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ABSTRACT

Glaucoma is characterized by a slow and progressive degeneration of optic nerve, including retinal ganglion cell (RGC) axons in the optic nerve head (ONH), leading to visual impairment. Despite the high prevalence, the biological basis of glaucoma pathogenesis still is not yet fully understood, and the factors contributing to its progression are currently not well characterized. Intraocular pressure (IOP) is the only modifiable risk factor, and reduction of IOP is the standard treatment for glaucoma. However, lowering IOP itself is not always effective for preserving visual function in patients with primary open-angle glaucoma. The second messenger cyclic adenosine 3′,5′-monophosphate (cAMP) regulates numerous biological processes in the central nervous system including retina and optic nerve. Although recent studies revealed that cAMP generated by adenylyl cyclases (ACs) is important in regulating aqueous humour dynamics in ocular tissues such as ciliary body and trabecular meshwork as well as cell death and growth in retina and optic nerve, the functional role and its significance of the cAMP in the eye of glaucoma remain to be elucidated. In this review, we will discuss the functional role of cAMP in aqueous humour dynamics and IOP regulation, and review the current medications, which are related to cAMP signaling pathway, for glaucoma treatment. Also, we will further focus on cAMP signaling in RGC growth and regeneration by soluble AC as well as ONH astrocytes by transmembrane ACs to understand its potential role in the pathogenesis of glaucoma neurodegeneration.

INTRODUCTION

Glaucoma is an optic neuropathy and the main cause of irreversible blindness worldwide (1-3). It has been estimated that glaucoma will affect more than 80 million individuals worldwide by 2020, with at least 6-8 million individuals becoming bilaterally blind (1, 2). Primary open-angle glaucoma (POAG), the most common form of open-angle glaucoma, is characterized by a slow and progressive degeneration of retinal ganglion cell (RGC) axons in the optic nerve head (ONH) and retinal nerve fiber layer, leading to an excavated appearance of the optic disc and visual impairment (1, 3). Regardless, the biological basis of glaucoma pathogenesis is not yet fully understood, and the factors contributing to its progression are currently not well characterized.

Cyclic adenosine 3′,5′-monophosphate (cAMP) is the first discovered second messenger for the signal transduction (4). Its signaling pathway exists in all type of cells and contributes to numerous biological processes such as cell growth, differentiation, death, gene expression and inflammatory cytokine secretion, and neurotransmission (5-7) in the central nervous system (CNS). Upon stimulation, cAMP synthesis and its degradation are tightly regulated by adenylyl cyclases (ACs) and cyclic nucleotide phosphodiesterases (PDEs), respectively (6). The activation of cAMP signaling exerts opposite effects on cell survival in a cell type-specific manner (8) due to that it exerts its effect through various effectors such as cAMP-dependent protein kinase A (PKA) (9, 10), guanine-nucleotide exchange proteins activated by cAMP (11, 12) and cyclic-nucleotide-gated ion channels (13, 14).

Among the key regulators of cAMP signaling pathway, ACs are enzymes that catalyze the synthesis of cAMP from adenosine 5′-triphosphate (ATP). To date, ten distinct AC genes (AC1-10) has been identified by molecular cloning techniques and these genes encode nine mammalian transmembrane ACs (tmACs; AC1-9), and a soluble AC (sAC; AC10), respectively (15-17). Each AC has various functional roles and distribution pattern in tissues (18, 19). The activity of tmACs is regulated by physical and functional interaction with G-protein coupled receptors (GPCRs) in the plasma membrane (19-21). In contrast, sAC does not have transmembrane domains and is localized in the cytoplasm compartments and within distinct organelles such as nuclei and mitochondria (17, 22). While tmACs except AC9 are sensitive to forskolin but not to bicarbonate, sAC is sensitive to bicarbonate but not to forskolin and requires a divalent cation such as Ca^{2+} for its activity (6, 17).

ACs have been thought as potential drug targets in many neurodegenerative disorders including glaucoma (23, 24). Since the activation of cAMP signaling pathway by forskolin, a tmACs activator, has been reported to be involved in the reduction of intraocular pressure (IOP) (25, 26), a recent clinical trial for POAG treatment have demonstrated that 1% forskolin eye drop can be used as a safe alternative to β-adrenergic receptor blockers (β-blockers)and prostaglandin analogues (27), which are mostly used for glaucoma treatment although they have several side effects (2, 28). Considering the evidence demonstrating that RGC survival and axon growth are enhanced via activation of sAC-mediated cAMP signaling pathway (29-32), the therapeutic strategy for modulating cAMP signaling pathway in glaucoma treatment is considered to rescue RGCs against glaucomatous insults. However, the effect of the cAMP pathway activation on IOP regulation, RGC and ONH degeneration remains poorly understood. In this review, we will discuss recent literature for the role of cAMP in the eye, addressing its possible relationship with glaucoma protection or degeneration.

cAMP in IOP regulation

IOP regulation by aqueous humour dynamics: IOP is currently the only proven treatable risk factor in glaucoma (1, 28). Aqueous humour that is secreted to the iris by the ciliary body in the posterior chamber not only regulates IOP by a balance between the secretion and drainage but also provides nutrients to the iris, lens, and cornea by circulation in the anterior chamber (1). The outflow of aqueous humour is controlled via a conventional pathway through trabecular meshwork (TM) and Schlemm's canal (SC), and an independent uveoscleral outflow pathway through the ciliary body and iris root (33, 34). In this regards, the therapeutic strategies that reduce aqueous humour inflow and/or increase its outflow have been thought to be important to treat IOP-related glaucomatous optic neuropathy.

The role of cAMP on aqueous humour inflow: Lowing or stabilizing IOP is considered to be an effective approach to reducing glaucoma progression (2, 35). Previous clinical studies have reported that the adrenergic agents, such as epinephrine and phenylephrine, lower IOP in patients with POAG (36, 37). Variations of aqueous humour inflow in IOP changes are associated with the 24 h circadian IOP profile and the body posture (35, 38). Since Neufeld *et al*. have first reported that adrenergic agents, including epinephrine and phenylephrine, increased cAMP

concentration in the aqueous humour (39), treatment of timolol, the first FDA approved β-blocker for the treatment of glaucoma (40), decreased IOP in normal volunteer and glaucoma patient (41, 42). These findings led to an attention of the adrenergic control on IOP and the therapeutic potential of the cAMP signaling pathway in glaucoma treatment. Since then several studies have identified adrenergic receptor-AC complex in the ciliary process (43-46), supporting the functional role of cAMP in aqueous humour formation. The activation of ACs-linked receptors by several endogenous or exogenous factors not only increases intracellular cAMP level but also decreases net aqueous humour flow and lower IOP (37, 39, 47-51). Furthermore, an increase of cAMP level by a topical suspension of 1% forskolin lowered IOP in rabbits and monkeys, as well as normal human volunteers (25), suggesting that increasing cAMP may decrease the net rate of aqueous humour inflow (46).

Due to the discrepancy between adrenergic agonists and blockers (e.g., epinephrine and timolol) on IOP regulation, however, it is difficult to conclude whether increasing cAMP level reduces IOP inflow. Using molecular and cellular biological techniques, recent evidence addresses that adrenergic receptors are GPCRs that are classified into two main categories, α and β type, and these are further grouped according to their isotypes (α1, α2, β1, β2, and β3) that are linked to different G_α subunit (Table1). Epinephrine, also known as adrenaline, is a nonselective agonist of all adrenergic receptors and timolol is a non-selective β-blocker. Currently, the agonists which are selectively targeted to the α2 subclass are most commonly prescribed to lower IOP in patients with glaucoma (52). The activation of α2 adrenergic receptor reduces cAMP production due to it is linked to $G_{\alpha i}$, inhibitory G_α subunit. Indeed, adrenergic receptor agonists (e.g. apraclodine and brimonidine) decrease aqueous humour production (53-55). However, β adrenergic receptors are mainly linked to $G_{\alpha s}$, stimulatory G_α subunit, (Table 1) and β2 adrenergic receptor is predominantly present in human ciliary processes from donor eyes (56). Also, timolol decreases the aqueous humour formation in ciliary epithelium by a cAMP-dependent manner (57, 58). Together, these findings support the notion that reducing cAMP, but not increasing cAMP, lowers aqueous humour formation and IOP. Although current studies do not provide a clear conclusion whether the increase or decrease of cAMP level reduces aqueous humour formation, it is possible that cAMP plays a critical role in the regulation of aqueous humour production and IOP inflow.

The role of cAMP on aqueous humour outflow: Aqueous humour outflow facility decreases with aging and glaucoma progression (59). Elevated IOPs in glaucoma are results from the predominantly reduced capacity of outflow in the conventional pathway rather than disruption of IOP-maintaining strategies through decreasing both inflow and uveoscleral outflow without changes in conventional outflow facility in healthy aging eye (59, 60).

Increasing outflow facility through the elevated cAMP level by adrenergic agents has also been reported (48, 61, 62); however, it has not been explained the precise effect of cAMP until sAC is discovered to play a role in the outflow control. Carbonic anhydrases are a family of enzymes that catalyze the rapid interconversion of carbon dioxide and hydrogen peroxide (H_2O_2) to bicarbonate (HCO_3^-) and H^+, and its inhibition lowers IOP in patient with glaucoma (63). Since an HCO_3-sensitive AC activity has been reported in the ciliary body of rabbit eye (64), sAC expression was identified in the non-pigmented epithelium of the ciliary body and the sAC was characterized as a responsible enzyme to control the activity of cAMP in the ciliary body (65). Although carbonic anhydrase inhibitors including acetazolamide are known to lower IOP due to a diminished rate of aqueous humour formation in the ciliary epithelium (63, 66), the relationship between carbonic anhydrase-generated HCO_3^- and cAMP signaling pathway has yet to be characterized in IOP regulation. Furthermore, it is unknown whether sAC contributes to aqueous humour formation in the eye.

If so, how does sAC regulate IOP? Shahidullah *et al*. examined the influence of carbonic anhydrase inhibitors on sAC and found that acetazolamide increases sAC-generated cAMP level in ciliary epithelium, suggesting the possibility that sAC-mediated increasing cAMP level can lower IOP (67). Previous studies revealed that sAC contributes to the regulation of conventional outflow (68). In these studies, Bestropin 2 (Best2), an anion channel, was characterized as a bicarbonate channel (69) and Best2 was only present in the non-pigmented epithelium of the ciliary body in the eye (68, 70). Furthermore, Best2 knockout mice show a significant IOP lowering than wild-type (WT) control littermates (71, 72). Because sAC plays a role as an evolutionary conserved HCO_3^- sensor (73), it was hypothesized that sAC may contribute to downstream function of Best2 in non-pigmented epithelium. Interestingly, they found that sAC knockout mice showed a higher IOP with a lower outflow facility than WT controls (65). Collectively, these studies suggest that sAC is critical to regulate IOP. Because no sAC expression is observed in drainage-associated tissues such as the TM/SC complex of the mouse

(65), it is proposed that there may be an unknown biochemical pathway for communication between the ciliary body and drainage tissues, which is regulated by HCO_3^- and cAMP (65, 68). However, the precise mechanism of the IOP regulation by sAC remains unknown.

Cholinergic drugs, also known as cholinomimetics, miotics, parasympathomimetics, and acetylcholine receptor agonists, are the first class of drugs that are used to treat glaucoma (74). Cholinergic drugs including pilocarpine and carbacol have been used to increase outflow through the conventional pathway (75, 76). Cholinergic drugs can act directly by binding to muscarinic acetylcholine receptors, which are GPCRs (77). These receptors have five isoforms (M1~M5) and all types of these receptors are expressed in the eye (77). Although cholinergic drugs have been reported to increase outflow facility of aqueous humour via M3 that is linked to $G_{\alpha q}$, a G_α subunit which activates phospholipase/Ca^{2+} pathway (77), some types of these receptors (M1, M2 and M4) are also linked to $G_{\alpha s}$ or $G_{\alpha i}$ subunit that can stimulate or inhibit AC activity, respectively (Table 1). Interestingly, AC2 and 4 are expressed in the human outflow tissues, and carbachol treatment increases outflow facility that is mediated by cAMP (78).

Prostaglandin analogs are the newest class of drugs that are the most efficacious to lower IOP in patients with POAG (28, 79). Prostaglandins are a group of physiologically active lipid compounds acting like the hormone and exert their effects by binding to ten known prostaglandin receptors such as type I, E and F, which are GPCRs liking to various G_α subunit including $G_{\alpha s,}$ $G_{\alpha i,}$ and $G_{\alpha q}$. Since $G_{\alpha q}$-linked prostaglandin F receptor has been mostly targeted and used for glaucoma treatment, little is known about the effect of prostaglandin analogs through cAMP signaling pathway in IOP regulation. However, several studies have intriguingly demonstrated that $G_{\alpha s}$-linked prostaglandin EP4 receptor is expressed in eye tissues including cornea, iris, ciliary body, TM/SC complex and retina, and activation of this receptor with its agonists (3,7-di-thia PGE1 and PF-04475270) reduces IOP in experimental animal models of glaucoma (80). Although there may be limited opportunity to develop EP4 agonists for clinical evaluation in patients due to the risk of corneal neovascularization and persistent ocular hyperemia (80), these results also strongly support the notion that cAMP is a key regulator of IOP control in glaucoma. To date, there is no direct evidence that sAC-mediated cAMP signaling pathway is involved in the IOP-lowering effect of cholinergic drugs and prostaglandin analogs. Considering the recent evidence that GPCR-mediated Ca^{2+} increment can also directly activate sAC (81), however, it is possible that the effect of IOP lowering by these drugs might be from sAC activation via $G_{\alpha q}$-

mediated Ca^{2+} signaling. Further studies to examine the relationship between sAC-mediated cAMP signaling and these drugs may provide important insight to understand the functional role of cAMP in IOP regulation.

cAMP in RGCs

RGCs communicate the information from visual processing in the retina to the brain. RGCs are the most predominant cell type in the ganglion cell layer, which is the innermost retinal layer. The cell body of the RGC extends an axon that runs along the nerve fiber layer of the optic disc (also known as ONH). In the human, RGC axons terminate mostly in the lateral geniculate nucleus and some in the superior colliculus to complete visual system (1). Because RGC and its axon loss are a major pathological phenotype during visual impairment in glaucoma (1, 28), current studies focus on the direct or indirect prevention of the loss of RGC and its axon for glaucoma treatment. Currently, several studies have demonstrated that cAMP is involved in RGC survival (29, 82-86) and differentiation (87), as well as its axonal growth (82, 83) and regeneration (30).

Glutamate excitotoxicity has been implicated as an important pathophysiological mechanism underlying RGC death in glaucomatous neurodegeneration (88-91). Brimonidine, a selective α2 adrenergic receptor agonist, provides a significant evidence that links cAMP signaling pathway and glutamate excitotoxicity to protect RGCs directly against glaucomatous damage. The potential mechanisms for brimonidine-mediated RGCs protection have been implicated as inhibition of glutamate release, upregulation of brain-derived neurotrophic factor expression, regulation of cytosolic Ca^{2+} signaling and modulation of *N*-methyl-D-aspartate receptors (NMDARs) function (92-94). Since dexmedetomidine, a α2 adrenergic receptor agonist, has been reported to be neuroprotective in animal models of focal cerebral ischemia (95), several studies have demonstrated that α2 adrenergic receptor is present in the retina (96-98) including human RGCs (99) and its activation protects RGCs in an animal model of glaucoma (97). Furthermore, brimonidine clinically preserved visual function in glaucoma patients with high pressure or low pressure (100, 101), suggesting an important evidence that brimonidine may also be involved in neuroprotection by the independent manner with IOP-lowering action. Indeed, brimonidine has been reported to protect RGCs against glutamate excitotoxicity *in vitro* as well as in rodent models of experimental ischemia or glaucoma (92, 97, 102-106). How does cAMP

signaling pathway regulate the brimonidine-mediated RGCs protection? Of interest, brimonidine protects RGCs through preventing the increase in intracellular calcium concentration ($[Ca^{2+}]_i$) induced by activation of NMDARs (92, 94, 105). Furthermore, brimonidine reduces NMDA-evoked $[Ca^{2+}]_i$ increase, while isoproterenol, a β adrenergic receptor agonist, enhances NMDA-evoked $[Ca^{2+}]_i$ increase via a cAMP/PKA signaling pathway-dependent manner (107). These results importantly suggest that brimonidine-mediated inhibition of cAMP/PKA pathway could be an important mechanism to protect RGCs against glutamate excitotoxicity-induced glaucomatous neurodegeneration.

Although the excessive Ca^{2+} influx in the excitotoxicity condition causes RGCs death, Ca^{2+} homeostasis in normal condition is essential for RGC function and survival. Furthermore, the elevated Ca^{2+} level has been reported to exert the protective effect on RGCs through the activation of cAMP signaling pathway (82, 83, 86, 108-110). Surprisingly, a recent study has demonstrated that RGC death was not exacerbated by overstimulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor-mediated Ca^{2+} influx in purified RGCs *in vitro*. Instead, this stimulation improved RGC survival in contrary to NMDAR activation-mediated cell death (111). How does the elevated Ca^{2+} influx protect RGCs? Previous studies have demonstrated that RGC's response to neurotrophic factors is weak unless they are depolarized, or intracellular cAMP level is elevated (82, 83). Furthermore, electrical activity-mediated depolarization promotes RGC survival and axon growth by increasing intracellular cAMP level (82, 83, 86, 108). Also, the depolarization of RGCs activates a cAMP/PKA pathway in a Ca^{2+} dependent manner (110). If so, what are the key regulators of Ca^{2+}-dependent activation of the cAMP/PKA in RGCs? Screening analysis for AC isotypes in RGCs identified that total six tmACs (AC1-3, 5, 8 and 9) and sAC are expressed in RGCs (18, 24, 112). Among them, AC1, 3, 8, and sAC are characterized to be activated by Ca^{2+} (109, 113). Moreover, recent studies have demonstrated that sAC, but not AC1 and 8, is necessary for RGC survival and axon growth *in vitro* or *in vivo* (29), and this effect is related to Ca^{2+}-dependent cAMP/PKA activation (29, 109). These findings suggest a substantial possibility that sAC modulation has a therapeutic potential for glaucoma treatment (29). Considering the effects of α2 adrenergic receptor agonists and β-blockers on cAMP signaling pathway (see Table 1), it is likely that reducing cAMP level can improve visual function in patients with glaucoma. However, the precise effect of the cAMP signaling pathway in glaucomatous RGC degeneration has yet elucidated in the aspect of direct

neuroprotection. Future studies will be needed to investigate the functional role of cAMP on RGC protection and degeneration in glaucoma.

cAMP in ONH astrocytes

In the adult human ONH, approximately one million nerve fibers are converged and exited from the eye to the optic nerve through the lamina cribrosa (LC) region (1, 28). LC preserves a pressure gradient between the intraocular and extraocular space, forming the cribriform plates with astrocytes and LC cells (114, 115). Elevated IOP triggers optic disc cupping in the LC region and remodels the extracellular matrix (ECM), and in turn, leads to RGC axonal degeneration in glaucoma (28). Astrocytes are predominant cells in the ONH (116, 117) and their processes ensheath axon bundles in the prelaminar and LC region (118). ONH astrocytes not only provide cellular support to unmyelinated RGC axons by interfacing between connective tissue surfaces and surrounding blood vessels but also play a fundamental role in the mechanical stability of the LC by modulating ECM remodeling in the most mammal (116, 117). Upon glaucomatous injuries, activated astrocytes in the ONH induce reactive astrogliosis, which is characterized by morphological alteration of astrocytes by hypertrophy with thickened enlarged processes and by the increase of glial fibrillary acidic protein (GFAP) expression (115). Importantly, we and others have demonstrated that ONH astrocyte dysfunction that is accompanied by RGCs axon loss is closely associated with the pathogenesis of glaucomatous ONH degeneration in patients with glaucoma (116, 119-121) as well as in experimental animal models of glaucoma (116, 122-125).

Although ONH astrocytes play a critical role in RGC and its axon protection against glaucomatous damages, little is known about the relationship between cAMP and ONH astrocytes in glaucomatous neurodegeneration. Previous studies have demonstrated that the basal level of cAMP was significantly higher in the unstimulated glaucomatous ONH astrocytes from donors of Caucasian American (CA) and African American (AA) donors with POAG compared with their unstimulated ONH astrocytes from normal healthy counterparts (120). In addition, transcriptome analysis for cAMP signaling pathway-related genes showed that while regulator of G-protein signaling 5 (*RGS5*), two tmACs (*AC3 and 9*) and PDE4D interacting protein (*PDE4DIP*) gene expression are upregulated, β-adrenergic receptor kinase 2 (*ADRBK2*) gene expression is downregulated in the ONH astrocyte from the AA, a population at higher risk by

3times for POAG than CA (126, 127). Furthermore, elevated hydrostatic pressure, a mimetic of high IOP *in vitro*, upregulated the mRNA expression of two tmACs genes, *AC3* and *9*, in the ONH astrocytes from AA donors (121), suggesting an intriguing possibility that the tmACs-mediated cAMP signaling pathway may play a role in the pathogenesis of glaucomatous ONH astrocytes.

Since the expression of α and β adrenergic receptors has been found in cultured astrocytes from the cerebral cortex of rats (128, 129), only α1 and β2 adrenergic receptors are found to be expressed in the astrocytes of the rabbit, rat and human optic nerve *in vivo*, suggesting that β2 adrenergic receptor may provide a therapeutic target for regulation of astrocyte functions in response to neuronal injury (130). AC3 and nine are coupled to the β-adrenergic receptors that are linked G_s subunit (113, 131, 132). The response of β-adrenergic receptor is regulated by GPCR kinases (GRKs) that phosphorylate the agonist-activated GPCRs and promote its desensitization, a process that inhibits further signaling transduction in response to repeated or prolonged agonist stimulation of many GPCRs (133). In the olfactory system, β adrenergic receptor kinase 2 (also known as GRK3) knockout mice showed the loss of odorant-induced desensitization of cAMP responses (134). The alteration of GPCR desensitization by GRKs malfunction have also been reported to be associated with another ocular disease. For example, null mutation in rhodopsin kinase (GRK1) gene leads to Oguchi disease, a recessively inherited form of stationary night blindness due to the malfunction of the rod photoreceptor caused by the prolonged activity of photoactivated rhodopsin (135). Also, RGS5, a negative regulator of G-protein-mediated signaling through promoting GTP hydrolysis, interacts with $G_{\alpha i}$, but not with $G_{\alpha s}$ (136, 137), suggesting that the increased expression of RGS5 in AA astrocytes inhibits $G_{\alpha i}$ activity, enhances ACs activation and consequently increases cAMP accumulation (121, 127). Together, these findings strongly suggest that the abnormal regulation of adrenergic receptors-mediated cAMP signaling pathway in ONH astrocytes may contribute to glaucomatous ONH degeneration.

Oxidative stress has been thought to be an important pathophysiological mechanism in many neurodegenerative diseases including glaucoma (116, 138-141). In the CNS, neurons are most vulnerable cells to oxidative stress due to their low reactive oxygens species detoxifying capacity; therefore its survival is highly dependent on the capacity of neighboring astrocytes during oxidative stress-induced neurodegeneration (142, 143). Furthermore, astrocytes are the

responsible cell type that is mostly related to oxidative stress-mediated glaucomatous ONH degeneration (116, 122, 138, 144). Indeed, we have demonstrated that oxidative stress-mediated mitochondrial dysfunction or alteration could be an important pathophysiological mechanism in the dysfunction of ONH astrocytes (144). Further, we have found that coenzyme Q10, an essential cofactor of the electron transport chain and a potent antioxidant, protected cultured ONH astrocytes from H_2O_2-induced oxidative stress (144) as well as RGCs and their axons in experimental rodent models of retinal ischemia or glaucoma (145-147). However, the relationship between cAMP signaling pathway and oxidative stress in ONH astrocyte dysfunction and degeneration remains unknown. Previous studies have demonstrated that tmAC5 knockout mice show resistance to oxidative stress (148) and activation of tmACs-mediated cAMP/PKA signal pathway induced by forskolin is associated with increased vulnerability to H_2O_2-induced oxidative stress in rat neocortical astrocytes *in vitro* (149). Collectively, these findings suggest an important possibility that tmACs activation-mediated cAMP/PKA signaling pathway may contribute to astrocyte dysfunction in glaucomatous ONH degeneration.

Brimonidine protects not only RGC somas but also their axons in the optic nerve of rats with elevated IOP induced by laser cauterization of the episcleral veins (104). We also found that brimonidine prevents the increased GFAP expression in müller cells, the most predominant retinal glial cells, as well as protects RGCs in ischemic retina (105), suggesting the possibility that brimonidine-mediated protection may also be involved in modulation of glial responses against pressure-induced ischemic insults. Our previous report has demonstrated that functional NMDARs are present in human ONH astrocytes and its expression levels are increased in cultured ONH astrocytes from patients with glaucoma (122). Because brimonidine-mediated tmACs inhibition protects RGCs against NMDARs-mediated glutamate excitotoxicity (107), these findings suggest another possibility that brimonidine may also protect astrocytes by inhibiting tmACs activation in glaucomatous ONH degeneration. Although future studies need to investigate the effect of brimonidine on ONH astrocytes, this idea is supported by the evidence that the activation of metabotropic glutamate receptors 3, a GPCR linked to $G_{\alpha i}$ subunit, protects cultured astrocytes against hypoxic/ischemic damage by tmACs inhibition (150, 151). Therefore, it would be important to know whether the tmACs activation contributes to ONH astrocyte dysfunction in glaucomatous neurodegeneration.

Conclusion

Glaucoma is the leading cause of irreversible blindness worldwide. Despite the high prevalence, the biological basis of POAG still is not yet fully understood. Since adrenergic agents such as brimonidine have beneficial effects on IOP-lowering and RGC protection in POAG, the current understanding of cAMP signaling pathway regulated by adrenergic agents may provide a therapeutic potential for glaucoma treatment. In this regards, inhibition of tmACs activation by adrenergic receptors reflects an important explanation for the utilization of adrenergic agents, such as α2 adrenergic receptor agonists and β blockers, in glaucoma treatment. On the other hand, activation of cAMP signaling pathway by sAC has been shown its dual action in the IOP lowering and RGCs protection (Figure 1). Therefore, it is possible that cAMP signaling pathway by tmACs and sAC activation may have distinct roles in various cell types of the eye. Moreover, because the functional role of tmACs or sAC in ocular tissues is yet to be characterized, it would be important to investigate the functional role of cAMP signaling pathway induced by tmACs or sAC activation not only in these ocular tissues but also in specific cell types of neurons and glial cells. Future studies into the pathogenic or protective mechanisms of the cAMP signaling pathway will provide new therapeutic strategies to understand aqueous humour dynamics and IOP regulation, and to enhance the survival of RGC and its axon, as well as ONH astrocytes in glaucoma and other optic neuropathies.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interests.

FIGURE LEGENDS

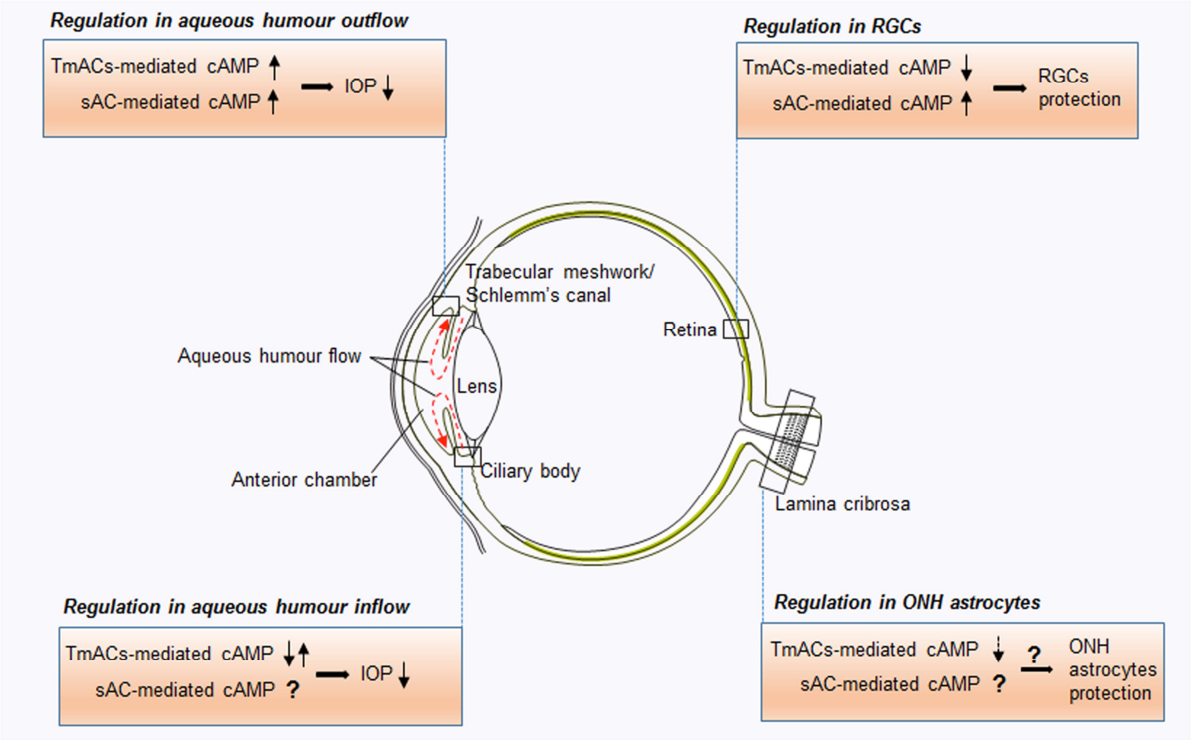

Figure 1. Schematic diagram for proposed functional role of cAMP in the eye of glaucoma. The differential effects of cAMP generated by tmACs or sAC are shown on the aspect of IOP regulation, and RGCs and ONH astrocytes protection. Black arrows with solid or dotted lines are experimentally confirmed or inferred from other types of astrocytes, respectively (see more detail in the text). Question marks represent what should be experimentally confirmed in future studies. cAMP, cyclic adenosine 3′,5′-monophosphate; IOP, intraocular pressure; RGCs, retinal ganglion cells; ONH, optic nerve head; sAC, soluble adenylyl cyclase; tmACs, transmembrane adenylyl cyclases.

Table 1. cAMP signaling pathway-related IOP reducing drugs used in glaucoma treatment.

	Drug target	Subtype	GPCR type	ACs type	Available Drugs	Drug type	Mechanisms of action
Inflow	α-ARs (2, 52)	α1	G_q	-	Apraclonidine Brimonidine	Agonists	Decrease inflow
		α2	G_i	tmAC			
	β-ARs (2, 152)	β1	G_s	tmAC	Timolol, betaxol, carteolol and levobunolol	Blockers	Decrease inflow
		β2	G_s and G_i	tmAC			
		β3	G_s	tmAC			
	CA (2, 63)		-	sAC?	Dorzolamide, brinzolamide, acetazolamide and methazolamide	Inhibitors	Decrease inflow
Outflow	CRs (2, 77)	M1	$G_{q(153)}$ and $G_{s(154, 155)}$	tmAC	Pilocarpine, carbachol	Agonists	Increase outflow
		M2	G_i	tmAC			
		M3	$G_{q(154-156)}$	-			
		M4	$G_{i(157)}$	tmAC			
		M5	$G_{q(158)}$	-			
	PGR (EP4) (80)		Gs	tmAC	-	Agonists	Increase outflow
	PGR (F) (2, 79)		Gq	-	Latanoprost, travoprost,bimatoprost and tafluprost	PGF2α analogues	Increase outflow

ARs, adrenergic receptors; CA, Carbonic anhydrase; CRs, Cholinergic receptors; PGR, Prostaglandin receptor; sAC, soluble adenylyl cyclase; tmACs, transmembrane adenylyl cyclases.

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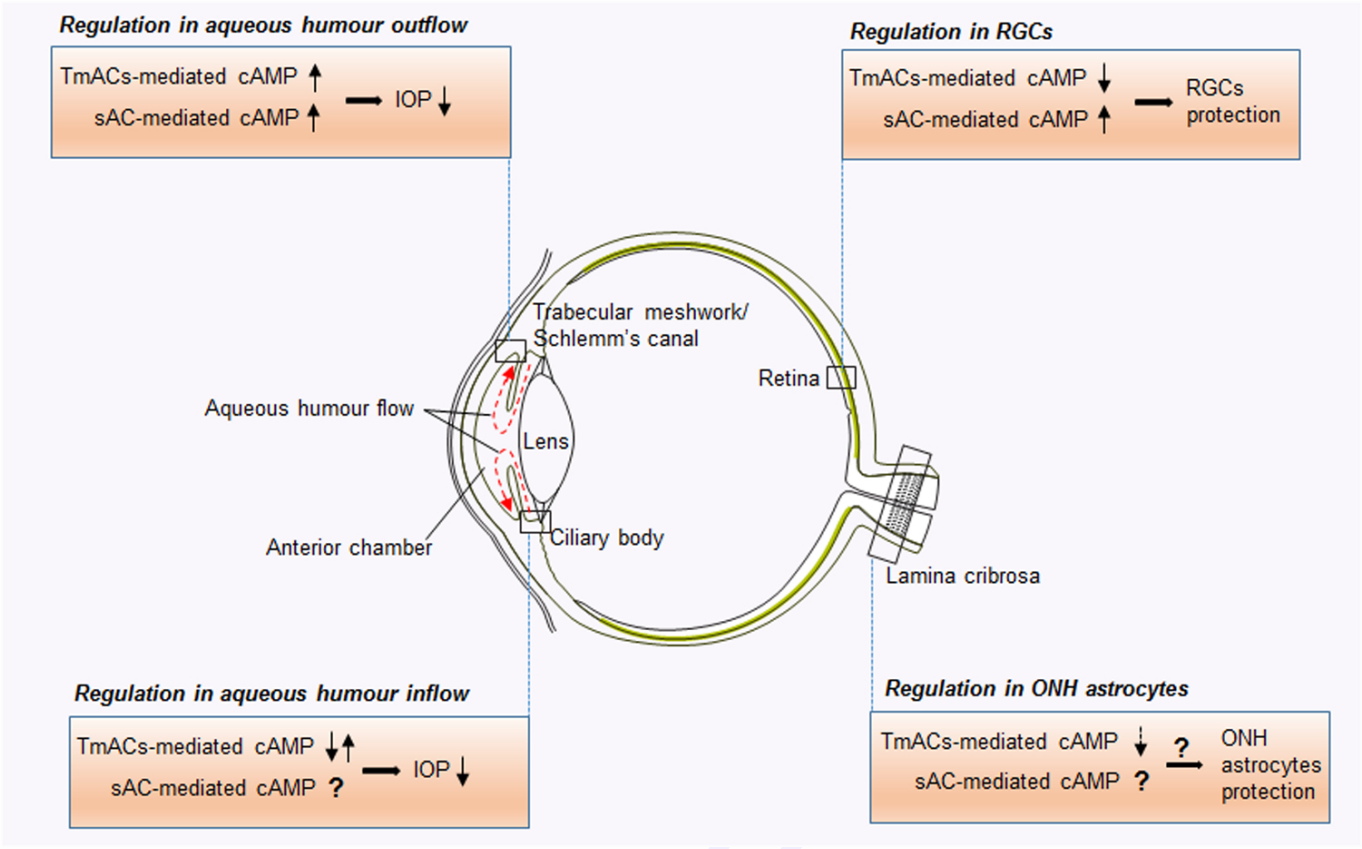

Fig. 1

Table 1. cAMP signaling pathway-related IOP reducing drugs used in glaucoma treatment.

	Drug target	Subtype	GPCR type	ACs type	Available Drugs	Drug type	Mechanisms of action
Inflow	α-ARs (2, 52)	α1	G_q	-	Apraclonidine Brimonidine	Agonists	Decrease inflow
		α2	G_i	tmAC			
	β-ARs (2, 152)	β1	G_s	tmAC	Timolol, betaxol, carteolol and levobunolol	Blockers	Decrease inflow
		β2	G_s and G_i	tmAC			
		β3	G_s	tmAC			
	CA (2, 63)		-	sAC?	Dorzolamide, brinzolamide, acetazolamide and methazolamide	Inhibitors	Decrease inflow
Outflow	CRs (2, 77)	M1	$G_{q(153)}$ and $G_{s(154, 155)}$	tmAC	Pilocarpine, carbachol	Agonists	Increase outflow
		M2	G_i	tmAC			
		M3	$G_{q(154-156)}$	-			
		M4	$G_{i(157)}$	tmAC			
		M5	$G_{q(158)}$	-			
	PGR (EP4) (80)		Gs	tmAC	-	Agonists	Increase outflow
	PGR (F) (2, 79)		Gq	-	Latanoprost, travoprost,bimatoprost and tafluprost	PGF2α analogues	Increase outflow

ARs, adrenergic receptors; CA, Carbonic anhydrase; CRs, Cholinergic receptors; PGR, Prostaglandin receptor; sAC, soluble adenylyl cyclase; tmACs, transmembrane adenylyl cyclases.