, since carbonic anhydrase is directly involved in the molecular production of AQH, inhibitors of this enzyme in the ciliary epithelium block its activity and thus lower IOP in animals and in OHT/POAG patients. Lastly, autotaxin is an ectonucleotide pyrophosphatase/phosphodiesterase 2 secreted enzyme that possess lysophospholipase D activity that converts lysophosphatidylcholine within cell membranes or from food into lysophosphatidyl acid (LPA) (Figure 2). Recent evidence suggests that inhibitors of autotaxin [60] can lower IOP by indirectly reducing the activation of rho kinase by preventing generation of LPA within ciliary muscle and TM cells. Previously, much research and clinical data have supported the role and use of rho kinase inhibitors (e.g. ripasudil; netarsudil; Figure 2) to treat OHT and glaucoma since these drugs relax CM and TM cells and thereby promote TM outflow of AQH to lower and control IOP.

Age-related macular degeneration and role of receptor-tyrosine-kinase signaling pathways

Visual impairment caused by wet age-related macular degeneration (wAMD) is a serious and highly prevalent ocular disease in older patients, especially in the Western developed nations. The loss of central vision associated with wAMD profoundly and negatively affects visual functioning such that tasks we take for granted such as reading and driving are rendered quite difficult. wAMD is due to damage of the retinal macula, a region that is highly enriched in rods and cones, and which only represents about 2% of the total retina but which requires almost half of the visual cortex to process macular-derived information transmitted from the eyes to the brain. wAMD afflicted 6.2 million people globally in 2015, and was the 4th leading cause of blindness after cataracts, preterm birth, and glaucoma [36]. Patients over the age of fifty often succumb to wAMD and in the US this is the most prevalent form of vision loss in this age bracket. Major risk factors for AMD/wAMD are smoking, a diet rich in fats, atherosclerosis, hypertension, obesity and extended UV exposure.

Whilst the exact etiology of wAMD remains largely unknown, oxidative stress, mitochondrial dysfunction and inflammatory processes appear to conspire to cause this disease at the outer retinal level [61]. Accumulation of yellow drusen (collection of extracellular proteins and lipids) in the macula between the retinal pigment epithelium (RPE) and the underlying choroid triggers the damage of the retina. Atrophic AMD (geographic atrophy) begins with a progressive and irreversible loss of the choriocapillaris (nutrient supplier to RPE cells), RPE (that absorbs light and phagocytoses dead photoreceptor cells), and the overlying photoreceptors. Decreased blood flow in the choriocapillaris causes hypoxia (Figure 9) and precedes atrophy of the RPE and the photoreceptors. Neovascular or exudative AMD/ wAMD causes vision loss due to abnormal new blood vessel growth (choroidal neovascularization) in the choriocapillaris through the Bruch's membrane. wAMD is usually, but not always, preceded by the dry form of AMD. The proliferation of abnormal blood vessels in the retina is stimulated by vascular endothelial growth factor (VEGF) released during hypoxic conditions at the back of the eye (Figure 7 and 9) [61,62]. Because these new blood vessels are abnormal, these are also more fragile than typical blood vessels, which ultimately leads to blood and protein leakage below the macula leading to local edema. Bleeding, leaking, and scarring from these blood vessels eventually cause irreversible damage to the photoreceptors and rapid vision loss if left untreated. Since VEGF and related growth factors, such as hypoxia-induced factor (HIF) (Figure 7 and 9) and angiopoietin-2 are responsible for the angiogenic response of the choroidal vasculature [61,62], inhibitors of the receptor-tyrosine-kinase for VEGF and/or binding and removal of this factor with antibodies represent the current treatment options for wAMD. Ranibizumab, aflibercept and brolucizumab are such approved biologics that remove VEGF from the retina and vitreous following intravitreal injection and thus effectively remove VEGF and prevent it from occupying and activating sufficient number of receptors and thereby halt downstream signaling (Figure 9) [61,62]. Other treatment strategies for wAMD involve blocking the receptor-tyrosine-kinase (RTK) binding sites for VEGF on the inner leaflet of the VEGF-receptor-kinase complex directly with small molecule drugs (e.g. axitinib; PAN-90806; RTKi-MPP; Brevanib [62]) and thus blocking the VEGF-receptor signal transduction downstream from the RTK to prevent formation of the new blood vessels as illustrated in figure 9.

Figure 9: The signal transduction pathway utilized by VEGF receptors involving recruitment of enzymatic activity of the receptor-tyrosine-kinase (RTK) component of the receptor and the downstream events leading to aberrant angiogenesis are shown in the left panel. The sequestering of VEGF by various antibody biologics to prevent VEGF binding to its receptors and thus inhibit angiogenesis is also depicted in the left panel. Conversely, the left panel shows how small molecule drugs bind directly to the catalytic units of the RTK to block the signaling ability of the VEGF receptor complex and thus prevent angiogenesis.

Relatively recent research has shown that drusen contains several inflammatory mediators which recruit complement factors from the circulation. Accordingly, abnormal activation of the immune and inflammatory systems also appear to play a significant part in the etiology of wAMD. Complement may thus also be a culprit in addition to oxidative stress, malfunctioning autophagic mechanisms, and inflammation in the development of wAMD encompassing the angiogenic processes [61,62].

Concluding Remarks

In conclusion, it is evident that receptors and their signaling pathways are pivotal in cellular communication in normal and disease states of the eye. Therapeutic intervention to treat the selected diseases discussed above necessitates administering drugs/antibodies (agonist/antagonists/activators/inhibitors) that either act at the receptor-binding sites of the endogenous ligands at the exterior cell membrane, or at the membrane/cytoplasm boundary at the receptor-kinase complex level or at second messenger/cytoplasmic protein/organelle level, or modulating gene expression within the cell nucleus. A number of examples have been provided to illustrate these aspects. Receptors, enzymes, ion-channels and their signal transduction mechanisms, which form the basis of majority of the health authority-approved drugs today, illustrate the importance of regulating and correcting signaling processes in health and disease.

Conflict of Interest Statement

All views and statements in this article are based on the author's personal experiences and perspectives. His whole intention is writing this review article is: 1). To help disseminate information and knowledge and to teach and inspire others; 2). To enhance the awareness of young students and new researchers about the covered subject matter; 3). To raise awareness about the research and science behind the drug discovery aspects of disease pathogenesis and their mitigation at the level of receptors, enzymes, etc. It is hoped that these scholarly aspirations will be appreciated in an effort to "let knowledge enrich your life so you can enlighten others' minds ".

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