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Invited Mini Review

Role of cyclic AMP in the eye with glaucoma

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Glaucoma is characterized by a slow and progressive degeneration of the optic nerve, including retinal ganglion cell (RGC) axons in the optic nerve head (ONH), leading to visual impairment. Despite its 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 the retina and the optic nerve. Although recent studies revealed that cAMP generated by adenylyl cyclases (ACs) is important in regulating aqueous humor dynamics in ocular tissues, such as the ciliary body and trabecular meshwork, as well as cell death and growth in the retina and optic nerve, the functional role and significance of cAMP in glaucoma remain to be elucidated. In this review, we will discuss the functional role of cAMP in aqueous humor dynamics and IOP regulation, and review the current medications, which are related to the 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. [BMB Reports 2017; 50(2): 60-70]

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 to 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 signal transduction (4). Its signaling pathway exists in all types of cells and contributes to numerous biological processes, such as cell growth, differentiation, death, gene expression, 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 causes opposite effects on cell survival in a cell-type-specific manner (8), because it exerts its effect through various effectors, such as cAMP-dependent protein kinase A (PKA) (9, 10), exchange protein directly activated by cAMP (Epac) (11, 12), and cyclic-nucleotide-gated ion channels (13, 14).

Among the key regulators of the 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) have 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 patterns 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).

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ACs have been thought of as potential drug targets in many neurodegenerative disorders, including glaucoma (23, 24). Since the activation of the 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 has demonstrated that 1% forskolin eye drops 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). Since the evidence demonstrates that RGC survival and axon growth are enhanced via activation of the sAC-mediated cAMP signaling pathway (29-32), the therapeutic strategy for modulating the cAMP signaling pathway in glaucoma treatment is considered to rescue RGCs from 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 on the role of cAMP in the eye, addressing its possible relationship to glaucoma protection or degeneration.

cAMP IN IOP REGULATION

IOP regulation by aqueous humor dynamics

IOP is currently the only proven treatable risk factor in glaucoma (1, 28). As an aqueous humor that is secreted to the iris by the ciliary body in the posterior chamber, it 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 the aqueous humor is controlled via a conventional pathway through a trabecular meshwork (TM) and Schlemm's canal (SC), and via an independent uveoscleral outflow pathway through the ciliary body and iris root (33, 34). In this regard, the therapeutic strategies that reduce aqueous humor inflow and/or increase its outflow have been thought to be important in treating IOP-related glaucomatous optic neuropathy.

The role of cAMP in aqueous humor inflow

Lowering 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 humor inflow in IOP changes are associated with the 24 h circadian IOP profile and body posture (35, 38). Since Neufeld *et al*. first reported that adrenergic agents, including epinephrine and phenylephrine, increased cAMP concentration in the aqueous humor (39), treatment with timolol, the first FDA-approved β-blocker for the treatment of glaucoma (40), decreased IOP in normal volunteer and glaucoma patients (41, 42). These findings led to attention on the adrenergic control of IOP and the therapeutic potential of the cAMP signaling pathway in glaucoma treatment. Since then, several studies have identified an adrenergic receptor-AC complex in the ciliary process (43-46), supporting the functional role of cAMP in aqueous humor formation. The activation of ACs-linked receptors by several endogenous or exogenous factors not only increases intracellular cAMP level, but also decreases net aqueous humor flow and lowers IOP (37, 39, 47-51). Furthermore, an increase of the cAMP level by a topical suspension of 1% forskolin lowered IOP in rabbits and monkeys, as well as in normal human volunteers (25), suggesting that increasing cAMP may decrease the net rate of aqueous humor inflow (46).

Because of 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 indicates that adrenergic receptors are GPCRs, which are classified into two main categories, α and β, and these are further grouped according to their isotypes (α1, α2, β1, β2, and β3), which are linked to different G_α subunits (Table 1). 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 because it is linked to $G_{\alpha i}$, the inhibitory G_α subunit. Indeed, adrenergic receptor agonists (e.g., apraclodine and brimonidine) decrease aqueous humor production (53-55). However, β adrenergic receptors are mainly linked to $G_{\alpha s}$, a 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 humor formation in the ciliary epithelium in a cAMP-dependent manner (57, 58). Together, these findings support the notion that reducing cAMP, not increasing cAMP, lowers aqueous humor formation and IOP. Although current studies do not provide a clear conclusion whether the increase or decrease of cAMP level reduces aqueous humor formation, it is possible that cAMP plays a critical role in the regulation of aqueous humor production and IOP inflow.

The role of cAMP in aqueous humor outflow

Aqueous humor outflow decreases with aging and glaucoma progression (59). Elevated IOPs in glaucoma result 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 a change in the conventional outflow facility in healthy aging eyes (59, 60).

Increasing the outflow facility by elevating the cAMP level by adrenergic agents has also been reported (48, 61, 62); however, the precise effect of cAMP was not explained until sAC was found to play a role in the outflow control. Carbonic anhydrases are a family of enzymes that catalyze the rapid interconversion of carbon dioxide (CO_2) and water (H_2O) to

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	Agonists	Decrease inflow
		α2	G_i	tmAC	Brimonidine		
	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)		G_s	tmAC	-	Agonists	Increase outflow
	PGR (F) (2, 79)		G_q	-	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.

bicarbonate (HCO_3^-) and hydrogen ion (H^+), and its inhibition lowers IOP in patients with glaucoma (63). Since an HCO_3-sensitive AC activity has been reported in the ciliary body of rabbit eyes (64), sAC expression was identified in the non-pigmented epithelium of the ciliary body and the sAC was characterized as an enzyme responsible for controlling the activity of cAMP in the ciliary body (65). Although carbonic anhydrase inhibitors, including acetazolamide, are known to lower IOP by diminishing the rate of aqueous humor formation in the ciliary epithelium (63, 66), the relationship between carbonic anhydrase-generated HCO_3^- and the cAMP signaling pathway has yet to be characterized in IOP regulation. Furthermore, it is not known whether sAC contributes to aqueous humor 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 the sAC-generated cAMP level in the ciliary epithelium, suggesting the possibility that sAC-mediated increasing of the 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 present only in the non-pigmented epithelium of the ciliary body in the eye (68, 70). Furthermore, Best2 knockout mice show a significant IOP lowering compared with wild-type (WT) control littermates (71, 72). Because sAC plays a role as an evolutionarily conserved HCO_3^- sensor (73), it was hypothesized that sAC may contribute to a downstream function of Best2 in the 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 for regulating 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, one that 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 carbachol, 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 the outflow facility of the aqueous humor via M3 that is linked to $G_{\alpha q}$, a G_α subunit which activates the phospholipase/Ca^{2+} pathway (77), some types of these receptors (M1, M2 and M4) are also linked to $G_{\alpha s}$ or $G_{\alpha i}$ subunits 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 for lowering IOP in patients with POAG (28, 79). Prostaglandins are a group of physiologically active lipid compounds that act like the hormone and exert their

effects by binding to ten known prostaglandin receptors, such as types I, E and F, which are GPCRs linking to various G_α subunits, including $G_{\alpha s}$, $G_{\alpha i}$, and $G_{\alpha q}$. Since a $G_{\alpha q}$-linked prostaglandin F receptor has been mostly targeted and used for glaucoma treatment, little is known about the effect of prostaglandin analogs through the cAMP signaling pathway in IOP regulation. However, several studies have intriguingly demonstrated that a $G_{\alpha s}$-linked prostaglandin EP4 receptor is expressed in eye tissues, including the cornea, iris, ciliary body, TM/SC complex, and retina, and that 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, because of 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 the 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 may result 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 into 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 humans, RGC axons terminate mostly in the lateral geniculate nucleus and some in the superior colliculus to complete the 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 significant evidence that links the cAMP signaling pathway and glutamate excitotoxicity to protect RGCs directly against glaucomatous damage. The potential mechanisms for brimonidine-mediated RGCs protection are thought to be 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) (92-94). Since dexmedetomidine, an α2 adrenergic receptor agonist, has been reported to be neuroprotective in animal models of focal cerebral ischemia (95), several studies have demonstrated that the 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 important evidence that brimonidine may also be involved in neuroprotection in an 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 the cAMP signaling pathway regulate the brimonidine-mediated RGCs protection? Of interest, brimonidine protects RGCs by 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 strongly suggest that brimonidine-mediated inhibition of the 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 RGC death, Ca^{2+} homeostasis in a normal condition is essential for RGC function and survival. Furthermore, the elevated Ca^{2+} level has been reported to protect RGCs by activating the 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 contrast to NMDAR activation-mediated cell death (111). How does the elevated Ca^{2+} influx protect RGCs? Previous studies have demonstrated that RGC response to neurotrophic factors is weak, unless they are depolarized, or the intracellular cAMP level is elevated (82, 83). Furthermore, electrical activity-mediated depolarization promotes RGC survival and axon growth by increasing the 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 a total of 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 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); 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 the cAMP signaling pathway (see Table 1), it is likely that reducing the 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 to be elucidated in terms 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 converge in and exit from the eye to the optic nerve through the lamina cribrosa (LC) region (1, 28). The 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 most mammals (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 Caucasian American (CA) and African American (AA) donors with POAG compared with unstimulated ONH astrocytes from normal healthy counterparts (120). In addition, transcriptome analysis for cAMP-signaling-pathway related genes showed that, while regulators of G-protein signaling 5 (*RGS5*), two tmACs (*AC3* and *AC9*) 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 three times for POAG than CA are (126, 127). Furthermore, elevated hydrostatic pressure, a mimetic of high IOP *in vitro*, upregulated the mRNA expression of two tmACs genes, *AC3* and *AC9*, 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 the β2 adrenergic receptor may provide a therapeutic target for regulation of astrocyte functions in response to neuronal injury (130). AC3 and AC9 are coupled to the β-adrenergic receptors that are linked to $G_{\alpha s}$ subunits (113, 131, 132). The response of the 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 has also been reported to be associated with another ocular disease. For example, null mutation in the 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 the 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 the cells most vulnerable to oxidative stress, because of their low reactive oxygen 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 the 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 the 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 the 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 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 its 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 the 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 the cAMP signaling pathway by sAC has been shown to have dual action in IOP lowering and RGCs protection (Fig. 1). Therefore, it is possible that the 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 the 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

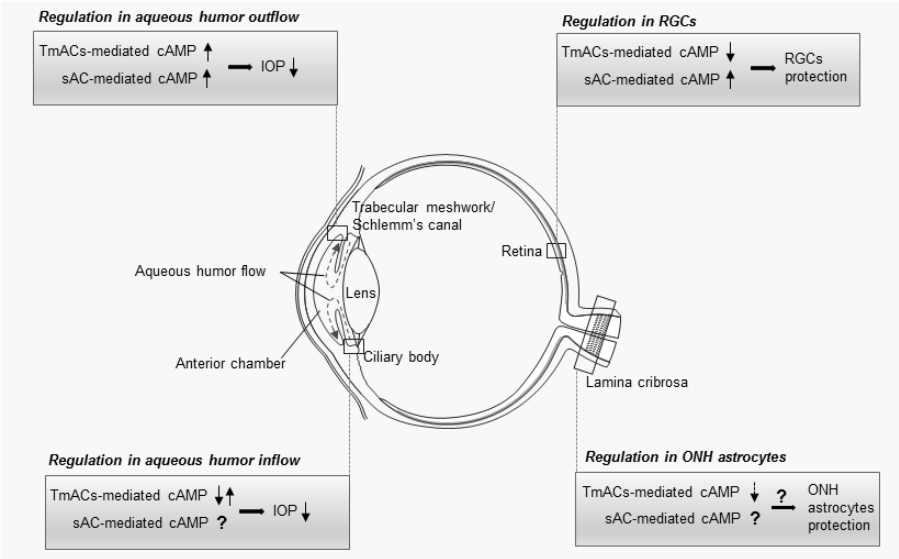

Fig. 1. Schematic diagram for proposed functional role of cAMP in glaucoma. The differential effects of cAMP generated by tmACs or sAC are shown in terms of IOP regulation and RGCs and ONH astrocytes protection. Black arrows with solid or dotted lines are experimentally confirmed or inferred from other types of astrocytes, respectively (see more detail in the text). Question marks represent what should be experimentally confirmed in future studies. Definitions: 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.

strategies to understand aqueous humor 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 have no conflicting financial interests.

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