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Review

Soluble adenylyl cyclase in the eye ☆

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ABSTRACT

Adenylyl cyclases (ACs) are a family of enzymes which convert ATP to cAMP, an essential intermediate in many signal transduction pathways. Of the 10 AC genes in man, 9 fall into the category of transmembrane ACs (tmACs), which associate with G-protein coupled receptors (GPCRs) and are activated by forskolin. The 10th AC, termed soluble AC (sAC) is neither activated by forskolin nor does it interact with GPCRs. Rather, sAC can be found in many compartments within the cell and is activated by bicarbonate. As such, sAC is considered a major sensor of bicarbonate in many tissues. The pathways involving sAC vary in different tissues and organ systems, and are as diverse as facilitating sperm capacitation and regulating pressure in the eye. The role of sAC in the eye has only recently begun to receive significant attention. Here we summarize what is known about the roles of sAC in the eye. This article is part of a Special Issue entitled: The role of soluble adenylyl cyclase in health and disease.

1. Introduction

ACs are a family of enzymes that catalyze the conversion of adenosine-5′-triphosphate (ATP) to cyclic adenosine 3′, 5′ monophosphate (cAMP) [1]. In mammals, there are 10 distinct AC genes [1]. Nine of these encode transmembrane adenylyl cyclases (tmACs) which exhibit differential expression patterns, are stimulated by forskolin, and physically and functionally interact with G-protein coupled receptors (GPCRs) [2–4]. The tenth AC gene encodes the most evolutionarily conserved but most recently identified member of the AC family; soluble adenylyl cyclase (sAC) [5]. Theodor Braun first reported soluble AC activity in rat testis in 1975 [6]. Initially this was predicted to result from the activity of the well-known tmACs. Over twenty years later, the laboratory of Jochen Buck and Lonny Levin succeeded in isolating sAC and cloning the gene demonstrating that sAC is a unique enzyme that does not interact with GPCRs and is not activated by forskolin [5]. Instead, sAC is activated by bicarbonate and requires a divalent cation such as Ca^{2+} for its activity [7–13]. There are at least two sAC isoforms generated through alternative splicing that have different regulatory characteristics. sAC is expressed in many different cell types and can be localized anywhere in the cell [2,6,7,14–20]. It's involvement in a wide array of signaling pathways in multiple organ systems has only recently begun to gain attention [14,17,20–24]. But in the end sAC is always functioning as a sensor of HCO_3^- and affecting the pathways it interacts with based on local HCO_3^- concentration [11].

In the eye the metabolic needs of the various tissues that form the optical system necessary for vision vary. Some tissues in the eye like the cornea and lens are avascular [25], others are among the most metabolically active in the body. The need for a HCO_3^- sensor in eye tissues is obvious, however, the role of sAC in the eye and the pathways in which it participates have not been extensively studied. One can imagine many reasons why various ocular tissues might require regulation in response to changes in the local concentration of HCO_3^-. For example, the concentration of CO_2/HCO_3^- in the retina varies greatly depending on light [26,27]. Our laboratory has been interested in understanding the many roles of sAC in the eye. Here we will review what is known about sAC and ocular physiology.

2. sAC expression in the eye

The first identification of sAC in the eye was in the corneal endothelia (CE) by Sun et al. in 2003 [28]. Since then several groups have reported on the expression of sAC in various ocular tissues [15,16,29–31]. However, no systematic study of sAC activity or expression has been undertaken in the eye to date. We performed a comprehensive sAC expression profile in the mouse eye by immunofluorescence staining. Staining of sAC was abundant in most ocular tissues including the cornea, the ciliary body, and the retina. In the lens, sAC was observed in the epithelial cells lining the anterior surface of the lens, but not in the fiber cells. We did not note significant staining of the choroid, or sclera, and staining was absent from drainage tissues (trabecular meshwork and Schlemm's canal) [16]. These data confirm that most of the major sites of sAC activity in the eye (ciliary body, cornea, and retina) have been identified and confirmed using enzymatic assays for sAC activity.

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Exceptions to this include the retinal pigment epithelium (RPE) and several layers of the neurosensory retina.

2.1. sAC in the cornea

The cornea is a clear avascular structure through which light enters the eye. It is comprised primarily of the stroma, a connective tissue which is rich in collagen and proteoglycans [32]. The outer layer of the cornea is a non-keratinized stratified epithelium which is continuously renewed [32]. Lining the inner surface of the stroma is the corneal endothelium (CE) [33]. The CE forms a monolayer of hexagonally shaped cells that play an indispensable role in maintaining the proper hydration of the stroma, without which, the transparency of the cornea cannot be maintained [32,34,35]. As shown in Fig. 1, sAC is expressed by cells in all three layers. The role of sAC in the cornea however, has been examined only for the CE [36].

The CE forms a leaky barrier allowing nutrients from the aqueous humor to pass into the corneal stroma [35]. Proper hydration however, requires the CE to continuously pump H_2O out of the stroma, a process that is stimulated by HCO_3^- and follows a Cl^- gradient [37]. In 2003, Sun et al., demonstrated the presence of sAC in primary cultures of bovine corneal endothelial cells [28]. In that study, sAC was found distributed evenly throughout the cytoplasm of CE cells [18]. HCO_3^- treatment resulted in an increase in cAMP production measured in the presence of the phosphodiesterase inhibitor rolipram [28]. This correlated with a 78% increase in apical Cl^- permeability in the cells, which was determined to occur through the activation of the cystic fibrosis transmembrane conductance regulator (CFTR) [28]. As fluid transport is coupled to both HCO_3^- and Cl^- gradients, these data implicate sAC as a potentially important player in maintaining corneal transparency by regulating Cl^- secretion in the CE in response to changes in HCO_3^-.

CE cell density drops progressively with age. During one's lifetime the density of CE cells can drop from an average of 5500 cells/mm^2 at birth to as low as 400–700 cells/mm^2 without diminishing corneal transparency [38,39]. CE cells do not undergo mitosis *in vivo* [40]. Excessive loss of CE due to surgery, trauma, or disease, can cause a loss of corneal opacity. Li et al., demonstrated that HCO_3^- promotes corneal epithelial cell survival when the cells are exposed to a pro-apoptotic stimulus [36]. Sun et al. found that sAC expression in CE at both the mRNA and protein levels is regulated by HCO_3^- in a dose and time dependent manner [18]. In most cells cAMP is anti-apoptotic [41], and the levels of cAMP in CE are elevated in response to the HCO_3^- [29]. The anti-apoptotic effect of HCO_3^- was significantly reduced in cells in which sAC was reduced using siRNA, or treated with 2-hydroxyestradiol (2HE), a non-specific sAC inhibitor implicating sAC as important in the protection and survival of CE cells [29,36].

2.2. sAC in the retina

The retina is located in the posterior eye and is comprised of the photoreceptive cells and various layers of neurons and glia. These layers are shown in Fig. 2. Immunostaining of the retina for sAC indicates that the enzyme is expressed throughout its various cell layers. Although cAMP plays a multitude of roles in regulating various aspects of vision, notably on cell survival [42,43], the role of sAC in the retina is for the most part unknown. Most work on retinal sAC has centered on its function in retinal ganglion cells (RGCs) [15,31]. RGCs are the neurons that connect the retina to the brain. Their perikarya are found in the ganglion cell layer of the retina, and their axons comprise the optic nerve. Most RGCs synapse in the lateral geniculate nucleus, though RGCs involved in circadian functions and pupillary reflexes terminate elsewhere in the brain [44,45]. RGC death is the reason for vision loss in glaucoma [46], a disease that causes vision impairment in 2.7 million Americans over the age of 40.

A number of laboratories have reported that cAMP is critical to RGC survival and axonal growth [47–50]. The first report of a potential role of sAC in RGCs came in 2009. Dunn et al., examined the roles of specific ACs and phosphodiesterase inhibitors in Ca^{2+}-dependent protein kinase A (PKA) activation in RGCs [31]. Ca^{2+}-dependent PKA activation has been reported to be involved in neuronal survival, and axonal growth and fine tuning. They found that Ca^{2+}-dependent PKA transients are retained even during blockade of transmembrane ACs suggesting a role for sAC in PKA activation. However, the specific role of sAC was not investigated in that study.

Corredor et al., hypothesized that sAC generated increases in cAMP could underlie the survival and axon growth effects of cAMP in RGCs [15]. To test this they applied HCO_3^- or electrical stimulation to cultured RGCs and measured survival and axonal growth. HCO_3^- was found to increase axon growth but had no effect on survival. In contrast to HCO_3^- electrical stimulation had a positive effect on RGC survival. Similar results were obtained when sAC was inhibited with KH7 or 2HE, or knocked down using anti-sAC siRNA. Interestingly, inhibitors of tmACs had no effect on survival or axonal growth of RGC cultures from C1/AC8 double KO mice, though the competitive PKA inhibitor H89 did confirm the hypothesis of Dunn et al., [31] indicating that tmACs may be less important than sAC in mediating RGC cell growth and survival.

To further test this, Corredor et al., examined the effect of KH7 on RGC survival in adult rat retinas [15]. Injection of KH7 significantly reduced the number of RGCs in the retina after 5 days, although when the same experiment was performed following optic nerve crush injury, no effect of KH7 was observed. Examination of sAC expression 6 days after optic nerve crush indicated that sAC had been down-regulated in response to the injury. The authors conclude that sAC elevates cAMP in RGCs and is required for RGC survival.

Although all work on retinal sAC has centered on its role in RGCs, sAC is expressed by other retinal neurons including photoreceptors and by the RPE as well. Similar to RGCs, a role for cAMP in photoreceptor survival has been reported [51] though it is not known if sAC is the physiological source. Photoreceptor metabolic activity varies greatly in dark *versus* light, resulting in dramatic changes in CO_2/HCO_3^- concentration in the outer retina. Excess CO_2/HCO_3^- is transported out of the retina by the RPE [52–54]. Determining whether there is a significant role for sAC in helping the retina adjust to the changes in CO_2/HCO_3^- occurring in light and dark is a topic worthy of future investigation.

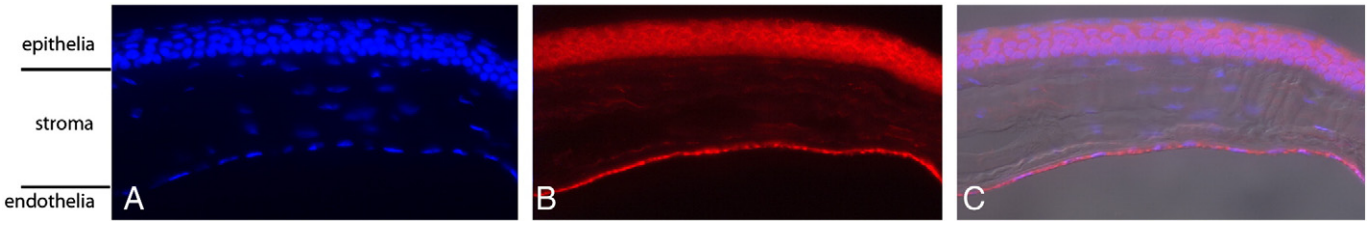

Fig. 1. Expression of sAC in the cornea. Nuclei were stained with DAPI (A). Staining for sAC in the cornea with a sAC specific monoclonal antibody (B). Merging of sAC and DAPI staining with a differential interference contrast image of the same field (C) reveals that sAC is expressed in cells throughout the cornea.

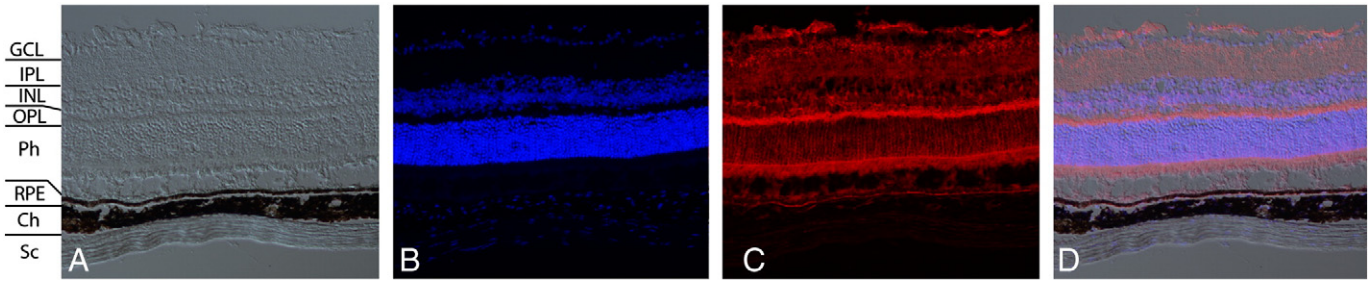

Fig. 2. Expression of sAC in the retina. Differential interference contrast image (A) shows various layers of the neurosensory retina, choroid (Ch), and sclera (Sc). Cellular components of these layers are identified by nuclear staining with DAPI (B). Staining for sAC using a sAC specific monoclonal antibody (C) reveals expression throughout the layers of the neurosensory retina and the retinal pigment epithelium (RPE). Merging the images (D) shows that sAC (red) is highly expressed in the retina and RPE, but little sAC staining is present in the choroid or sclera. Ph = photoreceptors; OPL = outer plexiform layer; INL = inner nuclear layer; IPL = inner plexiform layer; GCL = ganglion cell layer.

2.3. sAC in the ciliary body

The ciliary body is a triangular shaped region of the eye that lies between the scleral spur and the retina. It plays a role in accommodation and also the generation of aqueous humor (AH). The AH provides nourishment to the avascular tissues (cornea and lens) of the anterior chamber of the eye. AH is continually produced and drained from the eye. These pathways termed "inflow" and "outflow" pathways, respectively, generate intraocular pressure (IOP) through the resistance to the drainage of AH. A healthy IOP (~15 mm Hg) is required to maintain the shape and optical properties of the eye [55]. Although glaucoma is a disease that results from the death of retinal ganglion cells, the only effective treatment for glaucoma is to lower IOP [46]. For this reason the physiology of the ciliary body and the control of AH dynamics is an area that has received a great deal of attention.

It has long been known that HCO_3^- and cAMP play important roles in regulating IOP [56–62]. AH is generated by the pigmented epithelial cells (PEs) and non-pigmented epithelial cells (NPEs) of the ciliary processes (Fig. 3A). Among the many agents that reduce AH secretion is forskolin [63] suggesting that cAMP diminishes AH production. Similarly, cAMP is known to facilitate drainage by the conventional outflow pathway [57,64–68]. This pathway is pressure responsive and is formed by the trabecular meshwork and Schlemm's canal. In 1993, Mittag et al. reported a HCO_3^- sensitive adenylyl cyclase (AC) activity in the rabbit ciliary body [17]. Following our identification of sAC in the ciliary epithelia as the enzyme responsible for this activity [16] (Fig. 3), Shahidullah et al. [24] demonstrated that sAC participates in the regulation of pH in ciliary epithelial cells and that this function is altered in response to acetazolamide, a carbonic anhydrase inhibitor. Application of acetazolamide to the eye results in a decrease in IOP due to a diminished rate of AH production. While sAC is not linked to the formation of aqueous humor, Shahidullah et al., did show that cAMP was elevated in NPE cells following acetazolamide treatment and that this elevation of cAMP was sensitive to HCO_3^- and the sAC inhibitor KH7. The downstream effect of the sAC mediated increase in cAMP was enhanced H^+ transport.

We became interested in the role of sAC in the ciliary body as a result of our work on Bestrophin 2 (Best2) [69–71]. The bestrophins are a family of integral membrane proteins that function as anion channels [72–78]. There are 4 bestrophins in the human genome. Only two, Best1 and Best2 are known to be expressed in the eye, and Best2 is only expressed by the non-pigmented epithelium (NPE) of the ciliary body [69,70,79,80]. Since Best2 functions as a bicarbonate channel [71], we hypothesized that IOP in *Best2*$^{-/-}$ mice would be increased due to an increase in bicarbonate in NPE cells lacking Best2. However, Best2$^{-/-}$ mice displayed a significantly lower IOP than their Best2$^{+/+}$ WT littermate controls [70]. A comprehensive examination of aqueous dynamics in the mutant mice revealed that they do produce increased aqueous humor when compared to Best2$^{+/+}$ littermate controls [70]. However, conventional and uveoscleral drainage of AH in Best2$^{-/-}$ mice increased sufficiently to overcompensate for the increase in AH generated [70]. Since Best2 is found only in the ciliary body, and there were no noticeable anatomic changes in the anterior chamber of the Best2$^{-/-}$ mice, we concluded that there must be a mechanism for pressure-independent, biochemical communication between the inflow and outflow pathways.

Since Best2 is a HCO_3^- channel we reasoned that this activity is likely due to sAC and that it could play a role in the communication between the ciliary body and drainage tissues. Enzyme assays confirmed the presence of a HCO_3^- inducible, KH7 sensitive adenylyl cyclase activity indicating expression of sAC in the ciliary body, which was confirmed by immunoprecipitation and Western blot [16]. Immunofluorescence staining demonstrated that sAC is localized to the PEs and NPEs of the ciliary processes (Fig. 3).

To test the hypothesis that sAC in the ciliary body is a mediator of outflow resistance, we administered the sAC specific inhibitor KH7 to mice by intraperitoneal injection and measured changes in IOP and episcleral venous pressure (EVP) 2 h after the injection [16]. We found that wild-type mice treated with KH7 showed a >40% increase in IOP but no increase in EVP, indicating that the effect of KH7 was specific to the formation and/or drainage of aqueous humor [16]. Best2$^{-/-}$ mice injected with KH7 exhibited a >45% increase in IOP–EVP in comparison to controls [16]. To elucidate the mechanism behind this IOP increase we measured the effect of KH7 on inflow and outflow in mice. We found that KH7 caused a ~50% decrease in conventional outflow compared to controls. However, there was no statistically significant change in inflow. Uveoscleral outflow was increased about 16% in KH7 treated mice over controls, a difference within the range of our experimental error [16]. No sAC enzyme activity or immunoreactivity was observed in drainage tissues [16]. These data suggested that sAC in the ciliary body plays a significant role in the regulation of outflow resistance,

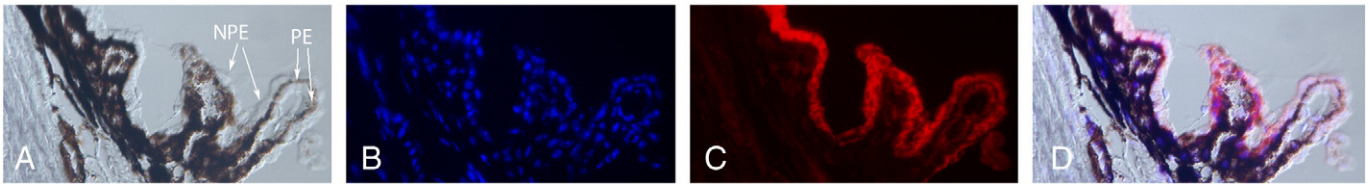

Fig. 3. Expression of sAC in the ciliary body. Differential interference contrast image of the mouse ciliary body (A) and cell nuclei stained with DAPI (B). Staining for sAC with a sAC specific monoclonal antibody (C). Merging of the images (D) reveals expression in the epithelial cells of the ciliary processes.

which had not been previously known to be a function of the ciliary body.

To further test the hypothesis that sAC in the ciliary body played a role in the regulation of outflow resistance, we studied aqueous humor dynamics in *Sacy*$^{tm1Lex/tm1Lex}$ mice which lack the C1 catalytic domain of sAC. We found that *Sacy*$^{tm1Lex/tm1Lex}$ mice exhibit a higher IOP and higher outflow resistance than wild type littermate controls [16]. However, as would be expected in mice lacking sAC, KH7 had no significant effect on these parameters in *Sacy*$^{tm1Lex/tm1Lex}$ mice [16]. These results strongly suggest that sAC is a critical regulator of IOP and provide very strong evidence for the existence of a biochemical pathway for communication between the CB and drainage tissues that is regulated by HCO_3^- and cAMP. The results of sAC inhibition on IOP and outflow facility show the clearest evidence to date that a communicative pathway must exist between the ciliary body and drainage tissues for regulating outflow facility independent of pressure, and suggest new areas for therapeutic intervention for the regulation of IOP in the treatment of glaucoma.

3. Concluding remarks

The eye is a complex sensory organ with tissues that form a living optical system capable of focusing and detecting light, and transmitting that information to the brain. The metabolic needs of the various ocular structures are highly variable. As such, one can easily envision the need for a cellular HCO_3^- sensor and how critical it would be in maintaining an environment conducive to vision. As we have seen, in CE cells and RGCs sAC potentially plays a fundamental role in cell survival. In the cornea and ciliary body sAC also seems to regulate fluid and ion transport, and in the ciliary body, sAC plays a role in a paracellular signaling pathway that regulates outflow resistance. The role of sAC in regulating outflow resistance and in the survival of RGCs and CE cells suggests that pathways dependent on sAC might be worthy targets for therapies in glaucoma and corneal disease.

sAC is broadly expressed in ocular tissues. It is likely that many of the roles of sAC in the eye have yet to be elucidated. In the cornea sAC is highly expressed in the stratified epithelia that form the outer corneal surface, but its role there is unknown. sAC is expressed in all layers of the retina but only in RGCs has its role been tested experimentally. Considering the changes in metabolic rate that occur in the retina in response to light, it would seem that sAC could play a number of important, but as yet unrecognized roles. The importance of the very few pathways already identified in the eye in which sAC is a player suggests that future work in this area could lead to an enhanced basic knowledge of cAMP signaling in these tissues and potentially to the identification of new pathways that could be therapeutic targets in a variety of blinding eye diseases.

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