Pituitary Adenylate Cyclase-Activating Polypeptide Role in the Eye

Subjects: Ophthalmology

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Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) is a neuropeptide with widespread distribution throughout the central and peripheral nervous system as well as in many other peripheral organs. It plays cytoprotective effects mediated mainly through the activation of specific receptors. PACAP is known to play pleiotropic effects on the eye, including the cornea, protecting it against different types of insult.

Keywords: PACAP ; eye ; cornea ; injury ; repair

1. Introduction

The cornea, the outermost part of the eye, is a transparent tissue with refractive and barrier functions [1]. Due to the direct connection of the cornea with the external environment, different types of insults, such as chemical, mechanical, and thermal damage, can cause its injury [2]. For this reason, corneal damage represents one of the major causes of blindness worldwide [3]. To date, corneal transplantation represents the most common and successful surgery by restoring good eyesight. However, the high cost, the high graft failure rate, the legal issues, and the lack of donors urge new options for treating, at least, some corneal lesions [4][5].

The neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) was isolated for the first time in 1989 from sheep hypothalamic extracts [6]. It exists in two active forms, PACAP27 and PACAP38, including 27 and 38 amino acid residues, respectively, and with mostly comparable functions. PACAP belongs to the vasoactive intestinal polypeptide (VIP)/secretin/glucagon family peptides and shows a high degree of homology (~70%) to VIP. PACAP and VIP share three different receptors: the PAC1 receptor (PAC1R), which has a high affinity to PACAP as compared to VIP, and the VPAC1 and VPAC2 receptors, showing a comparable affinity for both peptides [7][8][9][10]. Alternative splicing occurring in the PAC1R gene generates different variants (Null, Hip, Hop1, Hop2, Hiphop1, Hiphop2, short and very short isoforms) that can activate the adenylate cyclase (AC) pathway forming cAMP as well as phospholipase C (PLC) pathway promoting the formation of protein kinase C (PKC) [11]. VPAC receptors are coupled to Gs proteins resulting in the activation of AC as well as other signaling cascades [12][13]. Some of the protective effects of PACAP are also mediated by the stimulation of an intracellular factor known as activity-dependent neuroprotective protein (ADNP) [14][15][16]. In accord, peptide activity scanning identified NAP (NAPVSIPQ), the smallest active element of ADNP, acts in synergy with PACAP by showing neuroprotective effect [17][18]. PACAP is widely distributed in the nervous system and is consequently implicated in different neurodegenerative diseases [19][20][21][22][23][24][25][26]. In addition, it plays a controversial function in various types of tumors by promoting or inhibiting its progression [27][28][29][30][31][32][33][34][35][36][37].

2. Overview on the Cornea Anatomy

The human cornea, together with the surrounding sclera, constitutes the protective outer barrier of the eye. In particular, it represents the outer covering of the anterior portion of the eyeball by exerting two essential functions: it protects from external physical trauma and provides about 70% refractive power of the eye. To perform these functions, the corneal tissue is both mechanically strong and transparent.

The anterior surface of the cornea is convex and aspheric [38]. The cornea comprises five main layers: the epithelium, the Bowman's membrane, the stroma, the Descemet's membrane, and the endothelium [39][40] (**Figure 1**).

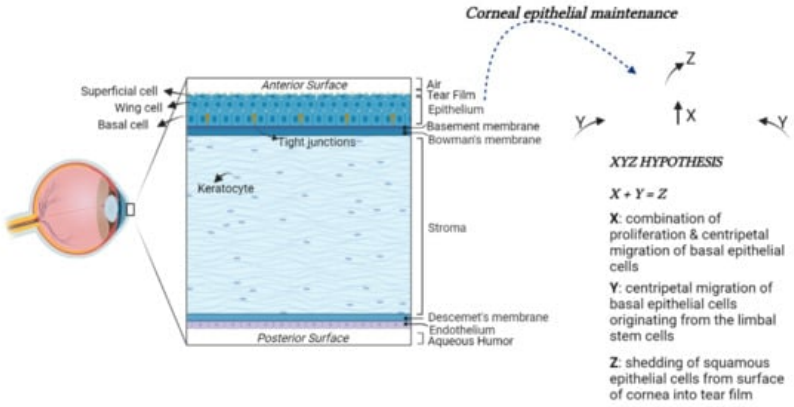

Figure 1. Structural anatomy of the human cornea. From left to right: (1) A diagram of human cornea structure; (2) The XYZ hypothesis. The asymmetric division of LESCs generates a stem-like daughter cell, remaining within the limbus, and a TAC, migrating in a centripetal direction (Y). TACs undergo multiple replications. In this process, they lose stemness, migrate anteriorly and differentiate to post-mitotic suprabasal wing cells (X), and progress in superficial squamous cells, which are lost during normal corneal surface exfoliation (Z) of the epithelial corneal maintenance.

The corneal epithelium is continuously subjected to a wide range of insults; therefore, its long-term maintenance is regulated by limbal epithelial stem cells (LESCs). The LESCs reside in an annular transition zone known as the limbus, laying at the junction area between the cornea and the sclera. They show typical characteristics of immature and undifferentiated cells [41][42]. In particular, they do not express the cytokeratin 3 and 12, commonly detected in mature, differentiated corneal epithelial cells, whereas they express cytokeratin 14 or TP63, which are stem markers of the immature or progenitor cells in various stratified epithelia. The LESCs give rise to transit-amplifying cells (TACs), which migrate and divide into basal corneal epithelial cells in normal homeostasis conditions or to replace those cells desquamated or lost by lesions (**Figure 1**) [43][44].

3. Role of PACAP and Its Receptors in the Eye

The presence of PACAP and its receptors has been largely shown in the eye [33][45]. PACAP positive expression was found in mammalian, teleost, turtle, and chicken retina [46]. In particular, in situ hybridization and immunohistochemical analysis have revealed the presence of PACAP in specific cell populations of retinal tissue samples. PACAP was positively expressed in the nerve fiber layer (NFL), the ganglion cell layer (GCL), the inner plexiform layer (IPL), and the pigment epithelium (PE). The immunoreactivity of PACAP appeared in the early phase of retinal development [47], as demonstrated by its presence in the chick inner nuclear layer (INL) from embryonic day 8 [48]. PACAP mRNA expression was detectable in the rat GCL at embryonic day 20 [49], whereas, in the zebrafish, PACAP immune-positive signal was found in the retina at 24 h post-fertilization [50]. PAC1R was strongly expressed in the GCL, in neuronal cell bodies of amacrine and horizontal cells localized in the INL and in the PE. On the contrary, PAC1R was weakly expressed in the IPL, outer plexiform layer (OPL), outer nuclear layer (ONL), and photoreceptor layer [51][52][53][54]. The expression profile of PAC1R splice variants (Null, Hip, Hop1, Hop2, Hiphop1, and Hiphop2) was described during retina development [11]. The expression of PAC1Rs at the subcellular level was identified at the plasma membrane, in the rough endoplasmic reticulum, in the cytoplasmic matrix of retinal ganglion cells (RGCs) and amacrine cells in the INL [55]. PAC1R immunoreactivity was also detected in retinal tissue and in rat primary cultures of Müller cells [56][57]. In the rat retina, the expression of VPAC1R and VPAC2R was demonstrated [58]. Moreover, Lakk et al. [11] showed the potential involvement of VPACRs at all stages of retinal development in the rat.

The protective effects of PACAP in the visual system have been widely studied in the neural and non-neuronal parts of the eye, including the cornea (**Figure 2**).

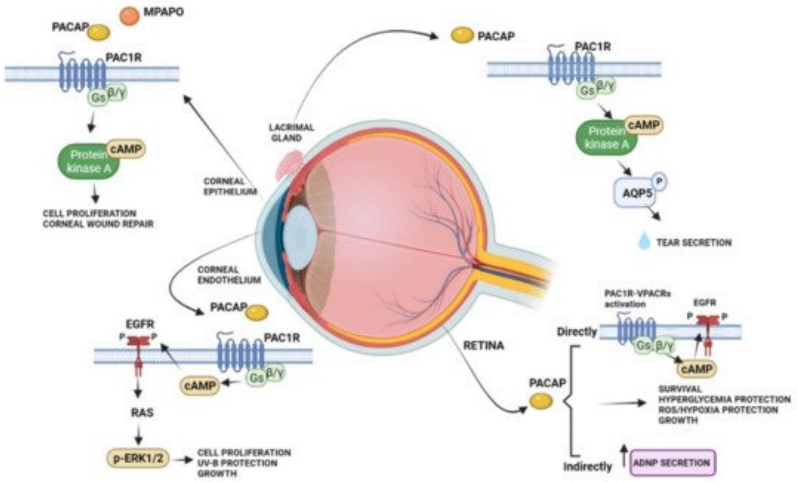

Figure 2. Schematic diagram showing the main pro-survival/antiapoptotic/protective intracellular pathways activated by PACAP in different ocular structures.

Several studies showed that PACAP has protective effects in the retina against toxic or ischemic insults, inflammation, hypoxia, oxygen-induced retinopathy, traumatic injuries, glaucoma, and diabetic retinopathy [59][60][61][62][63][64][65][66][67][68][69][70]. The protective role of PACAP was observed in different cell types, including bipolar neurons, amacrine, and pigment epithelial cells [71][72]. In particular, in the retinal pigment epithelial cells, PACAP counteracted oxidative stress and hypoxic insult by exerting protective and pro-survival effects [72][73][74][75].

4. The Role of PACAP in the Cornea

The expression of PACAP and its receptors has been shown in the cornea. Here, PACAP positive cells were found in rabbit and human corneal epithelium, particularly in the basal cells. Moreover, the expression of PACAP was detected in the corneal endothelial layer and weakly in the stroma [76][77]. High expression levels of PAC1R were identified in the stromal and basal cells of the epithelium. Furthermore, the VPACRs were strongly expressed in all layers of the epithelium and in stromal cells of the rabbit cornea [77]. Previously, Wang [78] et al. detected PACAP immunoreactivity in nerve terminals running in the stroma and sending off some branches into the epithelium. Corneal injury is frequently associated with damage of the epithelium and its innervating fibers. In an in vivo experimental model of laser-assisted in situ keratomileuses (LASIK) surgery, PACAP showed to accelerate recovery of corneal sensitivity after the creation of a corneal flap. In more detail, the administration of 10 μM PACAP27 increased up to 75% the corneal sensitivity eight weeks after the operation [79]. In accord, it has been demonstrated that PACAP induced the growth of neuronal processes in cultured trigeminal ganglion cells. These neurons secrete various biologically active molecules enhancing the proliferation and differentiation of corneal epithelial cells as well as collagen VII production, important to maintaining and repairing the corneal epithelium [80]. The protective effect of PACAP was confirmed by Wu et al., 2015 [81], by showing that the peptide alone or in combination with the receptor protein of laminin, known as N-terminal agrin domain (NtA), significantly accelerated the process of repairing the mechanically injured corneal epithelial cells. It is well known that tear fluid contains different antibacterial proteins, growth factors, and secretory mucin important for corneal maintenance and its repairing [80][82]. For this reason, tear fluid reduction, occurring in dry eye syndrome, is an inducing factor in corneal keratinization. PACAP played an important role in protecting the corneal surface by stimulating tear secretion [83]. As described above, in the lacrimal gland, PACAP is an endogenous modulator of AQP5, involved in tear production [84]. In accord, PACAP null mice showed a reduction in the AQP5 expression, whereas the eye treatment with PACAP drops stimulated its transcription. Furthermore, PACAP null mice exhibited the dry eye syndrome phenotype with a corneal disorder associated with the reduction in tear volume [84].

5. Conclusions

The direct contact of the cornea with the external environment makes it frequently exposed to various types of injuries. The surgical replacement of lesioned cornea with healthy donor tissue is the frequently used therapeutic approach. To date, the actual challenge is linked to recruiting a sufficient number of donors, requiring alternatives to decrease this persistent demand. PACAP has shown important corneal protective and regenerative effects. Therefore, the development of innovative nanoformulation platforms for topical PACAP or PAC1R agonists delivery, as well as the synthesis of molecules able to increase PACAP endogenous expression, might represent a valid strategy for the treatment of some corneal diseases.

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