Review: Role of cAMP signaling in diabetic retinopathy

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Despite decades of research, diabetic retinopathy remains the leading cause of blindness in working age adults. Treatments for early phases for the disease remain elusive. One pathway that appears to regulate neuronal, vascular, and inflammatory components of diabetic retinopathy is the cyclic adenosine 3', 5'-monophosphate (cAMP) pathway. In this review, we discuss the current literature on cAMP actions on the retina, with a focus on neurovascular changes commonly associated with preproliferative diabetic retinopathy models.

Diabetic retinopathy is the leading cause of blindness in working age adults. Although a large number of therapeutics have been tested in animal models, none has led to a successful treatment for non-proliferative diabetic retinopathy. Anti-VEGF is effective for some cases of proliferative diabetic retinopathy and macular edema [1,2]. Reactive oxygen species, increased inflammatory factors, imbalanced growth factors, dyslipidemia, and dysfunctional insulin signal transduction have all been suggested to be involved in the pathogenesis of diabetic retinopathy [3-7]. In addition, altered cAMP signaling can regulate many of these same pathways, leading to retinal damage.

Cyclic adenosine 3', 5'-monophosphate (cAMP) is a G-protein mediated signaling pathway that can regulate several downstream pathways, including those related to gluconeogenesis, muscle contraction, a large number of transcription factors, as well as many others [8]. Once activated, cAMP can lead to activation of protein kinase A (PKA), exchange protein activated by cAMP (Epac), and popeye domain containing proteins (Popdc) [9-11], as well as ion channels. Although cAMP can regulate a plethora of signaling cascades, its role in diabetic retinopathy has been less well studied. In diabetic retinopathy, changes in permeability, neurons, and vasculature, and inflammatory proteins have been recorded in animal models of non-proliferative diabetic retinopathy (Figure 1) [12-14].

cAMP signaling in permeability changes in the diabetic retina: Original studies from bovine retinal cells showed that beta-adrenergic receptors increase cAMP, leading to decreased permeability [15]. More recent studies on bovine retinal endothelial cells found cAMP was key in the maintenance of the retinal barrier, and that TNFα decreased intracellular cAMP levels [16]. We developed a novel beta-adrenergic receptor agonist, Compound 49b, and reported that Compound 49b regulated key barrier proteins, occludin and zonula occludens 1 (ZO-1), in retinal endothelial cells grown in high glucose [17]. In that study, we used Epac1 siRNA to demonstrate that Compound 49b required Epac1 to maintain the barrier [17]. To further investigate the role of Epac1 in retinal permeability in diabetes, we made diabetic Epac1 floxed and cdh5Cre-Epac1 mice to eliminate Epac1 in endothelial cells and used fluorescein angiography and Evan’s blue studies to demonstrate that Epac1 is key to reduced retinal permeability in the diabetic retina [18]. Similarly, work by Ramos et al. demonstrated that Epac1 activation of Rap1 reverses cytokine-induced increases in permeability in bovine retinal endothelial cells [19]. The work with Epac1 mice and bovine retinal endothelial cells agrees with a review article by Wilson and Ye, suggesting that Epac1 and Rap1 regulate retinal permeability [20]. Less has been done to focus on PKA in retinal permeability in diabetes. However, studies in healthy mice showed that PKA can phosphorylate connexin 36 to reduce retinal permeability in amacrine cells [21]. There remains a need to further understand the specific mechanisms by which Epac1 and PKA can regulate permeability in the diabetic retina. Taken together, data suggest that cAMP signaling is key to barrier maintenance.

Role of cAMP signaling in vascular damage in diabetic retinopathy: Few groups have explored the role of cAMP signaling in the diabetic retinal vasculature. We showed that Compound 49b, which likely increased Epac1 and PKA, protects against the formation of degenerate capillaries in the diabetic retina [22]. We also recently reported that Epac1 endothelial cell knockout (KO) mice have increased numbers of degenerate capillaries when exposed to diabetes [18] and ischemia/reperfusion (I/R) at 10 days post-ischemia [23]. An additional study of retinal pericytes in culture confirmed that PKA is key to retinal pericyte contractility [24]. In contrast to work in the diabetic mouse models, a recent study showed...
that Epac1 inhibition reduces retinal angiogenesis in the oxygen-induced retinopathy model [25]. Other studies in cancer models also showed that Epac1 promotes angiogenesis through various signaling cascades [26,27], suggesting that Epac1 may have different actions on the vasculature depending on the cellular milieu. Thus, more studies on the role of Epac1 in the diabetic retinal vasculature are needed.

**cAMP signaling actions on neuronal changes in the diabetic retina:** Retinal ischemia can produce neuronal changes similar to diabetes [28], including reduced retinal thickness and loss of cell numbers in the ganglion cell layer. Using the I/R model with measurements at 2 days post-ischemia, studies have shown that increasing cAMP signaling can increase retinal ganglion cell (RGC) regeneration following damage [29]. Similarly, we found that Epac1 prevents the loss of retinal thickness and cell numbers in diabetic mice [18] and in the I/R model using endothelial cell–specific Epac1 KO mice [23]. These findings agree with those of a study that used the I/R model showing that Epac2 protects against neuronal damage in the retina [30]. In contrast, another group using whole animal Epac1 KO mice found that Epac1 promotes retinal neurodegeneration [31]. These authors performed their measurements at 24 h post-ischemia and measured apoptosis, not retinal thickness. Few studies have investigated PKA actions on neuronal changes in the diabetic retina. Therefore, additional work is needed to clarify cAMP actions on retinal neuronal changes in response to diabetes.

**Actions of cAMP signaling in inflammatory markers in the diabetic retina:** Work in healthy rodents showed that norepinephrine and cAMP are key to regulation of a large number of night and day genes, including a large number of inflammatory genes [32]. Work in Epac2 knockout mice exposed to I/R showed increased glial fibrillary acidic protein (GFAP) in the retina [30]. Increased GFAP signaling is often associated with increased inflammatory mediators in the retina [33]. Linking cAMP signaling to diabetic retinopathy, one group showed that Epac1 regulates O-GlyNAcylation in mice on a high-fat diet [34]. These changes included reduced Mas signaling and measurements of mitochondrial superoxide dismutase [35].

In previous work, we showed that Compound 49b reduces TNFα levels in retinal endothelial cells exposed to high glucose, which is associated with decreased retinal damage in response to diabetes [22]. Recently, we reported that Epac1 decreases inflammatory mediators in human primary retinal endothelial cells grown in high glucose [37]. Taken together, the data strongly suggest that cAMP signaling can reduce inflammatory mediators in multiple retinal cell types, which protects the retina against stressors.

**Conclusions:** Despite knowledge of the actions of the cAMP pathway on a large number of signaling cascades, much less work has been conducted to investigate actions of cAMP and its downstream mediators, PKA and Epac, on the diabetic retina. Most studies have shown that cAMP signaling is protective to the diabetic retina, reducing neuronal, vascular, permeability, and inflammatory changes; however, other studies showed detrimental effects. Additional work is needed to determine the cellular mechanisms by which cAMP can act...
on the retina, as well as optimizing cAMP-based therapies for systemic delivery.

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