Role of cyclic AMP in the eye with glaucoma

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Glucoma 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

Glucoma 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 Ca2+ for its activity (6, 17).
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, 36). 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 Go 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 β2 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 Goβ, the inhibitory Go, subunit. Indeed, adrenergic receptor agonists (e.g., apraclodine and brimonidine) decrease aqueous humor production (53-55). However, β adrenergic receptors are mainly linked to Goα, a stimulatory Go, 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 (CO2) and water (H2O) to...
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_\alpha 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

### 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   | α1        | G_\alpha | Apraclonidine   | Agonists  | Decrease inflow      |
|             |         | α2        | G_\alpha | Brimonidine     |           |                      |
|             | β-ARs   | β1        | G_\alpha | Timolol, betaxol,| Blockers  | Decrease inflow      |
|             |         | β2        | G_\alpha | carteolol and levobunol |           |                      |
|             |         | β3        | G_\alpha |                |           |                      |
|             | CA      |           | sAC      | Dorzolamide, brinzolamide, acetazolamide and methazolamide | Inhibitors| Decrease inflow      |
| Outflow     | CRs     | M1        | G_α(153) and G_α(154, 155) | Pilocarpine, carbachol | Agonists  | Increase outflow     |
|             |         | M2        | G_\alpha | tmAC            |           |                      |
|             |         | M3        | G_α(154-156) | -                | Agonists  | Increase outflow     |
|             |         | M4        | G_α(157) | tmAC            |           |                      |
|             |         | M5        | G_α(158) | -               |           |                      |
|             | PGR (EP4) | G_\alpha | tmAC     | Latanoprost, travoprost, bimatoprost and tafluprost | PGF2α analogues | Increase outflow     |
|             | PGR (F) |           | sAC      |                |           |                      |

ARs, adrenergic receptors; CA, Carbonic anhydrase; CRs, Cholinergic receptors; PGR, Prostaglandin receptor; sAC, soluble adenylyl cyclase; tmACs, transmembrane adenylyl cyclases.
effects by binding to ten known prostaglandin receptors, such as types I, E, and F, which are GPCRs linking to various Gs subunits, including G_{ai}, G_{ar} and G_{aq}. Since a G_{ar}-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_{aq}-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_{aq}-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.

\section*{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 \(\alpha_2\) 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 desmethylmediate, an \(\alpha_2\) adrenergic receptor agonist, has been reported to be neuroprotective in animal models of focal cerebral ischemia (95), several studies have demonstrated that the \(\alpha_2\) 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\(^{2+}\)]\text{c}) induced by activation of NMDARs (92, 94, 105). Furthermore, brimonidine reduces NMDA-evoked [Ca\(^{2+}\)]\text{c} increase, while isoproprenal, a \(\beta\) adrenergic receptor agonist, enhances NMDA-evoked [Ca\(^{2+}\)]\text{c} increase via a \(cAMP/\text{PKA}\) signaling pathway dependent manner (107). These results strongly suggest that brimonidine-mediated inhibition of the \(cAMP/\text{PKA}\) 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 \(\alpha\)-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 (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\(^{2+}\)]\text{c}) induced by activation of NMDARs (92, 94, 105). Furthermore, brimonidine reduces NMDA-evoked [Ca\(^{2+}\)]\text{c} increase, while isoproprenal, a \(\beta\) adrenergic receptor agonist, enhances NMDA-evoked [Ca\(^{2+}\)]\text{c} increase via a \(cAMP/\text{PKA}\) signaling pathway dependent manner (107). These results strongly suggest that brimonidine-mediated inhibition of the \(cAMP/\text{PKA}\) pathway could be an important mechanism to protect RGCs against glutamate excitotoxicity-induced glaucomatous neurodegeneration.

### References

1. Myoung Sup Shim, et al. Role of cAMP in glaucoma. BMB Reports 63.
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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 Gαs 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 Gαq, but not with Gαs (136, 137), suggesting that the increased expression of RGS5 in AA astrocytes inhibits Gαq 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 

H₂O₂-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 H₂O₂-
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 
triamcinolone-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 glau-
comatous 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 G₃₉ 
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 
triamcinolone 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 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.

REFERENCES

1. Weinreb RN and Khaw PT (2004) Primary open-angle glaucoma. Lancet 363, 1711-1720
2. Zhang K, Zhang L and Weinreb RN (2012) Ophthalmic drug discovery: novel targets and mechanisms for retinal diseases and glaucoma. Nat Rev Drug Discov 11, 541-559
3. Weinreb RN, Leung CK and Crowston JG et al (2016) Primary open-angle glaucoma. Nat Rev Dis Primers 2, 16067
4. Rall TW and Sutherland EW (1958) Formation of a cyclic adenine ribonucleotide by tissue particles. J Biol Chem 232, 1063-1076
5. Huneycutt BS and Benveniste EN (1995) Regulation of astrocyte cell biology by the cAMP/protein kinase A signaling pathway. Adv Neuroimmunol 5, 9281-9289
6. Ladirov Y and Appukuttan A (2014) Role of soluble adenylyl cyclase in cell death and growth. Biochim Biophys Acta 1842, 2646-2655
7. Martinez J, Stessin AM and Campana A et al (2014) Soluble adenylyl cyclase is necessary and sufficient to overcome the block of axonal growth by myelin-associated factors. J Neurosci 34, 9281-9289
8. Insel PA, Zhang L, Murray F, Yokouchi H and Zambon AC (2012) Cyclic AMP is both a pro-apoptotic and anti-apoptotic second messenger. Acta Physiol (Oxf) 204, 277-287
9. Walsh DA, Perkins JP and Krebs EG (1968) An adenosine 3',5'-monophosphate-depicting protein kinase from rabbit skeletal muscle. J Biol Chem 243, 3763-3765
10. Taylor SS, Zhang P, Steichen JM, Keshwani MM and Kornev AP (2013) PKA: lessons learned after twenty years. Biochim Biophys Acta 1834, 1271-1278
11. de Rooij J, Zwartkruis FJ, Verheijen MH et al (1998) Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. Nature 396, 474-477
12. Kawasaki H, Springett GM, Mochizuki N et al (1998) A family of cAMP-binding proteins that directly activate Rap1. Science (New York, N.Y.) 282, 2275-2279
13. Kaupp UB and Seifert R (2002) Cyclic nucleotide-gated ion channels. Physiol Rev 82, 769-824
14. Matulef K and Zagotta WN (2003) Cyclic nucleotide-gated ion channels. Annu Rev Cell Dev Biol 19, 23-44
15. Sunahara RK, Dessauer CW and Gilman AG (1996) Complexity and diversity of mammalian adenylyl cyclases. Annu Rev Pharmacol Toxicol 36, 461-480
16. Patel TB, Du Z, Pierre S, Cartin L and Scholich K (2001) Molecular biological approaches to unravel adenylyl cyclase signaling and function. Gene 269, 13-25
17. Buck J, Sinclair ML, Schapal L, Cann MJ and Levin LR (1999) Cytosolic adenylyl cyclase defines a unique signaling molecule in mammals. Proc Natl Acad Sci U S A 96, 79-84
18. Hanoune J and Defer N (2001) Regulation and role of adenylyl cyclase isoforms. Annu Rev Pharmacol Toxicol 41, 145-174
19. Defer N, Best-Belpomme M and Hanoune J (2000) Tissue specificity and physiological relevance of various isoforms of adenylyl cyclase. Am J Physiol Renal Physiol 279, F400-416
20. Tang WJ and Gilman AG (1992) Adenylyl cyclases. Cell 70, 869-872
21. Cooper DM (2003) Regulation and organization of adenylyl cyclases and cAMP. Biochem J 375, 517-529
22. Valsecchi F, Ramos-Espiritu LS, Buck J, Levin LR, and Manfredi G (2013) cAMP and mitochondria. Physiology (Bethesda, Md.) 28, 199-209
23. Pierre S, Eschenhagen T, Geisslinger G and Scholich K (2009) Capturing adenylyl cyclases as potential drug targets. Nat Rev Drug Discov 8, 321-335
24. Lee YS, Marmorstein LY and Marmorstein AD (2014) Soluble adenylyl cyclase in the eye. Biochim Biophys Acta 1842, 2579-2583
25. Caprioli J and Sears M (1983) Forskolin lowers intraocular pressure in rabbits, monkeys, and man. Lancet 1, 958-960
26. Burstein NL, Sears ML and Mead A (1984) Aqueous flow in human eyes is reduced by forskolin, a potent adenylate cyclase activator. Exp Eye Res 39, 745-749
27. Wajeeel M, Nagabhushanam K, Natarajan S, Vaidyanathan P, Kari SK and Jose JA (2015) Efficacy and safety of 1% forskolin eye drops in open angle glaucoma - An open label study. Saudi Med J 35, 197-200
28. Weinreb RN, Aung T and Medeiros FA (2014) Cyclic AMP is both a pro-apoptotic and anti-apoptotic second messenger. Adv Neuroimmunol 5, 261-269
29. Pierre S, Eschenhagen T, Geisslinger G and Scholich K (2009) Capturing adenylyl cyclases as potential drug targets. Nat Rev Drug Discov 8, 321-335
30. Hellstrom M and Harvey AR (2014) Cyclic AMP and the glial cell modulator ibudilast attenuates neuroinflammation and enhances retinal ganglion cell viability in glaucoma through protein kinase A signaling. Neurobiol Dis 50, F400-416
31. Cueva Vargas JL, Belforte N and Di Polo A (2016) The complexity and diversity of mammalian adenylyl cyclases. Annu Rev Pharmacol Toxicol 36, 461-480
32. de Lima S, Habboub G and Benowitz LI (2012) Cyclic nucleotide-gated ion channels. Annu Rev Cell Dev Biol 19, 23-44
33. Defer N, Best-Belpomme M and Hanoune J (2000) Tissue specificity and physiological relevance of various isoforms of adenylyl cyclase. Am J Physiol Renal Physiol 279, F400-416
34. Cooper DM (2003) Regulation and organization of adenylyl cyclases and cAMP. Biochem J 375, 517-529
35. Pierre S, Eschenhagen T, Geisslinger G and Scholich K (2009) Capturing adenylyl cyclases as potential drug targets. Nat Rev Drug Discov 8, 321-335
36. Lee YS, Marmorstein LY and Marmorstein AD (2014) Soluble adenylyl cyclase in the eye. Biochim Biophys Acta 1842, 2579-2583
37. Caprioli J and Sears M (1983) Forskolin lowers intraocular pressure in rabbits, monkeys, and man. Lancet 1, 958-960
38. Burstein NL, Sears ML and Mead A (1984) Aqueous flow in human eyes is reduced by forskolin, a potent adenylate cyclase activator. Exp Eye Res 39, 745-749
39. Wajeeel M, Nagabhushanam K, Natarajan S, Vaidyanathan P, Kari SK and Jose JA (2015) Efficacy and safety of 1% forskolin eye drops in open angle glaucoma - An open label study. Saudi Med J 35, 197-200
40. Weinreb RN, Aung T and Medeiros FA (2014) The pathophysiology and treatment of glaucoma: a review. JAMA 311, 1901-1911
41. Corredor RG, Trachtenberg EF, Pita-Thomas W, Jin X, Hu Y and Goldberg JL (2012) Soluble adenylyl cyclase activity is necessary for retinal ganglion cell survival and axon growth. J Neurosci 32, 7734-7744
42. Hellstrom M and Harvey AR (2014) Cyclic AMP and the regeneration of retinal ganglion cell axons. Int J Biochem Cell Biol 56, 66-73
43. Cueva Vargas JL, Belforte N and Di Polo A (2016) The glial cell modulator ibudilast attenuates neuroinflammation and enhances retinal ganglion cell viability in glaucoma through protein kinase A signaling. Neurobiol Dis 53, 156-171
44. de Lima S, Habboub G and Benowitz LI (2012) Combinatorial therapy stimulates long-distance regeneration, target reinnervation, and partial recovery of vision after optic nerve injury in mice. Int Rev Neurobiol 106, 153-172
33. Tamm ER (2009) The trabecular meshwork outflow pathways: structural and functional aspects. Exp Eye Res 88, 649-655
34. Alm A and Nilsson SF (2009) Uveoscleral outflow—a review. Exp Eye Res 88, 760-768
35. Crowston JG and Weinreb RN (2005) Glaucoma medication and aqueous humor dynamics. Curr Opin Ophthalmol 16, 94-100
36. Grant WM (1955) Physiological and pharmacological influences upon intraocular pressure. Pharmacol Rev 7, 143-182
37. Lee PF (1958) The influence of epinephrine and phenylephrine on intraocular pressure. AMA Arch Ophthalmol 60, 863-867
38. Ericson LA (1958) Twenty-four hourly variations in the inflow of the aqueous humour. Acta Ophthalmol (Copenh) 36, xxx
39. Neufeld AH, Jampol LM and Sears ML (1972) Cyclic-AMP in the aqueous humor: the effects of adrenergic agents. Exp Eye Res 14, 242-250
40. Reautil N (2011) A history of glaucoma pharmacology. Optom Vis Sci 88, 36-38
41. Katz IM, Hubbard WA, Getson AJ and Gould AL (1976) Intraocular pressure decrease in normal volunteers following timolol ophthamlic solution. Invest Ophthalmol 15, 489-492
42. Zimmerman Tj and Boger WP 3rd (1979) The beta-adrenergic blocking agents and the treatment of glaucoma. Surv Ophthalmol 23, 347-362
43. Neufeld AH and Page ED (1977) In vitro determination of the ability of drugs to bind to adrenergic receptors. Invest Ophthalmol Vis Sci 16, 1118-1124
44. Bromberg BB, Gregory DS and Sears ML (1980) Beta-adrenergic receptors in ciliary processes of the rabbit. Invest Ophthalmol Vis Sci 19, 203-207
45. Nathanson JA (1980) Adrenergic regulation of intraocular pressure: identification of beta 2-adrenergic-stimulated adenylate cyclase in ciliary process epithelium. Proc Natl Acad Sci U S A 77, 7420-7424
46. Caprioli J and Sears M (1984) The adenylate cyclase receptor complex and aqueous humor formation. Yale J Biol Med 57, 283-300
47. Eakins KE and Eakins HM (1964) Adrenergic mechanisms and the outflow of aqueous humor from the rabbit eye. J Pharmacol Exp Ther 144, 60-65
48. Neufeld AH and Sears ML (1974) Cyclic-AMP in ocular tissues of the rabbit, monkey, and human. Invest Ophthalmol 13, 475-477
49. Gregory D, Sears M, Bausher L, Mishima H and Mead A (1981) Intraocular pressure and aqueous flow are decreased by cholerin toxin. Invest Ophthalmol Vis Sci 20, 371-381
50. Ross RA and Drance SM (1970) Effects of topically applied isoproterenol on aqueous dynamics in man. Arch Ophthalmol 83, 39-46
51. Sears M and Mead A (1983) A major pathway for the regulation of intraocular pressure. Int Ophthalmol 6, 201-212
52. Arthur S and Cantor LB (2011) Update on the role of alpha-agonists in glaucoma management. Exp Eye Res 93, 271-283
53. Gharagozloo NZ, Relf SJ and Brubaker RF (1988) Aqueous flow is reduced by the alpha-adrenergic agonist, apraclonidine hydrochloride (ALO 2145). Ophthalmology 95, 1217-1220
54. Toris CB, Gleason ML, Camras CB and Yablonski ME (1995) Effects of brimonidine on aqueous humor dynamics in human eyes. Arch Ophthalmol 113, 1514-1517
55. Bausher LP and Horio B (1995) Regulation of cyclic AMP production in adult human ciliary processes. Exp Eye Res 60, 43-48
56. Nathanson JA (1981) Human ciliary process adrenergic receptor: pharmacological characterization. Invest Ophthalmol Vis Sci 21, 786-804
57. Chen S, Inoue R, Inomata H and Ito Y (1994) Role of cyclic AMP-induced Cl conductance in aqueous humour formation by the dog ciliary epithelium. Br J Pharmacol 112, 1137-1145
58. Do CW and Civan MM (2004) Basis of chloride transport in ciliary epithelium. J Membr Biol 200, 1-13
59. Gabelt BT and Kaufman PL (2005) Changes in aqueous humor dynamics with age and glaucoma. Prog Retin Eye Res 24, 612-637
60. Toris CB, Yablonski ME, Wang YL and Camras CB (1999) Aqueous humor dynamics in the aging human eye. Am J Ophthalmol 127, 407-412
61. Neufeld AH, Dueker DK, Vegge T and Sears ML (1975) Adenosine 3’-5’-monophosphate increases the outflow of aqueous humor from the rabbit eye. Invest Ophthalmol 14, 40-42
62. Neufeld AH and Sears ML (1975) Adenosine 3’5’-monophosphate analogue increases the outflow facility of the primrate eye. Invest Ophthalmol 14, 688-689
63. Pfeiffer N (1997) Dorzolamide: development and clinical application of a topical carbonic anhydrase inhibitor. Surv Ophthalmol 42, 137-151
64. Mittag TV, Guo WB and Kobayashi K (1993) Bicarbonate-activated adenyl cyclase in fluid-transporting tissues. Am J Physiol 264, F1060-1064
65. Lee YS, Tresguerres M, Hess K et al (2011) Regulation of anterior chamber drainage by bicarbonate-sensitive soluble adenylyl cyclase in the ciliary body. J Biol Chem 286, 41353-41358
66. Hett JA and Docklater CA (1992) Topical carbonic anhydrase inhibitors: a new perspective in glaucoma therapy. Optom Clin 2, 97-112
67. Shahidullah M, Mandal A, Wei G, Levin LR, Buck J and Delamere NA (2014) Nonpigmented ciliary epithelial cells respond to acetazolamide by a soluble adenylyl cyclase mechanism. Invest Ophthalmol Vis Sci 55, 187-197
68. Lee YS and Marmostein AD (2014) Control of outflow resistance by soluble adenylyl cyclase. J Ocul Pharmacol Ther 30, 138-142
69. Yu K, Lujan R, Marmorstein A, Gabriel S and Hartzell HC (2010) Bestrophin-2 mediates bicarbonate transport by goblet cells in mouse colon. J Clin Invest 120, 1722-1735
70. Zhang Y, Patil RV and Marmostein AD (2010)
Bestrophin 2 is expressed in human non-pigmented ciliary epithelium but not retinal pigment epithelium. Mol Vis 16, 200-206

71. Bakall B, McLaughlin P, Stanton JB et al (2008) Bestrophin-2 is involved in the generation of intraocular pressure. Invest Ophthalmol Vis Sci 49, 1563-1570

72. Zhang Y, Davidson BR, Stamer WD, Barton JK, Marmorstein LY and Marmorstein AD (2009) Enhanced inflow and outflow rates despite lower IOP in bestrophin-2-deficient mice. Invest Ophthalmol Vis Sci 50, 765-770

73. Chen Y, Cann MJ, Litvin TN et al (2000) Soluble adenylyl cyclase as an evolutionarily conserved bicarbonate sensor. Science 289, 625-628

74. Donegan RK and Lieberman RL (2016) Discovery of Molecular Therapeutics for Glaucoma: Challenges, Successes, and Promising Directions. J Med Chem 59, 788-809

75. Migdal C (2000) Glaucoma medical treatment: philosophy, principles and practice. Eye 14, 515-518

76. Medeiros FA and Weinreb RN (2002) Medical backflow and outflow pathways. JAMA Arch Ophthalmol 120, 797-803

77. Wheeler LA, Gil DW and WoldeMussie E (2001) Role of intravitreal injection of forskolin, homotaurine, and adrenoreceptor agonists: effects on ocular function. Handb Exp Pharmacol 208, 263-298

78. Zhang X, Wang N, Schroeder A and Erickson KA (2000) Expression of adenylate cyclase subtypes II and IV in the human outflow pathway. Invest Ophthalmol Vis Sci 41, 998-1005

79. Cracknell KP and Grierson I (2009) Prostaglandin analogues in the anterior eye: their pressure lowering action and side effects. Exp Eye Res 88, 786-791

80. Prasanna G, Li B, Mogi M and Rice DS (2016) Pharmacology of novel intraocular pressure-lowering targets that enhance conventional outflow facility: Pitfalls, promises and what lies ahead? Eur J Pharmacol 787, 47-56

81. Inda C, Dos Santos Claro PA, Bonfiglio JJ et al (2016) Different cAMP sources are critically involved in G protein-coupled receptor CRHR1 signaling. J Cell Biol 214, 181-195

82. Shen S, Wiertel AP, McMorris FA and Barres BA (1999) Retinal ganglion cells lose trophic responsiveness after axotomy. Neuron 23, 283-295

83. Goldblum J, Espinosa JS, Xu Y, Davidson N, Kovacs GT and Barres BA (2002) Retinal ganglion cells do not extend axons by default: promotion by neurotrophic signaling and electrical activity. Neuron 33, 689-702

84. Russo R, Adornetto A, Cavaliere F et al (2015) Intravitreal injection of forskolin, homotaurine, and L-carnosine affords neuroprotection to retinal ganglion cells following retinal ischemic injury. Mol Vis 21, 718-729

85. Rehen SK, Varella MH, Freitas FG, Moraes MO and Linden R (1996) Contrasting effects of protein synthesis inhibition and of cyclic AMP on apoptosis in the developing retina. Development 122, 1439-1448

86. Meyer-Franke A, Wilkinson GA, Kruttgen A et al (1998) Depolarization and cAMP elevation rapidly recruit TrkB to the plasma membrane of CNS neurons. Neuron 21, 681-693

87. Shaw PX, Fang J, Sang A, Wang Y, Kapiloff MS and Goldberg JL (2016) Soluble Adenylyl Cyclase Is Required for Retinal Ganglion Cell and Photoreceptor Differentiation. Invest Ophthalmol Vis Sci 57, 5083-5092

88. Lucas DR and Newhouse JP (1957) The toxic effect of sodium L-glutamate on the inner layers of the retina. AMA Arch Ophthalmol 58, 193-201

89. Olney JW and Ho OL (1970) Brain damage in infant mice following oral intake of glutamate, aspartate or cysteine. Nature 227, 609-611

90. Olney JW (1969) Glutamate-induced retinal degeneration in neonatal mice. Electron microscopy of the acutely evolving lesion. J Neuropathol Exp Neurol 28, 455-474

91. Lipton SA (2003) Possible role for memantine in protecting retinal ganglion cells from glaucomatous damage. Surv Ophthalmol 48 Suppl 1, S38-46

92. Dong CJ, Guo Y, Agey P, Wheeler L and Hare WA (2008) Alpha2 adrenergic modulation of NMDA receptor function as a major mechanism of RGC protection in experimental glaucoma and retinal excitotoxicity. Invest Ophthalmol Vis Sci 49, 4515-4522

93. Gao H, Qiao X, Cantor LB and WuDunn D (2002) Up-regulation of brain-derived neurotrophic factor expression by brimonidine in rat retinal ganglion cells. Arch Ophthalmol 120, 797-803

94. Dong CJ, Guo Y, Wheeler L and Hare WA (2007) Alpha2 adrenergic receptor-mediated modulation of cytosolic Ca++ signals at the inner plexiform layer of the rat retina. Invest Ophthalmol Vis Sci 48, 1410-1415

95. Maier C, Steinberg GK, Sun GH, Zhi GT and Maze M (1993) Neuroprotection by the alpha 2-adrenoceptor agonist dexmedetomidine in a focal model of cerebral ischemia. Anesthesiology 79, 306-312

96. Matsuo T and Cynader MS (1992) Localization of alpha-2 adrenergic receptors in the human eye. Ophthalmic Res 24, 213-219

97. Wheeler LA, Gil DW and WoldeMussie E (2001) Role of alpha-2 adrenergic receptors in neuroprotection and glaucoma. Surv Ophthalmol 45 Suppl 3, S290-294; discussion S295-296

98. Woldemussie E, Wijono M and Pow D (2007) Localization of alpha 2 receptors in ocular tissues. Vis Neurosci 24, 745-756

99. Kalapesi FB, Coroneo MT and Hill MA (2005) Human ganglion cells express the alpha-2 adrenergic receptor: relevance to neuroprotection. Br J Ophthalmol 89, 758-763

100. Evans DW, Hosking SL, Gherghel D and Bartlett JD (2003) Contrast sensitivity improves after brimonidine therapy in primary open angle glaucoma: a case for neuroprotection. Br J Ophthalmol 87, 1463-1465

101. Krupin T, Liebmann JM, Greenfield DS, Ritch R and Gardiner S (2011) A randomized trial of brimonidine versus timolol in preserving visual function: results from the Low-Pressure Glaucoma Treatment Study. Am J Ophthalmol 151, 671-681

102. Goldenberg-Cohen N, Dadon-Bar-El S, Hasanreisoglu M et al (2009) Possible neuroprotective effect of brimonidine in a mouse model of ischaemic optic neuropathy. Clin
103. Lee KY, Nakayama M, Aihara M, Chen YN and Araie M (2010) Brimonidine is neuroprotective against glutamate-induced neurotoxicity, oxidative stress, and hypoxia in purified rat retinal ganglion cells. Mol Vis 16, 246-251
104. Lambert WS, Ruiz L, Crish SD, Wheeler LA and Callkins DJ (2011) Brimonidine prevents axonal and somatic degeneration of retinal ganglion cell neurons. Mol Neurodegener 6, 4
105. Lee D, Kim KY, Noh YH et al (2012) Brimonidine blocks glutamate excitotoxicity-induced oxidative stress and preserves mitochondrial transcription factor a in ischemic retinal injury. PLoS One 7, e47098
106. Donello JE, Padillo EU, Webster ML, Wheeler LA and Gil DW (2001) alpha(2)-Adrenoceptor agonists inhibit vitreal glutamate and aspartate accumulation and preserve retinal function after transient ischemia. J Pharmacol Exp Ther 296, 216-223
107. Han Y and Wu SM (2002) NMDA-evoked [Ca2+]i increase in salamander retinal ganglion cells; modulation by PKA and adrenergic receptors. Vis Neurosci 19, 249-256
108. Miotke JA, MacLennan AJ and Meyer RL (2007) Immunohistochemical localization of CNTRFalpha in adult mouse retina and optic nerve following intraorbital nerve crush: evidence for the axonal loss of a trophic factor receptor after injury. J Comp Neurol 500, 384-400
109. Dunn TA, Storm DR and Feller MB (2009) Calcium-dependent increases in protein kinase-A activity in mouse retinal ganglion cells are mediated by multiple adenylyl cyclases. PLoS One 4, e7877
110. Dunn TA, Wang CT, Colicos MA et al (2006) Imaging of cAMP levels and protein kinase A activity reveals that retinal waves drive oscillations in second-messenger cascades. J Neurosci 26, 12807-12815
111. Park YH, Mueller BH 2nd, McGrady NR, Ma HY and Yoro T (2015) AMPA receptor desensitization is the determinant of AMPA receptor mediated excitotoxicity in purified retinal ganglion cells. Exp Eye Res 132, 136-150
112. Nicol X, Bennis M, Ishikawa Y et al (2006) Role of the calcium modulated cyclases in the development of the retinal projections. Eur J Neurosci 24, 3401-3414
113. Sunahara RK and Taussig R (2002) Isoforms of mammalian adenylyl cyclase: multiplicities of signaling. Mol Interv 2, 168-184
114. Jonas JB, Mardin CY, Schlotzer-Schrehardt U and Naumann GO (1991) Morphometry of the human lamina cribrosa surface. Invest Ophthalmol Vis Sci 32, 401-405
115. Schneider M and Fuchshofer R (2016) The role of astrocytes in optic nerve head fibrosis in glaucoma. Exp Eye Res 142, 49-55
116. Hernandez MR, Miao H and Lukas T (2008) Astrocytes in glaucomatous optic neuropathy. Prog Brain Res 173, 353-373
117. Hernandez MR (2000) The optic nerve head in glaucoma: role of astrocytes in tissue remodeling. Prog Retin Eye Res 19, 297-321
118. Anderson DR (1969) Ultrastructure of human and monkey lamina cribrosa and optic nerve head. Arch Ophthalmol 82, 800-814
119. Varela HJ and Hernandez MR (1997) Astrocyte responses in human optic nerve head with primary open-angle glaucoma. J Glaucoma 6, 303-313
120. Lukas TJ, Miao H, Chen L et al (2008) Susceptibility to glaucoma: differential comparison of the astrocyte transcriptome from glaucomatous African American and Caucasian American donors. Genome Biol 9, R111
121. Chen L, Lukas TJ and Hernandez MR (2009) Hydrostatic pressure-dependent changes in cyclic AMP signaling in optic nerve head astrocytes from Caucasian and African American donors. Mol Vis 15, 1664-1672
122. Ju WK, Kim KY, Noh YH et al (2015) Increased mitochondrial fission and volume density by blocking glutamate excitotoxicity protect glaucomatous optic nerve head astrocytes. Glia 63, 736-753
123. Son JL, Soto I, Oglesby E et al (2010) Glaucomatous optic nerve injury involves early astrocyte reactivity and late oligodendrocyte loss. Glia 58, 780-789
124. Sun D, Iye-Barthel M, Musland RH and Jakobs TC (2010) Structural remodeling of fibrous astrocytes after axonal injury. J Neurosci 30, 14008-14019
125. Dai C, Khaw PT, Yin ZQ, Li D, Raisman G and Li Y (2012) Structural basis of glaucoma: the fortified astrocytes of the optic nerve head are the target of raised intraocular pressure. Glia 60, 13-28
126. Leske MC (2007) Open-angle glaucoma – an epidemiologic overview. Ophthalmic Epidemiol 14, 166-172
127. Miao H, Chen L, Riorian SM et al (2008) Gene expression and functional studies of the optic nerve head astrocyte transcriptome from normal African Americans and Caucasian Americans donors. PLoS One 3, e2847
128. Trimmer PA and McCarthy KD (1986) Immunochemistry of defined astroglial from fetal, newborn and young adult rat glia: beta-adrenergic receptors are expressed by glia in vivo in the normal and injured central nervous system in the rat, rabbit, and human. J Neurosci 15, 152-164
129. Small KM, Brown KM, Theiss CT et al (2003) An Ile to Met polymorphism in the catalytic domain of adenylyl cyclase type 9 confers reduced beta2-adrenergic receptor stimulation. Pharmacogenetics 13, 535-541
130. Lopez-Canales OA, Castillo-Hernandez MC, Vargas-Robles H, Rios A, Lopez-Canales JS and Escalante B (2016) Role of adenylyl cyclase in reduced beta-adrenergic receptor-mediated vasorelaxation during maturation. Braz J Med Biol Res 49, e5285
131. Sato PY, Chuprun JK, Schwartz M and Koch WJ (2015) The evolving impact of g protein-coupled receptor kinases in cardiac health and disease. Physiol Rev 95, 377-404
132. Peppel K, Boekhoff I, McDonald P, Breer H, Caron MG and Lefkowitz RJ (1997) G protein-coupled receptor kinase 3 (GRK3) gene disruption leads to loss of odorant
receptor desensitization. J Biol Chem 272, 25425-25428
135. Yamamoto S, Sippel KC, Berson EL and Dryja TP (1997) Defects in the rhodopsin kinase gene in the Oguchi form of stationary night blindness. Nature Genet 15, 175-178
136. De Vries L, Zheng B, Fischer T, Elenko E and Farquhar MG (2000) The regulator of G protein signaling family. Annu Rev Pharmacol Toxicol 40, 235-271
137. Wang Q, Liu M, Mullah B, Siderovski DP and Neubig RR (2002) Receptor-selective effects of endogenous RGS3 and RGS5 to regulate mitogen-activated protein kinase activation in rat vascular smooth muscle cells. J Biol Chem 277, 24949-24958
138. Tezel G (2006) Oxidative stress in glaucomatous neurodegeneration: mechanisms and consequences. Prog Retin Eye Res 25, 490-513
139. Beal MF (1995) Aging, energy, and oxidative stress in neurodegenerative diseases. Ann Neurol 38, 357-366
140. Jenner P (1991) Oxidative stress as a cause of Parkinson’s disease. Acta Neurol Scand Suppl 136, 6-15
141. Coyle JT and Puttfarcken P (1993) Oxidative stress, glutamate, and neurodegenerative disorders. Science 262, 689-695
142. Dringen R, Pawlowski PG and Hirrlinger J (2005) Peroxide detoxification by brain cells. J Neurosci Res 79, 157-165
143. Fernandez-Fernandez S, Almeida A and Boloans J (2012) Antioxidant and bioenergetic coupling between neurons and astrocytes. Biochem J 443, 3-11
144. Noh YH, Kim KY, Shim MS et al (2013) Inhibition of oxidative stress by coenzyme Q10 increases mitochondrial mass and improves bioenergetic function in optic nerve head astrocytes. Cell Death Dis 4, e820
145. Lee D, Shim MS, Kim KY et al (2014) Coenzyme Q10 inhibits glutamate excitotoxicity and oxidative stress-mediated mitochondrial alteration in a mouse model of glaucoma. Invest Ophthalmol Vis Sci 55, 993-1005
146. Lee D, Kim KY, Shim MS et al (2014) Coenzyme Q10 ameliorates oxidative stress and prevents mitochondrial alteration in ischemic retinal injury. Apoptosis 19, 603-614
147. Kim SY, Shim MS, Kim KY, Weinreb RN, Wheeler LA and Ju WK (2014) Inhibition of cyclophilin D by cyclosporin A promotes retinal ganglion cell survival by preventing mitochondrial alteration in ischemic injury. Cell Death Dis 5, e1105
148. Yan L, Vatner DE, O’Connor JP et al (2007) Type 5 adenylyl cyclase disruption increases longevity and protects against stress. Cell 130, 247-258
149. Ogura M, Taniura H, Nakamichi N and Yoneda Y (2007) Upregulation of the glutamine transporter through trans-activation mediated by cAMP/protein kinase A signals toward exacerbation of vulnerability to oxidative stress in rat neocortical astrocytes. J Cell Physiol 212, 375-385
150. Ciccarelli R, D’Alimonte I, Ballerini P et al (2007) Molecular signalling mediating the protective effect of A1 adenosine and mGlu3 metabotropic glutamate receptor activation against apoptosis by oxygen/glucose deprivation in cultured astrocytes. Mol Pharmacol 71, 1369-1380
151. Durand D, Camiglia L, Caruso C and Lasaga M (2011) Reduced cAMP, Akt activation and p65-c-Rel dimerization: mechanisms involved in the protective effects of mGluR3 agonists in cultured astrocytes. PLoS One 6, e22235
152. Seifert R (2013) Functional selectivity of G-protein-coupled receptors: from recombinant systems to native human cells. Biochem Pharmacol 86, 853-861
153. Berstein G, Blank JL, Smrcka AV et al (1992) Reconstitution of agonist-stimulated phosphatidylinositol 4,5-biphosphate hydrolysis using purified m1 muscarinic receptor, Gq/11, and phospholipase C-beta 1. J Biol Chem 267, 8081-8088
154. Buck MA and Fraser CM (1990) Muscarinic acetylcholine receptor subtypes which selectively couple to phospholipase C: pharmacological and biochemical properties. Biochem Biophys Res Commun 173, 666-672
155. Burford NT and Nahorski SR (1996) Muscarinic m1 receptor-stimulated adenylyl cyclase activity in Chinese hamster ovary cells is mediated by Gs alpha and is not a consequence of phosphoinositidase C activation. Biochem Biophys Res Commun 173, 666-672
156. Jones SV, Heilman CJ and Brann MR (1991) Functional responses of cloned muscarinic receptors expressed in CHO-K1 cells. Mol Pharmacol 40, 242-247
157. Caulfield MP (1993) Muscarinic receptors—characterization, coupling and function. Pharmacol Ther 58, 319-379